nederlandse vereniging voor Medische Microbiologie

NEDERLANDS TIJDSCHRIFT VOOR MEDISCHE MICROBIOLOGIE

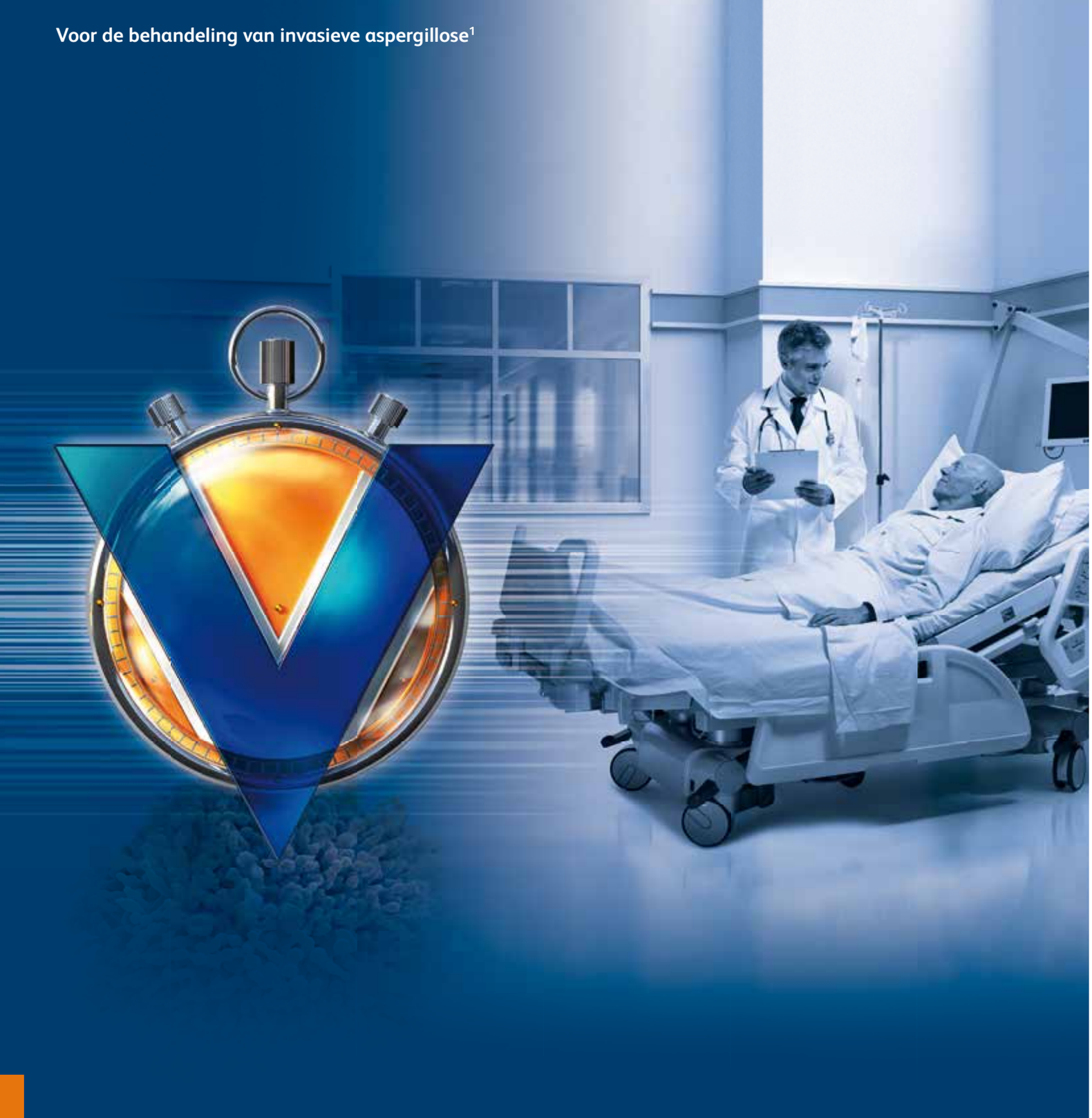
Supplement bij tweeëntwintigste jaargang, april 2014

Voorjaarsvergadering van de Nederlandse Vereniging voor Medische Microbiologie (NVMM) en de Koninklijke Nederlandse Vereniging voor Microbiologie (KNVM)

Papendal, 15 & 16 april 2014
Programma-overzicht
Abstracts
Auteursindex

22e jaargang . april 2014 . Supplement

Voor de behandeling van invasieve aspergillose[1]



Kom nu in actie tegen invasieve aspergillose [2,3,4]

Voor referenties en samenvatting productkenmerken zie elders in deze uitgave.

14.VFE.21.13. Aanmaakdatum: Maart 2014.

Pfizer Anti-Infectives

IV/PO
VFEND®
(voriconazole)

Redt levens[9]

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The Scientific Spring Meeting is organized by the Dutch Society of Medical Microbiology (NVMM) and the Royal Dutch Society of Microbiology (KNVM).

The Scientific Spring Meeting KNVM & NVMM has been financially supported by:

NWO
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Meeting secretariat

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P.O. Box 2428
5202 CK 's-Hertogenbosch
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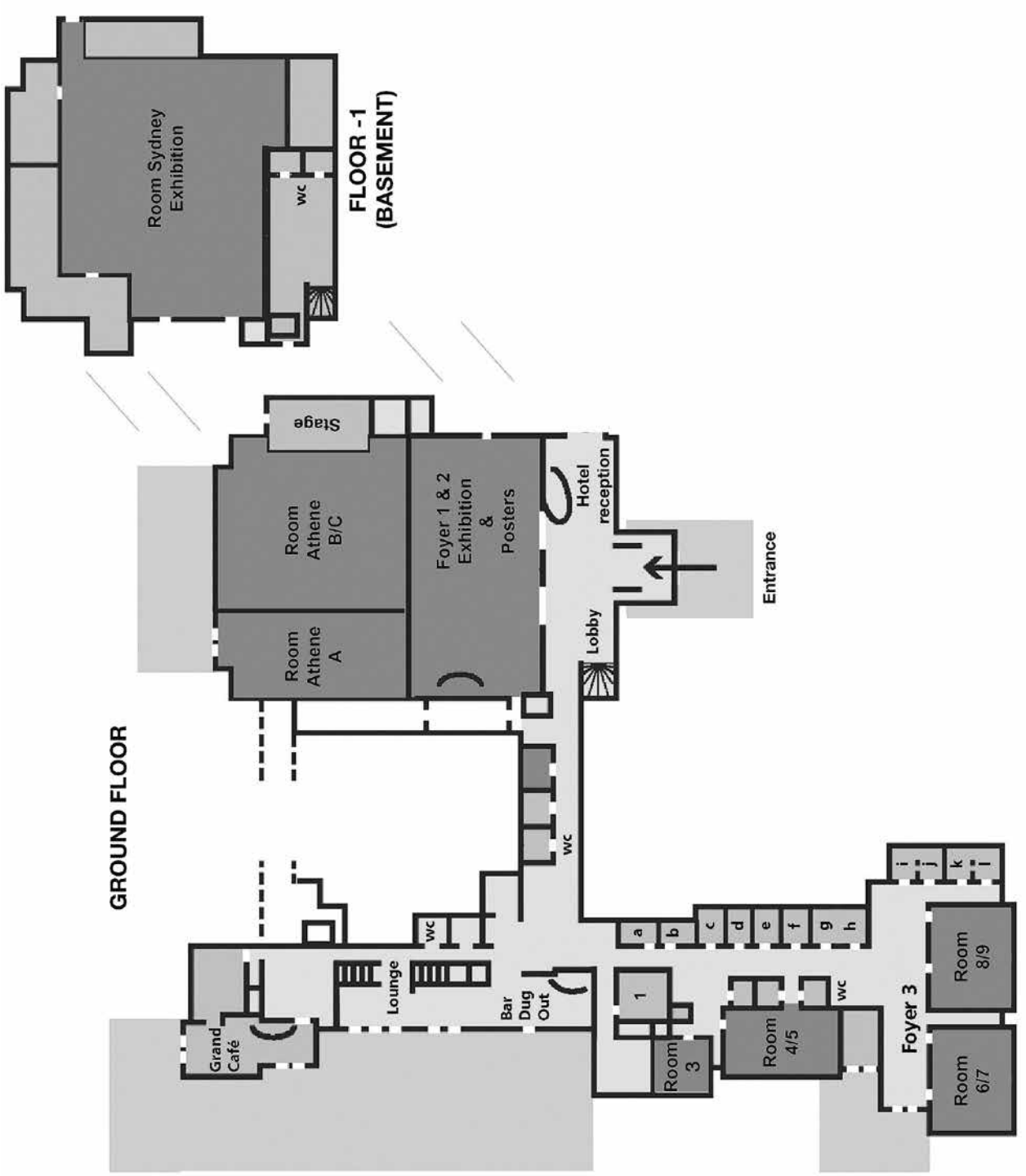
TUESDAY APRIL 15, 2014							
	EXHIBITION	ROOM ATHENE B/C	ROOM ATHENE A	ROOM 3	ROOM 4/5	ROOM 6/7	ROOM 8/9
09:00 - 09:30	Registration						
09:30 - 11:00		Plenary session					
11:00 - 11:30	Coffee/tea						
11:30 - 12:45		Plenary session & Award Ceremony					
12:45 - 14:00	Lunch		KNVM Business Meeting				
14:00 - 15:30		Developments in antiviral therapy and antiviral resistance	Staphylococcal virulence	Campylobacter: latest insights into epidemiology, pathogenesis and intervention	Determining the value of surveillance programs of healthcare-associated infections and antimicrobial resistance: what are we missing?	New insights in epidemiology and treatment of *Clostridium difficile* infections (CDI)	Antoni van Leeuwenhoek: Scientist or Clever Burgher?
15:30 - 16:00	Coffee/tea						
16:00 - 17:30		Recent discoveries in anaerobic methane oxidation	Use of Next Generation Sequencing (NGS) for diagnostics and epidemiology in clinical practice	Bacterial competence of human pathogens	Opportunistic parasitic infections and their atypical clinical presentation	Microbe-macrobe symbioses - section microbial ecology	Improvement of vaccines against seasonal influenza
17:30 - 18:30	Drinks						
18:30 - 20:30	Dinner (restaurant)						
20:30 - 22:15		Poster session & Poster award ceremony					
22:15 - 01:30	Party						

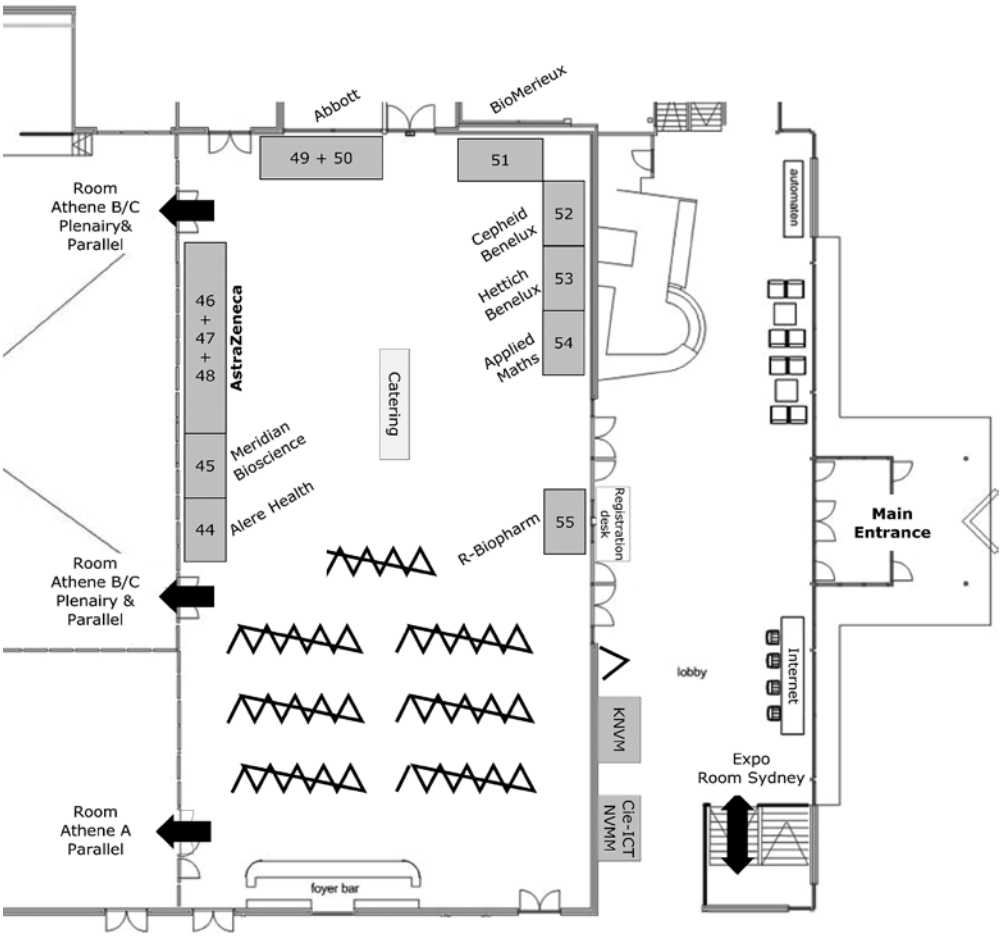
PROGRAMME

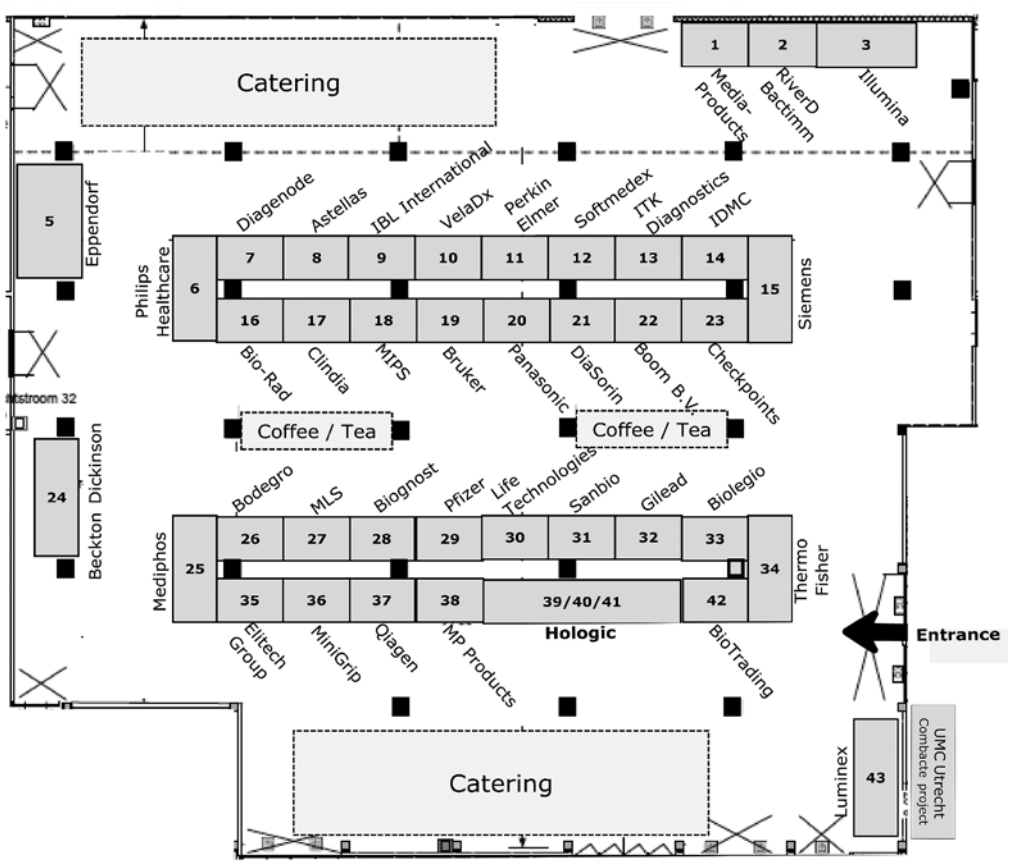
WEDNESDAY APRIL 16, 2014							
	EXHIBITION	ROOM ATHENE B/C	ROOM ATHENE A	ROOM 3	ROOM 4/5	ROOM 6/7	ROOM 8/9
08:30 - 09:00	Registration						
09:00 - 10:30		Ecology and evolution of antibiotic resistance	Werkgroep MALDI-TOF & NVMy: Update on microbial diagnostics – application of MALDI-TOF		Glycobiology in health and disease	Bachelor and Master (BAMA) Symposium	Pathogenesis 1
10:30 - 11:00	Coffee/tea						
11:00 - 12:30		Pathogenesis 2	NWKV & WAMM: Clinical cases in medical microbiology: an interactive session	Tuberculosis: New NVMM Diagnostic Guideline and nontuber-culous mycobacteria	Microbiome	Bachelor and Master (BAMA) Symposium	Viral infections in immunocompromised patients
12:45 - 13:45		Lunch Symposium: Experience the possibilities with BD MAX™	BBC-MMO business meeting				
14:00 - 15:30		General microbiology	WAMM, WMDI & NWKV: Laboratory of the Future	Risk Management: another step ahead!	A closer look at the Far East: medical micro-biology in South-East Asian 'Tigers'	*Dientamoeba fragilis* infections: clinical relevance, diagnosis and treatment	Bacterial vaccines: from failures to promises
15:30 - 16:00	Coffee/tea						
16:00 - 18:00		NVMM Business Meeting					

FLOORPLAN PAPENDAL



EXHIBITION ROOMS





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Vela Diagnostics

MONDAY 14 APRIL 2014

Room 6/7 & 8/9

13:00 - 17:15 **National examination for medical microbiologists in training**

Restaurant

19:00 - 21:00 **Dinner**

TUESDAY 15 APRIL 2014

09:00 - 09:30 Registration

09:30 - 11:00 Plenary session

Athene B/C *Chair: Menno de Jong*

09:30 - 10:15 **Haploid genetics in human cells unravels portals for pathogens**

O001 Thijn Brummelkamp (The Netherlands)

10:15 - 11:00 **Disecting the global dissemination of antibiotic resistance genes**

O002 Morten Sommer (Denmark)

11:00 - 11:30 Coffee/tea break

11:30 - 12:45 Plenary session & award ceremony

Athene B/C *Chair: Wilbert Bitter*

11:30 - 12:15 **A full quantitative understanding of a living system: Is this possible?**

O003 Louis Serrano (Spain)

12:15 - 12:45 **Award ceremony**

12:45 - 14:00 Lunch

Athene A

13:00 - 14:00 KNVM Business meeting

14:00 - 15:30 Parallel sessions

Athene B/C **Developments in antiviral therapy and antiviral resistance**

Chair: Katja Wolthers

14:00 - 14:30 **Antiviral therapy, a lot has been achieved, still many viruses to go**

O004 Johan Neyts

14:30 - 15:00 **Host targeted antiviral therapy to combat enteroviruses?**

O005 Frank van Kuppeveld

15:00 - 15:15 **Acyclovir prophylaxis predisposes to antiviral resistant recurrent herpetic keratitis**

O006 Georges Verjans

15:15 - 15:30 **Treatment of chronic Hepatitis E virus genotype 3 in solid organ transplant recipients**

O007 Annelies Riezebos-Brilman

Athene A **Staphylococcal virulence**

Chairs: Maarten van Dijl, Jos van Strijp & Willem van Wamel

14:00 - 14:30 ***Staphylococcus aureus* bi-component leukotoxins and their role in the disarmament of innate and adaptive immunity**

O008 Victor Torres (USA)

14:30 - 14:45 **The staphylococcal cassette chromosome mec type v from *Staphylococcus aureus* ST398 is packaged into bacteriophage capsid**

O009 Girbe Buist

14:45 - 15:00 **The Extracellular Adherence Protein from *Staphylococcus aureus* inhibits the classical and lectin complement pathways by blocking formation of the C3 convertase**

O010 Daphne Stapels

15:00 - 15:15 **CCR1, CCR2 and CCR5 are receptors for *Staphylococcus aureus* leukotoxin LukM/LukF**

O011 Manouk Vrieling

15:15 - 15:30 **Phagocytosis escape by a *Staphylococcus aureus* protein that connects complement and coagulation proteins at the bacterial surface**

O012 Annemarie Kuipers

Room 3 ***Campylobacter*: latest insights into epidemiology, pathogenesis and intervention**

Chairs: Wilma Hazeleger & Wilma Jacobs

14:00 - 14:30 **Controlling campylobacteriosis by reducing the contamination of broiler chicken meat**

O013 Arie Havelaar

14:30 - 14:45 ***Campylobacter* virulence: the Omics perspective**

O014 Arnoud van Vliet

14:45 - 15:00 **Interventions to prevent *Campylobacter* in primary production, new developments and dilemma's**

O015 Jaap Wagenaar

15:00 - 15:15 **Time series analysis of genes expressed in epithelial cells during infection by *Campylobacter jejuni* 11168 and its avirulent cas9 deletion mutant**

O016 Peter van Baarlen

15:15 - 15:30 **Combined analysis of whole genome MLST data and whole genome mapping data for clinical *Campylobacter jejuni* and *C. coli* isolates**

O017 Martine Rijnsburger

Room 4/5 **Determining the value of surveillance programs of healthcare-associated infections and antimicrobial resistance: what are we missing?**

Chair: Marc Bonten

14:00 - 14:30 **What counts? Producing data on CVC-BSIs for quality improvement: An ethnographic study.**

O018 Carolyn Tarrant (United Kingdom)

14:30 - 15:00 **Surveillance of hospital-associated infections (HAI) and antimicrobial resistance (AMR) in the Netherlands and rankability of their rates**

O019 Akke van der Bij

15:00 - 15:15 **Carbapenemase producing bacteria in travellers constitute a potential threat for current hospital infection control programs**

O020 Roel Nijhuis

15:15 - 15:30 **Re-emerging hepatitis E virus in the Netherlands**

O021 Hans Zaaijer

Room 6/7 **New insights in epidemiology and treatment of *Clostridium difficile* infections (CDI)**

Chair: Ed Kuiper

14:00 - 14:30 **Whole genome sequencing reveals diverse sources for *Clostridium difficile* infection**

O022 Sarah Walker (United Kingdom)

14:30 - 14:45 **Changes of the epidemiology of CDI in the Netherlands and Europe**

O023 Sofie van Dorp

14:45 - 15:00 **Update of European guidelines for the treatment of *Clostridium difficile* infection (CDI)**

O024 Sylvia Debast

15:00 - 15:15 **Characteristics of hospitalised acute Q fever patients during a large epidemic, the Netherlands**

O025 Lieke Wielders

15:15 - 15:30 **Whole genome sequencing revealed transmission of *Clostridium difficile* between humans and farm animals in the Netherlands**

O026 Wilco Knetsch

Room 8/9 **Antoni van Leeuwenhoek: Scientist or Clever Burgher?**

Chair: Joop van Doorn

14:00 - 14:30 **Antoni van Leeuwenhoek: The Myth, The Man**

O027 Douglas Anderson (USA)

14:30 - 15:00 **Through van Leeuwenhoek's eyes - his microscopes then and now**

O028 Lesley Robertson

15:00 - 15:30 **Small things considered. Observations and theories concerning minuscule objects made by Antoni van Leeuwenhoek.**

O029 Jantien Backer

15:30 - 16:00 **Coffee/tea break**

16:00 - 17:30 **Parallel sessions**

Athene B/C **Recent discoveries in anaerobic methane oxidation**

Chair: Mike Jetten

16:00 - 16:30 **Time travel with methanogens**

O030 Alexander Zehnder (Switzerland)

16:30 - 17:00 **Oxygen-producing 'anaerobes' & denitrifying 'methanogens' - surprises in nitrate-/nitrite-dependent methane oxidation**

O031 Katharina Ettwig

17:00 - 17:15 **Novel anaerobes for a biobased economy: succinate production from glycerol by *Ercella succinogenes* gen. nov., sp. nov.**

O032 Diana Sousa

17:15 - 17:30 **Abundance and activity of marine anammox bacteria in marine sediments of the southern North Sea**

O033 Laura Villanueva

Athene A **Use of Next Generation Sequencing (NGS) for diagnostics and epidemiology in clinical practice**

Chairs: John Rossen & Paul Savelkoul

16:00 - 16:30 **Towards standardized bacterial whole genome sequencing for global surveillance in routine practice**

O034 Dag Harmsen (Germany)

16:30 - 16:45 **Sequence-based epidemiology of vancomycin-resistant *Enterococcus faecium* in the Netherlands as a putative standard application**

O035 Rob Willems

16:45 - 17:00 **The use of local whole genome sequencing analysis during hospital outbreaks of highly resistant microorganisms**

O036 John Rossen

17:00 - 17:15 **High transmission rate of livestock-associated MRSA isolates between veterinarians and their household members**

O037 Thijs Bosch

17:15 - 17:30 **Combining enrichment culturing and metagenomic sequencing: characterization of populations of enterococci and Enterobacteriaceae during antibiotic therapy**

O038 Xinglin Zhang

Room 3 **Bacterial competence of human pathogens**

Chairs: Jan-Willem Veening & Dirk-Jan Scheffers

16:00 - 16:30 **Regulatory and mechanistic aspect of natural transformation in the human pathogen *Vibrio cholerae***

O039 Melanie Blokesch (Switzerland)

16:30 - 16:45 **The evolution of pneumococcal sex**

O040 Daniel Rozen

16:45 - 17:00 **Feedback control of a bimodal developmental process**

O041 Leendert Hamoen

17:00 - 17:15 **Control of competence for DNA transformation in *Streptococcus suis***

O042 Edoardo Zaccaria

17:15 - 17:30 **Antibiotic-induced increase of origin-proximal gene copy numbers triggers bacterial competence**

O043 Jelle Slager

Room 4/5 **Opportunistic parasitic infections and their atypical clinical presentation**

Chairs: Titia Kortbeek & Bert Mulder

16:00 - 16:30 **Toxoplasmosis in HIV and stamceltransplant patients**

O044 Karin Schurink

16:30 - 16:45 **Hyperstrongyloidiasis**

O045 Bibi Werdmuller

16:45 - 17:00 **Haematological parasitology: leishmaniasis**

O046 Bram Goorhuis

17:00 - 17:30 **American trypanosomiasis (Chagas disease) in The Netherlands**

O047 Aldert Bart

Room 6/7 **Microbe-macrobe symbioses - section microbial ecology**

Chair: Guus Roeselers

16:00 - 16:30 **Tales from the underground: symbiotic associations of cave-dwelling amphipods**

O048 Sharmishtha Dattagupta (Germany)

16:30 - 16:45 **Co-evolution of mammals with their gut symbiont *A. muciniphila***

O049 Janneke Ouwerkerk

16:45 - 17:00 **The Seagrass Microbiome Project**

O050 Catarina Cúcio

17:00 - 17:15 **Sponge microbiota as a reservoir for antibiotic resistance**

O051 Dennis Versluis

17:15 - 17:30 **The role of fungal volatiles in plant growth and development**

O052 Viviane Cordovez

Room 8/9 **Improvement of vaccines against seasonal influenza**

Chairs: Han van den Bosch & Anke Huckriede

16:00 - 16:30 **Improvement of vaccines against seasonal influenza**

O053 Geert Leroux-Roels (Belgium)

16:30 - 17:00 **The importance of T-cell responses in immunity to influenza**

O054 Xavier Saelens (Belgium)

17:00 - 17:15 **Efficacy of influenza vaccines**

O055 Eelko Hak

17.15 - 17.30 **Spray freeze-dried influenza vaccines for pulmonary administration**

O056 Anke Huckriede

Sydney

17:30 - 18:30 **Drinks**

Restaurant

18:30 - 20:30 **Dinner**

Foyer

20:30 - 21:15 **Poster session - Even poster numbers**

21:15 - 22:00 **Poster session - Odd poster numbers**

22:00 - 22:15 **Poster award ceremony**

Athene A

22:15 - 01:30 **Party**

WEDNESDAY 16 APRIL 2014

08:30 - 09:00 **Registration**

09:00 - 10:30 **Parallel sessions**

Athene B/C **Ecology and evolution of antibiotic resistance**

Chairs: Arjan de Visser & Willem van Schaik

09:00 - 09:30 **Non-lethal selection of antibiotic resistance**

O060 Dan Andersson (Sweden)

09:30 - 09:45 **Parallel evolution of cefotaxime resistance in experimental populations of *Escherichia coli***

O061 Martijn Schenk

09:45 - 10:00 **The epidemic spread of Extended-Spectrum Beta-Lactamase-carrying plasmids among *Escherichia coli* from different hosts**

O062 Mark de Been

10:00 - 10:15 **Decrease of antimicrobial resistance in *E. coli* from animal husbandry reflects the reduction of antibiotic usage in animals in The Netherlands**

O063 Dik Mevius

10:15 - 10:30 **Mining microbial metatranscriptomes for expression of antibiotic resistance genes under natural conditions**

O064 Dennis Versluis

Athene A **Werkgroep MALDI-TOF & NVMy: Update on microbial diagnostics – application of MALDI-TOF**

Chair: Karola Waar

09:00 - 09:30 **The role of MALDI-TOF MS in the diagnosis of invasive fungal infections**

O065 Maurizio Sanguinetti (Italy)

09:30 - 10:00 **Typing below the species level using MALDI-TOF mass spectrometry - applications and limits**

O066 Oliver Bader (Germany)

10:00 - 10:15 **Performance of MALDI-TOF MS in identification of yeasts: a multi-center study**

O067 Anneloes Vlek

10:15 - 10:30 **Potential misidentification of *Staphylococcus pseudintermedius* using MALDI-TOF MS and the development of a real time PCR**

O068 Koen Verstappen

Room 4/5 **Glycobiology in health and disease**

Chairs: Clara Belzer & Nina van Sorge

09:00 - 09:30 **The intestinal mucus and its crosstalk with bacteria, stimulation and protection**

O069 Malin Johansson (Sweden)

09:30 - 10:00 **C-type lectins shape innate and adaptive immunity to bacteria and fungi**

O070 Theo Geijtenbeek

10:00 - 10:15 **Sortase-based pull downs identify proteins involved in Dectin1 signaling**

O071 Karin Strijbis

10:15 - 10:30 **Structural information of the complex of staphylococcal superantigen-like protein 3 and toll-like receptor 2**

O072 Kirsten Koymans

Room 6/7 **Bachelor and Master (BAMA) Symposium**

Chair: Bas Zaat

09:00 - 09:15 **Phosphate stress and induction of the stringent response cause upregulation of mycobacterial capsular polysaccharides**

O073 Maroeska Burggraaf

09:15 - 09:30 **Molecular mechanisms of LukM/F' interaction with its G-protein coupled receptor CCR1**

O074 Dani Heesterbeek

09:30 - 09:45 **A Lipooligosaccharide Silencing RNA regulates *Campylobacter jejuni* pathogenicity**

O075 Dior Beerens

09:45 - 10:00 **Using culturomics to isolate travellers-related antibiotic resistant bacteria from stool samples**

O076 Joyce Aarts

10:00 - 10:30 **Detection and killing of resistant *S. aureus* using antimicrobial peptides**

O077 Joep Kooijman

Room 8/9 **Pathogenesis 1**

Chair: Pieter-Jan Haas

09:00 - 09:15 **Matrix metallopeptidase 9 is induced by muramyl dipeptide in an NOD2 dependent manner and can be counteracted by lipopoly-saccharide stimulation**

O078 Marloes Vissers

09:15 - 09:30 **Proteolytic events involved in the regulation of CSS ECF sigma factor activity in *Pseudomonas aeruginosa***

O079 Karlijn Bastiaansen

09:30 - 09:45 **Characterization of YajC a transmembrane protein involved in cell wall stability and biofilm formation in *Enterococcus faecium***

O080 Fernanda Paganelli

09:45 - 10:00 **Lack of pAp phosphatase reduces *Streptococcus pneumoniae* virulence by negatively affecting de novo lipid synthesis and thus membrane homeostasis**

O081 Kirsten Kuipers

10:00 - 10:15 **The C5 convertase is the docking site for Membrane Attack Complex assembly on the bacterial surface**

O082 Evelien Berends

10:15 - 10:30 **Efficient phagocytosis of *Aspergillus fumigatus* through antibody-dependent complement activation**

O083 Steven Braem

10:30 - 11:00 **Coffee/tea break**

11:00 - 12:30 **Parallel sessions**

Athene B/C **Pathogenesis 2**

Chair: Wilbert Bitter

11:00 - 11:15 **T-Cell tropism of simian Varicella virus during primary infection**

O084 Werner Ouwendijk

11:15 - 11:30 **Human TLR10 is an anti-inflammatory pattern recognition receptor**

O085 Marije Doppenberg-Oosting

11:30 - 11:45 **Identification of the staphylococcal Haemolysin-gamma receptors**

O086 András Spaan

11:45 - 12:00 **The role of the mycosin protease in type VII secretion of pathogenic *Mycobacteria***

O087 Vincent van Winden

12:00 - 12:15 **A passenger or the driver? A PPE substrate of the Mycobacterial secretion system ESX-5 is crucial for protein secretion via this system.**

O088 Louis Ates

12:15 - 12:30 **Modelling tuberculous meningitis in zebrafish using *Mycobacterium marinum***

O089 Lisanne van Leeuwen

Athene A **NWKV & WAMM: Clinical cases in medical microbiology: an interactive session**

Chairs/moderators: Ed Kuijper, Saskia Kuipers, Ann Vossen & Rolf Vreede

11:00 - 11:30 **Alcohol and blisters?**

O090 Madelon Engel

11:30 - 12:00 **Out of Africa with a headache**

O091 Daphne Scoop

12:00 - 12:30 **Acid-fast?**

O092 Jakko van Ingen

Room 3 **Tuberculosis: New NVMM Diagnostic Guideline and nontuberculous mycobacteria**

Chairs: Jakko van Ingen & Bert Mulder

11:00 - 11:15 **Drug susceptibility testing of non-tuberculous mycobacteria: useful for treatment guidance?**

O093 Ed Kuiper

11:15 - 11:30 **Quick molecular diagnosis of (resistant) *M. tuberculosis* according to the revised NVMM guidelines**

O094 Sarah Vainio

11:30 - 11:45 **The secondary laboratory diagnosis of tuberculosis in the Netherlands**

O095 Dick van Soolingen

11:45 - 12:00 **Identification of nontuberculous myco-bacteria by MALDI-TOF**

O096 Arjan Jansz

12:00 - 12:15 **Utility of rpoB gene sequencing for identification of nontuberculous mycobacteria in the Netherlands**

O097 Rina de Zwaan

12:15 - 12:30 **Development and evaluation of the detection of Mycobacterium tuberculosis complex on the BD-MAX system in a European multicenter study**

O098 Ingrid op den Buijs

Room 4/5 **Microbiome**

Chair: Birgitta Duim

11:00-11.15 **Dysbiosis of upper respiratory tract microbiota in elderly pneumonia patients**

O099 Debby Bogaert

11:15-11.30 **The intestinal microbiome of phytopathogenic root fly larvae**

O100 Cornelia Welte

11:30-11:45 **Congruency of phylogenetic composition and activity patterns in the human small intestine**

O101 Tom van den Bogert

11:45-12:00 **Functional metagenomic analysis of genes conferring resistance to the disinfectant benzalkonium chloride in the human gut microbiota**

O102 Elena Buelow

12:00-12:15 **Gut microbiota continues to evolve during first five years of life**

O103 Anat Eck

12:15-12:30 **Effects of selective digestive decontamination on the gut microbiota in Intensive Care Unit patients**

O104 Teresita de Jesus Bello Gonzalez

Room 6/7 **Bachelor and Master (BAMA) Symposium**

Chair: Marie-Monique Immink

11:00 - 11:15 **The generation of a highly effective model to study the convertases of the complement system**

O105 Hatice Orhan

11:15 - 11:30 **Chlamydial load in patients whom have naturallycleared *Chlamydia trachomatis* compared to non-cleared patients**

O106 Marieke Franssen

11:30 - 11:45 **Butyrate-producing bacteria related to intestinal diseases**

O107 Denise Vodegel

11:45 - 12:00 **Reducing turn-around time in positive blood cultures**

O108 Mandy Kuijstermans

12:00 - 12:15 **Enrichment and isolation of sulfate-reducing bacteria associated with methanotrophic archaea**

O109 Daan van Vliet

12:15 - 12:30 **The immunomodulatory effect of probiotic bacteria fractions**

O110 Olaf Perdijk

Room 8/9 **Viral infections in immunocompromised patients**

Chair: Anne Wensing

11:00 - 11:30 **Importance of immunereconstitution and viral infections: Towards more predictable immune-reconstitution after HCT**

O111 Jaap-Jan Boelens

11:30 - 11:45 **Human herpesvirus-6 DNAemia is a sign of impending primary CMV infection in CMV sero-discordant renal transplantations**

O112 Coretta van Leer-Buter

11:45 - 12:00 **Central nervous system infections with JC virus in immunocompromised patients**

O113 Jean-Luc Murk

12:00 - 12:15 **Gene expression analysis reveals an important role for neutrophils in infants with severe viral respiratory tract infections**

O114 Inge Ahout

12:15 - 12:30 **Human Polyomavirus 9 infection emerges in immunocompromized patients**

O115 Herman Wunderink

12:30 - 14:00 **Lunch**

Athene B/C

12:45 - 13:45 **Lunch Symposium: Experience the possibilities with BD MAX™**

Paul Smits
Ingrid op den Buijs
Marijo Parcina (Germany)

Athene A

13:00 - 14:00 **BBC-MMO Business meeting**

14:00 - 15:30 **Parallel sessions**

Athene B/C **General microbiology**

Chair: Stanley Brul

14:00 - 14:15 **Unraveling the environmental significance of nitrite-dependent anaerobic methane oxidation: a lipid biomarker approach**

O116 Dorien Kool

14:15 - 14:30 ***Streptococcus pneumoniae* modulates human respiratory syncytial virus infection *in vitro* and *in vivo***

O117 Tien Nguyen

14:30 - 14:45 **Time resolved monitoring of Bacillus spore coat protein cross-links, spore germination and spore thermal resistance**

O118 Stanley Brul

14:45 - 15:00 **Rare earth metals are essential for methanotrophic life in volcanic mudpots**

O119 Huub op den Camp

15:00 - 15:15 **No gain, but also no pain? Selective disadvantage of carrying an IncI1 plasmid with blaCTX-M-1 genes in absence of antimicrobials**

O120 Egil Fischer

15:15 - 15:30 **TnSeq in *Streptococcus suis* demonstrates that NADH oxidase is essential during infection of piglets**

O121 Astrid de Greeff

Athene A **WAMM, WMDI & NWKV: Laboratory of the Future**

Chairs: Rolf Vreede & Els Wessels

14:00 - 14:30 **Automated microbiological diagnostics: the next step?**

O122 Ed IJzerman

14:30 - 15:00 **Molecular microbiology 7 day testing, triple the workload and half the staff but managing to deliver faster service**

O123 Kate Templeton (United Kingdom)

15:00 - 15:15 **Development of a rapid molecular test for diagnosis of bloodstream infections**

O124 Martine Bos

15:15 - 15:30 **Automated monitoring of bacterial microcolony growth**

O125 Alice den Hertog

Room 3 **Risk Management: another step ahead!**

Chair: Eric van der Vorm

14:00 - 15:00 **Integral risk management**

O126 John Prooi

15:00 - 15:30 **Infection prevention; same goal and together strong!**

O127 Greet Vos

Room 4/5 **A closer look at the Far East: medical microbiology in South-East Asian 'Tigers'**

Chairs: Menno de Jong & Henri Verbrugh

14:00 - 14:30 **Challenges and priorities in Southeast Asian medical microbiology**

O128 Heiman Wertheim

14:30 - 15:00 **Novel remedies for deficiencies in medical microbiological services in SE Asia**

O129 Constance Schultsz

15:00 - 15:15 **Acquisition of ESBL- and carbapenemase-producing Enterobacteriaceae in a large prospective cohort of healthy travellers: The Carriage of Multiresistant Bacteria After Travel (COMBAT) study**

O130 Maris Arcilla

15:15 - 15:30 **Targeted resistome detection reveals high acquisition rates of ESBL and quinolone resistance genes in the gut microbiota after international travel**

O131 Christian von Wintersdorff

Room 6/7 ***Dientamoeba fragilis* infections: clinical relevance, diagnosis and treatment**

Chair: Titia Kortbeek & Bert Mulder

14:00 - 14:30 ***Dientamoeba fragilis* in children in Denmark: aspects on transmission, epidemiology and management**

O132 Dennis Röser (Denmark)

14:30 - 15:00 **Treatment of *Dientamoeba fragilis*: Indications and possibilities**

O133 Tom van Gool

15:00 - 15:15 **Molecular detection of *Dientamoeba fragilis* shows a significantly higher rate in healthy controls than in gastroenteritis cases**

O134 Bert Mulder

15:15 - 15:30 **Case-Control study Gastro Enteritis: The value of molecular detection in diagnosing gastro enteritis**

O135 Lesla Bruijnesteijn van Coppenraet

Room 8/9 **Bacterial vaccines: from failures to promises**

Chairs: Wouter Jong & Ben van der Zeist

14:00 - 14:30 ***Staphylococcus aureus vaccines: from failure to promise***

O137 Fabio Bagnoli (Italy)

14:30 - 15:00 **Novel vaccines based on bacterial outer membrane vesicles**

O138 Peter van der Ley

15:00 - 15:15 **An autotransporter display platform for the development of recombinant outer membrane vesicle vaccines**

O139 Maria Daleke-Schermerhorn

15:15 - 15:30 **Antibody responses against non-covalent cell surface-bound staphylococcal proteins**

O140 Francisco Romero Pastrana

15:30 - 16:00 **Coffee/tea break**

Athene B/C

16:00 - 18:00 **NVMM Business meeting**

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Voor productinformatie zie elders in deze uitgave.

13.ZYX.21.2

Verkorte productinformatie ECALTA (september 2012). De volledige productinformatie (SPC van 23 augustus 2012) is op aanvraag verkrijgbaar. **Samenstelling:** ECALTA bevat 100 mg anidulafungin per injectieflacon, overeenkomend met een sterkte van 3,33 mg/ml oplossing na reconstitutie met water voor injecties. De verdunde oplossing bevat 0,77 mg/ml anidulafungin. **Indicaties:** Behandeling van invasieve candidiasis bij volwassen niet-neutropenische patiënten. ECALTA is hoofdzakelijk onderzocht bij patiënten met candidemie en slechts bij een beperkt aantal patiënten met diepgelegen Candida infecties of met abcesvorming. **Farmacotherapeutische groep:** Antimycotica voor systemisch gebruik, andere antimycotica voor systemisch gebruik, ATC-code: J02 AX 06. **Dosering:** De behandeling met ECALTA moet worden uitgevoerd door een arts die ervaring heeft met de behandeling van invasieve schimmelinfecties. De eenmalige aanvangdosis van 200 mg dient op dag 1 te worden toegediend, daarna gevolgd door dagelijks 100 mg. Er zijn onvoldoende gegevens beschikbaar om een behandeling van langer dan 35 dagen met de 100 mg dosis te onderbouwen. De veiligheid en werkzaamheid van ECALTA bij kinderen jonger dan 18 jaar zijn niet vastgesteld. Op basis van de momenteel beschikbare gegevens kan geen doseringsadvies worden gedaan. Het wordt aanbevolen om ECALTA toe te dienen met een infusiesnelheid die niet hoger is dan 1,1 mg/minuut (overeenkomend met 1,4 ml/minuut wanneer gereconstitueerd en verdund conform instructies). ECALTA mag niet worden toegediend als een bolusinjectie. **Contra-indicaties:** Overgevoeligheid voor het werkzame bestanddeel of voor één van de hulpstoffen; overgevoeligheid voor andere geneesmiddelen uit de groep van echinocandinen. **Waarschuwingen en voorzorgen:** De werkzaamheid van ECALTA bij neutropenische patiënten met candidemie en bij patiënten met diepgelegen Candida infecties of intra-abdominaal abces en peritonitis is niet vastgesteld. De klinische werkzaamheid is hoofdzakelijk beoordeeld bij niet-neutropenische patiënten met C. albicans infecties en bij een kleiner aantal patiënten met niet-albicans infecties, voornamelijk C. glabrata, C. parapsilosis en C. tropicalis. Patiënten met Candida-endocarditis, -osteomyelitis of -meningitis en bekende C. krusei infectie zijn niet onderzocht. Verhoogde waarden van leverenzymen zijn waargenomen bij gezonde personen en patiënten die met anidulafungin werden behandeld. Bij een aantal patiënten met een ernstige onderliggende medische aandoening die gelijktijdig meerdere geneesmiddelen kregen naast anidulafungin, zijn klinisch significante leverafwijkingen opgetreden. Gevallen van significante leverstoornis, hepatitis en leverfalen kwamen soms voor tijdens klinische onderzoeken. Bij patiënten met verhoogde leverenzymen tijdens behandeling met anidulafungin dient te worden gecontroleerd op tekenen van verslechterende leverfunctie en dient het risico/voordeel van voortzetting van behandeling met anidulafungin geëvalueerd te worden. Anafylactische reacties, waaronder shock, zijn gemeld bij het gebruik van anidulafungin. Indien deze reacties voorkomen, dient de behandeling met anidulafungin te worden stopgezet en dient passende behandeling te worden gegeven. Infusiegerelateerde bijwerkingen zijn gemeld bij het gebruik van anidulafungin, waaronder uitslag, urticaria, blozen, pruritus, dyspneu, bronchospasmen en hypotensie. Infuusgerelateerde bijwerkingen komen weinig voor wanneer de snelheid waarmee anidulafungin wordt geïnfundeerd niet hoger is dan 1,1 mg/minuut. In een onderzoek bij ratten is verergering van infusie-gerelateerde reacties door gelijktijdige behandeling met anesthetica waargenomen waarvan de klinische relevantie onbekend is. Men dient voorzichtig te zijn bij het gelijktijdig toedienen van anidulafungin en anesthetica. Patiënten met een zeldzame erfelijke fructose-intolerantie dienen dit geneesmiddel niet te gebruiken. **Bijwerkingen:** Bijwerkingen in klinische studies waren meestal licht tot matig en leidden zelden tot stopzetting van de behandeling. De meest gerapporteerde, vaak voorkomende bijwerkingen (≥1/100 tot <1/10) zijn: coagulopathie, convulsies, hoofdpijn, diarree, braken, misselijkheid, verhoogd creatininegehalte in het bloed, uitslag, pruritus, hypokaliëmie, flushing, verhoogde alanine-aminotransferase, verhoogde alkalische fosfatase in het bloed, verhoogde aspartaat-aminotransferase, verhoogd bilirubine in het bloed, verhoogde gamma-glutamyltransferase. Soms (≥1/1000, < 1/100) zijn waargenomen: pijn in de bovenbuik, urticaria, hyperglykemie, hypertensie, opvliegers, pijn op de infusieplaats, cholestase. Bijwerkingen uit spontane meldingen met frequentie niet bekend (kan met de beschikbare gegevens niet worden bepaald) zijn: anafylactische shock, anafylactische reactie (zie "Waarschuwingen en voorzorgen"), hypotensie, bronchospasmen, dyspneu. **Afleveringsstatus:** UR. **Verpakking en Registratienummer:** ECALTA, 100 mg poeder voor concentraat voor oplossing voor intraveneuze infusie: EU/1/07/416/002 (1 injectieflacon met 100 mg poeder). **Vergoeding en prijzen:** ECALTA wordt vergoed volgens de 'Beleidsregel dure geneesmiddelen in ziekenhuizen'. Voor prijzen wordt verwezen naar de Z-Index taxe. **Voor medische informatie over dit product belt u met 0800-MEDINFO (6334636). Registratiehouder:** Pfizer Limited, Ramsgate Road, Sandwich, Kent CT13 9NJ, Verenigd Koninkrijk. **Neem voor correspondentie en inlichtingen contact op met de lokale vertegenwoordiger: Pfizer bv, Postbus 37, 2900 AA Capelle a/d IJssel.**

1. Reboli AC et al; Anidulafungin Study Group. Anidulafungin versus fluconazole for invasive candidiasis. New England Journal of Medicine 2007;356(24):2472-82.* **2.** Glöckner et al, Treatment of invasive candidiasis with echinochandines. Mycoses. 2009 Nov;52(6):476-86. **3.** Ecalta 2011 Summary of Product Characteristics **4.** Stichting Werkgroep Antibioticabeleid (SWAB), Optimaliseren van het antibioticabeleid in Nederland XII, SWAB-richtlijnen voor de behandeling van invasieve schimmelinfecties, September 2008. **5.** Joseph J.M et al; Anidulafungin: a drug evaluation of a new echinocandin; Expert Opin Pharmacother. 2008 Sep;9(13):2339-48.

*In deze studie werd anidulafungin-IV vergeleken met fluconazol-IV bij 245 patiënten met invasieve candidiasis. Het primaire eindpunt was globale respons (microbiologisch en klinisch) aan het eind van de IV-behandelperiode.

12.EDL.21.9

Pfizer

Ecalta (anidulafungin IV)

Verkorte productinformatie VFEND (opgesteld: november 2013). **De volledige productinformatie (SPC) is op aanvraag verkrijgbaar. Samenstelling:** VFEND 50 mg en 200 mg filmomhulde tabletten bevatten respectievelijk 50 mg en 200 mg voriconazol. VFEND 200 mg poeder voor oplossing voor infusie, bevat 200 mg voriconazol per flacon, overeenkomend met een 10 mg/ml oplossing na reconstitutie. VFEND 40mg/ml poeder voor orale suspensie bevat per ml 40 mg voriconazol. **Indicaties**: Voor volwassenen en kinderen in de leeftijd van 2 jaar en ouder voor de behandeling van invasieve aspergillose, candidemie bij niet-neutropenische patiënten, fluconazol-resistente ernstige invasieve *Candida*-infecties, ernstige schimmelinfecties, veroorzaakt door *Scedosporium* spp en *Fusarium* spp. VFEND dient in eerste instantie te worden toegediend aan patiënten met progressieve, mogelijk levensbedreigende infecties **Farmacotherapeutische groep:** Antimycotica voor systemisch gebruik; triazoolderivaten; ATC code: J02A C03. **Contra-indicaties**: Overgevoeligheid voor het werkzame bestanddeel of voor één van de hulpstoffen; gelijktijdige toediening met CYP3A4-substraten terfenadine, astemizol, cisapride, pimozide, kinidine, en van rifampicine, carbamazepine, fenobarbital, ergotamine-alkaloïden (ergotamine, dihydro-ergotamine), efavirenz doses van 400 mg eenmaal daagsof hoger, ritonavir (in een dosering van tweemaal daags 400 mg en hoger) en sirolimus; gelijktijdige toediening met sintjanskruid. **Waarschuwingen en voorzorgen**: Voorzichtigheid bij toediening aan patiënten met een overgevoeligheid voor andere azolen. Voriconazol is geassocieerd met een verlenging van het QT-interval. Er deden zich zeldzame gevallen voor van torsade de pointes bij patiënten behandeld met voriconazol, die risicofactoren vertoonden en die gelijktijdig geneesmiddelen toegediend kregen die mogelijk aan deze voorvallen hadden bijgedragen. Voorzichtigheid is geboden bij de toediening van voriconazol aan patiënten met potentieel pro-aritmische factoren. Elektrolytstoornissen dienen voor aanvang van de behandeling met VFEND te worden gecontroleerd en gecorrigeerd. Ernstige hepatische reacties, die meestal reversibel zijn na staken van de VFEND toediening, kunnen optreden. Patiënten die VFEND krijgen, moeten nauwgezet worden gecontroleerd op hepatische toxiciteit. De klinische behandeling dient te bestaan uit laboratoriumbeoordeling van de leverfunctie (specifiek ASAT en ALAT) bij de start van de behandeling met VFEND en tenminste wekelijks gedurende de eerste maand van de behandeling. De behandeling dient zo kort mogelijk te zijn, maar indien op basis van de baten-risico-beoordeling de behandeling wordt voortgezet, kan de controlefrequentie worden verminderd tot maandelijks als er geen veranderingen zijn in de leverfunctietesten. Als de leverfunctietesten opvallend verhogen, dient VFEND te worden gestopt, tenzij de medische beoordeling van de baten versus het risico van de behandeling voor de patiënt voortzetting van het gebruik rechtvaardigt. Controle van de leverfunctie dient zowel bij kinderen als bij volwassenen te worden uitgevoerd. Er zijn meldingen geweest van langdurige bijwerkingen met betrekking tot het zicht, inclusief troebel zicht, optische neuritis en papiloedeem. Acuut nierfalen kan voorkomen, daarom is een controle van de nierfunctie noodzakelijk. Patiënten, vooral kinderen, met risicofactoren voor acute pancreatitis dienen nauwkeurig gecontroleerd te worden tijdens behandeling met VFEND. Controle van serumamylase of -lipase kan worden overwogen. Patiënten ontwikkelden zelden exfoliatieve huidreacties tijdens VFEND behandeling. Bij uitbreiding van deze reacties dient VFEND toediening te worden gestopt in geval laesies verergeren. Daarnaast is VFEND geassocieerd met fototoxiciteit en pseudoporfyrie. Alle patiënten inclusief kinderen dienen tijdens de behandeling intense of langdurige blootstelling aan direct zonlicht te mijden en maatregelen te nemen zoals beschermende kleding en zonnebrandcrème met een hoge zonbeschermingsfactor (SPF). Langetermijnbehandeling: De volgende ernstige bijwerkingen zijn gemeld in verband met langdurige behandeling met VFEND. Artsen dienen daarom de noodzaak te overwegen om de blootstelling aan VFEND te beperken. Bij patiënten werd plaveiselcelcarcinoom van de huid gemeld, sommige van deze patiënten hebben eerdere fototoxische reacties opgetreden, dient multidisciplinair advies te worden ingewonnen en de patiënt doorverwezen te worden naar een dermatoloog. Stopzetting van de behandeling met VFEND dient overwogen te worden. Er dient systematisch en regelmatig dermatologische beoordeling plaats te vinden, wanneer de behandeling met VFEND wordt voortgezet ondanks het optreden van fototoxisch gerelateerde laesies om premaligne laesies vroegtijdig te kunnen detecteren en behandelen. De behandeling met VFEND dient te worden gestopt indien premaligne huidlaesies of plaveiselcelcarcinoom worden vastgesteld. Bij transplantatiepatiënten is niet-infectieuze periostitis met verhoogde gehalten fluoride en alkalische fosfatase gemeld. Als een patiënt skeletpijn en radiologische bevindingen ontwikkelt die passen bij periostitis, dient na multidisciplinair advies de stopzetting van de behandeling met VFEND overwogen te worden. Onder de leeftijd van twee jaar zijn de veiligheid en werkzaamheid van VFEND niet aangetoond. Voriconazol is geïndiceerd voor pediatrische patiënten van twee jaar of ouder. De orale biologische beschikbaarheid kan beperkt zijn bij pediatrische patiënten van 2 tot <12 jaar met malabsorptie en een voor de leeftijd zeer laag lichaamsgewicht. In dat geval is de intraveneuze toediening van voriconazol aanbevolen. Bij gelijktijdig gebruik met fenytoïne of rifabutine wordt een zorgvuldige controle van de fenytoïnespiegels of volledige bloedceltelling (bij rifabutine) aanbevolen. Gelijktijdig gebruik van voriconazol en fenytoïne / rifabutine dient vermeden te worden, tenzij het voordeel opweegt tegen het risico. Bij gelijktijdig gebruik met efavirenz dient de dosis voriconazol verhoogd te worden tot 400 mg om de 12 uur en dient de dosis efavirenz verlaagd te worden tot 300 mg om de 24 uur. Gelijktijdig gebruik met een lage dosis ritonavir (100 mg tweemaal daags) en everolimus dient vermeden te worden. Een frequente controle op methadongerelateerde ongewenste bijwerkingen en toxiciteit, waaronder QTc-verlenging, wordt aanbevolen bij gelijktijdige toediening van voriconazol. Een dosisvermindering van methadon kan noodzakelijk zijn. Verlaging van de dosis alfentanil, fentanyl en andere kortwerkende opiaten die een op alfentanil gelijkende structuur hebben en door CYP3A4 gemetaboliseerd worden, dient te worden overwogen bij gelijktijdige toediening met voriconazol. Aangezien de halfwaardetijd van alfentanil viervoudig verlengd wordt wanneer alfentanil gelijktijdig met voriconazol wordt toegediend en aangezien het gelijktijdig gebruik van voriconazol met fentanyl resulteert in een verhoging van de gemiddelde AUC 0-∞ van fentanyl, kan het nodig zijn de opioïdgerelateerde bijwerkingen regelmatig te controleren (inclusief een langer toezicht op de ademhaling). Verlaging van de dosis oxycodon en andere langwerkende opiaten die door CYP3A4 gemetaboliseerd worden, dient te worden overwogen bij gelijktijdige toediening met voriconazol. Het kan nodig zijn de opioïdgerelateerde bijwerkingen regelmatig te controleren. De gelijktijdige toediening van oraal voriconazol en oraal fluconazol resulteerde in een significante verhoging van de Cmax en AUCτ van voriconazol bij gezonde proefpersonen. Controle van de met voriconazol geassocieerde bijwerkingen is aanbevolen als voriconazol opeenvolgend na fluconazol wordt gebruikt. De tabletten bevatten lactose en mogen niet gebruikt worden bij Lapp-lactase deficiëntie of glucose-galactose-malabsorptie patiënten. De orale suspensie bevat saccharose en mag niet gebruikt worden bij patiënten met zeldzame, erfelijke problemen van fructose-intolerantie, sucrase-isomaltase-deficiëntie of glucose-galactose-malabsorptie. **Bijwerkingen**: De meest gerapporteerde, zeer vaak voorkomende bijwerkingen (≥1/10) zijn: perifeer oedeem, hoofdpijn, visusstoornis (inclusief troebel zicht, chromatopsie en fotofobie), buikpijn, misselijkheid, braken, diarree, huiduitslag en pyrexie. Verder zijn vaak (≥1/100, <1/10) waargenomen: verhoogde leverfunctiewaarden (met inbegrip van ASAT, ALAT, alkalische fosfatase, gammaGT, LDH, bilirubine), verhoogde bloedcreatininespiegel, pancytopenie, beenmergdepressie, leukopenie, trombocytopenie, purpura, anemie, duizeligheid, verwardheid, tremor, agitatie, pareisthesie, 'Acute respiratory distress'-syndroom, longoedeem, ademnood, thoraxpijn, acuut nierfalen, hematurie, exfoliatieve dermatitis, aangezichtsoedeem, fototoxische reactie, maculo-papulaire huiduitslag, maculaire huiduitslag, papulaire huiduitslag, cheilitis, pruritus, alopecia, erytheem, rugpijn, hypoglykemie, hypokaliëmie, gastro-enteritis, griepachtige symptomen, hypotensie, tromboflebitis, flebitis, reactie/ontsteking op de injectieplaats, rillingen, asthenie, sinusitis, geelzucht, cholestatische geelzucht, depressie, angst, hallucinatie. Soms (≥1/1.000, < 1/100) zijn waargenomen: verlengd gecorrigeerd QT interval op het electrocardiogram, verhoogde bloedureumspiegel, verhoogde bloedcholesterolspiegel, ventrikelfibrillatie, ventriculaire aritmie, supraventriculaire aritmie, supraventriculaire tachycardie, tachycardie, bradycardie, diffuse intravasculaire coagulatie, agranulocytose, lymfadenopathie, eosinofilie, hersenoedeem, ataxie, diplopie, vertigo, hypesthesie, papiloedeem, oogzenuwstoornis (inclusief optische neuritis), nystagmus, scleritis, bifaritis, pancreatitis, peritonitis, duodenitis, gingivitis, glossitis, gezwollen tong, dyspepsie, constipatie, nefritis, proteïnurie, Syndroom van Stevens-Johnson, angioneurotisch oedeem, allergische dermatitis, urticaria, geneesmiddelovergevoeligheid, psoriasis, artritis, bijnierschorsinsufficiëntie, anafylactoïde reactie, overgevoeligheid, leverfalen, hepatitis, hepatomegalie, cholecystitis, cholelithiasis, hyponatriëmie. Zelden (≥1/10.000, <1/1.000) komen voor: torsade de pointes, ventriculaire tachycardie, volledig atrioventriculair blok, bundeltakblok, nodaal ritme, convulsie, encefalopathie, Syndroom van Guillain-Barré, extrapyramidale symptomen, perifere neuropathie, slaperigheid tijdens infusie, retinale bloeding, optische atrofie, oogdraaien, corneatroebeling, hypoacusis, tinnitus, dysgeusie, tubulaire necrose van de nier, toxische epidermale necrolyse, erythema multiforme, discoïde lupus erythematodes, pseudoporfyrie, hypertonie, hyperthyreoïdie, hypothyreoïdie, pseudomembraneuze colitis, lymfangitis, hepatisch coma, insomnia. Niet bekend (kan met de beschikbare gegevens niet worden bepaald) komt voor: plaveiselcelcarcinoom, periostitis. **Afleveringsstatus:** U.R. **Verpakking en Registratienummer:** VFEND, filmomhulde tabletten 50 mg: EU/1/02/212/006 (30 stuks), filmomhulde tabletten 200 mg: EU/1/02/212/018 (30 stuks) VFEND, poeder voor oplossing voor infusie 200 mg: EU/1/02/212/025 (1 injectieflacon) VFEND, poeder voor orale suspensie: EU/1/02/212/026 (1 flacon), VFEND 200 mg poeder en oplosmiddel voor oplossing voor infusie: EU/1/02/212/027. Niet alle genoemde presentaties worden in de handel gebracht. **Vergoeding en prijzen:** VFEND, filmomhulde tabletten en poeder voor orale suspensie worden volledig vergoed binnen het GVS, VFEND 200 mg poeder voor oplossing voor infusie wordt vergoed volgens de lijst "add-on". Voor prijzen wordt verwezen naar de Z-Index taxe. **Voor medische informatie over dit product belt u met 0800-MEDINFO (6334636). Registratiehouder:** Pfizer Limited, Ramsgate Road, Sandwich, Kent CT13 9NJ, Verenigd Koninkrijk. **Neem voor correspondentie en inlichtingen contact op met de lokale vertegenwoordiger: Pfizer bv, Postbus 37, 2900 AA Capelle a/d IJssel.**

Referenties: 1. VFend®, samenvatting van de productkenmerken, EMA. 2013. 2. Walsh TJ, et al. Clin Infect Dis. 2008;46(3):327-60. 3. Wingard JR, et al. Cancer 2008; 112:2309-12. 4. Neofytos D, et al. Clin Infect Dis 2009;48:265-73. 5. Herbrecht R, et al. N Engl J Med. 2002;347(6):408-15.

Pfizer

IV/PO VFEND (voriconazole)

14.VFE.21.13

Verkorte productinformatie ZYVOXID (opgesteld februari 2014). **De volledige productinformatie (SPC) is op aanvraag verkrijgbaar. Samenstelling:** ZYVOXID 600 mg, filmomhulde tabletten bevatten 600 mg linezolid. ZYVOXID 2 mg/ml, oplossing voor infusie, bevat 2 mg/ml linezolid. De 300 ml infuuszakken bevatten 600 mg linezolid. Hulpstoffen: elke 300 ml bevat 13,7 g glucose en 114 mg natrium. **Indicaties:** Nosocomiale pneumonie, buiten het ziekenhuis verworven pneumonie indien veroorzaakt of vermoedelijk veroorzaakt door gevoelige grampositieve bacteriën. Niet werkzaam tegen infecties veroorzaakt door gramnegatieve pathogenen. Gecompliceerde huid- en weke delen infecties: alleen geïndiceerd wanneer uit microbiologisch onderzoek is gebleken dat de infectie wordt veroorzaakt door gevoelige grampositieve bacteriën. Uitsluitend toepassen bij patiënten met gecompliceerde huid- en weke delen infecties bij een bewezen of vermoede gelijktijdige infectie met gramnegatieve pathogenen, als er geen andere behandelmogelijkheden meer zijn. Onder deze omstandigheden moet gelijktijdig worden gestart met behandeling gericht tegen gramnegatieve pathogenen. Behandeling uitsluitend starten in een ziekenhuisomgeving en na overleg met een relevante specialist zoals een microbioloog of infectioloog. **Officiële richtlijnen over passend gebruik van antibacteriële middelen in overweging nemen. Farmacotherapeutische groep:** Overige bacteriële middelen; ATC-code: J01 XX08. Dosering: 600 mg (oraal, intraveneus) tweemaal per dag gedurende 10-14 dagen. De maximale behandelingsduur is 28 dagen. De veiligheid en effectiviteit van linezolid zijn niet vastgesteld, wanneer langer dan 28 dagen toegediend. **Contra-indicaties:** Overgevoeligheid voor het werkzame bestanddeel linezolid of één van de hulpstoffen. Niet toepassen bij patiënten die geneesmiddelen gebruiken die de monoamino-oxidasen A of B inhiberen (bv. fenelzine, isocarboxazide, selegiline, moclobemide) of binnen twee weken na gebruik van dergelijke geneesmiddelen. Niet toedienen aan patiënten met de volgende onderliggende klinische aandoeningen of aan patiënten die gelijktijdig behandeld worden met de volgende geneesmiddelen, tenzij er mogelijkheden zijn voor een nauwkeurige observatie van de patiënt en voor controle van de bloeddruk: patiënten met ongecontroleerde hypertensie, feochromocytoom, carcinoïd, thyrotoxicose, bipolaire depressie, schizoaffectieve stoornis, acute verwardheid, patiënten die één van de volgende geneesmiddelen innemen: serotonine heropnameremmers, tricyclische antidepressiva, serotonine 5-HT1 receptor agonisten (triptanen), direct en indirect werkende sympathicomimetische stoffen (inclusief de adrenerge broncho-dilatatoren, pseudo-efedrine en fenylpropanolamine), vasopressieve stoffen (bv. adrenaline en noradrenaline), dopaminerge stoffen (bv. dopamine en dobutamine), pethidine of buspiron. **Waarschuwingen en voorzorgen:** *Myelosuppressie:* Myelosuppressie (inclusief anemie, leukopenie, pancytopenie en trombocytopenie) werd gerapporteerd bij patiënten die linezolid kregen. In gevallen met bekende afloop stegen de getroffen hematologische parameters bij stopzetten van linezolid opnieuw naar de waarden vóór de start van de behandeling. Het risico op deze effecten lijkt gebonden te zijn aan de behandelingsduur. Oudere patiënten kunnen een groter risico op bloeddyscrasie lopen dan jongere patiënten. Trombocytopenie kan vaker optreden bij patiënten met ernstige nierinsufficiëntie, ongeacht of zij dialyse ondergaan. Nauwgezet monitoren van bloedwaarden is daarom aangeraden bij: patiënten met bestaande anemie, granulocytopenie of trombocytopenie; patiënten die tegelijkertijd geneesmiddelen krijgen die de hemoglobinespiegels kunnen laten dalen, het aantal bloedcellen kunnen onderdrukken of het aantal bloedplaatjes of hun functie negatief beïnvloeden; patiënten met ernstige nierinsufficiëntie; patiënten die langer dan 10 tot 14 dagen behandeld worden. Linezolid zou alleen aan deze patiënten toegediend moeten worden als de mogelijkheid bestaat hemoglobinespiegels, bloedwaarden en het aantal bloedplaatjes nauwgezet te monitoren. Als significante myelosuppressie optreedt, dient de behandeling gestaakt te worden tenzij voortzetten als absoluut noodzakelijk wordt beschouwd. Wanneer dit het geval is dienen intensief monitoren van de bloedwaarden en geschikte behandelstrategieën geïmplementeerd te worden. Aangeraden wordt om wekelijks de volledige bloedwaarden (inclusief hemoglobinespiegels, bloedplaatjes- en totale en gedifferentieerde leukocytaantallen) te monitoren, ongeacht de baselinewaarden. In "compassionate use"-studies werd een hogere incidentie van ernstige anemie gemeld bij patiënten die langer behandeld werden met linezolid dan de maximaal aanbevolen duur van 28 dagen. Deze patiënten vereisten vaker een bloedtransfusie. Gevallen van anemie die een bloedtransfusie vereiste, zijn eveneens postmarketing gemeld; daarbij deden zich meer gevallen voor bij patiënten die een linezolidbehandeling toegediend kregen gedurende meer dan 28 dagen. Gevallen van sideroblastische anemie zijn postmarketing gemeld. Wanneer hettijdstip van ontstaan bekend was, hadden de meeste patiënten een linezolidbehandeling gedurende meer dan 28 dagen toegediend gekregen. *Antibioticumgeassocieerde diarree en colitis:* Pseudomembraneuze colitis is gemeld. Bij patiënten die diarree vertonen na de toediening van een antibacterieel middel dient deze diagnose in overweging genomen te worden. In gevallen van vermoede of vastgestelde antibioticum-geassocieerde colitis, kan stopzetting van linezolid nodig zijn. Geschikte behandelstrategieën dienen ingesteld te worden. Antibioticumgeassocieerde diarree en antibioticageassocieerde colitis, inclusief pseudomembraneuze colitis en *Clostridium difficile*-geassocieerde diarree zijn gemeld, waarvan de ernst kan variëren van lichte diarree tot fatale colitis. Deze diagnose dient overwogen te worden bij patiënten die ernstige diarree krijgen tijdens of na het gebruik van linezolid. In dat geval de lopende antibioticumbehandeling, inclusief linezolid, stopzetten en onmiddellijk gepaste therapeutische maatregelen nemen. Geneesmiddelen die de peristaltiek onderdrukken zijn dan gecontra-indiceerd. *Lactaatacidose:* Lactaatacidose is gemeld bij gebruik van linezolid. Patiënten die verschijnselen en symptomen van metabole acidose ontwikkelen tijdens behandeling met linezolid, dienen onmiddellijk medische zorg te krijgen. Als lactaatacidose optreedt, dienen de voordelen van het voortzetten van linezolid te worden afgewogen tegenover de potentiële risico's. *Mitochondriale disfunctie:* Linezolid remt de mitochondriale proteïnesynthese. Bijwerkingen, zoals lactaatacidose, anemie en neuropathie (optisch en perifeer) kunnen optreden als gevolg van deze remming; deze voorvallen zijn frequenter wanneer het geneesmiddel langer dan 28 dagen wordt gebruikt. *Serotoninesyndroom:* Bij gelijktijdige toediening van linezolid en serotonerge middelen, waaronder antidepressiva zoals selectieve serotonineheropnameremmers (SSRI's), werden spontane meldingen van serotoninessyndroom gerapporteerd. Gelijktijdige toediening van linezolid en serotonerge middelen is gecontra-indiceerd, behalve wanneer de gelijktijdige toediening van linezolid en serotonerge middelen noodzakelijk is. In deze gevallen de patiënt nauwgezet observeren voor verschijnselen en symptomen van serotoninessyndroom. Als deze optreden, de stopzetting van één of beide middelen overwegen; als het serotonerge middel stopgezet wordt, kunnen ontwenningsverschijnselen optreden. *Perifere neuropathie en optische neuropathie:* Perifere neuropathie, optische neuropathie en optische neuritis, soms overgaand tot visusverlies zijn gemeld. Deze meldingen deden zich voornamelijk voor bij patiënten die langer dan 28 dagen behandeld werden. Alle patiënten dient geadviseerd te worden om symptomen van visusstoornissen te melden. In dergelijke gevallen is een onmiddellijke evaluatie aanbevolen met een eventuele verwijzing naar een oogarts. Bij patiënten die langer dan 28 dagen met Zyvoxid behandeld worden, dient de visuele functie regelmatig gecontroleerd te worden. Als perifere of optische neuropathie voorkomt, dient het voortzetten van het gebruik van Zyvoxid te worden afgewogen tegenover de potentiële risico's. Er kan een verhoogd risico op neuropathie zijn wanneer linezolid wordt gebruikt bij patiënten die tegelijkertijd of recent antimycobacteriële middelen voor de behandeling van tuberculose innemen, respectievelijk innamen. *Convulsies:* Convulsies zijn gemeld bij patiënten tijdens behandeling met Zyvoxid. In de meeste gevallen was een voorgeschiedenis van epileptische aanvallen of risicofactoren voor epileptische aanvallen gemeld. Patiënten dient geadviseerd te worden hun arts te informeren indien zij een voorgeschiedenis van epileptische aanvallen hebben. *Monoamino-oxidase-inhibitoren:* Linezolid is een reversibele, niet-selectieve monoamino-oxidase-inhibitor (MAOI); in de doseringen die gebruikt worden in de antibacteriële therapie, heeft het echter geen antidepressief effect. Er zijn erg weinig gegevens uit geneesmiddelinteractiestudies en over de veiligheid van linezolid indien toegediend aan patiënten met onderliggende aandoeningen of middelen waardoor een interactie kan zouden kunnen lopen op MAO-inhibitie. Linezolid wordt bijgevolg niet aanbevolen voor gebruik onder deze omstandigheden tenzij nauwkeurige observatie en controle van de ontvanger mogelijk is. *Gebruik met tyraminerijk voedsel:* Patiënten moet worden ontraden grote hoeveelheden tyraminerijk voedsel te gebruiken. *Superinfectie:* De effecten van linezolidbehandeling op de normale flora zijn niet geëvalueerd in klinische studies. Indien superinfectie tijdens de behandeling voorkomt, dienen aangepaste maatregelen genomen te worden. *Oplossing voor infusie:* De oplossing bevat glucose. Hiermee moet rekening worden gehouden bij patiënten met diabetes mellitus of andere omstandigheden geassocieerd met glucose-intolerantie. De oplossing bevat ook natrium. Bij patiënten op een gecontroleerd natriumdieet dient er rekening gehouden te worden met de hoeveelheid natrium. **Voor een volledige beschrijving van waarschuwingen en voorzorgen wordt verwezen naar de SPC. Bijwerkingen:** Bijwerkingen die met een frequentie ≥ 0,1% of die ernstig geacht werden in klinische onderzoeken (> 2000 volwassen patiënten die de aanbevolen dosering ontvingen, max 28 dagen). Ca. 22% van de patiënten ondervond bijwerkingen; de meest gemelde bijwerkingen waren hoofdpijn (2,1%), diarree (4,2%), misselijkheid (3,3%) en candidose (voornamelijk orale [0,8%] en vaginale [1,1%] candidose). De meest gemelde bijwerkingen die leidden tot stoppen van de behandeling: hoofdpijn, diarree, misselijkheid en braken. Ongeveer 3% van de patiënten staakte de behandeling om deze reden. Extra bijwerkingen gemeld tijdens postmarketing ervaring zijn opgenomen in de tabel met de frequentie categorie 'Niet bekend', omdat de daadwerkelijke frequentie niet vastgesteld kan worden op basis van de beschikbare gegevens. De volgende bijwerkingen werden opgemerkt en gemeld met de volgende frequenties: vaak (≥1/100 tot <1/10): candidiasis, orale candidiasis, vaginale candidiasis, schimmelinfectie, hoofdpijn, smaakverandering (metaalsmaak), diarree, misselijkheid, braken, abnormale leverfunctietesten, verhoogde AST, ALT of alkalische fosfatase, verhoogd BUN, verhoogde LDH, creatinekinase, lipase, amylase of niet-nuchtere glucose, verlaagd totaal proteïne, albumine, natrium of calcium, verhoogd of verlaagd kalium of bicarbonaat, verhoogde neutrofielen of eosinofielen, verlaagde hemoglobine, hematocriet of aantal rode bloedcellen, verhoogd of verlaagd aantal trombocyten of witte bloedcellen. Soms (≥1/1.000 tot <1/100): vaginitis, leukopenie, neutropenie, trombocytopenie, eosinofilie, slapeloosheid, duizeligheid, hypo-esthesie paresthesie, wazig zien, tinnitus, hypertensie, flebitis, tromboflebitis, pancreatitis, gastritis, plaatselijke of algemene buikpijn, obstipatie, droge mond, dyspepsie, glossitis, weke ontlasting, stomatitis, tongverkleuring of stoornis, verhoogde totaal bilirubine, urticaria, dermatitis, diaphoresis, pruritus, rash, polyurie, verhoogde creatitine, vulvovaginale stoornis, koude rillingen, vermoeidheid, koorts, toegenomen dorst, plaatselijke pijn, verhoogd natrium of calcium, verlaagd niet-nuchtere glucose, verhoogd of verminderd chloride, verhoogd aantal reticulocyten, verlaagd aantal neutrofielen. Zelden (≥1/10.000 tot <1/1.000): aritmie (tachycardie), voorbijgaande ischemische aanvallen, nierfalen. Niet bekend (kan met de beschikbare gegevens niet worden bepaald): antibioticum geassocieerde colitis, waaronder pseudomembraneuze colitis, myelosuppressie, pancytopenie, anemie, sideroblastische anemie, anafylaxie, lactaatacidose, hyponatremie, serotonine-syndroom, convulsies, perifere neuropathie, optische neuropathie, optische neuritis, visusverlies, veranderingen in de gezichtsscherpte of in het kleuren zien, veranderingen van gezichtsveld-stoornissen, oppervlakkige tandverkleuring, bulleuze huidaandoeningen zoals beschreven bij het Stevens-Johnson syndroom en toxische epidermale necrolyse, angio-oedeem, alopecia. **Afleveringsstatus:** U.R. **Verpakking en Registratienummer:** ZYVOXID, 600 mg filmomhulde tabletten: RVG 29569; ZYVOXID 2 mg/ml oplossing voor infusie: RVG 29567. Vergoeding en prijzen: ZYVOXID 600 mg, filmomhulde tabletten worden volledig vergoed binnen het GVS, ZYVOXID 2 mg/ml, oplossing voor infusie wordt niet vergoed binnen het GVS of de lijst add-on. Voor prijzen wordt verwezen naar de Zindex taxe. **Voor medische informatie over dit product belt u met 0800-MEDINFO (6334636). Neem voor correspondentie en inlichtingen contact op met de registratiehouder: Pfizer bv, Postbus 37, 2900 AA Capelle a/d IJssel.**

13.ZFX.21.2

Pfizer Anti-Infectives

Referenties: 1. Wunderink RG, et al. CID 2012;54:621-629
2. Itani KMF, et al. Am J Surg 2010;199:804-816
3. SPC Zyvoxid® 5 februari 2014

IV/PO ZYVOXID (linezolid)

O001

Haploid genetics in human cells unravels portals for pathogens

T. Brummelkamp
NKI, Amsterdam

Experimental genetics provides a powerful window into complex biological processes. Even though human cells are a widely used experimental tool, the human genome has for a long time been refractory to efficient mutagenesis due to its diploid nature. Recently we have developed an entirely novel genetic model system to expand the toolbox for genetics in human cells. This method enables efficient inactivation of human genes by a single mutation using insertional mutagenesis in cells that are haploid or near-haploid. Combined with parallel sequencing approaches, this method generates high-density genetic overviews of genes required for nearly any selectable cell trait. We have used these genetic screens to identify genes required for drug action, noes components of biosynthetic pathways and receptors for bacterial toxins. Recently, haploid genetic screens have been used to dissect the entry routes used by deadly hemorrhagic fever viruses.

O002

Dissecting the global dissemination of antibiotic resistance genes

M. Sommer[1,2]
[1]*DTU, Dept. of Systems Biology, Lyngby, Denmark,* [2]*Novo Nordisk Foundation Center for Biosustainability, Hørsholm, Denmark*

The increasing levels of multi-drug resistance in human pathogenic bacteria are compromising our ability to treat infectious disease. Since antibiotic resistance determinants, often encoded on mobilizable elements, can be readily transferred between bacteria, we must understand the relative abundance and diversity of reservoirs of resistance genes encoded within microbial communities from different environments and their accessibility to clinically relevant pathogens.
Using metagenomic functional selections we have characterized the resistomes of a variety of environments spanning from the soil to the human gut microbiome. We find that the human gut microbiome constitutes and important reservoir of antibiotic resistance genes that is part of large gene exchange networks comprising most human pathogens; however, other environments also participate in these environments. Based on these results and the rapidly growing DNA sequence data a framework is emerging for describing how antibiotic resistance genes are exchanged between microbial communities and which resistance reservoirs are most accessible to pathogens.

O003

A full quantitative understanding of a living system: Is this possible?

L. Serrano
CRG-Centre Regulació Genòmica, Barcelona, Spain

The goal of Systems Biology is to provide a quantitative and predictive description of a living system to the extent that it can be fully simulated in a computer. We have undertaken such Endeavour using as a model the small bacterium, *M. pneumoniae*, a human pathogenic bacterium causing atypical pneumonia as model system for our study. Containing a reduced genome with only 690 ORFs, this bacterium is an ideal organism for exhaustive quantitative and systems-wide studies, avoiding technical limitations due to exceeding sample complexity, constrained by limitations in dynamic range and resolution of current generation mass spectrometers. Available data on the transcriptome, on protein complexes, as well as on metabolic pathways facilitate the integration of the data generated for this study into an organism-wide context. Additionally, *M. pneumoniae* represents a relevant organism to study stochastic noise in living systems. The cells are significantly smaller than other bacteria, such as *Eschericha coli* (0.05 mm^3 and 1 mm^3, respectively) resulting in principle in an increased susceptibility to abundance fluctuations of cellular molecules. Our analysis shows that even apparently simpler organisms have a large hidden layer of complexity and that for every question we have answered we have got two new ones. We are still far away to be get a full understanding of a cell.

O004

Antiviral therapy, a lot has been achieved, still many viruses to go

J. Neyts
Rega Institute for Medical Research, University of Leuven (KU Leuven), Leuven, Belgium

Today, small molecule antiviral drugs are available for the treatment of infections with herpes viruses, HIV, HBV and HCV as well as with influenza viruses. Ribavirin, a broad-spectrum (but a specific) antiviral, has been approved for the treatment of infections with respiratory syncytial virus, HCV and Lassa virus. Yet, for many other viruses

that cause life-threatening infections [most of which are considered emerging and/or neglected] there are no drugs available. Ideally, potent and broad-spectrum (i.e., pan-genus or pan-family virus activity) antiviral drugs should be developed whereby one drug could be used for the treatment of a number of such viral infections

O005

Host targeted antiviral therapy to combat enteroviruses?

F.J.M. van Kuppeveld
UMC Utrecht University, Dept. of Infectious Diseases and Immunology, Utrecht

The genus *Enterovirus* of the family *Picornaviridae* include several medical and socio-economic important pathogens of humans. These include poliovirus (the causative agent of poliomyelitis), coxsackievirus and echovirus (which can cause meningitis, encephalitis and myocarditis), and the common cold causing rhinoviruses which are increasingly in the spotlight as exacerbating agents in asthma and chronic obstructive pulmonary disease.
In addition, this genus includes enterovirus 71, a virus that causes large epidemics in South-East Asia associated with hand-foot-and-mouth disease but also with severe neurological disease and significant number of deaths. A vaccine is available for poliovirus but development of vaccines against other enteroviruses is essentially impossible given the multitude of serotypes (e.g. up to date already more than 150 rhinovirus types have been identified). Hence, antiviral drugs are urgently needed to prevent or treat enterovirus infections. Despite extensive efforts, however, no approved antiviral therapy targeting enteroviruses is available and treatment options remain limited to supportive and symptomatic treatment. Here, an overview of the latest developments in the finding for inhibitors of enterovirus replication (e.g. capsid binders, protease inhibitors, and polymerase inhibitors) will be discussed. In addition, I will focus on the identification of host factors that are essential for enterovirus replication and the potential of inhibitors of host factors as broad-spectrum antivirals against this important group of pathogens.

O006

Acyclovir prophylaxis predisposes to antiviral resistant recurrent herpetic keratitis

G.M.G.M. Verjans, M. van Velzen, D.A.M.C. van de Vijver, F.B. van Loenen, A.D.M.E. Osterhaus, L. Remeijer
Erasmus MC, Dept. of Viroscience, Rotterdam

Introduction: Long-term acyclovir (ACV) prophylaxis, recommended to prevent recurrent herpes simplex virus type 1 (HSV-1) ocular disorders, may pose a risk for ACV-refractory disease due to ACV-resistance. We determined the effect of ACV prophylaxis on the prevalence of corneal acyclovir-resistant (ACV^R) HSV-1 and clinical consequences thereof in patients with recurrent HSV-1 keratitis (rHK).
Methods: Frequencies of ACV^R viruses were determined in 169 corneal HSV-1 isolates from 78 rHK patients with a history of stromal disease. The isolates' ACV susceptibility phenotypes were correlated with clinical parameters to identify risk factors predisposing to ACV^R rHK.
Results: Corneal HSV-1 isolates with > 28% ACV^R viruses were defined as ACV^R isolates. Forty-four isolates (26%) were ACV-resistant. Multivariate analyses identified long-term ACV prophylaxis (= 12 months) (odds ratio [OR] 3.42, 95% confidence interval [CI] 1.32-8.87) and recurrence duration of = 45 days (OR 2.23, 95% CI 1.02-4.87), indicative for ACV-refractory disease, as independent risk factors for ACV^R isolates. Moreover, ACV^R HSV-1 was a risk factor for ACV-refractory disease (OR 2.28, 95% CI 1.06-4.89).
Conclusions: The data suggest that ACV prophylaxis for = 12 months predisposes to ACV-refractory disease due to the emergence of corneal ACV^R HSV-1. The data warrant ACV-susceptibility testing during follow-up of rHK patients.

O007

Treatment of chronic Hepatitis E virus genotype 3 in solid organ transplant recipients

A. Riezebos-Brilman, H. Blokzijl, J.S.F. Sanders, E.A.M. Verschuuren, H.G.M. Niesters
University Medical Center Groningen, Dept. of Medical Microbiology, Groningen

Introduction: Non-travel related Hepatitis E virus (HEV) hepatitis is an under diagnosed emerging infection in developed countries. The screening of healthy blood donors in the Netherlands recently showed an overall seroprevalence of 27%. Due to its asymptomatic course it remains unrecognized in the general population, but can be of great clinical importance in immune-compromised patients such as solid-organ transplant (SOT) recipients. Also, it may be the cause of an acute hepatitis within a normally healthy population. Increasing numbers of reports show that HEV genotype 3 infection can cause chronic hepatitis in SOT recipients. This chronic hepatitis may lead to rapidly progressive cirrhosis, necessitating early therapeutic intervention.
Methods: Since the second half of 2007, SOT recipients with unexplained hepatitis were screened for HEV and clinical data were collected. HEV was further characterized by sequencing of the ORF1 and ORF2 regions.

Results: In total 20 SOT recipients, presenting with elevated ALTs, tested positive for HEV RNA genotype 3. Of these 5 were lung-, 1 heart-, 6 liver- and 6 kidney-transplant recipients; 2 received multiple SOTs. At the time of HEV detection, most patients showed elevated ALT, ranging from 36-1001U/L (median of 128U/L). A chronic infection could be diagnosed in 13 of these 20 SOT recipients (65%). Two kidney-transplant recipients, were able to resolve the infection within 6 months upon reduction of immunosuppression. So far, 10 SOT recipients were successfully treated. Two liver transplant patients were treated with pegylated interferon alpha-2b. Eight patients, 3 lung-, 2 liver-, 1 heart- and 2 multiple organ transplant recipients were treated with oral ribavirin.
Conclusion: Early recognition of HEV infection as a cause of post transplant hepatitis is crucial to minimize liver damage and may play a role in clinical decision making in liver transplant recipients whether or not initiating anti-rejection treatment. An increasing awareness of the existence of chronic HEV infection among transplant clinicians and medical microbiologists is needed.Low grade liver function test abnormalities after SOT should trigger suspicion of chronic HEV infection. The favorable outcome upon antiviral treatment in 8 SOT recipients suggests that oral ribavirin might be an effective treatment of chronic HEV infection. However, further studies are needed to investigate the effectiveness and optimal duration of antiviral treatment in different groups of SOT recipients.

O008

Staphylococcus aureus bi-component leukotoxins and their role in the disarmament of innate and adaptive immunity

V.J. Torres
New York University School of Medicine, Dept. of Microbiology, New York, USA

Staphylococcus aureus (*S. aureus*) is a Gram-positive bacterium responsible for a wide spectrum of diseases worldwide, ranging from minor skin and soft tissue infections to more invasive and deadly infections such as sepsis, pneumonia, osteomyelitis, and endocarditis. The ability of *S. aureus* to thrive as a human pathogen depends on the production of a large arsenal of virulence factors that promote evasion of the host immune response. Among these factors, *S. aureus* strains associated with human infections can produce up to five bi-component pore-forming toxins known as leucocidins or leukotoxins. These toxins, which include LukSF-PV or PVL, HlgAB, HlgCB, LukED, and LukAB, are thought to contribute to *S. aureus* pathogenesis by killing immune cells, hampering the ability of the host to contain infections. These toxins have long been presumed to be redundant virulence factors, as each leukotoxin is able to kill human polymorphonuclear leukocytes (neutrophils), the first line of defense against *S. aureus* infection. Notably, it is now clear that each toxin has the potential to contribute independently to *S. aureus* pathogenesis via unique cell-type and species-specific targeting of cellular receptors. Due to their unique receptor affinities, many of the bi-component toxins expand their repertoire of cellular targets to an increasingly diverse array of leukocytes. Moreover, two toxins, PVL and LukAB, exhibit potent species specificity, resulting in preferential targeting of human leukocytes over that of other mammals. Altogether, these observations support the notion that specific cellular receptors dictate the susceptibility of host cells to the leukotoxins. In this lecture, I will highlight the recent discoveries of bi-component leukotoxin receptors and how receptor-targeting *in vivo* influences *S. aureus* pathogenesis during bloodstream infection.

O009

The staphylococcal cassette chromosome mec type v from *Staphylococcus aureus* ST398 is packaged into bacteriophage capsid

G. Buist[1], M.A. Chlebowicz[1], I. Mašlanová[2], L. Kuntová[2], H. Grundmann[1], R. Pantucek[2], J. Doškar[2], J.M. van Dijl[1]
[1]*University Medical Center Groningen, Medical Microbiology – Molecular Bacteriology, Groningen,* [2]*Masaryk University, Experimental Biology, Brno, Czech Republic*

Introduction: Bacterial determinants for antibiotic resistance can be mobilized from one strain to another via natural transformation, conjugation, or transduction. Even though competence and conjugation have been reported for *Staphylococcus aureus*, genome sequencing studies suggest that especially phages have had a major impact on the evolution of this bacterium. This is evidenced by the presence of phage genome sequences that have inserted at different genomic positions. These prophages can be induced by DNA damage, resulting in the synthesis and dissemination of many phage particles by the host cells. Importantly, not only phage DNA is packaged into the newly synthesized phage particles, but also plasmids or chromosomal DNA of the bacterial host. Previous studies have shown that certain serological group B bacteriophages of *S. aureus* are capable of generalized transduction. The present studies were therefore aimed at assessing the transducing capabilities of the phages from strain UMCG-M4.
Methods/Results: Genome sequencing of a clinical methicillin-resistant *S. aureus* (MRSA) isolate of the sequence type 398 (UMCG-M4) revealed the presence of four prophages belonging to the serological groups A, B and Fa. We show that these phages can indeed transduce plasmid pT181 to the recipient *S. aureus* strain RN4220.

To identify routes for transmission of the Staphylococcal Cassette Chromosome *mec* (SCC*mec*) between *S. aureus* strains, we also investigated the possible involvement of transducing phages in the transfer of this mobile genetic element from strain UMCG-M4 to other strains. While no transduction of the complete SCC*mec* element was observed, we were able to demonstrate that purified phage particles did contain large parts of the SCC*mec* element of the donor strain.

Conclusion: This shows that staphylococcal phages can encapsulate important resistance determinants, such as *mecA*, which may lead to their transfer to other staphylococci.

O010

The Extracellular Adherence Protein from *Staphylococcus aureus* inhibits the classical and lectin complement pathways by blocking formation of the C3 convertase

D.A.C. Stapels[1], J.L. Woehl[2], B.L. Garcia[2], K.X. Ramyar[2], M. Ruyken[3], A. Keightley[4], M. Syriga[5], E. Reis[5], D. Ricklin[5], J.D. Lambris[5], S.H.M. Rooijakkers[3], B.V. Geisbrecht[2]

[1]UMC Utrecht, Dept. of Medical Microbiology, Utrecht, [2]Kansas State University, Dept. of Biochemistry & Molecular B, Manhattan, USA, [3]University Medical Center Utrecht, Medical Microbiology, Utrecht, [4]University of Missouri-Kansas City, School of Biological Sciences, Kansas City, USA,[5]University of Pennsylvania, Dept. of Pathology & Lab. Medicine, Philadelphia, USA

The Gram-positive bacterium *Staphylococcus aureus* is an opportunistic pathogen that can cause severe infections like pneumonia and sepsis. It owes part of its success to the numerous, small inhibitors of the innate immune system it secretes. These target many processes like complement activation, neutrophil activation and neutrophil protease activity. The complement system can be activated via the classical (CP) and lectin pathway (LP) that both result in the formation of the C3 convertase C4b2a. Later on, an alternative pathway (AP) C3 convertase can be formed to amplify the initial signal. Most of the known complement inhibitors of *S. aureus* specifically target components within the AP. Since also the CP and LP are also known to be important during *S. aureus* infection, we screened a collection of recombinant, secreted staphylococcal proteins in search for specific inhibitors of these pathways. Here we identify the extracellular adherence protein (Eap) as a potent, specific inhibitor of both the CP and LP. In an ELISA setup we found that Eap blocked CP/LP-dependent activation of C3, but not C4. Flow-cytometric analysis showed that Eap likewise inhibited deposition of C3b on the surface of *S. aureus* cells. In turn, this significantly diminished the extent of *S. aureus* opsonophagocytosis by neutrophils in CP/LP-dependent assays. This combination of functional properties suggested that Eap acts specifically at the level of the CP/LP C3 convertase. Indeed, we demonstrated a direct, nanomolar-affinity interaction of Eap with C4b using isothermal titration calorimetry. Competition assays showed that Eap binding to C4b inhibited binding of both full-length C2 and its C2b fragment, suggesting that Eap disrupts formation of the CP/LP C3 pro-convertase C4bC2. Eap binding also diminished interaction of C4b-binding protein (C4bBP) with C4b, but required a large stoichiometric excess to block C4bBP-dependent proteolysis of C4b by factor I. As a whole, our results demonstrate that (1) *S. aureus* inhibits the two most potent routes of complement activation by expression of a single protein, and (2) that this protein binds C4b, which prevents subsequent association of C2 and thereby the formation of the C3 convertase C4b2a. This novel mechanism of immune evasion brings us one step closer to understanding the pathogenicity of *S. aureus*.

O011

CCR1, CCR2 and CCR5 are receptors for *Staphylococcus aureus* leukotoxin LukM/LukF'

M. Vrieling[1], K.J. Koymans[1], C.P.M. van Kessel[1], C.J.C. de Haas[1], P.C. Aerts[1], A.P. Koets[2], V.P.M.G. Rutten[2], J.A.G. van Strijp[1]

[1]UMC Utrecht, Dept. of Medical Microbiology, Utrecht, [2]Utrecht University, Faculty of Veterinary Medicine, Dept. of Infectious Diseases and Immunology, Utrecht

Introduction: *Staphylococcus aureus* is a ubiquitous pathogen commonly associated with bovine mastitis. Among secreted virulence factors of *S. aureus* are bi-component pore forming toxins, competent of killing a broad range of immune cells. These toxins consist of two distinct subunits (S and F components) of which the S-component binds to a specific receptor on the cell surface. Recently, it has been discovered that these receptors belong to the G-protein coupled receptor family (GPCRs). Genes encoding the leukotoxin pair LukM/LukF' seem to be exclusively harboured by mastitits causing strains. This toxin pair is described to be the most active leukotoxin on bovine neutrophils, but is also active on monocytes and a small subpopulation of lymphocytes. In this study we set out to identify the molecular targets of LukM/LukF' on bovine leukocytes.

Methods: All described bovine CXC- CC-, CX3X- and XC- chemokine receptors were cloned and transiently expressed in 293T cells. Transfected cells were incubated with recombinant LukM/LukF' toxin and pore-formation was determined by measuring DAPI-fluorescence in a flowcytometer. Binding of recombinant LukM was assessed using an anti-HIS-tag antibody. Human and bovine peripheral blood leukocytes were isolated from venous

blood of healthy donors using a Ficoll/Histopaque gradient. Pore-formation of human and bovine leukocytes was measured as described above. CCR1 expression on bovine peripheral blood cells was determined with a cross-reactive anti-human CCR1 antibody.

Results: Screening of the transfected cells showed that LukM specifically binds to bovine CCR1, CCR2 and CCR5 expressing 293T cells. Although the LukM/LukF' gene is rarely found in strains of human origin, human CCR1, CCR2 and CCR5 transfected cells also bind LukM. Addition of LukF' results in pore-formation allowing entry of DAPI into transfected cell expressing either bovine or human receptor variants. Therefore, it is expected that the bovine and human CCR1, CCR2 and CCR5 exhibit a common interaction site for LukM. In human peripheral blood CCR1, CCR2 and CCR5 are constitutively expressed on monocytes and specific T cell subsets but not on neutrophils. This corresponds with the subsets targeted by LukM in human peripheral blood, where -in contrast to the bovine situation- the neutrophil population remains unaffected. Interspecies differences in chemokine receptor expression could possibly explain the differences in LukM-sensitivity. In contrast to human neutrophils, bovine neutrophils express significant levels of CCR1 which can explain their susceptibility to LukM/LukF' toxin.

Conclusion: CCR1, CCR2 and CCR5 are receptors for *Staphylococcus aureus* leukotoxin LukM/LukF'. When anti-bovine CCR2 and CCR5 antibodies become available, we hope to characterize all cell subsets targeted by LukM/LukF' and reveal the functional *in vivo* relevance of this toxin in bovine mastitis.

O012

Phagocytosis escape by a *Staphylococcus aureus* protein that connects complement and coagulation proteins at the bacterial surface

A. Kuipers, Y.P. Ko, C.M. Freitag, I. Jongerius, E. Medina, W.J. van Rooijen, A.N. Spaan, K.P.M. van Kessel, M. Hook, S.H.M. Rooijakkers
UMC Utrecht, Dept. of Medical Microbiology, Utrecht

Upon contact with human plasma, bacteria are rapidly recognized by the complement system that labels their surface for uptake and clearance by phagocytic cells. *Staphylococcus aureus* secretes the 16 kD Extracellular fibrinogen binding protein (Efb) that binds two different plasma proteins using separate domains: the Efb N-terminus binds to fibrinogen, while the C-terminus binds complement C3. In this study, we show that Efb blocks phagocytosis of *S. aureus* by human neutrophils. *In vitro*, we demonstrate that Efb blocks phagocytosis in plasma and in human whole blood. Using a mouse peritonitis model we show that Efb effectively blocks phagocytosis *in vivo*, either as a purified protein or when produced endogenously by *S. aureus*. Mutational analysis revealed that Efb requires both its fibrinogen and complement binding residues for phagocytic escape. Using confocal and transmission electron microscopy we show that Efb attracts fibrinogen to the surface of complement-labeled *S. aureus* generating a capsule'-like shield. This thick layer of fibrinogen shields both surface-bound C3b and antibodies from recognition by phagocytic receptors. This information is critical for future vaccination attempts, since opsonizing antibodies may not function in the presence of Efb. Altogether we discover that Efb from *S. aureus* uniquely escapes phagocytosis by forming a bridge between a complement and coagulation protein.

O013

Controlling campylobacteriosis by reducing the contamination of broiler chicken meat

A.H. Havelaar, A.N. Swart
RIVM, CIb, Bilthoven

Campylobacteriosis is one of the most important foodborne diseases worldwide. In the Netherlands, the incidence of human campylobacteriosis has increased by 25% between 2003 and 2011, and correlated with increasing use of proton-pump inhibitors, in particular in the elderly population. In 2011, the disease burden (3,600 Disability Adjusted Life Years) was highest among 14 food-related pathogens, while the cost-of-illness (82 million euros) was second only to norovirus. Molecular studies using Multi Locus Sequence Typing suggest that approximately 65% of strains infecting humans originate from the poultry reservoir. By combining molecular and epidemiological studies, it is estimated that approximately 30% of all human cases is related to the handling and consumption of poultry meat. Hence, major public health benefits are expected from reduction of the contamination of poultry meat.

Risk assessment studies suggest that in particular removing highly contaminated broiler meat batches from the fresh meat market will reduce human disease incidence. To leave flexibility to the industry, a process hygiene criterion (PHC) is a useful instrument to communicate the necessary level of control. Using monitoring data provided by the Dutch poultry industry, a risk assessment model was developed to evaluate PHC with different levels of stringency. A PHC with a critical limit of 1,000 cfu/g (breast skin samples after cooling) is most efficient. If all batches of broiler meat consumed in the Netherlands would meet such a criterion, the incidence of human illness would be reduced by approximately 70%. Economic analysis suggests that the costs to implement such an intervention are less than the averted

cost-of-illness, while additionally there would be benefits in reduced disease burden.

O014

Campylobacter virulence: the Omics perspective

A.H.M. van Vliet
Institute of Food Research, Gut Health and Food Safety Programme, Norwich, United Kingdom

The foodborne bacterial pathogen *Campylobacter* is the most common cause of bacterial infectious intestinal disease in the Western world. Surprisingly, despite a strong research effort on *Campylobacter* epidemiology, pathogenesis and biology, and the early availability of several genome sequences, there is still a relatively limited understanding of its virulence and transmission. As a consequence, *Campylobacter* continues to defy public health and food safety approaches to its control, and reduction of *Campylobacter* illness is a key target for regulators, retailers, producers and industry.
The rapid developments in the different omics technologies, especially high-throughput sequencing, and the small genome size of *Campylobacter* have allowed for a rapid increase of available genome sequences of *Campylobacter* isolates. Repositories like BIGSdb, with > 2,000 *C. jejuni* and *C. coli* genome sequences, now allow for comparative genomics of *Campylobacter* isolates from different sources, such as clinical isolates, agricultural and environmental isolates. Such analyses highlight the significant genomic variability between isolates, with strains differing in plasmid content, and in the presence or absence of clusters of genes or insertion elements conferring metabolic or virulence properties. The aim of this presentation is to highlight the potential of omics technologies to further the research in in virulence, epidemiology and biology of *Campylobacter*.

O015

Interventions to prevent *Campylobacter* in primary production, new developments and dilemma's

J.A. Wagenaar[1,2]
[1]*UMC Utrecht, Dept. of Infectious Diseases and Immunology, Utrecht,* [2] *WHO Collaborating Center for Campylobacter and OIE Reference Laboratory for Campylobacteriosis*

Campylobacteriosis is one of the most important bacterial food borne illnesses in humans worldwide (see 'The Global view on *Campylobacter*' report of a WHO-expert meeting on *Campylobacter* http://www.who.int/iris/bitstream/10665/80751/1/9789241564601_eng.pdf)
Attribution studies have identified poultry as the major reservoir responsible for up to 80% of the human infections. However, only an estimated 30% of the human infections is associated with consumption and handling of poultry meat. Other, yet unknown, routes of transmission of *Campylobacter* from the poultry reservoir (living animals on farms) towards humans contribute considerably. Control of *Campylobacter* in the poultry meat production chain will reduce the human burden of illness; the control of the *Campylobacter* colonization in broilers (primary production) would be the best approach. Broilers can easily become colonized with *Campylobacter* and, although risk factors for flocks to become colonized with *Campylobacter* have been identified, interventions in the primary production cannot be easily implemented effectively. In lack of effective measures in slaughterhouses to prevent contamination of meat from colonized animals, focus should be on prevention of introduction of *Campylobacter* into living broilers. Options for increased biosecurity and in particular the control of flies will be presented. There is general shift towards production systems with slower growing animals and outdoor access of birds. This is conflicting with *Campylobacter*-free production. The dilemma between the production of safe food and consumers' preferences for increased animal welfare and organic production will be discussed.

O016

Time series analysis of genes expressed in epithelial cells during infection by *Campylobacter jejuni* 11168 and its avirulent cas9 deletion mutant

P. van Baarlen[1], R. Louwen[2], H.P. Endtz[2], J.M. Wells[2]
[1] *Host-Microbe Interactomics Group, Wageningen University, Wageningen,* [2] *Erasmus MC, Dept. of Medical Microbiology and Infectious Diseases, University Medical Centre, Rotterdam*

A repetitive DNA fragment with small variable sequences in between, CRISPR, and a set of associated genes (Cas), together forming an adaptive immune system in diverse bacteria, have shown to be crucial regulators of pathogenicity of the zoonotic human intestinal pathogen *Campylobacter jejuni*. We previously showed that disruption of *cas9* leads to near-loss of the ability of some *C. jejuni* isolates to cause damage to intestinal epithelial cells. Since *C. jejuni* adheres onto and invades into intestinal epithelial cells within 4 hours, we hypothesized that in this time period cellular pathways leading to cell damage might be activated by wild-type but not *cas9* deletion mutant *C. jejuni*. We therefore performed Caco-2 infection assays using the virulent wild-type 11168 isolate and its isogenic avirulent *cas9* deletion mutant that we had generated previously and extracted eukaryotic RNA at 6 different timepoints in order to obtain transcriptomes at these timepoints. We analysed transcriptomes using time-series statistics and gene ontology enrichment calculations and

found genes and biological processes that were differentially regulated in Caco-2 cells over time upon infection by 11168 and its *cas9* deletion mutant. Using network analysis, we were able to identify the main cellular processes and identify a set of about 200 genes, including major gene regulators such as *TP53* and *JUN*, that were differentially regulated during infection by wild-type and deletion mutant. These regulators could explain the differential induction of cell damage by the 11168 wild-type and *cas9* deletion mutant strain and shed light on genetic factors determining *C. jejuni*-induced disease and cell death induction. We concluded that time series transcriptomics were suitable to describe the dynamic infection process of Caco-2 cells by *C. jejuni* and to find the main pathways and genes mediating cell damage and cell death that were induced by *C. jejuni* within the first 4 hours after infection. This study also highlighted the importance of CRISPR-Cas and specifically, Cas9, for *C. jejuni* pathogenicity.

O017

Combined analysis of whole genome MLST data and whole genome mapping data for clinical *Campylobacter jejuni* and *C. coli* isolates

M.C. Rijnsburger[1], K. de Bruyne[2], R. Burggrave[3], A.J. Cody[4], M.C.J. Maiden[4], B. Pot[2], M. Bos[5], P. Savelkoul[5], H. Pouseele[2]

[1]VU University Medical Center, Dept. of Medical Microbiology and Infection Prevention, Amsterdam, [2]Applied Maths NV, Sint-Martens-Latem, [3]PiEXT BV, Rosmalen, [4]University of Oxford, Oxford, United Kingdom,[5]VU University Medical Center, Amsterdam

Introduction: For many years, pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST) based on a small set of housekeeping genes, have been applied as standards for epidemiological analyses of *Campylobacter*. Recently developed methods such as whole genome mapping (WGM), a technique generating ordered restriction maps across entire microbial genomes and whole genome MLST (wgMLST), in this instance utilizing whole genome data from up to 1647 loci, have been jointly evaluated as replacement techniques.

Methods: Whole genome maps and wgMLST data were generated for 103 strains of *Campylobacter jejuni* and 10 strains of *C. coli*. The whole genome maps were obtained using the Argus™ Whole Genome Mapping System (OpGen< sup > < /sup >), using the NheI restriction enzyme. Overlapping single-molecule maps were assembled with a minimum of 30X coverage over any part of the genome, yielding highly accurate consensus whole genome maps. *Campylobacter* strains with known assembled genome sequences were subjected to WGM for validation of the technique.

The whole genome sequence data ware obtained from the Bacterial Isolate Genome Sequence Database (BIGSdb, http://pubmlst.org/campylobacter). The wgMLST profiles were extracted from these sequences by a newly developed algorithm implemented in the BioNumerics software suit (patent pending). Both data types, the whole genome maps and wgMLST data were analysed using the BioNumerics< sup > < /sup > Seven software (Applied Maths NV).

Results: Whole genome maps obtained in vitro and in silico for a number of *C.jejuni* reference strains were compared to validate the WGM technique.

Representative strains of the major clonal complexes of *C. jejuni* were investigated in order to compare the epidemiological resolution power of the different techniques. Additional isolates belonging to ST-21 complex, the most prevalent complex found clinically, were also investigated by both wgMLST and WGM. Genetic variation arising during infection was investigated by comparison of isolates collected from the same patient during the course of their illness, using both procedures.

Overall, data obtained by wgMLST and WGM were highly reproducible and of greater resolution than that obtained by PFGE. The combination of the two techniques facilitated correlation of WGM with DNA sequences. Comparison of WGM data from all isolates identified those known to belong to a confirmed outbreak. The congruence between wgMLST and WGM results was calculated at 81%.

Conclusion: Whole genome MLST data and WGM are important analysis tools for real-time investigations and proved valuable alternatives for PFGE analysis of *Campylobacter*. The BioNumerics< sup > < /sup > Seven software offers a platform where both techniques can be analysed and the results can be linked.

O018

What counts? Producing data on CVC-BSIs for quality improvement: An ethnographic study.

C. Tarrant

SAPPHIRE, Dept. of Health Sciences, University of Leicester, United Kingdom

Background: Data on hospital-acquired infection rates are seen as key performance indicators, and play an important role in quality improvement efforts. The credibility of these data has, however, been called into question. Little is known about how hospitals go about generating performance data for quality improvement, or the challenges in producing valid and reliable data. We explored the factors impacting on the quality and comparability of data on rates of central venous catheter bloodstream infections (CVC-BSIs) reported to a large-scale patient safety programme in England.

Methods: We conducted an ethnographic study involving observations and collection and analysis of documents in 17 English intensive care units. We also conducted face-to-face interviews with 93 staff in participating ICUs, and telephone interviews with 29 staff from other ICUs who had attended programme training events. The study focused on gaining an understanding of the features of local systems to collect data on CVC-BSIs that were used to generate data for reporting to the central programme database.

Findings: We found a great deal of variability within and between ICUs in their approach to collecting and reporting data on CVC-BSIs. Although the programme provided staff with clear definitions of CVC-BSIs, these definitions were seen as subjective and as admitting the possibility of unfairness. Hospitals varied in: how they applied inclusion and exclusion criteria, the data collection systems they established, practices in sending blood samples for analysis, microbiological support and laboratory techniques, and procedures for collecting and compiling data on possible infections.

Hospitals faced mundane challenges in coordinating their data collection systems, including poor alignment of clinical practices and the tasks of generation of data for auditing purposes, and difficulties engaging staff in data recording.

This resulted in a data set that had limited comparability: it reflected local context and local social practices, rather than being standardized across all ICUs.

Conclusion: Rather being objective and reliable measures of incidence, reported rates to some extent reflected variation in underlying local practices: differences in the ways that ICUs made sense of definitions, the features of the data systems they designed, and local variability in clinical and administrative practices.

Unless hospitals are deploying the same methods to generate the data, using their reported rates to produce league tables for performance or impose financial sanctions is probably not appropriate.

O019

Surveillance of hospital-associated infections (HAI) and antimicrobial resistance (AMR) in the Netherlands and rankability of their rates

A.K. van der Bij
RIVM, Centre for Infectious Disease Control, Bilthoven

Antimicrobial resistance (AMR) and healthcare-associated infections (HAI) are among the most serious public health problems. The issues of AMR and HAI overlap widely, but are not synonymous. HAI are often due to antibiotic-resistant bacteria, but not always. Inversely, antibiotic-resistant bacteria, including multidrug resistant types, are not only responsible for HAI but are also responsible for infections in outpatients and found as part of the flora of healthy individuals, in pet animals and in the environment. They are also isolated from food-producing animals and from food products. The estimated burden of AMR in HAI in the European Union is on a yearly basis; 25 000 deaths, 2,5 million extra hospital days and EUR 900 million extra costs. Surveillance provides important information that allows for the identification of trends in pathogen and infection incidence and AMR, including identification of emerging pathogens. Additionally, routine surveillance is important for the control of AMR and HAI and for guiding clinician decisions regarding empiric therapy. In this presentation the value of surveillance programs, including criteria for their evaluation on AMR and HAI will be discussed. Examples of Dutch and international surveillance programs on AMR and HAI will be presented and limitations in the data collection will be discussed. Besides the role of national surveillance programs in control of infections and resistance, information of these programs can also be used as an indicator of quality of care by assessing hospital performance. However, the random variation and case-mix differences must be taken into account when judging individual hospitals since only a small part of observed unadjusted differences between hospitals in HAI rates is likely to be attributable to quality of care differences. The presentation will conclude with future challenges in AMR and HAI surveillance and the opportunities for improvement of current national surveillance programs that will help to assess the impact of rational prescribing programs for both current and new antibacterial agents in humans and animals and the identification of new and emerging threats.

O020

Carbapenemase producing bacteria in travellers constitute a potential threat for current hospital infection control programs

R.H.T. Nijhuis[1], P.H.M. Savelkoul[2], A.A. van Zwet[1]
[1]*Rijnstate, Dept. of Medical Microbiology and Immunology, Velp,* [2]*MUMC+, Dept. of Medical Microbiology, Maastricht*

Introduction: The increasing prevalence of carbapenemase producing bacteria (CPB) is a challenge for infection-control workers. Despite the endemic situation of CPB in some countries, hospital infection-control screening programs in The Netherlands are only aimed for detection of CPB in patients that had been hospitalized abroad. In this study, we investigated the prevalence of CPB in fecal samples of patients travelling abroad, that were not included in the aforementioned screening approach. Simultaneously, these results were compared with a control-group of patients that had not been abroad.

Methods: In total, 400 fecal samples that were sent to the laboratory for investigation on pathogens causing gastroenteritis were included in this study. Two-hundred came from patients that had been abroad and the other 200 came from patients with no travelling history. All samples were tested using two selective agars (ChromID Carba and ChromID OXA-48, Biomerieux) and a molecular method able to detect carbapenemase-genes KPC, NDM/VIM and OXA-48 (Check-Direct CPE, Check-Points).
Results: Both patient groups included 111 female and 89 male patients, with a mean age of 33.1 and 33.7 for the patients from abroad and the control group respectively. Most patients travelled to Turkey, India/Nepal, France, Morocco, Egypt and Italy (21, 18, 13, 11, 10, 8 respectively). On the selective agars, 13 fecal samples (of which 7 from abroad) showed carbapenemase suspected isolates as interpreted by the manual. After confirmation using Vitek2 (Biomerieux) and (if necessary) the Check-Direct CPE, none of these isolates turned out to be a carbapenemase producer. The molecular method on the other hand, gave a positive result in 2 fecal samples. Both samples were included in the group with patients from abroad and came from patients that had travelled to Morocco and India/Nepal without any history of hospitalization. The carbapenemase genes detected were OXA-48 (Morocco) and NDM/VIM (India/Nepal), with Ct-values of 37.7 and 23.1 respectively. Only the carbapenemase producing isolate of the India/Nepal sample could be subcultured after overnight incubation in a broth. Of both fecal samples, no growth could be detected using the selective media.
Conclusion: This study shows that CPB can be detected directly from fecal samples from patients that have been abroad without hospitalization, especially in patients that visited countries where CPB is endemic (such as Morocco and India). The current screening approach (CPB detection only in patients that have been hospitalized abroad) might not be sufficient enough. Moreover, we experienced inadequate detection of CPB when selective media were used. Therefore molecular methods should be an essential element in each CPB screening program.

O021

Re-emerging hepatitis E virus in the Netherlands

H.L. Zaaijer, M. Molier, E. Slot, B.M. Hogema
Sanquin, Blood-borne Infections, Amsterdam

Introduction: In Europe, the dynamics of endemic hepatitis E virus (HEV) infection remain enigmatic. The present study aims to determine anti-HEV seroprevalence changes over time, using archived and recent samples from Dutch blood donors.
Methods: Archived Dutch donor samples collected between 1988 and 2004 were tested for presence of HEV IgG antibodies (Wantai anti-HEV EIA). In addition, 5,239 donors who donated throughout the Netherlands in 2011 and 2012 were tested for presence of anti-HEV IgG and IgM; and for HEV RNA when IgM-positive; and 40,176 blood donations collected in 2011 and 2012 were tested for HEV RNA in 459 pools of 48 or 480 donations.
Results: The age-weighed anti-HEV seroprevalence among donors aged 18-64 declined from 46.6% in 1988 to 27.3% in 2000 and to 20.9% in 2011. Seroprevalence strongly increased with age at each time point. The highest anti-HEV seroprevalence (76%) was found among the oldest donors in 1988. The course of HEV incidence is best reflected by the seroprevalence in younger donors, who have been exposed shorter: Among donors aged 18-21 years the anti-HEV seroprevalence declined from 19.8% in 1988 to 7.0% in 1995 and to 4.3% in 2000, increasing to 12.7% in 2011. Regarding the current infection pressure of HEV in the Netherlands, the screening of 40,176 recent donations for HEV RNA identified 13 viremic donors. Among 5,239 recent blood donors, 1,401 (27%) tested repeat-positive for HEV IgG, of which 49 (3.5%) also tested positive for HEV IgM; 4 IgM-positive donors tested positive for HEV RNA. Sixteen of the 17 (13+4) HEV strains could be sequenced and were found to be HEV genotype 3.
Conclusion: The finding of 17 HEV RNA-positive donations among 45,415 recent donations corresponds to at least one HEV viremic blood donation per day in the Netherlands. Surprisingly, the current high incidence of endemic HEV infection may be less than it was before 1988, when anti-HEV seroprevalence among oldest donors reached 76%. Apparently, after an extended period of low incidence of HEV infection in the Netherlands, HEV re-emerged in the last decade.

O022

Whole genome sequencing reveals diverse sources for *Clostridium difficile* infection

S. Walker
University of Oxford, Oxford, United Kingdom

Clostridium difficile infection (CDI) has traditionally been considered predominantly transmitted within hospitals. However, endemic spread hampers identification of sources of infections and the assessment of intervention efficacy. High-throughput whole-genome sequencing (WGS) methods are revolutionizing bacterial genomics, potentially providing sufficient resolution to determine which cases within temporo-spatial clusters are likely to have been transmitted. The assessment of whether two *C. difficile* WGS are sufficiently genetically "close" for transmission between cases to have been plausible can only be made within the context of the evolution and genetic diversity of the organism within individual patients over

time. Within this framework, WGS can identify outbreaks of closely genetically-related cases, including highlighting potential genetic links between cases not previously known to be related based on epidemiological data. In other situations, WGS can refute transmission between cases that are epidemiologically linked (for example through shared space and time on a hospital ward), including refuting transmission between cases sharing the same genotype – indicating the additional benefit WGS may provide over existing typing strategies.

All symptomatic hospital/community CDI cases from Oxfordshire, UK, September 2007-March 2011, underwent WGS. Single nucleotide variants (SNVs) between cases were compared using *C. difficile* evolution rates estimated from 145 serially-sampled patients, which showed that 0-2 SNVs could be expected between transmitted isolates < 124 days apart. Plausible epidemiological links between genetically-related cases were identified from hospital admission/community location data. Of 957 CDI from April 2008-March 2011, only 333 (35%) were within 2 SNVs of ≥ 1 previous case since September 2007; 428 (45%) were > 10 SNVs from all previous cases. Of the 333 cases ≤ 2 SNVs from a prior case (consistent with transmission), 126 (38%) shared ward-based contact within plausible limits on infectious/incubation periods, but 120 (36%) had no hospital/community contact. Distinct subtypes (cases > 10 SNVs from all previous cases) continued to be identified consistently throughout the study, suggesting cases arise from a considerable reservoir of *C. difficile*. Surprisingly, over the study period reductions in the incidence of genetically-related CDI (≤ 2 SNVs from a previous case) were similar to those in genetically-distinct (> 10 SNVs) CDI, suggesting interventions targeting transition from exposure to disease, rather than just transmission, likely played a major role in recent CDI declines.

Thus, over a 3 year period, 45% of Oxfordshire CDI was genetically-distinct from all previous cases. Genetically diverse sources, in addition to symptomatic patients, play a major part in *C. difficile* transmission. With the advent of rapid bench-top sequencers, WGS will soon be provided in a format and at a cost accessible to reference laboratories and routine hospital laboratories, enabling sources of outbreaks to be identified in clinically relevant timescales, and demonstrating the potential of WGS to transform infection control practice over the coming decade.

O023

Changes of the epidemiology of *CDI* in The Netherlands and Europe

S.M. van Dorp[1], D.W. Notermans[2], G.A. Kampinga[3], A.G. Buijting[4], Ecdis[5], E.J. Kuijper[1]

[1]LUMC, Dept. of Medical Microbiology, Leiden, [2]RIVM, Centre for Infectious Disease Control, Bilthoven, [3]University Medical Center Groningen, Dept. of Medical Microbiology, Groningen, [4]St. Elisabeth Hospital, Dept. of Medical Microbiology, Tilburg, [5]European Clostridium difficile infection surveillance network

Introduction: *Clostridium difficile* is a major cause of health-care associated diarrhoea in Europe. The incidence of *C. difficile* infections (CDI) increased in more than half of the European countries in the last five years (Point-Prevalence Study ECDC, 2013) but standardized European-wide surveillance of CDI has not been implemented yet. Since 2009, a national sentinel CDI surveillance program is performed in The Netherlands. Simultaneously, we participate in a European network to support capacity building for pan-European CDI surveillance. As part of this initiative, we tested the feasibility of a new standardized surveillance program in fourteen European countries.

Methods: Nineteen hospitals, distributed equally among the Netherlands, participate in a sentinel surveillance of CDI and continuously collect clinical, epidemiological and microbiological data. Of these nineteen hospitals, three tested a new European surveillance program for a period of three months. This pan-European survey was performed from May-October 2013 in thirty-seven hospitals in fourteen European countries. Epidemiological hospital data and a minimum set of clinical data from all patients with CDI diagnosed were collected. For the first ten cases per hospital, extended clinical data and faeces samples were collected. Samples were cultured for the presence of *C. difficile*. All *C. difficile* isolates were characterized and PCR ribotyped at the National Reference Laboratory in Leiden.

Results: Since 2009, a stable CDI incidence of 15 per 10,000 admissions is observed in The Netherlands. In 2013, 37% of all CDI patients had an onset of symptoms in the community. Ribotype 014 (16%) and ribotype 001 (14%) were most frequently isolated. The more virulent ribotype 078 (12%) was the third most common type. *C. difficile* ribotype 027 accounted for 3% of all surveyed CDI patients, and caused an outbreak in one of the nineteen participating hospitals. In contrast, 17 laboratories submitted 296 *C. difficile* samples from patients with severe CDI or from outbreaks; ribotype 027 was found in 20%. The overall CDI incidence across the thirty-seven European hospitals was much higher; 26,2 per 10,000 discharges or admissions (IQR 15,4-41,2). Half of the patients had symptoms present at admission; 87% of the patients had healthcare-associated CDI. Most frequently found types were ribotype 027 (30%), 014/020 (14%) and 001 (6%), whereas ribotype 078 was only found in 2.2%.

Conclusions: The CDI sentinel surveillance in The Netherlands indicates that *C. difficile* ribotype 027 does not belong to the seven most frequently endemic strains, but is

still associated with outbreaks. In contrast, type 027 is an important ribotype in many European countries, especially in Eastern-Europe. Implementation of a standardised CDI surveillance in acute care hospitals is indispensable for estimating the incidence and for tracking important epidemiological trends and changes.

O024

Update of European guidelines for the treatment of *Clostridium difficile* infection (CDI)

S.B. Debast[1], M.P. Bauer[2], E.J. Kuijper[2]

[1]*Radboudumc, Dept. of Medical Microbiology, Nijmegen,* [2]*Leiden University Medical Center, Dept. of Infectious Diseases, Leiden*

Introduction: In 2009 the first European Society of Clinical Microbiology and Infection (ESCMID) treatment guidance document for *Clostridium difficile* infection (CDI) was published. The guideline has been applied widely in clinical practice. In 2013, a new European study group was formed to update and review newly available treatment modalities of CDI.

Methods: A computerized literature search was carried out to analyse randomized and non-randomized trials investigating the effect of an intervention on the clinical outcome of CDI. The Grades of Recommendation Assessment, Development and Evaluation (GRADE) system was used to grade the strength of our recommendations and the quality of the evidence. The ESCMID and an international team of experts from 11 European countries supported the process. Treatment options that were reviewed include: antibiotics, toxin-binding resins and polymers, immunotherapy, probiotics, and faecal or bacterial intestinal transplantation. To improve clinical guidance in the treatment of CDI, treatment recommendations were specified for various patient groups, e.g. initial non-severe disease, severe CDI, first recurrence or risk for recurrent disease, multiple recurrences, and treatment of CDI when oral administration is not possible.

Results and recommendations: Except for very mild CDI, which is clearly induced by antibiotic usage, antibiotic treatment is advised. The main antibiotics that are recommended are: metronidazole, vancomycin and fidaxomicin. The choice for one of these antibiotics depends mainly on the stage and severity of disease. Faecal transplantation is one of the main advances in combined non-antibiotic and antibiotic therapies for multiple recurrent CDI. In case of perforation of the colon and/or systemic inflammation and deteriorating clinical condition despite antibiotic therapy, total abdominal colectomy or diverting loop ileostomy combined with colonic lavage is recommended.

O025

Characteristics of hospitalised acute Q fever patients during a large epidemic, the Netherlands

C.C.H. Wielders[1,2], A.M.H. Wuister[2,3], V.L. de Visser[4], M.G. de Jager-Leclercq[3], C.A.R. Groot[5], F. Dijkstra[2], A.B. van Gageldonk-Lafeber[2], J.P.G. van Leuken[2,6], P.C. Wever[1], W. van der Hoek[2], P.M. Schneeberger[1,2]

[1]*Jeroen Bosch Hospital, Dept. of Medical Microbiology and Infection Control, 's-Hertogenbosch,* [2]*National Institute for Public Health and the Environment (RIVM), Centre for Infectious Disease Control, Bilthoven,* [3]*Bernhoven Hospital, Dept. of Internal Medicine, Uden,* [4]*Jeroen Bosch Hospital, Dept. of Pulmonary Diseases, 's-Hertogenbosch,* [5]*Bernhoven Hospital, Dept. of Pulmonary Diseases, Uden,* [6]*Utrecht University, Faculty of Veterinary Sciences, Institute for Risk Assessment Sciences, Utrecht*

Introduction: From 2007 through 2009, the Netherlands experienced a major Q fever epidemic with unaccountable high hospitalisation rates (20-46%; overall 22%), mostly for pneumonia. This is much higher than the 2-5% hospitalisation rate for acute Q fever pneumonia and hepatitis reported in the international literature. We analysed epidemiological and clinical features of hospitalised acute Q fever patients and compared patients presenting with Q fever pneumonia with patients admitted for other forms of community-acquired pneumonia (CAP). We also examined whether proximity to infected ruminant farms as an indicator of infection dose was a risk factor for hospitalisation.

Methods: A retrospective cohort study was conducted for all patients diagnosed and hospitalised with acute Q fever from 2007 through 2009 in one general hospital situated in the high Q fever incidence area in the south of the Netherlands (Bernhoven Hospital). Patient characteristics, clinical symptoms and follow-up data were obtained from the clinical patient files for the 183 consecutively hospitalised acute Q fever patients. In a sub analysis, we compared these acute Q fever patients which were hospitalised with a pneumonia (defined as an infiltrate observed on a chest X-ray) with data from 254 hospitalised CAP patients (with aetiology other than *Coxiella burnetii*) by using two pneumonia severity scores, the Pneumonia Severity Index (PSI) and the Confusion, blood Urea nitrogen, Respiratory rate, Blood pressure, age = 65 score (CURB-65). Hepatitis was defined as > twofold increase of the reference value for alanine aminotransferase in combination with > twofold increase in bilirubin.

Results: The most frequent clinical signs upon presentation were fever, cough and dyspnoea. Pneumonia was the clinical presentation of the acute Q fever infection in 154 (84.2%) patients. Elevated liver enzymes were found (alanine aminotransferase in 32.3% of the patients and bilirubin in 25.6%), though none of the patients met our

definition of hepatitis. Twelve percent of the acute Q fever patients living in the catchment area of the Bernhoven Hospital were hospitalised with a median duration of five days. Acute Q fever pneumonia patients were younger, had less co-morbidity, and had lower PSI and CURB-65 scores than other CAP patients (all $p < 0.001$). Anecdotal information from attending physicians suggests that some patients were admitted because of severe subjective dyspnoea, which is not included as an item in the scoring systems. Proximity to an infected ruminant farm was not associated with hospitalisation. Additional microbiological tests hardly revealed any other aetiological pathogens.

Conclusions:

1. Hospitalised Dutch acute Q fever patients mostly presented with fever and pneumonia.
2. Patients with acute Q fever pneumonia were hospitalised despite young age, low PSI and CURB-65 scores, and limited co-morbidity.
3. Proximity to an infected ruminant farm, reflecting level of exposure to *C. burnetii*, does not influence admission rates.

O026

Whole genome sequencing revealed transmission of *Clostridium difficile* between humans and farm animals in the Netherlands

C.W. Knetsch[1], T. Connor[2], A. Mutreja[3], I. Sanders[1], S. Dorp[1], L. Lipman[4], L. Keessen[4], J. Corver[1], E. Kuijper[1], T. Lawley[3]

[1]*Leiden University Medical Center, Dept. of Medical Microbiology, Leiden,* [2]*Cardiff School of Biosciences, Cardiff, United Kingdom,* [3]*Wellcome Trust Sanger Institute, Bacterial Pathogenesis Laboratory, Hinxton,United Kingdom,* [4] *Utrecht University, Institute for Risk Assessment Sciences, Utrecht*

Objectives: *Clostridium difficile* is an important cause of antibiotic associated diarrhea in the developed world. The incidence of CDI caused by *C. difficile* PCR ribotype 078 is increasing within the European healthcare system. The source of this increase remains to be elucidated However, farm animals are suggested as a potential reservoir by which *C. difficile* is entering the human population, thereby suggesting zoonosis. We used whole genome sequencing combined with single-nucleotide polymorphism (SNP) analysis to compare pairs (n = 12) of *C. difficile* 078 samples isolated from farmers and pigs collected at the same farm.

Methods: In total, 65 *C. difficile* 078 isolates were sequenced and SNP typed including clinical isolates (n = 31), farm isolates (15 farmer and 14 pig), supplemented with pig isolates (n = 5) representing a nation-wide sampling. Whole genome sequencing was done on extracted genomic DNA using Illumina Hiseq. The short DNA sequence reads were bioinformatically mapped against a high-quality reference genome, after which SNPs were called. The phylogenetic SNPs together with year of isolation of the samples were used to estimate the mutation rate of type 078. Sequenced genomes were scanned to identify potential antimicrobial resistance determinants that were linked to an antimicrobial resistance phenotype.

Results: *C. difficile* 078 isolated from humans and farm animals are in 3 out of twelve human-pig pairs genetically indistinguishable (zero SNP differences). A mutation rate of 1.1 SNPs per genome per year for type 078 was estimated. Our study revealed that clonal spread (here defined as = 1 SNP difference) of *C. difficile* between animals and humans is much more commonly observed than expected. This is illustrated by the percentage of farm sites (˜42%) where clonal spread was observed. In addition, we observed that identical antimicrobial resistance determinants carried on mobile genetic elements are present in human and animal *C. difficile* 078 isolates. The presence of the tetM determinant correlated with a tetracycline resistant phenotype.

Conclusion: Our results demonstrate a transmission network for *C. difficile* 078 between the community and farm animals that is spreading genetic determinants of antibiotic resistance. Zoonotic transmission from farm animals is probable partially responsible for the increase in human *C. difficile* infections due to type 078.

O027

Antoni van Leeuwenhoek: The Myth, The Man

D. Anderson

Humanities, Medaille College, Buffalo, USA

Antoni van Leeuwenhoek, born in 1632, was a world-famous citizen of Delft, one of the leading cities in what was then the world's most healthy, prosperous, and learned country, the Dutch Republic. Coming from a prosperous family with a heritage of public service, Leeuwenhoek continued that tradition by serving in paid municipal offices. Self-taught, he did not begin his scientific career until he was in his forties, but he continued it for the half century until his death in 1723. He made hundreds of tiny single-lens microscopes, the limitations of which forced him to pioneer the use of now-common microscopic techniques such as sectioning. He was the first human to see microbes and microscopic structures in animals, plants, and minerals. Over fifty years, he wrote only letters, more than three hundred of them, and published half of them himself. More than a hundred were published in translation in Philosophical Transactions, the journal of England's Royal Society, of which he was elected a fellow in 1680. Today, Leeuwenhoek is considered in the lesser rank of scientists and is not well known outside of his homeland.

Over the years, the few documented facts about Leeuwenhoek's life have been fleshed out with a story about the city janitor who got lucky with a lot of help from his betters. Fleshing out makes a good story, or at least a coherent one. As a result, biographies of Leeuwenhoek, especially short biographical sketches, are full of error.
To help contextualize his science, this talk will respond to the myth of Leeuwenhoek with documented facts about his heritage, his family, his means of support, his intelligence, his professional secrets, and his relationship to the scientific community, especially the Royal Society. Recent archival research in Delft has contributed new information in these areas, but much remains to be learned.

O028

Through van Leeuwenhoek's eyes – his microscopes then and now

L.A. Robertson
TU Delft, BT, Delft

For obvious reasons, most of the people interested in the work of Antonie van Leeuwenhoek focus on his published results. Indeed, more attention is given to his microbiology than any of the wide range of other subjects he studied, including microfossils in chalk and cells in plant and animal tissue. This talk will feature the aspect of van Leeuwenhoek's life without which nothing would have been possible, his microscopes. They were much simpler than most microscopes in use at that time, and the secret of van Leeuwenhoek's success seems to have come from two factors – his keen eye and the quality of his lenses.
Van Leeuwenhoek usually used glue to mount his specimans on the sample pin on his microscope. It is known that he preferred to make a new microscope rather than destroy a particularly good sample. Descriptions of the microscopes written at the time of the auction after his daughter's death and at the Royal Society after his bequest arrived, specify what samples can be seen with specific instruments. By the time of his death, he had made around 500 microscopes from brass, silver and gold. Most of them had glass lenses, but a few were made from quartz.
Only 10 microscopes with claims to be originals are now known. Although doubts about the provenance of one or two of them have been raised, they are all different (and thus not copies of each other) and cannot be traced back to known makers of replicas. A policy of 'innocent until proved guilty' seems most appropriate. Van Leeuwenhoek adapted his microscope design to allow the observation of the circulation of blood circulation in living eels and fish – only one of these now survives.
It has only been possible to determine what probably happened to a few of the missing microscopes, although it is easy to imagine the fate of most of the examples made of gold and silver once their significance had been forgotten.
Although the actual structure of the microscopes is very simple and easy to copy, thanks to his secrecy about his methods, a debate has gone on since his death about how he produced his lenses. Were they blown, or were they ground and polished (or both)? Most of the surviving microscopes have ground lenses, but the strongest one in Utrecht) has a blown lens. It seems likely that he did both. Studies of the magnification and image quality to be obtained by the two techniques have been made, and good quality replicas are now available.
The second half of the lecture will deal with ways that the microscopes can be used. With the appearance of digital cameras that allow single-spot metering, it has become possible to take photographs and make short movies using replica van Leeuwenhoek microscopes provided that they have good quality lenses.

O029

Small things considered. Observations and theories concerning minuscule objects made by Antoni van Leeuwenhoek.

J.A. Backer[1], J. van Doorn[2]
[1]*Wageningen UR, CVI lelystad, Lelystad,* [2]*Enza Zaden, Seed Technology, Physiology and Seed Health, Enkhuizen*

Introduction: Antoni van Leeuwenhoek analyzed many objects using his self-made microscopes. His technical skills made his observations possible. But he wasn't just a skilled technician making precise observations and didn't stop by just describing his findings. His contribution to science in the late 17th and early 18th century was also inserting his observations into frameworks and theories.
Van Leeuwenhoek became famous as he was the first person to observe bacteria. Less well known is, that he also visualized microscopic structures, invisible to the naked eye, in plants, animals and minerals.
Material and methods: In this presentation, an overview will be given concerning the observation of very small objects other than microbes, and how this was interpreted by Van Leeuwenhoek.
Results and discussion: Van Leeuwenhoek looked at a great variety of specimen. Among these were small eukaryotic organisms like algae, and *Giardia* spp. But he also observed spermatozoon ('zaaddiertjes') and red blood cells, and developed theories about the functioning in reproduction and blood circulation, respectively. To estimate the size of these minuscule objects Van Leeuwenhoek used a very clever (3D !) size reference system.
Finally the ranking of Van Leeuwenhoek will be discussed. What was the opinion of Van Leeuwenhoek's fellow scientists in The Netherlands and Europe concerning his

findings and theories? Was he recognized as a scientist, or just as a clever burgher?

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O030

Time travel with methanogens

A.J.B. Zehnder

Nanyang Technological University (NTU), Singapore, Alberta Innovates – Energy and Environment Solutions (AI-EES), Alberta Canada & Triple Z Ltd, Switzerland

The Persians used methane leaking out of the ground to sustain fires in their temples, the Chinese very early on evaporated with natural gas fed fires water from salt solutions to produce salt. Will-o'-the-Wisps occasionally seen at night over marshes are thought to be methane flames. Humans were fascinated throughout history by the "combustible air" coming from swamps or leaking from the ground. It was not until the end of the 18th century that methane was described by Volta as odourless combustible gas; he called it "aria infiammabile". Already in the mid-19th century methane was identified by a number of scientists as a product of microbial fermentation. Interestingly, before even the theories of thermodynamics were fully developed, microbiologists were wondering how methane bacteria could grow on such little amount of energy released during cleavage of acetate. It was not until 1906 that Nicolaas Louis Soehngen described for the first time two highly purified cultures of microbes producing methane from acetate. One was a sarcina type, the other was called for long just simply "fat rod". It became *Methanothrix soehngenii*, renamed later *Methanosaeta concilii*. At the turn to the 20th century the centres for methanogenic research were Strasbourg, St. Petersburg, and Delft. During World War II, most of the research on these fascinating bacteria shifted to the US. It was only in the early 1970s when groups in Europe started again looking into methanogens, namely in the Netherlands, Switzerland, Germany, and the Soviet Union (Moscow). With more sophisticated anaerobic technics, the better understanding of biochemistry and the upcoming molecular analytics, our knowledge about methanogens increased almost exponentially. Among some bacteria living in extreme environments also a methanogen isolated in Switzerland and later called Methanobrevibacter arboriphilus lead to the discovery of the domain of Archaea.

O032

Novel anaerobes for a biobased economy: succinate production from glycerol by Ercella succinogenes gen. nov., sp. nov.

D.Z. Sousa, A.H. van Gelder, I. Sánchez-Andrea, J.M. Stams

Wageningen University, Laboratory of Microbiology, Wageningen

Innovative technologies are needed to produce energy-rich compounds from waste streams and non-food agricultural crops. The work presented in this study was performed within the ERC project '*Novel Anaerobes for a biobased economy*', in which microbial diversity is exploited for biotechnological purposes. The aim of this project is to isolate and study novel anaerobic microorganisms that can degrade biopolymers and produce valuable energy-rich organic compounds (e.g. organic acids, ethanol, methane), hydrogen or electricity. One of the targets is to obtain novel anaerobes that use glycerol and produce succinate. Succinate is a valuable energy-rich compound used for the synthesis of, for example, 1,4 butanediol, tetrahydrofuran, ?-butyrolactone, or adipic acid. Crude glycerol is an important side product of biodiesel production. Due to the massive production of biodiesel, the price of bulk glycerol dropped considerably in the past decade, and presently glycerol is an interesting substrate for biotechnological applications. Here we describe the isolation of a novel anaerobic bacterium, *Ercella succinogenes* strain ZWB^T, that is able to produce succinate from glycerol.

Sludge collected from a biogas desulfurization bioreactor (Eerbeek, The Netherlands) was incubated in bicarbonate-buffered anaerobic medium supplemented with 0.1 g L^{-1} of yeast extract and 20 mM glycerol as carbon and energy source. Cultures were incubated statically at 30C in the dark. Upon growth serial dilutions were performed and a pure culture of strain ZWB^T was obtained. Strain ZWB^T is a non-spore former, cells are motile and stained Gram-negative. The temperature range for growth was 25 to 40C, with an optimum at 37C. The pH range for growth was 7.0 to 9.0, with an optimum at pH 7.5. Strain ZWB^T ferments glycerol and several carbohydrates to mainly H_2 succinate, and acetate. On the basis of 16S rRNA gene sequence similarity, strain ZWB^T belongs to the clostridial cluster III of *Firmicutes* and it is distantly related to *Saccharofermentans acetigenes* (92% sequence similarity of the rRNA genes). Based on the physiological features and phylogenetic analysis, strain ZWB^T represents a novel species of a new genus, for which we propose the name *Ercella succinogenes* gen. nov., sp. nov. Genome sequencing of strain ZWB^T is currently ongoing. Future work will include bioreactor operation and differential proteomic studies to get a complete picture of the functional metabolic pathways.

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O033

Abundance and activity of marine anammox bacteria in marine sediments of the southern North Sea

L. Villanueva, N. Bale, Y. Lipsewers, E. Hopmans, S. Schouten, J.S. Sinninghe Damsté
Royal Netherlands Institute for Sea Research, Dept. of Marine Organic Biogeochemistry, Den Burg

The marine nitrogen cycle has been redefined in recent years by the discovery of anammox bacteria involved in the anaerobic oxidation of ammonia with nitrite to form N_2 released back to the atmosphere. Here, we evaluated the potential role of this process in coastal marine sediments by investigating the seasonal and spatial abundance and activity of anammox bacteria in several stations with differences in sedimentological characteristics and total organic carbon (TOC) in the southern North Sea.
The abundance and activity of anammox bacteria was measured by quantifying the anammox specific phosphocholine (PC)-monoether ladderane lipid, as well as copy numbers and gene expression of the anammox bacteria 16S rRNA and the hydrazine synthase (*hzs*A) gene involved in the anammox reaction. Anammox activity was also measured as the production of N_2 from ^{15}N-labeled substrates. In addition, we analyzed the seasonal abundance and activity of anammox bacteria using the biomarkers mentioned above in muddy sediments of the Oyster grounds (OG) station in fine resolution (1 cm) up to 12 cm depth.
Higher abundance and activity of anammox bacteria were detected in sediments with higher TOC (muddy stations, e.g. OG), and also in the summer when both the lower oxygen penetration into the sediment and higher temperature favor the anammox bacteria metabolism. However, anammox bacteria 16S rRNA and *hzs*A gene abundance were also relatively high in winter (4-fold less than in summer) throughout the upper 12 cm of the sediment in the OG station, where oxygen is expected to be high due to intense bioturbation and higher oxygen penetration depth in the winter. This suggests that anammox bacteria can tolerate the presence of oxygen and potentially have an important role in the nitrogen cycle in marine sediments also during winter months.
The potential rate of anammox agreed well with the abundance of anammox bacteria 16S rRNA and *hzs*A gene copies, and the transcriptional activity of anammox bacteria 16S rRNA gene indicating that gene-based anammox bacteria markers are good proxies of anammox activity. However, the increase of anammox bacteria 16S rRNA gene transcriptional activity was not observed for the *hzs*A gene, which remained stable across sediment depth and also in the different seasons suggesting that the expression of this gene is low and constitutive in these conditions. We also observed a general lack of correlation between PC-monoether ladderane, which remained stable within the sediment, and gene-based biomarkers of anammox bacteria suggesting that the PC-ladderane lipid could be preserved in the sediment potentially indicating a long-term presence of anammox bacteria.
In conclusion, the abundance and activity of anammox bacteria, even in sandy sediments with low TOC content, were similar to those reported for other marine coastal sediments, indicating that anammox bacteria are important contributors to the nitrogen cycle in sediments of shallow continental shelf areas of the North Sea.

O034

Towards standardized bacterial whole genome sequencing for global surveillance in routine practice

D. Harmsen
University Münster, Münster, Germany

The health of animal and human populations world-wide is confronted with the threat of existing, new or emerging infectious diseases and epidemics spreading faster and appearing more frequently than ever before. Many of these infections are zoonoses, thus necessitating an integrative approach to research and public health measures in the human and veterinary field including the food chain ('One Health' approach). This raises new challenges beyond national borders to public health, veterinary and food safety scientists and experts, policymakers, and populations.
A variety of bacterial epidemiological typing methods have been developed to generate isolate-specific fingerprints for following transmission and to detect outbreaks. However, until now there is no single typing method available to address different bacterial population structures (clonal vs. non-clonal) and all study types (e.g., evolutionary/phylogenetic or transmission chain/outbreak investigation). For decades, the common typing method has been based on multi locus sequence typing (MLST) of 5-7 housekeeping genes or pulse field gel electrophoresis (PFGE) and multi locus VNTR analysis (MLVA). Although widely adopted, these methods either lack discriminatory power or are labour-intensive and difficult to standardize. With fast and affordable benchtop microbial whole genome shotgun (WGS) next generation sequencing (NGS) and automatized software analysis, microbiologists can use now one method that fits all bacterial species and study types ('disruptive technology'). Therefore, the next big challenge will be to harmonize analysis of WGS NGS data so that micro-

biologist speak one language world-wide, i.e. 'molecular Esperanto', that allows them to compare their data quickly with data not generated by them.
To achieve this goal we propose taking a genome-wide gene by gene (core genome MLST [cgMLST] or MLST+) approach. In contrast to the highly popular single nucleotide polymorphism (SNP) analysis the MLST+ approach allows for an additive expandable nomenclature that is an easy portable, storable and retrievable barcode of every bacterial isolate sequenced by WGS NGS world-wide.

O035

Sequence-based epidemiology of vancomycin-resistant *Enterococcus faecium* in the Netherlands as a putative standard application

R.J.L. Willems
UMC Utrecht, Dept. of Medical Microbiology, Utrecht

The first vancomycin-resistant *Enterococcus faecium* (VRE) outbreaks in Dutch hospitals occurred more than ten years ago. After 2000 such outbreaks remained uncommon. From 2011 Dutch hospitals were suddenly confronted with a nation-wide epidemic rise of VRE which is still ongoing. To obtain insight in this epidemic rise the molecular epidemiology of VRE emerging in Dutch hospitals was studied in detail.
VRE were initially typed by multilocus sequence typing (MLST). The genetic relatedness of 23 ST117 isolates, representing the most prevalent ST, from 12 Dutch hospitals was further determined by whole genome sequencing (WGS). A phylogenetic tree was constructed using the variable positions from a core genome alignment. Also the insertion site of the *vanB* gene cluster was determined to further characterize ST117 subclones.
In total, 358 VRE, carrying *vanA* (n = 167), *vanB* (n = 190) or *vanA* and *vanB* (n = 1) from 30 hospitals were typed by MLST. This revealed 24 STs of which 12 STs were found in > 1 hospital. The most wide-spread STs were ST117 (15 hospitals), ST203 (10 hospitals), and ST18 (7 hospitals). In 13 hospitals more than 1 STs was found. WGS determined the genetic relatedness of 23 ST117 isolates from 12 Dutch hospitals (5 vancomycin-susceptible, 5 *vanA*-VRE, 12 *vanB*-VRE and 1 *vanA-vanB*-VRE) in more detail. A phylogenetic tree was reconstructed based on 2090 variable positions in a total core alignment of 1.69 Mb obtained after filtering for SNPs that are affected by recombination. Characterization of the insertion site of the *vanB* gene cluster suggested that this cluster was acquired at least 5 times by ST117. Combining *vanB* insertion site, numbers of SNPs and date of isolation suggested that ST117 was composed of multiple different subclones, of which one was found in 6 Dutch hospitals dispersed over the country, indicating inter-hospital spread.
VRE in Dutch hospitals are highly polyclonal suggesting that the epidemic rise of VRE is not the result of spread of a single clone. WGS of 23 ST117 revealed multiple independent acquisitions of *van* genes and the presence of multiple ST117 subclones. Despite high-level of heterogeneity there are indications of inter-hospital transmission of VRE subclones. Construction of Plasmid Constellation Networks (PCNs), as a method to visualize plasmids in Next-Generation Sequencing data, and further characterization of other mobile genetic elements carrying the *van* genes will disclose the impact of horizontal gene transfer of *van* genes on the epidemic rise of VRE in The Netherlands.

O036

The use of local whole genome sequencing analysis during hospital outbreaks of highly resistant microorganisms

J.W.A. Rossen, A.W. Friedrich
University of Groningen, University Medical Center Groningen, Dept. of Medical Microbiology, Groningen

Next generation sequencing (NGS) is changing the face of microbiology. Mapping of bacterial genomes by sequencing started less than 20 years ago, when the Sanger method was used to sequence the first complete bacterial genome sequence. Over the past decade, the advent of next-generation techniques has dramatically increased the speed and reduced the costs of sequencing, such that it is now possible to sequence a bacterial genome within days.
While initial NGS was used in research settings to investigate the molecular basis of host-pathogen interactions, it now starts to penetrate into clinical and public health laboratories.
In these settings NGS may provide molecular typing data with the highest possible level of resolution for pathogen surveillance and outbreak detection as well as information on the antimicrobial resistance and virulence potential of potential pathogens. Furthermore, NGS may provide insight into the composition of bacterial populations and may rapidly identify the introduction of new highly virulent or resistant variants in hospitals and the population.

O037

High transmission rate of livestock-associated MRSA isolates between veterinarians and their household members

T. Bosch, E. Verkade, M. van Luit, F. Landman, J. Kluytmans, L. Schouls
RIVM, Centre for Infectious Disease Control, Bilthoven

Introduction: In 2003, livestock-associated MRSA (LA-MRSA) emerged and since 2007 has been the

predominant lineage found among the MRSA isolated from patients in the Netherlands. Research has shown that human-to-human transmission of LA-MRSA is less likely to occur compared to other MRSA lineages. However, these reports describe the transmissibility of the ST398 clade based on epidemiological data and low-resolution molecular typing, making it difficult to interpret whether transmission-events really had occurred. In the study presented here, we investigated whether transmission of LA-MRSA between humans occurred using the high-resolution typing technique whole genome mapping (WGM). To assess this, we used LA-MRSA isolates originating from a 2-year prospective longitudinal cohort study in which livestock veterinarians and their household members were sampled for the presence of *S. aureus*.

Methods: A total of 181 LA-MRSA isolates, 122 originating from 17 epidemiologically unrelated veterinarians and 59 LA-MRSA isolates from their household members, were subjected to *spa*-typing, MLVA and WGM. Whole genome maps were created using DNA that was digested with restriction enzyme *Afl*II in a micro-fluids system. The resulting restriction fragments were sized in the whole genome mapper and assembled into a whole genome map and subsequently imported into a Bionumerics database for further analysis.

Results: Based on previously established cut-off values for WGM of LA-MRSA we assessed that transmission had likely occurred within 15 of the 17 veterinarians and their household members (similarities per household ranging from 96%-99%). In these 15 households, only one LA-MRSA type per household appeared to be involved in transmission. However, these LA-MRSA types differed considerably between households. In contrast, in one of the two remaining households, there was likely transmission between the veterinarian and his household members with 2 different LA-MRSA strains. The WGMs of isolates from the last household were quite different, indicating that transmission had not occurred between the veterinarian and his household member.

WGM also showed that, although persistent carriage of LA-MRSA was found in every veterinarian, multiple LA-MRSA strains were isolated from either veterinarian and/or contact(s) in 6 different households. These findings were corroborated by *spa*- and MLVA-typing results in 5 of the 6 cases, but in the other case, *spa*- and MLVA-typing results were indistinguishable. Furthermore, a comparison of 37 WGMs of isolates originating from 4 veterinarians and household members all yielding *spa*-type t011/MLVA-type 398 revealed a different cluster for each household (range 92.0-93.1%), showing the higher discriminatory power of WGM.

Conclusions: Our results suggest persistent carriage of LA-MRSA in veterinarians, lasting for a time period up to 14 months. Alternatively, carriage with the same LA-MRSA type may reflect a repeated re-colonization with the same LA-MRSA strain. Furthermore, this study shows that transmission of LA-MRSA between veterinarians and their household members occurred in high frequency, which sheds new light on the potential of LA-MRSA to transmit between humans.

O038

Combining enrichment culturing and metagenomic sequencing: characterization of populations of enterococci and *Enterobacteriaceae* during antibiotic therapy

X. Zhang, M. Rogers, J.L. Willems, W. van Schaik
University Medical Center Utrecht, Dept. of Medical Microbiology, Utrecht

Enterococci and *Enterobacteriaceae* are part of the human intestinal flora but are also important opportunistic pathogens that are responsible for serious nosocomial infections. Shotgun metagenomic sequencing has been widely used to investigate the composition and dynamics of microbial populations in the human gut. However, shotgun metagenomic sequencing of microbial DNA that is extracted from fecal samples rarely provides sufficient sequence data on enterococci and *Enterobacteriaceae*, due to their low relative abundance in the gut microbiota. In this study, we aimed to decipher genomic dynamics, at the level of species and strains, of enterococci and *Enterobacteriaceae* during hospitalization and antibiotic therapy by combining selective enrichment culturing with metagenome analysis of the cultured bacterial population. Three commercially available enrichment media (for enterococci: Kanamycin Esculin Azide broth and Enterococcosel broth; for *Enterobacteriaceae*:Enterobacteria Enrichment broth (Mossel)) were compared with the rich medium Brain Heart Infusion broth for their ability to enrich enterococci and *Enterobacteriaceae* from fecal samples of four healthy volunteers. The relative abundance of enterococci and *Enterobacteriaceae* among the microbiota were determined by 16S rRNA gene-based analysis. The V4 region of the 16S rRNA genes was amplified by PCR and sequenced using an Illumina Miseq system, and QIIME was used to compute microbial diversity and relative abundance of the different microbial groups. Enterococcosel broth showed the best enrichment efficiency for enterococci, with an average relative abundance of 0.01% in the fecal samples and 65% after enrichment. Mossel could be used to enrich *Enterobacteriaceae* (0.03% in the fecal samples and 90% after enrichment). Fecal samples of 4 ICU patients were collected at the start of hospitalization and at ICU discharge. DNA isolated from these fecal samples and the DNA isolated from enrichment cultures in Enterococcosel and Mossel are currently being analyzed by metagenomic shotgun sequencing.

O039

Regulatory and mechanistic aspects of natural transformation in the human pathogen *Vibrio cholerae*

M. Blokesch

Global Health Institute, School of Life Sciences, Swiss Federal Institute of Technology Lausanne (EPFL), Lausanne, Switzerland

Introduction: *Vibrio cholerae*, the causative agent of cholera, is a member of aquatic habitats where it is often found associated with zooplankton and more specifically with their chitinous exoskeletons. Upon growth on such chitinous surfaces, *V. cholerae* enters into the state of natural competence for transformation.
Methods: In this study we used molecular and cellular microbiology-based approaches to elucidate the regulatory network of natural competence and to visualize proteins of the DNA uptake machinery.
Results: We demonstrate that natural competence for transformation of *V. cholerae* depends not only on the main inducer chitin but also on the abundance of species-specific quorum sensing autoinducers and the absence of preferred carbon sources. However, only a subset of competence genes is coregulated by QS. Moreover, the DNA uptake process occurs in at least two steps.
Conclusion: Our results illustrate that for *Vibrio cholerae*:
the fate of surrounding DNA is mainly determined by quorum sensing and species-specific autoinducers;
the DNA uptake process itself is not species-specific;
the DNA uptake machinery of *V. cholerae* includes a type IV pilus-like structure;
the periplasmic DNA-binding protein ComEA plays a major role in the DNA uptake process.

O041

Feedback control of a bimodal developmental process

P. Gamba[1], L.W. Hamoen[1,2]

[1]*Centre for Bacterial Cell Biology, Institute for Cell and Molecular Biosciences, Newcastle University, UK,* [2]*Swammerdam Institute for Life Sciences (SILS), University of Amsterdam*

In *Bacillus subtilis*, competence for genetic transformation only develops in a subset of the population and depends on the cellular levels of ComK, which activates its own transcription as well as the transcription of more than a hundred genes. Levels of ComK are generally kept very low by a number of mechanisms, and all factors involved in *comK* regulation are themselves part of extensive regulatory networks. We have found a novel regulator that influences the bimodal induction of ComK. Interestingly, the expression of this unknown gene is repressed in competent cells, possibly directly by ComK. This finding reveals a unique nested regulation loop in the complex bimodal ComK regulation pathway.

O042

Control of competence for DNA transformation in *Streptococcus suis*

E.Z. Zaccaria, J.W. Wells, P. van Baarlen, A.d.G. Greef de, H.S. Smith

Wageningen University, Host-Microbe Interactomics, Wageningen

Streptococcus suis (*S. suis*) is one of the major bacterial pathogen of pigs worldwide and is considered an important zoonotic pathogen due to the high mortality observed in recent human outbreaks in China. It can cause meningitis, endocarditis and septicaemia thanks to its ability to disseminate rapidly to the organs after entering the bloodstream. Recently several highly virulent and drug resistant strains have been isolated both in pigs and human making cross-protective vaccines all the more urgent. At the moment little is known about the factors contributing to *S. suis* virulence especially if compared with other streptococci. This is also due to the few molecular tools available to study this important pathogen. Even if this species does not fall within any of the phylogenetic clusters of streptococci previously shown to regulate competence via peptide pheromones, we observed that *S. suis* genome sequences show the presence of a gene homologous to the *S. pneumoniae* and *S. mutans comX* gene which mediates the expression of late competence genes that enable uptake of foreign DNA.
We identified in virulent *S. suis* serotype 2 genomes the set of genes involved in competence including the alternative sigma factor *comX*, core gene for competence induction and conserved in all naturally competent streptococci. We identified a homologue of *comR*, the peptide signal-dependent regulator of *comX* in *S. mutans* and *S. thermophilus*, in the *S. suis* genome sequences. A small open reading frame (ORF) was found just downstream of *comR* suggesting the possible role of it as *comX*-inducing peptide (XIP). We synthesised a series of truncated peptides encoded by this unknown ORF and one of these synthetic peptides was able to induce competence for genetic transformation. To understand better the mechanism of competence development we tested the XIP at different growth condition and optical density, with a number of DNA and peptide concentration. Different alleles of the gene encoding for the XIP (*comS*) are present among strains of *S. suis*. These *comS* alleles are not functionally equivalent and appear to operate in conjunction with a cognate comR to regulate *comX* through a conserved *comR*-box promoter. In order to demonstrate that the XIP is produced by *S. suis* a MALDI TOF mass spectrometry followed by RP-HPLC was performed.

By optimising the conditions for XIP-induced genetic competence we have opened up new avenues for genetic studies in this important zoonotic pathogen. Moreover our approaches to identify and optimize peptide-induced competence may also assist other researchers wishing to identify natural competence in other bacteria

O043

Antibiotic-induced increase of origin-proximal gene copy numbers triggers bacterial competence

J. Slager, M. Kjos, L.R.J. Attaiech, J.W. Veening
University of Groningen, Molecular Genetics, Groningen

Streptococcus pneumoniae (pneumococcus) kills nearly 1 million children annually and the emergence of antibiotic-resistant strains poses a serious threat to human health. Since pneumococci can take up DNA from their environment by a process called competence, genes associated with antibiotic resistance can rapidly spread. Remarkably, competence is activated in response to several antibiotics. Here, we demonstrate that antibiotics targeting DNA replication cause an increase in the copy number of genes proximal to the origin of replication (*oriC*). As the genes required for competence initiation are located near *oriC*, competence is thereby activated. Transcriptome analyses show that DNA replication targeting antibiotics also upregulate origin-proximal gene expression in other bacteria. This mechanism is a direct, intrinsic consequence of replication fork stalling. Our data suggest that evolution has conserved the *oriC*-proximal location of important genes in bacteria to allow for a robust response to replication stress without the need for complex gene-regulatory pathways.

O044

Toxoplasmosis infection in immunocompromised patients

C.A.M. Schurink, J.J. van Hellemond
Erasmus MC, Dept. of Medical Microbiology and Infectious Diseases, Rotterdam

Toxoplasma gondii is an ubiquitous obligate intracellular parasite. Following oral infection the parasite crosses the intestinal barrier and disseminates throughout the body. After the acute phase a latent infection is established in which the parasite remains in a dormant state in muscle and central nervous tissues. In immunocompromised patients the clinical presentation is divers and may lead to life threatening infections.
In the presentation I will focus on the clinical presentation and diagnostics of *Toxoplasma gondii* infections in different groups of immunocompromised patients, among which transplantation and HIV-AIDS patients.

O045

Hyperstrongyloidiasis

B.F.M. Werdmuller
COMICRO, Dept. of Microbiology, Zaandam

A patient with ATLL an HTLV1 infection is described. During chemo- and antiretroviral therapy the patient developed a hyperstrongyloidiasis. The association between Strongyloides and HTLV1 is discussed. The therapy and the consequences for the laboratory are evaluated

O046

Haematological parasitology: leishmaniasis

A. Goorhuis
AMC, Dept. of Infectious Diseases, Amsterdam

In the developed world, the occurrence of a clinical syndrome with fever, weight loss, hepatosplenomegaly and pancytopenia is usually associated with malignancy. However, anyone who has lived in or has travelled to *Leishmania*-endemic areas is at risk of late infection, especially if he or she becomes immunocompromised. Timely diagnosis requires apt clinical suspicion by treating physicians. This session covers the key aspects of visceral Leishmaniasis.

O047

American trypanosomiasis (Chagas disease) in The Netherlands

A. Bart
AMC, Dept. of Parasitology, Amsterdam

About 7-8 million persons worldwide are estimated to be infected with *Trypanosoma cruzi*, the causative agent of Chagas disease or American trypanosomiasis.
Chagas disease used to be rare in Europe, but due to increased migration from South and Central America, where the infection is endemic, numbers of infected persons in Europe have increased in the past decennium. In Spain, this has led to infections due to congenital transmission, through blood transfusion and via transplantation.
Estimates for numbers of infected persons in The Netherlands, Belgium, France, Germany, Switzerland, the UK, Italy, Spain and Portugal combined range from 68,000 to 123,000. Only few of these infected persons are identified: 89.2-99.9% underdiagnosis is estimated in these countries.
In this presentation an overview will be given about current developments in The Netherlands: estimated number of infected persons, numbers of identified patients, diagnostic possibilities and safety of bloodtransfusions with regard to Chagas disease.

O048

Tales from the underground: symbiotic associations of cave-dwelling amphipods

S. Dattagupta, J. Bauermeister, J.F. Flot, N. Cerveau, L. Groeneveld
University of Göttingen, Courant Centre Geobiology, Germany

Sulfidic ecosystems supported by chemoautotrophy are found in marine environments such as deep-sea vents and also in terrestrial limestone caves. The best-studied examples of the latter are Frasassi caves in Italy and Movile cave in Romania, which are located more than 1200 km apart from each other. Both cave ecosystems contain water bodies rich in sulfide, where microbial mats formed by chemosynthetic microbes as well as several species of macroinvertebrates are found. Among these macroinvertebrates are groundwater niphargid amphipods, of which we identified several species in each cave ecosystem by morphological and molecular characterizations. We further found filamentous ectosymbionts belonging to the sulfur-oxidizing bacterial clade *Thiothrix* on all niphargid species occupying these caves. Although Frasassi and Movile niphargids are distant relatives, some of their *Thiothrix* ectosymbionts are indistinguishable based on their 16S rRNA gene phylogeny. Our results imply that the Niphargid – *Thiothrix* ectosymbiosis could be widespread in groundwater ecosystems. In addition to describing the ecology and distribution of this ectosymbiosis, we will report on our latest insights on the physiology and genome evolution of the *Thiothrix* ectosymbionts.

O049

Co-evolution of mammals with their gut symbiont *A. muciniphila*

J.P. Ouwerkerk[1], W.M. de Vos[1,2], C. Belzer[2]
[1]*Laboratory of Microbiology, Wageningen University, Wageningen,* [2]*University of Helsinki, Dept. of Basic Veterinary Medicine, Division of Microbiology and Epidemiology, Helsinki, Finland*

Akkermansia muciniphila is an abundant member of the healthy human intestine, and has its habitat within the mucin layer that lines the gut. Deeply rooted within the Verrucomicrobia phylum, this gut symbiont is the only representative cultured from the mammalian gastrointestinal tract. *Akkermansia* 16S rDNA sequences are universally distributed throughout the mammalian tree, both in domesticated and wild mammals. We would like to elucidate this broad colonization capacity of *Akkermansia* spp. within the mammalian host.
Faecal samples were obtained from a wide range of mammals. Verrucomicrobia were sequenced and the presence and abundance of *Akkermansia* spp. was determined. From each faecal sample an effort was made to isolate new *Akkermansia* species.
This study shows that apart from *A. muciniphila* there are other Verrucomicrobia within the gastrointestinal tract of mammals. On top of this our results indicate that colonization of *Akkermansia* spp. among mammalians can be extended from what was previous known. The new isolates that were obtained underline the mucus degrading capacity of these different strains but at the same time have some different characteristics that might be connected to the host environmental differences. In conclusion, the gastrointestinal tract of mammals is colonised with other Verrucomicrobia and frequently with *A. muciniphila* strains that can use host mucus as an energy source but have adapted to their host gut physiology.

O050

The seagrass microbiome project

A.C. Cúcio[1], A.H. Engelen[2], G.M. Muyzer[1]
[1]*University of Amsterdam, IBED – Aquatic Microbiology, Amsterdam,* [2]*University of Algarve, CCMAR, Faro, Portugal*

Seagrasses are a group of marine flowering plants worldwide distributed. This functional group occurs mainly in coastal areas and is often associated with mangroves, coral reefs and salt marshes. Seagrasses provide food and habitat for many species either in tropical or temperate regions, and form extended meadows of high importance due to their buffering capacity. They reduce wave dynamics in coastal areas and enhance sediment stabilization, and are also an important sink of nutrients. Carbon mineralization and other key biogeochemical processes occur at the rhizosphere level and are the result of complex interactions between a consortium of bacteria and the plant itself. A steep redox potential is created around the roots through the diffusion of oxygen, and allows the distribution of specific groups of bacteria according to the availability of the most favorable electron acceptors. In the presence of high levels of organic matter, the anaerobic mineralization is enhanced by sulfate reducing bacteria. This reaction occurs with the production of hydrogen sulfide, a highly toxic compound that is detoxified by other types of microorganisms, the sulfur oxidizing bacteria.
This study is based on a multiphasic approach composed of both culture-dependent and -independent analysis. The rhizosphere of three seagrass species (*Zostera marina, Z. noltii* and *Cymodocea nodosa*), bulk sediment and overlying seawater were sampled from Faro, Portugal, and Roscoff, France. Enrichment cultures were prepared using specific culture media for the growth of sulfate-reducing, sulfur oxidizing and nitrogen fixing bacteria, and isolation of both aerobic and anaerobic strains followed traditional microbi-

ology methods. It was possible to anaerobically isolate a strain from the rhizosphere of *Z. noltii* using diazotrophic medium, which is potentially related to bacteria previously described to promote plant growth. Several studies performed on terrestrial plants reveal the presence of important bacteria playing an active role on antimicrobial protection and production of plant growth promoting factors, for instance. For the second part of this study, DNA was purified from the same samples previously mentioned, and analysed using a MiSeq Illumina sequencing platform. The species-specificity of rhizosphere bacteria from these seagrasses will be enlightened and discussed, as well as the comparison with the bulk sediment surrounding these meadows.

O051

Sponge microbiota as a reservoir for antibiotic resistance

D. Versluis[1], K. McPherson[1], M.W.J. van Passel[2], D. Sipkema[1], H. Smidt[1]

[1]*Wageningen University, Laboratory of Microbiology, Wageningen,* [2]*RIVM, Bilthoven*

Introduction: The increasing emergence of antibiotic resistant bacterial pathogens poses a serious threat to human health. Even though it is well known which genes confer antibiotic resistance, little is known about resistance gene origin and dissemination. The current consensus is that most resistance genes found in pathogens originate from soil bacteria. Nevertheless, resistance genes have been detected in any biological niche associated with complex microbial communities. Here, we investigate the marine sponge microbiota as a reservoir for antibiotic resistance. Marine sponges are particularly interesting because of their capacity to produce a variety of secondary metabolites, which also include antimicrobial substances.
Methods: A high throughput cultivation-based resistance screen was performed with samples from three marine sponge species (*Petrosia ficiformis, Aplysina aerophoba* and *Corticium candelabrum*) in which bacteria where cultivated on media containing either one or two of 14 different types of antibiotics. The selected cultivation media were Mueller Hinton agar (adapted, classic and 10-fold diluted), Marine agar (classic and 10-fold diluted) and Mucin agar. Samples were inoculated directly on agar, and on filter membranes on top of each medium. (Combinations of) the following fourteen antibiotics were used in the resistance screen: polymyxin B, linezolid, daptomycin, vancomycin, penicillin, erythromycin, ciprofloxacin, tetracycline, chloramphenicol, sulfamethoxazole, trimethoprim, lincomycin, kanamycin and rifampycin. Growth was monitored for 3 months after inoculation during which time colonies were picked. A multidrug resistant isolate (resistant to = 3 antibiotics) was selected for genome sequencing.
Results: A library of over 600 isolates was obtained, and species identification by barcoded pyrosequencing indicated the presence of more than 35 different species, some of which are new species (< 97% 16S rRNA gene sequence identity with known species). Most species belonged to the Proteobacteria, but also different Bacteroidetes, Actinobacteria and Firmicutes were isolated. Resistance profiling of individual isolates showed resistance against all antibiotics that were used in the resistance screen, except for cefotaxime and rifampycin. A number of isolates was multidrug resistant, most notably a *Pseudovibrio* isolate that was resistant to polymyxin B, penicillin, tetracycline, lincomycin and ampicillin. Interestingly, *Pseudovibrio spp.* have the potential to produce antimicrobial compounds themselves. Genome sequencing and analysis of the *Pseudovibrio* isolate in question indicated the presence of a putative b-lactamase resistance gene.
Conclusion: A collection of strains has been isolated that are resistant to a range of antibiotics. Some of these strains can serve as a reservoir for antibiotic resistance genes that could spread to bacterial pathogens. However, antibiotic resistance can also result from intrinsic resistance or points mutations at the antibiotic target sites. Additional functional studies and genome analyses have to be performed to investigate the presence of resistance genes, and to be able to tell more about the risk for dissemination by looking at their genomic context. At this moment one putative b-lactam resistance gene was identified in a *Pseudovibrio sp,* but its activity still has to be tested.

O052

The role of fungal volatiles in plant growth and development

V. Cordovez[1], L. Mommer[2], M. Mumm[2], J. Raaijmakers[1]

[1]*NIOO-KNAW, Microbial Ecology, Wageningen,* [2]*Wageningen University and Research Centre, Nature Conservation and Plant Ecology, Wageningen*

Introduction: Plant-associated microorganisms produce an array of secondary metabolites, including volatile organic compounds (VOCs). VOCs are molecules with low molecular weight and high vapor pressure. Because of these properties, volatiles can travel long distances in soil, facilitating the interactions between plants and their surroundings above and below ground without direct contact. Several bacterial volatiles have been shown to affect plant growth, to induce systemic resistance and to inhibit growth of plant pathogenic fungi. Although fungi are known to produce a large range of VOCs, not much is known about the role of these compounds. Here, the effects of fungal volatiles on plant growth and on plant development were investigated.

Methodology: The soil-borne fungi *Rhizoctonia solani* and *Verticillium dahliae* were used to study the effect of volatiles on plant growth and plant root architecture. *Arabidopsis thaliana* seedlings were grown *in vitro* physically separated from the fungal cultures to allow only the exchange of volatiles. For the detection of fungal volatiles, the fungal headspace was trapped on cartridges filled with an adsorbent (Tenax-TA) and analysed by gas chromatography-mass spectrometry (GC-MS).
Results: Plants exposed to fungal volatiles developed more lateral roots and showed a significant increase in both shoot and root biomass. Although both fungi were shown to produce ethylene, a hormone involved in plant growth related processes, *in vitro* assays with ethylene insensitive Arabidopsis mutants showed no increase in plant biomass upon exposure to fungal volatiles. In total, 27 (16 known and 9 unknown) compounds were emitted by the fungi. To test their role in plant growth and development, synthetic compounds that were commercially available were used for *in vitro* assays. Plants were exposed to different concentrations of individual VOCs or to a mixture of compounds. So far, tested compounds did not mimic the growth promotion observed with the fungal cultures. These compounds were: 1-octen-3-ol, 3-octanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 2-methyl-1-propanol and 3-octanone.
Conclusions: 1. The soil-borne fungi *R. solani* and *V. dahliae* change plant root architecture and promote plant growth via the production of volatile compounds.
2. Both soil-borne fungi produce ethylene; however, this volatile appears not to be responsible on its own for the growth promotion observed *in vitro*.
3. Compounds tested here were not able to mimic plant growth promotion suggesting that other yet unknown fungal VOCs are involved in plant growth promotion and/or alteration of root architecture.

O053

Improvement of vaccines against seasonal influenza

G. Leroux-Roels
Center for Vaccinology, Ghent University & University Hospital, Ghent

Infections with influenza viruses are causing extensive morbidity and mortality worldwide. People of all ages are infected with influenza viruses but adults over the age of 65 are most at risk of severe influenza-related complications. Yearly vaccination for all older adults (generally at age ≥ 65 years) is therefore recommended in many developed countries. Consequently no randomized studies have been conducted in elderly populations in the past decades, leaving us without sound data for vaccine efficacy or effectiveness for these populations. The substantial burden of influenza in older adult populations despite widespread vaccination suggests that vaccines with increased efficacy are needed.
Several factors are known to determine the efficacy of influenza vaccines. The best protective efficacy is observed when there is an antigenic fit between circulating and vaccine strains. Important also are the immune competence, age and general health status of the vaccine recipient. Estimations of vaccine efficacy are influenced by the choice of study endpoint (virus detection via rtPCR or culture, serology, clinical outcomes) and by the time elapsed after vaccination (waning immunity).
Recent efforts to improve influenza vaccines have focused on the aforementioned factors that determine vaccine efficacy. Commonly used trivalent inactivated vaccines (TIV) contain one influenza B lineage (Victoria or Yamagata) and may be ineffective against viruses of the other lineage. In the past decade, predictions about which lineage will predominate in an upcoming influenza season have been correct only half of the times. The inclusion of both lineages in a quadrivalent inactivated vaccine (QIV) would eliminate B lineage mismatch. Clinical studies have demonstrated non-inferiority of QIV vs. TIV for antibody responses against both influenza A strains and the shared B strain. QIV was efficacious in preventing influenza in children; its impact on influenza in older adults needs further investigation. Other efforts to overcome antigen mismatch encompass the development of "universal flu vaccines" that aim at eliciting protective antibodies or disease-modulating T cell responses against conserved B cell or T cell epitopes, respectively. Immunosenescence and comorbidities render influenza vaccines less immunogenic and less effective in older than in younger adults. To increase the vaccine-induced immune responses, humoral as well as cellular, TIV have been formulated with oil-in-water adjuvants (MF59 or AS03), have been administered at higher doses or intradermally rather than intramuscularly. Although these interventions improve immunogenicity, their effect on vaccine effectiveness remains to be established. During the 2008-09 and 2009-10 influenza seasons an RCT was conducted to compare the effectiveness of AS03-adjuvanted versus non-adjuvanted TIV in over 43 000 older adults. This trial could not demonstrate the superiority of the adjuvanted vaccine in protecting against laboratory-proven influenza infections of all types (primary objective). It showed however that the AS03-adjuvanted TIV was more efficacious in protecting against laboratory-proven influenza A/H3N2 infections and reduced the severity of disease, the incidence of all-cause death and pneumonia. Because the primary objective was not met the development of AS03-adjuvanted TIV was discontinued. The TIV formulated with MF59 is licensed and widely used in older people but no prospective RCT of vaccine efficacy of this formulation has been done in people of ≥ 65 years.

While efforts to improve effectiveness of seasonal influenza vaccines in older adults are continuing, it remains equally challenging to define the outcomes that need to be measured to best demonstrate vaccine effectiveness in this population.

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O054

The importance of T-cell responses in immunity to influenza

X. Saelens

VIB Inflammation Research Center, Department of Molecular Biomedical Research, Ghent University, Ghent, Belgium

Humoral and cellular immunity are both associated with clinical protection against human influenza. Antibodies that can prevent attachment of influenza virions to epithelial cells are considered one of the best correlates of protection against disease caused by influenza A and B viruses. Such antibodies are induced upon infection with influenza viruses and are also aimed for by administration of licensed flu vaccines. Most of these antibodies are highly strain-specific. In contrast, T cell-based immunity against influenza is directed predominantly against conserved viral epitopes and hence is often cross-reactive. CD4 and CD8 influenza virus-specific T cells can protect against influenza virus infection in experimental animal models and have been shown to correlate with protection against disease caused by influenza viruses in humans. The principle mode of action of T cell immunity directed against influenza viruses is killing of infected host cells, resulting in control of virus production and, inevitably, a degree of tissue damage. This implies that memory CD8 T cells have to operate in a sea of viruses and have likely evolved mechanisms to protect themselves from infection. In addition, negative feedback mechanisms are operating to prevent extensive inflammation and damage due to cytotoxic T cell activity in the lungs.

Influenza vaccines that primarily aim at inducing cross-reactive T cell based immunity are considered promising "universal" influenza vaccine candidates. However, current vaccination practices are still limited in their potential to boost and maintain the number of antigen-specific T cells up to levels that are sufficiently high and can assure fast enough homing of these T cells into the infected lung. Natural infection with influenza viruses is associated with very strong T cell immune responses in immunologically naïve individuals. Therefore, the use of life attenuated influenza viruses to induce T cell immunity next to B cell immunity directed against the surface antigen has been explored to broaden immune protection against influenza. We have proposed an approach that is based on the use of an infection permissive vaccination strategy. Such a vaccine allows a controlled degree of replication by naturally circulating influenza viruses, to a level that is sufficient to induce strong T cell responses but in the absence of overt disease. Preferentially, such a vaccine should be broadly protective and could have advantages for certain target groups such as very young children.

Epidemiological studies have provided strong support for an important and broadly protective role for T cells against disease caused by influenza viruses. The challenge is now to develop a safe and effective vaccine that can elicit such T cells to control acute infection by influenza viruses in the lung compartment.

O056

Development of a spray freeze-dried whole inactivated virus influenza vaccine for pulmonary administration

H.P. Patil[1], S. Murugappan[2], J. de Vries-Idema[1], T. Meijerhof[1], J. Wilschut[1], H.W. Frijlink[2], W.L.J. Hinrichs[2], A. Huckriede[1]

[1]*University of Groningen, Dept. of Medical Microbiology, Molecular Virology Section, University Medical Center Groningen, Groningen,* [2]*University of Groningen, Dept. of Pharmaceutical Technology and Biopharmacy,Groningen*

Influenza virus is well known for its ability to cause seasonal epidemics and occasional pandemics, both with very large impact on society. Vaccination has proven to be effective in controlling influenza epidemics and pandemics but current vaccines are still suboptimal: they have to be administered by tedious intramuscular injection, they are highly vulnerable to degradation when not stored within a very tight temperature range (2-8), and they fail to elicit immunity in the nose and throat and thus do not prevent early stages of the infection.

To overcome these drawbacks we converted liquid vaccine to a dry powder and delivered this powder vaccine directly to the lungs (pulmonary immunization). Dry powder vaccines produced by spray freeze-drying of the antigens with suitable sugars as stabilizer were stable at temperatures of 30°C for at least 3 months. Two administrations of these powder vaccines to the lungs of mice elicited influenza specific immunity comparable to that achieved by conventional intramuscular injection. The immune responses could be further improved and IgA and IgG titres in the airways could be enhanced by including suitable adjuvants in the vaccine. Mice that received two pulmonal immunizations with dry powder vaccine adjuvanted with the saponin-

derived compound GPI-0100 showed the strongest systemic and mucosal antibody responses and were partially protected against challenge with a heterologous virus strain.
Our findings demonstrate that formulation of influenza vaccines as stable dry powders is feasible and imply that these vaccines will elicit strong local and systemic immunity when administered to the lungs e.g. by simple inhalation. The stability of the vaccine and the ease of administration makes dry powder vaccines especially suitable for use in resource-poor countries and for mass vaccination campaigns as required in case of pandemics.

O060

Evolution of antibiotic resistance at very low antibiotic levels

D.I. Andersson
Dept. of Medical Biochemistry and Microbiology, Uppsala University, Uppsala, Sweden

The widespread human and agricultural use of antibiotics results in the generation of antibiotic concentration gradients, and as a consequence bacteria are frequently exposed to non-lethal (sub-inhibitory) concentrations of drugs. Sub-MIC concentrations of antibiotic allow for continued growth of susceptible strains, but at a slower rate than in the absence of the drug, and they are found in many natural environments (e.g. sewage water and sludge, rivers, lakes) and in patients and in livestock during antibiotic therapy
Recent evidence suggests that this non-lethal exposure has an important role in the evolution of antibiotic resistance. These low, sub-inhibitory antibiotic concentrations have effects that operate on at least at three different levels: as a (i) selector of resistance (enriching for pre-existing resistant bacteria as well as selecting for *de novo* resistance); (ii) generator of genetic and phenotypic variability and (iii) signaling molecules (influencing, for example, virulence, biofilm formation and gene expression). Together these effects may act to accelerate the emergence and spread of antibiotic-resistant bacteria among humans and animals.

O062

The epidemic spread of Extended-Spectrum Beta-Lactamase-carrying plasmids among *Escherichia coli* from different hosts

M. de Been, V.F. Lanza, M. de Toro, J. Scharringa, W. Dohmen, Y. Du, J. Hu, Y. Lei, N. Li, D.J.J. Heederik, A.C. Fluit, M.J.M. Bonten, R.J.L. Willems, F. de la Cruz, W. van Schaik
University Medical Centre Utrecht, Dept. of Medical Microbiology, Utrecht

Incidences of infections caused by Extended-Spectrum Beta-Lactamase (ESBL)-producing *Enterobacteriaceae* are rapidly increasing worldwide and results from recent studies have suggested that these bacteria and their antibiotic resistance genes spread from food-producing animals, through the food-chain, to humans. We used whole-genome sequencing (WGS) to study the relatedness of ESBL-producing *E. coli* from humans, chicken retail meat, poultry and pigs. One strain collection included pairs of human and poultry-associated strains that had previously been considered to be identical based on Multi Locus Sequence Typing (MLST), plasmid typing (pMLST) and ESBL gene sequencing. The second collection included isolates from farmers and their pigs. In the first collection, phylogenomic and core genome analyses revealed considerable heterogeneity between human and poultry-associated isolates. The most closely related pairs of strains from both sources carried over 1000 SNPs per Mbp core genome. Among epidemiologically linked strains from humans and pigs only six SNPs per Mbp core genome were detected. WGS-based plasmid reconstructions revealed virtually identical ESBL-carrying IncI1/ST3 and IncI1/ST7, as well as AmpC-type beta-lactamase-carrying IncK plasmid backbones that were shared by genetically unrelated human, poultry and pig isolates. Our findings failed to demonstrate evidence for recent clonal transmission of ESBL-producing *E. coli* from poultry to humans, as has been suggested based on traditional, low-resolution typing methods. Instead, our data suggest that ESBL genes are mainly disseminated via distinct plasmids.

O063

Decrease of antimicrobial resistance in *E. coli* from animal husbandry reflects the reduction of antibiotic usage in animals in The Netherlands

D.J. Mevius, K.T. Veldman, C. Dierikx, J. Testerink, M. Japing, A. van Essen,
Central Veterinary Institute part of Wageningen UR, Dept. of Bacteriology and TSE's, Lelystad

Introduction: The purpose of this study was to determine trends in antimicrobial resistance in animals in association with a marked decrease in antibiotic usage in animals in The Netherlands. The study focused at the resistance rates of commensal indicator *E. coli* from the gastro-intestinal tract for critically important antibiotics comprising 3rd generation cephalosporins and fluoroquinolones.
Methods: Since 1998,a national monitoring program on antibiotic resistance in animals has been implemented in The Netherlands involving broilers, slaughter pigs, veal calves and dairy cattle. The monitoring includes both indicator bacteria (*E. coli* and enterococci) and food-borne pathogens (*Salmonella* and *Campylobacter*). Each year, bacterial isolates are obtained from fecal samples and tested for antimicrobial susceptibility with broth micro-dilution according to ISO standards (ISO 20776-1: 2006).

Proportions of acquired resistance are determined using epidemiological cut-off values as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Moreover, data on consumption of antibiotics in animals in the period 1999 -2012 provided by the federation of the Dutch veterinary pharmaceutical industry (FIDIN) are included.

Results: Data on the sales of antibiotics for veterinary use in The Netherlands demonstrated a continuous increase from 2000 to 2007, followed by more than 50% decrease from 2008 onwards. In the period2004-2010 the resistance rates of commensal *E. coli* for 3rd generation cephalosporins (cefotaxime) in broilers were high (range 9.7 - 20.9%), but they remarkably decreased from 18.3% in 2010 to 8.1% in 2011, 5.8% in 2012 and 2.7% in 2013. In slaughter pigs, veal calves and dairy cows in *E. coli* resistance to 3rd generation cephalosporins was continuously low in the last five years (0-3.7%). In contrast, resistance to fluoroquinolones (ciprofloxacin) was not affected. In broilers resistance to ciprofloxacin remained stable at a high level (50 - 60%). In veal calves resistance decreased from 23.5% in 2011 to 5.6% in 2012, but 2013 increased again in 2013 to 8.5%. In both slaughter pigs and dairy cows resistance to ciprofloxacin remained low in the last 4 years (0 - 1.7%). Furthermore, we saw an overall decrease in resistance for most antibiotics in broilers, slaughter pigs and veal calves.

Conclusion: (1) The recent decrease of resistance in *E. coli* for 3rd generation cephalosporins in broilers is associated with the stop of administration of ceftiofur to day-old chickens in hatcheries since spring 2010. This, extra label preventive use of a 3rd generation cephalosporins in poultry is not legal in the EU and also highly inappropriate. It has selected for ESBL-producing bacteria in broilers and has facilitated the transmission of ESBLs in the poultry production pyramid.

(2) Moreover, the decrease in consumption of antibiotics in food-producing animals in The Netherlands is probably the main reason for the overall decline in resistance rates in all animal species. However, resistance against fluoroquinolones is still undesirable high in some animal species, particularly broilers.

O064

Mining microbial metatranscriptomes for expression of antibiotic resistance genes under natural conditions

D. Versluis[1], M.W.J. van Passel[2], H. Smidt[1], D. Sipkema[1], M. Leimena[1], J. Ramiro Garcia[1], M.M. D'Andrea[3], F. Hugenholtz[1], J. Zhang[1], B. Öztürk[1], L. Nylund[4], W. van Schaik[5], W. de Vos[1], M. Kleerebezem[1]

[1]Wageningen University, Laboratory of Microbiology, Wageningen, [2]RIVM, Bilthoven, [3]University of Siena, Dept. of Medical Biotechnologies, Siena, Italy [4]University of Turku, Functional Foods Forum, Turku, Finland, [5]University Medical Centre Utrecht, Dept. of Medical Microbiology, Utrecht

Introduction: Metagenome studies can reveal the presence of resistance genes in samples that consist of a community of microbes. To date, this approach has been used to detect resistance genes in environments as different as gut microbiota, soil, seawater, caves and glaciers. This approach, however, cannot be used to identify active functional resistance-encoding genes. Metatranscriptomics, on the other hand, can provide such information, but has not yet been comprehensively applied in the study of antibiotic resistance. Here, the aim is to investigate which antibiotic resistance genes are expressed in different antibiotic naive microbial communities using metatranscriptome data, and furthermore, to study how such expression would relate to the circumstances in that particular biological niche. In addition, we examined the potential for antibiotic production by assessing the expression of relevant secondary metabolite biosynthesis gene clusters.

Methods: Metatranscriptome datasets that contain > 10^6 reads were screened for the expression of resistance genes. Therefore, metatranscriptome reads were compared by megaBLAST to genes in the resistance determinants database (http://www.fibim.unisi.it/REDDB/Default.asp). For a gene to be considered expressed at least three reads were required to align with an alignment length of = 200 bp, and for at least two reads to not have overlapping alignments. The analysed metatranscriptome datasets were generated from the intestinal microbiota of four human adults, one human infant, six pigs and 15 mice, as well as from sea bacterioplankton, a marine sponge, forest soil and sub-seafloor sediment.

Results: We show that resistance genes are expressed in all studied ecological niches, albeit with niche-specific differences in relative expression levels and diversity of transcripts. In the gut microbiota of human adults and pigs the cumulative relative expression level of resistance genes was more than 20-fold higher than in mice. Furthermore, in mice microbiota tetracycline resistance genes were predominantly expressed (in the human infant gut exclusively) whereas in pigs and human adult microbiota the spectrum of expressed genes was significantly different, and also included b-lactam, aminoglycoside, macrolide and glycopeptide resistance genes. In gut microbiota very little indication for potential antibiotic biosynthesis was observed whereas in sea and soil expression of these genes was considerably higher. In sea bacterioplankton and the marine sponge, only -lactam resistance genes were detected. Expression of resistance genes in forest soil and sub-seafloor sediment mainly consisted of those providing resistance against -lactams, chloramphenicols, aminoglycosides and tetracyclines.

Conclusion: In the gut microbiota, expression of antibiotic resistance genes was detected even though the hosts were not treated with antibiotics and very little indication for potential antibiotic biosynthesis was observed. Therefore, we suggest that their expression is either a remnant of prior application of antibiotics within this niche, or that these genes serve other roles besides antibiotic resistance. Furthermore, it is tempting to speculate that their expression is either constitutive, or controlled at least in part independently of the presence of antibiotics. In conclusion, the diversity of expressed resistance genes in gut microbiota and other environmental niches suggests a broad reservoir of functional genes that can potentially be transferred to pathogenic bacteria.

O065

The role of MALDI-TOF MS in the diagnosis of IFI

M. Sanguinetti
Institute of Microbiology, Catholic University of Sacred Heart, Rome, Italy

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is becoming an essential in most clinical microbiology laboratories throughout the world. Its successful use is mainly attributable to the low operational costs, the universality and flexibility of detection, as well as the specificity and speed of analysis. Based on characteristic protein spectra obtained from intact cells, by means of simple, rapid, and reproducible preanalytical and analytical protocols, MALDI-TOF MS allows a highly discriminatory identification of yeasts and filamentous fungi starting from colonies. Whenever used early, direct identification of yeasts from positive blood cultures has the potential to greatly shorten turnaround times and to improve laboratory diagnosis of fungemia. More recently, but still at an infancy stage, MALDI-TOF MS is used to perform strain typing and to determine antifungal drug susceptibility. In this article, we discuss how the MALDI-TOF MS technology is destined to become a powerful tool for routine mycological diagnostics.

O066

Typing below the species level using MALDI-ToF mass spectrometry – applications and limits

A.E. Zautner, C. Folba, M. Bernhard, U. Groß, M. Weig, O. Bader
Institute for Medical Microbiology and UMG Labor, University Medical Center Göttingen, Göttingen, Germany

Species identification of pathogenic microorganisms is a critical step in clinical microbiology and a prerequisite for proper interpretation of antibiotic resistance patterns. Over the past few years, intact cell mass spectrometry (ICMS) employing MALDI-ToF devices has been introduced into routine diagnostics. This method is applicable to bacteria as well as to yeasts and molds, and presents a major paradigm change in clinical and diagnostic microbiology. While species identification with this method is very robust, subtyping within a species is a more subtle process. Phylogenies derived from cluster analyses mass spectra only partially correlate with those from other typing methods, e.g. MLST, however, they may be useful for the analysis of outbreak situations or predict certain clinically relevant subgroups. Next to algorithmic issues of the clustering method employed, also experimental setup is of relevance here since clustering algorithms take peak abundance into account, along with peak identity.
For deeper phylogenetic analyses, charting individual biomarker ions has successfully been used. Using *Campylobacter jejuni* as a model, we demonstrate how this process can be formalized and eventually be used for typing systems in a process we have termed 'mass spectrometric phyloproteomics' (MSPP).

O067

Performance of MALDI-TOF MS in identification of yeasts: a multi-center study

A.L.M. Vlek[1], A. Kolecka[2], M. Groenewald[2], C.H.E. Boel[3], T. Boekhout[2]
[1]*Diakonessenhuis Utrecht, Dept. of Microbiologie, Utrecht,* [2]*CBS Fungal Diversity Centre, Utrecht,* [3]*University Medical Centre Utrecht, Dept. of Medical Microbiology, Utrecht*

Introduction: fast and reliable identification of fungal pathogens is important as this will accelerate diagnosis and contribute to appropriate treatment choice. MALDI-TOF MS might deliver more accurate yeast identifications compared to conventional methods in reduced turnaround times. However, MALDI-TOF MS yeast identification results are variable and difficult to compare between laboratories as different sample preparation methods are used.
We performed a multi-center study to evaluate the performance of MALDI-TOF MS regarding the identification of yeasts, to evaluate different cut-off values and to compare different methods of sample preparation and different databases.
Methods: a blinded, heterogeneous set of 35 reference yeast strains was sent to 13 laboratories for duplicate MALDI-TOF MS identification using 3 different preparation methods (direct transfer, extended direct transfer and ethanol/formic acid extraction) and 2 different databases (a commercially available and an extended database containing 510 additional main mass spectra).

To determine the optimal score cut-off value, MALDI-TOF MS results were categorized as correct identification, no correct identification, no peaks found or not performed/ missing. Based on the optimal cut-off value, the percentage of correct identification was compared between the databases and the sample preparation methods.

Results: 5460 MALDI-TOF MS results were available for analysis (13 laboratories, 35 strains, 2 databases, 3 extraction methods, 2 duplicates). For evaluation of cut-off values, results from the new database and the full extraction method (n = 910) were used, after exclusion of missings (n = 87) and 'no peaks found' (n = 35). For a cut-off value of 2.0, 71.4% of results show scores above the cut-off, of which 99.6% correct identifications and 0.4% errors at species or genus level. When the cut-off is lowered to 1.6, 92.5% of scores are above the cut-off, with 99.0% correct identifications and 1.0% errors. 1.7 was considered the optimal cut-off value with 90.6% of results showing scores above the cut-off, of which 99.3% was correctly identified and 0.7% was incorrect (0.5% error at species level, 0.1% error at genus level). The cut-off of 1.7 was used for further analyses. The percentage of correct identification using the old database was 61.5% versus 86.8% with the new database (full extraction method). The number of main mass spectra in the database was significantly associated with a correct MALDI-TOF MS identification (OR 1.10; 95% CI 1.05-1.15, $p < 0.01$). Proportions of correct identifications when comparing the three sample preparation methods were 39.8%, 68.1% and 86.8% respectively (extended database). However, large differences in performance between laboratories were observed. When only *C. albicans* and *C. glabrata* were included in the analysis, proportions of correct identification increased to 46.2%, 75.4% and 98.5% of strains.

Conclusions: MALDI-TOF MS is a reliable and appropriate method for identification of yeast pathogens. A cut-off value of 1.7 is adequate. Differences in sample preparation methods and choice of database have significant impact on the accuracy of identifications in routine practice.

O068

Potential misidentification of *Staphylococcus pseudintermedius* using MALDI-TOF MS and the development of a real time PCR

K.M. Verstappen, E.M. Broens, A.J. Timmerman, N. Helmond, J.A. Wagenaar, J.G. Kusters, B. Duim

UMC Utrecht, Dept. of Infectious Diseases and Immunology, Utrecht, [2]University Medical Centre Utrecht, Dept. of Medical Microbiology, Utrecht

Introduction: Coagulase-positive staphylococci other than *Staphylococcus aureus* – i.e. *S. intermedius, S. pseudintermedius, S. hyicus, S. lutrae, S. delphini,* and *S. schleiferi* – cancolonize animals and humans, but are difficult to distinguish from each other by routine diagnostic methods as they lack discriminatory phenotypic characteristics. Sequence-based techniques (e.g. on 16S or *tuf* genes) and PCR-Restriction Fragment Length Polymorphism are suitable to correctly identify staphylococcal species, but are too laborious for routine diagnostics. *S. pseudintermedius* is rarely observed in humans, but is frequently isolated as a pathogen from dogs and cats. MALDI-TOF MS is a relatively new, convenient routine identification method but it is unclear if it can correctly identify *S. pseudintermedius*. The aim of this study was i) to determine if MALDI-TOF MS is able to correctly identify *S. pseudintermedius* and ii) to design a real time PCR (RT-PCR) for the identification of *S. pseudintermedius*.

Methods: Whole genome sequences of all staphylococcal species present in Genbank (n = 244) were downloaded and stored in a local database. A sliding frame (size: 300 bp; shift: 100 bp) of one of the *S. pseudintermedius* genomes was used in a local alignment (BLAST) against all other individual genomes of *S. pseudintermedius,* and against all other staphylococci. A Perl-script was written to submit the queries to an offline BLAST program and write the results to a MS Access database. Potential targets were checked for specificity in the online version of BLAST (full nt database). DNA extraction and RT-PCR were performed using standard methods. MALDI-TOF MS analyses was performed on 10 strains of *S. pseudintermedius* (confirmed with PCR-RFLP) using the Bruker microflex LT and the biotyper database version 4613) on duplicate samples extracted with ethanol and formic acid, as recommended by Bruker.

Results: The BLAST search revealed that a 300 bp frame – encoding a phosphoesterase – was uniquely present in *S. pseudintermedius* genomes and absent in all other genomes. A subsequent BLAST search revealed no homology with any of the sequences present in the NCBI database. RT-PCR primers against this gene were designed with a product size of 206 bp. When this RT-PCR was tested on DNA isolated from *S. pseudintermedius* (n = 36), *S. aureus* (n = 6), *S. chromogenes* (n = 3), *S. hyicus* (n = 3), *S. schleiferi* (n = 2), *S. intermedius* (n = 1), and *S. sciuri* (n = 1) it was only positive for the *S. pseudintermedius* samples. Testing against a larger panel of strains is currently ongoing. MALDI-TOF MS analysis classified the majority of the 10 tested *S. pseudintermedius* isolates as < i> S. intermedius *e. assuming that highest score > 2.0 represents correct species ID)*. Further analysis of the score values strongly suggests the presence of a misidentified *S. intermedius* entry in the Bruker database.

Conclusions: Our data indicate that i) it is feasible to design a convenient RT-PCR for routine identification of *S. pseudintermedius*. ii) MALDI-TOF has problems with the distinction of *S. pseudintermedius* and *S. intermedius*; and hence it cannot reliably identify these two staphylococcal species.

O069

The intestinal mucus and its crosstalk with bacteria, stimulation and protection

M.E.V. Johansson
University of Gothenburg, Dept. of Medical Biochemistry, Inst. Biomedicine, Sweden

The gastrointestinal tract is fascinating in that it is able to digest food but does not digest itself and that it can harbour more bacteria than there are human cells, and yet it does not allow bacteria to take over despite their rapid multiplication. It can also handle hydrochloric acid and bicarbonate at extreme pH without denaturing the tissue. The mechanisms behind these amazing skills are several but a major reason is the uttermost defence line of the gastrointestinal tract, the MUCUS.

The main mucus component in the intestine is the MUC2 mucin, a large highly O-glycosylated multimeric glycoprotein. The production and release of this molecule is from the goblet cells, a cell type specialized for this purpose. These large oligomers form netlike structures that are densely packed in granulaes and are expanded over 1000 times upon secretion. In the small intestine the mucus covering the epithelium is freely movable over the surface and allows penetration, features important to mediate nutritional uptake. The bacterial exposure is limited by distal propulsion and anti-microbial mechanisms. The challenge in colon is the enormous number of constantly dividing bacteria that we have evolved to co-exist with. The epithelium is here covered by mucus that forms a well-organized layer that is attached to the epithelium. This inner mucus layer acts as a physical barrier and restricts the access of bacteria to the epithelium by. The turnover of mucus is very rapid to maintain its protective function. This inner mucus is however changed into a more voluminous mucus layer at a distance from the epithelium and this creates a good habitat for the bacteria where the mucin glycans provide an unlimited source of energy.

Disruption of the inner mucus barrier generates abnormal bacterial contact as seen in several spontaneous colitis models and also in ulcerative colitis patients. This inner mucus is however changed into a more voluminous mucus layer at a distance from the epithelium and this creates a good habitat for the bacteria where the mucin glycans provides a source of energy.

The mucus secretion and formation is dependent on bacterial stimuli as germ free animals have a very thin inner layer that is penetrable to small beads. The importance of the flora to develop a functional protective barrier was shown in experiments on separately housed mouse strains. The mucus layer development was also studied over time after colonization with bacteria from animals with a well-developed mucus layer. The process to develop a functional protective mucus layer was revealed to take extensive time.

Pathogens that use the colon as target for infection need to overcome this protective gel and there are examples were secretion of specific enzymes can be used to disrupt this normally protease resistant main structural component, MUC2.

O070

C-type lectins shape innate and adaptive immunity to bacteria and fungi

T.B.H. Geijtenbeek
AMC, Dept. of Experimental Immunology, Amsterdam

Adaptive immune responses by dendritic cells (DCs) are controlled by pattern recognition receptors such as Toll-like receptors (TLRs), RIG-I like receptors and C-type lectin receptors (CLRs). CLRs interact with carbohydrate structures on pathogens and facilitate pathogen uptake for antigen processing and presentation. However, it is becoming evident that CLRs are also important in inducing adaptive immunity to specific pathogens. Upon pathogen recognition, CLRs trigger signaling pathways that induce specific cytokines to dictate T cell polarization. However, several pathogens have evolved to not only escape immune recognition but also to subvert recognition towards for their replication and/or dissemination.Here I will discuss how innate recognition by DCs is crucial for induction of pathogen-tailored immune responses and how certain pathogens such as HIV-1, Measles virus, different fungi and M. tuberculosis subvert innate receptors and DCs for their benefit.

O071

Sortase-based pull downs identify proteins involved in Dectin1 signaling

K. Strijbis[1], A. Esteban[2], G.R. Fink[2], H.L. Ploegh[2]
[1]*UMC Utrecht, Dept. of Infectious Diseases and Immunology, Utrecht,* [2]*Whitehead Institute for Biomedical Research, Cambridge, United Kingdom*

Candida albicans is a commensal yeast in the human gastro-intestinal tract, but also the most common cause of human fungal infections. The -glucan receptor Dectin1 is the major receptor involved in innate immune responses to *C. albicans*. Dectin1-mediated phagocytosis is a tightly orchestrated multi-step process, but only a limited number of Dectin1 interacting proteins have been identified. Amongst these proteins are the tyrosine kinase Syk and tetraspanin CD37. We hypothesized that a new pull down method based on the sortase enzyme might identify novel Dectin1 interactors. Dectin1 was expressed

in RAW macrophages with a sortase recognition motif (LPETG) at the extracellular C-terminus. Incubation of the RAW-Dectin1-LPETG macrophages with the sortase enzyme and a biotin nucleophile resulted in specific labeling of the Dectin1 plasma membrane population with biotin. The labeled cells were then used in phagocytosis assays with the yeast cell wall particle Zymosan or live *C. albicans* and a large-scale pull down was performed for biotinylated Dectin1 and its interacting proteins. Our pull down experiment revealed that Dectin1 interacts with the -galactoside-binding lectin Galectin-3 in the presence of Zymosan. Galectin-3 contributes to TNF production in the presence of *C. albicans*, but not in the presence of *Saccharomyces cerevisiae*. Our data suggest that the Dectin1/Galectin-3 interaction contributes to differentiating pathogenic from non-pathogenic yeasts. Our pull down experiments also identified Bruton's Tyrosine Kinase (BTK) and Vav1 oncogene as interaction partners of Dectin1 during phagocytosis of live *C. albicans*. We confirmed these interactions in phagocytosis time course experiments with the RAW-Dectin1 cell line and live *C. albicans*. Confocal microscopy experiments showed that Vav1-mCherry and BTK-mCherry localize to the *Candida*-containing phagocytic cup. Using confocal microscopy in combination with biosensors, we characterized the lipid composition of the phagocytic cup and early phagosome during *C. albicans* phagocytosis. BTK and Vav1 were recruited to membranes rich in phosphatidylinositol 3,4,5-trisphosphate (PIP_3). PIP_3 production and BTK and Vav1 recruitment are most pronounced when macrophages engage the hyphal or filamentous form of *C. albicans* that is associated with virulence traits. BTK and Vav1 knockout macrophages have reduced phagocytic capacity of both Zymosan and live *C. albicans*. BTK and Vav1 knockout mice are more sensitive to systemic *C. albicans* infection compared to wild type mice. We therefore conclude that BTK and Vav1 play an important role in innate anti-fungal immune responses.

O072

Structural information of the complex of staphylococcal superantigen-like protein 3 and toll-like receptor 2

K.J. Koymans[1], L.J. Feitsma[2], P.C. Aerts[1], T.H.C. Brondijk[2], C.P.M. van Kessel[1], E.G. Huizinga[2], C.J.C. de Haas[1], J.A.G. van Strijp[1]

[1]UMC Utrecht, Dept. of Medical Microbiology, Utrecht, [2]UMC Utrecht, Dept. of Crystal and Structural Chemistry, Utrecht

Introduction: In recent years *Staphylococcus aureus* has become a major health threat to both humans and domestic animals. Increased antibiotic resistance and a high amount of virulence factors secreted by *S. aureus* contribute to this. Amongst these secreted virulence factors there are numerous immune evasion molecules that the bacterium uses to prevent recognition by the immune system. Staphylococcal superantigen-like protein 3 (SSL3) was recently identified to be a potent inhibitor of the pattern recognition receptor toll-like receptor 2 (TLR2). TLR2 recognizes lipopeptides found on bacteria and has an important role in the defense against *S. aureus*. SSL3 is the first molecule that has ever been described to block TLR2 through a direct interaction with the extracellular part of the receptor. Furthermore it was found that its family member superantigen-like protein 4 (SSL4) also has TLR2 inhibitory capacity, although to a lesser extent than SSL3. We set out to further study the interaction between SSL3 and TLR2 through crystallization of the complex.

Methods: For crystallization studies the extracellular part of murine TLR2 (residues Q25-A588) was mixed with SSL3 (residues 134-326) in a 1:1 ratio. To confirm the binding interface SSL3 and SSL4 mutants were cloned and expressed in *E. coli* Rosetta Gami. Mutants were tested on their ability to inhibit IL-8 production by HEK TLR2/6 cells after MALP-2 stimulation.

Results: The 3.2A-resolution crystal structure of murine TLR2 in complex with SSL3-134-326 was solved. Comparing the binding interface with the previously elucidated TLR1-TLR2 and TLR2-TLR6 heterodimers in complex with tri- and diacetylated lipopeptides reveals that: 1) dimerization of TLR2 with TLR1 and TLR6 is sterically inhibited by binding of SSL3 and 2) binding of bacterial lipopeptides to TLR2 is prevented by SSL3 binding. The TLR2-SSL3 complex revealed a number of aromatic amino acids in SSL3 that are involved in hydrophobic interactions with TLR2: F156, F158, and W163. Furthermore, N174, R175, P194, and L211 might play an important role in formation of the complex. Using the information from the binding interface we created several SSL3 mutants. Mutating F156 and F158 together results in a dramatic loss of activity. Mutating F156 and F158 in combination with P194 and INRF172 result in complete loss of inhibitory activity. The structure of SSL3 and SSL4 is similar and mainly differs in the flexible loops of the proteins that make contact with TLR2, which might result in the reduced activity of SSL4 compared to SSL3. Several amino acids of SSL4 were replaced by amino acids of SSL3 to see if SSL4 function can be enhanced. We found that including three regions in SSL4 results in activity of SSL4 comparable to that of SSL3.

Conclusion: We found that SSL3 makes contact with TLR2 in a highly hydrophobic interface. The mechanism of inhibition takes places through a) steric hindering of dimer formation of TLR1-TLR2 and TLR2-TLR6 and b) through shielding of the ligand binding pocket. Mutants of SSL3 and SSL4 confirmed essential amino acids in the binding interface.

O073

Phosphate stress and induction of the stringent response cause upregulation of mycobacterial capsular polysaccharides

M.J. Burggraaf[1], R. Weerd, van de[2], J.J. Maaskant[2], B.J. Appelmelk[2]

[1]VU Amsterdam, Dept. of Medical Microbiology and Infection Control, Amsterdam, [2]VU Medical Center, Dept. of Medical Microbiology and Infection Control, Amsterdam

Tuberculosis, an infectious disease caused by the bacterium *Mycobacterium tuberculosis*, is one of the world's major infectious diseases, with 1.4 million deaths each year. The outermost surface of *M. tuberculosis* is known as the capsule, which is mainly composed of the polysaccharides a-glucan and arabinomannan. In a previous study we performed a transposon screen and isolated mutants in the inorganic phosphate specific transport (Pst) system that all showed enhanced production of capsular a-glucan. This mutant phenotype could be mimicked by growing wild-type bacteria under phosphate stress, indicating that reduced phosphate uptake by the Pst system causes enhanced production of capsular a-glucan. During infection *M. tuberculosis* is suggested to encounter phosphate stress in macrophages and hence upregulation of capsular polysaccharides might play an important role in host-pathogen interaction.

The goal of the present study is to investigate the role of the phosphate acquisition system Pst in capsule biosynthesis in further detail. First of all, since transposon insertions can have strong (polar) effects on adjacent genes, we genetically complemented a phosphate transporter *pstS* mutant in order to prove the direct involvement of this gene in the phenotype. Finally, as many bacterial stress responses are signaled through the so-called 'stringent response' (a general stress response pathway that downregulates cellular metabolism in order to survive under severe stress conditions), the involvement of this major pathway was also investigated.

Our data show that a *pstS* phosphate transporter mutant has enhanced capsular polysaccharide levels. This effect was neutralized by complementing the mutant with the *pstS* gene, thereby proving the direct involvement of *pstS* in the enhanced production of capsular polysaccharides. This contributes to previous findings that showed enhanced capsule production upon phosphate limitation. The involvement of the stringent response was investigated by use of serine hydroxamate, a specific, chemical inducer of the stringent response. Induction of the stringent response caused upregulation of a-glucan and arabinomannan in mycobacterium, suggesting that capsule production is stringent response dependent.

In conclusion, the presented data showed direct involvement of phosphate acquisition in upregulation of mycobacterial capsular a-glucan and arabinomannan and suggests that this effect is stringent response dependent. This indicates a direct link between P_I limitation and upregulation of capsular polysaccharides. Further research is needed to elucidate the role for the capsule *in vivo* and to investigate if it can serve as a novel drug target.

O074

Molecular mechanisms of LukM/F' interaction with its G-protein coupled receptor CCR1

D.A.C. Heesterbeek, M. Vrieling, K.J. Koymans, P.C. Aerts, C.P.M. van Kessel, C.J.C. de Haas, J.A.G. van Strijp

UMC Utrecht, Dept. of Medical Microbiology, Utrecht

Introduction: *Staphylococcus aureus* is a pathogen commonly associated with bovine mastitis. Among secreted virulence factors of *S. aureus* are bi-component pore forming toxins, able to kill a broad range of immune cells. These toxins consist of two distinct subunits (S and F components) of which the S-component binds to a specific receptor on the cell surface followed by binding of the F subunit. Together the two subunits induce pore formation, with subsequent cell lysis. It has recently been identified that LukMF' lyses cells through interaction with the G-protein coupled receptor CCR1.

Methods: The cow genome encodes a chemokine receptor called CCR1L which is highly similar to CCR1 but does not interact with LukMF'. We generated gain of function and loss of function mutants using the CCR1 and CCR1L receptor as backbone. We introduced different loops of CCR1 (gain of function) or CCR1L (loss of function) to determine which parts are responsible for binding or lysis. Mutants were expressed on 293T cells and the activity of LukM/F' was evaluated using fluorescent markers and flowcytometry.

Results: We have shown that the extracellular part of CCR1 loop three is essential for the ability of LukM/F' to lyse cells. Expression of CCR1 loop two and three together increases lysis-efficiency, while expression of loop two alone does not provoke lysis. Additionally, substitution of the CCR1 N-terminus with the Human CXCR1 N-terminus decreases lysis-efficiency and especially binding affinity of LukM to CCR1. This suggests that the CCR1 N-terminus has a role in the interaction with LukM, but is not essential in the process of pore-formation. The fact that CCR1L (not susceptible) and CCR1 (fully susceptible) have an identical N-terminus adds up to this conclusion.

Conclusion: CCR1 loop three defines LukM binding and LukMF' pore-formation. In future experiments, we hope to pinpoint the amino acids responsible for this effect. We expect that the cysteine of CCR1L in the external part of loop three is involved in the blockage of activity by the formation of disulfide bonds with the cysteine in the N-terminus.

O075

A Lipooligosaccharide Silencing RNA regulates *Campylobacter jejuni* pathogenicity

D. Beerens[1], R. Staals[2], J. van der Oost[2], H. Endtz[1], P. van Baarlen[2], R. Louwen[1]

[1]*Erasmus MC, Dept. of Medical Microbiology and Infectious Diseases, Rotterdam,* [2]*Wageningen University, Laboratory of Microbiology, Wageningen*

The bacterial cell envelope is the most important structure to counteract in early stages biotic stressors. Independent studies have shown that modification of the bacterial cell envelope can both function as a defense mechanism, i.e. against bacteriophages and as a virulence factor, i.e. in transcytosis (apical invasion and basolateral translocation). Bacteriophages and travelling across epithelial cells both induce stress on the *C. jejuni* cell envelope for which is shown that sialylated LOS is required to resist such stresses. Envelope stress have been indirectly linked to the *C*lustered *R*egularly *I*nterspaced *S*hort *P*alindromic *R*epeat and *C*RISPR *A*ssociated genes (CRISPR-Cas) system. CRISPR-Cas encodes a bacterial defense mechanism against invading foreign nucleic acids in a RNA interference like manner and can be divided into three main groups (Type I-III). Type II CRISPR-Cas systems are mainly present in pathogenic bacteria that are able to decorate their lipooligosaccharide (LOS) structures on their cell envelope with sialic acid. *Campylobacter jejuni*, a gram-negative zoonotic bacterial pathogen, harbors a Type II CRISPR-Cas system and is able to incorporate sialic acid residues on its cell envelope exposed LOS structures. Presence of sialylated LOS makes *C. jejuni* isolates highly pathogenic, provokes a more severe colitis and induces post-infectious complications in susceptible patients. CRISPR-Cas and sialylated LOS have both been linked to viral defense and virulence features in *C. jejuni*. Recently, a molecular mechanism between CRISPR-Cas and bacterial virulence was revealed. In *Francisella novicida,* CRISPR-Cas was found to regulate the expression of a lipoprotein involved in host cell entry in an antisense RNA like manner. Based on acknowledged functions of CRISPR-Cas and sialylated LOS structures on the *C. jejuni* cell envelope involved in viral defense, virulence features and the ability of CRISPR-Cas systems to regulate the expression of cell envelope structures through a RNA interference mechanism lead us to hypothesize that a dual function between the two systems might exist.

Recently, bioinformatics revealed that a potential small RNA molecule is present in the CRISPR-Cas system harboring identity to an endogenous gene involved in biosynthesis of sialylated LOS structures, which we termed as LOS silencing RNA (LSRNA). RNA analysis has shown in different *C. jejuni* isolates (n = 4) that this LSRNA is transcribed under specific stress conditions. To examine the function of LSRNA and the role herein of each separate part of the *C. jejuni* CRISPR-Cas system, a strain is used that lacks the CRISPR-Cas system, but is able to express sialylated LOS. The generated CRISPR-Cas variants in this strain will be further analysed under different biotic and abiotic stressors in combination with RNA sequencing to determine whether LSRNA, or any other CRISPR-Cas controlled RNAs, regulate the expression of sialylated LOS. When our results show that small interfering RNAs such as the LSRNA indeed regulate the sialylated LOS expression *in vitro*, animal models will be used to further analyze *C. jejuni* CRISPR-Cas/LOS dependent virulence features. Regulation of sialylated LOS expression via this novel LSRNA might be beneficial to regulate *C. jejuni* bacteriophage defense and virulence features.

O076

Using culturomics to isolate travellers-related antibiotic resistant bacteria from stool samples

J. Aarts, C.J.H. von Wintersdorff, J.H.J. Maes, P.H.M. Savelkoul, J. Penders, P.F.G. Wolffs

Maastricht University Medical Centre, Dept. of Medical Microbiology, Maastricht

Introduction: International travelling provides an opportunity for antibiotic resistant bacteria to migrate between geographical areas. Our previous research has shown an increased level of fluoroquinolone resistance encoding *qnrB* and *qnrS* genes, as well as ESBL encoding $bla_{CTX\text{-}M}$ genes in the human gut microbiota after international travel. However, since in our previous research the human gut microbiome was screened using a targeted metagenomic approach, the host organisms of these resistance genes were unknown. In this study, we aimed to identify the bacteria harbouring these antibiotic resistance genes by using culturomics and screening for several antimicrobial resistance genes using quantitative PCR.

Methods: Stool samples (post-travel) of 26 healthy Dutch volunteers were selected, which tested positive for at least one of the three resistance genes *qnrB*, *qnrS* and $bla_{CTX\text{-}M}$. These samples were cultured on 9 different ways using different growth conditions and selective and elective media, also called culturomics. Individual colonies were isolated and cultured. Quantitative PCRs were performed to screen for the *qnrB*, *qnrS* and $bla_{CTX\text{-}M}$ resistance genes. The positive colonies for one or more antimicrobial resistance genes were then analysed by 16S rDNA sequencing and/or MALDI-TOF MS.

Results: A total of 400 colonies were screened and 42 colonies (10,5%) were positive for one of the three resistance genes. Twenty colonies were positive for *qnrS* and 22 were positive for $bla_{CTX\text{-}M}$. Six colonies were already identified using sequencing the 16S rDNA. Preliminary

results from sequencing the 16S rDNA showed that the majority of colonies harbouring resistance genes were *Escherichia coli*. However, preliminary results suggest that also *Bacillus subtilis* was found which contained the *qnrS* resistance gene. Thus far the presence of *qnrS* in *Bacillus subtilis* has not been described before.

Conclusion: 1) Culturomics is a good approach to isolate the bacteria from the stool samples of travellers in order to identify the travel-related antibiotic resistant bacteria using sequencing the 16S rDNA and/or using the MALDI-TOF MS.

O077

Peptidor: Detection and killing of resistant *S. aureus* using antimicrobial peptides

J.F. Kooijman, iGEM-team: M. Krishnan, B. Kumar, J.D. te Winkel, D. Zafeiropoulou, A.S. Meyer, C. Plesa, E. Yildirim
TU Delft, Delft

Methicillin-Resistant *Staphylococcus aureus* causes major problems, especially in hospitals, leading to over half a million infections annually in the US alone. Of the alternative treatments currently under investigation one of the more promising is through antimicrobial peptides (AMPs). These small, highly-specific peptides attack the membrane of target organisms. Thousands of AMPs are known to exist and little resistance against them has been developed. The Peptidor project consists of an *E. coli* that can detect *S. aureus*, using *S. aureus*' native quorum sensing system, in order to locally produce and deliver AMPs. Upon detection, peptides inactivated by a SUMO-tag fusion, are overexpressed. After a delay period, introduced through a negative transcriptional cascade, a SUMO protease is expressed cleaving off the inactivating tag. Using this mechanism, high concentrations of peptide are delivered at the infection to efficiently kill *S. aureus*. As a safety mechanism, the timer also activates an *E. coli* kill-switch.

O078

Matrix metallopeptidase 9 is induced by muramyl dipeptide in an NOD2 dependent manner and can be counteracted by lipopolysaccharide stimulation

M. Vissers, Y. Hartman, L. Groh, M.I. de Jonge, G. Ferwerda
Radboudumc, Dept. of Pediatrics, Nijmegen

Matrix metallopeptidase 9 (MMP-9) is a protease that is able to cleave extracellular matrix gelatin and collagen. Several studies have suggested that induction of MMP-9 by *Streptococcus pneumoniae* could be important for developing invasive disease. However, not much is known about the induction of MMP-9 and the regulatory processes involved.

We obtained peripheral blood mononuclear cells (PBMCs) from healthy volunteers and Crohn patients who have a homozygous mutation in their nucleotide-binding oligomerization domain-containing protein 2 (NOD2) gene, rendering it non-functional. These PBMCs were stimulated with different bacteria and bacterial ligands and MMP-9 induction was measured by ELISA.

Gram-positive bacteria induce a high amount of MMP-9 and a low amount of IL-1, whereas Gram-negative bacteria show an opposite effect and induce low amounts of MMP-9 and high amounts of IL-1. When ligands for specific pattern recognition receptors (PRRs) were used, the highest induction of MMP-9 was seen with NOD2 stimulation by muramyl dipeptide (MDP). MDP is an important pathogen-associated molecular pattern (PAMP) for Gram-positive bacteria whereas lipopolysaccharide (LPS) is an important PAMP for Gram-negative bacteria. Dose response curves for MDP show an increasing MMP-9 induction with increasing MDP dose. Interestingly, the dose response curve for LPS shows an opposite trend, which suggest high doses of LPS can inhibit MMP-9 production. Experiments using PBMCs from Crohn patients with a non-functional NOD2 show that MMP-9 induction by *Streptococcus pneumoniae* and MDP is NOD2 dependent. Moreover, the induction of MMP-9 by MDP can be counteracted by simultaneously adding LPS. mRNA data show that the inhibitory effect of LPS is post-transcriptional. The inhibitory effect of LPS is not mediated by TIMP-1, an endogenous inhibitor of MMP-9. Currently, ongoing experiments focus on studying how LPS inhibits MDP-dependent MMP-9 induction and whether the MMP-9 that is induced by MDP is active.

Collectively, these data show that Gram-positive bacteria are able to induce high amounts of MMP-9 in an NOD2 dependent manner. This MMP-9 induction can be counteracted by LPS stimulation. *Streptococcus pneumoniae* could potentially use this high MMP-9 induction to enhance its virulence and cause invasive disease.

O079

Proteolytic events involved in the regulation of CSS ECF sigma factor activity in Pseudomonas aeruginosa

K.C.J.T. Bastiaansen[1], W. Bitter[1], M.A. Llamas[2]
[1]*VU University Amsterdam, Dept. of Molecular Microbiology, Utrecht,* [2]*EEZ-CSIC, Dept. of Environmental Protection, Granada, Spain*

Extracytoplasmic function (ECF) sigma factors form the largest and most diverse group of alternative sigma

factors. An important mechanism used by Gram-negative bacteria to activate these sigma factors is the so-called Cell-Surface Signalling (CSS) cascade. In general, a CSS system is comprised of the cytosolic sigma factor, a transmembrane anti-sigma factor and an outer membrane receptor. An activated ECF sigma factor associates with the RNA polymerase core enzyme and drives transcription of a specific set of genes. As CSS is mainly involved in the regulation of iron uptake, the inducing signal is usually a specific iron-siderophore complex that binds to the outer membrane receptor. Somehow, this signal has to be transduced via the transmembrane anti-sigma factor to the cytosol. Recent data shows that proteolytic degradation of the anti-sigma factor in response to the signal is key to sigma factor activity.

The CSS anti-sigma factor in general consists of a cytosolic tail that binds the sigma factor, a single transmembrane domain and a large periplasmic region. An interesting recent observation is that these anti-sigma factors are in fact already processed prior to signal recognition. For example, the full-length form of the ~36 kDa FoxR anti-sigma factor of the *P. aeruginosa* ferrioxamine-mediated CSS pathway is hardly detected, but an N-terminal domain of ~21 kDa and a C-terminal domain of ~15 kDa can be observed, implying a single proteolytic event. The question that remains is whether signalling occurs through the full-length anti-sigma factor or through the two separate domains. Localization experiments show that both N- and C-domains associate with cell envelope preparations. In order to examine the initial cleavage event of CSS anti-sigma factors in more detail we subjected purified C-terminal domain of FoxR to N-terminal sequencing and revealed the exact cleavage site. This site is conserved in other CSS anti-sigma factors, indicating that a conserved process could be responsible for the separation of the N-and C-terminal domains. However, so far the protease responsible for this processing step has not been identified. To examine whether a specific periplasmic protease is involved we expressed the periplasmic fragment of FoxR in the cytosol. Interestingly, also this FoxR variant is processed, indicating that FoxR might contain autoproteolytic activity. By site-directed mutagenesis we created several FoxR mutants in which initial cleavage was blocked. These constructs retained the ability to activate sigma factor activity in response to the signal. Therefore, the processing event does not seem to be essential for the regulation of CSS activity. Currently, we are testing whether an active signalling pathway can be constructed using two separate FoxR domains. Together, our data show that the proteolytic cascade regulating FoxR activity is even more complex than anticipated and might involve an autoproteolytic step.

O080

Characterization of YajC a transmembrane protein involved in cell wall stability and biofilm formation in *Enterococcus faecium*

F.L.P. Paganelli[1], P.A.H. Hendrickx[1], X.Z. Zhang[1], D.W. Wobser[2], J.H. Huebner[3], J.B. Boeren[4], J.T. Top[1], I.B. Braat[1], R.J.L.W. WIllems[1], H.L.L. Leavis[1]

[1]UMC Utrecht, Dept. of Medical Microbiology, Utrecht, [2]University Medical Center Freiburg, Dept. of Environmental Health Sc, Freiburg,Germany [3]Hauner Children´s Hospital Ludwigs-Maximilian Universität München, Division of Pediatric Infectious Dise, München, Germany, [4]Wageningen University, Laboratory of Biochemistry, Wageningen

The multi-drug resistant nosocomial pathogen *Enterococcus faecium* (Efm)is the third most frequent pathogen causing nosocomial bacteremia and biofilm (BF) associated infections. BF formation is a critical step in these difficult-to-treat infections, and increased understanding of the role of virulence factors in this process may lead to more effective targeted drugs. In this study we used Microarray-based Transposon Mapping (M-TraM) to identify genes essential for BF. Mutants affected in BF were selected from the transposon-mutant by repetitively subculturing of the planktonic cells from a semi-static BF model. Using microarrays, the composition of the mutant library recovered from the planktonic phase after four rounds of subculturing was compared to the entire transposon mutant library. This revealed 26 genes that were significantly enriched in the planktonic phase. A markerless double crossover mutant of *yajC*, identified in the screen as most essential in BF formation, was constructed to confirm the phenotype and to perform additional functional assays. The *yajC* gene is conserved in several gram negative and gram positive bacteria and it is predicted to encode a transmembrane protein. YajC deficient mutant showed a significant decrease in BF formation compared to the wild-type in a semi-static model and was attenuated in a rat endocarditis model, confirming the relevance of this gene *in vivo*. In addition, initial adherence of the mutant was reduced (in polystyrene assays) and several LPxTG surface proteins (i.e. pili, Esp, von Willebrand factor type protein) were detached from the cell wall of the mutant after exposure to limited external stress, unlike the WT. Mass spectrometry analysis on the detached proteins revealed apart from several surface proteins and transporters, mainly intracellular proteins. Moreover, the mutant was more sensitive to -lactams antibiotics and lysozyme; however susceptibility to lysis by triton X100 remained unchanged and growth rate was not different from wild-type. Based on these results, we hypothesized that YajC plays a role in the cell wall stability and anchoring of surface proteins in *E. faecium*, and therefore, is a promising candidate for targeted-drug development against *E. faecium* infections.

O081

Lack of pAp phosphatase reduces *Streptococcus pneumoniae* virulence by negatively affecting de novo lipid synthesis and thus membrane homeostasis

K. Kuipers[1], M. Rohde[2], D. de Gouw[1], S. van der Beek[1], H. Venselaar[3], J. Gloerich[4], P. Hermans[1], A. Zomer[1,2], H. Bootsma[1], M.I. de Jonge[1]

[1]*Radboudumc, Laboratory of Pediatric Infectious Diseases, Nijmegen,* [2]*Helmholtz Centre for Infection Research, Dept. of Medical Microbiology, Braunschweig, Germany,* [3]*Radboudumc, CMBI, NCMLS, Nijmegen,* [4]*Radboudumc, Nijmegen Proteomics Facility, Dept. of Laboratory Medicine, Nijmegen*

Introduction: The specific human pathogen *Streptococcus pneumoniae* is responsible for life threatening invasive diseases such as meningitis, bacteraemia and pneumonia. The current vaccines are highly effective but only against a limited number of serotypes. Therefore multiple efforts are being undertaken to develop more broadly protective pneumococcal vaccines. Genomic array or sequence footprinting was used to identify conditionally essential genes involved in virulence. One of the genes identified encodes a highly conserved protein, SP1298, belonging to the DHH family 1 proteins, known for their phosphatase activity. Here, we aim to elucidate the function of SP1298.
Methods: The TIGR4 wildtype, SP1298 deletion mutant, and complemented deletion mutant were compared in various morphological and functional assays. Scanning and transmission electron microscopy analyses were performed to study cell morphology. Cell integrity was studied in an ethanol killing assay and lipid amount was qualitatively analyzed using fluorescent microscopy.
Results: Based on the high percentage of homology with *S. mutans* SMU.1297 (68% similarity), SP1298 is predicted to be a 3'-phosphoadenosine-5'-phosphate (pAp) phosphatase, converting pAp into adenosine 5'-phosphate and inorganic phosphate. In *S. pneumoniae,* pAp is a byproduct formed during panthothenate and CoA synthesis, precursors required to initiate lipid production. Furthermore, the metabolite pAp is known to inhibit ACP synthase (AcpS) activity, the critical enzyme responsible for lipid precursor formation. Considering this, pAp levels will accumulate as a result of SP1298 inactivation, thereby hampering *de novo* lipid synthesis and thus affecting cell integrity. Indeed, the latter was confirmed by the aberrant morphology of the SP1298 deletion mutant, illustrated by shorter chain formation and misshaped cocci, and the high sensitivity of TIGR4 fjSP1298 to ethanol killing in comparison with the TIGR4 wildtype strain. Additionally, preliminary data showed that lipid staining qualitatively differed between TIGR4 wildtype and TIGR4 fjSP1298 using fluorescence microscopy, supporting the hypothesis that the absence of SP1298 may (indirectly) hamper the formation of fatty acids.
Conclusion: Taken together, in absence of pAp phophatase SP1298, pAp levels will increase thereby inhibiting AcpS and precursor formation required to start *de novo* lipid production, thereby putting fatty acid formation to a hold. In turn, *S. pneumoniae* is not able to maintain membrane and cell homeostasis and is therefore highly vulnerable for environmental stress, explaining the strongly attenuated phenotype as previously observed in *in vivo* infection studies. For the first time, the current study provides key information about the importance of pAp phophatase in bacterial membrane and cell homeostasis. Taking together, gaining insight into *S. pneumoniae* homeostasis and virulence will aid the development of an universal vaccine to prevent pneumococcal infection and disease in both developed and developing countries.

O082

The C5 convertase is the docking site for Membrane Attack Complex assembly on the bacterial surface

E.T.M. Berends, M. Ruyken, J.A. Soppe, H. Orhan, S.H.M. Rooijakkers

UMC Utrecht, Dept. of Medical Microbiology, Utrecht

The plasma proteins of the complement system are essential in the host's innate immune response against bacterial infections. Complement labels bacteria for phagocytic uptake and directly kills bacteria via a Membrane Attack Complex (MAC or C5b-9) that inserts into bacterial membranes. Formation of the MAC is initiated by surface-bound convertase enzymes that cleave C5 into C5b. According to the general hypothesis, C5b is then released from the surface to form a soluble C5b,6 complex. Upon binding of C7, this complex becomes hydrophobic and integrates into the lipid membrane. Finally, the incorporation of C8 and multiple copies of C9 results in a ring-structured complex that perturbs the bacterial membrane.
In this study we provide evidence that MAC assembly on bacteria exclusively takes place on the surface without a soluble-phase transition step. First, we showed that C5b mediates initial binding of MAC to the surface, since C5b can bind to bacteria in C6 and C7 deficient serum. Second, we showed that purified C5b-9 can only bind to bacteria when they are coated with convertases. Third, we found that C3b-coated bacteria or magnetic beads have a high affinity for C5.
In summary, this study shows that MAC assembly is a surface-localized process, just like all other steps in the complement cascade. We hypothesize that surface-bound C5 convertases are the docking site for C5 and C5b-9 on the bacterial surface. This explains why soluble regulators of MAC do not control MAC formation on bacteria. These findings provide a novel view on the molecular mechanisms of bacterial killing by complement.

O083

Efficient phagocytosis of *Aspergillus fumigatus* through antibody-dependent complement activation

S.G.E. Braem[1], J.J.P.A. de Cock[2], H.A.B. Wösten[2], J.A.G. van Strijp[1], P.J.A. Haas[1]

[1]*UMC Utrecht, Dept. of Medical Microbiology, Utrecht,* [2]*Utrecht University, Microbiology and Kluyver Centre, Utrecht*

Introduction: *Aspergillus fumigatus* is an important fungal pathogen in invasive fungal infections. Infected individuals encounter different morphologic forms of *A. fumigatus*. Airborne dormant conidia are constantly inhaled and reach the lung alveoli. When the innate immune response is inadequate, conidia will swell, germinate and proliferate into hyphal structures, causing invasive growth. Neutrophils are essential in controlling *A. fumigatus* infection. Upon infection, neutrophils migrate to the lung, where they recognize the invading pathogen and clear the infection by phagocytosis and reactive oxygen species-dependent killing. Efficient neutrophil activity is dependent on the opsonisation of *A. fumigatus*. Main opsonins in blood serum are antibodies and proteins from the complement system. The complement system can be activated via three different pathways, namely the alternative (low spontaneous hydrolysis), classical (antibody dependent) and lectin pathway (recognition of sugars). All pathways lead to opsonisation of the microbe with C3b. Current literature demonstrates that all morphotypes initiate the complement system via the alternative pathway, whereas complement activation on hyphae is partly classical pathway dependent. Moreover, neutrophil phagocytosis of dormant conidia is thought to be complement dependent and fully initiated via the alternative pathway. The objective of the present work was to elucidate the importance of complement and antibodies in the clearance of *A. fumigatus*.

Methods: To study the different morphological forms of *A. fumigatus*, dormant conidia were isolated and grown for 4h and 8h in transformation medium, to obtain swollen conidia and germ tubes respectively. To study the effect of complement, normal human sera or heat-inactivated sera were used. Sera of healthy volunteers were used to investigate difference in antibody titers. C3b and antibody binding on the different morphotypes was measured by flow cytometry. A fluorescent *A. fumigatus* strain was used to study phagocytosis and killing. Different deficient sera were used to elucidate the most important initiator of the complement pathway on the different morphotypes.

Results: C3b and antibodies are deposited on all morphological forms of *A. fumigatus*. Interestingly, differences in antibody binding to swollen conidia were measured between sera from different healthy donors. Complement is essential for phagocytosis by neutrophils of all morphotypes. Although, antibodies are deposited on all morphological forms, phagocytosis induced by Fc-receptors was not observed. In addition, serum-opsonised *A. fumigatus* are more effectively killed by neutrophils. Experiments with deficient sera illustrate that opsonisation and phagocytosis of swollen conidia and germ tubes is mainly dependent on the activation of the classical pathway.

Conclusion: Complement is necessary for opsonisation, phagocytosis and killing of all morphological forms of *A. fumigatus*. The classical complement pathway plays a pivotal role in the opsonisation and phagocytosis of swollen conidia and germ tubes. Since the classical pathway is initiated via antibody-antigen complexes on the surface of *A. fumigatus*, differences in antibody levels may influence the capacity of neutrophils to clear infection.

O084

T-Cell tropism of simian varicella virus during primary infection

W.J.D. Ouwendijk[1], R. Mahalingam[2], R.L. de Swart[1], B.L. Haagmans[1], G. van Amerongen[1], S. Getu[1], D. Gilden[2], A.D.M.E. Osterhaus[1], G.M.G.M. Verjans[1]

[1]*Erasmus MC, Dept. of Viroscience, Rotterdam,* [2]*University of Colorado School of Medicine, Dept. of Neurology, Denver, USA*

Varicella-zoster virus (VZV) causes varicella (chickenpox), establishes life-long latent infection in sensory ganglia and reactivates years later to cause zoster (shingles). VZV is acquired via the respiratory route, and skin rash occurs up to 3 weeks after exposure. The pathogenesis of VZV infection is largely unknown, mostly due to the long incubation period and highly restricted host range of the virus. Simian varicella virus (SVV) infection of non-human primates mimics clinical, pathological and immunological features of human VZV infection. In this study we have used SVV infection of African green monkeys as a model to identify the cell types involved in virus dissemination during primary infection.

Five African green monkeys were intratracheally infected with recombinant SVV expressing enhanced green fluorescent protein (SVV-EGFP; n = 3) and wild-type SVV (SVV-wt; n = 2) as a control. Blood and alveolar lavage samples were collected and animals were euthanized at 9 (n = 2), 13 (n = 2) and 20 (n = 1) days after infection (dpi). Samples were analyzed by virus isolation, qPCR, flow cytometry and immunohistochemistry.

All monkeys developed fever and (fluorescent) skin rash. SVV replication in the lungs peaked at 5 dpi and mainly involved viral infection of epithelial cells, alveolar macrophages, dendritic cells and T-cells. SVV replication in blood peaked at 5 - 7 dpi and the virus preferentially infected memory T-cells, initially central memory T-cells and subsequently effector memory T-cells. High levels of

SVV DNA and extensive tissue damage in lymph nodes suggest that SVV-infected alveolar macrophages or lung-derived DC may transfer the virus to memory T-cells at these sites. The use of SVV-EGFP allowed detection of SVV in perivascular infiltrates in early non-vesicular skin lesions, implicating hematogenous spread. In ganglia, SVV was found primarily in neurons and occasionally in T-cells adjacent to neurons.
Collectively, the data presented implicate the role of memory T-cells in the inter-organ dissemination of SVV during primary infection of its natural immunocompetent host.

O085

Human TLR10 is an anti-inflammatory pattern recognition receptor

M. Doppenberg-Oosting, S. Cheng, G.P. Zom, J.M. Bolscher, R. Vestering-Stenger, T.P. Plantinga, P. Arts, A. Garritsen, H. van Eenennaam, M.M.J. Willems, D.V. Fillipov, P. Sturm, B. Kullberg, A. Hoischen, G.J. Adema, F. Ossendorp, J.W.M. van der Meer, M.G. Netea, L.A.B. Joosten
Radboudumc, Dept. of Internal Medicine, Nijmegen

Introduction: Toll-Like Receptor (TLR) 10 is the only pattern recognition receptor without known ligand specificity and biological function. Here we demonstrate that TLR10 is an inhibitory receptor.
Methods: Blocking with antagonistic antibodies, siRNA, overexpression assays, ELISA, polymorphisms, gene expression analysis.
Results: Blocking TLR10 either by antagonistic antibodies or by siRNA enhances proinflammatory cytokine production specifically after exposure to TLR2 ligands. This inhibitory role of TLR10 is dual: on the one hand, co-transfection experiments showed that TLR10 acts as an inhibitory receptor when forming heterodimers with TLR2; on the other hand, cross-linking experiments showed specific induction of the anti-inflammatory cytokine IL-1 receptor antagonist (IL-1Ra), while proinflammatory cytokines were not produced. Furthermore, individuals bearing *TLR10* polymorphisms display an increased capacity to produce proinflammatory cytokines upon ligation of TLR2, but not of TLR4, in a gene-dose dependent manner.
Conclusion: We demonstrate for the first time that TLR10 suppresses TLR2 function and does so partly by competitive binding to TLR2 ligands and partly by inducing the production of IL-1Ra.

O086

Identification of the staphylococcal Haemolysin-gamma receptors

A.N. Spaan[1], T. Henry[2], M. Vrieling[1], M. Perret[2], T. Reyes-Robles[3], F. Alonzo III[3], C.J.C. Haas[1], K.P.M. van Kessel[1], V. Torres[3], F. Vandenesch[2], G. Lina[2], J.A.G. van Strijp[1]
[1]UMC Utrecht, Dept. of Medical Microbiology, Utrecht, [2]CIRI, International Center for Infectiology Research, Lyon, France, [3]New York University School of Medicine, Dept. of Microbiology, New York, USA

Introduction: *Staphylococcus aureus* expresses up to five different bi-component pore forming toxins, known as leukocidins. For both Panton-Valentine Leukocidin (PVL) and LukED, the molecular mechanism of cell selectivity was recently eludicated by the discovery of the involved host target cell receptors, which all belong to a family of chemokine receptors. Here, we identify the receptors of both Haemolysin-gamma AB (HlγAB) and CB (HlγCB).
Methods and results: PVL and Hl?CB are predicted to bind shared receptors. In contrast, Hl?CB and Hl?AB are predicted to bind different receptors. Using an over expression system, we tested the interaction of Hl?AB and Hl?CB with a panel of chemokine receptors.
We identified the human C5aR and C5L2 as the receptors mediating Hl?CB binding and lysis. This finding is in line with predictions. For Hl?AB, the human chemokine receptors CXCR1, CXCR2 and CCR2 were identified to mediate binding and lysis.
The receptors targeted by Hl?AB and Hl?CB are highly expressed on phagocytes, as objectified by semi-quantification of receptor expression levels on freshly isolated leukocytes.
Conclusion: The identification of the major receptors for Hl?AB and Hl?CB explains cell selectivity of both toxins. Just as for PVL and LukDE, the receptors exploited by Hl?AB and Hl?CB belong to the family of chemokine receptors with a seven-transmembrane spanning composition. This suggests convergent evolution between staphylococcal leukocidins and their host target cell counterparts.
Identification of the involved receptors offers directions to further investigate the contribution of leukocidins to staphylococcal pathophysiology. However, this is challenged by incompatibility of the toxins with the murine receptor orthologues.

O087

The role of the mycosin protease in type VII secretion of pathogenic *Mycobacteria*

V.J.C. van Winden[1], R. Ummels[1], W. Bitter[1], E.N.G. Houben[2]

[1]VU University Medical Center, Dept. of Medical Microbiology and Infection Control, Amsterdam, [2]Institute of Molecular Cell Biology, VU University, Dept. of Molecular Microbiology, Amsterdam

Introduction: The family of *Mycobacteria* contains several notorious pathogens such as *Mycobacterium leprae* and *Mycobacterium tuberculosis*. The latter pathogenis the causative agent of tuberculosis (TB), a disease responsible for over 1.3 million deaths in 2012. Due to an increasing number of drug-resistant strains that are emerging, novel anti-TB drugs are required for an effective treatment of TB. A promising new group of novel drug targets are the type VII secretion (T7S) systems, which are present in all known mycobacteria. T7S systems are used by mycobacteria to secrete proteins across its highly impermeable cell envelope. *M. tuberculosis* has five of these systems, ESX-1 to ESX-5, of which at least three are crucial for the virulence and/or viability of this pathogenAmong the most conserved components of T7S systems is mycosin (MycP), a membrane-associated subtilisin-like protease that is essential for T7S secretion. Due to the proteolytic activity mycosins are promising targets for development of novel drugs against TB. In this study, we set out to functionally dissect the role of mycosin proteases in T7S and especially its role in the ESX-5 system.

Methods: First, a *mycP5* deletion strain was created in *Mycobacterium marinum*. Subsequently, various MycP5 variants, including a protease inactive version and a variant in which the transmembrane domain was deleted, were introduced in the *mycP5* deletion strain. Functional complementation by these variants was verified by the secretion analysis of ESX-5 substrates and by analysing mycobacterial virulence in the zebrafish infection model.

Results: As expected, deletion of *mycP5* resulted in complete blockage of ESX-5 secretion, which could be reversed by introduction of an intact copy of the gene. Surprisingly, while the transmembrane domain mutant of MycP5 was not able to complement the secretion defect, the protease-inactive variant restored secretion comparable to wild-type levels. In addition, we could not observe any difference in the processing of secreted substrates. We are currently testing the various MycP5 mutants in the zebrafish infection model to investigate the effect on virulence.

Conclusion: MycP5 needs to be membrane associated in order to carry out its essential function in T7S, but the proteolytic activity of MycP5 is not essential for ESX-5 dependent secretion.These results indicate a dual function for mycosins, with a putative proteolytic role and a second, so-far unknown, role in the regulation of the secretion process. We are currently pinpointing which domain in MycP5 is involved in this second function in T7S.

O088

A passenger or the driver? A PPE substrate of the Mycobacterial secretion system ESX-5 is crucial for protein secretion via this system.

L.S. Ates[1], R. Ummels[2], M. Kok[3], S.R. Piersma[2], J.R. Jimenez[2], E.N.G. Houben[4], W. Bitter[2]

[1]VU University Medical Center, Dept. of Medical Microbiology and Infection Prevention, Amsterdam, [2]VU University Medical Center, Dept. of Medical Microbiology, Amsterdam, [4]VU University Amsterdam, Dept. of Molecular Microbiology, Amsterdam

Introduction: Mycobacteria are the causative agent of several serious diseases, such as tuberculosis which kills 1.4 million people per annum. These pathogens possess a hydrophobic impermeable cell envelope, consisting of long chain lipid and sugar moieties, which protects the bacteria from the host's immune response. To cause disease, mycobacteria need to transport proteins across this highly impermeable cell envelope. For this, mycobacteria have developed specialised secretion systems, the type VII secretion systems (T7SS). *Mycobacterium tuberculosis* and the closely related fish pathogen *Mycobacterium marinum* possess five of these T7SSs; ESX-1 to ESX-5. ESX-5 is the most recently evolved T7SS and is only present in slow-growing mycobacterial species that include most pathogenic species. The ESX-5 system is responsible for the secretion of more than a hundred different substrates, called the PE and PPE proteins, of which the function remains largely unknown. The ESX-5 secretion system consists of an inner membrane complex (EccBCDE), a protease with an unknown function ($MycP_5$) and several intracellular chaperones. How the ESX-5 system translocates proteins over the mycobacterial outer membrane is not known however. To elucidate this process we set out to find genes essential for ESX-5 secretion outside the ESX-5 locus, but instead found a crucial role for a PPE protein, one of the substrates of the ESX-5 system.

Methods: Protein secretion in *M. marinum* was studied by transposon mutagenesis followed by a screen for PE_PGRS secretion mutants. The identified mutant was tested extensively using immunoblotting techniques as well as quantitative LC/MS/MS of the surface associated proteome. Complementation was performed with different constructs to elucidate the role of specific domains. Targeted knock-outs in *M. tuberculosis* were created and clinical strains of *M. tuberculosis*, with mutations in this region were investigated.

Results: By screening a transposon mutant library for mutants deficient in PE_PGRS, one mutant was identified which showed defective PE_PGRS secretion, comparable to mutants in the ESX-5 system itself. Surprisingly the gene in question was a PPE protein, a substrate of the ESX-5 system. This mutant was defective both in native PE_PGRS secretion as well as overexpressed PE_PGRS proteins of *M. tuberculosis* (Rv1818c & Rv2615c). Other known ESX-5 substrates LipY and PE25-PPE41 were secreted when overexpressed. To investigate the full extent of the secretion defect in the PPE-mutant, we analyzed the surface associated proteome by LC/MS/MS. The secretion of a wide range of PE_PGRS, PE and PPE proteins was affected by the PPE-mutation, suggesting a general role in ESX-5 protein secretion for this single PPE. Interestingly, clinical strains of *M. tuberculosis* show high genomic variability in this PPE gene. We are currently investigating the role of this PPE in *M. tuberculosis* by testing these clinical strains and mutated laboratory strains on ESX-5 secretion.
Conclusion: A mutation in a mycobacterial PPE protein leads to a dramatic reduction in secreted ESX-5 proteins. This is the first time that an ESX-5 secretion mutant is described outside of the ESX-5 genomic region. Mutations in this gene in clinical strains indicate that this might be a clinically relevant phenomenon.

O089

Modelling tuberculous meningitis in zebrafish using *Mycobacterium marinum*

L.M. van Leeuwen, M. van der Kuip, W. Bitter, A.M. van Furth, A.M. van der Sar
VU University Medical Center, Dept. of Medical Microbiology and Infection Control, Dept. of Pediatric Infectious Diseases, Amsterdam

Tuberculous meningitis (TBM) is one of the most severe extra-pulmonary manifestations of tuberculosis with a high morbidity and mortality. Characteristic pathological features of TBM are Rich foci, i.e. brain and spinal cord-specific granulomas formed after hematogenous spread of pulmonary tuberculosis. Little is known about early pathogenesis of TBM and the role of Rich foci. To study this we have adapted the zebrafish – *Mycobacterium marinum* model. First, we analyzed whether TBM can occur in adult zebrafish and showed that intraperitoneal infection resulted in the formation of granulomas in the meninges in 20% of the cases, with an occasional granuloma in the parenchyma. In zebrafish embryos, bacterial infiltration and clustering of infected phagocytes was observed after infection via three different inoculation routes, i.e. parenchyma, hind-brain ventricle and caudal vein. Infection via the bloodstream resulted in the formation of early brain parenchyma granulomas in 70% of the cases. In these zebrafish embryos, infiltrates were located in the proximity of blood vessels. Interestingly, no differences were observed when embryos were infected before or after early formation of the blood-brain barrier (BBB), indicating that bacteria are able to cross this barrier with relatively high efficiency. In agreement with this observation, infected zebrafish larvae with a fully developed BBB also showed infiltration of the brain tissue. Upon infection of embryos with a *M. marinum* ESX-1 mutant small clusters and scattered single phagocytes with a high bacterial load were observed in the brain tissue. Importantly, the lower levels of extracellular mutant bacteria did not seem to affect traversal across the BBB, indicating that mycobacteria can reach the central nervous system (CNS) by hitchhiking in phagocytic host cells. Preliminary results of infection experiments with zebrafish embryos lacking phagocytic cells, suggest that mycobacteria migrate to the CNS without the presence of phagocytes. Possibly, mycobacteria are able to use more than one method to cross the BBB. In conclusion, our adapted zebrafish – *M. marinum* infection model for studying granuloma formation in the brain, will allow for the detailed analysis of both bacterial and host factors involved in TBM. It will help solve longstanding questions on how mycobacteria are able to reach the CNS and the role of Rich foci. This model can potentially contribute to development of better diagnostics and therapeutics.

O093

Drug susceptibility testing of non-tuberculous mycobacteria: useful for treatment guidance?

E.J. Kuijper[1], J. van Ingen[2]
[1]*LUMC, Dept. of Medical Microbiology, Leiden,* [2]*Radboudumc, Dept. of Medical Microbiology, Nijmegen*

Background: Nontuberculous mycobacteria (NTM) comprises environmental mycobacteria that are present in soil and both natural as well as treated water. Most NTM infections are opportunistic in nature and affect patients with impaired local or systemic immunity. Pulmonary NTM disease is the most frequent disease manifestation, followed by lymphadenitis, skin infections or disseminated infections, typically in the severely immunocompromised. As the incidence of NTM disease continues to increase, drug treatment is becoming more of interest.
Mechanisms of resistance: In NTM, key drug targets such as rpoB for rifampicin, 16S for streptomycin and aminoglycosides, are similar or identical to those in *M. tuberculosis*. Since NTM has higher MICs in vitro, it is hypothesized that impermeability of the cell wall is a strong driver of drug resistance. The broad repertoire of efflux pumps in NTM form a second line of defense against antimi-

crobial compounds. Interestingly, blocking of efflux pumps increased susceptibility to rifampicin, isoniazid, tetracyclines, quinolones and macrolides, in both slowly and rapidly growing NTM.

Clsi recommendation: The methodology for drug susceptibility testing (DST) varies and is not standardized. The current Clinical Laboratory Standards Institute (CLSI) recommendations are built on a series of comparative studies of the various methods and experience of a small number of reference laboratories. CLSI recommends the broth microdilution with cation-adjusted Mueller-Hinton medium for the drug susceptibility testing (DST) of rapidly growing mycobacteria (RGM), *M. kansasii* and non-fastidious slowly growing mycobacteria (SGM). For *M. avium-intracellulare,* broth microdilution using 10% OADC-enriched cation-adjusted Mueller-Hinton medium is an alternative. In the absence of guidelines from the European Committee on Antimicrobial Susceptibility Testing, the CLSI approved methods can for now be regarded as the current gold standard.

Epsilon test: Application of *Epsilon tests* (E-tests) has been introduced in most of the medical microbiology laboratories, also for DST of mycobacteria. Most laboratories assessed this methodology with Mueller Hinton blood solid media, although for *M. marinum* Middlebrook 7H11 solid medium was preferred. MICs determined by E-tests were similar to those measured by the proportion method or absolute concentration method on Lwenstein-Jensen or Middlebrook 7H10 medium for most drugs. For E-test of *M. marinum* however, MICs for clarithromycin were in general 2-3 fold lower whereas MICs for ethambutol were > 3 fold lower. Testing at multiple laboratory sites showed that reproducibility of E-tests was inferior to that of broth microdilution particularly for susceptibility testing to amikacin, imipenem and ciprofloxacin, three key drugs.

Molecular methods: Commercial assays are not available for detection of resistance-conferring mutations in NTM. In-house methods based on sequencing of the target gene and comparisons with wild type strains of the same species have been developed, mostly for MAC, *M. kansasii* and *M. abscessus*. These include 23S rRNA and *erm* gene mutation analysis to assess macrolide susceptibility in MAC and rapid growers, *rpoB* gene mutation analysis to assess rifamycin susceptibility in *M. kansasii* and 16S rRNA gene mutation analysis to assess aminoglycoside resistance in MAC and *M. abscessus*. Molecular analysis of macrolide susceptibility in MAC and *M. abscessus* have received most attention, since macrolides are the cornerstone of treatment NTM disease. and the fact that MAC and *M. abscessus* group organisms are the most frequent causative agents.

Clinical validation.:The lack of clinical validation remains an impediment to wider implementation of DST for NTM. The best evidence for relationships between MICs and outcomes of treatment has been collected for disseminated infections by MAC, indicating that only drug susceptibility testing results for clarithromycin predicted outcome of treatment with this drug.

Perspectives: The relative rarity of NTM diseases requires an international collaborative effort that is more likely to be based in academia than in industry.

O094

Quick molecular diagnosis of (resistant) *M. tuberculosis* according to the revised NVMM guidelines

S.J. Vainio
VU University Medical Center, Dept. of Medical Microbiology and Infection Control, Amsterdam

In comparison to conventional microbiological methods, molecular techniques have many advantages, like being quick and requiring only small amounts of sample material. In the case of tuberculosis (TBC), it is especially the fastness that makes these methods attractive, and there are several commercial and non-commercial molecular assays for detecting TBC. However, the molecular methods also have disadvantages, like variable sensitivity and specificity and the detection of non-viable bacteria. Adequate controls must be included, as well, to confirm proper technical performance. While molecular methods are routinely used in many Dutch laboratories for TBC diagnostics on auramine/ZN positive respiratory material, the practices with auramine/ZN negative and non-respiratory material are less established. In the new NVMM guideline for TBC diagnostics, these issues are addressed. The most commonly used methods and their performance on different types of material will be discussed, along with the caveats involved in using molecular techniques and their position in relation to the conventional methods. In addition to the detection of the presence of mycobacteria, molecular methods can also be used for detecting mutations conferring resistance to certain anti-tuberculosis drugs. These methods and their practical application will be conferred.

O095

The secondary laboratory diagnosis of tuberculosis in the Netherlands

D. van Soolingen, T. van der Laan, A. Mulder, M. Enaimi, R. de Zwaan, J. de Beer, M. Kamst
RIVM, IDS, Bilthoven

Worldwide, tuberculosis (TB) is still the second most important infectious disease after HIV/AIDS. Resistance is an emerging problem and especially multidrug resistant TB (MDR-TB) is a threat to TB control. In the Netherlands, the incidence of TB is declining rapidly and

in fact we have reached the elimination phase. In 2013, around 850 new cases were notified, of which 75% were diagnosed in foreign-borns. In the last years around 12% of the cases revealed a form of resistance to at least one drug and in between 10 to 20 cases of MDR-TB were diagnosed annually. Treatment of MDR-TB cases is not only cumbersome and often associated with serious side effects, but also involves a long-term hospitalization in one of the two referral TB centers. The RIVM serves as a (inter-) national TB reference laboratory offering identification, drug susceptibility testing and molecular typing of mycobacterial isolates.

Identification of *Mycobacterium* species is performed using reverse line blot hybridization methodologies and, if required, sequencing of the 16S- or the *rpoB* gene. Also information of the DNA fingerprinting can be used to recognize rarely encountered sub-species of the *M. tuberculosis* complex. Whole Genome Sequencing of *Mycobacterium* isolates will in the future add to our knowledge on the taxonomy of nontuberculous mycobacteria (NTM) and will provide a new basis for research on the clinical relevance of these prevalent bacteria.

Rapid molecular detection of rifampicin and isoniazid resistance has a high negative- and positive predictive value and is important for screening on MDR-TB. In case of suspected multidrug resistance, the clinical sample can be tested directly. Screening for phenotypic resistance against first line drugs is for 25% of the cases performed at regional laboratories, and for the other 75% at the RIVM by the MGIT method. If rifampicin resistance is detected, the susceptibility to alternative drugs is determined by the MGIT method or the classical 7H10 agar dilution method. On request, also the susceptibility of NTM to a variety of drugs is determined.

The RIVM uses first-, second- and third-line controls to consolidate the quality. The WHO is responsible for yearly proficiency studies on resistance testing in the international context and this yields important insights in the quality of WHO supra-national laboratories. It also adds to international standardization and further improvement of the resistance testing.

All *M. tuberculosis* complex isolates in the Netherlands are sent to the RIVM for epidemiological typing by Variable Number of Tandem Repeat (VNTR) typing. Clustering of isolates and the respective cases is reported to Municipal Health Services. This information is used directly to support contact tracing and source case finding. With this routine typing activity also rarely occurring laboratory cross contaminations are detected and reported.

The quality of this DNA typing technique is controlled by an international worldwide proficiency study, annually organized by the RIVM for the ECDC in Stockholm.

Currently, the possibility for typing *M. tuberculosis* isolates by Whole Genome Sequencing is being explored. The initial studies reveal a higher resolution in typing by this technique and this will improve the utility of DNA typing in the molecular epidemiology and will facilitate the surveillance of TB transmission. This becomes more and more important in the TB elimination phase.

O097

Utility of rpoB gene sequencing for identification of nontuberculous mycobacteria in the Netherlands

C.C. de Zwaan[1], J. van Ingen[2], D. van Soolingen[1]

[1]*National Institute for Public Health and the Environment (RIVM), Diagnostic Laboratory for Bacteriology and Parasitology (BPD), Unit Mycobacteria, Bilthoven,* [2]*Radboudumc, Dept. of Medical Microbiology, Nijmegen*

In the Netherlands, clinical isolation of nontuberculous mycobacteria (NTM) has increased over the past decade. Proper identification of isolates is important, as NTM species differ strongly in clinical relevance and required treatment. Most of the currently applied identification methods like reverse line blot assays and 16S rDNA gene sequencing cannot distinguish between all different Mycobacterium species and groupings within species. RpoB gene sequencing reveals a promising level of discrimination among rapid growing mycobacteria (RGM) and slow growing mycobacteria (SGM) including the Mycobacterium avium complex (MAC). In this study we have prospectively compared rpoB gene sequencing with our routine algorithm of reverse line blot identification combined with partial 16S rDNA gene sequencing in 455 consecutive NTM isolates, submitted to the Mycobacteria reference laboratory in the Netherlands in the January 2010 to Arpil 2011 period. RpoB gene sequencing identified 403 isolates to species level as 45 different known species, 44 isolates were to complex- or group level and 8 isolates remained unidentifiable to species level. In contrast, our reference reverse line blot assay with adjunctive 16S rRNA gene sequencing identified 390 isolates to species level, as 30 distinct species, 58 isolates to complex- or group level and seven isolates remained unidentified. The higher discriminatory power of rpoB gene sequencing largely results from the distinction of separate species within the M. avium complex, M. terrae complex, M. fortuitum-M. peregrinum complex and the distinction of M. abscessus subspecies. Also, M. gordonae, M. kansasii and M. interjectum were separated into multiple groupings with relatively low sequence similarity (98-94%), suggesting these are complexes of closely related species. We conclude that rpoB gene sequencing is a more discriminative identification technique than the combination of reverse line blot and 16S rDNA gene sequencing and could introduce a major improvement in clinical care of NTM disease and the research on epidemiology and clinical relevance of NTM.

O098

Development and evaluation of the detection of *Mycobacterium tuberculosis* complex on the BD-MAX system in a European multicenter study

I.O.M. op den Buijs[1], A.L. Seagar[2], P. Baltar[3], R.T.J.M. Roymans[1], K.E. Templeton[2], I.F. Laurenson[2], G. Barbeito[3], J.H.B. van de Bovenkamp[1]

[1]*Stichting PAMM, Dept. of Medical Microbiology, Veldhoven,* [2]*Royal Infirmary of Edinburgh, Scottish Mycobacteria Reference Laborato, Edinburgh,Scotland,* [3]*Clinical Hospital of Santiago, Dep. of Clinical Microbiology, Santiago De Compostella, Spain*

Introduction: The ability to rapidly diagnose *Mycobacterium tuberculosis* complex (MTB) is a priority for healthcare worldwide. Furthermore, there is a need for automated tests which can be done in non-specialised settings. The purpose of this study was to evaluate an in-house qPCR for the detection of MTB on the BD-MAX system (Becton Dickinson). To demonstrate multi-user acceptability custom made BD-MAX sealed snap-in tubes were made containing dried primers and probes which were evaluated for MTB assay performance in 3 laboratories.

Methods: The MTB2012 QCMD panel was tested on the BD-MAX at three laboratory sites. This panel of ten specimens with 100-10,000 cells/sample of MTB, was treated with proteinase K and inactivated for 20 minutes at 100°C. The total sample volume was transferred to a Sample Buffer Tube and loaded onto the BD-MAX instrument. The extraction was performed using the ExK DNA-1 extraction kit and qPCR was performed with BD MMK (SPC) mastermix.

The analytical limit of detection (LoD) of the assay was determined by testing several concentrations of the target sequence cloned into a plasmid. Each concentration was tested in replicas and the LoD was defined as the lowest concentration at which all replicates tested positive. A retrospective evaluation of 242 BAL and sputum samples was performed. Specimens were treated according to the customary pre-processing protocol at the specific laboratory sites and tested in the MTB assay. Pre-treatment for laboratories 1, 2 and 3 were, respectively, proteinase K treatment (81 samples); 1:1 sputasol treatment followed by decontamination with a 2% NaOH-NALC solution and phosphate buffer (100 samples); BD Mycoprep decontamination treatment (61 samples). qPCR results were compared to culture using Mycobacteria Growth Indicator Tube (BD) which was performed at all sites.

Results: Eight QCMD specimens containing 100-10,000 cells/sample were positive and 2 with no cells were negative in the MTB assay at all laboratory sites. This is in complete concordance with the final QCMD report. All plasmid-dilutions with a concentration of 16 target copies in the PCR provided a MTB-positive result in the assay. In addition, 1 target copy per PCR was negative in all replicas. Thus, this study shows a 100% LoD for 16 target copies per PCR reaction. Furthermore, the retrospective clinical evaluation has shown an overall sensitivity, specificity, positive and negative predictive values of 91.9% (86.7-100%), 97.9% (95.8-100%), 95.8% and 95.9% using different pre-treatment methods.

Conclusion: A sensitive assay has been developed for the detection of *Mycobacterium tuberculosis* complex on the BD-MAX. The utilisation of the dried down primer/probes on the automated BD-MAX offered considerable practical advantages in terms of walk away functionality, minimal hands on time and ease-of-use. This type of assay set up on BD-MAX could enable a wider use of PCR for MTB diagnosis.

O099

Dysbiosis of upper respiratory tract microbiota in elderly pneumonia patients

W.A.A. de Steenhuijsen Piters[1], E.G.W. Huijskens[2], A.L. Wyllie[1], G. Biesbroek[1], X. Wang[1], M.R. van den Bergh[1], A. van der Ende[3], R. Veenhoven[4], K. Trzcinski[1], M. Bonten[1], J. Rossen[2], E.A.M. Sanders[1], D. Bogaert[1]

[1]*UMC Utrecht, Dept. of Pediatric Immunology and Infectious Diseases, Utrecht* [2]*St. Elisabeth Hospital, Laboratory of Medical Microbiology and Immunology, Tilburg,* [3]*Academic Medical Center, Dept. of Medical Microbiology, Amsterdam,* [4]*Linnaeus Institute, Spaarne Hospital, Hoofddorp*

Background: Lower respiratory tract infections are a major cause of morbidity and mortality in elderly. Potential pathogens of such infections are regular residents of the upper respiratory tract, where they generally reside asymptomatically. We hypothesize that dysbiosis of the respiratory microbiome is involved in pathogen overgrowth and consequently disease. We therefore compared oropharyngeal microbiota of healthy elderly with elderly pneumonia patients.

Methods: We determined the microbial density and composition of oropharyngeal samples of elderly (> 60 years) pneumonia patients (n = 100) and healthy elderly (n = 91) by barcoded sequencing of the V5-V7 regions of the 16S-rRNA gene. In addition, we verified our findings in a cohort of young adult pneumonia patients (n = 27) and healthy young adults (n = 187).

Results: The overall bacterial density was significantly higher in pneumonia patients compared to controls (median 503.8 vs 154.7 pg/L, p = 0.0012). Bacterial diversity was significantly lower in elderly pneumonia patients compared to elderly controls (number observed species 120 vs 159, p < 0.0001). The most frequently encountered genera were *Streptococcus*, *Rothia*, *Prevotella*, *Veillonella*, *Actinomyces*, *Neisseria* and *Lactobacillus*.

Microbiota profiles differed significantly between healthy elderly and elderly pneumonia patients (Nonmetric Multidimensional Scaling, $p < 0.05$); the abundance of *Streptococcus (pseudo)pneumoniae* (24.9% vs 9.7%) and *Rothia* (15.3 vs 9.3%) was significantly higher, while the abundance of lactic acid bacteria like Gemellales (11.2% vs 27.7%) and *Prevotella melaninogenica* (0.9 vs 6.0%) and anaerobes including *Veillonela dispar* (2.4% vs 6.1%) were significantly lower in elderly pneumonia patients compared to healthy controls (FDR-corrected p-value: $q< 0.05$). However less outspoken, the observed changes in microbiota profiles in elderly pneumonia patients and healthy controls were mirrored in our adult cohorts.

Conclusions: These results suggest that pneumonia in elderly is accompanied by dysbiosis of the oropharyngeal microbiome accompanied by bacterial overgrowth of specific bacteria. Whether this imbalance is either a causative factor in the pathogenesis of pneumonia or an epiphenomenon has yet to be determined.

O100

The intestinal microbiome of phytopathogenic root fly larvae

C.U. Welte, R. de Graaf, N. van Dam, M.S.M. Jetten
Radboudumc, Dept. of Microbiology, Nijmegen

Brassica plants produce various toxic compounds such as isothiocyanates in response to herbivore damage. Despite their toxicity, some insects cope well with these compounds. One example is the larva of the cabbage root fly (*Delia radicum*) which is a serious agricultural pest. The mechanism by which these root feeding insects detoxify isothiocyanates has not yet been explored. Our hypothesis is that microorganisms residing in the gut contain enzymes that break down the isothiocyanates and are thus crucial for survival and phytopathogenicity of the root fly larvae.

We substantiated this hypothesis by isolating 15 representative microbial species from the *D. radicum* gut that were highly resistant to isothiocyanates as judged by disc diffusion assays with the root volatile 2-phenylethyl isothiocyanate. A subset of these microorganisms was also able to break down isothiocyanates to a variety of different volatile compounds. We characterized the isolates physiologically and genetically to unravel their isothiocyanate breakdown mechanism. It appeared that four isolates belonging to the genera *Acinetobacter, Serratia, Pectobacterium* and *Providencia* were able to rapidly break down isothiocyanates whereas isolates of the genus *Pseudomonas* only showed very high resistance but no breakdown under the tested conditions.

Furthermore, we performed a metagenomic survey of the microbial gut content of *D. radicum* larvae to assess the total genetic content of the insect gut microbiome. Using the Ion Torrent technology we were able to sequence about 4.5 million reads with an average read length of about 250 bp. Within these 1.1 billion bp of sequence information, about 5000 reads mapped to 16S rRNA genes. The analysis of those revealed a moderate diversity of gut microbes in *D. radicum* larvae with the majority of bacterial lineages falling into the class of Gammaproteobacteria. Sequencing reads mapping to the 16S rRNA genes from the genera *Providencia, Morganella, Acinetobacter* and *Pseudomonas* dominated this fraction of the metagenome. This is dissimilar to many other investigated insect metagenomes that are dominated by members of the Firmicutes which are well known for their fermentative capabilities.

Additionally, the metagenome was analysed with respect to functional gene content. One aspect of these analyses is that roots where *D. radicum* larvae feed on have a low nitrogen content; microorganisms residing in the gut may have beneficial effects on the nitrogen supply to the larvae. Surprisingly, the number of bacterial nitrogenases retrieved was low indicating that not nitrogen gas is the main biological nitrogen source. However, physiological studies of one of the isolates indicated that it was able to use 2-phenylethyl isothiocyanate as a nitrogen source and might liberate biologically usable nitrogen to the insect.

Taken together, the results of this study provide for the first time an in-depth analysis of the microbial content of the root fly larval gut. Both the metagenome analyses and the isolation experiments indicate clearly that the *D. radicum* gut contains a highly specialized microbiota that helps the root fly larvae to survive on their toxic host plants.

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O101

Congruency of phylogenetic composition and activity patterns in the human small intestine

B. van den Bogert[1], M.M. Leimena[1], J. Boekhorst[2], E.J. Smid[1], E.G. Zoetendal[1], M. Kleerebezem[1]
[1]*Wageningen University, Laboratory of Microbiology, Wageningen,* [2]*NIZO Food Research BV, Ede*

Introduction: Ribosomal RNA (rRNA) gene and metagenomics based technologies have been extensively used to profile and catalogue the genetic content of the human intestinal microbiota to elucidate its role in health and disease. Metatranscriptomics may complement these analyses by identifying the active microbial members and unravelling their response to environmental conditions. Since the major part of nutrient digestion and absorption occurs in the small intestine, its microbiota is expected to have an important influence on the digestion of food and host physiology.

Here, samples collected from ileostomy subjects (subjects without a colon) were used to study the phylogenetic dynamics of total and active fractions of the small intestinal microbiota through pyrosequencing of 16S rRNA gene (rDNA) and rRNA combined with elucidation of the specific activity patterns through metatranscriptomics.

Methods: Samples were collected from four ileostomy subjects who each donated four samples, collected on two distinct time points during the day (morning and afternoon), two days apart. Samples were mixed with RNAlater to stabilize microbial RNA.

For bacterial composition and activity profiling, barcoded amplicons corresponding to the V1-V2 region of 16S rRNA gene copies (rDNA) and their transcripts (rRNA) were pyrosequenced using a Genome Sequencer FLX in combination with titanium chemistry. The pyrosequencing data analysis was carried out employing the Quantitative Insights Into Microbial Ecology (QIIME) pipeline.

Metatranscriptome datasets were generated by Illumina sequencing of sample-specific cDNA libraries derived from mRNA enriched samples of the ileostomy effluent microbiota, and were analyzed using the bio-it pipeline described in Leimena and Ramiro-Garcia et al., 2013.

Results: The community composition as assessed from rDNA, rRNA, and mRNA patterns appeared to be similar, albeit that species richness and diversity metrics of the active ileostoma microbiota were reduced as compared to the overall community composition assessment (rDNA). This finding implies that low abundance populations may represent less active members of the microbial community. Although each subject displayed a distinct microbial community, the genera *Streptococcus, Veillonella* as well as clostridial community members were encountered in all ileostoma samples and were strongly represented in the activity profiles. While the phylogenetic assignment of specific metabolic functions was clearly different in the different samples, the overall activity patterns obtained for each of the samples was relatively similar.

Conclusion: 1) The predominant small intestinal microbiota also displays a high level of activity. 2) Bacteria belonging to *Streptococcus, Veillonella,* and clostridia are typical and active commensals of the human small intestine. 3) The human small intestinal microbiota displays a high degree of functional redundancy among the community members in different individuals. 4) This study integrates the complementary 16S rRNA and metatranscriptome data to establish a functional community reconstruction, thereby expanding our knowledge of the functional biodiversity and metabolic interactions of the human small intestine microbiome.

O102

Functional metagenomic analysis of genes conferring resistance to the disinfectant benzalkonium chloride in the human gut microbiota

E. Buelow[1], D. Versluis[2], M.W.J. van Passel[2], H. Smidt[2], R.J.L. Willems[1], M.J.M. Bonten[1], W. van Schaik[1]

[1]*University Medical Center Utrecht, Dept. of Medical Microbiology, Utrecht,* [2]*Wageningen University, Laboratory of microbiology, Wageningen*

Introduction: Resistance to antimicrobial agents in nosocomial pathogens has become a major public health concern. The emergence of multi-resistant pathogens has been attributed to the extensive use of antibiotics and other antimicrobial agents in domestic, agricultural and clinical settings. The application of disinfectants, such as quaternary ammonium compounds (QACs), in clinical environments may enhance the selection of strains that are less-susceptible to these compounds and, potentially, to other antimicrobials.

Methods: We constructed six fosmid libraries using DNA isolated from feces of two patients (collected at different timepoints during and after hospitalization) to identify genes involved in conferring resistance to the QAC benzalkonium chloride (BC). These libraries were screened for clones that were resistant to BC. Genes on the fosmid insert that conferred resistance to BC were identified using *in vitro* transposon mutagenesis. Fosmid inserts were sequenced by Illumina sequencing on the HiSeq 2000 system.

Results: Four out of the six libraries yielded clones that were resistant to BC. These four libraries had been constructed using fecal samples that were collected throughout hospital stay. Two fosmid libraries that were constructed using fecal samples obtained 4 days after the start of hospitalization and 9 months after hospital discharge did not yield any BC-resistant clones.

The genes that conferred BC-resistance to *Escherichia coli* during growth in LB broth, were predicted to encode i) an ABC transporter (most closely related to a protein from *Bifidobacterium adolescentis*), ii) a subunit of a multi-drug efflux pump (*acrB*-like gene predicted to originate from the *Pseudomonas aeruginosa* plasmid pOZ176), and iii) an UDP-4-glucose-epimerase (predicted to originate from *Eggerthella lenta*). The clone carrying the *acrB*-likegene also showed reduced sensitivity towards the antimicrobials triclosan and ampicillin. Complete fosmid sequencing revealed that the *acrB*-like gene and the accessory genes of the predicted multi-drug efflux system were located on a mobile genetic element.

Conclusion: This study identified three different genes and mechanisms that are involved in conferring resistance to BC. Our study suggests that the use of QACs in clinical

settings may co-select for antibiotic resistance among bacteria of the patient gut microbiota.

O103

Gut microbiota continues to evolve during first five years of life

A. Eck[1], N.B.M.M. Rutten[2], I. Besseling-van der Vaart[3], G.T. Rijker[4], A.M. de Vlieger[2], P.H.M. Savelkoul[1], A.E. Budding[1]
[1]VU University Medical Center, Dept. of Medical Microbiology, Amsterdam, [2]St Antonius Hospital, Dept. of Pediatrics, Nieuwegein, [3]Winclove Bio Industries BV, Amsterdam, [4]St Antonius Hospital, Dept. of Medical Microbiology and Immunology, Nieuwegein

Introduction: The intestinal microbiota undergoes dynamic change during development, with the most dramatic changes in composition believed to occur throughout infancy and childhood. The growing recognition that intestinal microbiota may contribute fundamentally to infant and childhood development and immunity is creating an impetus to understand the dynamics that lead to acquisition and colonization of intestinal microbiota in children. Contrary to the current belief, recent studies suggest that the gut microbiota of school-age and adolescent children differ significantly from that of adults, indicating that the human microbiota may be evolving during childhood and adolescence. Here we followed children from birth over a 5-year period and examined the development of their gut microbiota.
Methods: Fecal samples were collected from 99 children over a 5-year period with the following time points: first week, second week, first month, 3 months, first year, 18 months, 2 years and 5 years. To obtain gut microbial profiles, we used the IS-Pro technique: a high throughput PCR based profiling technique which combines bacterial species differentiation by the length of the 16S-23S rDNA interspace region with instant taxonomic classification by phylum specific fluorescent labeling of PCR primers.
Results: As expected, a clear trend of increasing diversity was evident in the cohort with the rise of age. Differences in overall bacterial community composition were assessed and principal coordinate analysis indicated that samples were grouped by their microbiota profile into their corresponding age group. Early time points showed high inter-individual variability while later time points showed convergence to a more stable profile. Furthermore, samples were predominately grouped by time point and not by individual.
Conclusion: These findings lead us to conclude that the gut microbiota continues to evolve at least until 5 years of age and does not reach stability by the second year of life as was previously described.

O104

Effects of selective digestive decontamination on the gut microbiota in Intensive Care Unit patients

T.D.J. Bello Gonzalez[1], M.W.J. van Passel[1], E. Buelow[2], W. van Schaik[2], H. Smidt[1]
[1]Wageningen University, Dept. of Microbiology, Wageningen, [2]University Medical Center Untrecht, Dept. of Medical Microbiology, Utrecht

Introduction: During hospitalization in ICUs, patients are at risk for infections caused by antibiotic-resistant pathogens, and these infections are associated with high morbidity and mortality. Recent studies based on conventional microbiological cultures of rectal swabs taken during selective digestive decontamination (SDD), showed that SDD improves patient survival by preventing infections caused by opportunistic pathogens. However, it cannot excluded that resistant bacteria are present in the gut in low quantities but that the administered antibiotics suppress their growth leading to negative cultures. The aim of this study was determine the effect of SDD on the gut microbiota in ICU patients during different time points by phylogenetic profiling of faecal samples using the Human Intestinal Tract Chip (HITChip), in order to identify changes in the gut microbiota that could occur in patients during and after discontinuation of SDD.
Methods: To determine the effect of SDD on the phylogenetic profile and epertoire of the gut microbiota of ICU patients, we collected faecal samples at different time points from 10 patients hospitalized in the ICU at Utrecht Medical Centre in 2011-2013. Total DNA were extracted using standardized protocol in our laboratory and the phylogenetic profiles of the gut microbiota were determine by 16S ribosomal RNA gene-targeted HITChip analysis.
Results: Phylogenetic profiling of faecal samples revealed changes in the gut microbiota before, during and after ICU stay. The relative abundance of *Bacteroidetes* and *Firmicutes* (*Clostridium cluster* IV and XIVa) representing the majority of the gut microbiota present during the hospitalization changed considerably during hospitalization. Furthermore, during and after hospitalization, the *Bacilli* were significantly increased whereas the *Proteobacteria* were suppressed.
Conclusions: The SDD therapy affects the dynamics of the gut microbiota in ICU patients, with pronounced differences in the composition of the microbiota between the different patients.

O105

The generation of a highly effective model to study the convertases of the complement system

H. Orhan[1], E.T.M. Berends[2], J.A. Soppe[2], M. Ruyken[2], S.H.M. Rooijakkers[2]

[1]*University Medical Center Utrecht, Dept. of Medical Microbiology, Utrecht,* [2]*University Medical Center Utrecht, Dept. of Medical Microbiology, Utrecht*

One of the essential mechanisms in innate immune defense is the complement system, which has a key role in tagging and eliminating microbial intruders. The complement system can be initiated via the classical (CP), lectin (LP), and alternative pathway (AP). Activation by any of these pathways results in a hierarchical downstream cascade of catalytic reactions generating activated complement proteins with important functions including opsonization of bacteria, release of chemoattractants, and direct killing of Gram-negative bacteria by Membrane Attack Complex (MAC) formation. The C5 convertase is a serine protease enzyme complex generated on the bacterial surface that cleaves C5 into the chemoattractant C5a and C5b which initiates MAC assembly. C3 convertases are bimolecular enzymes of C4b and C2a (CP and LP) or C3b and Bb (AP). Subsequently, C5 convertases are formed when additional C3b molecules assemble together with the C3 convertases. Studying the complement C5 convertases is complicated by the fact that the enzyme complex has a short half-life (~90s), a similar enzyme complex can cleave C3 in the AP, and in fluid phase a-specific low-affinity' C5 convertases are formed. Therefore we developed model systems using purified proteins which enable us to characterize the complement C3 and C5 convertases in a controllable environment.

Our model is based on streptavidin coated beads which are used as a replacement of the bacterial surface. We generated biotinylated C4b (CP/LP) and C3b (AP) that are the non-catalytical components of the C3/C5 convertases. By flow cytometry, we verified deposition of C3b and C4b to the beads. In addition we analyzed the binding of C5 and found that on the beads bearing C4b and C3b (CP, LP) or C3b alone (AP), C5 could stably bind after incubation with C5 only. By adding C2 together with MASP-2 (to cleave C2 into C2a) or factor B and factor D (to cleave factor B into Bb) in the presence of C3 or C5, we could generate C3 and C5 convertases. Convertase activity was analyzed by evaluating the supernatant for the presence of C3a and C5a in a calcium mobilization assay using HEK-Ga16 cells transfected with the C3a receptor (HEK-Ga16-C3aR) and U937 cells transfected with the C5a receptor (U937-C5aR). In our models, we formed active C3 and C5 convertases. Activity was dose-dependent on the amount of C4b or C3b present on the surface.

Taken together, we generated functional model systems that will allow us to further characterize the molecular structure and function of the complement convertases (1). We found that the binding of the non-catalytical subunits of the convertases, C3b and C4b, have affinity for their substrates (2) and that activity is dose-dependent on the amount of C3b and C4b (3). Within our models all factors are controllable, thereby making it possible to study the interactions of the complement proteins independently under different conditions. Because of the high flexibility of this model, it could also provide us with new insights into the enzyme's mechanism of switching from cleaving C3 to C5, which is yet waiting to be unraveled.

O106

Chlamydial load in patients whom have naturallycleared *Chlamydia trachomatis* compared to non-cleared patients

M.J.V. Franssen[1,3], A. Dirks[1,2], G.A. van Liere[1,2], N.H. Dukers-Muijrers[1,2], P.F.G. Wolffs[1], C.J. Hoebe[1,2]

[1]*Maastricht University Medical Centre, Dept. of Medical Microbiology, CAPHRI, Maastricht,* [2]*South Limburg Public Health Service, Dept. of Sexual Health, Dept. of Infectious Diseases and Environmental Health, Heerlen,* [3]*Fontys University of Applied Sciences, Eindhoven*

Introduction: *Chlamydia trachomatis* (CT)is the most common bacterial sexually transmitted infection (STI) worldwide. More than half of people with an urogenital CT infection are asymptomatic but may eventually experience complications. This study investigates the CT load, partly because little is known about the natural course of the Chlamydial load during a CT infection. Another reason of studying CT load is because approximately 20% of CT positive patients clear urogenital CT naturally. Hypothesized is that patients whom have cleared CT naturally possibly had a low initial load.

Methods: Two samples (obtained from STI-clinic South-Limburg) per location (urine, vaginal and/or anal swabs) per person were used (some patients > 1 location). One sample was obtained at the first STI clinic consult and one sample prior to treatment. The average time between these two samples was ~ two weeks. CT load was measured for 249 locations (67% women); urine (n = 64) and vaginal (n = 153)and anal (n = 32)swabs. 18 of those patients cleared naturally (3 urine samples, 12 vaginal and 3 anal). The CT load was determined with quantitative PCR, targets were; outer membrane protein A (ompA), which is a single-copy CT gene and Human Leucocyte Antigen (HLA) gene to detect human cells. HLA was used to normalize CT load.

Results: The initial load of patients whom have cleared naturally had an average of 0.003 CT/HLA. The average initial load of patients whom have not cleared was 2.0 CT/

HLA. In 57% of non-cleared samples the CT load remained unchanged over time (n = 131, range: -0.97 - 2.0-fold load change). In 43% of non-cleared samples CT load increased more than 2-fold (n = 100, average 920-fold increase). Vaginal load increased most with an average of 1544-fold (n = 59). Decreases in CT load over 0.97-fold were not observed.

Conclusions: This study showed that 1) the initial load of naturally cleared patients was low (0.003 CT/HLA average). 2) Non-cleared patients had a higher initial load (2.0 CT/HLA average). 3) 57% of non-cleared samples remained unchanged over time. 43% of non-cleared samples increased more than 2-fold (average 920-fold increase). Decreases in CT load over 0.97-fold were not observed. Future studies on clearance should include more patients.

O107

Butyrate-producing bacteria related to intestinal diseases

D. Vodegel[1], J. Penders[2]

[1]*Fontys, Dept. of Applied Science, Eindhoven,* [2]*Maastricht University Medical Centre, Dept. of Medical Microbiology, Maastricht*

Introduction: Inflammatory Bowel Diseases (IBD) and Irritable Bowel Syndrome (IBS) are disorders of the gastro-intestinal tract. Both IBD and IBS have been associated with perturbations in the indigenous microbiota compositions. Butyrate is a short chain fatty acid that is produced by several groups of intestinal bacteria. Given the anti-inflammatory activity of butyrate, it is thought that these bacteria are less prevalent among patients with IBD and potentially also in patients with IBS. The aim of the present study was to set up, optimize and validate a quantitative PCR to enumerate butyrate-producing bacteria, and subsequently to examine whether the abundance of butyrate producing bacteria in feces differs between healthy volunteers, patients with IBD and patients with IBS. Because of the anti-inflammatory activity of butyrate, it is hypothesize that the abundance of butyrate-producing bacteria is lower in the intestinal microbiota of IBD and IBS patients as compared with healthy volunteers.

Methods: Fecal samples from 10 patients with Irritable Bowel Syndrome, 6 patients with Inflammatory Bowel Disease and 13 healthy volunteers were collected and stored at -80 C until further analysis. The samples were analyzed with an optimized and validated quantitative PCR for butyrate-producing bacteria.

Results: The results showed a significant difference (p = 0.046) in the number of butyrate-producing bacteria in the fecal samples of healthy subjects (median 12.2 log10 copies/gram feces, range 11.1 to 12.9) as compared to IBD patients (median 11.7, range 8.9-12.1) and between IBD and IBS patients (median 12.6, range 11.1-13.1, p = 0.031). To control for the variations in the consistency of the stool samples, normalization for the number of 16S rDNA copies/gram feces was performed. After normalization for total bacterial counts (based upon 16S rDNA) the significant difference between healthy and IBS patients disappeared (p = 0.101). However, the statistically significant difference between IBD and IBS patients remained after adjusting for total bacterial counts (p = 0.031). The median relative abundance of butyrate-producing bacteria was 4.0% (range 0.2-12.1%) in healthy volunteers as compared to 6.0% (3.1-11.2%) in IBS patients

Conclusion: The lower abundance of butyrate-producing bacteria in IBD patients as compared to healthy controls was likely due the looser (more watery) stool samples in IBD patients as this difference disappeared after adjusting for total bacterial counts. The significant difference between IBS and IBD remained after adjusting for total bacterial counts.

O108

Reducing turn-around time in positive blood cultures

M.A. Kuijstermans

UZ Antwerpen / Avans Hogeschool, Dept. of Microbiology, Antwerpen

Introduction: Sepsis is a major cause of mortality. Early diagnosis may contribute to the increase of survival rates in sepsis patients. The aim of this study is to reduce the Turn-around time (TAT) in the analysis of positive blood cultures.

Methods: The study was performed over a 4-month period at the University Hospital of Antwerp. Clinical blood samples were cultivated in the Biomrieux BacT/ALERT 3D. A total of 200 blood cultures which became positive between 21:00 and 15:00 the next day, were cultivated on blood agar plates. Cultures were checked for growth at 4,6 and 7 hours of incubation (based on results from an spiking experiment). Any microbial growth was identified with Brker MALDI-TOF MS. In case of reliable identification within the same day between 08:00 and 15:00, standardized susceptibility tests were performed. Results were compared with results of the routine microbial analysis. Mycobacteria were excluded from the study.

Results: As this is an ongoing study, data shown are collected between September 30th 2013 and December 24th 2013. 9,00% of all samples (18/200) were polymicrobial and were excluded from analysis. Strains in positive blood cultures were identified within 7 hours in 78,02% (142/182) of the remaining cases, whereof 58,79% (107/182) within 4 hours. The average decrease in TAT for identification was 16,62 hours. If identified before 15:00, antibiotic susceptibility testing was performed. Discordance in

susceptibility testing was 8,33% and the average decrease in TAT was 13,79 hours.

Conclusions: Using MALDI-TOF MS (and immediate inoculation), it is possible to obtain a reliable identification of a majority of microbes cultured from blood within 7 hours. Turn-around time for identification of these strains is decreased by 16,62 hours. Moreover, if performed, antibiotic susceptibility testing was obtained 13,79 hours earlier. Final results and conclusions of the complete study will be given during the symposium.

O109

Enrichment and isolation of sulfate-reducing bacteria associated with methanotrophic archaea

D.M. van Vliet, H.F. Treppke, C.M. Plugge, A.J.M. Stams, P.H.A. Timmers

Wageningen University, Dept. of Microbial Physiology, Wageningen

Introduction: Wastewater rich in sulfate and flue gas containing sulfur-dioxide can be cleaned either through chemical precipitation to gypsum using limestone, or through biological treatment. Sulfate-reducing bacteria (SRB) reduce the sulfate to sulfide, after which the sulfide is biologically oxidized to elemental sulfur. The sulphur precipitates and is reusable in industry. Currently hydrogen is often used as electron donor for SRB, though methane used to produce hydrogen by steam-reforming could also serve directly as a cheap and environmentally friendly substrate. Anaerobic oxidation of methane (AOM) was thought impossible until recently. Now, after more than a decade of bioreactor studies and phylogenetic analyses on several marine sediment samples, we know that AOM is mediated by anaerobic methanotrophic archaea (ANME) in consortia with groups of autotrophic SRB, all thus far uncultivated. While the syntrophic relation is deemed obligate, increasing evidence shows that there is not a strict preference for one group of SRB.[1] Slow growth of the consortia limits physiological studies and isolation and application in wastewater treatment. The aim of this study is the use of alternative substrates to grow SRB associated with AOM. By isolating SRB on substrates other than methane with sulfate as electron acceptor and reintroduction of isolated SRB to active AOM consortia, we aim to increase methane oxidation rates and therefore biomass production rates so that application in wastewater treatment is more feasible.

Methods: In previous studies at our laboratory, AOM-active sediment from Eckernfrde Bay (Baltic Sea) has been enriched for ANME and associated SRB for 355 days by feeding methane with only sulfate as electron acceptor. This sediment was stored in two subsequent dilution series over the next 5 years. The most diluted culture still showing sulfide production as proxy for AOM activity was used as inoculum for this study. Cultures were incubated in batch with sulfate and 20 mM of 10 different potential substrates, including hydrogen/CO_2 (80% v/v hydrogen), acetate, lactate, pyruvate, propionate, formate, butyrate, methanol, methanethiol (1 mM) and polysulphide (1 mM). Growth and activity were monitored by microscopy, sulfide measurements, GC analysis for methane or hydrogen concentration and HPLC/IC analysis for substrate and sulfate concentrations. 16S rRNA clone libraries were constructed and sequenced for phylogenetic analysis.

Results and conclusion: In cultures containing hydrogen, lactate, pyruvate, methanol, formate and butyrate, growth was found and phylogenetic analysis was done. Methanogens were dominant in the methanol cultures. The heterotrophic *Desulfovibrio desulfuricans* (98% similarity) was dominant in lactate and pyruvate cultures. In hydrogen, formate and butyrate cultures clone sequencing revealed organisms related to autotrophic *Desulfotignum* spp. (93-98% similarity) and *Desulfosarcina* spp. (90-97%). These potential AOM-associated SRB were similar (< 99%) to clones from AOM reactors from several different previous studies. It is thus likely these SRB are capable of taking the role of syntrophic partner in AOM. Further enrichment in dilution series is in progress.

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O110

The immunomodulatory effect of probiotic bacteria fractions

O.I.J. Perdijk, A.J. Thiele-Taverne, M. Meijerink, J.M. Wells, A. Mercenier

Wageningen University, Dept. of Host-microbe interactomics, Wageningen

Background: Probiotics are defined as 'live micro-organisms which, when administered in adequate amounts, confer a health bene?t on the host'. Specific strains have been shown to mitigate symptoms from gastrointestinal tract disturbances, including infections and inflammation. However, maintaining probiotics alive in certain products represents a technological challenge. Therefore, heat-treated (HT) probiotics might provide an alternative solution owing their longer shelf life and decreased sensitivity to storage and transport conditions, provided they still deliver the expected health benefit. Previous studies have established that non-replicating microorganisms possess intact components known to interact with host pattern recognition receptors on immune and other cell types such as peptidoglycan,

lipoproteins, lipoteichoic acid and unmethylated CpG motifs. This study investigated the *in vitro* immune effect of different bacterial fractions derived from live or heat-treated *Bifidobacterium* and *Lactobacillus* strains that were previously shown to impact on the immune system *in vitro* and *in vivo*.

Methods: Cell wall and supernatant fractions purified (Biofocus) from three *Bifidobacterium* and two *Lactobacillus* strains were used in this study, i.e. *B. lactis* NCC2818 (live andHT), *B. longum* NCC2705 and NCC3001 (live), *L. johnsonii* NCC533 (HT) and *L. rhamnosus* NCC4007 (live). The fractions were kindly supplied by the Nestl Research Center. The effect of the bacterial fractions on immune cells was tested by *in vitro* stimulation of human peripheral blood mononuclear cells (PBMCs) and Caco-2 cells. Cytokine release was measured by cytometric bead array and ELISA. Furthermore, bacterial fractions were tested for their capacity to signal to toll-like receptors (TLR) in human TLR transfected HEK-293 cells.

Results: Interestingly, the preliminary results clearly showed strain and fraction specific signaling and immunomodulatory patterns. Compared to lipid, peptidoglycan or peptide fractions, or spheroplasts, the fractions enriched in cell wall constituents were those inducing the highest TLR2 signaling in HEK-293 cells and cytokine levels after PBMC stimulations. Caco-2 cells secreted high levels of IL-8 only after stimulation with the fraction enriched in polar lipids, independently of the strain.

Conclusions: The results show that both the signaling and immune responses to *Bifidobacterium* and *Lactobacillus* derived fractions are highly strain and fraction specific. Future studies should assess the immunomodulatory effects of the most active fractions *in vivo* with the aim to correlate these results with the known effect of the corresponding bacterium in mouse models. The ultimate goal of this approach is to identify the probiotic active compounds that underlie the cross talk between a given strain or bacterial preparation and the immune system of the host.

Keywords: Probiotics, *Lactobacillus*, *Bifidobacterium*, bacterial fractions, immunomodulation, cytokines, TLR signaling.

O111

Importance of immunereconstitution and viral infections: Towards more predictable immune-reconstitution after HCT

J.J. Boelens

UMC Utrecht, Dept. of Blood and Marrow Transplantation, Utrecht

In allogeneic hematopoietic cell transplantation (HCT) is a currative treatment option in various diseases in children and adults, such as cancer, metabolic disorders, hemoglobinopathies and immune deficiencies. The outcome of HCT is highly variable among patients; understanding the sources of variability could significantly improve HCT outcome in terms of efficacy and tolerability of treatment. Relapse and transplantation-associated mortality (mainly due to GvHD and viral reactivations) are the major limitations of HCT. The conditioning regimen given prior to HCT is one of the variables that may be optimized. Individualizing the conditioning regimen resulting in a predictable immune-reconstitution may significantly impact the outcomes.

In the conditioning regimen for HCT, serotherapy (e.g. ATG/Campath) is combined with a variety of cytostatic drugs (or TBI; total body irradiation). It is hypothesized that the exposure to the drugs used in the conditioning phase is a major determinant of this variability in outcomes. For Busulfan, it has clearly been demonstrated that targeting to a predefined exposure and thereby reducing the variability in exposure substantially improves the outcomes: less graft-rejection, lower toxicity (e.g. VOD, aGvHD). More recent Fludarabine has replaced Cyclofosfamide since it has a more favorable efficacy-toxicity profile. Therefor, nowadays FluBu is used more and more as a conditioning platform in HCTs. Probably the most component in the conditioning is the use of serotherapy such as Thymoglobuline (ATG; anti-thymocyte globuline). ATG is added to prevent 'graft-versus-host disease (GvHD)' and rejection. ATG is a polyclonal antibody depleting T-cells. The therapeutic window is however critical as over-exposure may result in delayed reconstitution of donor T-cells and increased risk of viral infections. We recently showed that the timing of ATG (early or late prior to HCT) impacts viral reactivations ($p = 0.011$) in cord blood transplantation. Furthermore we (in collaboration with the pediatric program in the LUMC and LACDR) recently described the population pharmacokinetics (PK) of Thymoglobulin as a first step towards an 'evidence-based' dosing regimen of Thymoglobulin for HCT. Thereafter we did an extensive Pharmaco-dynamic analyses ($n = 264$ pediatric patients: LUMC + UMCU). We showed that in multivariate analyses the 'overall survival' and 'event free survival' were higher in patients with a low Thymoglobulin exposures after HCT ($p = 0.0002$). This was associated with successful T-cell reconstitution defined as having 100 CD3+/uL twice within 100 days, while aGvHD was not significantly increased. Poor immune-reconstitution was associated more viral reactivations (EBV, CMV, Adeno-virus and HHV6) and associated transplantation-associated mortality.

The conditioning regimen prior to HCT is an essential part of the HCT. PK and PD studies have taught us to optimize the outcomes of HCT. Individualizing the conditioning regimen will improve the outcomes of HCT due to a better predictable immune-reconstitution resulting in less virus-associated problems after HCT.

O112

Human herpesvirus - 6 DNAemia is a sign of impending primary CMV infection in CMV sero-discordant renal transplantations

C.C. van Leer, J.S.F. Sanders, H.E.J. Vroom, A. Riezebos-Brilman, H.G.M. Niesters
UMCG, Dept. of Medical Microbiology, Groningen

Introduction: Human herpesvirus-6 (HHV6) and cytomegalovirus (CMV) are both members of the beta-herpesviridae. These viruses are usually acquired asymptomatically, remain latently present after primary infections and may reactivate in imuunocompromized individuals.
In patients who have received an organ transplant, reactivations of these viruses frequently cause complications. Although few dispute the potential of HHV-6 reactivations to cause disease such as pneumonitis and encephalitis, HHV-6 DNA is frequently detected without evident organ localized disease, the clinical significance of this HHV-6 DNAemia is not clear.
What is almost universally associated with the detection of HHV-6 DNA post-organ transplantation, is concurrent or subsequent CMV reactivation This has given rise to the hypothesis that HHV-6 reactivations trigger CMV reactivations. Nevertheless, due to the high seropositivity for both viruses in most study populations, it is impossible to conclude that either virus has any effect on the other, or whether reactivation of HHV-6 and more severe reactivations of CMV are both independently caused by the immunocompromized condition of the transplantation patients.We report the results of is the first cohort study investigating the complexities between CMV and HHV-6 in which a relatively large number of CMV-seronegative recipients have been included
Methods: 92 consecutive patients receiving a renal transplantation were included. All available stored whole blood samples were tested for HHV-6 DNA by quantitative PCR. Details including CMV serostatus of donor and recipient were recorded.
Results: CMV-seropositive recipients have a 68% chance of developing HHV-6 DNAemia if the kidney came from a CMV-seropositive donor, compared to 26% if the kidney came from a CMV-seronegative donor. CMV-seronegative recipients, who are bound to undergo primary CMV infections following transplantation with a renal graft from a CMV-seropositive donor, have 88% chance of developing HHV-6 DNAemia. If they receive a graft from a CMV-seronegative donor the chance of developing HHV-6 DNAemia is 22%.
Conclusions: Receiving a renal transplant from a CMV-seropositive donor increases the chance of developing HHV-6 DNAemia. HHV-6 DNAemia is a sign of impending primary CMV infections following sero-discordant renal transplantations.

O113

Central nervous system infections with JC virus in immuno-compromised patients

J.L. Murk
UMC Utrecht, Dept. of Medical Microbiology, Utrecht

Progressive multifocal leukoencephalopathy (PML) is a rare life threatening disease of the brain caused by an infection with JC polyomavirus (JCPyV). The incidence of PML in the general population is < 0.3 cases per 100.000 person years. With an incidence of 2.4 per 1000 person years, PML used to be mainly a disease of AIDS patients. When combined antiretroviral (cART) therapy became available the incidence in HIV patients dropped more than 10 times. There has, however, been a resurgence of PML cases after the introduction of several immunomodulating monoclonal antibodies and due to the increasing numbers of transplant recipients. Risk groups with the highest incidence of PML are now multiple-sclerosis patients treated with natalizumab, AIDS patients, transplant recipients, patients with hematological malignancies, and patients treated with rituximab and possibly efalizumab (treatment of psoriasis). As a consequence, physicians from various disciplines encounter PML cases. In this presentation the current understanding of risk factors for PML and diagnostic challenges are discussed.

O114

Gene expression analysis reveals an important role for neutrophils in infants with severe viral respiratory tract infections

I.M.L. Ahout[1], A. Zomer[1], H.K. Brand[1], E. Simonetti[1], A. Andeweg[2], V.L. Jong[3], M.J.C. Eijkemans[3], M.A. Bijl[2], M.I. de Jonge[1], R. de Groot[1], G. Ferwerda[1]
[1]*Radboudumc, Dept. of Pediatrics, Nijmegen,* [2]*Erasmus University Medical Center, Dept. of Virology, Rotterdam,* [3]*University Medical Center Utrecht, Julius Center for Health Sciences, Dept. of Biostatistics and Research, Utrecht*

Introduction: The pathophysiology of severity of viral respiratory infections in infants remains to be elucidated. In micro-array studies it was shown that neutrophils, inflammation and interferon genes were up regulated during RSV infections. In this study we aim to extend these findings and focus on differences in the immune response of infants with a severe course of disease. Methods Affymetrix micro-array on whole blood of 51 patients < 2 years of age with a PCR confirmed viral lower respiratory tract infection and 12 age-matched healthy controls was performed. The course of disease was retrospective determined. The data was analyzed with a modular described by Chaussebel et.al.[1] A bandwidth of health and subsequent distance to health were calculated.

Subsequently, IL-6, IP-10, IL-8 and gCSF levels in plasma of the same patients were measured and blood smears were analyzed.

Results: The modular distance to health correlated significantly with disease severity, duration of supplemental oxygen and length of stay in the hospital. Moreover these patients clustered together based on disease severity. Especially up regulation of the neutrophil module is discriminative for patients with a severe course of disease. Four of the five patients who appeared to be misclassified were reported to have repeated apnea or extremely dyspnoe. The other misclassified patient's recovery sample was an outlier as well. The neutrophil up regulation was partly due to cell shift (severe patients had increased numbers of banded neutrophils), but also a result of neutrophil activation (increased plasma levels of gCSF and IL-8). We have performed micro-arrays on neutrophils as well and these results will be available soon.

Conclusion: Neutrophils seems to have an unexpected role during severe respiratory viral infections in children and might offer new targets to reduce the burden of respiratory viral infections.

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O115

Human Polyomavirus 9 infection emerges in immunocompromized patients

H.F. Wunderink[1], P.Z. van der Meijden[1], C.S. van der Blij-de Brouwer[1], H.L. Zaaijer[2], J.I. Rotmans[1], J.N. Bouwes Bavinck[1], M.C.W. Feltkamp[1]

[1]*Leiden University Medical Center, Dept. of Medical Microbiology, Leiderdorp,* [2]*Sanquin Blood Supply Foundation, Dept. of Blood-borne Infections, Amsterdam*

Several new human polyomavirus have recently been identified, one of them is human polyomavirus 9 (HPyV9). Whether HPyV9 is pathogenic and commonly found, for instance in immunosuppressed individuals, is unknown.

A cohort of 101 immunosuppressed kidney transplant patients was systematically screened for the presence of HPyV9 infection over time. A total of 541 patient sera, collected every three months until 18 months after transplantation, were analyzed for the presence of HPyV9 DNA (viremia) and IgG seroreactivity by quantitative PCR and immunoassay, respectively. Sera collected one year apart from a cohort of 87 age and sex-matched healthy blood donors were included as a control.

In 21% of the immunosuppressed individuals, viremia was detected at some time point after transplantation. The prevalence of HPyV9 viremia peaked at 3 months post-transplantation. The highest mean viral loads were measured just after transplantation. In the control group, no HPyV9 viremia was detectable, just as in sera from the transplantation cohort collected prior to transplantation.

HPyV9 seroprevalence significantly increased from 33 to 46% during follow-up, while it remained stable at 30% in the control group. No correlation between presence of HPyV9 viremia and seropositivity was observed. Further analysis revealed a significant association between HPyV9 viremia and viremia by the related BK polyomavirus, an established pathogen in these patients, but not by cytomegalovirus.

In conclusion, our data show that infection with HPyV9 is frequently observed in kidney transplant patients in the first year after transplantation. The nature of this infection, whether endogenous or donor-derived, is not known and deserves further study, as well as its pathogenic potential.

O116

Unraveling the environmental significance of nitrite-dependent anaerobic methane oxidation: a lipid biomarker approach

J.S. Sinninghe Damste[1], D.M. Kool[1], H.M. Talbot[2], D. Rush[2], O. Rasigraf[3], K. Ettwig[3], M.S.M. Jetten[3]

[1]*NIOZ, Dept. Of marine organic biogeochemistry, Den Burg,* [2]*University of Newcastle, School of Civil Engineering and Geoscien, Newcastle,United Kingdom,* [3]*Radboudumc, Dept. of Microbiology, Nijmegen*

The newly identified bacterium *Candidatus* Methylomirabilis oxyfera' oxidizes methane in the absence of oxygen, by coupling it to nitrite reduction. Although *M. oxyfera* thrives in anoxic environments, it employs a special intra-aerobic' pathway through which it internally produces its own oxygen for the oxidation of methane. Only little is known about the occurrence and significance of this new' methane sink in the environment, partly because the identification of *M. oxyfera* in ecosystems primarily relies on molecular techniques targeting DNA and RNA. To enhance our ability to detect and study this organism in the environment, we are investigating the potential of new biomarkers for *M. oxyfera* based on its lipid composition. Core lipid analyses of *M. oxyfera* enrichment cultures revealed two characteristic C17 fatty acids, a $10MeC_{16:0}$ and an unprecedented $10MeC_{16:1?7}$ fatty acid, which we were able to detect in the environment as well. At present, we are further investigating the lipid biomarker potential for *M. oxyfera* focusing on hopanoid lipids and the complementary use of stable carbon isotopes.

Our hopanoid analyses revealed that *M. oxyfera* synthesizes significant amounts of fairly uncommon bacteriohopanepolyols, BHPs, namely BHP-hexol, -pentol, -tetrol,

and 3-methyl homologues of all three. None of the C-35 amino-BHPs that are more commonly observed in (aerobic) methanotrophs were detected. Our findings provide the first ever account of a 3-methyl-BHP-hexol, and only the second known source organism for BHP-hexol after *Alicyclobacillus acidoterrestris*. As the genes required for C3-methylation seem to be exclusively present in micro-organisms with an aerobic metabolism, the abundant presence of 3-methyl-BHPs in *M. oxyfera* denotes for the first time the potential production of 3-methyl-hopanoids in anoxic environments.

Furthermore, with ^{13}C-labeling experiments we show that *M. oxyfera* does not assimilate methane-carbon, but rather C from bicarbonate/CO_2, into its BHPs. This implies that methanotroph-derived hopanoids do not necessarily exhibit a strongly depleted carbon isotopic signature, which is commonly anticipated with methanotrophy. This has implications for the interpretation of the presence and isotope composition of geohopanoids, the geological degradation products of the BHPs, in the paleorecord.

Our new findings on *M. oxyfera* hopanoid lipids and carbon assimilation may improve our ability to detect and study this organism in the environment, and to assess the significance of nitrite-dependent methane oxidation in the past and present global methane cycle.

O117

Streptococcus pneumoniae modulates human respiratory syncytial virus infection *in vitro* and *in vivo*

D.T. Nguyen[1], R. Louwen[1], G. van Amerongen[1], K.E. Elberse[2], S. Yüksel[1], A.D.M.E. Osterhaus[1], W.P. Duprex[3], R.L. de Swart[1]

[1]Erasmus MC, Dept. of Viroscience, Rotterdam, [2]RIVM, CIB, Bilthoven [3]Boston University, Dept. of Microbiology, Boston, USA

Introduction: Globally, human respiratory syncytial virus (HRSV) and *Streptococcus pneumoniae* are important causative agents of respiratory tract infections. For both pathogens the disease burden is highest in the pediatric population. Significant numbers of children are colonized with *Streptococcus pneumoniae* in the nasopharynx. From here the pneumococcus can spread to other parts of the body, causing disease or even death. Almost all infants are infected by HRSV before the age of two years, but only a small proportion develops severe disease. Bacterial pathogens such as *Streptococcus pneumoniae* can often be cultured from children hospitalized with HRSV. Interactions between viral and bacterial pathogens have been described both *in vitro* and *in vivo*, but causal relationships are poorly understood. The aim of the current study was to study the influence of pneumococcal colonization on subsequent HRSV infection.

Methods: *S. pneumoniae* serotypes were obtained from colonized children of a large cohort study. We used a new recombinant subgroup B HRSV strain that expresses enhanced green fluorescent protein (EGFP): rHRSV^{B05}EGFP. For *in vitro* studies primary well-differentiated normal human bronchial epithelial (wd-NHBE) cells with tight-junctions and cilia were used. Briefly, wd-NHBE cells were coinfected with *S. pneumoniae* and rHRSV^{B05}EGFP. Two days post-infection (d.p.i.) EGFP$^+$ cells were semi-automatically counted. For *in vivo* studies we used the HRSV cotton rat (*Sigmodon Hispidus*) model. Groups of animals were colonized with different *S. pneumoniae* serotypes. Three days later animals were inoculated intranasally with rHRSV^{B05}EGFP. After 5 days animals were euthanized. For our transmission study two groups of each six animals were mock-treated or inoculated with *S. pneumoniae* serotype and infected with HRSV as described above. *S. pneumoniae* and HRSV nave contact animals were placed with the infected animals. *S. pneumoniae* and rHRSV^{B05}EGFP titers were determined in both the upper and lower respiratory tract.

Results: In wd-NHBE cells coinfection with pneumococcus serotype 8, 15A, or 19F enhanced HRSV infection compared to mock and other serotypes. Animals colonized with pneumococcus serotype 19F and 23F enhanced HRSV infection significantly compared to mock-treated. With use of EGFP$^+$ cells HRSV infection could be real-time monitored. Syncytia were observed in nasal septa and conchae microscopically. Significantly more syncytia were observed in the group colonized with serotype 19F compared to mock. We hypothesized that higher virus loads facilitates transmission of virus or bacteria. This hypothesis was tested in a *S. pneumoniae* / HRSV cotton rat transmission model. Again, more virus was isolated in the colonized group compared to mock. However, no HRSV or pneumococcus was transmitted from the index animals to the contact animals.

Conclusion: In the present study we have studied interactions between *S. pneumoniae* and HRSV *in vitro* and *in vivo*. In wd-NHBE cells coinfected with HRSV and pneumococcus serotype 8, 15A or 19F statistically significant more epithelial cells were infected. In animals with successful induction of pneumococcus carriage HRSV infection was significant enhanced, but this was serotype specific. Also pneumococcus carriage with serotype 19F resulted in significant more syncytia formation within the nasal septum.

O118

Time resolved monitoring of Bacillus spore coat protein cross-links, spore germination and spore thermal resistance

W. Abhyankar[1,2], R. Pandey[1,3], A. ter Beek[1], S. Stelder[1,2], S. Omardien[1,3,4], L. Zheng[1,2], L.J. de Koning[2], C.G. de Koster[2*], S. Brul[1*]

[1]*Dept. of Molecular Biology & Microbial Food Safety,* [2]*Mass spectrometry of Biomacromolecules,*[3]*Van Leeuwenhoek Centre for Advanced Microscopy Section of Molecular Cytology, Swammerdam Institute for Life Sciences, University of Amsterdam,* [4]*Academic Medical Center, Dept. of Medical Microbiology University of Amsterdam, Amsterdam*
**Equal contribution*

Introduction: Bacterial spores allow Bacilli and Clostridia to survive adverse environmental conditions such as thermal stress. Spores are well known foes of human health compromising amongst others food preservation. A recent study by Setlow and co-workers (Sanchez-Salas et al. 2011. Applied & Environmental Microbiology 77, 6746-6754) concluded that spore maturation, after release from the mother cell, is an important factor in acquiring wet-heat resistance. Cross-linking levels of spore surface component during spore maturation were previously studied as putative determinants of thermal stress resistance. It was concluded that levels of cortex peptidoglycan cross-linking are not discriminatory in terms of thermal stress resistance (Atrih and Foster. 2001. Journal of Applied Microbiology 91, 364-372). Here we hypothesized that increased levels of cross-linking of specific coat proteins might be an important element in spore heat survival. The kind of peptide cross-linking found in spore coats are dityrosine links, e-(?)-gltamyl-lysine isopeptide linkages and disulfide bonds (Henriques and Moran. 2007. Annual reviews of Microbiology 61, 555-588). From a proteomics point of view we expect that the more coat proteins are cross-linked, the lower is their protease digestibility. Using ^{15}N-labeled 8-day mature spores as a reference, we monitored the loss of protease digestion efficiency of ^{14}N-spore coat proteins as a function of their tryptic peptide ^{14}N/^{15}N isotopic ratios. Simultaneously we assessed the levels of spore thermal resistance and spore germination time.

Materials and method essentials: *Bacillus subtilis* wild-type strain PY79 (Eichenberger, New York University, USA) was used for preparing ^{14}N and ^{15}N-labeled spores. Bacilli were pre-cultured in defined medium till OD 0.4 (Abhyankar et al. 2011. Proteomics11, 4541-4550) and inoculated into in-house built well aerated bench fermenters. The cultures were allowed to sporulate and mature at 37 C for maximally 10 days. Query cultures were grown and sporulated in the presence of $^{14}NH_4Cl$ while reference cultures contained $^{15}NH_4Cl$ as the sole nitrogen source. Biologically independent triplicates were analyzed. LC-MS/MS data were acquired with a Bruker ApexUltra Fourier transform ion cyclotron resonance mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with a 7 T magnet and a nano-electrospray Apollo II DualSource coupled to an Ultimate 3000 (Dionex, Sunnyvale, CA, USA) HPLC system. Spore germination time was assessed with our live-imaging system SporeTracker (Pandey et al. 2013. PlosOne 8, e58972)

Results, conclusions and discussion: The data showed that spore maturation is coupled to protein cross-linking indicated by increased heat-resistance and loss of trypsin digestibility of spore outer coat- and crust layer proteins CotG, YurS, CotU, CotI, CotZ, CotY, CotB and CotC. Except for SpoIVA and SafA in young spores within the mother cell, inner coat proteins are not involved in spore maturation. Initial data suggest that mature spores are less prone to germinate compared to young spores reflecting perhaps their more dormant resilient nature. This difference is not apparent after a thermal stress treatment. Future studies will focus on detailed time resolved analyses of spore germination and outgrowth characteristics in relation to the positioning of the coat protein peptide cross-links and heat resistance development during *B. subtilis* spore maturation.

O119

Rare earth metals are essential for methanotrophic life in volcanic mudpots

H.J.M. op den Camp[1], A. Pol[2], T.R.M. Barends[3], A. Dietl[3], A.F. Khadem[2], J. Eygensteyn[2], M.S.M. Jetten[2]
[1]*Radboudumc, Dept. of Microbiology, Nijmegen,* [2]*Radboudumc, Dept. of Microbiology, Nijmegen,* [3]*Max-Planck Institute for Medical Research, Dept. of Biomolecular Mechanisms, Heidelberg, Germany*

Verrucomicrobial methanotrophs, representatives of the genus *Methylacidiphilum*, are micro-aerophilic methanotrophs outside of the Proteobacteria phylum. They are by far the most acidophilic (< pH 1) bacteria capable of methane oxidation. Growth of *Methylacidiphilum fumariolicum* SolV, an extremely acidophilic methanotrophic microbe isolated from an Italian volcanic mudpot, is shown to be strictly dependent on the presence of lanthanides, a group of rare earth elements (REE) such as lanthanum (Ln), cerium (Ce), praseodymium (Pr) and neodymium (Nd). REE are essential for many high-tech devices like solar cells, mobile phones, and computers but an exact role for them in biology is not yet known. REE are abundantly present in the earth's crust but due to their low solubility their concentration in most ecosystems does not exceed the nanomolar range. After fractionation of the bacterial cells and crystallization of the methanol dehydrogenase (MDH), it was shown that lanthanides were present as cofactor in a homodimeric MDH comparable to one of the MDHs of *Methylobacterium extorquens* AM1. We hypothesize that the lanthanides provide superior catalytic properties to PQQ-dependent MDH, which is a key enzyme for both methanotrophs and methylotrophs. Thus far, all isolated MxaF-type MDH's contain calcium as a catalytic cofactor. The gene encoding the MDH of strain SolV was identified

to be a *xox*F-ortholog, phylogenetically closely related to *mxa*F. Potential REE-binding motifs in XoxF enzymes of many methylotrophs were identified through analysis of the protein structure and alignment of amino acids. This suggests that there may be much more lanthanide-dependent MDHs in nature. Our findings will have major environmental implications since metagenome studies showed (lanthanide-containing) XoxF type MDH to be much more prominent in nature than MxaF type enzymes.

O120

No gain, but also no pain? Selective disadvantage of carrying an IncI1 plasmid with blaCTX-M-1 genes in absence of antimicrobials

E.A.J. Fischer[1], C.M. Dierikx[1], A. van Essen – Zandbergen[1], H.J.W. van Roermund[1], D.J. Mevius[1], J.A. Stegeman[2], D. Klinkenberg[2]

[1]*Central Veterinary Institute, part of Wageningen UR, Dept. of Epidemiology, Crisis Organisation and Diagnostics, Lelystad,* [2]*Utrecht University, Faculty of Veterinary Medicine, Utrecht*

Background: Bacteria producing Extended Spectrum Beta-Lactamases (ESBL), conferring resistance to important antimicrobials, are found with high prevalences in Dutch broiler chickens. The $bla_{CTX\text{-}M\text{-}1}$ genes on IncI1 plasmids are most common. We aimed to determine the fitness cost of this plasmid for the bacterium, using both *in vitro* experiments and a mathematical model. This model was used to investigate the possible *in vivo* dynamics.

Methods: We modelled the population dynamics of IncI1 plasmids carrying $bla_{CTX\text{-}M\text{-}1}$ genes in a batch culture with three *E. coli* populations: donors (D), recipients (R) and transconjugants (T). Bacterial growth was described by logistic growth with parameters: intrinsic growth-rate, carrying capacity and lag-phase, and the conjugation process by a mass-action process.

Parameters were estimated from experiments with pure culture of D, R, or T and mixed culture of D and R. The long-term behaviour was compared to a 3-months experiment in which a mixed culture of R and T was regularly diluted in new medium.

The model was adapted to *in vivo* conditions by including continuous outflow of bacteria and lowering the overall carrying capacity. This model was used to analyse the dynamics of the plasmid within the ranges of the estimated parameters.

Results and discussion: In the batch-culture experiments, no differences in estimated growth parameters were found. The conjugation rate of T was 10^4 times larger than that of the D. In the 3-months experiment, the proportion of T did not decrease. In these experiments, IncI1 plasmid carrying $bla_{CTX\text{-}M\text{-}1}$ genes imposed no or a very small fitness cost on its host in the absence of antimicrobials.

Extrapolation of the results to the *in vivo* system showed that only a difference in the carrying capacity could lead to extinction of the plasmid within a reasonable time frame. This extinction would occur within one broiler production round (6 weeks).

References

- This presentation is based on two manuscripts Fischer et al under revision and Klinkenberg et al. in prep, and the poster Fischer et al. at the Epidemics4 conference November 2013.

O121

TnSeq in *Streptococcus suis* demonstrates that NADH oxidase is essential during infection of piglets

A. de Greeff[1], H.J. Bootsma[2], S.F. Harders-Westerveen[1], N. Stockhofe-Zurwieden[1], E. Zaccaria[3], J.M. Wells[3], A. Zomer[2], M.I. de Jonge[2], H.E. Smith[1]

[1]*Central Veterinary Institute, part of Wageningen UR, Dept. of Infection Biology, Lelystad,* [2]*Radboudumc, Dept. of Pediatric Infectious Diseases, Nijmegen,* [3]*Wageningen UR, Dept. of Host Microbe Interactions, Wageningen*

Background: *Streptococcus suis* is the causative agent of severe infections in piglets. Sows are symptomless carriers of *S. suis*, young piglets develop symptoms like meningitis, arthritis and sepsis. In South-East Asia *S. suis* is an important human pathogen, whereas in Western countries humans are occasionally infected. Control of the infection is hampered by the lack of effective vaccines. As a result *S. suis* is the most prevalent cause for antibiotic use in the pig industry. Therefore, a new method to identify novel vaccine candidates was applied to *S. suis*. TnSeq was used to select for genes essential for survival of *S. suis* under *in vivo* mimicking conditions. Genes involved in oxidative stress, metal stress and survival in porcine serum were identified.

Methods: A *mariner* T7 transposon mutant library was constructed in *S. suis* using the newly discovered competence peptide ComS. The library was used to select for genes conditionally essential under peroxide stress metal stress and growth in porcine serum, in comparison to growth in Todd Hewitt medium (THM). Negatively selected mutants were identified by profiling the relative abundance of each mutant using transposon insertion site sequencing and the ESSENTIALS data analysis pipeline. To confirm the results, directed mutants were generated by allelic exchange. These mutants were tested for their ability to grow under the selective conditions. Virulence of mutants was tested in an experimental infection in piglets.

Results: TnSeq screening yielded 61 genes that were negatively selected from the mutant library after growth in porcine serum, 16 genes that were negatively selected under metal stress, and 14 genes that were negatively selected under peroxide stress. To confirm the role of

these genes in these processes, 10 directed knock-out mutants were constructed. Growth attenuation of these mutants under the selective conditions was confirmed for 7 mutants. Four of these were tested for attenuation in virulence during experimental infection. These included mutants in NADH oxidase, a copper transporter, an ATPase exporter and an ABC transporter lipoprotein. Mutants were tested in competition with the wild-type strain; piglets were infected with a 1:1 mixed inoculum of wild-type and mutant strain. Analysis of bacteria re-isolated from affected organs showed that the ratio of wild type and mutant strain was skewed for 2 out of 4 mutants, whereas the other 2 mutants could be reisolated in the same ratio as in the inoculum. The *nox* mutant and the mutant in the ABC transporter lipoprotein were severely attenuated for survival in blood.

Conclusion: Using TnSeq in *S. suis* 77 transposon mutant were identified as conditionally essential under *in vivo* mimicking conditions. Oxidative stress and metal homeostasis turned out to be very important and intertwined processes that are essential under *in vivo* mimicking conditions. During an experimental infection in piglets, we showed that 2 of 4 tested mutants were severely attenuated for virulence under *in vivo* conditions as well. Taken together, the TnSeq approach was successfully used for *S. suis* and enables selection of transposon mutants essential during natural infection of the host.

O122

Automated microbiological diagnostics: the next step?

E. IJzerman
Streeklaboratorium Kennemerland, Haarlem

Until recently it seemed rather impossible in medical microbiology to automate the handicraft of technicians. However, with ongoing mechanisation and with the introduction of high-quality digital camera's the image of conventional bacteriology is changing rapidly. The process of visual inspection of agar plates can nowadays be replaced by judging digital images of the plates. Computer supported follow-up steps may in the near future lead to robotised determination and susceptibility testing of isolated micro-organisms.

The introduction of digital imaging of culture results has lead to a change in work-flow by separating (in place and time) the process of evaluation the necessary follow-up steps, based on the images, from the actual performance of these steps. This allows the development of a business model in which the activities of plate reading and performing the skills of determination and susceptibility testing are situated at different locations. Bacteriology skills, molecular diagnostics and automated serology for several hospitals can be clustered in one production facility while reading rooms for culture images are located in each of these hospitals with a staff of medical microbiologists and highly qualified technicians close to patient care. With this combination of reading rooms in participating hospitals and a centralised production facility each hospital has his own, nearby, high quality, low costs (virtual) laboratory.

This is still a theoretical model. The next step will be to implement this model as a logical consequence of the ongoing mechanisation and automation. Who will take this step?

O123

Molecular Microbiology -7 day testing, triple the workload and half the staff but managing to deliver a faster service

K. Templeton
NHS Lothian, Royal Infirmary, Edinburgh, United Kingdom

Background: Diagnosis of viral infections used to rely on techniques that took several weeks to obtain results. As a consequence of the time taken to produce these results they had little impact on patient acute management and were often only available once the patient was home and fully recovered or had died. Additionally, the sensitivity of many assays and complexity of the testing meant that patients were undiagnosed. In the past few years there has been a dramatic change in the technology able to produce results for infections with the use of real-time PCR. This has enabled results to be delivered on the same day as the sample is collected.

The timely aspect of results has been most notably recognised during the H1N1 influenza outbreak in 2009. Molecular testing was put at the forefront of diagnostics and through necessity laboratories adapted to enormously increase their workload and to extend the time periods for service delivery.

The ability for these results to be available and deliverable in a useful and clinical relevant timeframe came to the notice to a wider group of clinical specialities who had not previously been aware of rapid molecular diagnosis.

As well as the demand for an increased service there has also been since 2010 an increased emphasis on cost efficiency and within NHS Lothian there was a 5 year plan created to deliver 20% cost reduction.

Aim: In 2012 NHS Lothian planned to deliver a 7-day service for molecular microbiology within the overall budget and financial plan.

The plan: The whole process went through organisational change process with full consultation with all affected staff. The overall statement and motivation was to improve the clinical service.

There was reconfiguration of staffing levels with an increase in healthcare association practioners (non-qualified staff without a university level degree) and

a decrease in by 50% in qualified staff. There was also a complete overview of the processes from the sample to the result back to the patient looking at LEAN processing and as much use d of automation and automatic downloads of electronic information from one system to another.

The service: The service runs from 08.30 to 20.00 Monday to Friday and from 09.00 to 16.00 Saturday and Sunday. The aim of the molecular testing was to be able to perform same day testing for respiratory screens, Cerebrospinal fluid (CSF) screens, Norovirus and Measles. The respiratory testing included a full respiratory viral PCR screen, pneumocystis, legionella, mycoplasma and pertussis. The CSF screen included *Neisseria meningitis*, *Haemophilis influenza* and *Streptococcus pneumoniae* as well as full viral CSF PCR screen.

Some tests were only performed Monday to Friday and batched into two runs per week, e.g. Human immunodeficiency viral load testing and cytomegalovirus quantification. The cut off time of 09.00 was used as time to ensure same day delivery of results. In order to process samples as efficiently, two sample streams were processed. The respiratory samples on easyMAGs and the noroivrus samples on the BDMax. The results were available by 14.00 in 80% of cases and this gave time for treatment decisions, infection control decisions and other clinical management decision to be made. Prior to adoption of this service only 15% of samples were available to be acted on the same day. The workload has increased accorss all the sample results. In the case of Norovirus the workload was 2500 in 2012 and 6000 in 2013. The future, it is planned to look at more point of care options, increase the level of automation and increasingly move away from batch processing. In addition, there is now a strong emphasis on quality improvement and how deliver of these faster results have had positive impacts on clinical areas with separate budgets. This collaborative team working is ensuring improved diagnosis for patients while recognising the role that the molecular service as played in this outcome.

O124

Development of a rapid molecular test for diagnosis of bloodstream infections

M.P. Bos[1], B. van Meerbergen[2], A. Rubenjan[3], A. Catsburg[1], S. Berghmans[2], G. Maertens[2], P. Savelkoul[3]

[1]*Microbiome, Dept. of Medical Microbiology, Amsterdam,* [2]*Biocartis, Mechelen,* [3]*VU University Medical Center, Dept. of Medical Microbiology, Amsterdam*

Introduction: Blood culture (BC) is still the gold standard for diagnosis of bloodstream infections (BSI), despite its time-consuming nature. As patient outcome may improve from faster diagnosis, alternative diagnostics are eagerly awaited. Sensitive detection of pathogens in blood samples from patients with BSI requires large input volumes since pathogen loads are often low. This poses a problem for molecular assays, as the excess of human DNA in such samples inhibits the pathogen-specific PCR. To solve this obstacle, we developed a method, dubbed Polaris,[1] to specifically remove human DNA from blood thus allowing the use of large volumes (5 ml) of blood as input for molecular assays. Furthermore, we developed a multiplex realtime PCR assay to detect the most prevalent bacterial and fungal pathogens found in BSI, in a species-specific manner. The multiplex also includes a molecular Gram-stain PCR, i.e. a broad PCR to detect the presence of either Gram-negative or Gram-positive bacterial pathogens not included in the species-specific assays.

Methods: The multiplex BSI PCR was validated using 5 ml healthy human blood samples spiked with 12 different pathogens in different concentrations. Samples were processed by the Polaris method and DNA was isolated by automated easyMAG extraction. Clinical blood samples, derived simultaneously with BC from critically ill patients that were included in the MARS (Molecular Diagnosis and Risk Stratification of Sepsis) study, were processed in a similar manner.

Results: Sensitivity of the multiplex BSI PCR was high, with most pathogens detected at 1-10 genome copies per PCR. Thirty-two clinical blood samples (5 ml each) with corresponding positive BC containing in total 34 pathogens were processed by the Polaris procedure and tested with species-specific realtime PCRs. We detected *P. aeruginosa* (6x), *E. coli* (3x), *E. faecalis* (7x), *E. faecium* (2x), *Klebsiella spp* (1x), *S. aureus* (4x), coagulase-negative staphylococci (CNS) (3x) and Enterocci (1x). Thus, 27 (79%) PCR results were concordant with blood culture results. Seven pathogens reported in the BC were not detected in the molecular assay: *E. coli* (1x); *E. faecalis* (3x); CNS (3x). Two of the CNS-negative samples and the negative *E. coli* sample were associated with low load BC (i.e. only 1 out of 2 bottles was positive). One of the missed *E. faecalis* samples was inhibited in the PCR.

Conclusion: In conclusion, we demonstrate that a novel pathogen DNA enrichment method coupled with species-specific PCR confirmed positive BC results in 79% of clinical samples examined. A highly sensitive multiplex realtime PCR assay was developed to detect the most prevalent pathogens causing BSI. Since the combination of enrichment and PCR procedures can be completed in less than 4 hours after blood withdrawal, this strategy offers great potential for a much faster diagnosis of BSI.

Reference

- Loonen *et al.* PLoS One, 2013, e72349.
- Acknowledgement: this research was supported by the Center for Translational Molecular Medicine MARS

O125

Automated monitoring of bacterial microcolony growth

A.L. den Hertog[1], C. Ingham[2], F. Fey[3], V. Monteban[4], R.M. Anthony[1]

[1]*Koninklijk Instituut voor de Tropen (KIT), Dept. of Biomedical Research, Amsterdam,* [2]*Microdish BV, Utrecht,* [3]*CCM BV, Nuenen,* [4]*Lumibyte, Nuenen*

Introduction: The increasing prevalence of *Mycobacterium tuberculosis* (Mtb) strains resistant to one or more antibiotics makes drug susceptibility testing (DST) a high priority. A complete overview of the susceptibility of strains to available drugs is required both to ensure the provision of the least toxic and most effective therapy to the individual patient and for interrupting the transmission of drug resistant pathogens. Unfortunately DST for tuberculosis is slow, complex and dangerous and difficult to effectively implement in all setting.

Methods: We developed a (myco)bacterial culture method for monitoring the growth from individual CFUs, using a microscope system to image growing colonies. As the mycobacteria are inoculated on porous aluminum supports (PAO) on agar, the growing colonies can be transferred to different media, e.g. containing antibiotics, while growth monitoring is continued. This procedure avoids subculturing and a new lag phase, this allows rapid drug susceptibility testing of a whole population of microcolonies individually. Growth rate analysis enables detection of mixed susceptible/resistant populations and subtle effects such as partial inhibition of growth (den Hertog et al 2010 PLoS ONE 5(6):e11008.doi:10.1371/journal.pone.0011008).

Results: Using this method, we tested the susceptibility for the antibiotic ethambutol of a blinded panel of 20 Mtb strains. In this study the growth of in total more than 100,000 automatically identified microcolonies were tracked. Classification of 18 strains corresponded to the gold standard and one misclassification was due to contamination. Results were achieved after only 2 days of exposure, and within 9 days after inoculation, which is at least as rapid as standard DST methods for TB, which has a division time of more than 20 hours.

Conclusion: This approach could be applied to drug susceptibility testing, not only in mycobacteria but also bacteria from other genera. Other applications of growth rate monitoring could be in industry e.g. for compound testing. To make the method more accessible and supportive of a wider variety of assays both for research and routine use, we are developing a user friendly system with integrated image analysis software in a consortium of KIT Biomedical Research, MicroDish bv, CCM bv and LumiByte bv, The Netherlands. A first generation research system is expected to be available in 2014.

O126

Integral risk management

J. Prooi

Safecaregroup, Rotterdam

In many organisations there is not sufficient coherence (at all) between the various types of risks. Staff departments have developed their own system e.g. information, quality, finance, security, patient safety, laboer safety (arbo), social safety, fire safety, etc.

This fragmented approach can lead to ineffective and inefficient management of risks Integral Risk Management (IRM) can help to avoid this.

What is IRM?

It is the identification, assesment and prioritization of risks followed by coordinated application of resources to minimize, monitor and control these risks (or the probability and/or impact of unfortunate events).

This process has continuously to be executed which has to result in -a self-learning and self-directing organisation, -in which every leader feels responsable managing the risks in his/her department, -which is aware of the impact of the various risks and their coherence but also of the various options to control including its consequences.

O127

Infection prevention; same goal and together strong!

M.C. Vos

Erasmus MC on behalf of the working- and steeringgroup "Handen uit de Mouwen"

Introduction: Recently, in de region Rijnmond a very special initiative on patient safety has been created. This initiative "Handen uit de Mouwen" or "Roll up your sleeves" is taken by "De Stichting Samenwerkende Rijnmondse Ziekenhuizen, SRZ". SRZ is a cooperation of elf ziekenhuizen en een revalidatiecentrum in de regio Rijnmond. Overall aim of SRZ is to promote the field of cross-institutional issues, the quality of health care in the Rijnmond region and everything connected therewith. The goal is to improve the quality of the hospital care, and the increase of the efficiency of the patients in the Rijnmond region.

The SRZ region aims for being the safest hospital region of Netherlands in close cooperation. Together we are stronger and we can achieve more. The premise is that we support and help each other, not compete. The SRZ has chosen infection prevention as main task, which is why since the end of 2012 the subject hygiene, including dress codes, is on the agenda.

The guidelines for hand hygiene in Dutch hospitals are followed in only 20% of all opportunities observed. This leads to avoidable outbreaks and nosocomial infections,

associated with increased morbidity and mortality, longer hospital stay and a clear demonstrable increase in cost. For the patients concerned and their families, this means a lot of extra " distress and inconvenience" and extra disease burden with a preventable death as the most dramatic outcome.

Methods: The substantive goal is prevention of nosocomial infections and related health impacts and costs by improving compliance with general precautions: no hand and wrist jewellery, no artificial nails / nail polish, long hair up, uniforms worn correctly and hand hygiene cf WHO guidelines (5 times).

Target: The " Roll up your sleeves ' program focuses on the approximately 25,000 health care workers of the 11 SRZ hospitals. These hospitals provide care to the 1.2 million inhabitants of the Rijnmond area and region of rising health care for specific medical problems

We set up a structure program;

1. Master plan of action: agreements that apply to all SRZ hospitals

2. Optional program for each hospital: depending on the initial situation and the results run hospitals complementary interventions.

The objective was: 100% compliance clothing protocol per now and 100% hand hygiene compliance by 1-1-2016.

Strict definition for measuring compliance on dress code and hand hygiene were made. The Australion observation tool, audit form and coding classification sheet was used. Observatios were done by using Ipads.

Results: The program started with two pilot observations in two selected hospitals, the Erasmus MC and the Maasstad Hospital. The results are not available yet.

Conclusions: By cooperation of hospitals, a program can be initiated to join goals, knowledge and implementation on patient safety issues

O128

Challenges and priorities in Southeast Asian medical microbiology

H.F.L. Wertheim

Oxford University, OUCRU Hanoi, Hanoi, Vietnam

Southeast (SE) Asia is very heterogeneous in many aspects and there is no simple answer to the question: What are the challenges and priorities in SE Asian Medical Microbiology?'. The kind of medical microbiology service needed depends on the local setting, as that determines both resources available for providing diagnostics and the infectious disease epidemiology, which informs what diagnostics and services are needed. During this presentation we will assess the current status and developments of medical microbiology in Asia, following the typical job description of a medical microbiologist in the Netherlands. In this abstract a sketch is provided of the heterogeneity that exists in Asia, as background information for this presentation.

SE Asia consists of two geographic regions: mainland Southeast Asia, with Cambodia, Laos, Myanmar, Thailand, and Vietnam; and Maritime Southeast Asia, comprising Brunei, Malaysia, East Timor, Indonesia, Philippines, and Singapore. The climate in SE Asia is mainly tropical with plentiful rainfall (exception: northern Vietnam has a subtropical climate with a cold winter). The increase in species richness or biodiversity that occurs from the poles to the tropics and the high population and livestock densities, impacts the regional infectious disease epidemiology. Also cultural/religious diversity is large within the region with several countries being largely Islamic and others Buddhist, leading for example to different food consumption practices and thus exposure to different pathogens (e.g. no *Streptococcus suis* meningitis in Islamic countries).

More than 60% of the world population lives in Asia, a region with some of the fastest developing economies in the world, but also contains some of the poorest nations. GDPs can vary from 950 USD per capita in 2012 in Myanmar to 50,000 USD in Singapore. Therefore there are huge disparities in resources in this region. Yet, despite tremendous advances, infectious diseases still remain a major burden for the human population in Asia. Of the estimated 2.1 million deaths in children aged less than five years in Southeast Asia in 2010, 47% are attributable to infectious causes. Asia is a global focus of major social and environmental change that may facilitate the emergence of new pathogens. There is a large interest in doing research in Asia, resulting now in huge increases in investment in research in Asia, while this is generally in decline in Western nations.

O129

Novel remedies for deficiencies in medical microbiological services in SE Asia

C. Schultsz

Academic Medical Center, University of Amsterdam, Dept. of Global Health and Dept. of Medical Microbiology, Amsterdam

Whilst economic development and increased investments in health care have allowed for major improvements in health systems, clinical microbiology remains an area for improvement in many countries in SE Asia, as much as in other parts of the world. Quality and accreditation systems have been introduced at primary, secondary and tertiary care levels in both private and public laboratories, but assessment of the quality of diagnostic services remains difficult. In the end, completed paper work cannot guarantee the delivery of high quality

diagnostics in daily practice. Microbiologists in low- and middle-income countries are almost always working as a single head of a laboratory without opportunities to interact with colleagues. Feasible approaches are needed to monitor activities and further develop clinical microbiology including strengthening of professional organization and the provision of networks which allow laboratory professionals to communicate with each other and strengthen their position, in the laboratory, in their health care setting, and at policy levels.

Antimicrobial resistance is a major concern in SE-Asia, driven by uncontrolled usage and over the counter sales of antimicrobial drugs. The concept of antimicrobial stewardship is only viable with the availability of surveillance data, which can inform empirical treatment, as well as a functional microbiology laboratory, which can generate accurate test results for individual patients. However, surveillance of pathogens and their antimicrobial susceptibility requires large investments, in terms of financial-, human- and other resources, which are not available in most countries in SE Asia. Novel approaches to antimicrobial resistance surveillance, which generate timely data relevant for the local setting, should allow for informed empirical therapy. Improved diagnostic capacity, including detection of novel and emerging pathogens, which often emerge in this geographical region, is needed. Allocation of resources during low and high epidemic seasons, based on the local epidemiology of the relevant pathogens, is also required.

Examples of above challenges and innovative approaches to tackle these will be presented.

O130

Acquisition of ESBL- and carbapenemase-producing Enterobacteriaceae in a large prospective cohort of healthy travellers: The carriage of multiresistant bacteria after travel (COMBAT) study

J.M. van Hattem[1], M.S. Arcilla[2], M.C.J. Bootsma[3], P.J. van Genderen[4], A. Goorhuis[1], C. Schultsz[1], E.E. Stobberingh[5], H.A. Verbrugh[2], M.D. de Jong[1], D.C. Melles[2], J. Penders[5]

[1]Academisch Medisch Centrum, Dept. of Medical Microbiology, Amsterdam, [2]Erasmus Medisch Centrum, Dept. of Medical Microbiology, Rotterdam [3]Utrecht University, Mathematics, Utrecht, [4]Havenziekenhuis, Dept. of. tropical medicine and travel medicine, Rotterdam, [5]Maastricht UMC+, Dept. of Medical Microbiology, Maastricht

Background: International travel provides an opportunity for resistant bacteria or plasmids to transfer rapidly between regions. Recent prospective studies reported significant acquisition rates of extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-E) among healthy travellers, whereas acquisition of carbapenemase-producing Enterobacteriaceae (CP-E) was not observed.

Objective: The COMBAT-study aims to study the influence of international travel and travel-associated risk factors on the acquisition, persistence and transmission of ESBL-E and CP-E. Here we present the first results of this study.

Design: A multicenter longitudinal cohort following healthy Dutch travellers prior to intercontinental travel until 12 months after return. Post travel household transmission was also studied.

Methods: Adult volunteers travelling abroad for 1-12 weeks were recruited from November 2012 to November 2013 at travel clinics run by the Academic Medical Center (Amsterdam), Havenziekenhuis (Rotterdam) and Maastricht University Medical Center (Maastricht) in the Netherlands. Faecal samples were collected before and immediately after travel and 1 month after return. Follow-up faecal samples were collected 3, 6 and 12 months after return from travellers (and their non-travelling household members) who acquired ESBL-E and/or CP-E. Questionnaires were collected from all participants at each time-point. Faecal samples were selectively enriched and sub-cultured on chromID ESBL. ESBL phenotype was confirmed by the combination disk diffusion test. Positive post-travel isolates from travellers with negative pre-travel samples are currently analysed for ESBL- and carbapenemase genes with Alere microarray and gene sequencing. In a sub-study 500 travellers were screened post-travel for OXA-48-producing Enterobacteriaceae with chromID OXA-48.

Results: 1961 travellers and 202 non-travelling household members were included. So far, data on ESBL-E and CP-E in pre-and post-travel samples are complete for 1729 travellers. The median age was 50.0 years (range 18-82) and 929 (53,8%) were women. 145 (8,4%) participants carried ESBL-E before travel and were excluded from the analysis. Out of the remaining travellers 'at risk' 547 (34.5%, CI_{95} 32.2-37.0%) acquired ESBL-E during travel (453 *Escherichia coli*, 21 *Klebsiella pneumoniae*, 31 other species, and 42 two or more different species (mostly *E. coli* + *K. pneumoniae*). Acquisition rates were highest for South (73.0%), East (55.4%) and West Asia (48.5%) and lowest for Southern Africa (6.2%). Four travellers acquired CP-E during travel (2 *E. coli* NDM, 1 *E. coli* OXA-48, 1 *K. pneumoniae* OXA-48) without contact with foreign medical-care.

Conclusions: This study found a very high acquisition rate of 34.5% of ESBL-E among Dutch travellers. Asia is the continent with the highest risk. Moreover, this is the first large prospective study that shows travellers can acquire CP-E in the absence of admission to health-care facilities.

Keywords: antimicrobial resistance, Enterobacteriaceae, travel

O131

Targeted resistome detection reveals high acquisition rates of ESBL and quinolone resistance genes in the gut microbiota after international travel

C.J.H. von Wintersdorff[1], J. Penders[1], E.E. Stobberingh[1], A.M.L. Oude Lashof[1], C.J.P.A. Hoebe[2], P.H.M. Savelkoul[1], P.F.G. Wolffs[1]

[1]*Maastricht University Medical Center, Dept. of Medical Microbiology, Maastricht,* [2]*Public Health Service South Limburg, Geleen*

Introduction: International travelling provides an excellent opportunity for the carriage of antibiotic resistant micro-organisms from one location to another. Previous studies have linked traveling to areas with high rates of antimicrobial resistance to the acquisition of extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae, but information on the acquisition of other antimicrobial resistance genes or resistant organisms is largely lacking. We investigated the effect of international travel on the gut resistome by using a targeted (PCR-based) metagenomic approach.

Methods: Pre- and post-travel stool samples of 122 healthy Dutch long-distance travellers were directly submitted to DNA extraction, followed by molecular detection for the presence and relative abundance of *cfxA*, $bla_{\text{CTX-M}}$ -, bla_{NDM}, *tetM, tetQ, ermB, aac(6')-aph(2'), qnrA, qnrB* and *qnrS* genes.

Results: Our results confirm high acquisition rates of the extended-spectrum beta-lactamase encoding gene $bla_{\text{CTX-M}}$, as prevalence rose from 9.0% pre-travel to 33.6% post-travel ($p < 0.001$). However, additionally, the prevalence of quinolone resistance encoding genes *qnrB* and *qnrS* rose from 6.6% and 8.2% pre-travel to 36.9% and 55.7% post-travel respectively (both $p < 0.001$). Travel to South-East Asia and the Indian subcontinent was associated with the highest acquisition rates of *qnrS* and both $bla_{\text{CTX-M}}$ and *qnrS* respectively. Associations between the acquisitions of the $bla_{\text{CTX-M}}$ and *qnr* genes were investigated, but showed that the acquisition of a $bla_{\text{CTX-M}}$ gene was not associated to that of a *qnrB* ($p = 0.305$) or *qnrS* gene ($p = 0.080$).

Conclusion: 1) Significant increases in the prevalence of ESBL and quinolone resistance encoding genes $bla_{\text{CTX-M}}$ -, *qnrB* and *qnrS* were observed after travelling.

2) Travel to South-East Asia and the Indian subcontinent were the highest risk factors for acquisition of these antibiotic resistance genes.

3) These findings further support the increasing evidence that travellers are aiding in the spread of antimicrobial resistance.

O132

Dientamoeba fragilis in Children in Denmark: Aspects on transmission, epidemiology and management

D. Röser

Staten Serums Institute, Dept. of Microbiology and Infection Control, Sector for Diagnostics and Infection Control, Copenhagen, Denmark

Background: *Dientamoeba fragilis* is a common intestinal protozoan of unresolved clinical significance. Despite its discovery nearly a century ago, research into *D. fragilis* has been relatively scarce. As a consequence, our knowledge of *D. fragilis* is gapped in several key areas, adversely affecting our ability to address the protozoan in the clinical setting. Mode of transmission, risk factors for infection, and treatment efficacy all remain inadequately described. We have approached these three topics in a series of studies, applying molecular diagnostics, epidemiological tools and a randomized controlled treatment trial.

Methods and results: In study 1, we applied molecular diagnostics for *D. fragilis* and *E. vermicularis* (pinworm) on surface-sterilized pinworm eggs, successfully amplifying DNA of both organisms, thereby supporting the nematode egg vector hypothesis for transmission of *D. fragilis*. In study 2 and 3 we examined a cohort of 9,945 Danish patients who had submitted a total of 22,484 faecal samples for investigation for *D. fragilis*. Conducting both a cohort and a case-control analysis, we showed a positive association between *D. fragilis* infection and the following parameters: age (children and parental age adults), gender (parental age females), and prior intake of drugs exclusively used to treat pinworm infection (mebendazole). We also showed a negative association between *D. fragilis* infection and intake of several antimicrobial drug groups, incl. metronidazole, broad spectrum penicillin, fluoroquinolones and macrolides, and with similar trends for all other investigated antimicrobials. In study 4 we conducted a double-blinded, placebo-controlled randomized clinical trial on the efficacy of metronidazole for *D. fragilis* infection in 3-12 year old Danish children with chronic (> 4 weeks) gastrointestinal symptoms. Here, we showed no greater clinical effect of metronidazole than that of placebo, and we also demonstrated a microbiological efficacy that was initially only moderate (2-week eradication rate; 62.5%) and declining rapidly (8-week eradication rate; 24.9%).

Conclusions: We find it likely that *D. fragilis* is transmitted by pinworm eggs, and suggest this as a possible explanation for the generally high prevalence of *D. fragilis*, as compared with other endemic protozoans, which utilize other means of transmission, and also the particularly high prevalence in children, who harbor pinworm most often. We consider the general detrimental effect of antimicrobials on the intestinal flora a likely explanation for the observed negative association with *D. fragilis* infections.

Finally, we find *D. fragilis* an unlikely candidate for routine investigation and treatment in the clinical setting, due to a lack of clinical and microbiological effect of treatment.

O134

Molecular detection of *Dientamoeba fragilis* shows a significantly higher rate in healthy controls than in gastroenteritis cases

B. Mulder[1], L. Bruijnesteijn[2], T.A. Schuurs[3], T. Mank[4], J.F.L. Weel[5], G.J.H.M. Ruijs[6], A.G.M. van der Zanden[7]

[1]LabMicTA, Dept. of Parasitology, Enschede, [2] ISALA Clinics, Dept. of Microbiology, Zwolle, [3] IZORE Microbiology Laboratory, Dept. of Molecular Biology, Leeuwarden, [4]Regional Laboratory, Dept. of Microbiology, Haarlem, [5] IZORE Laboratory, Dept. of Microbiology, Leeuwarden, [6]Isala Clinics, Dept. of Microbiology, Zwolle, [7]LABMICTA/ ZGT, Dept. of Molecular Biology, Hengelo

Molecular detection of *Dientamoeba fragilis* has been introduced in many Dutch laboratories. After this introduction questionable high positivity rates of detection are seen in patients with gastro-enteritic complaints. A case control study to evaluate the application of highly sensitive molecular diagnostics in gastro enteritis (GE) was performed to further elucidate the clinical significance of this finding.

During two years, in four representative regions of the Netherlands, stool samples were collected from patients who visited their general practitioner because of gastrointestinal complaints. Matched control samples were collected from patients without complaints or from family of laboratory technicians. Matching criteria were age group (0-4, 5-20, 21-50 and > 51), month of sample collection, sex and region. All samples were analysed by multiplex real-time PCR.

A total of 2745 materials was collected by the four participating laboratories, comprising of 1543 GE case stool samples and 1202 control samples. We found a significant higher overall rate (39%) of *Dientamoeba fragilis* in controls than in cases (25%) consistently in all age groups. No difference in median Ct value is observed for cases and controls. If the laboratory staff and family are excluded as controls the difference is smaller but still significant. Among laboratory staff prevalence rates of over 60% were observed.

We conclude that a positive finding of *Dientamoeba fragilis* in gastroenteritis patients should be interpreted with caution in view of the higher prevalence in controls. Working in a microbiology laboratory consists a risk factor for *Dientamoeba fragilis* infection. The relevance of genotypes will be investigated in future research in this multicenter initiative.

O135

Case-Control study Gastro Enteritis: The value of molecular detection in diagnosing gastro enteritis.

L.E.S. Bruijnesteijn van Coppenraet[1], G.J.H.M. Ruijs[1], W.A. van der Reijden[2], L. Mulder[3], A.G.M. van der Zanden[3], J.F.L. Weel[4], T.A. Schuurs[4]

[1]Isala, LMMI, Zwolle, [2]Streeklaboratorium Kennemerland, Dept. of Microbiologie, Haarlem, [3]Labmicta, Dept. of Microbiology, Hengelo, [4]Izore, Dept. of Microbiology, Leeuwarden

Following the introduction of molecular techniques for the detection of infectious organisms causing gastroenteritis (GE), questions remained concerning the clinical value of finding a) a very low amount of DNA of bacteria and / or parasites causing GE and b) pathogens of questionable pathogenic repute. Therefore we decided to perform a multiregional study targeting these questions. Stool samples of cases and matched controls were examined for the presence of bacterial and parasitic causes of GE and the clinical value of the (semi)-quantitative results was analysed.

For two years, stool samples were collected in four different regions of the Netherlands from patients visiting their general practitioner because of gastrointestinal complaints. Control samples, matched for age group, gender, season and region, were collected from persons without complaints.

A total of 2745 stool samples were included consisting of 1543 GE case samples and 1202 control samples. All samples were tested for the presence of *Dientamoeba fragilis*, *Giardia lamblia*, *Cryptosporidium parvum/hominis*, *Entamoeba histolytica*, *Campylobacter* spp, *Salmonella* spp, pathogenic *Yersinia enterocolytica*, pathogenic *E. coli*, *Shigella* spp. and toxigenic *Clostridium difficile* using multiplexed real-time PCR, and data from questionnaires was analysed.

Overall, in 1403 stool samples one or more target organisms were detected. *Campylobacter spp.* was significantly more often found in cases as compared to controls, 11 vs 2%. The same was observed for *G. lamblia*, *Salmonella spp*, *C. difficile* and several pathogenic *E. coli* types. By contrast, *D. fragilis* was found more frequently in controls than in cases (39% vs. 25%). Also, a higher occurrence of typical enteropathogenic *E. coli* (tEPEC) and shiga-like toxigenic *E. coli* (STEC) was seen in healthy controls. For *Campylobacter*, *Salmonella* and in some age categories for *Clostridium* and *Cryptosporidium*, average Ct-values were lower in cases than in controls.

The number and the severity of GE complaints was greater in *Campylobacter spp.* and *Salmonella spp.* compared to the other targets.

In conclusion, the findings of this study create a better understanding of molecular diagnostic results in

infectious gastro enteritis and reveal some interesting insights for the debate on clinical relevance of some gastro enteric 'pathogens'.

O137

Staphylococcus aureus vaccines: from failure to promise

F. Bagnoli
Novartis Vaccines & Diagnostics, Siena, Italy

Multidrug resistant *Staphylococcus aureus* strains are emerging and current antibiotics are not efficacious against such strains. Therefore, there is an urgent need to develop vaccines to target this pathogen. However, both active and passive immunization strategies have thus far failed to show efficacy in humans. There are several potential reasons behind the disappointing results of clinical trials, however we believe that just a few of them, which are common to all the trials, determined their downfall. First of all, preclinical results obtained with antigens tested in clinical trials were likely overestimated by vaccine manufacturers. Furthermore, vaccines tested in humans to date, since they all targeted single antigens, were probably disproportionate to the complex pathogenic mechanisms of the bacterium. What is more, the lack of known correlates of protection in humans has severely limited the ability to interpret both preclinical and clinical data.

Herein we present a newly designed vaccine, which targets four conserved antigens with different roles in *S. aureus* pathogenesis. The vaccine was shown to be highly efficacious against a panel of epidemiologically relevant *S. aureus* strains in four mouse models. It gave greater and broader protection than any single antigen tested and appeared to generate functional antibodies. We are now investigating the contribution to protective immunity of new generation adjuvants, which may be critical in augmenting antibody production and steering the T-cell response towards the proper profile of cytokine production.

O138

Novel vaccines based on bacterial outer membrane vesicles

P.A. van der Ley
InTraVacc, Research, Bilthoven

Outer membrane vesicles (OMVs) are released spontaneously during growth by many Gram-negative bacteria. They have natural properties like immunogenicity, self-adjuvation and uptake by immune cells which make them attractive for application as vaccines against pathogenic bacteria. In particular with *Neisseria meningitidis*, they have been investigated extensively for suitability as vaccines, including large-scale clinical testing. An OMV-containing meningococcal vaccine has recently been approved by the EMA. For other pathogens, OMV vaccines are also under investigation, an example being *Bordetella pertussis* where the recent increase in pertussis incidence has underscored the need for next generation vaccines that can induce a broader and more sustained protection than the ones which are currently used. In all cases, genetic engineering of the OMV-producing bacteria can be used to improve their usefulness as vaccines. Such modifications include (i) modifying the lipopolysaccharide (LPS) biosynthesis pathway in order to obtain less endotoxic and reactogenic variants, (ii) overexpression of crucial antigens, (iii) simultaneous expression of multiple antigenic variants, (iv) outer membrane retention of normally secreted antigens, (v) increased OMV production by removing outer membrane anchor proteins, (vi) removal of immune-modulating components which may trigger the wrong type of immune response, (vii) expression of heterologous antigens from other pathogens than the host OMV producing strain. Examples of these approaches will be presented.

O139

An autotransporter display platform for the development of recombinant outer membrane vesicle vaccines

M.H. Daleke-Schermerhorn, C.M. ten Hagen-Jongman, W.S.P. Jong, J. Luirink
Abera Bioscience AB / VU, R&D, Amstelveen

Gram-negative bacteria naturally release outer membrane vesicles (OMVs) from their surface, which due to their immunogenicity are interesting as vaccines. By packaging foreign peptides into OMVs, specific immune responses can be raised towards heterologous antigens in the context of a natural adjuvant. Recent data suggest that antigens exposed at the OMV surface elicit superior immune responses, but thus far, robust methods for targeting heterologous proteins to the vesicle surface are lacking. We recently reported on the successful engineering of the *Escherichia coli* autotransporter hemoglobin protease (Hbp) into a platform for high-density surface display and secretion of heterologous antigens in *Salmonella (*Jong *et al. Microb Cell Fact* 2012). Here we exploit this platform for display of heterologous proteins at the surface of *Salmonella* OMVs, using several mycobacterial and pneumococcal proteins as model antigens.

To allow for high-level production of OMVs, a hypervesiculating derivative of an attenuated *Salmonella enterica* serovarTyphimurium strain was generated. This was achieved by inactivating the *tolR* and *tolA* genes, which code for proteins involved in maintaining stability of the cell envelope. Next, we created Hbp fusion constructs for targeting model antigens to the OMV surface. Autotransporters such as Hbp consist of a secreted

passenger domain and a beta-domain that mediates translocation of the passenger across the bacterial cell envelope. Translational fusions were created between the passenger domain of Hbp and the *Mycobacterium tuberculosis* antigens ESAT-6, Ag85B and Rv2660c, both individually and simultaneously. Additional fusion proteins were created using using *Streptococcus pneumoniae* antigens. These fusion constructs were expressed in the hypervesiculating *Salmonella* strain and OMVs produced by this strain were isolated by high-speed centrifugation. Analysis of the isolated OMVs showed that the mycobacterial and pneumococcal antigens were efficiently displayed at the surface of the OMVs.

In conclusion, we show high-density display of several sizeable *M. tuberculosis* and *S. pneumoniae* antigens separately and simultaneously at the surface of *Salmonella* OMVs. Importantly, this demonstrates the potential of our autotransporter platform for the development of OMV vaccines. *In vitro* and *in vivo* studies are underway to evaluate the immunogenicity and protective efficacy of our vaccine candidates.

O140

Antibody responses against non-covalent cell surface-bound staphylococcal proteins

F. Romero Pastrana[1], J. Neef[1], D. de Graaf[1], D.G.A.M. Koedijk[1], J. de Groot[1], S. Engelmann[2], J.M. van Dijl[1], G. Buist[1]

[1]*UMCG, Dept. of Medical Microbiology, Groningen,* [2]*Universität Greifswald, Institut für Mikrobiologie, Greifswald, Germany*

Introduction: Most candidate targets that have so far been selected for studies on passive or active immunization against *Staphylococcus aureus*are covalently cell wall-bound proteins. Much less attention has been attributed to non-covalently cell wall-associated proteins. Although some of these are retained in the cell wall by specific domains, the wall-binding characteristics for most of these proteins are yet unknown. Immunoproteomic analyses have shown that quite a few of the non-covalently cell wall-bound proteins and also non-predictably wall-bound proteins are immunogenic. These proteins are thus potential targets to combat *S. aureus*infections via active or passive immunization approaches.

Methods: Available literature data combined with computer searches using MEME and BLAST were used to predict the covalently and non-covalently cell wall-bound proteins. Using a proteomics approach several non-covalently wall-bound proteins were identified. These proteins were produced in and isolated from *Lactococcus lactis*as Histidine-tagged fusions and used for cell wall-binding studies and immunogenicity testing.

Results: Different groups of non-covalently cell wall-bound proteins were distinguished. These include proteins with specific wall-binding domains and the 'secretable expanded repertoire adhesive molecules'. The *S. aureus*strains RN4220, SH1000 and Newman were grown and the profiles of the extracellular proteome and the wall proteomes were compared using SDS-PAGE. By extraction of the wall proteins and subsequent rebinding to purified cell wall-fragments, specific non-covalently bound wall proteins from both groups were identified. Heterologous production of these proteins in *L. lactis,*which maintained their biological activity, resulted in non-covalent binding to the cell wall of this bacterium. For most of the proteins also a strong non-covalent binding to different *S. aureus*strains was observed. The group of non-covalently cell wall-bound proteins, containing specific wall-binding domains, has been shown to specifically bind peptidoglycan. Using antibodies directed against the histidine-tag of the fusion proteins, it was demonstrated that these proteins bind to specific regions on the surface of *S. aureus*cells. Using Western blotting analyses and ELISA's with sera from *S. aureus*carriers or heavily colonized patients, potentially relevant immunogenic *S. aureus*surface proteins that are recognized by human antibodies were identified and compared.

Conclusion: This study shows that the groups of non-covalently cell wall-bound staphylococcal proteins are immunogenic and, thus, possible candidates for active or passive immunization.

P001

Surveillance cultures in Intensive Care Units: a nationwide survey on current practice providing future perspectives

J.B.J. Scholte[1], W.N.K.A. van Mook[1], C.F.M. Linssen[2], H.A. van Dessel[1], D.C.J.J. Bergmans[1], P.H.M. Savelkoul[1], P.M.H.J. Roekaerts[1]

[1]*Maastricht UMC, Intensive Care, Maastricht,* [2]*Atrium Medical Center, Dept. of Microbiology, Heerlen*

Introduction: Evidence for obtaining surveillance cultures (SC) in intensive care units (ICUs) is scarce. This study explored SC implementation, underlying motives for obtaining SC and the clinical decision making process based on SC results.

Methods: A questionnaire was distributed to Heads of Department (HOD) and microbiologists of all Intensive Care Departments in the Netherlands. Furthermore, a literature research on all aspects of SC was performed.

Results: In total 75 of 95 (79%) HOD and 38 of the 59 (64%) microbiology laboratories filled out at least one questionnaire. SC were routinely obtained according to 55/75 (73%) HOD and 33/38 (87%) microbiologists. SC were more often obtained in higher level ICUs. Most frequently,

SC were obtained twice weekly (88%) and sampled from trachea (74 and 87%), pharynx (74 and 88%), and rectum (68 and 84%), according to HOD and microbiologists, respectively. Selective digestive decontamination was used in 50% of the ICUs. Major reasons to obtain SC included perceived optimization of treatment of the individual patient (58% and 73%), prevention of multiple drug resistant micro-organisms (28% and 35%), and resistance monitoring (both 27%) according to HOD and microbiologists, respectively. On suspicion of infection of a yet unknown source, micro-organisms identified by SC were generally targeted, while in absence of signs of infection, these micro-organisms were not targeted. A third of HOD targets micro-organisms identified by SC in absence of signs of infection at the sampled site. Microbiologists were more reluctant to target these micro-organisms.

Conclusions: SC implementation is common practice in Dutch ICUs and SC are presumed to optimize individual patients' treatment by targeting identified micro-organisms when infection with unknown origin is suspected. Consensus is lacking on how to interpret SC results when the focus of infection is not at the sampled site. Therefore, SC may sometimes create more upheaval than intelligibility.

P003
Historical Microbiology – a Tool for Education

L.A. Robertson
TU Delft, Biotechnology, Delft

Introduction: It can be difficult to find experiments which catch the imagination of students, avoid the 'cookbook' approach, and yet stay within requirements for biological safety. In Delft, we have developed short projects for use by 1st year students, based on repeating and expanding historical experiments. The project presented here uses Antonie van Leeuwenhoek's famous pepper water experiment and involves an attempt to establish the materials and methods that would deliver his results, and then to examine the microorganisms he might have found.

Methods: Pepper is added to water and allowed to incubate at room temperature over a period of 4 weeks, with weekly checking. The students can choose from black or white pepper, whether it is whole or ground, and the concentrations to be used. They can select from a range of water types including sterile and non-sterile demineralized, tap, rain, snow and canal, depending on availability. For the isolation phase, they must choose from a short list of selective agar types including those for nitrogen fixers, heterotrophs, autotrophs, etc, with or without ground pepper. Analysis is kept simple by using Merck dip sticks for nitrate, nitrite and ammonium and pH paper. Taxonomy is also kept simple by using the API-10S set of physiological tests as well as standard Gram stain, oxidase and catalase tests and phase contrast microscopy.

Results: This practical has now run 8 times, with approximately 250 students. To summarize the results, the white pepper cultures grew more slowly than the black. The composition of the microbial communities depended on the colour and concentration of the pepper. The number and variety of protozoa present was especially dependant on concentration, and they failed to appear in the strongest mixtures. The type of water also influenced the variety of protozoa and the speed of community development.

A suggestion from M.W. Beijerinck that nitrogen fixing bacteria should be present was shown to be correct in nearly all cultures. Notably, some of the black pepper cultures contained extremely large, rod-shaped bacteria. 4 weeks is not long enough for proper isolation and identification of the bacteria.

Conclusion: This experiment is always the most popular in the post-practical evaluation by the students, followed closely by another historical microbiology project repeating M.W. Beijerinck's work with bioluminescent bacteria and oxygen. Students frequently visit their cultures for both experiments on non-practical days.

It is tempting to speculate that the giant bacteria found in certain cultures are the ones drawn by van Leeuwenhoek in his pepper water letter, but of course we can't prove it.

Van Leeuwenhoek provided little or no experimental detail, and the students know that they are doing real research. As well as a copy of van Leeuwenhoek's published letter, they are provided with a summary of the results from previous practicals, and thus gain an overview of the project. They learn to plan experiments to test a hypothesis, the use of enrichments and selective isolation, aseptic work and fundamental physiology and taxonomy.

P004
Lighting samples while using a van Leeuwenhoek microscope

L.A. Robertson
TU Delft, Biotechnology, Delft

Introduction: The quality of the lenses in modern replicas of van Leeuwenhoek microscopes has improved to the point where they can be used to repeat or extend van Leeuwenhoek's experiments. This poster describes the lighting of samples being viewed with such a replica to give better control of the light, and even to allow a primitive form of dark field microscopy.

Methods: The replica microscopes were made by Hans Loncke using methods available to van Leeuwenhoek. One microscope is permanently fitted with a sample containing fossilized diatoms. Living samples came from a pond in the Delft Botanic Garden and an algal bioreactor infected with *Paramecium* sp. in the Dept. of Biotechnology at TU

Delft. Replica 17th century magnifying glasses came from the Mueseum Boerhaave in Leiden.

Results and Conclusion: In his letters, van Leeuwenhoek frequently mentions using a candle to light his work. It has proved possible to do this (see http://tinyurl.com/p2gak30 for a movie clip showing a worm filmed by candlelight). However, the Inventory of the van Leeuwenhoek house made after the death of his daughter, Maria, shows that he owned a number of 'fire glasses', these days more usually called magnifying glasses. This poster shows that including a magnifying glass in the light path dramatically improves the level of lighting provided by a single candle, and that it can be adapted to provide dark field microscopy, something that van Leeuwenhoek sometimes appears to describe. However, using this dark field technique gives a different view, but it is not better than bright field lighting. It is unlikely that, as some have speculated, it was the use of dark field lighting that allowed van Leeuwenhoek to see bacteria. Rather, he simply used a set of microscopes with different magnifications, depending on the sample he wished to observe.

P006

Rapid Identification of respiratory viruses using liquid chromatography-tandem mass spectrometry

J.M.K. Majchrzykiewicz-Koehorst[1], H.T. Trip[1], N.S. Sedee[1], F.C. Coenjaerts[2], E.H. Heikens[2], M.V. Viveen[2], A.H. Hulst[1], J. van der Plas[1], A.P. Paauw[1]

[1]TNO, CBRN Protection, Rijswijk, [2]University Medical Center Utrecht, Dept. of Medical Microbiology, Utrecht

Introduction: Rapid detection and identification of pathogenic viruses is highly important for an adequate and fast response to biological warfare agents as well as in combating infections, natural outbreaks, and epidemics. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a generic method that can rapidly detect and identify microorganisms. Thus far, this technique has not been used for virological diagnostics.

Methods: Cultures of ten human influenza A (8 H1N1 and 2 H3N2), four human metapneumovirus (hMPV) and four human respiratory syncytial virus (hRSV) strains, were tested.The viral sampleswere analysed with LC-MS/MS using a nano-Advance LC system (Bruker) coupled to a Q-TOF mass spectrometer (maXis impact, Bruker).

Results: We investigated the applicability of LC-MS/MS for the identification of viruses from viral cultures. Therefore, a generic, simple, and fast sample preparation procedure compatible with MS was developed. Cultures of ten human influenza A, four hMPV and four hRSV strains, were analyzed by LC-MS/MS, and identified based on peptide mass fingerprinting using the Mascot search algorithm for protein identification. Additionally, two 1:1 mixed viral samples, mixA; H3N2 and hMPV and mixB; H1N1 and hRSV, were tested to determine if it is possible to identified multiple and different viruses from mixed infections.

In all samples the nucleoprotein (NP) of the tested viruses was detected. Based on these NPs, it was possible to identify all the tested hMPV and hRSV strains and the influenza A viruses to the subtype level (H1N1 or H3N2) within less than 5 hours. In addition, the two viruses from both mixtures were identified as well, based on the NP peptides. The sensitivity of LC-MS/MS based assays was approximately 6 x 10^7 genome copies/ml.

Conclusion: With generic, simple, and fast sample preparation method, respiratory viruses were identified using LC-MS/MS. Possibly, in the near future LC-MS/MS – based methods will be commonly used in diagnostics for the rapid identification of viruses from clinical specimen samples.

P008

Anaerobic microbiology in the Soehngen institute

M.S.M. Jetten

Radboudumc, Dept. of Microbiology, Nijmegen

The Soehngen institute of anaerobic microbiology has recently been funded by N.W.O and OCW. It is named after Nicolaas Soehngen who was one of the founding fathers of anaerobic microbiology in the Netherlands. A graduate from Beijerink in Delft who later became professor and Vice chancellor at Wageningen University. With Beijerinck, Kluyver, van Niel and Soehngen, there is a long tradition of excellence in microbiology, and this tradition has been maintained and presently microbiology is still one of the centers of excellence in Dutch Biology. The quality of microbiology in the Netherlands is internationally recognized by no less than 6 ERC AG grants and many other grants and prizes.

In the Soehngen institute, the complementary expertise centered on anaerobic microbiology will be extended in a synergistic approach. In the past the participating groups have already had many collaborations resulting in more in many joint papers of very high quality. Furthermore this joint effort will be extended in educating a new generation of leading, excellent microbiologists with a balanced gender composition and a large international contribution.

In the Soehngen institute for anaerobic microbiology we will engineer microbial ecosystems for our bio economy, improved human health and a better environment in several complementary workpackages.

Human health.The conversion of various polymers into volatile fatty acids (VFA) such as acetate, propionate and butyrate is one of the most important biotransformations catalyzed by anaerobes – not only in natural ecosystems but also in our own intestinal tract! Our intestines are

home to trillions of mainly anaerobic bacteria that not only convert our food, but also produce vitamins and stimulate our immune system. Propionate and butyrate are involved in specific signaling – the bacteria talk to us ! Furthermore we produce bile and mucus that contain sulfate groups that are recycled. We want to study this process as some undesired anaerobes convert the sulfate into H_2S that causes inflammation. Ultimately, anaerobic bacteria such as Akkermansia, may have potential as therapeutic bacteria that contribute to treating metabolic diseases such as type 2 diabetes and obesitas. Transplantation studies have already shown that microbiota can treat some diseases, such as recurrent Clostridium difficile infections, more efficiently that antibiotics.

Environment. To better understand the biogeochemical cycles we will have joint expeditions to alkaline soda lakes and marine ecosystems. We will bring the most promising samples to the laboratory to enrich new methane producing and oxidizing anaerobes. We will identify new lipid biomarkers for tracing these organisms. We will use high through put sequencing to unravel their genomic potential. In this way we will discover new enzymes and organisms for application in biofuel production and waste treatment.

Bio-based economy. Finally we will search for the microbial potential to produce valuable chemicals. Non-biodegradable waste can be gasified to syngas (H2/CO) after which anaerobes can produce organic acids. Biodegradable resources can be directly converted by the anaerobes. The organic acids can be used to synthesize new building blocks for the biobased society.

P009

Clinical case of an Ambler class D OXA-48-type Beta-lactamase in a *Klebsiella pneumonia* strain in a Dutch Hospital in 2007

I. Willemsen[1], A. van Gent[1], C. Verhulst[1], K. Verduin[1], J. Kluytmans[2]

[1]*Amphia ziekenhuis, Laboratory for microbiology and infection prevention, Breda,* [2]*VU University Medical Center, Laboratory for Microbiology and Infectious Diseases, Amsterdam*

The class D -lactamase OXA-48 gene was firstly identified in a *Klebsiella pneumoniae* isolate in Turkey in 2004. Since then, several other OXA-48-producing isolates of various Enterobacteriaceae have been reported all over the world. This case report describes what is believed to be the first carbapenem-resistant OXA-48 producing *K. pneumoniae* and *Escherichia coli* in the Netherlands.

In August 2006, a 63-year-old woman from Turkish origin, was seen in the emergency room of our hospital with abdominal pain, absence of defecation and weight loss of 8 kg in 2 months. She reported having been admitted in a Turkish hospital recently for a short duration (< 24 hours) during her holiday. She was diagnosed as having a large B-cell lymphoma of the stomach, for which chemotherapy was started.Multiple admissions had occurred since then and abdominal pain in the right upper quadrant was reported frequently. December 12th 2006, an abscess in the wall of the stomach was diagnosed. In January the patient developed an second abscess in the liver and a drain was placed. Fluid from her liver drain grew *K. pneumoniae*, with a MIC for meropenem of 1mg/L and for imipenem of 8mg/L. Four months later, rectal swabs also revealed an ESBL positive *E. coli* with a MIC for meropenem of 8mg/L and for imipenem of 8mg/L. At that time, the isolates were not recognised as OXA-48 producers because detection guidelines for carbapenemases were lacking.

The isolates were included in a historical collection, used for validation purposes, and by coincidence, in 2013, the Check-MDR CT103 microarray (Check-Points) revealed the presence of *bla* in both *E. coli* and *K. pneumoniae*. This case would have been recognised as a possible carbapenemase producer using the current Dutch National guideline for the detection of resistant micro-organisms; the MIC screening breakpoint for meropenem has been set at > 0.25 mg/L for all Enterobacteriaceae, and for Imipenem at > 1 mg/L for *E. coli*, *Klebsiella spp.*, *Salmonella spp.*, *Enterobacter spp.* and *Citrobacter spp.*

The first report of an OXA-48 producer, in The Netherlands, dated to 2010. This case report shows that OXA-48 carbapenemase can easily be missed and that it probably was present in the Netherlands earlier than currently known.The relation between our patient and her visit to Turkey is likely. This case illustrates the importance of good guidelines for the detection of carbapenem resistance, also at low MICs.

P010

Climate factors as important determinants of dengue incidence in Curaçao

T.D.K. Thai[1], M. Limper[2], I. Gerstenbluth[3], A.D.M.E. Osterhaus[4], A.J. Duits[5], E.C.M. van Gorp[2]

[1]*Erasmus Medisch Centrum, Dept. of Medical Microbiology and Infectious Diseases, Rotterdam,* [2]*Erasmus University Medical Center, Dept. of Internal Medicine, Rotterdam,* [3]*Medical & Public Health Service (GGD) of Curaçao, Epidemiology and Research Unit, Curaçao,* [4]*Erasmus University Medical Center, Dept. of Viroscience, Rotterdam,* [5]*Red Cross Blood Bank Foundation Curaçao, Curaçao*

Background: Macro- and microclimate may have variable impact on dengue incidence in different settings. We estimated the short-term impact and delayed effects of climate variables on dengue morbidity in Curaao.

Methods: Monthly dengue incidence data from 1999-2009 were included to estimate the short-term influences of climate variables by employing wavelet analysis, generalized additive models (GAM) and distributed lag non-linear models (DLNM) on rainfall, temperature, relative humidity in relation to dengue incidence.
Findings: Dengue incidence showed a significant irregular 4-year multi-annual cycle associated with climate variables. Based on GAM, temperature showed a U-shape, while humidity and rainfall exhibited a dome-shaped association, suggesting that deviation from mean temperature increases, and deviation from mean humidity and rainfall decreases dengue incidence, respectively. The cumulative RR for a 10-mm increase in monthly rainfall (up to 350 mm) with a 9-month maximum lag was 13.1% (95% CI: 10.3-16.8%) based on DLNM. Rainfall was associated with an immediate increase in dengue incidence of 4.1% (95% CI: 2.2-8.1%) after a 10 mm increase, with a maximum increase of 6.5% (95% CI: 3.2-10.0%) after 1.5 month lag. A 1 C decrease of mean temperature was associated with a RR of 17.4% (95% CI: 11.2-27.0%); the effect was inversed for a 1 C increase of mean temperature (RR = 0.457, 95% CI: 0.278-0.752).
Interpretation: Climate variables are important determinants of dengue incidence and provide insight into its short-term effects. An increase in mean temperature was associated with lower dengue incidence, whereas lower temperatures were associated with higher dengue incidence.

P011

The adult nasopharyngeal microbiome as a determinant of pneumococcal acquisition

A.J.H. Cremers[1], A.L. Zomer[1], J.F. Gritzfeld[2], S.A.F.T. Hijum[1], J.R. Shak[3], K.P. Klugman[3], J. Boekhorst[4], H. Timmerman[4], M.I. de Jonge[1], S.B. Gordon[2], P.W.M. Hermans[1]
[1]Radboudumc, Laboratory of Pediatric Infectious Diseases, Nijmegen, [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom, [3]Emory University, Atlanta, USA, [4]NIZO food research B.V., Ede

Several cohort studies have indicated associations between *S. pneumoniae* and other microbes in the nasopharynx. To study causal relationships between the nasopharyngeal microbiome and pneumococcal carriage, we employed an experimental human pneumococcal carriage model. Healthy adult volunteers were assessed for pneumococcal carriage by culture of nasal wash samples (NWS). Those without natural pneumococcal carriage received an intranasal pneumococcal inoculation with serotype 6B or 23F. The composition of the nasopharyngeal microbiome was longitudinally studied by 16S rDNA pyrosequencing on NWS collected before and after challenge. Among 40 selected volunteers 10 were natural carriers and 30 were experimentally challenged. At baseline, five distinct nasopharyngeal microbiome profiles were identified. The phylogenetic distance between microbiomes of natural pneumococcal carriers was particularly large compared to non-carriers. A more diverse microbiome prior to inoculation was associated with the establishment of pneumococcal carriage. Perturbation of microbiome diversity upon pneumococcal challenge was strain specific. Shifts in microbiome profile occurred after pneumococcal exposure, and those volunteers who acquired carriage more often diverted from their original profile. *S. pneumoniae* was little prominent in the microbiome of pneumococcal carriers. In conclusion, pneumococcal acquisition in healthy adults is more likely to occur in a diverse microbiome and appears to promote microbial heterogeneity.

P012

Rapid detection and semi-quantification of IgG-accessible *Staphylococcus aureus* surface-associated antigens using a multiplex competitive Luminex assay

S. Hansenova Manaskova[1], F.J. Bikker[2], E.C.I. Veerman[2], A. van Belkum[1], W.J.B. van Wamel[1]
[1]Erasmus MC, Dept. of Medical Microbiology and Infectious Diseases, Rotterdam, [2]Academic Centre for Dentistry Amsterdam, Dept. of Oral Biochemistry, Amsterdam

Introduction: The surface characterization of *Staphylococcus aureus* is currently labor intensive and time consuming. Therefore, we developed a novel method for the rapid yet comprehensive characterization of *S. aureus* cell-surface-associated proteins and carbohydrates, based on a competitive Luminex assay.
Methods: We used eleven *S. aureus* bacteraemia isolates and 8325-4 *S. aureus* WT strain with its isogenic *clfB*, *spa* and *srtA* KO variants. *S. aureus* bacteria or culture supernatant dilutions were exposed to human pooled serum. *S. aureus* specific IgGs from the serum were captured to the IgG-accessible staphylococcal antigens present on the bacterial surface, or in supernatant, in a dose dependent manner. The remaining non-captured IgGs were then quantified by incubation with Luminex beads to which the individual recombinant *S. aureus* surface proteins and carbohydrates were covalently linked. Using the competitive Luminex assay, log dose-response curves of specific antigens were generated for different growth conditions/phases and bacterial strains. In each assay, exponentially grown WT 8325-4 *S. aureus* (10^8 bacteria) were included as an internal reference control. The relative signal for the antigens tested in the test samples was calculated by comparing the signals of the test samples with that of the reference control (normalized to 1 unit per bacterium).

Results: The signals obtained are inversely proportional to the antigen concentration measured, and binding patterns were dependent on the particular *S. aureus* isolate used, growth conditions and growth phase. Since we did not find significant differences between the 8325-4 WT and its isogenic *spa* KO *S. aureus* strain concerning cell-surface-associated proteins, we concluded that our approach is valid. The detection of cell wall teichoic acid (WTA) and peptidoglycan (PG) carbohydrate structures increased significantly when bacteria entered the stationary growth phase. We observed almost 10 times more WTA in the stationary growth phase in comparison to the same bacterial strains from the exponential phase, this increase was significant ($p = 0.0028$; $p < 0.0001$; $p < 0.0001$) in three strains. The signal for ClfA decreased on average 7-fold in the stationary growth phase in three strains tested ($p = 0.0082$; $p < 0.0001$; $p = 0.0004$) in comparison to bacteria from the exponential growth phase. Furthermore, we detected an almost 20-fold increase of iron surface determinant A (IsdA) ($p = 0.0039$) under iron limiting conditions in comparison to iron rich conditions. Comparing a *srtA* KO strain with the WT strain did not show relevant differences. An SDS treatment of these two strains resulted in significant reduced signals for ClfA ($p = 0.0003$), ClfB ($p = 0.0003$) and IsdA ($p < 0.0001$) on *srtA S. aureus* KO strain in comparison to WT strain.
Conclusions/Discussion: Multiplex competitive Luminex assay is a suitable method for the rapid and simultaneous determination of known *S. aureus* IgG-accessible cell-surface-associated proteins and carbohydrates in various growth phases and growth conditions in different *S. aureus* strains. We propose that the sortaseA-dependent cell-surface-associated proteins of the *S. aureus srtA* KO strain can be non-covalently associated to the surface. The competitive Luminex assay is surface Protein A independent.

P013
Evaluation of a FRET-peptide Substrate to Predict Virulence in *Pseudomonas aeruginosa*

W.E. Kaman, N. El Arkoubi-El Arkoubi, S. Roffel, H.P. Endtz, A. van Belkum, F.J. Bikker J.P. Hays
Erasmus MC, Dept. of Medical Microbiology and Infectious Diseases, Rotterdam

Pseudomonas aeruginosa produces a number of proteases that are associated with virulence and disease progression. A substrate able to detect *P. aeruginosa*-specific proteolytic activity could help to rapidly alert clinicians to the virulence potential of individual *P. aeruginosa* strains. For this purpose we designed a set of *P. aeruginosa*-specific fluorogenic substrates, comprising fluorescence resonance energy transfer (FRET)-labeled peptides, and evaluated their applicability to *P. aeruginosa* virulence in a range of clinical isolates. A FRET-peptide comprising three glycines (3xGly) was found to be specific for the detection of *P. aeruginosa* proteases. Further screening of 97 *P. aeruginosa* clinical isolates showed a wide variation in 3xGly cleavage activity. The absence of 3xGly degradation by a *lasI* knock out strain indicated that 3xGly cleavage by *P. aeruginosa* could be quorum sensing (QS)-related, a hypothesis strengthened by the observation of a strong correlation between 3xGly cleavage, LasA staphylolytic activity and pyocyanin production. Additionally, isolates able to cleave 3xGly were more susceptible to the QS inhibiting antibiotic azithromycin (AZM). In conclusion, we designed and evaluated a 3xGly substrate possibly useful as a simple tool to predict virulence and AZM susceptibility.

P014
Galactomannan present in blood products before 2012

M. Knoester[1], D. de Korte[2], E.J. Kuijper[1]
[1]*Leiden University Medical Center, Dept. of Medical Microbiology, Leiden,* [2]*Sanquin Blood Bank, Research and Development, Amsterdam*

Introduction: In 2012, Martin-Rabadan *et al.* showed that two solutions used in blood and platelet donations of the firm Fresenius Kabi AG (Bad Homburg, Germany) contained the *Aspergillus* antigen galactomannan (GM). This led to false-positive GM results in patients receiving blood products prepared with these solutions. In response to this publication, Fresenius Kabi changed suppliers for one of the ingredients and now guarantees GM-free products. We investigated if blood products used in our tertiary care hospital containing solutions of Fresenius Kabi have been GM positive in the past, and are now GM negative.
Methods: GM was tested with the Platelia Aspergillus enzyme immunoassay (Bio-Rad Laboratories B.V., Veenendaal, the Netherlands), using a GM optical density index (GM-ODI) of 0.5 as a cutoff point.The blood product solutions that were tested were the anticoagulant citrate-phosphate-dextrose (CPD) and Composol platelet additive solution of Fresenius Kabi B.V. (Zeist, the Netherlands). Both were tested undiluted: one sample from a batch before the supplier change (2012) and one sample after. The blood products from Sanquin Blood Bank that were tested from before 2012 were: supernatant from thrombocyte concentrates preserved in plasma containing CPD ($n = 3$) and supernatant from thrombocyte concentrates preserved in Composol ($n = 2$). Plasma from whole blood donations ($n = 3$) was tested as a CPD containing blood product after 2012. No Composol containing blood product was tested after 2012 as Composol had only been used for experimental use and Sanquin never supplied platelet concentrates in Composol to the hospitals. As negative controls, plasma samples from apheresis were used ($n = 3$). In this product

tri-sodium-citrate is used as an anticoagulant instead of CPD. All tests were done in duplicate.

Results: All products containing CPD were highly GM positive before 2012 (n = 4, GM-ODI > 5.5) and negative after the supplier change (n = 4, GM-ODI = 0.2). All products containing Composol were highly GM positive before 2012 (n = 3, GM-ODI> 7). The sample of pure Composol from a recent batch was found GM negative (n = 1, GM-ODI < 0.5). The negative control samples were GM negative (n = 3, GM-ODI < 0.2).

Conclusion: Up to 2012, blood products supplied by Sanquin were GM positive due to the GM containing anticoagulant CPD of Fresenius Kabi. This may have induced false-positive patients' serum sample results. A change of suppliers for one of the ingredients of CPD and Composol introduced in 2012 resulted in GM negative solutions and blood transfusion products.

Reference

- Martin-Rabadan P, et al. False-positive Aspergillus Antigenemia Due to Blood Product Conditioning Fluids. CID 2012;55:e22-7.

P015

Mycoplasma genitalium more prevalent than Neisseria gonorrhoeae and *Trichomonas vaginalis* when routinely tested in women

D.R. Petermann Smits, R. Nijhuis, N. van Maarseveen, A.A. van Zwet

Rijnstate Hospital, Dept. of Medical Microbiology, Velp

Introduction: *Mycoplasma genitalium* (MG) is an emerging sexually transmitted disease (STD) and in contrast to *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG), it is usually not routinely tested. It is responsible for non-gonoccocal urethritis (NGU) in men, and there is an association with cervicitis, urethritis, possibly pelvic inflammatory disease (PID) and infertility in women.

To gain more insight in the prevalence of MG infection in our region, we routinely tested for MG in all women who were being tested for a STD. Here we describe the prevalence of MG compared to the prevalence of CT, NG and *Trichomonas vaginalis* (TV). Furthermore, since the detection of CT infection in non-invasive samples (urine) is sensitive, but less suitable compared to invasive samples (cervical or vaginal), we speculated whether this could also hold true for MG on the basis of Ct-values.

Methods: From September 16, 2013 until December 11, 2013, a total of 2449 samples were routinely tested in women for CT, NG, TV, and MG. DNA extraction for all samples was performed using the Abbott *m*Sample Preparation System*DNA* on the Abbott *m2000sp* system. For the detection of CT and NG the Abbott RealTime CT/NG assay was used. For the detection of TV and MG an internally-controlled in-house multiplex real-time PCR assay was used.

The different sample types received were urine, and swabs obtained from urethra, fluor, portio, vagina or cervix. Cervical swabs were sometimes provided in combination with urethral swabs. To determine whether there was a difference between the Ct-values of different sample types, we conducted a two-tailed unpaired T-test.

Results: Out of the 2449 samples tested, 147 were positive for CT (6.0%), 70 for MG (2.9%), 26 for TV (1.1%) and 17 for NG (0.7%), coming from 141, 68, 25 and 17 different women respectively. Notably, 14 out of 70 positive MG samples, were also positive for CT (12) or TV (2).

When comparing the Ct-values according to the site of sampling, we found a significant difference between invasive samples (urethra, fluor, portio, vagina or cervix) and non-invasive samples (urine), p = 0.009. The median Ct-values for invasive vs non-invasive samples were 32.66 and 36.39 respectively (range 25.58 - 39.13 and 29.3 - 39.3 respectively).

Conclusions: Our study shows a prevalence of 2.9% for MG within women being tested for STDs in our region. This prevalence is higher than the prevalence of NG and TV, which are more often routinely tested in combination with CT. This shows that testing for MG infection as part of a STD screening, might be of higher importance than considered until now. Furthermore, we assessed the different sample types for their MG Ct-values. Based on Ct-values, it seems that invasive samples have a better diagnostic value compared to non-invasive samples for the detection of MG.

P016

Interferon-gamma adjunctive immunotherapy in invasive fungal infections: a case series

M.S. Gresnigt[1], J. Leentjens[1], C.E. Delsing[1], F. Allantaz Frager[2], M. Kox[1], G. Monneret[2], F. Venet[2], C.P. Bleeker-Rovers[1], F.L. van de Veerdonk[1], P. Pickkers[1], A. Pachot[2], B.J. Kullberg[1], M.G. Netea[1]

[1]*Radboudumc, Dept. of Internal Medicine, Nijmegen,* [2]*Hôpital Edouard Herriot, Joint Unit « Sepsis » Hospices Civils de Lyon-Biomérieux, Lyon, France*

Introduction: The incidence of fungal infections is increasing at an alarming rate, despite development of new classes of antifungal agents. Invasive fungal infections remain associated with unacceptable high mortality rates and represent a major cause of death worldwide. The evolution of mechanisms for significant resistance to the current antifungal drugs further emphasizes the need for novel approaches to treat invasive fungal infections. As invasive fungal infections are most commonly observed in individuals with immune defects or dysfunction and the number of immunocompromised patients is steadily

increasing. Adjunctive immunotherapy, to improve anti-fungal host defense, is an attractive strategy to improve the outcome of patients with disseminated fungal infections.
Methods: In a case series we included eight patients with severe invasive *Candida* and/or *Aspergillus* infections who were treated with recombinant interferon-gamma(IFN?) for 2 weeks. Additionally, 3 other patients with invasive *Candida* infections were treated with placebo medication. The study medication was administered next to standard antifungal treatments. Blood was drawn at several timepoints during the course of treatment to measure the *ex-vivo* responsiveness of to fungal stimulation, HLA-DR expression and lymphocyte subsets.
Results: Treatment with IFN? significantly boosted *ex-vivo* IL-1 and TNFa responses to *C. albicans* and LPS at the first days after initiation of treatment. Additionally, an increase in the capacity to induce T-cell cytokines IL-17 and IL-22 was observed after initiation of IFN? therapy. Treatment with IFN? also modulated the leukocyte subsets in the peripheral blood. We were unable to find significant changes in HLA-DR expression which might be explained by the fact that most of the patients were not immunoparalysed. However, patients that demonstrated decreased HLA-DR upon inclusion showed a trend towards increasing HLA-DR expression on monocytes after IFN? treatment.
Conclusion: Collectively these data provide a first immunological proof-of-principle that adjunctive immunotherapy with IFNg enhances anti-fungal immunity and therefore could represent a promising intervention to improve outcome in patients presenting with fungal infections, but larger trials are required to assess the impact of IFN-? treatment on clinical outcome. Biomarkers of impaired anti-fungal immunity should be further investigated in order to identify patients who will benefit most from immunostimulatory therapy.

P017

Virological and clinical differences between mumps genotype G strains

S. Gouma[1], D. Gijselaar[1], J. Cremer[1], H. Boot[1], S. Hahné[1], M. Koopmans[2], R. van Binnendijk[1]
[1]*RIVM, Centre for Infectious Disease Research, Diagnostics and Screening (IDS), Bilthoven,* [2]*Erasmus MC, Dept. of Viroscience, Rotterdam*

Introduction: Phylogenetic analysis has shown that during the recent mumps outbreak in the Netherlands (September 2009 - August 2012) the majority of mumps patients were infected with genotype G (98.51%). In the course of the outbreak, two large clusters within genotype G were distinguished based on sequencing results of multiple genes, including the gene encoding the small hydrophobic (SH) protein. The first variant MuVs/Delft.NLD/03.10 (variant 1) was predominant during the first outbreak season (2010), whereas variant MuVs/Scheemda.NLD/12.10 (variant 2) emerged later and became dominant during the last two outbreak seasons (2011/2012). Because the predominance of variant 2 could not be explained by epidemiological factors, we have compared virological and clinical data between patients with mumps variants 1 and 2 to find possible explanations for the predominance of variant 2 after the first outbreak season.
Methods: Samples from 872 patients infected with mumps virus between 1 September 2009 and 31 August 2012 sent to the National Institute for Public Health and the Environment (RIVM) were used for analysis. Viral RNA was detected quantitatively in saliva samples obtained within six days after disease onset (n = 238) and qualitatively in urine specimens (n = 433), using real-time PCR. For mumps virus genotyping, the SH gene (316 bp) was sequenced. ANOVA was used for statistical analysis.
Results: Salivary viral loads in patients infected with mumps variant 2 declined significantly slower within the first six days after onset of parotitis as compared to viral loads in persons infected with variant 1 ($p = 0.047$). This difference was irrespective of age and vaccination status. For patients infected with variant 2, viral shedding was also more frequently detected in urine specimens (52.2%) as compared to variant 1 (35.8%, $p = 0.014$). Additionally, salivary viral loads were higher in patients with mumps virus detectable in their urine ($p < 0.001$). These results indicate that variant 2 is more viremic than variant 1 and causes a wider spread of mumps virus in vaccinated persons. In line with these findings, more orchitis cases were reported among males infected with variant 2 as compared to males infected with variant 1 (9.8% versus 3.3%, $p = 0.046$). Detection of mumps virus in urine appeared to be a strong predictor for orchitis, since all urine samples available from orchitis patients were tested PCR positive for mumps virus (n = 24).
Conclusion: Patients infected with mumps genotype G variant 2 shed mumps virus for a longer period via their saliva, had more often mumps virus detectable in their urine and had a higher risk for orchitis. These findings suggest that variant 2 might have an advantage in viral transmission.

P018

Report of a 5-month-old feverish child with petechiae caused by *Neisseria macacae* bacteraemia

M.A. Schouten[1], F. Suwandy[1], M. Quaak[1], F.A.G. Reubsaet[2]
[1]*Gelderse Vallei Hospital, Dept. of Medical Microbiology, Bennekom,* [2]*RIVM, Diagnostic Laboratory for Bacteriology, Bilthoven*

Parents of a 5-month-old boy observed a reddish rash on the legs. After three days he developed 39.3 degrees Celsius

fever. Meningococcal sepsis was highly suspected and the consulted general practitioner sent the child immediately to the Emergency Room.A crying infant was seen with nose flaring and a tachypnea with normal capillary refill and pulse. Body temperature was 39.5 degrees Celsius. Several petechiae and hematomas where diffusely distributed over the legs without signs of meningeal irritation. Treatment was started with i.v. cetriaxone given the suspicion of meningitis or meningococcal sepsis. Blood test showed leucocytosis (13.5 x 109/L) and a slightly elevated C-reactive protein (CRP) level (10 mg/L).Within 48 hours, the patient became afebrile and the petechiae disappeared. Blood tests on the second day showed further elevation of CRP to 62 mg/L, but the infant had no clinical signs of a serious infection anymore. A viral infection was suspected. Lumbar punctures failed so meningitis could not be excluded and treatment with i.v. ceftriaxone was continued.Blood culture showed gram negative diplococci 3 days after venapuncture. The strain was determined as Neisseria macacae using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS, Bruker, Germany). Identification was confirmed using 16S rRNA gene sequence analysis performed by the National Institute for Public Health and Environment (RIVM, The Netherlands). Disk diffusion (Oxoid, United Kingdom) showed susceptibility to ceftriaxone but resistance to penicillin and rifampicin. Neisseria macacae had never before been isolated in our laboratory, nor has there been a publication in literature of Neisseria macacae as a human isolate.This species has only been isolated from oropharynges of Rhesus monkeys. Neisseria macacae is genetically related to Neisseria sicca and Neisseria mucosa, human commensals seldom leading to infections. This lack of pathogenicity might explain why the infection in our patient showed a relatively mild course. Treatment with ceftriaxone i.v. could even be discontinued after 5 days and the patient was discharged from the hospital in good clinical condition. During a follow-up of 5 months, the patient did not have a new episode of fever or petechiae. Since Neisseria macacae thus far only has been isolated from monkeys we tried to find an epidemiological link between our patient and monkeys. Parents recollected their son had been bottle fed one month earlier by a female friend who works as a guide in a monkey zoo. The parents and other relatives and friends who had been in contact with the patient since his birth had no recent contact with monkeys in the Netherlands nor abroad.The zoo guide was screened for N. macacae carriership; a throat swab unfortunately showed no growth of N. macacae. This does not exclude this guide as the epidemiological link as we know that carriership can be transient. Given the fact that there could not be found another plausible explanation we believe our patient contracted the N. macacae strain from the zoo guide. This is to our knowledge the first report of Neisseria macacae as a possible zoonosis.

P019

Interferon 1 pathway component MDA5 (IFIH1) is highly upregulated after Candida stimulation and genetic variants are associated with systemic Candida infections

H.H.M. Jaeger, R. van der Lee, J. Cheng, T.S. Plantinga, S. Smeekens, M. Oosting, W. Barchet, V. Kumar, C. Wijmenga, F. van de Veerdonk, M. Huynen, B.J. Kullberg, J. Perfect, L. Joosten, M. Johnson, M.G. Netea
Radboudumc, Dept. of Internal Medicine, Nijmegen

Background and aim: The dimorphic and commensal fungus *Candida albicans* is found in about 80% of healthy individuals without causing any symptoms or signs of discomfort. Nevertheless, when the host immune system is diminished, *Candida* species in generaland *Candida albicans* in particular can cause severe infections such as systemic candidiasis. Although environmental factors have been associated with an increased susceptibility to systemic *Candida* infections, genetic factors are also believed to play an important role for the susceptibility and/or severity of the infection. We initiated a complementary transcriptomic and genetic approach to assess genes important for susceptibility to *Candida* infections.

Results: By assessing microarrays of macrophages stimulated with *Candida*, stimulation of a specific MDA5 pathway seemed to be induced by the fungus. In line with a role of MDA5 for antifungal defense, we identified two coding single nucleotide polymorphism (rs3747517, rs1990760) in this gene that were significantly associated with systemic blood infections with *C. albicans*. Furthermore these SNPs resulted in a upregulated cytokine response of IL-10 and in a reduced cytokine response of the anti-fungal cytokine IL-17. Additional in vitro studies have shown that MDA5 expression is defective in immune cells from patients suffering from chronic mucocutaneous infections (CMC). In an IFIH1 (MDA5) knock out mouse model we show that the cytokine response after *Candida* stimulation was specifically reduced compared to control mice.

Conclusions: Taken together, these observations point out towards a major role of MDA5 within the type I IFN pathway in the anti-*Candida* response. Genetic variations in this gene most likely lead to imbalances in the cytokine response against this fungus. These new insights may lead to a better understanding of the of the immune response against *C. albicans*.

P020

Development of a multiplex qPCR for the detection of atypical pneumonia using the automated BD MAX system

I.O.M. op den Buijs, R.T.J.M. Roymans, J.H.B. van de Bovenkamp
Stichting PAMM, Dept. of Medical Microbiology, Veldhoven

Introduction: A multiplex qPCR was developed for the detection of pneumonia causing bacteria: *Legionella pneumophila* (Lpn), *Mycoplasma pneumoniae* (Mpn) and *Chlamydophila pneumoniae* (Cpn) in respiratory specimens such as BAL and sputum on the BD MAX™ system (Becton, Dickinson and Company; BD). The BD-MAX™ is a fully automated molecular platform for the extraction of nucleic acids from various sample types followed by qPCR.
Methods: A multiplex qPCR was designed for the detection of Lpn, Mpn and Cpn. The analytical limit of detection (LoD) was estimated by testing a dilution of the target sequences cloned into a pUC57 plasmid. Each concentration was tested in triplicates using an input volume of 500l. The LoD was defined as the lowest concentration at which 100% of all replicates tested positive. The quality of the multiplex assay was confirmed by testing the MP.CP2013 and LPDNA2013 panels from the Quality Control for Molecular Diagnostics (QCMD). This panel consist of specimens with various concentrations of Lpn, Mpn or Cpn and are treated similar as clinical samples. Finally, to validate the assay, a retrospective clinical evaluation was performed. For this evaluation specimens were pre-treated using proteinase K. Consequently, DNA was extracted using the ExK DNA-1 extraction kit (BD) and amplification was performed using the BD MMK (SPC) mastermix (BD). Results were compared to our in-house reference method (EasyMAG, Biomerieux; ABI7500fast system, Life Technologies).
Results: Analytical sensitivity for Lpn, Mpn and Cpn were determined at respectively 4, 14 and 5 copies per PCR. In the QCMD CP.MP13 panel 4 specimens were positive for Cpn, and 2 specimens for Mpn. For Cpn 100% of the specimens were detected in concordance to the QCMD final report. Two educational Mpn positive samples were not detected in this multiplex assay. Nevertheless, all core samples were detected correctly as stated in the QCMD final report. For the QCMD LP13, 1 educational specimen had no Ct-value for the internal control (IC), therefore this sample was unresolved in the BD MAX™. Due to insufficient volume this specimen could not be retested. Furthermore, 6 out of 10 samples were detected positive for Lpn resulting in a 100% score for all core samples. During the retrospective clinical evaluation a total of 30 respiratory specimens were tested, of those specimens 5 (16%) were positive for Lpn, 3 (10%) were positive for Mpn and 2 (6%) were positive for Cpn. Furthermore, for 2 samples no Ct-value was found for the IC and could therefore not be used in the comparison.
Conclusion: A sensitive method for the detection of atypical pneumonia has been developed. The ease of use of the fully automated BD MAX system makes this assay applicable in a wide range of microbiological laboratories.

P021

C. DIFF QUIK CHEK COMPLETE® for Confirmation of Toxin Production by *Clostridium difficile* Isolates

M. Scholing, L. Thiel, G.B. Linde
GGD Amsterdam, Streeklaboratorium, Amsterdam

Introduction: Clostridium difficile associated disease (CDAD) is an urgent threat to public health. Reliable rapid toxin detection on stool samples and C. *difficile* isolates is necessary to control outbreaks. Toxogenic culture performed on unformed stool specimen is the gold standard for diagnosing *CDAD*. The method of confirming toxin production by *C. difficile* isolates is not specified in guidelines and many laboratories use either an enzyme immunoassay, *the cytotoxicity neutralization assay (CNA) or PCR detecting genes encoding for toxin A and B. To speed up toxicity confirmation we compared the* C. DIFF QUIK CHEK COMPLETE (Quik Chek) directly on *C. difficile* isolates with our current practice, VIDAS Clostridium difficile A & B (Vidas) on overnight liquid culture.
Methods: *C. difficile* culture was routinely performed on Cycloserine Cefoxitin Fructose Agar (bioMrieux, France). Isolates identified as *C. difficile* by colony morphology, Gramstaining and proline aminopeptidase were subsequently incubated overnight in Brain Heart Infusion (BHI) broth. To confirm toxin production 200l BHI broth was used for the Vidas assay (bioMrieux, France), an automated ELFA detecting *C. difficile* toxin A and B. This method was compared by subjecting five *C. difficile* colonies directly to the Quik Chek (Techlab, USA), a rapid enzyme immunoassay for the detection of glutamate dehydrogenase and *C. difficile* toxin A and B. In addition, direct testing of stool samples by Quik Check according manufacturers instructions was performed.
Results: We cultured *C. difficile* isolates from 39 stool samples. 26 Isolates were confirmed to produce toxin by the Vidas, 6 isolates revealed an equivocal result and 7 tested negative. The Quik Chek was positive in 32/32 strains that tested positive or equivocal in the Vidas (sensitivity 100%) and was negative in 7/7 strains that tested negative in the Vidas (specificity 100%). The sensitivity of the Quik Chek directly on stool samples was 25/32 (78%) if compared with toxogenic culture confirmed by the Vidas.
Conclusion: To our knowledge this is the first report of using the Quik Chek directly on *C. difficile* isolates for

confirmation of toxogenic culture. In this context the Quik Chek is a rapid and reliable alternative to the Vidas.

P022

Effects of N deposition on diazotrophic activity and distribution of microorganisms associated with Sphagnum magellanicum

M.A.R. Kox, C. Lüke, E. van Elzen, C. Fritz, M.S.M. Jetten, L.P.M. Lamers, K.F. Ettwig
Institute for Water and Wetland Research, Radboudumc, Dept. of Ecological Microbiology, Nijmegen

Introduction: Sphagnum-dominated peatlands are important carbon (C) sinks and greenhouse gas filters.[1] These ecosystems are generally N-limited, but increasing levels of atmospheric N-deposition due to anthropogenic activities have the potential to shift them from C-sinks to C-sources.[2] N-deposition effects on plant communities have been well studied, but little is known about the effects on the N-fixing microbial community associated with Sphagnum spp. in peatlands.[3]

Objectives: We aim to quantify the short-term and memory' effects of high N-deposition on diazotrophic activity and community associated with Sphagnum magellanicum in ombrotrophic bogs. Ultimately we want to understand the influence of N-deposition on ecosystem functioning

Materials and methods: N_2 fixation rates associated with S. magellanicum from pristine and high-N-deposition sites were measured by acetylene reduction assays, calibrated with $^{15}N_2$ (with and without C_2H_2). DNA was extracted from the same samples and diazotrophic community diversity was analysed by clone library construction of nifH genes and by amplicon sequencing of nifH genes (Ion Torrent PGMTM).

Results & Conclusion: N-fertilisation had a negative effect on N_2 fixation rates in mosses from both pristine sites and areas with high N-deposition. Labelling showed that acetylene decreased $^{15}N_2$ uptake, questioning the usefulness of this commonly used method. Community diversity analyses showed a diverse diazotrophic community, of which the majority of nifH sequences belonged to a cluster without cultured representatives and unclear affiliation.

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P023

The pathology of antibiotic-induced intestinal outgrowth by multi-drug resistant *Enterococcus faecium*

A.P.A. Hendrickx, J. Top, M.J.M. Bonten, R.J.L. Willems
University Medical Center Utrecht, Dept. of Medical Microbiology, Utrecht

Introduction: *Enterococcus faecium* was considered a commensal of the microbiota within the large intestine of the human gastrointestinal tract, but is now recognized as a multi-drug resistant (MDR) pathogen. In hospitals, patients are typically administered antibiotics that perturb and reduce the intestinal commensal microbiota, leading to outgrowth of MDR-*E. faecium*, subsequent spread, infections, and hospital outbreaks. However, intestinal blooming' byMDR-*E. faecium* has not been thoroughly studied. Therefore, the objective was to analyze the pathology of antibiotic-induced outgrowth of MDR-*E. faecium* within the large intestine.

Methods:. Four BALB/c mice were treated for 2 days with ceftriaxon prior inoculation with $1x10^{10}$ CFU's of MDR-*E. faecium* E1162 (Group A), and left on cefoxitin antibiotics during the experiment. Group B is similar as Group A, but animals did not receive antibiotics. Three mice were left untreated (Group C). Colonization of E1162was monitored by enumeration of CFU's from faeces. After 10 days intestinal parts were: (1) formalin fixed, paraffine embedded (FFPE), thin sectioned and subjected to Gram-, Hematoxylin and Eosin- (H&E), and Periodic acid-Schiff (PAS)-staining followed by light microscopy (LM); and (2) fixed in 2% glutaraldehyde, serially dehydrated, and analyzed by scanning electron microscopy (SEM).

Results: Enumerating CFU's demonstrated that Group A mice were highly colonized with E1162 ($10x10^{10}$ CFU/gram faeces), while Group B mice were low-level colonized ($1x10^{5}$ CFU/gram faeces), and Group C revealed no enterococci. SEM on cecum and colon tissue confirmed the presence of only cocci on the epithelial cells (Group A) in high levels, and Group B +C revealed dense microbiota consisting of various rod-shaped bacteria, cocci and spirochetes. In addition, LM on H&E-, Gram- and PAS-stained FFPE cecum and colon sections of Group A mice showed absence of microbiota and enrichment of enterococci aligned onto the thin mucus layer and surrounding the undigested faecal material. In the colon, enterococci were predominantly present in areas of mucus secreting Goblet cells and at the apical side of columnar epithelium. Group B + C mice had a very diverse Gram-negative and -positive microbiota separated by a thick mucus layer from the epithelium.

Conclusion: Antibiotics reduce the intestinal microbiota and mucus layer and allow MDR-*E. faecium* to bloom in the gut. The finding that enterococci predominate at the apical

side of the epithelium and surround faeces likely enables the bacteria to interact with host receptors and to spread after defecation.

P024

Capability of nitrification and denitrification by *Methylacidiphilum fumariolicum* SolV to handle nitrosative stress

S. Mohammadi, A. Pol, M. Jetten, H. op den Camp
IWWR Radboudumc, Dept. of Microbiology, Nijmegen

Methane is a powerful greenhouse gas, which is released to the atmosphere both from natural and anthropogenic sources. Understanding sources and sinks of methane is important for future models of climate change on our planet. Methane oxidizing microbes are one of the most important biological sinks of methane. Methanotrophic bacteria are distinctive in their ability to exploit methane as the only carbon and energy source. Recently, the new acidiphilic methanotrophic bacterium *Methylacidiphylum fumariolicum* SolV belonging to the Planctomycetes/ Verrucomicrobia/Chlamydiae superphylum was discovered, and it has been shown that its growth is dependent on the presence of lanthanides.

Methane and ammonia are structurally comparable molecules. Thus, methanotrophs and nitrifiers have many features in common. They use many similar important enzymes, most particularly the ammonia monooxygenase/ particulate methane monooxygenase enzyme family. The two groups are suggested to have a common evolutionary history. Nitrifiers are able to oxidize methane, and methanotrophs are capable of nitrification. In the draft genome of strain SolV, genes for nitrite reduction (*nirK*) and nitric oxide reduction (*norB*, *norC*), were identified but the gene to encode nitrous oxide reductase was absent. A *haoAB* gene cluster encoding hydroxylamine oxidase was also identified, suggesting the ability of nitrification and nitrosative stress handling.

In order to study the effects of nitrosative stress on strain SolV two chemostat bioreactors were used: (a) In the first bioreactor, cells were grown on methane, and nitrate was used as the nitrogen source. (b) In the second reactor, cells were grown on hydrogen, and ammonium was used as the nitrogen source. In both conditions, oxygen was limited.

Using ^{15}N stable isotopes in combination with a nitric oxide analyser and GC-MS, we showed that the cells grown on methane (nitrate limited condition; pH 6.2) are able to perform denitrification by converting nitrite to nitric oxide and further to nitrous oxide in the absence of oxygen. Moreover, washed cells from the same chemostat reactor were used in batch tests at pH 6.2. Once ammonium was added to these cells with methane concentration ranging from 0 to 5% (v/v), nitrite production was measured. Interestingly, we showed that nitrite production rate increases while methane concentration decreases, suggesting the capability of nitrification by strain SolV.

Furthermore, we demonstrated that the cells grown on hydrogen (N-source: ammonium) convert ammonium to significant amounts of nitrite at pH 5 - 5.5 in the oxygen limited condition. Moreover, a rapid production of nitric oxide and decrease of nitrite concentration were observed when oxygen was absent, suggesting the action of a denitrification pathway converting nitrite to nitric oxide. Additionally, a decrease in nitric oxide levels resulted in an increase in nitrous oxide concentrations, suggesting conversion of nitric oxide to nitrous oxide by nitric oxide reduction enzymes under anoxic condition.

This study showed that strain SolV, performs nitrification, when methane is limited or completely absent, and carries out denitrification by converting nitrite to nitric oxide and further to nitrous oxide under anoxic conditions. These observations also indicate that strain SolV is capable of handling nitrosative stress.

P025

Comparison of the Copan Eswab™ with the Medical Wire S-Transwab™ for microbial nucleic acid testing

N.M. van Maarseveen, M. Zegers, R. Neuteboom
Rijnstate, Dept. of Medical Microbiology and Immunology, Velp

Introduction: With the purchase of a new sample processing unit (Copan WASP) we had to replace our current sample collection swab (Medical Wire S-Transwab) with the Copan Eswab. The Medical Wire S-Transwab is a foam-tipped swab, whereas the Copan Eswab is a nylon flocked swab. Both swab systems contain modified Liquid Amies medium as transport medium.

In this study we compared the Copan Eswab with the Medical Wire S-Transwab for microbial nucleic acid testing by analyzing different storage times and temperatures.

Methods: Suspensions were made of several viral isolates (Influenza A, Influenza B, Respiratory Syncytial Virus, Herpes Simplex Virus, Varicella Zoster Virus) and bacterial strains (*Neisseria gonorrhoeae, Mycoplasma genitalium* and *Legionella pneumophila*). Each suspension was aliquoted (100l/ well) in a 96-well plate and subsequently absorbed by either the Eswab or the S-Transwab. After absorption, swabs were inserted in their respective transport medium and incubated at two different storage temperatures (room temperature and 4C) and different storage times (0 hours, 24 hours, 48 hours and 72 hours). After incubation, 200 l of transport medium was used for RNA/DNA extraction using the bioMrieux easyMAG extraction system followed by an in-house multiplex real-time PCR assay on an ABI 7500 system. For *Neisseria*

gonorrhoeae, DNA extraction was performed with the Abbott *m*Sample Preparation System*DNA* on the Abbott *m2000sp* system, followed by the Abbott RealTime CT/NG assay.

Results: For Influenza A, Influenza B, Herpes Simplex Virus, Varicella Zoster Virus, *Neisseria gonorrhoeae* and *Mycoplasma genitalium* the Eswab and the S-Transwab yielded similar results for up to 72 hours of storage, irrespective of the storage temperature.

For the Respiratory Syncytial Virus, Ct-values slightly increased over time with approximately 2-3 Ct-values in both the Eswab and the S-Transwab. This was observed for both storage temperatures.

Only for *Legionella pneumophila* a difference was observed between the two swabs. For the Eswab mean Ct-values were consistently lower than the S-Transwab, with a mean Ct-value of 32.1 for the Eswab and 34.5 for the S-Transwab. This difference in Ct-values was seen for all different storage times and temperatures.

Conclusion: For all targets, with the exception of Respiratory Syncytial Virus and *Legionella pneumophila*, the Copan Eswab and the Medical Wire S-Transwab performed equally well and yielded stable results for at least 72 hours, irrespective of the storage temperature. Thereby, both swabs seem suitable for use in microbial nucleic acid testing.

P026

Terminal anaerobic processes in hypersaline soda lakes

D.Y. Sorokin

TU Delft, Dept. of Biotechnology, Delft

Soda lakes represent a unique double extreme habitat characterized by high salt and alkalinity of its soda brines. Despite severe conditions, two decades of intense microbiological studies demonstrated a fully functional and very diverse prokaryotic system in soda lakes even close to soda saturation. Until recently, however, the two key terminal anaerobic processes of microbial sulfidogenesis and methanogenesis at extremely halo-alkaline conditions have been poorly understood.

Our 8 years study of anoxic sediments of hypersaline soda lakes is south-western Siberia in general demonstrated sulfidogenesis is a dominant terminal electron sink. The rates of sulfide production from sulfate, thiosulfate and sulfur were comparable with the rates in marine sediments and only significantly lowered at salt saturation. Sulfate reduction was most vulnerable to salt inhibition as compared to thiosulfate and sulfur reduction. Maximum activity was detected with formate as e-donor and elemental sulfur as e-donor, while sulfate reduction rates were the lowest. The key players were represented by obligately natronophilic SRB of the orders *Desulfovibrionales* and *Desulfobacterales*. The lithotrophic growth by thiosulfate and sulfite disproportionation was a common trait among the soda lake SRB. Acetate was directly utilized as electron donor only at sulfur-reducing conditions, while at sulfate-reducing conditions acetate was oxidized syntrophically. Bacterial sulfur reduction at moderate salinity was a function of a very specialized group of fatty acid-utilizing sulfur-reducing natronophilic bacteria from the class *Chrysiogenetes*, while at soda-saturated conditions sulfur-respiring natronoarchaea have been discovered for the first time.

Methanogenesis in soda lakes, similar to other saline sulfate-rich habitats was mostly active with methylated substrates. However, in case of limitation of SRB activity, also lithotrophic and even acetoclastic methanogenesis was manifesting. In the absence of SRB competition, the potential rates of methanogenesis from formate were close to the rates in marine sediments. Totally unexpected, however, was the fact that lithotrophic methanogenesis was possible in nearly saturated soda brines. The main players responsible for the methylotrophic process in soda lakes were identified as obligately natronophilic *Methanolobus* (moderate salinity) and *Methanosalsum* (extreme salinity), while obligate natronophilic representatives of the genus *Methanocalculus* were universally dominating the lithotrophic process from low to extreme salinity.

Overall results so far demonstrated that, despite generally very low energy efficiency, the secondary anaerobes still manage to function at doubly extreme conditions of soda brines. In that respect, the soda lakes seem to be fundamentally different from hypersaline habitats with neutral pH.

P027

Towards understanding the molecular basis of the foreign body response and biomaterial-associated infections

M. Riool, V. Jaspers, L. de Boer, O.W. Stockhammer, S.A.J. Zaat

Academic Medical Center, Dept. of Medical Microbiology, Amsterdam

Introduction: Infection of inserted or implanted medical devices ('biomaterials') can have disastrous consequences, including removal of the device. Implantation of a biomaterial provokes an inflammatory response known as the 'foreign body response'. *Staphylococcus epidermidis* is the major cause of biomaterial associated infection (BAI), which in absence of a foreign body hardly ever cause infection. Formation of biofilms on the biomaterial surface is generally considered the main reason for these persistent infections, but *Staphylococcus epidermidis* has been shown to survive inside macrophages around biomaterials implanted in mice (Boelens *et al*. 2000), and was retrieved

from peri-catheter tissue in humans (Broekhuizen *et al.* 2008), showing that the tissue around implants is a second niche for infection. In order to understand the high infection-susceptibility of tissue around biomaterials, we aimed to unravel the molecular basis of the foreign body reaction and of biomaterial-associated infection. We assessed the gene expression underlying the foreign body response to titanium over time and the influence of *S. epidermidis* on this response.

Methods: Four experimental groups were compared in the biomaterial-associated infection mouse model: a) sham surgery (no implantation of a biomaterial), b) implantation of a titanium biomaterial, c) sham surgery with an *S. epidermidis* infection, and d) implantation of a titanium biomaterial combined with an *S. epidermidis* infection. At 1 and 6 hours and 2, 4, 9, 14 and 21 days, bacterial colonization, histology and gene expression were analyzed. To characterize multiple cellular immune responses in single microscopic slides of mouse tissue with both implant and bacterial infection, an immunohistological staining protocol using multiple spectral imaging was developed. Gene expression was recorded using Affimetrix Mouse Gene-ST microarrays.

Results: The histology and gene expression patterns showed distinct differences between sham (i.e. only surgery) and biomaterial groups possibly related to the foreign body response, and between biomaterial without and with infection. The analysis of the expressed gene sets is presently ongoing.

Conclusion: These results are a powerful start towards understanding the molecular basis of the foreign body response and biomaterial-associated infection.

P028

Detection of oxacillinases and other beta-lactamases in Acinetobacter baumannii using LC-MS/MS

H. Trip[1], J.A. Majchrzykiewicz-Koehorst[1], A.G. Hulst[1], K. Mende[2], C.K. Murray[2], H.J. Jansen[3], A. Paauw[1]

[1]TNO, CBRN Protection, Rijswijk, [2]Brooke Army Medical Center, Fort Sam Houston, San Antonio, USA, [3]Ministry of Defence, CEAG

Introduction: For effective treatment of bacterial infections, rapid identification of antibiotic resistance against first line antibiotics has become of increasing importance, especially due to the emergence and fast spread of antibiotic resistance. The nosocomial pathogen *Acinetobacter baumannii,* intrinsically already resistant to first and second generation cephalosporins, has a high capacity of acquiring antibiotic resistance. Therefore the spread of resistance against carbapenems, a class of last resort beta-lactam antibiotics, has raised great concerns. Carbapenem resistance in *A. baumannii* is mainly caused by expression of oxacillinases, which can be subdivided in different subclasses such as OXA-23-like and OXA-54-like. In this study, LC-MS/MS was employed to detect and identify oxacillinases and other beta-lactamases in clinical isolates of *A. baumannii*, with the goal to evaluate its potential for detection of antibiotic resistance.

Methods: Thirty isolates, acquired from a military hospital (Brooke Army Medical Center,US), were tested for resistance against three carbapenems and ceftazidime, a third generation cephalosporin, by classical growth inhibition tests. Each isolate was PCR tested for 15 different beta-lactamase genes. LC-MS/MS analysis was performed on whole cell lysates, following a fast sample preparation procedure, including one hour trypsin digestion. The equipment used was a nano-Advance LC system (Bruker) coupled to a Q-TOF mass spectrometer (maXis impact, Bruker); LC gradient runtimes were 30 minutes. Data analysis was carried out using Mascot 2.0 software.

Results: Of the 30 tested isolates, 15 were resistant to all three carbapenems. LC-MS/MS analysis resulted in identification of 400 - 500 proteins per isolate, with protein coverage ranging from 5% to 76% (coverage by identified peptides). In each isolate that was carbapenem resistant, either OXA-23-like or OXA-24-like was detected. In these isolates only, the corresponding genes, $bla_{\text{OXA-24-like}}$ and $bla_{\text{OXA-23-like}}$, were also detected by PCR. OXA-51, endogenous to *A. baumannii*, was identified in only one, carbapenem sensitive isolate, whereas the $bla_{\text{OXA-51-like}}$ gene was detected by PCR in all isolates. In the isolate apparently expressing OXA-51 to a higher level, the insertion element IS*Aba1* was detected upstream of $bla_{\text{OXA-51-like}}$ by PCR. IS*Aba1* is known to increase expression by providing strong promoter elements. Other detected beta-lactamases were ADC (23 isolates), PER-1 (3 isolates), GES-1 (1 isolate) and CMY-2 (1 isolate). Their cognate genes were also detected by PCR in the same isolates.

Conclusions: Each oxacillinase likely responsible for carbapenem resistance, as well as other beta-lactamases were detected by LC-MS/MS. The identification of multiple antibiotic resistance proteins in one bacterial isolate in one LC-MS/MS run demonstrates the advantage over existing methods, which are more time-consuming or require resistance genes to be specifically targeted (PCR).

P029

Laboratory-based approach in surveillance of sexual transmitted infections: the Almere experience

C.P. van der Weijden[1], H. Kir[2], J.W. Dorigo-Zetsma[3]

[1]GGD Flevoland, AGZ, Almere, [2]Zorggroep Almere, Gezondheidscentrum Oost, Almere, [3]Centraal Bacteriologisch en Serologisch Laboratorium, Hilversum/Almere

Introduction: Sexually transmitted infections (STI) care in the Netherlands is primarily provided by general practi-

tioners (GPs) and specialized STI centres. GPs roughly provide 70-80% of the STI care in the Netherlands. There is no complete case-registration for STI, except for hepatitis B which is a notifiable disease. National STI surveillance is mainly based on data from the STI centres and therefore based on certain (high risk) populations. To gain better insight in the epidemiology of STI in the Almere-region, data from both GPs and the public health STI centre in Almere, Flevoland were compared and analysed.

Methods: Laboratory data on STI tests as requested by approximately 95% of GPs in Almere during the years 2011 and 2012 were anonymously analysed. Data were compared with STI surveillance data available from the STI centre. The analysis was primarily based on urogenital infections with *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoea* (GO) and included test result and test site, age, gender and 4 letter zip code area.

Results: A total of 15.930 CT- and 15.718 GO-test results were available for analysis. As expected GPs performed more STI screenings than the STI centre at a ratio of 3.7 to 1. The gender ratio differed between GPs and the STI centre (GPs 74% female; STI centre 54% female). Testing at extragenital sites was rarely done by GPs: only 0.1% of all tests was from the anorectal site, whereas at the STI centre this was 8%. Of the 57 anorectal infections (33 CT and 24 GO) approximately 50% would have been missed if only screened at the urogenital site.

The incidence for urogenital CT and GO was 11.0 respectively 2.3%. The incidence was slightly higher at the STI centre compared to GPs. This is mainly related to a lower incidence among screened women at the GPs. CT incidence GPs 10.2% (women 9.1%; men 13.4%), STI centre 13.8% (women 14.3%; men 13.3%). GO incidence GPs 2.0% (women 1.6%; men 3.3%). STI centre 3.4% (women 3.3%; men 3.4%). In the age group up to 25 years the highest incidence of CT was found. The urogenital CT incidence at both the GPs (16.4%) and the STI centre (18.2%) was higher than the national average for this age group in the two years included (2011 14.6%; 2012 14.9%). Two zip code areas with an above-average incidence of STI were identified.

Conclusion: This study provides new starting points in optimising STI screening and prevention.

-Urogenital STI in Almere were both at GPs and the STI centre primarily diagnosed with men and women below the age of 25 years with an incidence above the national average.

-(Young) men are underrepresented for STI screening at the GPs.

-Testing of extragenital sites appeared not to be standard practice for the GPs.

-Certain zip code areas show an elevated STI incidence.

How these findings can be used in STI control is currently being discussed with GPs and the STI centre.

P030

Evaluation of the Cepheid Xpert vanA/vanB assay in combining it with enriched inoculated broths for the direct detection of carriership with VanB-type VRE

X. Zhou, J.P. Arends, G.A. Kampinga, H.M. Ahmad, B. Dijkhuizen, P. van Barneveld, J.W.A. Rossen, A.W. Friedrich

University Medical Center Groningen, Dept. of Medical Microbiology, Groningen

Introduction: Rapid and accurate detection of VRE (vancomycin resistant enterococci) is needed for an adequate treatment and infection control. Previous studies using Cepheid's Xpert *vanA/vanB* assay showed good results for *vanA*, however many false positive values were found for *vanB* [1-3]. This could partially be explained by the fact that non-enterococcal *vanB* genes can be found in the gut [4,5]. We evaluated the use of Xpert *vanA/vanB* assay on enriched inoculated broths instead of using direct rectal specimens.

Methods: In the first evaluation, a total of 235 E-swabs were tested in parallel with enriched inoculated broths. First, 100 L E-swab medium was evaluated directly with the Xpert *vanA/vanB* assay. Further, E-swab medium was used to inoculate Brain Heart infusion (BHI) broth containing amoxicilline 16mg/L amphoterin-B 20 mg/L, aztreonam 20 mg/L and colistin 20 mg/L. Broths were incubated and again evaluated with the Xpert *vanA/vanB* assay. Broths were subcultured on VRE Brilliance agars (Oxoid). Growth of blue colonies was suspected for enterococci and identification was performed using MALDI-TOF Mass Spectrometry (Bruker). Confirmed growth of enterococci on the VRE agars was again evaluated with the Xpert *vanA/vanB* assay and these results were considered as 'golden standard' for VRE negativity or positivity. After the first evaluation, we evaluated 112 enriched inoculated broths to test our new algorithm.

Results: In the first evaluation 157 E-swabs as well as broths were negative (CT value 0 or > 36). For the remaining 78 E-swabs, 32 had a VRE *vanB* positive culture, but no *vanA* was found. Using the cut-off value of the GeneXpert system (= 36 for positivity) on the E-swabs, true positive and negative results were correct. However, false positive results were high (59%), which resulted in sensitivity, specificity, PPV (positive predictive value) and NPV (negative predictive value) of 100%, 77.3%, 41% and 100%, respectively. Therefore, we defined an own cut-off value for PCR on broth (= 25 for positivity). Now no false positive results were found, although 1 true positive VRE was missed (CT-value 25.9). This resulted in sensitivity, specificity, PPV and NPV of 96.9%, 100%, 100% and 99.5%, respectively. In the prospective 112 tested enriched inoculated broths, we again found no false positive results, one true-positive VRE was missed (CT-value 28.4).

Conclusion: This study shows the usability of Cepheid's Xpert *vanA/vanB* assay for *vanB* VRE in combination with enriched inoculated broths. Patients' samples with CT-values = 25 can be considered as true positive. In addition, samples with CT-values between > 25-30 (0.6% of all samples), should be confirmed to be negative by culture. Using the cut-off value of = 25 for positivity strongly improved the PPV compared to that found in previous studies in which the Xpert *vanA/vanB* assay was used on direct rectal swab specimens.
This work has been presented at the 4th ASM conference on Enterococci 2014

P031

The number of human commensal bacterium *Faecalibacterium prausnitzii* is significantly increased in the fecal samples of patients with active Crohn's disease

S.R. Konstantinov[1], E. Ester[2], M. Elizabeth[2], R.K.L. Lie[2], C.J. van der Woude[2], P. Maikel[2]
[1]*Erasmus MC, Dept. of Gastroenterology and Hepatology, Rotterdam,* [2]*Erasmus MC, University Medical Center Rotterdam*

Introduction: The causal relationship between the composition of the intestinal microbiota and Crohn's disease (CD) is increasingly recognized. Typically, a decreased intestinal microbiota diversity, together with a high incidence of different pathobionts and a low abundance of some commensal bacteria is concomitant with CD. At this point, however, it is unclear if there is a specific gut microbiota composition associated with an active CD.
Methods: We have first developed and validated a dysbiosis PCR-based assay allowing a tailored detection of the core intestinal pathobionts, commensal bacteria, and eukaryotic microorganisms in a single sample. Using this newly developed assay in our lab, we have examined DNA isolated from fecal samples of CD patients with both active (n = 10) and inactive (n = 14) disease and controls (n = 9). Follow up studies using a quantitative PCR (Q-PCR) technology was used to assess the abundance of a major CD pathobiont, an adherent and invasive *Escherichia coli* (AIEC),and the commensal bacterium, *Faecalibacterium prausnitzii,* in the different groups.
Results: Global analyses of the results using the dysbiosis PCR-based assay have revealed a number of different pathobionts present in the samples from the CD patients as compared with the healthy controls. AIEC in particular was detected in both active and inactive CD patients, but not in the control group. Further analyses using Q-PCR have shown that there was a significantly higher amount of AIEC in the samples of the inactive CD group as compared with the active CD group (p = 0.05). The commensal bacterium *F. prausnitzii* was detected in 7 out of 10 samples from the active CD group, 9 out of 14 samples from the inactive CD group, and in all samples from the control group. Based on the Q-PCR examination of the positive samples, we have found that the active CD group have harbored a significantly higher number of *F. prausnitzii* as compared with the inactive CD group and the controls (p = 0.05).
Conclusion: During the active CD there is an increase in the number of *F. prausnitzii* that is concomitant with a decreased abundance of AIEC in the fecal samples.

P032

Glycopeptide resistance among coagulase-negative Staphylococci in a tertiary care hospital: preliminary results

F.M.H.P.A. Koene, D.E.A. Potters and F.H. van Tiel
Maastricht University Medical Centre, Dept. of Medical Microbiology, Maastricht

Introduction: Central venous catheter associated bloodstream infections are an important complication in patients with haematological malignancy. Because of underlying diseases and received immunosuppressive (chemo)therapy, patients often are dependent on their central venous access. Central venous catheter associated bacteraemia, caused by coagulase –negative staphylococci (CoNS), is generally treated with vancomycin, pending the results of susceptibility testing. However, teicoplanin is an alternative antibiotic with fewer side-effects and a convenient dosage regimen. Our hypothesis is that the prevalence of teicoplanin resistant CoNS strains is too high to warrant empirical treatment with this glycopeptide. We present the preliminary results of our study.
Methods and materials: First blood culture isolates of CoNS from hospitalized patients between the 1st of January 2013 and the 1st of January 2014 were searched for in our hospital database. In all 343 isolates were included. Identification and susceptibility testing of isolates was performed on BD Phoenix™ Automated Microbiology System. This system generated the minimal inhibitory concentrations (MICs) of vancomycin and teicoplanin. Resistance to vancomycin was confirmed with an E-test.
Results: We analysed 343 isolates. The observed frequency of teicoplanin resistant strains among the included CoNS isolated strains was 22%. No vancomycin resistant strains were detected. The distribution of isolates according to hospital department was such that most CoNS isolates were found in the high care units, respectively the haematology-oncology ward (n = 63), the cardio-thoracic surgery ward (n = 53) and the intensive care unit (n = 36). The observed frequency of teicoplanin resistant CoNS strains on these wards was respectively 33%, 21% and 36%.

Conclusion: The frequency of occurrence of teicoplanin resistance among first CoNS blood culture isolates was shown to be high, namely 22%. None of the isolates was vancomycin resistant. Based on these preliminary results we would not advise teicoplanin as an empiric treatment on suspicion of CoNS bacteraemia.
We will study subsequent new episodes of CoNS isolates in blood cultures in this population and the presence of heterogeneously vancomycin resistant CoNS strains. Results of the comparison of vancomycin MIC and teicoplanin MIC are pending.

P033
Identification of the type II cytochrome c maturation pathway in anammox bacteria by comparative genomics

C. Ferousi[1], D.R. Speth[2], J. Reimann[2], H.J.M. op den Camp[2], J.W.A. Allen[3], J.T.M. Keltjens[2], M.S.M. Jetten[2]
[1]Radboudumc, Dept. of Ecological Microbiology, Nijmegen, [2]Radboudumc, Institute for Water and Wetland Research, Dept. of Ecological Microbiology, Nijmegen, [3]University of Oxford, Dept. of Biochemistry, Oxford,United Kingdom

Anaerobic ammonium oxidizing (anammox) bacteria may contribute up to 50% to the global nitrogen production, and are, thus, key players of the global nitrogen cycle. The molecular mechanism of anaerobic ammonium oxidation was recently elucidated and is suggested to proceed through a branched respiratory chain. This chain involves an exceptionally high number of *c*-type cytochrome proteins which are localized within the anammoxosome, a unique subcellular organelle.
During transport into the organelle, the *c*-type cytochrome apoproteins need to be post-translationally processed so that *b*-type hemes (Fe-protoporphyrin IX) become covalently attached to them, resulting in mature cytochrome *c*-type holoforms. This post-translational process is generally termed cytochrome *c* maturation and has been found to proceed *via* at least three distinct pathways (Systems I, II, and III) in a wide variety of organisms with a complex and unpredictable phylogenetic distribution.
In this study, comparative computational methods were applied to identify the cytochrome *c* maturation system employed by anammox bacteria. Draft genomes representative of four anammox genera were analyzed (*Kuenenia, Scalindua, Brocadia*, and strain KSU-1 representing *Jettenia* genus). Reference protein datasets for each of the three cytochrome *c* maturation Systems (I-III) were compiled, each comprising all indispensable and System-specific protein components. Anammox gene products were compared to the datasets utilizing blastP, HHpred, and HMMER annotation tools. Protein family matches, transmembrane helical domains, and signal peptides were predicted via Pfam, TMHMM, and SignalP, respectively. Conserved motifs and critical residues were procured from literature and probed in each gene product directly. Multiple alignments of identified anammox homologs were performed using ClustalW and phylogenetic trees were constructed based on the Maximum Likelihood algorithm utilizing the JTT matrix-based model.
Our results show that all tested anammox genome assemblies contain a complete type II cytochrome *c* maturation System. Our working model suggests that this machinery is localized at the anammoxosome membrane which is assumed to be the locus of anammox catabolism. These findings will stimulate further studies in dissecting the molecular and cellular basis of cytochrome *c* biogenesis in anammox bacteria.

P034
Anaerobic oxidation of methane and trace methane oxidation in a natural freshwater gas source (Berkhout, The Netherlands)

P.H.A. Timmers, M. van Rossem, C.M. Plugge, A.J.M. Stams
Wageningen University, Laboratory of Microbiology, Wageningen

Introduction: Anaerobic oxidation of methane (AOM) is a process widely found in freshwater environments where it is coupled to the reduction of nitrate. Other electron acceptors that could be coupled to microbial methane oxidation are iron and manganese, which was proposed to occur in laboratory incubations from marine sediments and in freshwater environments. Coupling of AOM to sulfate reduction (SR) has mainly been found in marine sediments and the responsible organisms were identified to be anaerobic methanotrophic archaea in consortia with sulfate reducing *Deltaproteobacteria*. AOM coupled to SR was found to a much lesser extent in terrestrial environments where sulfate concentrations are generally low. Trace methane oxidation (TMO) is the process of methane oxidation during net methanogenesis which was described extensively in methanogenic pure cultures and mixed communities. In this research, we investigated the co-occurrence of anaerobic methane oxidation (AOM) and trace methane oxidation (TMO) in a terrestrial natural gas source.
Methods: Long term incubations of gas source samples were done with ^{13}C-labeled methane ($^{13}CH_4$) and different electron acceptors possibly involved in methane oxidation such as nitrate, sulfate, humic acids and iron (ferrihydrite) with and without with humic acids. As control condition, no electron acceptor was added. During incubation, analysis of $^{13}CH_4$ and ^{13}C-labelled CO_2 ($^{13}CO_2$) and all substrates and products was done. After 323 days

of incubation, pyrosequencing of partial archaeal and bacterial 16S rRNA genes of the incubations with methane and sulfate and incubations fed only with methane or sulfate was done in parallel with the inoculum sample.

Results: Results gave evidence of both TMO and AOM during incubation. All conditions except the ones with sulfate and nitrate showed ^{12}C-methane production, indicating net methanogenesis. Conditions with ferrihydrite and the control showed highest $^{13}CO_2$ production as compared to other methanogenic conditions. Here, $^{13}CO_2$ simultaneously increased with methanogenesis which is typical for TMO. With humic acids as electron acceptor we found highest methane production with very low TMO activity. In the incubations with nitrate, no methane formation or methane oxidation was detected. Conditions with $^{13}CH_4$ and sulfate showed $^{13}CO_2$ production without any increase in ^{12}C-methane production. Also, when SR rates were higher, methane oxidation rates were also higher.

Conclusion: We found evidence for TMO during net methanogenesis and for AOM coupled to SR in freshwater samples. Methane oxidation was not a result of TMO and must have been coupled to SR.

Pyrosequencing results reveal which microorganisms are differentially growing when methane and sulfate are both present. Currently it is unknown if the communities involved in freshwater methane oxidation coupled to sulfate reduction are similar as those in marine environments.

P035

Glycosylation the S-layer protein of Kuenenia stuttgartiensis- the first glycoprotein described in the *Planctomycetes phylum*

M.C.F. van Teeseling[1], A. Hofinger-Horvath[2], P. Kosma[2], P. Messner[2], C. Schaeffer[2], M.S.M. Jetten[1], L. van Niftrik[1]
[1]*Radboudumc, Dept. of Microbiology, Nijmegen,* [2]*Universitaet fuer Bodenkultur, Division of Organic Chemistry, Vienna, Austria*

The cell plan of bacteria of the *Planctomycetes* phylum, to which the anammox bacterium *Kuenenia stuutgartiensis* belongs, is a highly debated subject. These bacteria have two or more compartments of which the identity depends on the location of the cytoplasmic membrane. Therefore, further knowledge concerning the composition of the cell envelope is crucial. Recently an S-layer (surface-layer) was discovered as the outermost component of the cell envelope in *K. stuttgartiensis*.[1] This S-layer is a crystalline layer surrounding the complete bacterium made up of many copies of the same protein. This protein was identified as a glycoprotein, thereby being the first glycoprotein identified within the Planctomycetes. This study focuses on determining the composition and structure of this glycan and the site and mode of attachment of the glycan to the S-layer protein.

Enriched S-layer glycoproteins were first treated with a protease cocktail and afterwards the glycopeptides were separated from the non glycosylated peptides via multiple chromatography techniques. Purified S-layer glycopeptides were subsequently analysed to determine which monosaccharides are present in the glycan. Mass spectrometry was performed to show the length and degree of branching of the glycans. NMR (nuclear magnetic resonance) analysis is being performed to probe the structure of the glycan in more detail. These techniques unravelled the composition of the glycan, showing multiple different (mono)saccharides present in the glycan, and gave insight in the structure of the glycan.

Via these techniques the first elucidation of a glycoprotein present in the *Planctomycetes* phylum has been made. This finding will hopefully lead to better understanding of the structure and function of the S-layer that has been found to be part of the cell envelope of the bacterium *K. stuttgartiensis*.

Reference

- van Teeseling MCF, de Almeida NM, Klingl A, et al. A new addition to the cell plan of anammox bacteria: '*Candidatus* Kuenenia stuttgartiensis' has a protein surface layer as the outermost layer of the cell. J. Bac. 2014;196:80-9.

P036

Genetic variability in the major capsid l1 protein of human papillomavirus type 16 (hpv16) in the Netherlands

A.J. King[1], J.A.J. Sonsma[1], E. van Logchem[1], R. Vriend[1], M. van der Sande[1], M. Feltkamp[2], H.J. Boot[1]
[1]*RIVM, CIb-IDS, Bilthoven,* [2]*LUMC, Leiden*

Objectives: Intratypic molecular variants of HPV type 16 and -18 are known to occur and are distributed differently within the five continents. In the Netherlands, a bivalent vaccine, composed of recombinant L1 proteins from HPV16 and -18, is used to prevent cervical cancer since 2009. Long term vaccination with L1 proteins, could lead to changes in HPV16 and 18 virus population. In order to be able to detect these changes, knowledge of the genetic diversity of L1 gene in HPV16 and -18 viruses circulating in the Netherlands at the start of vaccination is required.

Methods: Samples were obtained from swabs collected in 2009 and 2011 within the PASSYON (PApillomavirus Surveillance among STI clinic Youngsters) study among Dutch 16- to 24-year old male and female attendees of the sexually transmitted infection (STI) clinics. HPV DNA detection and genotyping was performed previously using the PCR-based reverse line blotting (SPF10-LiPA system version 1, DDL Diagnostic Laboratory). The entire L1 gene was amplified in 3 overlapping PCR products and

sequenced by classic Sanger sequencing in 213 HPV16 positive samples from women. Sequences were aligned using Bionumerics and compared with HPV16 European German reference sequence AF536179.
Conclusions: Sequencing of the entire HPV16 L1 gene revealed 95 SNPs (68 silent and 27 non-silent mutations) in all samples. The majority of the HPV16 isolates (198/213, 93%) clustered with the European/Asian types and 16/213 (7%) with the African variants. The most common L1 sequence found was detected in 31% of the samples and was very similar to the reference strain differing in only two positions with silent mutations. The majority of the non-silent mutations (17/27, 63%) was located in sequences encoding alpha helix, beta sheet or surface loops, in particular in the immunodominant FG loop, and may influence the protein secondary structure. Taken together, this study provides unique pre-vaccination data on the genetic variation of the L1 gene of HPV16 viruses circulating in the Netherlands among adolescents and young adults.

P037

Transformation of fluorescently labelled DNA and substrate specificity of the *Bacillus subtilis* competence machinery

M.B. Boonstra, O.P. Kuipers, J.W. Veening, A.G. Gruszka, A.H. Herrmann
University of Groningen, Dept of. molecular genetics, Groningen

Bacillus subtilis is a well-known naturally competent bacterium. Double-stranded DNA serves as a substrate for uptake. One strand is degraded on the outside of the membrane and the other is transported through the competence machinery into the cell. The role of most of the proteins involved in competence is known, and many have been studied using fluorescent labelling. However entrance of fluorescent DNA into the *B. subtilis* cytoplasm has not been observed. We have set up a system to follow DNA transportation and integration in real time. Fluorescent labelling of DNA and components of the competence system allow us to follow the uptake and localization of DNA during transformation. Introduction of a tetR-mCherry *tetO* operator system will allow us to determine localization of fluorescently labelled DNA in the cell. Moreover, by using specific end-labelling of DNA, with variably sized molecules, we will assess the pore width through which the substrate can pass. We will also study the role of proteins involved in recombination on the rate of integration of exogenous DNA during transformation. Much is known about the mechanism by which *B. subtilis* handles double stranded DNA. However, in its natural environment *B. subtilis* encounters not only double stranded DNA. We will therefore determine if *B. subtilis* can also use double stranded RNA and RNA/DNA hybrids as a substrate for transformation.

P038

Is there a difference in urogenital and anorectal Chlamydia load during concurrent *Chlamydia trachomatis* infection in women?

C.D.J. den Heijer[1], J.A.M.S. Dirks[1], G.A.F.S. van Liere[2], N.H.T.M. Dukers[2], C.J.P.A. Hoebe[2], P.F.G. Wolffs[1]
[1]*Maastricht University Medical Centre, Dept. of Medical Microbiology, Maastricht,* [2]*Public Health Service South Limburg, SIM, Geleen*

Introduction: *Chlamydia trachomatis* (CT) is a sexually transmitted infection (STI), that remains asymptomatic in the majority of the patients affected, although serious complications can develop during the course of the infection, such as pelvic inflammatory disease. Accurate knowledge of the pathogenesis is required to better understand the differences in clinical outcome. In this perspective, the bacterial load of the CT infection has the potential of being a factor of significance. In this study, we studied whether there were differences between the urogenital and anorectal load in women with concurrent CT infection. Moreover, anorectal loads were evaluated taking into account the patient's urogenital load.
Methods: Included swabs originated from women attending the STI clinic of South Limburg, Netherlands, from January 2012 to December 2013 who were diagnosed with an urogenital and anorectal CT infection concurrently. Initial samples were tested with the cobas 4800 (Roche Diagnostics) for CT presence. Subsequently, the bacterial load was determined by means of qPCR. For statistical analyses, loads were log-transformed to allow for parametric tests. In addition, patients were categorized into three categories based on the tertiles of the urogenital CT loads; the anorectal CT loads between these categories were compared. SPSS version 20 was used and a P-value < 0.05 was considered to be statistically significant.
Results: In total, 61 women were included in the present analyses. In 43 of these 61 women (70.5%) the urogenital load was higher than the anorectal load. Overall, the mean urogenital load was higher than the anorectal load (log CT load: 4.79 versus 3.66, respectively, p = 0.001). Anorectal CT load followed a similar trend as the urogenital CT load, with highest loads found in women within the highest tertile of urogenital CT load (mean log anorectal load: 3.91, 95% confidence interval (CI): 2.93-4.89), and lowest anorectal loads in women within the lowest tertile of urogenital loads (mean log anorectal load: 3.44, 95% CI: 2.56-4.31). However, these differences were not statistically significant (p > 0.05).

Conclusion: In this study, we found 1) a higher urogenital than anorectal load in women, attending a STI clinic in the southern part of the Netherlands, with a concurrent CT infection. 2) The relative levels of the urogenital and anorectal CT loads, i.e. high or low in comparison with other women, showed concordant results. Although current results present absolute Chlamydia loads, future analyses will take the number of human cells present in the swabs into account. The number of human cells could affect the load per sample, because CT is an obligate intracellular bacterium.

P039

Electronic surveillance for ventilator-associated events: a solution for variability?

M.S.M. van Mourik[1], P.M.C. Klein Klouwenberg[1], D.S.Y. Ong[1], J. Horn[2], M.J. Schultz[2], O.L. Cremer[1], M.J.M. Bonten[1]
[1]*UMC Utrecht, Dept. of Medical Microbiology, Utrecht,* [2]*Academic Medical Center, Dept. of Intensive Care Medicine, Amsterdam*

Introduction: Reliable surveillance methods are indispensable for benchmarking of healthcare-associated infection rates. In the United States, the National Healthcare Safety Network (NHSN) recently introduced surveillance of ventilator-associated events (VAE), including ventilator-associated conditions (VAC) as an alternative to conventional surveillance of ventilator-associated pneumonia. This new algorithm is amenable to automated implementation and strives for more consistent interpretation. We assess the feasibility and reliability of automated implementation in the Dutch ICU setting.
Methods: Retrospective analysis of an intensive care unit cohort with prospective assessment of VAP in 2 academic medical centers (January 2011 - June 2012). The VAE algorithm was electronically implemented as specified by NHSN using minute-to-minute ventilator data to detect VAC, infection-related VAC (IVAC) and ventilator-associated pneumonia (VAE-VAP). Several minor modifications were developed to improve stability and comparability with manual surveillance and assess the reliability of electronic implementation (hourly measurements, sustained settings and 10th percentile selection). Concordance was assessed between the algorithms and prospective surveillance. Attributable mortality of VAC was estimated by multivariable competing-risk survival analysis.
Results: 2080 patients contributed 2296 episodes of mechanical ventilation (MV). VAC incidence was 10.0/1000 MV days, IVAC 4.2/1000 MV days and VAE-VAP 3.2/1000 MV days. Prospective surveillance identified 8 VAP cases/1000 MV days. The original VAC algorithm detected 32% (38/115) of patients affected by VAP; positive predictive value was 25% (38/152). Applying the 10th percentile rule in the original algorithm did not change the overall incidence of VAC (158 events in 152 patients), but only 117 of the detected VAC episodes were identical. Using hourly (validated) measurements resulted in 159 episodes of VAC with a sensitivity of 30% and a positive predictive value of 21% for VAP. Applying a sustained settings rule resulted in 157 VACs with a sensitivity of 34% and a positive predictive value of 25%. Of the 158 episodes identified by the original algorithm, 104 (65%) were also identified by all other sensitivity analyses. Estimates of associated mortality for VAC identified using the various electronic implementations varied: original VAC subdistribution hazard ratio (SHR) 3.9 (95% confidence interval (CI) 2.9 - 5.3), 10th percentile rule SHR 6.3 (95% CI 4.8 - 8.4), SHR 5.2 (95% CI 3.9 - 6.9) for the sustained settings rule and SHR 6.3 (95% CI 4.7 - 8.5) for the hourly sampling scheme.
Conclusion: 1. Concordance between manual VAP surveillance and the VAE algorithm was poor. 2. Although electronic implementation of the VAE algorithm was feasible, small variations considerably altered the events detected and their effect on mortality. Using the current specifications, comparability across institutions using different electronic or manual implementations remains questionable

P040

Distinguishing disease severity of respiratory syncytial virus infections by nasopharyngeal gene expression

C.H. van den Kieboom, I.M.L. Ahout, A. Zomer, K.H. Brand, R. de Groot, G. Ferwerda, M.I. de Jonge
Radboudumc, Dept. of Pediatrics, Nijmegen

Introduction: Respiratory Syncytial Virus (RSV) causes in only mild infections in the vast majority of children. However, in some cases it leads to severe lower respiratory tract disease, such as bronchiolitis and pneumonia. The development of severe disease is host dependent underlining the importance of identifying associated markers. The current RSV diagnosis is based on viral detection in a nasopharyngeal sample, hence this material was analyzed for gene expression.
Methods: Severity of infection was defined by the degree of supportive care; no supportive care, solely supplemental oxygen support or mechanical ventilation. To assess the nasopharyngeal gene expression profiles, microarrays were performed. The analysis was based upon two comparisons, where the solely supplemental oxygen group was added either to mechanical ventilation (supportive care) or no supportive care (no mechanical ventilation), resulting in two gene selections. From these selections the overlapping gene and the only up-regulated gene were selected. The

expression profile of these genes was validated by real-time quantitative polymerase chain reaction (RT-qPCR) in a secondary cohort consisting out of acute and recovery samples.

Results: The microarray analysis resulted in a selection of 5 genes; GABA-B receptor 1 (*GB1*), tetraspanin 8 (*TSPAN8*), mucin 13 (*MUC13*), microseminoprotein (*MSP*) and the only up-regulated gene, chemokine ligand 7 (*CCL7*).

GB1 expression displays no significant differences between any of the groups. After reanalysis of the microarray probes, an overlap with ubiquitin D(*UBD*) was found. Therefore *UBD* was examined, the results show a significant difference between the recovery and acute patients, in addition distinguishing the no supportive care' from the supportive care' group.

TSPAN8, MUC13, MSP, CCL7 show a significant different expression between the recovery and acute patients, in addition distinguishing the no mechanical ventilation' from the mechanical ventilation' group.

Conclusion: Based upon differential expression of *UBD*, *CCL7*, *TSPAN8*, *MUC13* and *MSP*, the level of disease severity can be distinguished. This might be of prognostic value, predicting the development of severe disease.

By simultaneously predicting cause and course of the disease, current clinical judgement will become more objective and reproducible.

P041

Serotyping of *pneumococci* without culture directly from blood and urine samples

K.E.M. Elberse[1], S. van Mens[2], S. Meijvis[3], C. Blauwendraat[4], I. van de Pol[1], L. Schouls[1]

[1]*National Institute for Public Health and the Environment (RIVM), Centre for Infectious Disease Control Netherlands (CIb), Bilthoven,* [2]*Departments of Medical Microbiology and Immunology, Nieuwegein,* [3]*St Antonius Hospital, Dept. of Internal Medicine, Nieuwegein,* [4]*VU University Medical Center, Amsterdam*

Objectives: Detection of pneumococcal infections is increasingly performed using non-culture based techniques. Methods for serotyping directly from clinical material are scarcely available, although the need for serotyping to assess vaccine impact and effectiveness has increased with the introduction of nationwide pneumococcal vaccination. Here, we present 3 approaches for the detection and serotyping of pneumococci in patients with community acquired pneumonia (CAP) without culture directly from blood or urine.

Methods: The first approach is a quantitative PCR (qPCR) to detect pneumococci on blood samples and complemented with capsular sequence typing (CST) to identify their serotype. The second approach, a urinary antigen assay targeting 14 serotypes, designed as inhibition multiplex immunoassay (IMIA), is based on Luminex technology. The third approach is a multiplex immunoassay (MIA) also based on Luminex technology which detects serologic antibody responses against 14 serotypes. All assays were performed on materials obtained from 309 adult hospitalized CAP patients in 2007-2010. The results of the 3 assays were compared with the results obtained from conventional laboratory methods to detect pneumococcal CAP (blood cultures, sputum cultures and BinaxNOW urinary antigen tests).

Results: The three alternative assays in addition to the conventional techniques, were able to detect the pneumococcus as causative agent in 56% more patients (increase from 64 to 100 patients). We were able to assign a serotype to the pneumococcus causing infection in 68 patients using any of the alternative serotyping methods (CST, IMIA and MIA). The use of the IMIA resulted in the highest number of positive samples (n = 19, 11%) when conventional methods were negative, but the serological antibody response by MIA resulted in the highest percentage of positive samples (n = 18, 13%) when conventional methods were negative. The qPCR detected only 3 extra cases compared to conventional methods. We were able to assign a serotype using CST to 70% (11 of the 16) of the qPCR positive samples.

Conclusion: In this study we assessed the value of 3 additional methods to identify and concurrently serotype a pneumococcal isolate in patients hospitalized with CAP. We showed a marked increase in detection of pneumococcal infections using the additional methods. The MIA had the largest added value to conventional methods and the qPCR was most consistent with conventional methods. This study indicates the usefulness of additional molecular methods to conventional laboratory methods for the detection of pneumococcal pneumonia.

P042

Differences in pneumococcal populations from the Netherlands and Spain assessed by MLVA and MLST

K.E.M. Elberse[1], L. Selva[2], G. Pluister[1], N. Peris[2], A. van der Ende[3], C. Muñoz-Almagro[2]

[1]*National Institute for Public Health and the Environment (RIVM), Centre for Infectious Disease Control Netherlands (CIb), Bilthoven,* [2]*University Hospital Sant Joan de Deu, Molecular Microbiology Department, Barcelona, Spain,* [3]*Academic Medical Center, Reference Lab for Bacterial Meningitis, Amsterdam*

Introduction: In the era of global pneumococcal vaccination, selective pressure on the pneumococcal population may lead to changes in its composition. To monitor these alterations in the population, genotyping tools for large-scale applications are essential. For this,

we compared the multiple-locus variable number tandem repeat analysis (MLVA) with multilocus sequence typing (MLST) using 2 different pneumococcal populations, isolated from patients with invasive disease from Catalonia and the Netherlands.

Methods: The MLST and MLVA were performed as previously described (Enright & Spratt, Microbiology 1998 and Elberse et al. PLoS One 2011). Pneumococcal populations of 163 and 166 strains obtained in Catalan region in Spain and in the Netherlands, respectively, were genotyped. The population included 78 consecutive pediatric strains from Catalan pediatric patients and all Dutch pediatric strains (n = 71) of 2009-2012 and consecutive strains in the age group > 5 year from Catalonia (n = 85) and the Netherlands (n = 95) of 2009-2012.

Results: In total, the MLST and MLVA yielded 126 and 176 types, respectively. The MLST and MLVA performed on the Catalan strains yielded 73 and 92 types, respectively, and on the strains from the Netherland, 70 and 99 types, respectively. Major clones detected with MLST were ST306 (n = 22; 13.5%), ST191 (n = 15; 9.2%) and the multiresistant clone ST320 (n = 10; 6.1%) in Catalonia and ST191 (n = 17; 10.2%, ST306 (n = 15; 9.0%) and ST53 (n = 11; 6.6%) in the Netherlands. The Simpsons indices of diversity were high for both methods (MLST: 96.9% [95%CI: 96.0%-97.8%] and MLVA: 97.8% [95%CI: 97.0%-98.6%]) and comparable between the countries. Remarkably, the probability of 2 Catalan strains having the same MLVA type also sharing the same sequence type was 93.5% according to Wallace coefficient. However, in the Dutch strains this probability was only 59.7%. Differences in the clonality of the populations, for example high clonality in antibiotic resistant Catalan strains such as the high proportion of ST320, compared to the non-resistant Dutch strains, may offer an explanation for this phenomenon.

Conclusions: Both methods yield a high diversity index and congruence between the methods was high. Using MLVA, we could further distinguish highly clonal isolates when grouped by MLST. Although MLST is globally used as gold standard in genotyping of the pneumococcus, the MLVA yield comparable results and is the cheaper alternative.

P043

Validation of real-time-PCR assays for rapid and reliable identification of highly pathogenic bacteria potentially used in bioterrorism

M.C. de Vries, A.C.A. Hendriks, M.J.C. van den Beld, A.S.D. Harpal, E.M. Heerkens, B.J.A. Hoeve, F.A.G. Reubsaet

RIVM, IDS-BPD, Bilthoven

Introduction: The anthrax-letters in the USA in 2001 raised awareness in bioterrorism. The CDC (Centre for Disease Control) has made a list of potential agents to increase clinical and diagnostic alertness and preparation. This list includes bacteria like *Bacillus anthracis, Burkholderia mallei, B. pseudomallei, Francisella tularensis, Yersinia pestis*, and *Brucella* species. These agents, endemic in different parts of the world, occur occasionally as imported infections in The Netherlands. A swift and highly specific detection method for those organisms is essential to act appropriately to the situation or to prevent unnecessary actions and panic. Valuable methods could be MALDI-TOF MS and real-time PCR. Our laboratory validated both tests systematically to ensure their usefulness. The real-time PCR assays were validated as follows.

Methods: Real-time PCR assays were developed targeting more than one virulence gene and/or essential gene per organism.

Measurement trueness and specificity were tested *in silico* and validated using strains from culture collections, of which the identity was confirmed by sequence determination of the 16S-rRNA-gene, and wild type strains, whose identity was verified with conventional biochemical techniques, fatty acid analysis and 16S rDNA-sequencing. Genetically and phenotypically related strains, including possible false positive results based on literature, experience and *in silico* analyses, and the other bioterroristic bacteria were included to determine the specificity of the method. Total measurement trueness and 95% or more specificity was expected from all PCR assays.

Robustness of the assays was increased by using multiple targets per bacterium. Robustness was determined by the number of runs that were approved based on the Ct-values of the positive control compared to the total number of runs. This should be at least 90%. For determining the limit of detection (LOD), a panel of 8 different concentrations of target build in plasmids was setup in at least 8-fold. The LOD was determined by analysing the qualitative outcome of the PCR around the detection limit using Probit regression in SPSS. The LOD should be below the copy number of the target in lysates (about $7x10^8$ copies/reaction).

Results: The number of strains necessary to determine specificity depended on the relatedness with phylogenetic neighbours. For *Brucella* ssp. 7 strains of genetically related bacteria were tested as 29 strains were needed to confirm specificity of *B. anthracis*. However, 23 strains of phenotypically related strains were tested for *Brucella* ssp. and only 2 strains were verified for *B. anthracis*. A number of 16S rDNA sequences of *Burkholderia thailandensis* in Genbank were incorrect as demonstrated by sequence analyses of the same strains by our laboratory. Visualising product length on agarose gel facilitated differentiation of *Francisella tularensis* ssp. *holarctica* from the other subspecies.

All attributes of the assays performed according to the desired standards.

Conclusion: Thorough validation of those RT-PCR-assays confirmed the validity of the tests as fast and accurate methods for confirmation of DNA of *Bacillus anthracis, Burkholderia mallei, B. pseudomallei, Francisella tularensis, Yersinia pestis*, and *Brucella* species in lysates of pure cultures. Future work will be validating those tests in clinical samples.

P044

Cytokine responses of dendritic cells to *Staphylococcus aureus* can be modulated by biomaterials

P.P.S. Balraadjsing[1], E.C. Jong de[1], D.W. Grijpma[2], S.A.J. Zaat[1]
[1]*AMC, Dept. of Medical Microbiology, Amsterdam,* [2]*Twente University, Dept. of Biomaterial Science, Enschede*

Introduction: *Staphylococcus aureus* and *Staphylococcus epidermidis* are the major pathogens in medical device (biomaterial)-associated infection. The combination of a biomaterial and bacteria can provoke inflammatory reactions of non-expected nature. A major cell type orchestrating these immune responses are dendritic cells (DCs). To fully understand the immune responses in biomaterial-associated infection, insight needs to be obtained into maturation of DCs induced by staphylococci, by biomaterials and by the combination. The aim of this study is to assess how *S. epidermidis* and *S. aureus* influence DC cytokine secretion induced by three different materials; poly(L-lactic acid) (PLLA), poly (DL-lactic acid) (PDLLA) and poly(trimethylene carbonate) (PTMC).
Methods: Monocyte-derived DCs were cultured on PLLA, PDLLA or PTMC disks in the presence or absence of *S. epidermidis* or *S. aureus*. The level of cytokines secreted by DCs was assessed. The experiments were performed with cells of different donors.
Results: DCs cultured on PLLA, PDLLA and PTMC did not induce cytokine secretion. DCs cultured with *S. epidermidis* or *S. aureus* in absence of the biomaterials or on PLLA and PDLLA, secreted high levels of IL-10, IL-6 and IL-23. Significantly lower cytokine levels were measured when DCs were cultured on PTMC in presence of *S. aureus* and *S. epidermidis*.
Conclusion: In presence of *S. aureus* or *S. epidermidis* biomaterials differ in their capacity to induce DC cytokine secretion. The effect of the combined presence of biomaterials and staphylococci on DC maturation marker expression is in progress

P045

The KimTest, a simple phenotypic test to assess carbapenemase activity within 8 hours

W.K. van der Zwaluw, A. de Haan, M.E.O.C. Heck, E. Stobberingh, L.M. Schouls
RIVM, IDS-BSR, Bilthoven

Introduction: The emergence and spread of carbapenemase-producing Gram-negative rods is a worldwide emerging public health threat. Resistance to carbapenems is assessed in phenotypic susceptibility assays either on agar plates or in automated microbiology systems. However, high minimal inhibitory concentrations (MICs) do not necessarily reflect the production of carbapenemases. Therefore, PCRs to detect the genes encoding for carbapenemases are often employed. However, these methods can only detect known genes and their variants and the number of variants is expanding rapidly. In contrast, a phenotypic assay may detect carbapenemase activity irrespective of the underlying genetic mechanism. In 2012, Poirel et al. reported a new test to detect the capability of an isolate to hydrolyse carbapenems, the Carba NP test. Even though this method is a huge improvement over methods such as the Modified Hodge Test, the high costs and limited flexibility of this assay triggered us to develop an alternative bioassay designated as the KimTest.
Methods: For the KimTest, a susceptibility disk containing meropenem is immersed in a suspension of a bacterial isolate of interest and incubated for two hours. The disk is then removed from the suspension and placed on an agar plate inoculated with a susceptible indicator strain. After 6 hours of incubation the plates can be inspected. If the bacterial isolate produces carbapenemase, the meropenem in the susceptibility disk is inactivated allowing uninhibited growth of the susceptible indicator strain. Disks incubated in suspensions that do not contain carbapenemase yield a clear inhibition zone. To assess the performance of the assay, approximately 700 isolates submitted to the RIVM for the surveillance of the carbapenemase-producing *Enterobacteriaceae* during the first six months of 2012 and 2013 were tested in the KimTest and in a multiplex PCR that detects the predominant carbapenemase encoding genes (KPC, NDM, OXA-48, VIM and IMP).
Results: The results obtained with the KimTest showed a high concordance to those obtained by PCR and MICs, suggesting high sensitivity and specificity for detecting carbapenemase activity. Carbapenemases were detected in various species of *Enterobacteriaceae* (e.g. *E. coli, K. pneumoniae* and *Enterobacter* spp.), but also in non-fermenters *P. aeruginosa* and *A. baumannii*. The method was shown to be highly robust, being unaffected by changes in variables such as incubation temperature, disk manufacturer, lab staff and the age of the culture or bacterial suspension. Finally, this method was also success-

fully applied in a pilot to detect ESBL activity, using a disk containing cefepime instead of meropenem.
Conclusion: The KimTest is a cheap and robust phenotypic screening method that can yield results within a day. As it requires no complex equipment, reagents or skills, it can be applied in any microbiologic laboratory. All isolates for which there were discrepancies between PCR or MIC and the KimTest are under further investigation e.g. to assess the presence of carbapenemases encoded by genes not covered by the PCR. We are exploring the possible expansion of this method to assess enzymatic hydrolysis of other beta-lactam antibiotics, as demonstrated for cefepime.

P047
Effect of complement factor H deficiency on pneumococcal Infection in mice

E. van der Maten[1], D. Westra[1], S. van Selm[1], H.J. Bootsma[1], M.I. de Jonge[2], F.J.H. van Opzeeland[1], P.W.M. Hermans[1], R. de Groot[1], M.C. Pickering[3], L.P. van den Heuvel[1], N.C. van de Kar[1], M. van der Flier[1]
[1]Radboudumc, Laboratory of Pediatric Infectious Diseases, Nijmegen, [2]Radboudumc, Nijmegen, [3]Imperial College, Centre for Complement and Inflammation

Background: Complement factor H is a key inhibitor of the alternative complement pathway. *Streptococcus pneumoniae* binds human factor H to evade complement activation. However, *S. pneumoniae* is unable to bind mouse factor H, thus the use of murine infection models allows the analysis of the direct effect of complement factor H deficiency on the host response during pneumococcal infection. Importantly, complement factor H deficiency in mice causes secondary C3 deficiency as a result of uninhibited alternative complement pathway activation.
Methods: Wild-type (CfH+/+) and homozygous factor H deficient (CfH-/-) C57BL/6J mice were studied in a nasal colonization model and an intravenous inoculation bacteremia model. The *S. pneumoniae* TIGR4 strain was used in all experiments.
Results: In the colonization model no significant differences in bacterial counts in the nasal wash and nasal tissue were found between CfH+/+ and CfH-/- mice at 48 hours after infection. In the bacteremia model, the CfH-/- mice had higher disease scores, significantly higher serum Il-6 levels and a significantly higher bacterial load in blood compared to CfH+/+ mice at 19 hours post-infection.
Conclusion: In mice, factor H deficiency and the resulting secondary C3 deficiency has no effect on nasal colonization, but results in more severe invasive pneumococcal disease.
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P048
Identifying bacterial stress markers for the evaluation of (new) anti-mycobacterial drugs

M. Boot[1], W. Bitter[1], A.M. van der Sar[1], S. Commandeur[1], A.M. Abdallah[2], K. Chung[1]
[1]VU University Medical Center, Amsterdam, Dept. of Medical Microbiology and Infection Control, Amsterdam, [2]KAUST, Pathogen Genomics Group, Thuwal, Saudi Arabia

Mycobacterium tuberculosis is the causative agent of tuberculosis, an infectious disease that kills over 1.4 million people annually. Treatment of the disease consists of a combination of antimycobacterial drugs: ethambutol, isoniazid, rifampin and pyrazinamide. However, treatment takes a minimum of six months and causes severe side effects in patients. Importantly, a major drawback is the development of multi-drug resistance in strains of *M. tuberculosis*. To overcome this problem, new antibiotics have to be developed or existing compounds have to be tested in screens for their activity against *M. tuberculosis*. Current compound screens are analyzed in a binary fashion;.either a drug kills or inhibits the bacterium, or it remains viable and actively replicating. Monitoring the stress responses towards the compounds in such screens would gain more valuable and informative data as sub-optimal compounds can be qualitatively ranked and optimisation of promising, stress-inducing molecules can be achieved.
In this study we describe a method where we identified genes in *Mycobacterium tuberculosis* that are active upon treatment with sub-lethal concentration of the four first-line antibiotics. RNA sequencing led to the identification and selection of 20 genes that were up-regulated during antibiotic treatment *in vitro*. Promoter regions of these genes were cloned into a plasmid in front of a fluorescent reporter gene. These stress-marker constructs were transformed into *M. marinum*, the causative agent of tuberculosis in fish and frogs, and monitored for fluorescence during treatment with antibiotics. One of the stress constructs, containing a promoter region of the *iniBAC* operon, showed a specific induction upon treatment with ethambutol and isoniazid both on solid and in liquid medium. Furthermore, limited upregulation of the reporter gene was observed for this construct *in vivo* upon zebrafish embryos infection as determined by confocal microscopy, indicating antibiotic specificity. Another construct with the promoter of single-stranded binding (*ssb*) protein showed specific induction upon treatment with second-line antibiotic ciprofloxacin.
In conclusion, at least two of the stress-marker constructs can be used for compound screening *in vitro*, this will allow us to obtain important information about the cellular state of mycobacteria upon compound treatment. Moreover, these two stress markers constructs can be used to identify

specific cell wall stress (p*iniB*) and DNA damage (p*ssb*) that are caused by newly tested compounds. Lastly, this method can be used to study the role of compound-induced stress on population homogeneity, i.e. the effect on general populations or sub-populations of bacteria.

P049

Optimizing growth conditions of *Akkermansia muciniphila*

C.H. van der Ark
Wageningen UR, Dept. of Microbiology, Wageningen

Akkermansia muciniphila is a mucin-degrading intestinal microbe. It has been negatively correlated with gastrointestinal infections, obesity and type 2 diabetes, prompting it to belong to a new generation of therapeutic microbes. A well-defined and cost-efficient medium will enable large scale biomass production of *A. muciniphila*. To reach this goal, the challenges lie in the fact that *A. muciniphila* requires anaerobic conditions and mucin as a substrate. Mucin molecules have abundant O-linked glycosylation, and thereby serve as both N source and C source in the growth of *A. muciniphila*. We have developed new media and culturing approaches that allow the efficient growth of *A. muciniphila*. These have been tested in bioreactor conditions and proven to be able to provide *Akkermansia* with adequate nutrition. This brings the large-scale production of active cells of *A. muciniphila* within reach.

P050

The genome of the ammonia oxidizing archaeon *Nitrosoarchaeum limnia* strain Texel

C.L. Lüke, R. Speth, C.M. Haaijer, H.J.M. op den Camp, S.M. Jetten
Radboudumc, Dept. of Microbiology, Nijmegen

Oxidation of ammonia is the first and rate-limiting step in nitrification. Bacteria performing this reaction are known for more than a century, however, ammonia oxidizing archaea (AOA) were discovered only recently. They form an own phylum, the Thaumarchaeota. They are widespread in nature and outnumber their bacterial counterparts in most environments. Although they seem to be major players in global nitrification, only little is known about their niche differentiation and metabolic capacities. To date, only two AOA isolates and a few enrichment cultures are described. Here, a co-culture highly dominated by an AOA related to *Nitrosoarchaeum limnia* and a nitrite-oxidizing bacterium related to *Nitrospina gracilis* was sequenced using Ion Torrent technology. The culture was obtained from a marine water sample collected in the North Sea near the island Texel. We give first insights into the genome of *N. limnia* Texel.

N. limnia Texel is able to oxidize ammonia via the ammonia monooxygenase. The genes encoding the enzyme are arranged in a cluster, similar to their arrangement in other marine AOA, but different from AOA found in soils. No homolog of the bacterial hydroxylamine oxidoreductase could be detected, however, a variety of copper containing enzymes. Furthermore, genes encoding key enzymes of the 3-hydroxyproprionate/4-hydroxybutyrate pathway of CO_2 fixation were found. The genome of *N. limnia* Texel represents the forth described genome of a *Nitrosoarchaeum* species. They belong to the Marine Group-I, however, show a significant wider environmental distribution than its other members, *Nitrosopumilus* and *Cenarchaeum*. Comparative genomics will enable insights into the different mechanisms of niche adaptation within this closely related group of Thaumarchaeota.

P051

Human IgG does not block binding between the *S. aureus* virulence factor fibronectin binding protein A and fibrinogen

P.M. den Reijer, D. Allouch, H.A. Verbrugh, W.J.B. van Wamel
Erasmus MC, Dept. of Medical Microbiology and Infectious Diseases, Rotterdam

Introduction: The cell-wall associated fibronectin-binding protein A (FnBPA) mediates adhesion of *Staphylococcus aureus* (*S. aureus*) to fibronectin, elastin and fibrinogen. This promotes the colonization and invasion of diverse anatomical sites. It has previously been demonstrated that the fibrinogen-binding A domain of FnBPA differs substantially between *S. aureus* strains at the amino acid level, creating differences in surface-exposed epitopes and affecting immune-crossreactivity in animal models [1]. At the same time all FnBPA A domain isotypes, of which 7 have now been identified, appear to bind fibrinogen with similar affinity. It is currently unknown whether human patients produce isotype-specific antibodies following infection and whether these antibodies can block binding between FnBPA and fibrinogen.

In this study we determine the FnBPA isotype of 22 *S. aureus* strains, isolated from 22 bacteraemia patients, and characterize the FnBPA isotype-specific IgG antibody response of all 22 patients following infection. Furthermore, we investigate whether human IgG, including that of several patients, can interfere with the binding between different FnBPA A domain isotypes and human fibrinogen.

Methods: The FnBPA A domain of all *S. aureus* isolates was sequenced and aligned to 7 previously published, isotype-specific amino acid sequences.

IgG levels against recombinant proteins of 7 FnBPA isotypes were serially measured in patient serum using

a Luminex assay (median 10 samples per patient, interquartile range (IQR) 12), starting from the first positive blood culture (median 16 days, IQR 27 days). To determine the neutralization capacity of FnBPA isotype-specific antibodies, IgG was purified from human pooled serum and from 5 patients infected by strains with different isotypes. Recombinant FnBPA proteins either coupled to beads or directly labeled with R-phycoerythrin (R-PE) were pre-incubated with these antibodies and binding with purified human fibrinogen was measured using a Luminex setup.

Results: In total five different, earlier described FnBPA A domain isotypes were found in *S. aureus* isolates of 22 bacteremia patients. IgG in most patient sera was able to bind the four most common FnBPA isotypes (isotype I-IV). Isotype-specific IgG levels did generally not change significantly in patients following infection, with the exception of two patients who showed a more than 20-fold initial-to-peak increase in IgG specifically for the isotype of the infecting strain.

All recombinant FnBPA isotype proteins were able to bind human fibrinogen in a dose-dependent and saturable manner. This was confirmed by a competition assay using R-PE labeled and unlabeled fibrinogen. The specificity of the FnBPA-fibrinogen binding was confirmed by non-FnBPA protein controls. Interestingly, pre-incubation of all 7 FnBPA isotypes with any purified IgG did not affect the binding of this protein with fibrinogen.

Conclusion: Our results suggest that patients either have circulating IgG, that can bind the most common FnBPA isotypes, or develop *de novo* specific IgG following infection. However, none of these IgG antibodies appears to be able to block the binding between FnBPA and fibrinogen.

Reference

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P052

Assessing the environmental importance of nitrite-dependent anaerobic oxidation of methane

O. Rasigraf[1], D.M. Kool[2], M.S.M. Jetten[1], K.E. Ettwig[1]
[1]*Radboudumc, Dept. of Ecological Microbiology, Nijmegen,* [2]*NIOZ Texel, Marine Organic Biochemistry, Den Burg*

Anaerobic oxidation of methane coupled to nitrite reduction has recently been shown to be performed by a member of the NC10 candidate division, *Methylomirabilis oxyfera*. Though living under anoxic conditions, *M. oxyfera* oxidizes methane via the 'conventional' aerobic pathway starting with particulate methane monooxygenase. This is most probably accomplished by the intracellular dismutation of nitric oxide to molecular oxygen and nitrogen.

Here we report the fractionation factors for carbon and hydrogen during methane degradation and provide evidence for autotrophic carbon dioxide fixation via the Calvin-Benson-Bassham (CBB) cycle by an enrichment culture of *M. oxyfera* bacteria. In two separate batch incubation experiments with different absolute biomass and methane contents, the specific methanotrophic activity was similar and the progressive isotope enrichment identical. The isotope enrichment factors determined by Rayleigh approach were in the upper range of values reported so far for aerobic methanotrophs. In addition, two-dimensional specific isotope analysis ($? = (a_h^{-1}-1)/(a_h^{-1}-1)$) was performed and also the determined ? value was within the range determined for other aerobic and anaerobic methanotrophs. The results showed that in contrast to abiotic processes biological methane oxidation exhibits a narrow range of fractionation factors for carbon and hydrogen irrespective of the underlying biochemical mechanisms. In contrast to aerobic proteobacterial methanotrophs, *M. oxyfera* does not assimilate its cell carbon from methane. Instead, only the CBB cycle of autotrophic carbon dioxide fixation was shown to be complete in the genome, as well as transcribed and expressed. Cell-free extracts from the enrichment culture consisting of ~80% of *M. oxyfera* bacteria exhibited a specific ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) activity of ~0.2 nmol CO_2 mg^{-1} protein min^{-1}. This activity accounted for up to 10% of the total methane oxidation activity. In addition, stable isotope studies with whole cells in batch incubations supplied with ^{13}C-labelled methane and carbon dioxide were performed. The results showed a significant enrichment in ^{13}C of putatively specific *M. oxyfera*-like lipids after the incubation with labeled carbon dioxide and unlabeled methane but not vice versa. Altogether, these results indicate that CBB cycle is active and plays a major role in carbon assimilation by *M. oxyfera* bacteria, and show that autotrophy might be more widespread among methanotrophs than previously believed.

P053

Prediction of clinical status based on intestinal microbial profiling of patients with acute uncomplicated diverticulitis

A. Eck[1], L. Daniels[2], M.A. Boermeester[2], J.A. Bogaards[1], P.H.M. Savelkoul[1], A.E. Budding[1]
[1]*VU University Medical Center, Dept. of Medical Microbiology, Amsterdam,* [2]*Academic Medical Center, Dept. of Gastrointestinal Surgery, Amsterdam*

Objectives: There is increasing evidence of the role gut microbiota play in health and disease. Disease specific variations have been found for a number of intestinal disorders, such as inflammatory bowel disease and obesity.

Yet, little is known about microbiota variations in acute diverticulitis. The aim of this study was to characterize the baseline fecal microbiota composition in patients with a first episode of acute uncomplicated left-sided diverticulitis and to identify potential differences between diverticulitis patients and healthy controls.

Methods: Patients who were diagnosed with acute uncomplicated diverticulitis were sampled by rectal swabs. To obtain gut microbial profiles, we used the IS-Pro technique: a high throughput PCR based profiling technique which combines bacterial species differentiation by the length of the 16S-23S rDNA interspace region with instant taxonomic classification by phylum specific fluorescent labeling of PCR primers.

Results: We compared 31 diverticulitis patients with 25 healthy controls. For the phyla Bacteroidetes and Firmicutes, patients did not display significant differences in their fecal microbiota compared to healthy subjects. However, Shannon diversity of Proteobacteria was significantly higher in patients compared to controls ($p < 0.0001$). Differences in overall bacterial community composition were assessed and principal coordinates analysis (PCoA) revealed that in the Proteobacteria phylum patients were grouped separately from healthy subjects. Moreover, we were able to predict the clinical status of individuals by their Proteobacteria profile using a partial least squares discriminant analysis regression model (PLS-DA). By cross-validation of the model we reached a specificity of 76% and a sensitivity of 90%. In total, 84% of the samples were classified correctly. To summarize the performance of the PLS-DA model by means of predictive power, we used a receiver operating characteristic (ROC) curve.

Conclusions: The above findings indicate that diverticulitis patients demonstrate a dysbiosis signature in their intestinal microbiota, evident in the Proteobacteria phylum. This leads us to consider that specific members of this phylum play a role in the pathophysiology of diverticulitis. These results may further have clinical implications for treatment, as microbial stratification may guide antibiotic regimen and for diagnostics, as these species are potential biomarkers.

P054

Routine screening and typing of multidrug-resistant *Escherichia coli* in a large teaching hospital: monitoring a community-associated problem

P.M. den Reijer[1], S. van Burgh[2], A. Burggraaf[2], J.M. Ossewaarde[2], A. van der Zee[2]

[1]*Erasmus MC, Dept. of Medical Microbiology and Infectious Diseases Rotterdam,* [2]*Maasstad Hospital, Dept. of Medical Microbiology, Rotterdam*

Introduction: Multidrug-resistant *Escherichia coli* are detected at an increasingly alarming rate. Current WIP and NVMM guidelines recommend active screening for multidrug-resistant microorganisms (MDROs). However, in contrast to other MDROs, multidrug-resistant *E. coli* are believed to be mainly community-acquired and the optimal strategy for screening and transmission control remains under debate. More insight into the molecular epidemiology of this organism is needed.

In this study, we genetically characterized all multidrug-resistant *E. coli* identified in a large teaching hospital using an amplified fragment length polymorphism (AFLP) assay and real-time PCR for CTX-M genes. Our aims were to identify possible clonal spread of this organism, to gain more insight into possible transmission routes and to explore whether the source of multidrug-resistant *E. coli* is more likely community- or hospital associated.

Methods: All *E. coli* identified in our laboratory, classified as MDRO according to the NVMM guideline, were routinely typed using AFLP. Additionally, real-time PCR specific for five CTX-M groups was performed. Only the chronologically first isolated MDRO per patient was included.

Several patient characteristics including hospitalization status at time of MDRO isolation and, if applicable, the number of days between hospital admission and isolation were documented. Isolates were divided into two groups: the first group was obtained from non-hospitalized patients or from patients within 72 hours of hospitalization (group I) and a second group of isolates obtained 72 hours or later after admittance (group II).

Results: In a period of five months we identified 236 patientswith multidrug-resistant *E. coli*, of which 75% was hospitalized at the time of isolation. Seventy-eight percent (78%) of the isolates was obtained from rectal screening cultures. The number of days between hospital admission and isolation of the MDRO varied widely from 0 to 86 days (median 6 days, inter quartile range 9 days).

Fifty-five isolates (23%) of all multidrug-resistant *E. coli* showed a unique AFLP fingerprint, while 50 other isolates (21%) were grouped in one large cluster. Forty percent (40%) of isolates of group I contained a unique AFLP fingerprint, while this percentage was 28% for isolates of group II. The majority of isolates in both groups were clustered in small clusters of 2 to maximally 5 identical fingerprints.

Cluster analysis did not reveal distinct grouping of isolates in relation to number of days between hospital admission and strain isolation. The large cluster of 50 isolates contained isolates from both non-hospitalized patients and from patients obtained at variable times after hospital admission.

A CTX-M gene was detected in 137 isolates (58%), with CTX-M group I, including the endemic community-associated CTX-M 15, being the most prevalent (100 isolates, 42%)

Conclusion: We did not identify any clone of multidrug-resistant *Escherichia coli* that was typically isolated after a longer hospitalization period. Rather, our data suggests

that most isolates enter the hospital via a steady flow from the community. We observed one large cluster, possibly indicating the emergence of a clinically significant community-associated clone.

P055

Clinical validation of the Diagenode S-DiaMGTV qPCR kit for the detection of *Mycoplasma genitalium* and *Trichomonas vaginalis*

C.M.C. van Herk, J.H.B. van de Bovenkamp, P.T.W. van Alphen, R.T.J.M. Roymans
PAMM Laboratories, Veldhoven

Introduction: The S-DiaMGTV real time PCR kit from Diagenode is an assay allowing the qualitative detection and differentiation of *Mycoplasma genitalium* (MG) and *Trichomonas vaginalis* (TV) by qPCR in clinical specimens. This study was conducted in order to evaluate the performance of the S-DiaMGTV kit versus our own inhouse TV test and to investigate prevalence of MG. The kit has been tested during a retrospective and a prospective study in our laboratory. The retrospective study compared the results of a collection of selected TV-positive and TV-negative samples obtained with the S-DiaMGTV kit to the historical TV scores obtained with the in house reference method. The prospective study compared the results of samples tested in current routine between May and July 2013 by the reference methods for both microorganisms with the S-DiaMGTV kit.
Methods: For the retrospective study samples consists of 122 urogenital swabs (104 females, 18 males), and 32 urine specimens (9 females, 23 males). For prospective study 352 urogenital swabs (318 females, 34 males) and 183 urine specimens (37 female and 146 males) were tested. In addition, all positive MG samples and a selection of MG-negative samples detected during the prospective studies were tested by a MG-specific reference method and by sequencing in an external reference laboratory (Hpital Purpan, Toulouse, France). All samples were collected from Sexual Transmitted Infections (STI) clinics and general practitioners attendees.
Results: In the retrospective study, the percentage positive agreement of TV scores between the S-DiaMGTV and the reference method for swab and urine specimen study was 100% (91.6%-100%; CI: 95%) and 100% (61.0%-100%; CI: 95%), the negative agreement 97.5% (91.3%-99.3%; CI: 95%) and 100% (87.1%-100%; CI 95%), and the general agreements (Kappa coefficient) 0.96 and 1, respectively. In the prospective study, the S-DiaMGTV kit detected 6 out of 362 swabs TV-positive and 1 out of 183 urine specimens TV-positive. This represents a prevalence of 1.3% for TV. All TV-positives were confirmed by the reference method. In the same study, the S-DiaMGTV kit detected 13 out of 362 swabs MG-positive and 7 out of 183 urine specimens MG-positive. This represents a prevalence of 3.7% for MG. However, one MG-positive swab was not confirmed by the reference method and two could not be retested. After correction, the percentages of positive agreements of MG scores between the S-DiaMGTV and the reference method in the prospective study were 100% (72.3%-100%; CI: 95%) and 100% (64.6%-100%; CI: 95%), the negative agreements 98.0% (95.5%-99.7%; CI:95%) and 100% (94.5%-100%; CI 95%), and the general agreement 0.94 and 1 in swabs and urine specimens respectively. For comparison, the prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoea* in the same population was 6% and 1.1%, respectively.
Conclusion: An almost perfect agreement was shown between the S-DiaMGTV kit and reference methods for *Trichomonas vaginalis* and *Mycoplasma genitalium*. Prevalence of TV in the prospective study showed the same results as *Neisseria gonorrhoea*. However the prevalence of MG was much higher, reaching about 50% of the level of *Chlamydia trachomatis*.

P056

Characterisation of a novel butyrate producing bacterium in human gut

T.P.N. Bui, M. de Vos, M. Plugge
Wageningen University, Laboratory of Microbiology, Bennekom

A novel butyrate producing bacterium, strain *Anaerostipes* 1y-2^T, was isolated from a stool sample of a 1-year old, healthy Dutch infant. Lactate and acetate were used as carbon sources and energy sources for the isolation. The G + C composition of the strain was 44.5% and its major cellular fatty acids were $C_{12:00}$, $C_{19:1}$ iso I and $C_{16:00}$. The strain was Gram-variable, strictly anaerobic, spore-forming. Lactate, formate, butyrate, hydrogen and acetate were major metabolites from glucose fermentation. Both D and L-lactate were used to produce butyrate by strain 1y-2^T. The strain utilised D-galactose, D-fructose, D-mannose, L-rhamnose, dulcitol, D-mannitol, D-sorbitol, D-arabinose, D-maltose, D-saccharose, D-trehalose, xylitol, D-tagatose, D-arabinose but not inulin, laminarin, xylan. Butyrate was detected in all sugar fermentations. The isolate converted rhamnose mainly into 1,2 propanediol with traces of butyrate, acetate, formate. This metabolic trait is unique within the *Anaerostipes* genus. Strain 1y-2^T was phylogenetically closest to *Anaerostipes caccae* DSM14662^T (97% similarity of 16S rRNA) and their DNA-DNA relatedness was 33%. Based on a polyphasic approach, our data clearly show that strain 1y-2^T has distinct chemotypic and phenotypic criteria in comparison to the other *Anaerostipes* species.

P057

YycF/G and other antimicrobial drug targets in the emerging zoonotic pathogen *Streptococcus suis*

A.E. Bem[1], A. de Greeff[2], H.J. Bootsma[3], M.I. de Jonge[3], H. Smith[2], A. Zomer[3], A. Marina[4], P. van Baarlen[1], J.M. Wells[1]
[1]*WUR, Animal Sciences, Wageningen,* [2]*Central Veterinary Institute of Wageningen UR, Lelystad,* [3]*Radboudumc, Dept. of Pediatric Infectious Diseases, Nijmegen,* [4]*Instituto de Biomedicina de Valencia, Valencia, Spain*

Streptococcus suis is a major bacterial pathogen of swine, causing high mortality and economic losses. *S. suis* is considered worldwide to be a problem for pig industry but it is also emerging as an important cause of meningitis in humans in South East and East Asia.
The aim of this study was to identify novel antimicrobial drug targets in a high-throughput genome-wide screen using a mariner transposon mutant library of *S. suis* and identify obligate essential genes. Using transposon sequencing and bioinformatics tools we identified 281 essential genes and investigated their predicted function. Several genes were selected as attractive drug targets based on comparative studies in other pathogens. One of the validated essential targets is YycG, the response regulator of YycFG, the most conserved two-component system (TCS) in Gram-positive bacteria. Two-component systems (TCSs) are the main system for signal transduction in bacteria and are used to efficiently adapt to environmental changes such as temperature, osmolarity, chemoattractants and pH. YycFG proteins were expressed in *E. coli* and purified for *in vitro* assays with inhibitors identified by *in silico* docking of compounds to orthologous protein structures. Ultimately these compounds may be developed into novel antimicrobials to treat *S. suis* infections in young pigs.

P058

A 3-year's experience with a multiplex high-throughput molecular assay for the characterisation of genetic markers in the *Mycobacterium tuberculosis* genome

S. Sengstake[1], I. Bergval[1], A. Schuitema[1], J. de Beer[2], K. Tuin[3], E. Abadia[4], P. KLATSER[1], C. Sola[5], D. van Soolingen[2], R. Anthony[1]
[1]*KIT, Biomedical Research, Amsterdam,* [2]*Centre for Infectious Disease Control National Institute for Public Health and, Tuberculosis Reference Laboratory, Bilthoven,* [3]*MRC Holland, R&D, Amsterdam,* [4]*Venezuelan Institute of Scientific Research, Laboratory of Molecular Genetics, Caracas,Venezuela* [5]*CNRS/UPS11, Institute of Genetics and Microbiology, Orsay, France*

Introduction: Multiple techniques are required to fully characterise *Mycobacterium tuberculosis* (MTB) isolates. This is a concern with regard to costs, turn-around time, skills required and overall efficiency. The MTB genome is quite conserved and consequently genetic markers such as single nucleotide polymorphisms (SNPs) or large sequence polymorphisms (LSPs) are ideal for phylogenetic analysis. In addition drug resistance is exclusively conferred by genomic mutations. The simultaneous detection of pre-identified informative genetic markers in a single method, e.g. whole genome sequencing (WGS), could reduce time and errors and would thereby benefit control measures. As it is currently not feasible to screen the genome of every patient sample by WGS we aimed to develop a molecular tool and analysis strategy that facilitates multiplex but targeted genetic analysis and automated strain identification. The data generated with this method will be fully compatible with future sequence-based screening and typing methods.
Methods: We have developed a multiplex ligation-dependent probe amplification (MLPA) assay for screening 50 genetic markers in the MTB genome simultaneously. Genetic markers were extracted from the literature or derived from previous characterisation of representative strains. Using a liquid bead-based platform as a read-out allows the high-throughput analysis of multiple markers. Experimental data are standardised, streamlined and result in a binary output for each target included (absent/intermediate/present). Lineage types are defined by mutually exclusive markers resulting in profiles that can be checked for consistency.
Results: Within a three-year period we have analysed over a thousand MTB isolates, kindly provided by international partners, from seven countries by MLPA. The assay was also implemented and successfully run in a molecular research lab in Bulgaria and in parallel with the diagnostic work-flow in the country of Georgia. Good agreement was obtained between MLPA and other molecular methods regarding lineage identification. For specific MTB lineages MLPA had a higher discriminatory power. Sensitivity and specificity for drug resistance markers was dependent on the marker targeted. The current algorithm for data analysis identifies the presence or absence of a genetic marker and otherwise flags an intermediate signal. Genetic markers have been continuously adapted to target emerging epidemic TB strains and target novel drug resistance markers.
Conclusion: MLPA allows screening of dispersed genetic markers in the mycobacterial genome. This is of potential interest for the surveillance of drug resistant strains and/or clusters. At present this assay is performed on DNA from cultured isolates but developments in real time assays provide a possibility to optimize the assay toward increased sensitivity and faster turn-around time. MLPA is feasible also for other bacterial pathogens, even those with less conserved genomes as it targets both SNPs and LSPs.

P059

Nitrate-dependent anaerobic oxidation of methane by archaea

B. Zhu, D. Speth, F. Luesken, T. van Alen, B. Kartal, J. Keltjens, H. op den Camp, M.S.M. Mike, K.F. Ettwig
IWWR, RU Nijmegen, Dept. of Microbiology, Nijmegen

The first enrichment culture performing anaerobic oxidation of methane (AOM) coupled to denitrification was constituted for 80% of *Methylomirabilis oxyfera* bacteria and 10% archaea that were named AOM associated archaea (AAA). The culture of *M. oxyfera* and AAA was first assumed to function analogously to the sulfate-dependent AOM performing consortium of sulfate reducing bacteria and anaerobic methanotrophic archaea. However, after prolonged incubation with elevated nitrite the AAA disappeared from the enrichment culture and *M. oxyfera* was shown to be able to oxidize methane without an archaeal partner via an oxygenic pathway, by which NO is suggested to be dismutated into oxygen and nitrogen gas without nitrous oxide as an intermediate. The intra-cellular produced oxygen is used to oxidize methane via the conventional aerobic methane oxidation pathway. In order to study the function of AAA, nitrate and methane were provided as respective electron acceptor and donor, resulting in an enrichment dominated by AAA and *M. oxyfera* in about equal numbers. The enrichment oxidized methane to CO_2, and reduced nitrate to dinitrogen gas and ammonium. Metagenomic and transcriptomic analysis in combination with the physiological results of the community suggested that AAA oxidized methane via reverse methanogenesis and reduced nitrate to ammonium, with intermediate nitrite used by *M. oxyfera* to oxidize additional methane and produce dinitrogen gas. These findings expanded the known diversity of microbial AOM. The capability of performing methane-dependent dissimilatory nitrate reduction to ammonium (DNRA) in AAA forms a new link between nitrogen and carbon cycles. Due to the increasing presence of nitrate in the environments, AAA may also play important role in mitigating methane emissions.

P060

Finalisation of the revision of International Standard EN-ISO 10272 for detection and enumeration of *Campylobacter* in the food chain

W.F. Jacobs[1], E. de Boer[2], I. Jongenburger[2], I. Hansson[3]
[1]RIVM, CIB, Bilthoven, [2]NVWA, Wageningen, [3]SVA, EURL-Campylobacter, Uppsala, Sweden

Introduction: At its systematic review, it was considered necessary to revise ISO 10272:2006 - Horizontal method for detection and enumeration of *Campylobacter* spp. in food and animal feed. Lately, various published studies have recognized several shortcomings. Since the prevalence of extended-spectrum-beta-lactamase (ESBL) producing *Escherichia coli* in sample materials like poultry products is increasing, this results in rising problems to recover *Campylobacter* spp. using the ISO 10272:2006 enrichment method.

CEN/TC275/WG6/TAG19 elaborated the revision of ISO 10272. The first international voting round resulted in the updated version of March 2013, which is described below. This version was also used in the validation studies as asked for by the European Commission Mandate M/381. These validation studies include collaborative trials to establish the performance characteristics of the method. The collaborative trial on enumeration (15 participants) took place in June 2013. The collaborative trial on detection (17 participants) took place in November 2013. Data were generated on 5 different matrices: frozen minced meat (pork/beef), spinach, raw milk, broiler skin and broiler caecal material and calculations are currently performed. After input of the performance characteristics into the document, DIS/ISO 10272 will be ready for final technical and editorial international voting during summer 2014. Official publication of the finalised revised version of EN-ISO 10272 is expected to take place in 2015.

Part 1, Detection: Three different procedures are described, depending on expected numbers of campylobacters and the level of background microflora.

Part 1A: Enrichment in Bolton broth (4-6 h at 37C then 40-48 h at 41,5C), isolation on mCCDA and a second selective medium, with a selective principle different from mCCDA. E.g. cooked or frozen products.

Part 1B: Enrichment in Preston broth (22-26 h at 41,5C), isolation on mCCDA. E.g. raw meats, raw milk.

Part 1C: Direct isolation from sample material or a primary dilution onto mCCDA. E.g. faeces, poultry caecal contents or raw poultry meat.

Like for agar plates, all enrichment broths have to be incubated in microaerobic atmosphere.

Part 2, Enumeration: Plating on mCCDA for enumeration (in single, ISO 7218:2007) as before.

Part 3, Semi-quantitative estimation: ISO/TS 10272-3 was first published in 2010 and describes estimation of the *Campylobacter* level based on qualitative detection in a single range of ten-fold dilutions of a sample in Bolton broth. However, at the periodical review in 2013 it was decided to withdraw this part of the standard as this method is not reportedly being used.

Part 4, Samples from primary production: Originally, samples from primary production were to be described in a separate part 4, but the enlargement of the general scope of the method enabled these type of samples to be taken up into parts 1B and/or part 1C.

Confirmation: Confirmation tests in all parts of the standard are harmonised to include microscopic

examination (directly from the blood agar after purification), detection of oxidase and absence of aerobic growth at 25C. Alternatively, PCR tests or serological methods may be used. Optionally, *Campylobacter* species are identified by specific biochemical tests (catalase, hippurate hydrolysis, indoxyl acetate).

P061

Study of the performance of the Unyvero P50 Pneumonia application in broncho-alveolar lavage fluid.

H.A. van Dessel, I.H.M. Loo, van, P.H.M. Savelkoul, J.H.J. Maes, M.J. Mooij

University Hospital Maastricht, Dept. of Medical Microbiology, Maastricht

Introduction: The aim of the project is to test the performance of the Unyvero P50 Pneumonia application in broncho-alveolar lavage fluid (BALF) samples. The test combines all molecular testing steps in a disposable cartridge, targets 17 bacteria that cause pneumonia and 18 resistance gene markers, and can be used directly on relevant clinical samples.

Methods: Since 1997, BALF from patients admitted to the Maastricht University Medical Centre (MUMC) are performed and processed in a highly standardized way. Quantitative culture and differential cell count are routinely performed on all BALF samples. Other tests are performed on request. All BALF samples are stored in -80C. The performance of the Unyvero system was compared to results of quantitative culture, differential cell count, PCR or Groccot and Giemsa stain, depending on the target. For 'culturable' targets, sensitivity and specificity was evaluated by comparing results of the test with quantitative culture and/or differential cell count. Since microbiologically confirmed ventilated-associated pneumonia (VAP) is well defined as a quantitative culture of BALF which exceeds 10 colony forming units per ml (CFU/ml) and/or as a differential cell count with > = 2% cells that contain phagocytised organisms, the clinical cut-off value of the Unyvero system in BALF for diagnosis of VAP was evaluated.

Results: From 1997-2012, 3362 BALF samples were collected. The database was searched for samples positive for the Unyvero system targets. 'Culturable' targets are *Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter species, Stenotrophomonas maltophilia, Enterobacter sp., Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, Proteus sp., Serratia marcescens, Enterobacteriaceae, Streptococcus pneumoniae, Moraxella catarrhalis, Haemophilus influenza and Morganella morganii.* For each of these, five positive BALF specimens were selected of which three contain the target in > = 10 CFU/ml andtwo in < 10 CFU/ml.Three samples, positive for *Legionella pneumophila* by PCR were selected, as well as three positive for *Chlamydophila pneumonia* by PCR. For *P. jirovecii*, three samples positive by microscopy and PCR were selected as well as two samples only positive by PCR. Preliminary results based on three targets tested showed that micro-organisms at a concentration of < 10 CFU/ml in BAL fluid are not detected using the Unyvero system, apart from *Acinetobacter species* which is also detected at a lower concentration. Bacteria present in all specimens that contain > = 10 CFU/ml are detected using the molecular testing system. In addition, the Unyvero system also detects several antibiotic resistance genes, which will be confirmed by other methods. Results of the remaining targets will be available in the coming weeks.

Conclusion: Fast molecular testing systems become available as a tool to fasten the diagnosis of severe infections. The MUMC BALF collection is an ideal collection for clinical validation of such tests for the diagnosis of respiratory infections, especially VAP, since it contains more than 3000 samples, all performed, processed and stored in a highly standardised way. Preliminary results on three targets show promising results for the diagnosis of VAP.

P062

Pectin-derived acidic oligosaccharides improve *P. aeruginosa* infection outcome through Th1 polarization by modulation of the intestinal microbiota

L.P.J. Kleinjans[1], H. Bernard[2], N. Bartke[3], J.L. Desseyn[2], C. Belzer[1], J. Knol[1], F. Gottrand[2], M.O. Husson[2]

[1]Wageningen University, Laboratory of Microbiology, Wageningen, [2]Université Lille Nord de France, Unité Inserm 995, Faculté de médecine, Lille,France [3]Nutricia Research, Utrecht

Patients suffering from Cystic Fibrosis (CF) have an abnormally thick mucus layer that cannot exert its normal function of clearing bacteria. *Pseudomonas aeruginosa* is a major cause of chronic and often fatal broncho-pulmonary infection in CF patients. Severe *P. aeruginosa* pulmonary infections are associated with a Th2 predominant immune response. While a Th1 response might be more favourable. An increase in immunogenic Lactobacilli and Bifidobacteria in the intestinal microflora has been shown to be able to induce Th1 skewing. The abundance of these bacteria can be augmented by the administration of prebiotics such as pectin-derived acidic oligosaccharides (pAOS).

In this study we administered pAOS in order to improve *P. aeruginosa* infection outcome through Th1 polarization by modulation of the intestinal microbiota. Balb/c mice receiving a 5% pAOS-supplemented diet for 5 weeks prior to *P.aeruginosa* lung infection had a significantly changed intestinal microbiota composition compared to animals receiving a control diet. The phylogenetic

microarray MITchip' revealed that it takes about 3 weeks for the microbiota to adjust to the pAOS diet, and that *Allobaculum sp, Bacteroides vulgatus, Bifidobacterium sp, Ruminococcus obeum* and *Sutterella wadsworthia* and *Unclassified Clostridiales XIVa* – close to *Anaerostipes caccae* are significantly higher abundant in the pAOS fed mice. This shift in microbiota composition was accompanied by Th1 polarization, M1 macrophage activation and improved bacterial clearance during the first and second infection.
In summary, these results provide new grounds for the development of prebiotic strategies to modulate the immune system and to improve *P. aeruginosa* infection outcome in CF patients.

P063

Prediction of carriage with ESBL-producing bacteria at hospital admission; a cross-sectional study.

T.N. Platteel[1], J.W.T. Cohen Stuart[2], M.A. Leverstein-van Hall[3], E. Mascini[4], S. Thijssen[5], B. van Hees[6], J. Scharringa[1], A. Fluit[1], M.J.M. Bonten[1]
[1]UMC Utrecht, Dept. of Medical Microbiology, Utrecht, [2]MCA, Dept. of Medical Microbiology, Alkmaar, [3]Bronovo, Dept. of Medical Microbiology, Den Haag, [4]Rijnstate, Dept. of Medical Microbiology, Arnhem,[5]Diakonessenhuis, Dept. of Medical Microbiology, Utrecht,[6]Gelre ziekenhuizen, Dept. of Medical Microbiology, Apeldoorn

Objectives: With the rising incidence of ESBL-infections, identification of ESBL-carriage hospital admission is becoming more important for taking appropriate infection control measures and empirical therapy in case of infection. Identification from risk groups is necessary to identify ESBL-positive patients shortly after admission. Long-term care facilities (LTCFs) seem the ideal setting for accumulation of multiresistant bacteria with their relatively high antibiotic use, shortage on personnel and often few possibilities to undertake hygiene measures.
Aim of this study was to develop a prediction rule for ESBL-carriage at hospital admission based on previously identified risk factors. Besides, we wanted to determine whether patients from LTCFs in the Netherlands are indeed more often carrying ESBL-positive bacteria at admission, making them a possible target for screening at admission. Also, we estimated the risk of developing an infection with ESBL-positive bacteria during admission for carriers, compared to non-carriers.
Methods: A cross-sectional study was conducted in 4 hospitals in 3 regions in the Netherlands from January 2010 until December 2012, including all patients admitted from LTCFs in 4 internal medicine and surgical wards per hospital. Furthermore, patients admitted (> 18 yrs) from the community were included in 3 defined periods in each hospital.
A perianal swab was obtained within 48 hours after admission, which was inoculated on MacConkey agar and ESBL Brilliance plates (Thermo Fisher Scientific, UK). ESBL was confirmed by ESBL-microarray (Check-Points, Wageningen). Information was collected on antibiotic use, immunosuppressive therapy, previous surgery and hospital admissions, presence of external devices, travelling abroad and occupation. Medication use was confirmed with pharmacy databases. Univariate and binary logistic regression analysis were performed using SPSS 20. Missing data were replaced by multiple imputation.
Results: 1.351 patients were included; 579 (42.9%) from LTCFs and 772 (57.1%) admitted from the community. Patients were admitted from over 175 LTCFs. ESBL-carriage at admission was demonstrated in 109 patients (8.1%); 49 (8.5%) among admissions from LTCF and 60 (7.8%) from the community. The crude OR for ESBL-carriage at admission for the comparison of patients from LTCFs to patients from the community was 1.09 (95%CI 0.97-1.22). In multivariate analysis identification of ESBL-carriage within 1 year before admission, use of second-generation cephalosporins or broad-spectrum penicillins and hospital admission in the previous 6 months were identified as predictive factors. Age, gender and patient origin (LTCF or community) were added to the multivariate analysis. The adjusted OR for patients from LTCFs to patients from the community was 1.09 (95%CI 0.69-1.74). With these factors the AUC of the best possible prediction rule was 0.65 (95%CI 0.59-0.71). Carriers of ESBL-positive bacteria at admission had an infection-rate of 48.25/10000 admission days (95% CI 12.98/10000-123.5/10000), as compared to 1.678/10000 admission days (95%CI 0.1885/10000-6.058/10000) for non-carriers ($p < 0.001$).
Conclusion: It was not possible to reliably predict ESBL-carriage at hospital admission using previously identified risk factors. Prevalence of ESBL-carriage was comparable for patients admitted from the community or from LTCFs. Carriers of ESBL-positive bacteria have an increased risk of developing infections with ESBL-positive bacteria during admission, as compared to non-carriers.

P064

Microbial uptake of phosphate during anaerobic oxidation of methane

J. Graf, J. Milucka, T. Ferdelman, M. Kuypers
Max Planck Institute for Marine Microbiology, Dept. of Biogeochemistry, Bremen, Germany

Sulfate-coupled anaerobic oxidation of methane (AOM) is a major sink of methane in the ocean and plays an important role in sedimentary biogeochemical cycling of carbon and sulfur.[1] The *Deltaproteobacteria* (DSS) associated with the methanotrophic archaea (ANME) are capable of polysulfide

disproportionation and couple the carbon and sulfur cycles during AOM.[2] Investigations using transmission electron microscopy and energy-dispersive X-ray analysis (TEM-EDX) showed amorphous particles enriched in iron and phosphorus in the Deltaproteobacterial partner.[2] The function of these particles is yet unknown.

We investigated microbial uptake of phosphate during AOM using $^{33}P_I$ radiotracer experiments. In our studies we used highly enriched AOM cultures originating from sediments of the Mediterranean mud volcano Isis. These sediments were enriched during 9 years of continuous cultivation and consist mainly (> 95%) of ANME-2 and DSS cells as determined by CARD-FISH.[3] Radiotracer incubations on our AOM cultures showed a time-dependent accumulation of $^{33}P_I$ tracer in the biomass in the presence methane. This was accompanied by a corresponding decrease of $^{33}P_I$ in the medium In controls without methane $^{33}P_I$ uptake was negligible. Interestingly, the accumulation of $^{33}P_I$ in the biomass linearly correlated with the rate of sulfate reduction. The specific activity of $^{33}P_I$ in the medium decreased (phosphate concentration remained constant). Our data show that phosphate turnover during AOM can reach up to ~0.2% of the sulfate reduction rate. A substantial amount of the ^{33}P radiotracer (~7%) was recovered in the iron-bound fraction as determined by a sequential extraction of phosphorus (SEDEX) of the biomass. Without methane we only recovered a minor fraction of the ^{33}P radiotracer (~0.5%) in the Fe-bound fraction. It remains unclear if the Fe-bound phosphate fraction corresponds to the iron- and phosphorus rich intracellular particles found in the polysulfide disproportionating bacteria.

The observed rapid cycling of soluble and particulate phosphate fraction, most probably Fe-bound, suggests that AOM actively influences the phosphorus cycling in our AOM enrichment culture, and potentially in anoxic AOM-dominated sediments.

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P065

Development of novel Synthetic Antimicrobial Antibiofilm Peptides (SAAPs) using the thrombocidin-1 derived peptide L3 as a scaffold

P.H.S. Kwakman[1], L. de Boer[2], A. de Breij[2], P.H. Nibbering[2], J.W. Drijfhout[2], S.A.J. Zaat[1]

[1]AMC, Dept. of Medical Microbiology, Amsterdam, [2]LUMC, Leiden

Infection related to inserted or implanted medical devices ('biomaterials') is a major problem. These infections are mainly caused by staphylococci, which can form biofilms on these devices and are able to survive in tissue surrounding the implanted biomaterial. Biofilms are notoriously difficult to treat with antibiotics and there currently is no effective strategy to control biofilm formation. The EU consortium Biofilm Alliance (BALI) performs multidisciplinary research to design Synthetic Antimicrobial Antibiofilm Peptides (SAAPs) and a release system, which can be applied to the surface of biomaterials as a coating. In this study we aimed to develop novel SAAPs with optimized *in vitro* antimicrobial, anti-inflammatory and antibiofilm activities using peptide L3, a 15 amino acid peptide derived from thrombocidin(TC)-1, the major antimicrobial peptide of human blood platelets, as a scaffold. Twenty-three peptides were synthesized with substitutions throughout the peptide sequence of L3. We defined *in vitro* microbicidal activity in phosphate buffered saline (PBS) and in the presence of 50% human plasma in PBS as decisive for the therapeutic potential of such peptides. We determined the concentration of SAAPs required to kill at least 99.9% of the inoculum (lethal concentration; LC99.9) of the clinical isolate *Staphylococcus aureus* JAR060131 within 2 hours. While peptide L3 and most of the novel variants had LC99.9 values of > 60 M in the presence of PBS or 50% human plasma, peptide TC19 had substantially improved activity compared to L3 with LC99.9 values of 8 M and 15-60 M in PBS and in 50% plasma, respectively. The LC99.9 values of TC22 and TC23 in PBS were 60 M and 30-60 M, respectively, but these peptides lacked bactericidal activity in the presence of 50% plasma. In a more in-depth analysis, TC19 demonstrated activity at low-micromolar concentrations against a broad spectrum of microorganisms such as *Staphylococcus epidermidis, Enterococcus faecium, Pseudomonas aeruginosa, Escherichia coli, Candida albicans* and *Aspergillus niger,* including strains resistant to conventional antibiotics. The LC99.9 value of TC19 against *S. aureus* JAR060131 was not affected by a 2 hours pre-incubation of peptide in plasma, indicating that TC19 is not is not affected by plasma components. *In vitro* biofilm formation by *S. aureus* JAR060131 was reduced by > 50% and > 75% in the presence of 12.8 and 25.6 M TC19, respectively. Using *in vitro* whole blood assays, the concentration of TC19 required to reduce cytokine production upon stimulation with *S. aureus* or LPS by at least 50% (inhibitory concentration; IC50) was determined as a measure for anti-inflammatory activity. The IC50 for inhibition of IL-8 production after stimulation with 2.5xE06 CFU/ml of UV-killed *S. aureus* was 195 nM TC19, and the IC50 for reduction of IL12p40 production after stimulation with 1.25 ng/ml LPS was as low as 0.03 nM peptide. In conclusion,

peptide TC19 has broad spectrum antimicrobial activity in PBS and in 50% human plasma, and has potent antibiofilm and anti-inflammatory activity, indicating that it is a promising candidate to combat biomaterial-associated infection.

P066

Evaluation of the SERION, LIAISON and Luminex immunoassays for the detection of herpes simplex virus IgG antibodies

M.C. Wong, H.J. Gerritsen, A.C.T.M. Vossen, J.J.C. de Vries
LUMC, Dept. of Medical Microbiology, Leiden

Introduction: In an attempt to further automatize our Herpes Simplex Virus (HSV) IgG detection method, we compared the performances of the SERION HSV 1/2 IgG enzyme immunoassay (EIA) with the automated LIAISON HSV 1/2 IgG EIA and the semi-automated Luminex HSV 1 and 2 IgG assay.
Methods: The SERION *classic* HSV 1/2 IgG ELISA performed on the pipetting robot DSX, the DiaSorin LIAISON HSV 1/2 IgG assay, and the Zeus Scientific AtheNA Multi-Lyte HSV 1 & 2 IgG Plus assay were partially prospectively tested using 290 consecutive serum samples sent to the medical microbiology laboratory of the Leiden University Medical Center for HSV diagnostics from March 2009 till May 2009. Specimens showing contradictory results were repeatedly tested in the SERION and LIAISON HSV 1/2 assays and additionally analyzed with immunofluorescence (Focus Diagnostics' HSV IgG immunoblot HerpeSelect 1 and 2) and the DiaSorin LIAISON HSV 1- and HSV 2-type specific IgG EIA's.
Results: The overall agreement between the SERION HSV 1/2 IgG and LIAISON HSV 1/2 IgG assays was 91% and 96% when equivocal results were excluded. The percentage of equivocal test results was 2,4% for the SERION EIA, and 2,8% for the LIAISON EIA. Additional testing of the 19 discordant samples did not display a clear pattern to explain the discrepancy between both assays but they were generally very low positive and mainly from patients that had recently received blood products. The reproducibility of the SERION EIA performed on the pipetting robot DSX was 89%, while the reproducibility of the LIAISON EIA was 100%. When considering samples true positive if tested positive in at least 2 different assays, the sensitivity of the SERION, LIAISON and Luminex assays were 92-98%, the specificities were 75-95%.
Conclusion: These findings suggest that the LIAISON HSV 1/2 IgG EIA and the Luminex HSV 1 and 2 IgG assays have at least similar performances as the SERION HSV 1/2 IgG EIA.

P067

Yersinia ruckeri, an unusual infectious agent in human

S.D.K. de Keukeleire[1], A. de Bel[1], Y. Jansen[1], M. Janssens[2], G. Wauters[2], D. Piérard[1]
[1]*UZ Brussel, Laboratory of Microbiology, Brussel, Belgium,* [2]*Université Catholique de Louvain, National Reference Center for Yersinia, Brussels*

Introduction: *Yersinia ruckeri* was first described as the causative agent of enteric red mouth disease of freshwater and marine fish, especially known in rainbow trout (*Oncorhynchus mykiss*). The bacterium is shed in the feces of infected fish and the disease can be transmitted by water. We report the first case of Y. ruckeri in a 16-year-old male, presenting as a wound infection after hitting a stone while paddling in a river.
Methods: Wound swabs were cultured on MacConkey agar, TSA based medium enriched with 5% horse blood with 20 mg/L hemin (Hem side) or 40 mg/L nalidixic acid (NaI side) (Hem/NaL agar) and on an anaerobic agar enriched with 5% defibrinated horse blood (ANA). Identification took place using Matrix -assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Identification was confirmed by 16S rRNA sequencing at the National Reference Centre (NRC) for *Yersinia*. Phenotypic characteristics were determined using conventional methods. Susceptibility testing was performed on Mueller- Hinton agar by the EUCAST methodology.
Results: After 24 hours of incubation, cultures of the infected wound yielded a mixed culture. MALDI-TOF MS analysis of each colony type by direct transfer method and in combination with database version 3.3.1.0 (Bruker, Daltonik GmbH) and security relevant (SR) database revealed the presence of *Aeromonas spp., Lactobacillus spp., Clostridium perfringens* and *Yersina ruckeri*. The latter showed small non pigmented circular colonies on MacConkey agar and Hem/Nal agar and flat to convex colonies on anaerobic agar. The MALDI-TOF MS log score was: best match *Yersinia ruckeri* 1.969 and second best match *Yersinia pestis* log score 1.887. The identification was confirmed by the NRC *Yersinia*.
The strain fitted the general properties of Enterobacteriaceae, eg.: oxidase negative, catalase positive fermenting gram negative rods. Growth was optimal between 25C and 30C. Glucose and mannitol were acidified without gas production, but sucrose, xylose, rhamnose and melibiose were negative. Urease, esculin hydrolysis, indole and H2S production and VP reaction were negative. Simmons'citrate was delayed positive. In contrast to almost all other Yersinia species, lysine decarboxylase and gelatin hydrolysis were positive. It was sensitive to ampicillin, amoxillin/clavulanic acid, piperacillin/tazobactam,

temocillin, cefazolin, cefuroxime, ceftriaxone, ceftazidime, ceftazidime/clavulanic acid, cefepime, cefepime/clavulanic acid, meropenem, aztreonam, gentamicin, amikacin and ciprofloxacin. After three days of intravenous treatment with amoxicillin/clavulanic acid, the wound evaluated very favorably, the treatment was switched to an oral therapy and the patient discharged. At follow-up consultations at days 7 and 17, complete healing of the wound was observed.

Conclusion: Curiously, *Yersinia ruckeri* infections are only described in fish populations, as the causative agent of yersinosis or enteric red mouth disease of freshwater and marine fish. *Yersinia ruckeri* is able to survive and remains infective in an aquatic environment, mainly associated with poor water quality. This case report underlines the high discriminative power of MALDI-TOF MS and its utility for the identification of unusual causative agents. To the best of our knowledge, infection with *Yersina ruckeri* was never described in human.

P068

Multicenter study for evaluation and comparison of different methods for detection and/or differentiation of *Shigella* and *EIEC*

M.J.C. van den Beld[1], F.A.G. Reubsaet[1], R.F. Boer, de[2], J.W.A. Rossen[3], A.M.D. Kooistra-Smid[2], A.W. Friedrich[3]

[1]*RIVM, Infectious disease research, Dept. of Diagnostics and Screening, Bilthoven,* [2]*Certe, Dept. of Medical Microbiology, Groningen,* [3]*University Medical Center Groningen, Dept. of Medical Microbiology, Groningen*

Shigella and *Escherichia coli* are genetically closely related and should be classified as one species within the genus *Escherichia*. However, for clinical reasons, the disposition of *Shigella* and *E. coli* into two different genera is maintained. In general, *Shigella* can be distinguished from *E. coli* based on its physiological and biochemical characteristics, but this is not the case for Entero-invasive *E. coli* (EIEC). EIEC has some of the *E. coli* biochemical characteristics, while demonstrating the pathogenic behavior of *Shigella*. In addition, EIEC and *Shigella* both harbor the *ipaH*-gene, a multicopy gene situated on a large virulence plasmid and the chromosome. It is frequently used as target for detecting *Shigella* directly in feces, but it cannot distinguish *Shigella* from EIEC. *Shigella* infections are mandatory reportable towards health authorities in The Netherlands, while an infection caused by EIEC is not. Therefore, it is of high importance to differ between the two micro-organisms. Three different approaches are taken to develop a discriminating assay. The first is the development of a specific molecular assay based on comparing full genome sequences of EIEC and *Shigella* strains. The second approach is the use of a diagnostic screening algorithm using both molecular and culture methods, consisting of serogenotyping for the most prevalent serotypes of *Shigella*, after screening for the *ipaH*-gene in feces. In addition, *ipaH*-gene positive feces are cultured on selective media, colonies and plate streaks suspected to be *Shigella* or EIEC are confirmed with serogenotyping and classical serotyping with *Shigella* sppand EIEC polyvalent 7 and 8 antisera. The third approach is using an algorithm to differentiate between cultured *Shigella*, EIEC and non-enteroinvasive *E. coli* based on a PCR of the *ipaH*-gene in combination with biochemical and serological features. A well-defined set of *Shigella* and EIEC strains (in total approximately 90-100 strains) was selected and used to compare and evaluate the different methods.

P069

Natural course of *Chlamydia trachomatis* bacterial load in anogenital samples in the interval between screening and returning for treatment

J.A.M.C. Dirks[1], C.D.J. den Heijer[2], M. Franssen[1], N.H.T.M. Dukers-Muijrers[2], G.A.F.S. van Liere[2], P.F.G. Wolffs[1], C.J.P.A. Hoebe[2]

[1]*Maastricht University Medical Center, Dept. of Medical Microbiology, Maastricht,* [2]*Public Health Service South Limburg, Dept. of Sexual Health and Infectious Diseases, Geleen*

Introduction: Chlamydia trachomatis (CT) is the most common bacterial sexually transmitted infection (STI) worldwide, affecting over 5% of the general population in the Western world. Spontaneous clearance of CT-infections, without antibiotic treatment, has been reported after a year in > 50% of patients. This study investigated the natural course of the bacterial load in the interval between screening and returning for treatment as a possible factor in the clearance of CT.

Materials and methods: A total of 230 Ct-positives (32% men) were included from the STI-clinic in South Limburg in the Netherlands, with 31 (13%) anal-, 64 (26%) urine- and 153 (62%) vaginal infections. Quantitative PCR targeting both CT (omp-A gene) and eukaryotic cells (HLA-gene) was performed for two samples per location per patient; one diagnostic sample and one sample taken before receiving treatment. Statistical analyses were performed to investigate differences in the initial load of cleared and persisting CT infections and factors associated with clearance (sex, age, and time between samples). Moreover, the course of CT load in persisting CT infections was studied. Analysis are presented per sample type.

Results: Approximately 8% of all anogenital CT infections had been cleared in the sample before treatment, mostly in anal samples. In all three specimen types, the CT load

in the diagnostic sample was significantly ($p < 0.001$) lower in samples cleared of their CT infection, than those in which CT persisted. CT load in the initial sample was predictive of clearance only in vaginal samples ($p < 0.001$), most likely due to modest power in the other sample types, as a similar trend was seen in anal samples. Neither sex, age nor the time between samples (range: 3-35 days), was predictive of clearance. In the CT-persisting samples, no significant difference in CT load was seen between the two samples for genital samples. In anal samples a mean 0.5 log decrease in load was seen ($p = 0.04$). Load differences between samples was unrelated to sex, age, or the time between sampling.

Conclusion: 1) These preliminary results indicate that the bacterial load is an important factor in CT clearance, with a low CT load being predictive of clearance. 2) Clearance was not related to the age or sex of the patient, and unrelated to a larger time interval between samples. 3) In the case of persisting CT, the bacterial load appears stable over time. 4) The load difference between samples is unrelated to sex, age, or the time between samples. Further research, with the inclusion of more patients, is indicated to further elucidate the relation between the bacterial load and clearance.

P070

Metagenomic characterization of a full-scale nitritation/ anammox plant for nitrogen removal

D.R. Speth, M. In 't Zand, S. Guerrero Cruz, B.E. Dutilh, M.S.M. Jetten

Radboudumc, Dept. of Ecological Microbiology, Nijmegen

Anaerobic oxidation of ammonium (anammox) is a recently discovered process combining nitrite and ammonium to yield dinitrogen gas. When combined with partial nitritation, it can be used to efficiently remove ammonium from wastewater. This process is much cheaper then traditional nitrification/denitrification due to lower aeration costs and smaller installations. Although full-scale wastewater treatment plants employing this process are already in operation for over 5 years, the microbial community in these systems has never been thoroughly characterized.

Here we employ shotgun metagenomic sequencing to gain insight in the microbial community of the nitritation/ anammox reactor located at a wastewater treatment plant in Olburgen, the Netherlands. The dominant organisms were, as expected, a *Nitrosomonas europaea* strain and a *Candidatus* Brocadia sp.' accounting for the nitritation and the anammox activity respectively. Next to these organisms, abundant groups were *Anaerolineales* and *Sphingobacteriales*. Of these organisms draft genomes could be obtained. Additionally, a large number of sequences could be assigned to ciliates of the subclass *Peritrichia*. Further experiments will be required to establish if and how such organisms affect the system.

P071

Chlamydia trachomatis plasmid copy number in anogenital samples

J.A.M.C. Dirks[1], T.H.B. Geelen[1], N.H.T.M. Dukers-Muijrers[2], C.J.P.A. Hoebe[2], P.F.G. Wolffs[1]

[1]*Maastricht University Medical Center, Dept. of Medical Microbiology, Maastricht,* [2]*Public Health Service South Limburg, Geleen*

Introduction: Bacterial plasmids are extrachromosomal genetic elements that are capable of self-replication. They are generally present at stable copy numbers, which range from one to several hundred per cell. Several classes of plasmids exist, each conferring their own advantage to their host cell survival. Chlamydia trachomatis (CT) is an intracellular bacterium, showing high levels of conservation among strains, both of the chromosome and the cryptic plasmid. The plasmid is multifunctional, with effects on both the regulation chromosomal gene expression and virulence. In fact, plasmidless strains are rare and exhibit a diminished virulence. Reports show that the number of CT plasmids in laboratory strains is stable and ranges from 4-10 plasmid per bacterium. However, Pickett et al. (2005) demonstrated that CT can increase its plasmid numbers, paradoxically, when under stress. We investigated whether and how the natural situation in its host is stressful for CT and how the plasmid number correlates to this.

Materials and methods: Retrospectively, 1019 anogenital samples from the sexually transmitted infection (STI)-clinic in South Limburg (2010-2012) and the Dutch population-based screening were investigated for plasmid-per-bacterium ratios. The 1019 samples assessed (74% female; 42% STI-clinic) consisted of 719 cervico-vaginal swabs, 275 urine samples and 25 anal samples. Quantitative PCR targeting both the plasmid and chromosomal DNA (omp-A gene) was used to calculate the plasmid:bacterium ratio. For statistical analyses, loads were log-transformed to allow for parametric tests. Independent sample t-test and ANOVA were used to compare plasmid ratios between gender and sample-types.

Results: Plasmid-ratios ranged from 0.0002-57063. However, 708 samples (70%) had a ratio between 1 and 35. Stratified analysis showed a significantly higher plasmid-ratio in male urine (mean log. 1.6) than female urine (mean log. 1.3) ($p < 0.05$), but a non-significant trend in anal samples (mean log. men 1.8, women 1.6).

Anal samples contained significantly more plasmids per bacterium (mean log. 1.8) than cervicovaginal swabs (mean log. 1.3) ($p < 0.001$), but no more than in urine (mean log. 1.5).

Conclusion: 1) Plasmid-per-bacterium ratios in CT are much higher in clinical anogenital samples than is thus far reported in lab-cultured strains. It may be postulated that the human body is a stressor for CT which in turn upregulates its plasmid numbers. Currently, studies are being undertaken to investigate this process in further detail.

P072

Surface behaviour of *S. Typhimurium*, *S. Derby*, *S. Brandenburg* and *S. Infantis*

G.A.A. Castelijn, J. Parabirsing, M.H. Zwietring, R. Moezelaar, T. Abee

Wageningen University and TIFN, Wageningen

Cross-contamination due to *Salmonella* on the surface of processing equipment greatly contributes to contamination of pork products. Therefore, a clear understanding of surface and survival behaviour of relevant *Salmonella* serovars in pork processing environments is needed to develop better strategies for *Salmonella* control. Within this study the biofilm forming behaviour *S*. Typhimurium, *S*. Derby, *S*. Brandenburgand *S*. Infantis isolates was analyzed using the crystal violet assay. This assay, commonly used to analyse total biofilm formation, revealed variation in biofilm forming capacity between and within serovars. This has not been shown before for *S*. Derby, *S*. Brandenburgand *S*. Infantis. From each serovar, isolates with different biofilm forming capacity were selected to analyse biofilm formation on stainless steel. This revealed no significant differences between biofilm formation on polystyrene compared to stainless steel. Furthermore a relation was observed between biofilm forming capacity of an isolate and survival on stainless steel surfaces. On such surfaces, biofilms showed greater and longer survival than planktonic cells, and they were less susceptible to peracetic acid disinfection treatments. However, the latter effect was marginal and only observed in the presence of organic material, which drastically decreased the activity of peracetic acid. With the obtained results also a hierarchical cluster was performed to identify differences and similarities between the four different serovars. This indicated that the surface behaviour of *S*. Typhimurium was more comparable to *S*. Infantis than to *S*. Derby or *S*. Brandenburg.

P073

Isolation and characterization of resistant variants from *Salmonella* Typhimurium cell cultures treated with benzalkonium chloride

G.A.A. Castelijn, S. van der Veen, R. Moezelaar, T. Abee, M.H. Zwietering

Wageningen University and TIFN, Dept. of Food Microbiology, Wageningen

An efficient disinfection strategy within the food industry is needed to ensure food safety and prevent the spreading of foodborne pathogens such as *Salmonella* Typhimurium. However, several factors affect the efficiency of disinfection treatments resulting in low level exposure of the bacterial flora facilitating survival of bacterial cells. Therefore, this study analysed the effect of repeated exposure of *S*. Typhimurium cells to sub-lethal benzalkonium chloride (BKC) concentrations. One single BKC exposure showed tailing of the inactivation curve indicating variation in BKC susceptibility within the initial population. Repeated exposure resulted in the enrichment of a more resistant population and variants with increased BKC resistance were isolated. Phenotypical characterization of these resistant variants showed loss of motility, reduced biofilm forming capacity and reduced cell membrane permeability pointing to modification of cell surface properties. In conclusion, repeated exposure of *S*. Typhimurium to sub-lethal BKC concentrations rapidly selects for resistant variants. Occurrence and persistence of such variants in food processing environments can form a risk for public health.

P074

Pathogenic *Aspergillus* species are less immunogenic than non-pathogenic *Aspergillus* species

K.L. Becker[1], M.S. Gresnigt[1], R. Lubbers[1], M.G. Netea[1], J.F. Meis[2], F.L. van de Veerdonk[1]

[1]*Radboudumc, Dept. of Internal Medicine, Nijmegen,* [2]*Canisius-Wilhelmina Hospital, Dept. of Medical Microbiology and Infectious Diseases, Nijmegen*

Introduction: *Aspergillus* species differ in their pathogenicity. Aspergillosis is commonly caused by *A. fumigatus, A. flavus, A. niger* and *A. terreus*, while species like *A. glaucus* or *A. sydowii* never cause disease and *A. nidulans* only in certain diseases, like chronic granulomatous disease. We wanted to investigate whether these species induce different types of immune responses and whether they are differentially recognized, which might explain their diversity in pathogenicity.

Methods: Peripheral blood mononuclear cells (PBMCs) of healthy volunteers were stimulated with heat-killed or live conidia from pathogenic *Aspergillus* species (*A. flavus,*

A. fumigatus, A. niger, A. terreus) and non-pathogenic *Aspergillus* species (*A. glaucus, A. nidulans, A. sydowii)*. To investigate the role of Toll-like-receptor (TLR) 2 and 4 stimulations were done in the presence or absence of a anti- TLR2 antibody or Bartonella lipopolysaccharide (LPS). The innate cytokines TNFa, IL-1 and IL-6 were measured in the cell culture supernatant by ELISA.

Results: TNFa, IL-1 and IL-6 were significantly decreased after stimulation with pathogenic *Aspergillus* species compared to stimulation with non-pathogenic *Aspergillus* species (n = 8 donors). Inhibition of TLR4 with Bartonella LPS did not affect TNFa, IL-1 and IL-6 production of PBMCs stimulated with heat killed and live *A. fumigatus, A. sydowii* and *A. glaucus*. Blocking TLR2 had no effect on live *A. fumigatus, A. sydowii* and *A. glaucus,* however the TNFa response after stimulation with heat-killed *A. fumigatus, A. sydowii* and *A. glaucus* was comparably decreased (n = 4 donors).

Conclusion: Non-pathogenic *Aspergillus* species induced a significantly increased cytokine response compared to pathogenic *Aspergillus* species. We hypothesize, that the low immunogenicity allows the pathogenic *Aspergillus* species to evade the immune system and cause disease, while the non-pathogenic strains are recognized and cleared by the immune system sufficiently. However, we did not find any difference in the role of TLR2 or TLR4 in the recognition of pathogenic versus non-pathogenic species.

P075

The effect of nitrogen limitation on morphology and aroma formation in Pichia fabianii and P. kudriavzevii

I.M.H. van Rijswijck[1], J. Dijksterhuis[2], T. Abee[1], E.J. Smid[1]
[1]Wageningen University, Dept. of Food Microbiology, Wageningen, [2]CBS-KNAW Fungal Biodiversity Centre, Dept. of Applied and Industrial Mycology, Utrecht

Non-conventional yeast (NCY) species are found in many natural food fermentation processes. Nyanga, *et al.* (1), isolated various microorganisms from fermented *masau* fruits, a traditional Zimbabwean fermented product. Within this niche the yeast species *Saccharomyces cerevisiae, Pichia fabianii* and *Pichia kudriavzevii* dominated. The two *Pichia* species appeared to produce high levels of aroma compounds relative to ethanol formation (2). For *S. cerevisiae*, it is speculated that some of these aromatic alcohols (products of the Ehrlich pathway) act as quorum sensing molecules under conditions of nitrogen starvation and result in a switch in colony morphology (on solid medium) or trigger the transition to the stationary growth phase (in liquid medium) (3, 4). For the two *Pichia* species in this study, the role of these alcohols is not known yet. To understand the role of aroma production in NCY, we focus on three isolates; *S. cerevisiae* 131, *P. fabianii* 65 and *P. kudriavzevii* 129 (1).

All three isolates were grown on minimal agar plates supplemented with either 45.4 mM or 0.0454 mM $(NH_4)SO_4$. The colony morphology was monitored during 21 days and the volatile organic compounds (VOCs) produced by these colonies were measured by Gas Chromatography – Mass Spectrometry (GC-MS).

Within 21 days of incubation on nitrogen limited plates, all three species changed their morphology from a smooth colony into a colony with irregular surface and edges with structures resembling pseudohyphae. This change was not observed under conditions of nitrogen excess. The timing of the morphological switch was found to species specific. Using scanning electron microscopy (SEM) we analysed the structural organisation of the colony on cellular level. Under nitrogen limitation *P. kudriavzevii* 129 developed elongated cells in a pseudohyphae-like structure.

The VOCs produced by the three different species grown under various conditions were determined by GC-MS. Significant differences were observed between the strains when they were grown under nitrogen rich conditions. Additionally, significant differences in aroma profiles were found when the same strain was grown under either nitrogen rich or limited conditions. Under nitrogen limited conditions the variation between species decreased. Interestingly we do not observe an increase in compounds like tryrosol, tryptophol and phenylethanol when grown under nitrogen limited conditions. These compounds are suggested to be quorum sensing molecules under nitrogen limited conditions for *S. cerevisiae*.

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P076

Effects of lysozyme and penicillin on the growth and activity of anaerobic ammonium-oxidizing planctomycetes

Z. Hu, T. van Alen, M.S.M. Jetten, B. Kartal
Radboudumc, Dept. of Microbiology, Nijmegen

Anaerobic ammonium-oxidizing (anammox) Planctomycetes, oxidize ammonium in the absence of molecular oxygen with nitrite as the electron acceptor. Although Planctomycetes are generally assumed to lack pepti-

doglycan in their cell walls, recent genome data imply the presence of peptidoglycan-like cell wall structures in anammox bacteria. We investigated the effects of two antibacterial agents that target integrity and synthesis of peptidoglycan (lysozyme and penicillin G) on anammox bacterium *Kuenenia stuttgartiensis*. The effects of these compounds were determined in both short-term batch incubations and long-term (continuous cultivation) growth experiments in membrane bioreactors. 1 g/L lysozyme (20 mM EDTA) lysed anammox cells in less than 60 minutes, whereas Penicillin G did not have any observable short-term effects on anammox activity. Penicillin G (0.5, 1 and 5 g/L) reversibly inhibited the growth of anammox bacteria in continuous culture experiments. Furthermore, transcriptome analyses of PenicillinG-treated reactor and the control reactor revealed that Penicillin G resulted in 10-fold decrease in the ribosome levels of the cells. One of the cell division proteins (kustd1438) was down-regulated 25 fold. Our results suggest that anammox bacteria contain peptidoclycan-like components in their cell wall that can be targeted by lysozyme and Penicillin G sensitive proteins are involved in their synthesis. Finally, we showed that a continuous membrane reactor system with free-living planktonic cells was a very powerful tool to study the physiology of slow-growing microorganisms under physiological conditions.

P077

Novel insights in pneumococcal colonization in children from an infant mouse model

S.D. Kuil[1], N. Patterson[1], J. Ortega[1], A. Walduck[1], K. Short[2], O. Wijburg[3]

[1]Radboudumc, Faculty of Medicine, Nijmegen, [2]Erasmus MC, Rotterdam, [3]The University of Melbourne, Dept. of Microbiology and Immunology, Melbourne, Australia

Background: Globally, *Streptococcus pneumoniae* (the pneumococcus) is a major cause of bacterial pneumonia. Children form the highest risk group of invasive pneumococcal disease and nasopharyngeal colonization of *Streptococcus pneumoniae* in children occurs at increased incidence and duration compared to adults. Here, we develop an infant mouse model to study and understand nasopharyngeal colonization with *S. pneumoniae* in children.

Methods: Five-day old C57BL/6 mice and adult (6 weeks) mice were colonized with *S. pneumoniae* (type 19F) and clearance of bacteria from the nasopharynx was monitored by viable count. To determine the role of antibodies in controlling pneumonoccoal colonization in infant mice, B6.uMT-/- mice (which lack antibodies) were infected with *S. pneumoniae* and ELISAs were performed on B6 and B6.uMT-/- mice following infection.

Results: Following nasopharyngeal inoculation, 5-days old B6 mice remained colonized with approximately 10^5 CFU for approximately 40 days and complete clearance took 65 days. In contrast, in adult mice, bacterial counts were ~100 fold reduced, highly variable and complete clearance took 21-28 days. Differences in nasopharyngeal colonization between adult and infant mice were not due to anatomical differences. Interestingly, clearance of *S. pneumoniae* in infant B6.uMT-/- mice was significantly delayed with more than 400 fold increased bacterial load 49 days post-infection. A positive correlation was also observed between IgM titres in infant mice and the rate of pneumococcal clearance.

Conclusion: This study demonstrates that neonatal mice provide a useful model to study the prolonged pneumococcal colonization that is seen in children. Using this model, our data suggest that IgM may play an important role in controlling pneumococcal colonization in infants.

P078

A rapid genotyping tool for Polyomavirus BK by Real Time PCR

L. Rurenga Gard, A. Riezebos-Brilman, H.G.M. Niesters

UMCG, MMB/ Division of Clinical Virology, Groningen

Introduction: Polyomavirus BK (BKV) is ubiquitous and widely spread in adult human populations. Infection results in lifelong persistence of the virus. BKV may cause nephropathy in renal transplant recipients and hemorrhagic cystitis in bone marrow recipients. BKV can be classified in 4 main genotypes I-IV, which differs in prevalence and distribution. Until now it is not clear whether there is a difference among the various BKV genotypes and their causative role in development of nephropathy and hemorrhagic cystitis. The availability of a rapid, simple and cost-effective genotyping tool could be useful for studies, which investigate the impact of the role of BKV infection in relation to the genotype.

Methods: A multiplex of Real Time PCRs (RT-PCR) was developed to differentiate the 4 main genotypes of BKV. The RT-PCRs were designed on the VP1 gene and validated for precision by the intra- and inter-run variation, specificity and limit of detection. This was done by 10-fold dilution series of each genotype.

150 BKV positive samples (17 plasma, 133 urine), ranging from log 2 to 11 copies/ml, were tested with the specific genotype RT-PCRs. Of these 150 samples 50 were additionally confirmed by sequencing the 1630-1956 nucleotide fragment of VP1.

Results: For every genotype of BKV a 100% genotype specific RT-PCR was developed in multiplex with an internal control. The precision of the four RT-PCRs remained within 1SD and the limit of detection was log 3 copies/ml.

Of the 150 BKV positive samples, 105 (70%) was genotype I, 8 (5.3%) genotype II, 19 (12.7%) genotype IV and 3 (2%) genotype I+IV. In 15 (10%) genotyping by RT-PCR was not possible, due to a low viral load. The genotypes from the 46 of the 50 samples could be confirmed by sequencing. In the remaining 4 samples this was not possible, due to a low viral load. Two of the 3 samples with the double infection, could also be confirmed by sequencing.

Conclusion: This study describes a new multiplex RT-PCR for detection of the main subtypes of BKV. It showed to be a rapid, less expensive and even more sensitive genotyping tool compared to sequencing. Finally, it detects double infections with different BK genotypes. This method can be of value to gain further insight in the relation of BKV genotype and nephropathy and hemorrhagic cystitis.

P079

Coculturing anaerobic methane and ammonium oxidizing bacteria

C.L.M. Stultiens, S. Guerrero, H.J.M. op den Camp, B. Kartal, M.S.M. Jetten
Radboudumc, Dept. of Microbiology, Nijmegen

Ammonium and methane are major end products of anaerobic digestion, for instance in anaerobic treatment of waste water. Methane gas can be used as an energy source, yet dissolved methane contributes to the greenhouse effect upon discharge of the effluent to surface waters and is therefore undesirable. In addition, external input of organic carbon is needed in wastewater treatment for denitrification. Recently, nitrite-dependent anaerobic oxidation of methane (n-damo) performing bacteria and anaerobic ammonium oxidizing (anammox) bacteria were discovered. Combining these processes has great potential in the treatment of wastewater, as ammonium, nitrite and methane are removed simultaneously.

To assess the feasibility of coculturing anammox and n-damo bacteria an existing anaerobically methane oxidizing enrichment culture (1 liter) was used to inoculate a 3 liter sequencing batch reactor (SBR). After 50 days, ammonium was added to the medium to stimulate the growth of anammox bacteria. Two activity assays with the whole reactor were performed to determine the contributions of anammox and n-damo bacteria to nitrite removal. DNA was extracted at three different timepoints. Clone libraries based on the 16s rRNA genes and the pmoA gene of n-damo bacteria were constructed. Fluorescence *in situ* hybridization (FISH) was used to visualize n-damo and anammox bacteria in the culture at different timepoints.

Activity assays showed that the coculture consumed nitrite with 15.4 nmol min^{-1} mg $protein^{-1}$, and anammox bacteria were responsible of a nitrite conversion rate of 11.9 nmol min^{-1} mg $protein^{-1}$. During the enrichment of anammox bacteria, there was a shift from '*Candidatus* Kuenenia stuttgartiensis'-like 16s rRNA sequences to '*Candidatus* Jettenia asiatica' or '*Candidatus* Brocadia fulgida' related sequences. Also, within 50 days, anammox biomass increased from 5% of the total, to 50%. This shows that a stable coculture of n-damo and anammox bacteria converting nitrite at a rate of 0.1 kg-N/m^3/day (17.2 mmol day^{-1}) could be established. In this coculture, anammox bacteria represented 50% of the biomas, yet were responsible for 77% of the nitrite removal. This indicates that the application of such a coculture for nitrogen removal may be feasible in the near future.

P081

A diagnostic screening algorithm to optimize detection of *Shigella spp.* and entero-invasive *Escherichia coli*

R.F. de Boer, A. Ott, W. de Boer, M.C. Scholts, K. van Huisstede, A.M.D. Kooistra-Smid
Certe, Dept. of Medical Microbiology, Groningen

Objectives: *Shigella* spp. and entero-invasive *Escherichia coli* (EIEC) are causative agents of bacillary dysentery. A unique property shared by *Shigella* spp. and EIEC is a 220-kb plasmid (pINV) which contains virulence factors involved in invasion of intestinal epithelial cells. Diagnosis of infection caused by *Shigella* spp. or EIEC is hampered due to low sensitivity of culture and lack of unique geno-/phenotypic properties, rendering discrimination of these pathogens a challenge. This study describes a diagnostic screening algorithm using both molecular and conventional methods to optimize detection of *Shigella* spp. and EIEC infections.

Methods: Around 3275 stool samples from patients with presumed infectious gastroenteritis were prospectively screened using real-time PCR (qPCR) targeting the *ipaH* gene. Subsequently, qPCR positive stool specimens were cultured on selective media (SS, HEA, XLD and MacConkey) directly and after enrichment (> 16hrs) in GN broth. Identification of suspicious colonies was performed using biochemical methods and serotyping. Simultaneously, enriched GN broth was used for DNA isolation. Confirmatory qPCRs were performed on *ipaH* qPCR positive stools (direct/enriched/plate streaks) targeting O-serotype specific genes for *Shigella sonnei*, *Shigella flexneri* type 1-6 and *Shigella dysenteriae* type 1. Furthermore, cultured isolates of *Shigella* spp. and EIEC were confirmed with qPCR.

Results: The detection frequency of the *ipaH* gene was 1.0% (n = 34). The diagnostic algorithm was applied to 30 *ipaH* PCR positive samples (28 patients). Serotype specific qPCR identified *S. sonnei* (n = 11), *S. flexneri* (n = 13), and one co-infection (*S. sonnei* and *S. flexneri*). *S. dysenteriae* type 1 was not detected. Five samples were only *ipaH* PCR positive.

Culture was positive for *S. sonnei* (n = 8; 7 isolates and 1 plate streak), *S. flexneri* (n = 8; 1 isolate and 7 plate streaks), EIEC (n = 2), and *Pleisiomonas shigelloides* (n = 1). Furthermore, final identification was not possible for one cultured isolate due to cross-agglutination between *S. boydii* and EIEC.
Overall, there were no significant differences in *ipaH* qPCR *CT* values before and after enrichment. However, *CT* values were significantly lower for culture confirmed samples compared to culture negative ($p = 0.0007$).
Conclusion: The diagnostic algorithm enables fast and sensitive detection of, and discrimination between *Shigella* spp. and EIEC infections.
The majority of *ipaH* positive infections was caused by either *S. sonnei* or *S. flexneri*, whereas the detection frequency of EIEC appeared low.
The GN enrichment did not improve culture yield.

P082

Extraintestinal infections with *Vibrio cholerae* non-O1, non-O139: first case series from the Netherlands

D.W.L. Scoop[1], F.A.G. Reubsaet[2], E.J. Kuijper[1], D.W. Notermans[2]
[1]*Leiden University Medical Center, Dept. of Medical Microbiology, Leiden,* [2]*National Institute for Public Health and the Environment, CIb, Bilthoven*

Introduction: Over 500 serogroups of *Vibrio cholerae* are described, of which O1 and O139 are considered as enteropathogenic types, that can cause large epidemics. *V.cholerae* non-O1, non-O139 is present in surface water, both fresh and salt, also in Northern Europe. Contact with surface water or contaminated food products can incidentally cause either gastrointestinal or extraintestinal infection. Local small outbreaks have been described, but epidemics do not occur. Reports on infections with *V.cholerae* non-O1, non-O139 in the Netherlands are limited to case reports. Our aim was to retrospectively analyse the characteristics of patients with an extraintestinal *V.cholerae* non-O1, non-O139 infection in the Netherlands.
Methods: All human strains of *V.cholerae non-O1, non-O139*, sent between 1999 and 2013 to the Centre for Infectious Diseases Research, Diagnostics and Screening (IDS) of the Dutch National Institute for Public Health and the Environment (RIVM) were included in the analysis. All isolates were identified by polyphasic approach including phenotypic characteristics and 16s rRNA-gene sequencing, and serotyped for O1 and O139. The medical microbiologist who submitted the strain was contacted for clinical information and a possible source of infection, using a standardized questionnaire.
Results: Between 1999 and 2013, 42 strains of *V.cholerae* non-O1, non-O139 were collected. Extraintestinal infections occurred in 23, including five cases of septicaemia, 16 ear infections and two wound infections. The remaining 19 strains were isolated from fecal cultures of patients with with gastrointestinal disease.
Patients with extraintestinal infections varied in age from 7 to 72 yrs. Ten patients were male, five female (of the other eight unknown). Eight patients were immunocompetent (of the other 15 unknown).
Of 23 patients with extraintestinal infections, nine infections were contracted in the European Union (one in the United Kingdom, one in Hungary and seven in the Netherlands), whereas two patients developed symptoms directly after travel outside the European Union (Republic of Suriname and Thailand). Travel history of 12 patients is unknown.
Of seven extraintestinal infections acquired in the Netherlands, four directly followed recent exposition to recreational water and one patient developed septicaemia after consumption of fish (of two patients exposition unknown).
All tested isolates were reported susceptible to quinolones (n = 13), trimethoprim/sulfamethoxazole (n = 11) and carbapenems (n = 4). Resistance to second-generation cephalosporines was reported in one of eight tested isolates. Susceptibility to aminoglycosides was reported as intermediate in three of nine tested isolates.
One patient with sepsis died in spite of adequate antibiotic treatment. All other patients fully recovered.
Conclusion: This case series illustrates the infectious potential of *V.cholerae* non-O1, non-O139. In view of reported decreased susceptibility to empirical sepsis therapy, *V.cholerae* non-O1, non-O139 should be considered in infections following surface water contact. As no systematic surveillance of infections with *V.cholerae* non-O1, non-O139 is currently performed, the precise incidence and the source of infection remain unknown.

P083

Possible link between recent human and wildlife cases of tularemia in the Netherlands?

M.G.J. Koene[1], M.Y. Engelsma[1], J.M. Rijks[2], A. Leenders[3], M.J.L. Kik[2], J. IJzer[2], T. Oomen[4], J.W. van der Giessen[4], A. Gröne[2], H.J. Roest[1]
[1]*Central Veterinary Institute (CVI), Dept. of Bacteriology and TSEs, Lelystad,* [2]*Dutch Wildlife Health Centre (DWHC), Utrecht,* [3]*Jeroen Bosch Ziekenhuis, 's Hertogenbosch,* [4]*National Institute for Public Health and the Environment (RIVM), Bilthoven*

Tularemia is considered as an important emerging zoonotic pathogen in the Netherlands.[1] The causative agent *Francisella tularensis* has a very wide host range including mammals, birds, amphibians, fish and invertebrates. Four subspecies can be identified; *F. tularensis* subsp. *tularensis*, *F. tularensis* subsp. *holarctica*, *F. tularensis* subsp. *mediasiatica* and *F. tularensis* subsp. *novicida*.

Rapid diagnostic testing for tularemia is not widely available, as laboratory testing requires special diagnostic and safety procedures (BSL3 facilities). In the Netherlands, diagnostic testing on veterinary and human clinical medical samples is routinely carried out at CVI.

In recent years *F. tularensis* subsp. *holarctica* has been detected in wildlife in various European countries, including the neighbouring countries Germany and Belgium. In the Netherlands, non-targeted surveillance in brown hares was started in 2011 as a collaboration between CVI, DWHC, and RIVM. The first confirmedcase was a hare submitted in May 2013[2] from Limburg, near the site of the last documented case in 1953.[3] Necropsy findings were consistent with *F. tularensis* infection and TaqMan real-time PCR[4] analyses of spleen and lung samples were positive for pathogenic *F. tularensis*. Subspecies identification based on the concatenated partial sequences of five metabolic housekeeping genes[5] revealed the presence of *F. tularensis* subsp. *holarctica*. Culture on chocolate agar medium with cysteine and sodium sulphite provided negative results, which is a common finding in the laboratory confirmation of tularemia.

In August 2013, serum from a 16 year old patient was submitted to CVI for tularemia serology testing. The microagglutination test revealed a high titre (= 640) strongly suggestive of tularemia. Subsequent PCR analysis on purulent exudate from an inguinal lymph node was positive and further genotyping performed at the Swedish Defence Research Agency (FOI)[6] confirmed infection with *F. tularensis* subsp. *holartica*. Also in this case cultures remained negative.

Interestingly, in the days prior to the first clinical symptoms the patient had a history of insect bites while visiting the same Limburg region the positive hare originated from. It was attempted to confirm the epidemiological link between the hare and human case by genotyping[6], using clinical sample DNA. Although results showed both to belong to the same Iberian clone (subclade B.6/11) which is typical for Western Europe, higher resolution SNP-markers or sequencing is needed to be able to distinguish between B.6/11 isolates. Unfortunately, due to lack of sample material this could not be accomplished.

The information presented here highlights the importance of raising the awareness of physicians and veterinarians with regards to this disease. Continuation of tularemia surveillance in wildlife and more insight into the ecology of tularemia in the Netherlands is needed for proper risk evaluation.

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Po84

Detection of viral gastroenteritis with multiplex one-step RT-PCR using the Roche® Flow system

R. van Rhee-Luderer, A.N. Storm, E. Poelstra, A.J. Stellingwerff, S.J. Mulder, A.H. Brandenburg, J.F.L. Weel, T.A. Schuurs

Izore, Dept. of Medical Microbiology, Leeuwarden

Introduction: In our laboratory viral gastroenteritis (GE) is diagnosed based on rapid antigen detection of norovirus (predominantly genogroup II), rotavirus and adenovirus. ELISA-based antigen detection of astrovirus was performed in the past. Molecular detection of viral gastro-enteritis with an automated workflow was envisaged to allow easy parallel detection of more GE viruses with an enhanced sensitivity and specificity.

Methods: Two multiplex real-time one-step RT- PCR assays were developed for detection of norovirus genogroup I, norovirus genogroup II, rotavirus group A, adenovirus 40/41, astrovirus 1-8, sapovirus I, II, IV and V and an internal control virus. Primers and probes for the individual viruses were based on previously published assays. After pre-treatment, stool samples were fully automated processed with the Roche Flow system and PCR was performed in 384-well format. Results were validated with Aurora Flow software and uploaded to our laboratory information management system.

Results: Analysis of a reference panel of GE viruses confirmed that our multiplex assays detect all relevant subtypes. Flow based molecular detection of viral gastroenteritis was performed with 150 stool samples that had been subjected to antigen detection tests in the past. 30 norovirus positive samples were confirmed with PCR to be norovirus genogroup II positive and 3 extra norovirus genogroup II stool samples were identified. No norovirus genogroup I was detected in this set of samples. 32 rotavirus positive samples were confirmed with PCR. 26 out of 30 adenovirus positive samples were confirmed with PCR and 1 extra adenovirus positive sample was identified. The 4 adenovirus positive samples that were not detected with the adenovirus 40/41 PCR, were detected with a general adenovirus PCR, suggesting an increased specificity of the adenovirus 40/41 PCR compared to antigen detection. Of 30 astrovirus positive samples, 20 could be confirmed by PCR. The average OD of the astrovirus ELISA of the 10 samples that could not be confirmed with PCR was 1,9, compared to 14,4 for the PCR positive samples. Considering the analytical sensitivity of the PCR, the slightly elevated OD in the ELISA of these 10

samples was probably non-specific. In 4 samples sapovirus was detected.

Conclusion: Molecular detection of viral GE is more sensitive and more specific than antigen detection. The Roche flow system allows easy parallel detection of six different GE viruses with sample tracking throughout the complete analytical process. The automated workflow enables smooth processing of an increased number of samples during outbreaks without significant impact on hands-on time.

P085

Rapid and reliable identification of highly pathogenic Gram-negative bacteria using matrix-assisted laser desorption ionization-time of flight mass spectrometry

A.C.A. Hendriks, M.C. de Vries, M.J.C. Beld, A.S.D. Harpal, E.M. Heerkens, B.J.A. Hoeve, F.A.G. Reubsaet
RIVM, Infectieziekten onderzoek, Diagnostiek en Screening, Bilthoven

Introduction: Highly pathogenic bacteria, like *Burkholderia mallei, B. pseudomallei, Francisella tularensis, Yersinia pestis,* and *Brucella* species, endemic in different parts of the world, occur occasionally as imported infections in The Netherlands. Those organisms are listed by the Centre for Disease Control (CDC) as potential bioterroristic agents. A swift and highly specific detection method for those organisms is essential to act appropriately to the situation or to prevent unnecessary actions and panic. Valuable methods could be matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) and real-time PCR. Our laboratory validated both tests systematically to ensure their usefulness.

MALDI-TOF MS is a popular technique for identification of microorganisms in microbiology. Many laboratories use the MALDI Biotyper reference library (Bruker Daltonics, Germany) for identification. However, those highly pathogenic bacteria, all to be handled at biosafety level 3 (BSL-3), are only included in the Bruker's Security Relevant (SR) library. In this study, the use of MALDI-TOF MS for rapid and reliable identification of highly pathogenic Gram-negative bacteria, both with and without the SR-library, was investigated.

Methods: 42 strains of *Brucella* species, 10 *Burkholderia mallei*, 18 *Burkholderia pseudomallei*, 18 *Francisella tularensis,* and 18 strains of *Yersinia pestis* were included in this study. In addition, 93 genetically and phenotypically related strains of the described species were tested. All the strains were tested by Bruker's standard methods: formic acid extraction and direct transfer method. Growth controls were performed before the materials left the BSL-3 facility. The resulting spectra were compared with the Biotyper reference library and the SR-library. An in-house *Francisella* database was created. Bruker's FlexAnalysis software was used to check the presence of described biomarkers.

Results: Using the Biotyper reference library only, spectra from *Burkholderia mallei* and *B. pseudomallei* strains resulted in *Burkholderia thailandensis* (score > 2.000). *Yersinia pestis* resulted in *Yersinia pseudotuberculosis*, however all *Brucella* species and *Francisella tularensis* resulted correctly in no reliable identification' (score < 1.700). When the Biotyper reference library with the SR-library supplementation was used, in some cases *Burkholderia mallei* resulted in *Burkholderia pseudomallei* and vice versa. Some *Yersinia pestis* isolates scored *Yersinia pseudotuberculosis*. All the different *Brucella* species resulted in *Brucella melitensis,* the only *Brucella* species represented in the SR-library. The SR-library cannot distinguish between the different *Francisella tularensis* subspecies. When using the in-house *Francisella* database, all the *Francisella tularensis* subspecies were identified to the right subspecies level.

Conclusion: Since, using only the Biotyper reference library, *Burkholderia mallei/B. pseudomallei* or *Yersinia pestis* can result in the false identification of respectively *Burkholderia thailandensis* or *Yersinia pseudotuberculosis,* a warning should occur. Even in combination with the SR-library these respective organisms could not be distinguished. However the combined libraries are a useful tool for a fast indication of highly pathogenic Gram-negative bacteria. Confirmation by, for example Real-Time PCR assays, remains essential.

The in-house *Francisella* database is a useful addition to the MALDI-TOF MS libraries for identification of *Francisella tularensis* subspecies. Distinction of *Yersinia pestis* and *Yersinia pseudotuberculosis* can probably be solved by using *Yersinia pestis* specific biomarkers.

P086

Successful control of a large nosocomial VRE outbreak in a Dutch teaching hospital

F.N.J. Frakking, W.S. Bril, J. van 't Klooster, J.S. Sinnige, M. Tersmette
St Antonius Hospital, Dept. of Medical Microbiology, Nieuwegein

Objective: To describe the successful control of the first outbreak of Vancomycin-resistant Enterococcus faecium (VRE) in our 880-bed Dutch teaching hospital.

Methods: In March 2012, VRE was detected by culture in urines of three patients from two different wards. Regular infection control measures were taken. Amplified fragment length polymorphism (AFLP) patterns of the strains were identical. Screening (rectum swabs) resulted in several VRE-positive patients on multiple wards. A VRE outbreak

team was initiated. Health authorities, surrounding hospitals and the public were informed. Surveillance was started on high-risk wards. Meanwhile, molecular detection of *vanA* and *vanB* genes was developed for hospital-wide screening. Wards with multiple VRE-positive patients were closed, cleaned and disinfected. Patients previously admitted to these wards were electronically labeled as possible VRE carrier until five negative rectum swabs were obtained. Lectures were organized for hospital personnel. A VRE outpatient clinic was set up. Multiple Locus Sequence Typing (MLST) was performed. Temporarily, VRE-positive patients were nursed on a separate ward.

Results: Within 22 months, 248 VRE carriers were identified. Of these, 160 patients were detected only by hospital-wide surveillance. Almost all had been admitted for more than four days. Five patients had VRE bacteremias, in 20 patients VRE was cultured from other clinical sites, with a median of 26 days after the first positive screening culture. Spread of VRE was seen on 11 out of 23 wards on two different locations. Fogging these wards with H_2O_2 was effective in preventing further transmission. Apart from the initial *vanA* ST78 clone (n = 158), we detected two other epidemic MLST clones (*vanA* ST494, n = 23 and *vanB* ST117, n = 36). Furthermore, 31 patients had other, non-epidemic *vanA* and *vanB* clones. We identified 5433 possible contacts, of which < 1% was VRE positive. Until now, hospital-wide weekly screening has been maintained for early detection of incidental transmission from known VRE positive patients despite strict isolation precautions.

Conclusions: Standard infection control measures according to national guidelines were insufficient to detect a large VRE outbreak throughout our hospital.

Probably, frequent patient transfers between wards contributed to spread.

Intensified measures resulted in control of the outbreak, i.e. strict instead of contact isolation precautions, closing, cleaning and fogging of hospital wards, frequent audits by infection prevention personnel, termination of ciprofloxacin prophylaxis in hematology patients, and nursing of VRE-positive patients on a separate ward.

Surprisingly, three epidemic VRE strains and multiple patients with unidentical VRE clones were detected in a hospital and country that had not detected VRE in years. Probably, carriage of VRE has been underestimated in the Netherlands.

Acquisition of VRE mostly occurred in the hospital and not in the community

In 248 positive patients VRE carriage seldom resulted in VRE infection, but VRE bacteremia did occur

P087

Vancomycin cannot be solely dosed on estimated creatinine clearance

M.B. Haeseker[1], S. Croes[1], C. Neef[1], C.A. Bruggeman[1], L.M.L. Stolk[1], A. Verbon[2]

[1]*MUMC, Dept. of Medical Microbiology, Maastricht,* [2]*Erasmus MC, Rotterdam*

Introduction: Many methods based on the clearance of creatinine (CLcr) have been proposed to estimate the clearance of vancomycin (CLva). There are also many methods to estimate the CLcr. The aim of this study is to investigate which method (three new methods) based on the CLcr predicts the CLva most correctly. Additionally, we investigated which method to estimate the CLcr best correlates with the CLva and which other co-variates influence the CLva.

Methods: Patients > 18 years of age treated with vancomycin intravenously and hospitalised at non-Intensive Care Units in the Maastricht University Medical Centre were included from May 2011 until July 2013. Vancomycin serum levels were measured as standard clinical care with a fluorescence polarization immune assay of Roche Cobas Integra 800. Patients with at least two blood samples available were included. Pharmacokinetic parameters of individual patients, i.e. CLva and volume of distribution (Vd), were calculated with maximum a posteriori (MAP) Bayesian estimation (MW/Pharm 3.60, Mediware, the Netherlands). A two-compartment open pharmacokinetic model was used. The CLcr was estimated with the Cockroft-Gault, Modification of Diet in Renal Disease-4, -5, -6 and Chronic Kidney Disease Epidemiology Collaboration formula from serum creatinine, ureum and albumin. The estimated CLcr with the best correlation with CLva was used in three prediction models of the CLva. The three predicted CLva were compared with the measured CLva with MAP Bayesian Estimation. The influence of co-variates was determined using a predetermined set of predictors.

Results: A total of 171 patients were included. The mean (SD) serum creatinine was 90 (58) mol/L, Vd was 58 (30) l and CLva was 53 (24) ml/min. The best linear correlations between CLcr and CLva was R^2 0.51 with the C&G formula, this formula was used in all CLva prediction methods. The linear regression correlation between CLva calculated with MAP Bayesian Estimation and calculated with the prediction models were all similar (R^2 0.51). The prediction errors and the absolute prediction errors were large, ranging from (23-97%) and (36-98%), respectively. The CLva was significantly correlated to CLcr, creatinine, age, weight, gender and neutropenia. Indeed, patients above 70 years had lower CLva than patients younger than 70 years and patients with neutropenia had higher CLva.

Conclusion: Large prediction errors make the algorithms based on serum creatinine unsuitable for use in patient care. Other factors also influence the CLva individually, in our study age and neutropenia. Possible new predictive algorithms should be formulated which include all important co-variates. At this moment a reliable calculation of CLva is only possible with therapeutic drug monitoring.

P088

Molecular ecology and isolation techniques reveal the microbial ecology of sediments of an extreme acidic environment

I. Sánchez-Andrea, R. Amils, J.L. Sanz, A.J.M. Stams
WUR, Dept. of Agrotechnology, Wageningen

Tinto river (Huelva, Spain) is a natural acidic rock drainage (ARD) environment produced by the bio-oxidation of metallic sulfides from the Iberian Pyritic Belt. So far, and despite their ecological interest, the underlying sediments were studied only very sparsely. In this study, an extensive survey of the Tinto river anaerobic sediment microbiota is presented combining culture independent and dependent methods.

A geomicrobiological model of the different microbial cycles operating in the sediments has been developed through molecular biological methods.[1,2] Culture independent methods targeting the small subunit ribosomal RNA (SSU rRNA) such as denaturing gradient gel electrophoresis (DGGE), 16S rRNA gene sequencing (cloning) and catalyzed reporter deposition fluorescence *in situ* hybridization (CARD-FISH) were used. Microorganisms involved in the iron (*Acidithiobacillus ferrooxidans, Sulfobacillus* spp., etc.), sulfur (*Desulfurella* spp., *Desulfosporosinus* spp., *Thermodesulfobium* spp., etc.), carbon (*Acidiphilium* spp., *Bacillus* spp., *Clostridium* spp., *Acidobacterium* spp., etc.) and nitrogen (*Alcaligenes* spp.,*Pseudochrobactrum* spp., etc.) cycles were identified and their distribution correlated with physicochemical parameters of the sediments. Where the pH and redox potential are closer to those of the water column (pH 2.5 and +400 mV), the most abundant organisms were identified as iron-reducing bacteria: *Acidithiobacillus* spp. and *Acidiphilium* spp., probably related to the higher iron solubility at low pH. At higher pH (4.2-6.2) and more reducing redox potential (50, -210 mV) and therefore, lower solubility of iron, members of the sulfate-reducing genera *Syntrophobacter, Desulfosporosinus* and *Desulfurella* were dominant.

Additionally, targeted enrichment incubations were used to validate this model and prove the existence of the potential anaerobic activities detected in the acidic sediments of Tinto river. Methanogenic, sulfate-reducing, denitrifying, iron-reducing and hydrogen-producing enrichments yield positive results.[3] Within the detected anaerobic activities, acidic sulfate-reduction and the associated fermentation have high interest for their potential in bioremediation. Classical techniques for bacterial isolation were applied and some microorganisms were isolated such as acidophilic sulfate-reducing bacteria (two new species and a new genus, related to the *Desulfosporosinus/ Desulfitobacterium* cluster), fermenters (two new genus, far related to *Paludibacter* and *Propionispora*).[4] Related microorganisms are often found in molecular studies of ARD environments showing the ecological importance of the isolates. Their genomic and physiological characterization helps in the understanding of the anaerobic processes taken place in the underlying sediments of extreme acidic environments.

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P089

Spore germination responses of thirteen sequenced food-related isolates of *Bacillus subtilis*

A.O. Krawczyk, R.T. Eijlander, O.P. Kuipers
University of Groningen (RUG), Dept. of Molecular Genetics, Groningen

Introduction: In response to starvation, Bacillus subtilis can sporulate, what leads to the formation of highly resistant dormant endospores. Spores are found in a diversity of environments, including food stuffs. Due to their resistance, spores are difficult to remove from food products, causing problems for the food industry. In spite of their metabolic dormancy, spores can sense their environment and respond to various triggers through the process of germination and cell outgrowth. Vegetative cells subsequently cause food spoilage and a shorter product shelf-life. Improvement of food preservation could be achieved by accurate prediction of spore germination behavior.[1] However, this is a challenging task, since differences in germination responses are observed even among different strains of one species. For this reason the data obtained for the model strain, B. subtilis 168 cannot be directly translated to industrially-problematic strains.

Therefore, the purpose of this study was to elucidate germination behavior of B. subtilis isolates, which cause problems to the food industry and to couple the observed germination phenotypes with the isolates' genetic contents.
Methods: Thirteen different food isolates of B. subtilis were subjected to whole genome sequencing. Germination of their spores was induced under various conditions and analysed via absorbance measurements in microplate readers and phase-contrast microscopy.
Results: Striking differences in germination and outgrowth of the thirteen strains were observed. The spores differed not only in the responsiveness to specific nutrients but also in requirements for heat activation. The isolates varied significantly in presence of genes directly influencing germination.
Conclusions: 1. Defined germination triggers for the model strain, B. subtilis 168 do not necessarily promote efficient germination of industrial isolates of the same species.2. Specific heat activation temperatures are required for efficient spore germination of various strains of B. subtilis.3. Some of the observed phenotypes can be coupled to specific genetic traits of the strains.

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P090

High azole resistance rate of *Aspergillus fumigatus* at Intensive Care Unit in a Dutch tertiary hospital

A. Russcher, J. van Paassen, P.A. Dofferhoff, E.J. Kuijper
LUMC, Dept. of Medical Microbiology, Leiden

Introduction: The increase in azole resistance is emerging and complicates treatment of patients with invasive pulmonary aspergillosis. A recently completed two-years survey in 6 hospitals in The Netherlands revealed an overall prevalence rate of azole resistance in 6.8% of 921 *Aspergillus* isolates. In the survey, patients from various departments were included. We were interested in the azole prevalence rate in patients treated for (suspected) invasive pulmonary aspergillosis at the Intensive Care Unit (ICU) and performed a single-center retrospective cohort analysis.
Methods: Clinical and microbiological data were retrospectively collected from January 2010 to October 2013 on all ICU patients who received treatment for (suspected) invasive pulmonary aspergillosis. Diagnosis was classified according to EORTC/MSG criteria. If culture of respiratory samples was positive for *Aspergillus* species, *Aspergillus* colonies were subcultured on a specially developed 4-well azole-agar dilution plate (Nijmegen Reference Centre) containing no fungal agent (growth control), itraconazole (4 mg/L), voriconazole (1 mg/L), or posconazole (0.5 mg/L). If results were suggestive for azole resistance, the MIC was determined using the EUCAST broth microdilution method at the Nijmegen reference laboratory.
Results: In total, 146 patients who received treatment for (suspected) invasive pulmonary aspergillosis were included in the analysis. Of these, 38 patients had a positive culture with *Aspergillus* spp. All *Aspergillus* isolates were identified as *Aspergillus fumigatus*. One patient had proven invasive pulmonary aspergillosis, 29 patients had probable invasive pulmonary aspergillosis and in 8 patients a reliable diagnosis could not be made due to the absence of CT-scan or severe co-morbidity. The prevalence of patients with azole resistant *Aspergillus* varied between the years. Overall prevalence of azole-resistance was 26% (10/38). In 2010 no resistant *Aspergillus* was cultured (7 patients) whereas the prevalence of azole resistance was 50% in 2011 (5/10), 11% in 2012 (1/9) and 33% in 2013 (4/12). Of the patients harbouring a resistant isolate, 40% (4/10) were pretreated with azoles whereas 25% (7/28) of patients with susceptible isolates were pretreated. Crude 90-day mortality rates were 75% in patients with an azole-susceptible isolate (21/28) and 100% in patients with a resistant isolate (10/10).
Conclusion: The prevalence of azole resistance on the ICU varied widely over the past years but was remarkably high compared to previous observations and associated with high mortality. Resistance rates might be overestimated as resistant isolates are perhaps more likely to be cultured than susceptible isolates when azole therapy is already instituted at the time of sampling. Nevertheless, these results should encourage hospitals to monitor the prevalence of azole-resistant isolates and to (re-)evaluate treatment protocols accordingly.

P091

Antimicrobial stewardship in the general practice, a pilot

E. de Jong[1], C.M.A. Swanink[1], M.A. Lanting[2], R. Bosboom[1]
[1]*Ziekenhuis Rijnstate, Dept. of Medical Microbiology and Immunology, Arnhem,* [2]*General Practice Johan de Witt/ Cornelis, Dept. of General Practice, Arnhem*

Introduction: To reduce antibiotics (AB) used in the general practice -as set out in the SWAB vision document (June 2012)- we worked together with 6 general practitioners (GPs) in our region (Arnhem, Gelderland). This pilot project aimed to identify the potential of AB stewardship in the general practice.
Methods: To investigate the way a general practice prescribes AB we were actually present in the practice during the consultation of the GPs and the GP-assistants. During the period of 1 month (October 21st- November 21st) all ICPC-codes for urinary-tract infection (UTI) (U71) and (lower) respiratory tract infections. (LRTI) (R05-cough, R74-acute infection of upper respiratory tract,

R78-acute bronchitis, R81-pneumonia) were extracted from the GPs information system (HIS). We analysed all patients charts with one of the ICPC-codes regarding the concordance of AB prescription with the first choice AB in the NHG (Dutch GP-society) Standard. In LRTI's the role of C-reactive protein (CRP) was evaluated.

Results: 72 episodes of UTI in 71 patients were registered, 93% of the patients were female, the median age was 40 years (range 2-90). 45 (67%) patients were prescribed AB. In 55% of the episodes prescribing an antibiotic and/or the prescribed agent was in accordance with the NHG-standard. Most abnormalities were not prescribing the first choice agent and/or an excessive duration of the treatment.

118 episodes of LRTI in patients 116 were registered, 59% of the patients were female, the median age was 36 years (range 0-93). In 21 (19%) episodes in 19 patients (17%) AB were prescribed. In 5% of the episodes prescribing an antibiotic and/or the prescribed agent was in accordance with the NHG-standard. Most abnormalities were prescribing AB when the guideline stated you should not, and/or not prescribing the first choice AB. CRP was performed in 16 patients, none of which received AB.

These results were presented and discussed with the entire group of GPs (n = 6) in a meeting by the medical microbiologists.

Conclusion: This pilot shows that there is room for improvement in many aspects. CRP could help to reduce the amount of prescribed AB. Meetings with GPs to discuss the results can be the start of a more rational AB prescribing behaviour. The method of data collection and analysis should be made more convenient for the GP and the medical microbiologist.

P092

The Enzyme Immunoassay for detection of *Mycoplasma pneumoniae* IgM: is faster always better?

C.F.M. Linssen, F.S. Stals, M.F.H.A. van de Cruys, H.M.W. Bongers, E.I.G.B. de Brauwer

Atrium Medical Centre, Dept. of Medical Microbiology, Heerlen

Introduction: *Mycoplasma pneumoniae* causes respiratory infections, predominantly in youth. It has a worldwide distribution and approximately 20% of cases of atypical pneumonia are attributed to *M. pneumoniae*. Several tests can be used in the diagnostic workup of a *M. pneumoniae* infection such as polymerase-chain reaction (PCR), ELISA and CBR. Since PCR is not always available, a fast, easy to perform and reliable test for the detection of an acute *M. pneumoniae* infection is warranted.

Therefore the aim of the present study was to evaluate an Enzyme Immunoassay for the detection of *M. pneumoniae* IgM in day to day clinical practice in a large peripheral hospital.

Methods: In the period January 2013 - October 2013 all serum samples of patients suspected of an acute respiratory infection were included. The presence of IgM directed towards *M. pneumoniae* was determined using Immuno*Card*® Mycoplasma (Meridian, Bioscience Inc, Cincinnati, US). This 'fast test' generates a result within 9 minutes. Furthermore IgM and IgG directed towards *M. pneumoniae* were determined by fully automated chemiluminescence analyzer (Liaison XL, Diasorin, Saluggia, Italy). The clinical diagnosis for each patient was retrieved retrospectively.

Results: A total of 60 patients were included. Out of these 60 patients, 7 patients were diagnosed with *M. pneumoniae* infection, in one additional patient the diagnosis was suspected, but not confirmed. ImmunoCard® Mycoplasma yielded a positive result in 20 patients. Out of these 20 patients, 6 were eventually diagnosed with *M. pneumoniae* infection. In 2 patients diagnosed with *M. pneumoniae* infection, Immuno*Card*® Mycoplasma was negative. This resulted in both a sensitivity and specificity of 75%. The sensitivity and specificity of the IgM Liaison XL *M. pneumoniae* test was higher (87.5% and 88.4% respectively). The agreement between the ImmunoCard® Mycoplasma and the Liaison IgM was substantial, resulting in a kappa coefficient of 0.72. A disadvantage of Immuno*Card*® Mycoplasma was that the interpretation of the result was not always straight forward (any presence of color should be regarded as positive).

Conclusion: Immuno*Card*® Mycoplasma showed a lower sensitivity and specificity compared to the Liaison XL IgM test. However, since Immuno*Card*® Mycoplasma is easy to perform and results are available within 9 minutes, the test may have additional value at times when the Liaison XL is not available (e.g. during the weekend).

P093

Comparison of the AlereTM PBP2a test with the slide latex agglutination test for the identification of Methiciline Resistant *S. aureus*

C.F.M. Linssen, L. Martinussen, M. Vrancken, F.S. Stals, E.I.G.B. de Brauwer

Atrium Medical Centre, Dept. of Medical Microbiology, Heerlen

Rapid detection of Methiciline Resistant *S. aureus* (MRSA) in hospital setting is important. The slide latex agglutination test (Denka Seiken, Tokyo, Japan) is a rapid test with a specificity and sensitivity exceeding 99%. The disadvantage is the long hands on time of the test, including boiling and centrifuging. The AlereTM PBP 2a is an immunochromatographic qualitative assay for detection of penicillin binding

protein PBP2A with a specificity and sensitivity of 98%. Following the manufacturer instructions the test gives a clear and objective result within 6 minutes.
The aim of the present study was to compare the two tests in order to determine the value of the Alere™ PBP2A as rapid and reliable test for use in a routine clinical laboratory.
Method: a total of 50 previously identified MRSA strains (typed by reference laboratory RIVM) and 50 Methicilline Sensitive *S. aureus* (MSSA) were included. All strains were cultured on both blood agar and selective MRSA screen agar (Biomerieux, Marcy l'Etoile, France). Tests were performed on colonies taken from the blood agar and the MRSA screen agar. The sensitivity and specificity for each test was calculated. The inter-observer variability was defined by Cohen's kappa.
Results: both the AlereTM PBP2a and the latex agglutination test showed a sensitivity of 100% on colonies taken from either the blood agar or the MRSA ID agar. The specificity was 100% for the AlereTM PBP2a compared to 98% for the latex agglutination test on colonies taken from the blood agar. The inter-observer variability showed an excellent agreement (Cohen's kappa of 1)
Conclusions: 1) The Alere TM PBP2A test is a rapid, reliable test for use in a routine clinical laboratory with a short hands on time. 2)The test can be performed on colonies taken from either blood agar or MRSA screen agar.

P094

Comparison of HIV INNO-LIA™ with HIV Geenius™ HIV 1 and 2 for confirmatory serodiagnosis in patients with HIV 1 and 2

E. Koenraad, P. Daemen, J. Rahamat-Langendoen, F.F. Stelma
Radboudumc, Dept. of Medical Microbiology, Nijmegen

In the Netherlands the HIV Inno-Lia immunoblot is frequently used for confirmation of a serological positive HIV screening test. However the immunoblot is expensive and labor intensive. Due to efficiency reasons, samples are often tested in larger batches, resulting in longer turn-around times for confirmation. Recently BioRad introduced a point of care cassette, the Geenius HIV 1 / 2, that can be used for HIV 1 and 2 confirmation. This study describes the clinical evaluation performed in our laboratory of this test.
Population & methods:Blood samples with various clinical backgrounds were extracted from our local biobank (Dept. of Microbiology, Radboudumc). Samples came from the following patients: Chronic HIV1 n = 5, chronic HIV2 n = 2, recent HIV1 (< 3 months) n = 3 and other viral conditions n = 6 (Epstein Barr virus, Cytomegalovirus, Human T-Lymphotropic virus and influenza A virus infections). Mostly the material was serum, however in three cases whole blood was tested. In two cases of recent HIV, paired serum samples were tested. All samples were submitted to HIV Inno-Lia™ (Innogenetics, Gent, Belgium) and Geenius HIV 1 / 2 (BioRad Laboratories, France).
Results: Both confirmatory tests performed excellent in samples from patients with chronic HIV1 and chronic HIV2. None of the negative samples showed reactivity in either test. In samples from recently exposed HIV patients (n = 3) discrepant results were observed, however no structural differences could be observed that favors one test to the other. The HIV Geenius was less labor intensive compared to the HIV Inno-Lia, reducing the turnaround time for confirmation considerably.
Conclusion: In this evaluation the HIV Geenius and HIV Inno-Lia test performed equally well in chronic HIV 1 and 2 patients, however the confirmation of recent HIV infection showed variation between the tests. Therefore the performance in recent HIV has to be further evaluated. Further, the HIV Geenius test is easy to use and less labor intensive than the HIV Inno-Lia immunoblot.

P095

Real-time PCR for early detection of ganciclovir resistant human cytomegalovirus strains in immunocompromised hosts

I. Maat, N. Hamdiui, F.F. Stelma, L.B. Hilbrands, W.J.F.M. van der Velden, A.S. de Jong
Radboudumc, Dept. of Medical Microbiology, Nijmegen

Introduction: Human cytomegalovirus (HCMV) may cause longstanding and life-threatening tissue-invasive disease in immunocompromised solid organ or stem cell transplant recipients. Treatment consists of (val)ganciclovir (GCV) administration and immune reconstitution. GCV-resistant HCMV strains cause significant morbidity and mortality in 5-10% of the high-risk transplant patients.[1] Mutations in six codons of UL97 gene account for > 80% of the clinical GCV-resistant HCMV strains.[1] The standard genotypic method to detect GCV resistance is UL97 gene sequencing, which is time-consuming and has limited sensitivity. The aim of this study was to investigate whether screening for UL97 mutations using real-time PCR allows for more sensitive and more rapid detection of GCV resistance mutations.
Methods: For this retrospective analysis three allogeneic stem cell transplant patients and two kidney transplant patients were selected, who had received GCV prophylaxis and/or treatment, and in whom the presence of a GCV resistance mutation in UL97 gene had been detected in one or two samples during the course of infection. Of these five patients, a total of 97 consecutive plasma samples with known HCMV loads were subjected to nucleic acid isolation and real-time PCR/melting curve analysis,

to allow for the detection of the most common UL97 resistance mutations. In parallel, conventional UL97 gene sequencing was performed.

Results: Real-time PCR demonstrated the presence of 90 resistance mutations in 66 of 97 samples, distributed over the codons 460, 520, 592, 594, 595 and 603. Sixty-five (72%) resistance mutations were in both the real-time PCR and the convential UL97 gene sequencing PCR detected, and 20 (22%) mutations were only demonstrated by real-time PCR. Importantly, real-time PCR enabled the detection of mutations at lower viral loads, and by consequence earlier in the course of the infection than conventional sequencing. Interestingly, in four of the patients (80%), two or more resistance mutations were detected in the course of infection. In addition, in 24 instances more than one resistance mutation was detected in the same sample. These results indicate that GCV-resistance mutations may be replaced by others in time, suggesting that GCV-resistance in HCMV infection is the result of a dynamic process of generation and selection of mutant viruses. This is further substantiated by our finding that polymorphisms in the UL97 gene sequences appear and disappear over the course of infection, as demonstrated by conventional UL97 sequencing. The clinical relevance of this finding remains to be established.

Conclusion: We demonstrate that the use of real-time PCR/melting curve analysis allows for the detection of GCV-resistance mutations in HCMV at lower viral loads and at an earlier time point than conventional UL97 gene sequencing. We propose a screening strategy in which samples are first analyzed by real-time PCR and that samples in which none of the common mutations are detected are then subjected to UL97 gene sequencing, which requires samples with higher viral loads.

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P096

Disseminated cryptococcal infection in a renal transplant recipient presenting as a metastasized lung malignancy

C.M.A. Swanink, I. Maat, Y.P. de Jong, J.C. Verhave, R. Aliredjo, C.M.A. Swanink

Rijnstate Hospital, Dept. of Medical Microbiology and Immunology, Arnhem

Introduction: *Cryptococcus neoformans* is an encapsulated yeast, causing infections, especially in patients suffering from AIDS. Occasionally, patients undergoing immunosuppressive therapy or even immunocompetent patients are infected.

Case: A 73-year old man presented with unexplained weight loss, diarrhea, and pain in his right shoulder and buttock. He had a history of nicotine abuse and chronic obstructive pulmonary disease and received a renal transplant 17 years ago for which he was currently using tacrolimus and prednisone. Physical examination revealed a progressive swelling at the right sternoclavicular junction and normal breath sounds. The leukocyte count was 13.2 $X10^9$/L and the C-reactive protein concentration was 76 mg/L.

Chest radiography identified a pulmonary mass in the left lower lung lobe, showing FDG uptake on a subsequent FDG-PET scan. Other FDG positive lesions were identified in the right os ilium, right clavicle, proximal left femur, and transverse process of the second thoracic vertebra. Altogether, a metastasized pulmonary malignancy was suspected and biopsy of both the pulmonary lesion and the lesion in the right ilium was performed. Surprisingly, biopsies did not demonstrate malignancy but rather signs of fungal infection. The Grocott's stain showed round, yeast-like structures, suspect of *Cryptococcus spp.* A presumptive diagnosis of disseminated cryptococcal infection was made. The patient was hospitalized and treatment with liposomal amphotericin B 3mg/kg/day and flucytosine 100 mg/kg/day in 3 doses was started immediately. Cultures were taken from blood, urine and cerebrospinal fluid although there were no clinical signs of meningitis. Lumbar punction showed, clear spinal fluid, with 46 x 10^6/L leukocytes, 0.46 g/L protein, and 3.3 mmol/L glucose. Cultures of cerebrospinal fluid, blood and urine all revealed *Cryptococcus neoformans.*

Conclusion: Cryptococcal infection most frequently manifests as meningitis, but can affect virtually any organ (e.g. lungs, skin, urogenital tract). Although our patient had no clinical signs of meningitis, *Cryptococcus neoformans* was cultured from the cerebrospinal fluid. Lumbar puncture is warranted in any patient with disseminated cryptococcal infection, with or without neurological symptoms. In this case we present a renal transplant recipient, suffering from disseminated cryptococcosis, that mimicked a metastasized lung malignancy. Infection is an important differential diagnostic consideration in any immunocompromised patient with a pulmonary lesion.

P097

Respiratory systems in an anammox bacterium, a whole-membrane complexome approach

N.M. de Almeida, H.J.C.T. Wessels, J.T. Keltjens, M.S.M. Jetten, B. Kartal

Radboudumc, Nijmegen

Introduction: The anaerobic ammonium oxidizing (anammox) bacterium *Kuenenia stuttgartiensis* uses three reactions in its nitrogen catabolism with NO and hydrazine

as intermediates. The generated electrons are cycled through membrane-bound complexes, thereby establishing a proton motive force. In the genome there is a high redundancy of genes that encode proteins and protein complexes that are involved in the electron transport chain (ETC): 4 ATP synthase clusters, 3 NADH:quinone oxidoreductase clusters and 3 bc_1 complexes, that all show variations to the canonical bacterial *bc* complex. Previously, a whole cell proteomic approach did not reveal whether or not all ETC complexes are actually expressed and functionally present in the membranes of *K. stuttgartiensis*.
Methods: For the comprehensive analysis of the membrane complexes of *K. stuttgartiensis*, we applied two-dimensional gel electrophoresis, followed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Intact membrane complexes were loaded on a first dimension blue native (BN) PAGE, allowing separation by molecular mass. Gel lanes was either applied to a second dimension SDS PAGE for MALDI-TOF analyses of the protein spots, or cut into equally sized slices and analyzed via LC-MS/MS. Using advanced protein abundance profiling methods, we were able to detect and estimate the abundance of complexes associated with the membranes, and their subunit composition.
Results and conclusion: By combining BN-PAGE and high throughput proteomics with complexome migration profiling, we were able to have a look at the membrane protein complement of the cell under a defined growth condition. We were able to distinguish between highly abundant and less abundant redundant protein complexes and could verify subunit compositions of complexes so far only observed in genomes. By hierarchical clustering, we could identify previously wrongly annotated subunits, helping us to identify enzymes that were not obvious from the genome sequence. As such, we could use this study to verify and further specify our hypotheses without being dependent on activity assays, bringing us a step closer to understanding the anammox metabolism.

P098

Novel mono-, di-, and trimethylated ornithine membrane lipids in northern wetland planctomycetes

E.K.M. Moore, C. Rijpstra, C. Hopmans, L.V. Villanueva, N. Dedysh, H.W. Wienk, F.S. Schoutsen, A.M. Mets, S. Sinninghe Damste
Royal Netherlands Institute for Sea Research (NIOZ), Dept. of Biogeochemistry (BGC), 'T Horntje

Northern peatlands represent a significant global carbon store and commonly originate from *Sphagnum* moss-dominated wetlands. These ombrotrophic ecosystems are rain fed, resulting in nutrient-poor, acidic conditions. Members of the bacterial phylum *Planctomycetes* are highly abundant and appear to play an important role in the decomposition of *Sphagnum*-derived litter in these ecosystems. High-performance liquid chromatography coupled to high-resolution accurate-mass mass spectrometry (HPLC-HRAM/MS) analysis of lipid extracts of four isolated planctomycetes from wetlands of European north Russia revealed novel ornithine membrane lipids (OLs) that are mono-, di-, and trimethylated at the e-nitrogen position of the ornithine head group. Nuclear magnetic resonance (NMR) analysis of the isolated trimethylornithine lipid confirmed the structural identification.
Similar fatty acid distributions between mono-, di-, and trimethylornithine lipids suggest that the three lipid classes are biosynthetically linked, as in the sequential methylation of the terminal nitrogen in phosphatidylethanolamine to produce phosphatidylcholine. The mono-, di-, and trimethylornithine lipids described here represent the first report of methylation of the ornithine head groups in biological membranes. Various bacteria are known to produce OLs under phosphorus limitation or fatty-acid-hydroxylated OLs under thermal or acid stress. The sequential methylation of OLs, leading to a charged choline-like moiety in the trimethylornithine lipid head group, may be an adaptation to provide membrane stability under acidic conditions without the use of scarce phosphate in nutrient-poor ombrotrophic wetlands. Analysis of Russian and Swedish sediment cores from *Sphagnum* moss-dominated wetlands revealed that trimethylornithine lipids are present from the surface down to 50 cm depth with a sharp peak at the oxic/anoxic interface where *Sphagnum* degradation takes place. It appears that trimethylornithine lipids may be a useful proxy for *Sphagnum* degradation, and that planctomycetes may play an important role in this process.

P099

MALDI-TOF MS is unable to perform a reliable species level identification of clinical Aeromonas strains

W.S. Voskuil, W.V. Vries de, C.H.E. Boel, J.G. Kusters
University Medical Centre Utrecht, Dept. of Medical Microbiology, Utrecht

Introduction: *Aeromonas* is a gram-negative rod shaped bacterium commonly found in surface water. Of the 21 officially recognized species *A. hydrophila, A. caviae* and *A. veronii* (biovar *veronii* and *sobria)* are the most significant pathogens in humans. Identification of *Aeromonas* species based on biochemical reactions does not always result in unambiguous results. While too laborious for routine diagnostics sequence-based techniques can correctly identify *Aeromonas* species. MALDI-TOF MS is a convenient method for routine identification, but it has been suggested that MALDI-TOF MS is unable to distinguish *Aeromonas* at species levels. AIM OF THIS

STUDY: to evaluate MALDI-TOF MS for species level identification of *Aeromonas* isolates.

Methods: A collection of 2 type strains (ATCC) and 118 *Aeromonas* isolates from 11 Dutch hospitals was used. All strains (n = 120) were grown on TSA II agar (Becton Dickinson) for 16 hrs at 37°C. Separate colonies were picked and analysed (in duplicate with direct spot method) on a Bruker microflex LT with the Biotyper database version 4613 as recommended by Bruker. A cut-off of > 2.00 was used for reliable species identification. When scores were < 2.00 a protein extract was prepared and tested. When this still did not provide scores > 2.00, strains were reinoculated and retested as outlined above. DNA isolation, *dnaJ* gene sequencing, and phylogenetic trees were constructed using standard methods. The *dnaJ* gene sequences of the reference strains from the public databases were used to designate species names to the phylogenetic clusters.

Results: We were able to obtain reliable *dnaJ* sequences for 119/120 (99,2%) isolates. Based on the phylogenetic trees we were able to identify 39 strains as *A. veronii,* 30 as *A. caviae,* 26 as *A. hydrophila,* 16 as *A. media,* three as *A. veronii* biovar *veronii,* three as *A. salmonicida,* one as *A. bestiarum* and one as *A. jandaei.* When analysed by MALDI-TOF MS only for 114/120 (95%) isolates a score > 2.00 could be obtained. Repeated MALDI-TOF MS analysis, use of different media and and/or application of various extraction techniques did not improve these scores. When these 114 isolates were analysed in more detail it was observed that with the majority of the isolates matches were found to two or more different *Aeromonas* species with a score value > 2.0 and often the difference between two scores with different species names was < 0.1. This implies that a reliable identification was not possible for the majority of the tested isolates. If for the 114 isolates with a score of > 2.0 the highest score was regarded as the correct identification these strains consisted of 44 *A. veronii*, 31 *A. caviae,* 27 *A. hydrophila*, seven *A. media*, three *A. bestiarum*, one *A. eucrenophila*, and one *A. jandaei* isolate.

Conclusion: While the Bruker microflex LT is able to correctly identify *Aeromonas* isolates to the genus level, with the current version of the database it is not suitable to reliably assign *Aeromonas* strains to the species level.

P100

Susceptibility testing of Candida species isolates with the commercial MICRONAUT broth microdilution system

M. Heusinkveld, M. Gittelbauer, C. Vandenbroucke-Grauls, Y.J. Debets-Ossenkopp

VU University Medical Center, Dept. of Medical Microbiology and infection prevention, Amsterdam

Introduction: EUCAST prescribes a broth microdilution method as the preferred system to test susceptibility of *Candida* species to antifungals. However, most routine laboratories use the E-test, an agar diffusion method, which has a good categorical agreement with the broth microdilution methods. In our routine laboratory we use the E-test. This method is laborious, time consuming and the reading of the MICs is often a challenge due to 'trailing' when testing azoles. In search for an alternative we compared the performance of the Micronaut broth microdilution system (Merlin, Russelsheim, Germany) with the E-test.

Methods: Micronaut AM-MHK2 96-well plates contain nine vacuum dried antifungals in different concentrations. Rehydration with 100 ul yeast suspension in RPMI (2% glucose) broth results in twofold dilution series. Methylene blue is added for reduction of trailing. E-tests were carried out according to the manufacturers instructions on RPMI agar (2% glucose). The tests were run in parallel with a yeast culture grown overnight on Sabouraud dextrose agar, resuspended thereafter either in RPMI broth (Micronaut) or PBS (E-test) to achieve a final concentration of $1{,}5x10^5$ cfu/ml (Micronaut) and $1\text{-}5x10^5$ cfu/ml (E-test). Microdilution trays and E-tests were read after 24 hours of incubation at 37° C visually (Micronaut and E-test) and spectrophotometrically (Micronaut) and in case of insufficient growth after 48 hours. Reading and interpretation of the MICs was done according to EUCAST criteria.

Strains: ixty-three clinical isolates of *Candida* species (17 *C. albicans,* 9 *C. parapsilosis,* 8 *C. krusei,* 7 *C. glabratra, 6 C. dubliensis,* 6 *C. guilliermondii,* 6 *C. tropicalis,* 4 *C. kefyr,* 5 other candida species) from patients with an hematologic malignancy and 5 ATCC strains were tested.

Results: Micronaut system: 70% of the plates could be read spectrophotometrically after 24 hours, 20% after 48 hours and 10% had to be repeated because of no growth. In 7 Micronaut-plates the wells with anidulafungin showed irregular growth and the median MIC anidulafungin was considerably higher compared to the MIC as determined by E-test (median of all strains tested 0,062 vs 0,016 mg/L). For Amphotericin B the MICs were higher when determined with Micronaut (median of all strains tested 0,5 vs 0,064 mg/L). This was also seen with the azoles (median MIC for fluconazole of all strains tested 1,5 vs 0,5 mg/L). Categorical agreement between Micronaut and E-test MICs, according species-specific EUCAST clinical breakpoints, was for anidulafungin: 68%, for amphotericin B: 85%, and for fluconazol: 84%.

Conclusion: Antifungal susceptibility testing with the Micronaut broth microdilution system was in our laboratory easy to perform and less time consuming compared to E-test. However, Micronaut generated higher MICs compared to E-test for the same strain. The categorical agreement between the two methods did not exceed 85% and this can result in false resistant reports with the Micronaut system.

P101

Microbial community involved in anaerobic oxidation of methane in a rice paddy field

A. Vaksmaa, C. Lüke, T. van Alen, M.S.M. Jetten, K.F. Ettwig
Radboudumc, Dept. of Microbiology, Nijmegen

Methane is the second most relevant greenhouse gas in the atmosphere and is 25 times more potent in the radiative forcing than CO_2. Since the industrial age, the atmospheric CH_4 concentrations have increased about 150%.
Rice paddy fields contribute approximately 20% to the global methane emission, making them a relevant source of methane. Rice fields form several distinct habitats for microorganisms: the surface soil, the bulk soil, rhizosphere soil and roots. It is a dynamic ecosystem where (nitrogen) fertilization, rice cultivar, flooding and draining seasons affect the presence of microbial communities associated with methane production and consumption. An inbalance in the community may influence methane emission rates to atmosphere. Anaerobic oxidation of methane (AOM) coupled to nitrate and nitrite carried out by archaea or bacteria has recently been demonstrated.[1,2] Whereas most research efforts on rice fields have been targeted to methanogenesis and aerobic methane oxidation, only little is known about anaerobic methane oxidation. As rice field are heavily fertilized, nitrate-dependent AOM might be relevant in these ecosystems. So far only the different spatial distribution occurrence, but not significance of nitrate-dependent methane oxidizing bacteria has been demonstrated in rice paddy field soil. Here we collected rice paddy field bulk soil and rhizosphere samples from the fields of the Italian Rice Research Institute, Vercelli (Italy). The samples were taken in the flooded season (anaerobic conditions). Total microbial community was determined with Amplicon sequencing on the Ion Torrent PGM system by using 16S rRNA gene markers for bacteria and archaea. Community assessment was performed on two distinct compartments of paddy field soil – rhizosphere (roots and attached soil) and bulk soil. Assessment of the anaerobic methanotrophic community was performed using the mcrA gene encoding the methyl-coenzyme M reductase, the key enzyme for methanogenesis as well as archaeal methane oxidation. PCR amplification, cloning, sequencing and phylogenetic analysis was used to characterize the distribution of anaerobic oxidation of methane associated archaea (AAA) and the co-occurrence of other relevant phyla (*e.g.* methanotrophic bacteria of NC10 phylum). Quantification of total archaea, bacteria and AAA was carried out by Q-PCR. The communities that contribute to anaerobic oxidation of methane are different in the rhizosphere and soil compartment. Our experiments further demonstrate the presence of anaerobic methane oxidizing micro-organisms in the rice paddy field, showing that not only aerobic methanotrophs are important in these systems to counteract methane emissions.

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P102

Culturing human rhinovirus C on human airway epithelial cells

L. van der Linden, B.M. Westerhuis, R. Minnaar, M.D. de Jong, K.C. Wolthers
AMC, Dept. of Medical Microbiology and Clinical Virology, Amsterdam

Introduction: Human rhinovirus (HRV) species A, B, and C are members of the genus *Enterovirus* of the family *Picornaviridae*. They can cause a common cold, but also more severe diseases such as bronchiolitis, pneumonia or exacerbations of asthma and COPD. HRV-A and HRV-C appear to be associated with a more severe outcome than HRV-B. The discovery of the species HRV-C was delayed to 2007 due to the inability to culture these viruses on standard cell lines. Only recently people have reported successful culture of HRV-C on either snippets of sinus tissue or on human airway epithelial (HAE) cells, a culture system in which primary bronchial or nasal cells are differentiated under air-liquid interface. Our goal was to culture HRV-C clinical samples and to characterize the infection.
Methods: HAE cells were either obtained differentiated from Epithelix (bronchial or nasal) or differentiated in-house from residual surgical specimens (bronchial) and grown in transwells under air-liquid interface. These cells were inoculated with HRV-C-positive material from nose or throat swabs. After incubation, apical and basolateral viral loads were determined with qPCR.
Results: Clinical samples of HRV-C10, HRV-C15, and HRV-C44 were successfully propagated on HAE cells obtained from Epithelix both from bronchial or nasal origin. Virus was detected mainly at the apical side, but also at the basolateral side low viral loads could be detected. In contrast, culture of HRV-C on our in-house differentiated HAE cells proved unsuccessful.
Conclusion: (1) HRV-C can be propagated on HAE cells. (2) Virus is released from both the apical and the basolateral sides of the culture. (3) However, the permissiveness of HAE cells depends on the method of differentiation.

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Long-term gut colonization by multi-drug resistant *Enterococcus faecium* clones in patients

J. Baan, R.J.L. Willems, W. van Schaik
UMC Utrecht, Dept. of Medical Microbiology, Utrecht

The bacterium *Enterococcus faecium* is usually a harmless inhabitant of the human intestinal tract. However, *E. faecium* has emerged as an important cause of hospital-acquired infections. These infections are difficult to treat, because clinical *E. faecium* isolates have acquired resistance against numerous antibiotics. Interestingly, *E. faecium* strains that cause hospital-acquired infections are genetically distinct from antibiotic-resistant strains that are carried by healthy humans or livestock. Additional adaptations, such as the ability to colonize the intestinal tract of hospitalized patients for prolonged periods of time, appear to have evolved in clinical *E. faecium* strains.

In our hospital, regular diagnostic screening has identified ampicillin- and vancomycin-resistant enterococci (VRE and ARE) in patients that were hospitalized. Subsequent screening for colonization by VRE and ARE was performed whenever patients were subsequently hospitalized. Isolates from these patients were stored at -80C and resulted in a collection of strains that were isolated over an extended period of time (between 3 months and 7 years; collected between 2001 and 2008) from particular patients. Using pulsed-field gel electrophoresis, we analyzed the strain collections for 5 patients (consisting of a total of 100 strains) to study the similarity of the *E. faecium* strains found at different time points.

We observed high similarity between strains in the individual patients, with certain ARE strains appearing to be present in the patient for over 6 years. Furthermore, we found that patients may be colonized by multiple ARE strains simultaneously. Next, we plan to use next-generation whole-genome sequencing to characterize intra-clone genetic diversity and to identify genetic elements that these different *E. faecium* strains have evolved to allow long-term host colonization.

The results suggest that (i) certain *E. faecium* strains have the ability to colonize patients for extended periods of time. In addition, (ii) patients may be colonized with multiple antibiotic-resistant strains of *E. faecium* simultaneously. This finding may have implications for the future treatment of and infection control measures for patients colonized with ARE/VRE.

P104

Early respiratory microbiota composition determines bacterial succession patterns in children

G. Biesbroek[1], E. Tsivtsivadze[2], E.A.M. Sanders[1], R. Montijn[2], R.H. Veenhoven[3], B.J.F. Keijser[2], D. Bogaert[1]
[1]*UMC Utrecht, Dept. of Pediatric Infectious Diseases and Immunology, Utrecht,* [2]*TNO Earth, Environmental and Life Sciences, Research Group Microbiology and Systems, Zeist,* [3]*Spaarne Hospital, Research Center Linnaeus Institute, Hoofddorp*

Introduction: Many bacterial pathogens causing respiratory infections in children are common residents of the respiratory tract. Insight into bacterial colonization patterns and stability at young age might elucidate healthy or susceptible conditions for development of respiratory disease. We therefore studied the dynamics of microbiota profiles over time in healthy young children

Methods: We characterized the consecutive nasopharyngeal microbiota profiles of 60 healthy children at the ages of 1.5, 6, 12 and 24 months by 16S GS-flx-titanium-pyrosequencing, and analyzed the consecutive profiles using statistical machine-learning algorithms. Cross-sectional findings were validated in an additional group of 140 infants.

Results: Overall, we identified 6 distinct microbiota profiles represented by the dominant genera *Moraxella, Haemophilus, Streptococcus,* or *Staphylococcus,* a combination of *Dolosigranulum* and *Corynebacterium,* plus cluster-specific low abundant biomarker bacteria. We observed specific patterns of change over time, with more stable patterns marked by early presence and high abundance of the *Moraxella* and *Corynebacterium/Dolosigranulum* clusters, and less stable profiles marked by high abundance of the *Haemophilus* or *Streptococcus*-dominated clusters

Conclusions: The current study enabled us to gain insight in the dynamic nature of nasoparyngeal microbiota in infants. Our results suggest that the composition of early-life microbiota is associated with long-term stability and may predict susceptibility to disease

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P105

Recognition of *Coxiella burnetii* Nine Mile is mediated by TLR1/TLR2, while the Dutch outbreak isolate *Coxiella burnetii* 3262 is recognized by the TLR2/TLR6 heterodimer as well

A. Ammerdorffer[1], M.S. Gresnigt[1], T. Schoffelen[1], M. Oosting[1], M.H. den Brok[1], J.M. Rebel[2], M. van Deuren[1], M.G. Netea[1], H.I.J. Roest[2], L.A.B. Joosten[1], T. Sprong[3]
[1]*Radboudumc, Dept. of Internal Medicine, Nijmegen,* [2]*Central Veterinair Institute, Dept. of Infection Biology, Lelystad,* [3]*Canisius-Wilhelmina Ziekenhuis, Dept. of Medical Microbiology and Infectious Diseases, Nijmegen*

The bacterium *Coxiella burnetii* is the causative agent of Q fever. An unanswered question is how *C. burnetii* evades the immune system, and leads to chronic Q fever state in some cases. As pattern recognition receptors play an

important role in the first line defense against microorganisms, we investigated whether Toll like receptor (TLR) 1,2, 6, NOD1 and NOD2 are involved in the recognition of *C. burnetii*. Peripheral blood mononuclear cells (PBMCs) were isolated from healthy individuals and stimulated with *C. burnetii* Nine Mile and/or the Dutch outbreak isolate *C. burnetii* 3262. TLR2 and TLR4 were inhibited by using respectively a specific antibody and antagonist. The role of TLR1, TLR6, NOD1 and NOD2 was investigated using PBMCs of individuals genotyped for polymorphisms in these genes, and by using TLR1 and TLR6 knockout mice. Furthermore, PBMCs were isolated from NOD2 deficient individuals.

PBMCs preincubated with anti-TLR2 produced less IL-1beta and IL-6 upon challenge with *C. burnetii* Nine Mile, while blocking TLR4 in PBMCs had no effect. Bone marrow derived macrophages of mice lacking TLR1 and TLR2 produced less IL-6 after *C. burnetii* Nine Mile stimulation in comparison with the wild type mice. The production of IL-6 by TLR6 knockout mice was nearly completely diminished after *C. burnetii* Nine Mile stimulation. Individuals homozygous for SNPs in TLR1 genes showed an impaired TNF-alpha and IL-1beta response after stimulation with both *C. burnetii* Nine Mile and *C. burnetii* 3262. Interestingly, the cytokine production of individuals bearing the TLR6 SNP did not alter after *C. burnetii* Nine Mile stimulation, but a significant decreased cytokine response was observed in these persons after *C. burnetii* 3262 encounter. Cytokine production by PBMCs after *C. burnetii* stimulation was decreased in NOD2 deficient patients and in individuals with SNPS for NOD2. Among the individuals bearing the NOD1 SNP a declined production of cytokines was observed.

We conclude that NOD2 and TLR2 are important for the recognition and induction of an immune response against *C. burnetii* in humans. Furthermore, our results show that the Dutch outbreak strain *C. burnetii* 3262 is recognized by both TLR1 and TLR6, while *C. burnetii* Nine Mile is only recognized by TLR1.

P106

Lactobacillus friend or enemy? A case of an urinary tract infection

G.H.J. Wagenvoort[1], P. van Houten[2], D. Meier[1], T. Tersmette[1]
[1]*St Antonius Hospital Nieuwegein, Dept. of Medical Microbiology, Nieuwegein,* [2]*Zonnehuisgroep Amstelland, Amstelveen*

Introduction: This case report describes an urinary tract infection (UTI) with was caused by *Lactobacillus delbrueckii*.

Case report: A 92-year-old female in a nursing home was diagnosed with an UTI. Her relevant medical history included recurrent urinary tract infections and uterine prolapse (with a pessary, which was removed 4 days earlier). Her last UTI's were 4 and 6 months previously, from which no urine cultures were taken. Urinalysis which revealed no epithelial cells, many leukocytes and culture on Columbia-CNA Agar showed > 10E5 CFU/mL with green coloration. Examination with MALDI-TOF resulted in an identification of: *Lactobacillus delbruecki*.

These results were reported to the physician 3 days later. However, the complaints already disappeared after treatment with nitrofurantoin. Since *Lactobacillus delbrueckii* was resistance to this agent this suggested spontaneous clearance.

Discussion: Lactobacilli are commensal bacterial flora of the female genital tract and associated with a protective role in preventing UTI's. Although, they can be a cause of UTI. In medical literature, *Lactobacillus delbrueckii* is described as a cause of UTI, mainly in elderly woman. MALDI-TOF can indentify *Lactobacillus sp.* reliably. That, in combination with the appearance in microscopy and high leukocyte quantities, make it very likely that this was indeed a *Lactobacillus delbrueckii* UTI.

Conclusion: *Lactobacillus sp.* are generally considered a commensal and their role in UTI is likely underreported. Especially in elderly woman, laboratory personal and clinicians should be aware that they can also cause UTI.

P107

Experience on structured bedside consultations for patients with *Staphylococcus aureus* bacteremia in a large non-academic hospital

C.M.A. Swanink, E.M. Mascini, C. Lindeboom, C. Richter, E.H. Gisolf
Rijnstate Hospital, Dept. of Medical Microbiology and Immunology, Arnhem

Introduction: Staphylococcus aureus bacteremias (SAB) are often complicated with endocarditis, spondylodiscitis or other metastatic disease. These complications influence patient outcome and duration of antibiotic treatment. According to recent publications lower mortality rates are found in patients who received bedside consultation by an Infectious Diseases consultant. Even in uncomplicated SAB, guidelines on antibiotic treatment are not followed in a substantial percentage of patients. We therefore initiated bedside consultations in all patients with SAB in the beginning of 2012. With the introduction of the Antibiotic Stewardship team (A-team) in 2014, bedside consultations in SAB patients are presented as one of the five initial aims of the A-teams.

Methods: Since the beginning of 2012, all new SAB are not only reported to the treating physician, but also to the resident Infectious Diseases or consultative Internal

Medicine resident. Every week, an email is sent to re-check. The bedside consultation is done according to a written protocol. The visit is supervised by the Infectious Disease specialist. Also the cardiologists are involved in this protocol. As soon as the consultant finds an increased risk for complications or cardiac murmur, the cardiologist is consulted to perform an ultrasound. We retrospectively collected data on SAB patients in 2011 for comparison.

Results: In 2011, 61 patients were identified with a SAB, in 2012 75. Sixty percent of all these patients was male. Mortality was 26% in 2011 and 31% in 2012 (16 vs. 23 patients). In 2012, 10 of the 23 patients who died (43%), did not undergo additional diagnostic investigations. In 2012, more ultrasounds of the heart were performed in comparison to 2011 (44 vs. 64%). In 2012, more patients were treated correctly when compared to 2011 (57 vs. 81% of patients). Results of 2013 are pending.

Conclusion: Structured consultation in SAB patients results in an adequate treatment of SAB in a higher percentage of patients. So far we were not able to show a decrease in mortality rate. A large proportion of patients was already withdrawn from therapy because of severe comorbidity or died before additional investigations could be done. In these patients, no improvement of bedside consultation can be expected. The system of bedside consultations is vulnerable because of personnel changes and the involvement of many people. Continuous education is needed.

P108

Sonication of infected vascular prostheses followed by culture and polymerase chain reaction of sonication fluid

K.A. Heemstra, T.T. Severs, J.G. Kusters, P. Berger, C.E.H. Boel

UMC Utrecht, Dept. of Medical Microbiology, Utrecht

Introduction: Vascular prosthesis infections cause significant morbidity and mortality. Treatment consists of surgical debridement, graft replacement and long-term broad-spectrum antibiotic therapy. Adequate antibiotic therapy directed against the specific causative micro-organism is important. However detection of these micro-organisms is often difficult, as patients have frequently been treated with broad-spectrum antibiotics before specimens for culture are obtained. While the sensitivity of classical culture will suffer from this, polymerase chain reaction (PCR) targeting the bacterial 16S rRNA gene is probably not affected. In addition, the microorganisms are often firmly attached to the prosthesis in a biofilm. For this reason sonication, in which sound waves are used to disrupt the biofilm, is often used in orthopaedic surgery to enhance the success rate of microbial culture. The aim of this study was to test if sonication improves the detection of microorganisms on vascular prosthesis as compared to direct culture and to define the additional value of PCR of the sonication fluid.

Methods: We performed a prospective study in which vascular prosthesis were divided cross-sectional in three parts, one of which was used for direct culture, while the other two were sonicated followed by culture or 16S rRNA PCR of the sonication fluid respectively.

Results: Twenty-six vascular prosthesis of 19 patients were included in the analysis. Thirteen samples (50%) of eleven patient (58%) had been treated for > 24 hours with antibiotics before the samples were taken. Another 10/26 samples (38%) from 7 patients (37%) were treated with one dose of preoperative prophylactic cefazoline only.

Results of conventional culture of the vascular prosthesis were similar to results of cultures of sonication fluid in 17 samples (68%). In 8 samples (32%) results of conventional cultures were different from those from cultures of sonication fluid. In 4 samples cultures of sonication fluid were negative while in the corresponding conventional cultures microorganisms were found. In 3 samples that contained more than one species both techniques detected several microorganisms but with different results. In one sample two different species were found with both techniques, of which one was similar and one was different in both techniques. In one patient no conventional culture of the vascular prosthesis was performed.

Results of 16s rRNA were similar to the results of culture of sonication fluid in 13 samples (50%). In 9 samples (35%) the results of 16S rRNA were positive while results of culture of sonication fluid were negative. In this group we found two independent samples from the same patients in which the results of the 16S rRNA PCR were consistent. In two samples culture of sonication fluid was positive while results of 16S rRNA were negative. In one sample both techniques detected more than one species, of which only one was identical.

Conclusion: PCR amplification of the 16s rRNA gene of sonication fluid of suspected infected vascular prosthesis seems to be more sensitive than either direct culture or culture of sonication fluid of the vascular prosthesis. However relevance and clinical significance needs to be further investigated.

P109

Diagnosis of late onset sepsis in preterm infants with a novel multiplex PCR

M. van den Brand[1], F.A.M. van den Dungen[1], A. Rubenjan[1], A.M. van Furth[1], M.M. van Weissenbruch[1], M.P. Bos[2], R.P.H. Peters[3], P.H.M. Savelkoul[1]

[1]VU University Medical Center, Dept. of Medical Microbiology and Infection Control, Amsterdam, [2]Microbiome, Houten, [3]Maastricht University Medical Center, Dept. of Medical Microbiology, Maastricht

Objectives: Late onset sepsis (LOS) is a severe condition with high prevalence in preterm infants. The diagnostic standard, blood culture, suffers from suboptimal sensitivity and long turnaround time; hence, a rapid additional test would be highly valuable. The use of broad-range PCR showed fair results but implementation is hampered by increased time needed for species identification and associated costs. Multiplex PCR has the benefit of direct species identification, but is rarely used for diagnosis of LOS. We clinically evaluated the diagnostic performance of a novel multiplex PCR that quantitatively detects the 8 most prevalent bacterial pathogens in LOS. This assay is applicable on small blood volumes, requires limited hands-on time and provides species-specific results within 4 hours.

Methods: We prospectively included 85 suspected episodes of LOS, occurring in 71 preterm neonates (gestational age < 32 weeks) admitted to our NICU. A whole blood sample (0.2ml) for multiplex PCR was obtained together with blood culture prior to initiation of antibiotic therapy. Bacterial DNA was isolated using bacterial lysis buffer (Biocartis) and the EasyMag system, and subjected to the multiplex PCR (panel: coagulase negative staphylococci, *Staphylococcus aureus, Enterococcus faecalis, Streptococcus agalactiae, Escherichia coli, Klebsiella spp., Pseudomonas aeruginosa and Serratia marcescens*). Blood culture and PCR were compared.

Results: Blood culture was positive in 57 and negative in 28 episodes, while PCR was positive in 51. Coagulase negative staphylococci (n = 48) were most frequently detected by blood culture and/or PCR, followed by *S. aureus (*n = 13), *S. agalactiae* (n = 4), *E. faecalis* (n = 2), *E. coli* (n = 2), *Klebsiella* spp. (n = 2) and *Lactobacillus* spp. (n = 1). For monomicrobial infections (n = 78) PCR demonstrated a sensitivity of 80%, specificity 82%, PPV 89% and NPV of 70% compared to blood culture. PCR detected 5 additional episodes of possible LOS.

Seven episodes were polymicrobial, of which 4 were detected by PCR only. For example, the blood culture in one neonate with clinically necrotising enterocolitis revealed lactobacilli, while PCR detected DNA of *Klebiella spp.*, *E. faecalis* and *S. agalactiae*.

Conclusions: We clinically evaluated a newly developed multiplex PCR that detects the 8 most common bacterial pathogens causing LOS in a cohort of preterm neonates with a high incidence of positive blood culture.

1. PCR had a high positive predictive value of 89% and sensitivity of 80%
2. PCR detected additional cases of possible LOS

To conclude, this study demonstrated that multiplex PCR is a useful additional diagnostic tool for rapid identification of LOS.

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No indication for tissue tropism for *Chlamydia trachomatis* strains defined by using multilocus sequence typing

A.J. Versteeg, M.S. Rooijen van, M.F. Schim van der Loeff, H.J.C. Vries de, S.M. Bruisten
GGD Amsterdam, Cluster Infectious Diseases, Amsterdam

Introduction: A recently developed high resolution *Chlamydia trachomatis* multilocus sequence typing (CT-MLST) system has improved the characterization of different *C. trachomatis* strains infecting populations at risk. Studies using this CT-MLST method revealed separate transmission networks for men having sex with men (MSM) and heterosexuals [1] The different distributions of *C. trachomatis* strains may be a reflection of differences in sexual behavior of MSM and heterosexuals. However, in studies mentioned, samples from MSM were primarily obtained from the anorectal tract whereas samples from heterosexuals were obtained from the urogenital tract. Therefore another explanation might be tissue tropism, causing specific *C. trachomatis* sequence types to be preferentially associated with either the urogenital or the anorectal tract.

Methods: A retrospective analysis was performed using routinely collected data and samples (from 2012) of women diagnosed with chlamydia at the STI outpatient clinic of the PHS, Amsterdam.This analysis was restricted to women who were diagnosed with either concurrent *C. trachomatis* infections at multiple anatomic locations, or with a solitary rectal infection. Epidemiological data were retrieved from electronic patient records. Samples were typed using CT-MLST from which minimum spanning trees were generated. For the comparison of rectal infections between MSM and women, we selected samples from MSM with a rectal *C. trachomatis* infection from a previous study, for which full MLST data and epidemiological data were available.[1]

Results: Full MLST data were obtained for 207 MSM and 185 heterosexual women with rectal infections from which a minimum spanning tree was generated. This tree again showed a clear separation between samples from MSM and women which were dispersed over 6 large clusters. Of these, 3 clusters consisted predominantly of samples from women whereas the other 3 large clusters consisted of samples from MSM. So, in spite of the fact that only anal samples were used we observed the same phenomenon of separate transmission networks in MSM and heterosexuals.

Furthermore, we obtained full MLST data from 434 samples of 206 women with *C. trachomatis* concurrentinfections at multiple anatomical locations comprising 316 (72.8%) urogenital, 101 (23.3%) rectal, and 17 (3.9%) pharyngeal samples. Using the complete MLST profile of all 434 samples another minimum spanning tree was

generated in which 4 large clusters could be identified. This tree showed a heterogeneous distribution of STs found per anatomic location. If a cluster stands for a distinct *C. trachomatis* strain it was clear that each anatomical location could be infected with each of the strains defined by CT-MLST.

Conclusion: In rectal samples we still observed largely distinct *C. trachomatis* strains infecting MSM and women making tissue tropism unlikely.

We observed no significant differences in the proportion of urogenital, rectal or pharyngeal infections for each *C. trachomatis* strain, arguing against tissue tropism..

Most likely the separate transmission patterns in MSM and heterosexuals are due to network associated factors.

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P111

Evaluation of an algorithm for electronic surveillance of hospital acquired infections yielding serial weekly point-prevalence scores

H.R.A. Streefkerk, G.J.J.M. Borsboom, H.A. Verbrugh, R.P. Verkooijen, C.P. van der Hoeven
Erasmus MC, MMIZ, Rotterdam

Introduction: Insight in the epidemiology of hospital-acquired infections (HAI) is a prerequisite for infection control. Surveillance based on frequently performed point-prevalence surveys would be a way to be maximally informed. To reduce time spent we developed an electronic surveillance method. It is based on a selection algorithm that automatically differentiates between patients with and patients without high probability of having a HAI followed by detailed review of the patients with a high probability of HAI.

Methods: For each patient (n = 5,797) included in ten consecutive point-prevalence surveys all predictive parameters present from admission date to point-prevalence date were automatically gathered and stored in a database. Then each patient was automatically evaluated (marked HAI positive or HAI negative) based on the selection algorithm. The ten consecutive hospital-wide surveys were performed each March and October from 2008-2012. Consistency of the algorithm was analyzed using an exact likelihood ratio Chi-square test.

Results: Overall HAI prevalence was 8,1 HAI per 100 patients. Based on the selection algorithm 4,026 (69%) of the 5,797 patients were automatically marked as HAI negative. In the remaining 31% of the population 91% (429/471) of all types HAI, including 91% (120/132) of the SSI, 92% (103/112) of the BSI, 92% (102/111) of the LRTI, 86% (42/49) of the UTI were found. The performance of the algorithm was consistent, i.e. no significant trend indicated Similar consistent results have been observed with SSI, BSI, LRTI and UTI separately.

Conclusion: An electronic surveillance method based on our selection algorithm is a sensitive and reproducible way to automatically exclude the large majority of the patients from detailed assessment by the ICP. Among the automatically preselected patients > 90 percent of all HAI can be found. The predictors used by the algorithm are digitally available in most hospitals, thus gathering them and executing the algorithm can be fully automated. Time needed for the detailed reviewing of the selected patients' clinical and laboratory data is significantly reduced since all these data have already been gathered. Consequently, this electronic surveillance system allows for frequent serial surveys for HAI's, thereby significantly improving the value and robustness of HAI surveillance. Future near-real time automated feedback of the results of this type of surveillance to the medical and managerial staff may

P112

Hepatitis B virus 'anti-HBcore only' test results: occult, resolved or no HBV infection at all

T.J.W. van de Laar, T. Marijt-van der Kreek, M.W.A. Molenaar-de Backer, B.M. Hogema, H.L. Zaaijer
Sanquin Blood Supply, Dept. of Blood-borne Infections, Amsterdam

Introduction: Anti-HBcore reactivity in the absence of other HBV markers may indicate occult (HBsAg-negative) HBV infection, but also fits the serological profile of truly resolved HBV infection with declined anti-HBs titers. Alternatively, 'core-only' test results may be the result of false-positive anti-HBcore reactivity. To improve the detection of occult HBV-infection, anti-HBcore screening was included in the routine screening of blood donations in the Netherlands as of July 2011, enabling us to study the nature of isolated anti-HBcore reactivity.

Methods: The anti-HBcore test result (PRISM Abbott) of all donors (n = 453.481) during the first two years of anti-HBcore screening in the Netherlands (July 2011 - June 2013) were analysed, together with routine screening results for HBV DNA (in pools of 6 donation; Cobas Taqscreen, Roche), HBsAg and anti-HBs (PRISM, Abbott). The correlation between quantitative anti-HBcore results and other HBV laboratory markers, as well as age, sex and presumed ethnicity of the donors was studied to differentiate between true and false positive anti-HBcore reactivity. Donor ethnicity (Dutch, non-Dutch, ambiguous) was independently scored by three researchers based on surnames only. In case of ambiguous or discordant ethnicity scores, donor ethnicity was determined based on the 1947 Meertens Institute database of Dutch family names.

Results: Anti-HBcore reactivity was observed in 2901/453.481 (0.64%) of donors: 30 donors tested positive for HBV DNA and HBsAg, 1 donor tested HBsAg-negative with low-level HBV viremia, and 2870 donors had no marker of active HBV infection. In total, 444 donors tested anti-HBcore positive without detectable anti-HBs. Among these 444 'core-only' donors quantitative anti-HBcore results showed a bimodal distribution: either high (n = 113, 25,5%) or borderline (n = 273, 61,5%) anti-HBcore reactivity; intermediate reactivity was less common (n = 58, 13.1%). Non-Dutch ethnicity correlated significantly with high levels of anti-HBcore reactivity; increasing from 5.5% to 45.2% in the groups with borderline and high anti-HBcore reactivity, respectively ($p < 0.001$). The median age of donors with borderline' versus high' anti-HBcore reactivity increased from 52 to 57 years and 47 to 52 years among donors of Dutch and non-Dutch ethnicity, respectively. Prolonged anti-HBcore reactivity was observed in 230/262 (87%) of donors with borderline or intermediate reactivity, and in all donors with high-reactivity. Compared to 'core-only' donors, borderline (10.8% vs. 61.5%) and intermediate (8.6% vs. 13.1%) anti-HBc reactivity was less common among donors with detectable anti-HBs. Finally, false-positivity rates were estimated as 11-19%, and 62-74% among anti-HBs positive and anti-HBs negative donations, respectively.

Conclusion: In a Dutch low risk population, the anti-HBcore reactivity is extremely low (0.65%). The strength of the anti-HBcore signal correlates with the presence of other HBV markers and HBV risk factors, including non-Dutch ethnicity and older age, and is a useful marker to differentiate between true and false positive anti-HBcore test results.

P113

A concept NVMM Guideline for Laboratory detection of vancomycin resistant enterococcus

J.C. Sinnige[1], R.J.L. Willems[1], G.J.H.M. Ruijs[2], E.M. Mascini[3], J.P. Arends[4], M.J. Bruins[2], A. Troelstra[1]

[1]UMC Utrecht, Dept. of Medical Microbiology, Utrecht, [2]Isala, Dept. of Microbiology, Zwolle, [3]Rijnstate Hospital, Dept. of Medical Microbiology, Arnhem, [4]Universitair Medisch Centrum Groningen, Dept. of Medical Microbiology, Groningen

Objectives: The last two years an increased incidence of vancomycin resistant enterococcus (VRE) was observed in Dutch hospitals. The NVMM Guidelines on laboratory detection of highly resistant microorganisms are currently being re-evaluated and since these guidelines do not contain a section dedicated to the detection of VRE we developed recommendations for the laboratory detection of VRE in the Netherlands.

Methods: We did a search of relevant studies through pubmed and a review of the references of retrieved articles. Index searches included the following search term: ['vancomycin-resistant *Enterococcus faecium'* and 'detection']. The search was restricted to published English articles between 2000 and 2014. Relevant results of unpublished studies providing sufficient data were also included.

Results: In total 15 studies were reviewed. Based on this review stool or rectal swabs (RS) appeared a sensitive method for detection of VRE. The overall sensitivity of the RS culture was 58% (95% CI, 37-77); it ranged from 0% (at densities of 4.5 log10 cfu/g stool) to 100% (at VRE densities of 7.5 log10 cfu/g stool).[1] The sensitivity of four rectal swabs from 172 patients, each collected on separate days, was 92%, five rectal swabs resulted in a sensitivity of > 95%.[2] Laboratory methods include pooling 5 swabs in EB-Aztreonam media and culturing on selective solid agar media (data not published, tested on commercial available plates or in-house produced agar containing 4 mg/L vancomycin). The VRE-probable colonies detected on VRE selective plates were subjected to species and *van* gene confirmation by MALDI-TOF MS and PCR respectively. The minimum inhibitory concentrations (MICs) of vancomycin and teicoplanin for the VRE isolates were determined by Etest. VRE positive isolates are collected and representative isolates are typed using multi locus sequence typing (MLST).

Conclusion: Literature search offered several laboratory methods and clinical evidence for effective detection of VRE in rectal swab specimens. A common workflow helps laboratories with clinical decision making and prepares them for screening large groups of suspected carriers of higly resistant microorganisms.

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P114

Enterococcus faecalis endocarditis: acquired daptomycin resistance during treatment

V. Hira[1], C.C. Driessen[1], J.C.G.J. Peeters[1], I.H.M. van Loo[1], S. Lowe[1], F.H. van Tiel[1], P.H.M. Savelkoul[1,2] M.J. Mooij[1]

[1]Maastricht University Medical Centre, Dept. of Medical Microbiology, Maastricht, [2]VU University Medical Center, Dept. of Medical Microbiology and Infection Control, Amsterdam

Introduction: A 66-year old female with a history of aortic valve and mitral valve replacement and a pacemaker, was

admitted to our hospital after two weeks of malaise and fever. Three weeks earlier, she had undergone pacemaker replacement surgery. Blood cultures drawn on admission yielded growth of *Enterococcus faecalis*, and a subsequently performed transesophageal echo (TEE) showed vegetations on her mitral valve. Due to technical difficulties, the valve could not be replaced, and antibiotic treatment with amoxycillin 6dd 2000 mg iv and gentamicin 3 mg/kg iv was started. After five days, gentamicin was discontinued due to nephrotoxicity. Kidney function recovered after cessation of gentamicin, but four weeks later a TEE showed an increase in vegetation size on the mitral valve. Gentamicin was again added to the amoxycillin, after which her kidney function deteriorated again. Therapy was switched from amoxycillin and gentamicin to daptomycin 6 mg/kg iv, 3 weeks after reintroduction of gentamicin. This regimen was continued for another 9 weeks, until the patient was discharged in good health and without any clinical or laboratory signs of infection. However, two weeks after discharge she was readmitted with fever and blood cultures yielded a daptomycin resistant *E. faecalis*. In this study we investigated the nature of the *E. faecalis* strains isolated from blood cultures from this patient.

Methods: Isolation and antibiotic susceptibility testing of *E. faecalis* strains was performed using routine microbiological diagnostic methods and subsequently stored at -80°C. Molecular fingerprinting was performed using Multi Locus Sequence Typing (MLST) and Amplified Fragment Length Polymorphism (AFLP). These experiments were performed at UMC (Utrecht) and VUmc (Amsterdam), respectively. Attachment and biofilm formation were analysed using a microtiter plate assay, whereby attached cells were quantified using 0.5% (w/v) crystal violet upon 1 (attachment) and 24 hours (biofilm formation) of static culture in *Luria-Bertani media*, respectively. Data shown are from three independent biological experiments.

Results: Antibiotic susceptibility testing of the *E. faecalis* strains isolated from the blood cultures showed that the initial isolates were susceptible to daptomycin (MIC of 2 g/ml), whereas the *E. faecalis* strains isolated upon readmission were resistant to daptomycin (MIC of 12 g/ml). In order to differentiate relapse from reinfection, molecular fingerprinting of the strains was performed using both MLST and AFLP. MLST analysis showed that all strains belong to MLST sequence-type 21, and AFLP analysis confirmed that the daptomycin susceptible and resistant strains were identical. These findings demonstrate that a relapse occurred, whereby the *E. faecalis* strain acquired daptomycin resistance during daptomycin therapy. As these isolates were assumed to cause the endocarditis, we investigated the biofilm forming ability of both strains. The daptomycin resistant isolate showed a reduced biofilm formation compared to the susceptible strain in both early attachment and biofilm formation.

Conclusion: *E. faecalis* acquired daptomycin resistance during daptomycin therapy, which resulted in a relapse *E. faecalis* mitral valve endocarditis. Current work focuses on the identification of the daptomycin resistance mechanism and the link between this resistance mechanism and the reduced biofilm formation phenotype observed.

P115

Effect of simultaneous exposure of pigs to *Streptococcus suis* serotypes 2 and 9 on colonization and transmission of these serotypes, and on mortality

C.N.T. Dekker, A. Bouma, A.J.J.M. Daemen, J.C. Vernooij, L.A.M.G. van Leengoed, J.A. Wagenaar, J.A. Stegeman
Utrecht University, Faculty of Veterinary Medicine, Dept. of Infectious Diseases and Immunology, Utrecht

Introduction: *Streptococcus suis* (*S. suis*) is a major pathogen in pigs worldwide, causing meningitis, septicemia, arthritis, endocarditis, and mortality. *S. suis* in humans is considered as an emerging life-threatening infection, especially in Asia. Main risk factor for human infection is direct contact with infected pigs or their products.

In pigs, various serotypes of *S. suis* have been identified as cause of clinical infection. Comparable to e.g. *Streptococcus pneumoniae* in human, the presence of *S. suis* serotypes in pigs, however, differs between geographical areas, and varies over time. In several European countries, including The Netherlands, there has been a shift in predominant serotype from serotype 2 towards serotype 9 in the last two decades. We hypothesize a relation with one serotype affecting the other in colonization, transmission and invasion. The aim of this study was to evaluate whether simultaneous exposure of pigs to serotypes 2 and 9 affects the colonization and transmission of each serotype, and affects mortality.

Methods: Thirty-six caesarean-derived/colostrum-deprived piglets were randomly assigned to three groups, and there housed pair-wise. At 6 weeks-of-age one pig per pair was inoculated intranasally with either one (serotype 2 or 9; mono-group') or two serotypes simultaneously (dual-group'). Pigs in the mono-groups received $1x10^9$ CFU serotype 2 or 9, and in the dual-group a mixture of $1x10^9$ CFU serotype 2 and $1x10^9$ CFU serotype 9 (i.e. $2x10^9$ CFU *S. suis*/pig). The other pig of each pair was contact-exposed. Tonsillar brushing samples were collected from all pigs during three weeks post inoculation. Bacterial loads in the samples were quantified using multiplex real-time PCR. Transmission rates of the serotypes among pigs were estimated using a mathematical SI-model.

Results: The transmission rates for serotype 9 were 67/day (95%CI: 0-8) and 4.1/day (95%CI: 1.6-10.6), for the mono- and dual-group, respectively (p = 0.99). The transmission rates for serotype 2 were estimated at 29.4/day (95%CI: 0-8) in the mono-group, and 2.9/day (95%CI: 1.2-6.9) in the

dual-group (p = 0.99).Bacterial loads did not differ significantly between serotypes (p = 0.99). In the dual-group the average serotype 2 load in tonsillar samples from contact pigs was 1.4-1.8 ^{10}LogCFU/sample (i.e. 25-40 fold) reduced on days 1 to 4 and 6, in comparison to the mono-group (p < 0.01).Simultaneous exposure to the serotypes reduced the mortality hazard 6.3 times (95%CI: 2.0-19.8) compared to exposure to serotype 2 only, and increased it 6.6 times (95%CI: 1.4-30.9) compared to exposure to serotype 9 only.
Conclusions: Transmission rates for serotype 2 did neither differ significantly between serotypes, nor for a single serotype between the mono- and dual-group. This implies that simultaneous exposure to serotypes 2 and 9 does not affect the relative transmission rates of each serotype.Natural contact exposure to serotypes 2 and 9 simultaneously affects the clinical outcome of an infection of a particular serotype in a population, possibly by affecting the mucosal load. This might have contributed to the observed shift in distribution of clinical isolates in the field from serotype 2 to 9.

P116

Biological upgrading of syngas to fuels and chemicals

D.Z. Sousa[1], J.I. Alves[2], F.M. Pereira[2], C.M. Plugge[1], N.M. Reis[3], J.M. Stams[1], M.M. Alves[2]
[1]*Wageningen University, Laboratory of Microbiology, Wageningen,* [2]*University of Minho, Institute for Biotechnology and Bioengin, Braga, Portugal* [3]*Loughborough University, Chemical Engineering Department, Loughborough, United Kingdom*

Microbes can be used as biocatalysts to obtain added-value products from renewable sources. The big challenge of today is to efficiently convert lignocellulosic materials and wastes into fuels and other commodity chemicals, and a promising route for this is the combination of thermal and biological treatments. Presently cost-effective gasification technology exists to generate syngas (composed of CO, CO_2 and H_2) from biomass and wastes, and syngas fermentation to ethanol is performed at pilot and industrial scale. However, biological conversion of syngas has far more potential than this current application and innovative processes need to be explored. Here, we summarize our work on syngas conversion by anaerobic mixed microbial communities. We also addressed gas-liquid mass transfer limitations during syngas conversion by the utilization of a novel bioreactor configuration.
Stable thermophilic enrichments converting syngas (60% CO, 10% CO_2 and 30% H_2) and/or CO at 55C were obtained by incubation of a thermophilic anaerobic suspended sludge with these substrates. Cultures were successively transferred on syngas (cultures T-SYN) or pure CO (cultures T-CO). Initial CO partial pressure was increased from 0.09 to 0.88 bar over the duration of the enrichment experiment. T-SYN cultures produced mainly acetate, while hydrogen was the main product formed in T-CO cultures. *Desulfotomaculum* and *Caloribacterium* species were predominant in T-SYN cultures, while bacteria assigned to *Thermincola* and *Thermoanaerobacter* genera were abundant in T-CO cultures. A novel CO-tolerant bacterium, strain PCO, was isolated from the T-SYN culture. This bacterium is closest related to *Thermoanaerobacter thermohydrosulfuricus* (97% 16S rRNA gene identity). Although strain PCO does not utilize CO, it is able to grow in the presence of high CO concentrations (pCO = 1.7 bar). A new thermophilic hydrogenogenic carboxydotrophic bacterium, *Moorella stamsii*, could be isolated from the T-CO enrichment. This bacterium is able to utilize CO coupled to the production of hydrogen.
Mesophilic granular sludge was shown to efficiently convert syngas (60% CO, 10% CO_2 and 30% H_2) to methane. In batch assays, complete consumption of CO and H_2 was achieved in less than 72 h with syngas supplemented at 1 atm, and within 240 h for higher syngas pressures (up to 2.5 atm). Superior results were obtained using a novel multi-orifice baffled bioreactor (MOBB). A 10 L MOBB was fed with syngas and operated under oscillatory flow mixing, which is recognised to generate strong radial mixing and increased residence time of the gas-phase. Continuous syngas injection in the MOBB showed up to 15-fold enhancement of the CH production rate compared to the batch incubations. The overall performance of the novel MOBB in respect to syngas fermentation demonstrates its relevant impact on the anaerobic syngas fermentation process, opening perspectives for future technological development and industrial applications.
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P117

Decrease of the frequency of the mosaic penA gene in *Neisseria gonorrhoeae* between 2010 and 2012 was associated with less resistance to third generation cephalosporins

A.P. van Dam[1,2], I. Linde[2], S.M. Bruisten[2], H.J.C. de Vries[2]
[1]*OLVG General Hospital, Dept. of Medical Microbiology, Amsterdam,* [2]*Amsterdam Health Service, Public Health Laboratory, Amsterdam*

Introduction: Resistance of *Neisseria gonorrhoeae* against third generation cephalosporin is considered a major threat for public health. Increased MICs against cephalosporins are related to changes in the *penA* gene. A major determinant is the presence of a mosaic *penA* gene in

N.gonorrhoeae, partially derived from commensal *Neisseria* spp. We report resistance figures of *N.gonorrhoeae* from 2007 to 2013 and analyzed the frequency of *penA* mosaic strains in the period 2010-2012.

Methods: From all patients with high-risk sexual behavior visiting the STI outpatient of the GGD Amsterdam. MICs for cefotaxim were determined by E-test on 6857 clinical isolates from 2007-2013, MICs for cefixim and ceftriaxone were performed from 2010-13 (4191 strains). A specific qPCR was used to distinguish between strains with a mosaic *penA* gene and strains with a wild type gene. This qPCR was performed on 180 randomly chosen proctal isolates from males that were evenly distributed over the years 2010-2012 and on 346 cervical isolates from females obtained in the same years.

Results: The frequency of strains resistant to cefotaxim (MIC > 0.125) was 2.1% in 2007, peaked to 8.4 and 7.8% in 2008 and 2010, respectively and decreased to 3.0, 2.6 and 2.3% in 2011-13. The peak was most markedly seen in ano-rectal isolates from male patients, up to 12.3% and 12,1% in 2008 and 2010, respectively, and decreasing to 2.5% in 2013. In contrast, in urogenital strains from females, the frequency was never over 3.1%. Resistance against cefixime or ceftriaxone (MIC > 0.125) was not found. The frequency of strains with an increased MIC against cefixime (> 0.032) decreased also from 14.0% in 2010 to 6.8%, 5.4% and 5.5% in 2011-13. In contrast, the frequency of strains with an increased MIC (> 0.032) to ceftriaxone was 5.2% in 2010, dropped to 2.0 and 3.1% in subsequent years, but increased again to 7.8% in 2013. The frequency of the mosaic *penA* gene decreased from 35% to 3% in ano-rectal isolates from males between 2010 and 2012, and varied between 3 and 6% in isolates from females.

Conclusion: The decrease in the frequency of the mosaic *penA* gene in *N.gonorrhoeae* in the period 2010-2012, especially in ano-rectal isolates from males, correlated with a decrease in occurrence of strains with increased MICs against cefotaxim and cefixime, and, to a lesser extent, ceftriaxone. However, strains with increased MICs against ceftriaxone were more common in 2013. The same increase was not observed with cefixime or cefotaxime. Strains with resistance to ceftriaxone were not detected.

P118

Proteome analysis of the gut symbiont *Akkermansia muciniphila* confirm its specificity as a mucus-degrader

N. Ottman[1], M. Davids[3], H. Smidt[1], W.M. de Vos[1,2], C. Belzer[1]

WUR, Laboratory of Microbiology, Wageningen, [1]Wageningen University, Laboratory of Microbiology, Wageningen, [2]University of Helsinki, Dept. of Basic Veterinary Medicine, Division of Microbiology and Epidemiology, Helsinki, Finland, [3]Wageningen University, Laboratory of Systems and Synthetic Biology, Wageningen

The gut bacterium *Akkermansia muciniphila* resides in the mucus layer of the human intestine, where it very efficiently degrades mucins as substrates for growth. The genome of *A. muciniphila* further explains its mucosal preference with up to 11% of its putative secretome encoding genes being devoted to mucus degradation. This study focuses on defining mucus-specific protein production of *A. muciniphila* and determining the differences between growth conditions.

The bacteria were grown anaerobically either on mucin or glucose medium, and proteins were extracted from the cultures. Bacterial cell fractionation techniques were established to isolate membrane proteins and intracellular proteins. Enrichment for specific proteins was observed by SDS-PAGE and samples from each condition were analyzed by LC-MS/MS.

The proteome analysis shows many differences in the protein profiles between the tested conditions. Several iron metabolism proteins are present in higher numbers in mucin-grown bacteria. On top of this, glycosidases and sulfatases are found in higher levels in the mucin cultures, whereas proteases and sialidases are present in similar amounts in both conditions. One specific enzyme, beta-N-acetylhexosaminidase, is exclusively found in the mucin-grown cells. This enzyme is used to cleave N-acetylgalactosamine residues present in mucin, and the production seems to be highly dependent on the presence of mucin. Finally, the different cell fractions show that there is specific upregulation of membrane transporters in the mucin condition. In conclusion, *A. muciniphila* protein production is highly adapted to mucus degradation and consumption of mucin-derived sugars.

P119

Hydrazine and hydroxylamine conversion in anammox bacteria

J.R. Reimann, M.W. Maalcke, J.K. Keltjens, B.K. Kartal

Radboudumc, Dept. of Microbiology, Nijmegen

Anammox is a bacterial respiratory process in which nitrite serves as terminal electron acceptor for the anaerobic oxidation of ammonium, forming nitrogen gas as the end product. A key step in anammox is the combination of ammonia (NH_4) and internally produced nitric oxide (NO) catalyzed by hydrazine synthase (HZS) forming of one of nature's strongest reductant hydrazine (N_2H_4). The subsequent oxidation of hydrazine produces N_2 and H_2O and releases four low potential electrons. This redox reaction is catalyzed by the soluble enzyme hydrazine dehydrogenase (HDH), a homotrimeric octaheme protein

that belongs to the class of hydroxylamine oxidoreductases (HAO). Electrons originating from the oxidation of hydrazine are presumed to be fed into the (mena)quinone pool such that a proton-motif force is maintained.
In addition to HDH the genome of the anammox bacterium *Kuenenia stuttgarteinsis* encodes nine additional HAO-like proteins, some of which are highly expressed. Most of these proteins have an as yet unknown function, except for the protein encoded by ORF kustc1061. We recently purified and characterized kustc1061 and found this enzyme complex to be a dedicated hydroxylamine (NH_2OH) detoxification system. Hydroxylamine is a potent inhibitor of the anammox reaction due to an inhibitory effect of NH_2OH on N_2H_4 conversion by HDH. Preliminary data indicate that occasional incomplete conversion of NO and NH_4 by HZS leads to the release of NH_2OH, which needs to be rapidly converted.
Canonical HAOs, e.g. HAO from *Nitrosomonas europaea* (*Ne*HAO), catalyze the four-electron oxidation of hydroxylamine to nitrite as part of the nitrification process. Although kustc1061 shows high sequence similarity to *Ne*HAO, kustc1061 converts NH_2OH only to NO in a three-electron oxidation reaction. Comparison of the crystal structures of *Ne*HAO and kustc1061 show subtle differences in the active site architecture. These slight alterations appear to lead to two distinct NH_2OH catalytic routes explaining the formation of either nitrite or nitric oxide.

P120

Macrolide resistance and treatment failure in *Mycoplasma genitalium* infection in the Netherlands

D.S.J.M. van der Vegt[1], A.A. van Zwet[1], J.G. Kusters[2], T.T. Severs[2], R.H.T. Nijhuis[1]
[1]*Rijnstate, Dept. of Medical Microbiology and Immunology, Arnhem,* [2]*University Medical Center Utrecht, Dept. of Medical Microbiology, Utrecht*

Introduction: *Mycoplasma genitalium* (MG) is an emerging sexually transmitted organism responsible for non-gonococcal urethritis in men and associated with pelvic inflammatory disease and non-gonococcal cervicitis in women. The current first-line treatment for MG infection is the macrolide antibiotic azithromycin. As a consequence of macrolide resistance an increasing incidence of treatment failure (up to 33%) has been reported. Macrolide resistance in MG has been associated with point mutations in the 23S rRNA gene. The aim of this study was to determine the prevalence of macrolide resistance mutations in MG from the Netherlands, by detecting these mutations in a collection of MG-positive specimens.
Methods: Between January 2012 and October 2013, 1138 urogenital samples from 1006 patients were submitted to the microbiology laboratory of Rijnstate Hospital for MG testing. A total of 46 random selected archival samples that were tested positive in the routine PCR for MG were further analyzed for the presence of the macrolide associated mutations in the 23S rRNA gene using standard techniques.
Results: Among the 1138 samples tested during the study period, 623 samples were obtained from women and 515 samples from men. Of those, 132 samples (11.6%) tested positive for MG (58 males (44%), 74 females (56%)). Of the 46 samples that were selected for the detection of possible macrolide mutations, 43 (93%) were primary samples (25 males, 18 females), and 3 were secondary samples (2 males, 1 female) obtained for treatment follow-up. Of the 46 samples, 13 samples (28.2%; consisting of 11 initial samples and 2 secondary samples) from 11 unique patients (11/43 = 25.6%) possessed macrolide resistance mutations (A2058G (n = 6, 46% including one secondary sample, see below), A2059G (n = 6, 46%, including one secondary sample, see below), and A2058T (n = 1, 8%)). Of the patients with genotypic macrolide resistance, treatment advice was documented in six cases. All six were initially treated with an extended 5-day course of azithromycin (500 mg on day 1 followed by 250 mg on day 2-5). For one of these patients, the second test (performed 54 days post-treatment) remained positive and was shown to contain the same A2059G mutation as the initial sample. Subsequently the patient was treated with moxifloxacin (400 mg orally for 10 days) after which clinical symptoms disappeared. Another patient was initially treated with 1 g azithromycin for *Chlamydia trachomatis* infection 9 months before he was tested positive for MG. The test remained MG PCR positive 37 days after re-treatment with azithromycin (extended course) and harbored the same mutation type as the initial sample (A2058G). He was given moxifloxacin (400 mg orally for 10 days) and successfully cleared his MG infection as 67 days after treatment MG was no longer detectable.
Conclusion: To our knowledge, this is the first report describing macrolide resistance in MG from the Netherlands. The detected mutation profiles are associated with treatment failure. Since azithromycin a single dose is still the first-line treatment of choice for MG infections in many countries we believe current guidelines for empirical therapy should be modified to reduce treatment failure.

P121

Commensal *Streptococcus pneumoniae* in Europe: prevalence and antibiotic resistance

R.Y. Yahiaoui[1,7], C.D.J. den Heijer[1], E.M.E. van Bijnen[2], W.J. Paget[2,3], M. Pringle[4], H. Goossens[5], C.A. Bruggeman[1], F.G. Schellevis[2,6], E.E. Stobberingh[1] and the APRES Study Team
[1] *Maastricht University Medical Centre/CAPHRI, Maastricht,* [2] *NIVEL, The Netherlands Institute for Health Services*

Research, Utrecht, [3] Radboudumc, Dept. of Primary and Community Care, Nijmegen, [4] University of Nottingham, Division of Primary Care, Nottingham, United Kingdom, [5] Vaccine and Infectious Disease Institute, University of Antwerp, Wilrijk [6] VU University Medical Center, Dept. of General Practice/EMGO+ Institute, Amsterdam,[7] Haga hospital, Dept. of Medical Microbiology, The Hague

Introduction: The commensal microbiota is considered as the main reservoir for antibiotic resistant genes and resistant microorganisms. However, information on antibiotic resistance of *Streptococcus pneumoniae* has generally been obtained from invasive strains. In the framework of The Appropriateness of prescribing antibiotics in primary health care in Europe with respect to antibiotic resistance' study (APRES), we assessed the prevalence and antibiotics resistance of *S. pneumoniae* nasal carriage of general practice patients without an infection in nine European countries.

Methods: In each of nine participating European countries, 20 general practitioners (GP) were recruited by the national networks and aimed to provide 200 nasal swabs each, from November 2010 to August 2011, from patients older than four years, except for of United Kingdom where, due to ethical constraints, all patients were older than 18 years. To include in the study, patients should visit the practice for a non-infectious condition, did not use antibiotics and should not been hospitalized in the three months preceding the swab collection. Immunocompromised patients and nursing home residents were also excluded.

Isolation and identification of *S. pneumoniae* was performed in the national microbiological laboratory in the participating countries except for France. These were send to the central microbiological laboratory. Antibiotic resistance was performed at the central microbiological laboratory with microdilution for cefuroxime, cefaclor, ceftazidime, clarithromycin, clindamycin, ciprofloxacin, moxifloxacin, penicillin, tetracycline and trimethroprim-sulfothoxazoleaccording to the EUCAST guidelines.

Prevalence of nasal *S. pneumoniae* carriage and the antibiotic resistance werecalculated for each country separately and for the total study population.

Results: A total of 32770 nasal swabs have been collected. The overall prevalence of *S. pneumoniae* nasal carriage in the nine participating countries was 2.9% (n = 931/32182), ranged between 4.6%, (95% confidence interval: 4% - 4.3%) in France to 1.1%, (0.8% - 1.6%) in Austria. The highest resistance was observed to cefaclor, ranged between 30.6%, (15.6% - 45.7%) in the UK to 77.3%, (64.9% - 89.7%) in Belgium.

The antibiotic resistance for most common antibiotics in outpatients care, penicillin, macrolides and tetracycline ranged between 3.9%, (0.1% - 7.7%) in Sweden and 32.3%, (25.2% - 39.4%) in Spain for penicillin, 2%, (0% - 4.7%) in Sweden and 29.3%, (22.4% - 36.2%) in Spain for clarythromycin, and 2%, (0% - 4.7%) in Sweden and 29.9%, (23% - 36.8%) Spain for tetracycline. No resistance was observed for moxifloxacin and ciprofloxacin.

Conclusion: The prevalence of *S. pneumoniae* nasaal carriage prevalence was low among general practices patients in nine European countries. A large variation in prevalence of restistance was noted between the countries. Spain had the highest resistance prevalence to *S. pneumoniae* and the most multidrug resistant isolates, Sweden the lowest.

P122

Correlation between quality of life and ELISpot results in patients with persisting symptoms after successful treatment for Lyme neuroborreliosis

T. van Gorkom, W. Voet, S.U.C. Sankatsing, S.F.T. Thijsen
Diakonessenhuis, Dept. of Medical Microbiology and Immunology, Utrecht

Introduction: Despite successful antibiotic treatment, patients treated for Lyme neuroborreliosis (LNB) often report persisting symptoms, most of which are nonspecific, and which can impair quality of life. The reason for these symptoms remains unclear up to now.

Methods: We investigated the cellular immune response of 85 healthy volunteers, 12 patients who had been treated for LNB and 12 patients with active LNB. All 12 patients who were treated for LNB received ceftriaxone for 14 or 30 days intravenously and were included 1-9 years after treatment with a mean of 4.2 years (+/- 2.6 SD). For testing the cellular immune system, we used the Enzyme-Linked ImmunoSpot (ELISpot) assay, an interferon-γ release assay, by isolating peripheral blood mononuclear cells to see whether T-cells were able to produce interferon- γ when triggered with *Borrelia* antigen. We compared the results with the quality of life as measured by the RAND 36-Item Health Survey (version 1). The RAND-36 survey covers 8 different dimensions of health, which can be combined in 2 summary scores known as the physical component score (PCS) and the mental health component score (MCS).

Results: The RAND-36 survey was completed at time of inclusion in our study by 84 (98.8%) healthy volunteers and all 12 treated LNB patients. When comparing the RAND-36 surveys from the 84 healthy volunteers with the RAND-36 surveys from the 12 treated LNB patients, all 8 dimensions of health were significantly different (range $p < 0.0005$ - $p = 0.045$). The same was true for the PCS ($p = 0{,}050$) and the MCS ($p = 0{,}026$). The ELISpot results also differed significantly between the 85 healthy volunteers and the 12 treated LNB patients ($p = 0.023$) and between the 85 healthy volunteers and the 12 active LNB patients ($p < 0.0005$). Surprisingly, the ELISpot

results between treated LNB patients and active LNB patients didn't differ significantly (p = 0.443). When looking at the treated LNB patients, we found a significant correlation between the MCS and the ELISpot results (Spearman's correlation coefficient -0.617; p = 0.033), mainly caused by the dimensions social functioning' (Spearman's correlation coefficient -0.625; p = 0.030) and mental health' (Spearman's correlation coefficient -0.590; p = 0.043), which implies an ongoing immune activation.
Conclusion: the RAND 36-Item Health Survey as well as the ELISpot technique could be useful tools for patients with Post Lyme Disease Symptoms (PLDS), as results indicate that PLDS can be linked to an ongoing T-cell immune activation, however since the number of patients is very small, more research on this topic is required.

P123

Intestinal translocation contributes to *Streptococcus suis* infection in piglets

M.L. Ferrando[1], A. de Greeff[2], W.J.M. van Rooijen[1], V. Jaspers[1], Y. Pannekoek[1], A. van der Ende[1], N. Stockhofe-Zurwiede[2], H. Smith[2], C. Schultsz[1]
[1]*AMC, Dept. of Medical Microbiology, Amsterdam,* [2]*Central Veterinary Institute of Wageningen UR, Dept. of Infection Biology, Lelystad*

Streptococcus suis serotype 2 (SS2) is an emerging zoonotic pathogen which can cause severe disease with high mortality in human and pigs. SS2 is the most important cause of bacterial meningitis in adults in South-East Asia. Direct contact with pigs or contaminated pork meat in the presence of skin lesions is considered the main transmission route of SS2 to humans. However, consumption of pork meat contaminated with SS2 was recently identified as a novel risk factor for SS2 meningitis, indicating that SS2 should be considered a food borne zoonotic pathogen. The aim of our study was to develop and evaluate a non-invasive *in vivo* model of host-pathogen interaction at the intestinal mucosal surface in the piglet, the main host of *S. suis*, after oral infection. 6-9 weeks old specific pathogen free piglets, and pre-challenge free of SS2 tonsil carriage as determined by RT-PCR, were challenged via an orally administered gastric-acid resistant capsule containing 10^9 CFU/ml of *S. suis*, after mild stress induction. In 2 out of 15 infected piglets, clinical symptoms (arthritis, fasciitis) compatible with *S. suis* infection, were observed 24-48 hours after infection. *S. suis* translocated across the intestinal tract of 50% of infected pigs as indicated by positive culture and/or a positive RT-PCR result of intestinal lymph-nodes. Moreover, in piglets with positive intestinal lymph nodes, *S. suis* was detected in the intestinal mucosa as indicated by positive RT-PCR results of intestinal scrapings and by histological examination using type specific SS2 antibody staining, suggesting direct passage of *S. suis* in the intestinal mucosa. *S. suis* 2 was not detected in 4 control pigs who were challenged with capsules without bacteria.
Our results indicate that the gastro-intestinal tract should be considered as an entry site of *S. suis* infection in piglets. The recognition of this alternative route of infection may allow identification of novel targets and tools for intervention, resulting in more effective control of *S. suis* in pigs and as an emerging zoonotic disease.

P124

Mining of anaerobic microbial ecosystems

P. Worm
Wageningen University, Dept. of Microbiology – microbial Physiology, Wageningen

Molecular ecological research has shown that the majority of the microorganisms in nature has not yet been isolated and characterized. The role of these yet-uncultured microorganisms in geochemical processes is often not known. It is plausible to assume that many of these microorganisms have unique yet unknown metabolic properties, providing a largely hidden potential for exploitation. Insight into the physiology of such uncultured organisms is important to understand their role in their natural habitats and to get insight into their possible application in biotechnology. Especially anaerobic fermentative microorganisms are relevant for biotechnology as they are able to conserve chemical energy from complex organic polymers, and form relevant products, like organic acids and alcohols. The aim of the research is to develop and apply efficient and high throughput pipelines for the isolation and characterization of novel anaerobic microorganisms from anaerobic environments, including bacteria, archaea and fungi, using the MicroDish platform. This innovative technology is suited for high throughput isolation, but it has never been applied for isolation of strict anaerobes. The isolates obtained will be screened by rRNA gene sequencing and metabolic product profile. Selected strains will then be studied into depth using functional genomics approaches as well as further characterization of enzymes of interest. We focus our isolation studies on anaerobic microorganisms that are able to metabolize complex polymers, including ligno-celluloses and other polysaccharides and glycoproteins. The generic microscale and HTP screening pipeline furthermore allows for screening of a broad spectrum of anaerobic microbial source environments, differing in the occurrence of natural polymers, as well as microbial life-style, e.g. ranging from thermophiles to extremely slow-growing psychrophiles. This research (project 7.2.3.) is financed by BE-Basic.

P125

High throughput screening for syphilis in the Netherlands; a more efficient screening protocol is possible

F.F. Stelma, J. Rahamat-Langendoen
Radboudumc, Dept. of Medical Microbiology, Nijmegen

Syphilis has an incidence of 2/100.000 in the general population in the Netherlands (data Sanquin). However, in certain risk groups, like MSM (men having sex with men), the incidence is higher (about 2,2%; data RIVM). Screening for syphilis is therefore a frequent laboratory event. In the east of the Netherlands a large proportion of the screening is performed at the laboratory of Microbiology, Radboudumc, Nijmegen. The Dutch guideline for syphilis screening dictates that a positive screening result (TPPA or EIA), should be confirmed by FTA-ABS or immunoblot. Both FTA-ABS and immunoblot are labour intensive tests. We therefore evaluated the diagnostic properties of a more efficient test algorithm.

Population and methods: All serum samples presented for primary syphilis screening in 2013 were included in the diagnostic evaluation. Two-hundred-and-eighty-three samples showed positive in the EIA screening assay (Architect Syphilis TP, Abbott Diagnostics, Wiesbaden, Germany). These samples originated from regional STI centres n = 248 and various Radboudumc outpatient clinics n = 35. The positives were all submitted to TPPA (Fujirebio Inc, Tokyo, Japan), the non-treponemal rapid plasma reagin assay (RPR; Biomerieux, Boxtel, the Netherlands) and the Inno-Lia Syphilis immunoblot (Innogenetics NV, Ghent, Belgium).

Results: Of the 283 positive EIA screening tests, n = 243 (86%) were confirmed by a positive TPPA-assay. Only one sample showed a positive EIA screening test and TPPA with a negative Inno-Lia immunoblot. However this sample showed a positive RPR-assay. Consequently the immunoblot was regarded falsely negative. Forty samples (14%) showed discrepant results with a positive EIA screening test and a negative TPPA. For these samples the Inno-Lia immunoblot tested equivocal n = 14, positive n = 4 and negative n = 22. None of the samples with a negative or equivocal immunoblot result showed reactivity in the RPR-assay. Most of the equivocal and negative results, stayed equivocal when a second sample was submitted three weeks later. Only one patient seroconverted in the immunoblot, but not in the TPPA and the RPR-assay.

Conclusion: We propose to simplify the national screening algorithm as follows: When the EIA screening test is negative, the sample is regarded negative. When the EIA screening test is positive, the result is confirmed by TPPA and RPR. If the EIA screening test and TPPA results are concordant, the sample is regarded positive. If EIA and TPPA results are discrepant, an immunoblot is performed in order to confirm results further. This algorithm does not lead to more false negative samples compared to the current national algorithm. Further this algorithm decreases the use of immunoblot with 86%, thereby saving costs and decreasing the turnaround time in the laboratory.

P126

Outbreak of ST131 Extended-Spectrum beta-Lactamase (ESBL)-producing *Escherichia coli* in a long-term care facility (LTCF): duration of colonisation

I.T.M.A. Overdevest[1], J. Veenemans[2], Y. Hendriks[2], A. Mulders[3], W. Couprie[3], J. Johnson[4], J. Kluytmans[2]
[1]St. Elisabeth ziekenhuis, LMMI, Tilburg, [2]Amphia ziekenhuis, LMI, Breda, [3]Thebe, Long term care facilities, Breda, [4]Veterans Affairs Medical Centre and University of Minnesota, Minneapolis, USA

Introduction: The ST131 $bla_{CTX\text{-}M_{15}}$-carrying *E. coli* clone is pandemic in healthcare settings, where primarily the elderly are affected. Possibly more effective transmission and/or longer persistence are explanations for the success of this clone compared to other ESBL-producing *E. coli*. We performed a prospective cohort study to identify differences in the persistence of rectal carriage of ST131 versus other ESBL-producing *E. coli*.

Methods: During an infection control survey in a LTCF, an ongoing epidemic of rectal carriage with diverse ESBL-producing *E. coli* strains, including ST131 was discovered. To assess the size of the outbreak and to evaluate the measures to contain the outbreak, 4 consecutive cross-sectional surveys were performed during a 8-month period (March-October 2013) in which faecal samples or rectal swabs from all residents were collected. To find the transmission route, we sampled the hands of the health-care workers twice and the environment three times. Samples were selectively cultured for ESBL-producing organisms using antibiotic-supplemented Tryptic Soy Broth and selective agar plates. Species identification was done by MALDI-TOF spectroscopy, ESBL genotyping by a microarray, ST131 detection by ST131-specific PCR, and genomic profiling by amplification-fragment-length-polymorphism (AFLP) analysis. We used Kaplan-Meier survival analysis to compare the loss of faecal ESBL carriage of patients carrying ST131 *E. coli* with those carrying other ESBL *E. coli* types.

Results: The LTCF consisted of 9 wards. On 3 wards, an outbreak of colonisation with $bla_{CTX\text{-}M_{15}}$-carrying ST131 *E. coli* was going on (29 rectal carriers, all isolates belonging to the same AFLP cluster; prevalence 39-45%). On 3 other wards, smaller colonisation outbreaks were accompanied by sporadic carriage of other ESBL-producing *E. coli* (16 carriers, prevalence 11-23%). On the remaining 3 wards only sporadic carriage of ESBL-producing *E. coli* was discovered (prevalence < 6%). Improved emphasis on

hand hygiene and cleaning was introduces on all wards. With this regime, 10 of 16 non-ST131 carriers became ESBL-negative within 8 months and the outbreaks of non-ST131 ESBL-producing *E. coli* resolved. In contrast, only 7 of 29 patients carrying ST131 $bla_{CTX\text{-}M_{15}}$-positive *E. coli* became ESBL-negative, and the outbreak of ST131 $bla_{CTX\text{-}M_{15}}$-carrying *E. coli* on the first 3 wards persisted. The half-life of carriage for ST131 was > 8 months, whereas for approximately 50% of non-ST131 carriers, ESBL carriage could no longer be detected after 2 months. Using survival analysis, persistence of rectal carriage of the ST131 clone is significantly longer than that of other ESBL types (p < 0,001, log-rank test). Because repeated environmental cultures (n = 270) never showed ST131 *E. coli* and the epidemic strain was recovered from cultures of HCW hands (n = 243) only once, and because the acquisition rate among negative patients was low, we consider it unlikely that reinfections explain the longer persistence of the epidemic strain.

Conclusion: In this LTCF outbreak setting, rectal carriage of $bla_{CTX\text{-}M_{15}}$-carrying ST131 *E. coli* persisted more than four times longer than that of other ESBL-producing *E. coli* (> 8 months, vs. approximately 2 months). Prolonged duration of carriage may contribute to the epidemicity of ST131.

P128

Performance and cost effectiveness of IDM-Surveillance®, a software tool for automated detection of hospital acquired infections

I. ten Seldam, H.R.A. Streefkerk, C. van der Hoeven, R. Kamphuis, H.A. Verbrugh, A.W. Friedrich, J.L.V. Eriksson, N. Al Naiemi

ZiekenhuisGroep Twente/Labmicta, Dept. of Medical Microbiology and Infection Prevention, Hengelo

Introduction: To evaluate the performance and cost effectiveness of IDM-Surveillance (IDMC, Rotterdam, The Netherlands) in a large general hospital group in The Netherlands.

Methods: IDM-Surveillance is an algorithm-based registration tool that uses predictive clinical and diagnostic parameters to differentiate between patients having a HAI and those that do not. In a cross-sectional study we compared the performance and cost effectiveness of IDM-Surveillance with the classical point-prevalence surveys done by Infection Control Practitioners (ICP). The study population consisted of 2.526 patients previously included in six consecutive hospital-wide surveys performed each March and October from 2010-2013. All patients were retrospectively tested by IDM-Surveillance and results were compared with the classical survey outcomes. Two Infection Control experts using the Dutch national PREZIES criteria reviewed all patients again. Fisher's exact test was used to compare groups.

Results: IDM-Surveillance marked 2.098 patients (83%) automatically as not having a HAI. The remaining 17% of the population were reviewed by the ICP in detail using IDM-Surveillance. There were 54 patients (2.1%) with at least one HAI (39% SSI, 31% LRTI, 16% UTI, 11% BSI and 4% other). The sensitivity of IDM-Surveillance< b> < /b> and classical survey method for all types of HAI's was 91% and 69%, respectively (p = 0,0076). The average accuracy was 99.8% and 98.4%, respectively (p < 0.0001). Cases (n = 5) missed by IDM-Surveillance consisted of three urinary tract infections, one blood stream infection and one lower respiratory tract infection. Classical point-prevalence survey missed 17 patients and 23 patients were falsely scored positive. All SSI (n = 21) were detected by IDM-Surveillance while the classical survey method missed seven SSI (33%; p < 0.001). Total labour time of IDM-Surveillancewas approximately 3 hours, while the classical point-prevalence survey by ICP required 180 hours of labour for one point-prevalence population.

Conclusion: In conclusion, a computer-assisted point-prevalence survey with IDM-Surveillance software is more sensitive and accurate than classical point-prevalence survey for the detection van HAI and is highly cost effective.

P129

Extended-spectrum-beta-lactamase producing *Enterobacteriaceae* in household dogs: a longitudinal study in the Netherlands

V.O. Baede[1], J.A. Wagenaar[1,2], E.M. Broens[1], B. Duim[1], W. Dohmen[3], E.R. Nijsse[1], A.J. Timmerman[1], J. Hordijk[1]

[1]*Utrecht University, Faculty of Veterinary Medicine, Utrecht,* [2]*Central Veterinary Institute of Wageningen UR, Lelystad,* [3]*Utrecht University, Institute for Risk Assessment Sciences, Utrecht*

Introduction: A significant share of the global emergence of antimicrobial resistance is formed by extended-spectrum-beta-lactamase (ESBL) producing bacteria. Little is known about ESBL-epidemiology in companion animals. Living in close contact with humans, companion animals could be an important interspecies transmission route for ESBL-producing *Enterobacteriaceae*. A study of Hordijk et al. 2013, showed a prevalence of 45% in healthy dogs and 55% in diarrheic dogs in the Netherlands. The aim of this study was to investigate persistence of faecal carriage of ESBL-producing *Enterobacteriaceae* in household dogs in a longitudinal study.

Methods: Faecal samples from 38 dogs of 25 owners were collected monthly in the period July 2013-January 2014. From 7 of these 38 dogs, faecal samples were collected

for six consecutive weeks, to check for shifts in faecal shedding in between the monthly sampling moments. Shifts in faecal shedding were defined as changes in presence of ESBL-producing *Enterobacteriaceae* between consecutive faecal samples. Samples were cultured quantitatively on MacConkey agar, MacConkey agar supplemented with 1 mg/L cefotaxime (MacConkey+) and cultured in LB-enrichment broth supplemented with 1 mg/L cefotaxime with subsequent inoculation onto MacConkey+, enabling detection of 10^2 colony-forming unit/g faeces. ESBL-suspected CFU/g faeces were calculated based on the number colonies at the MacConkey+. The average ratio of ESBL-producing *Enterobacteriaceae* compared to the total number of *Enterobacteriaceae* was calculated as the mean of isolate ratios. Suspected ESBL-carrying isolates were tested for the presence of ESBL-genes by PCR and sequence analysis.
Results: From 38 dogs, a total of 270 faecal samples were collected. Faecal samples from 10 dogs contained ESBL-suspected isolates in each consecutive sample. In 6 dogs, in none of the samples ESBL-suspected isolates were found. In 22 dogs, shifts in faecal shedding were observed and the proportion of ESBL-producing *Enterobacteriaceae* varied between 7% and 86% of collected faecal samples per dog. These shifts were also seen between the weekly obtained samples. The pattern of faecal shedding shifts of dogs from the same owner was never exactly the same. From 99 faecal samples, all ESBL-suspected isolates were suspected to be *Escherichia coli*, based on distinctive growth on MacConkey agar (to be confirmed). Both *E.coli* and non-*E.coli* ESBL-suspected isolates were obtained from 22 faecal samples and 10 faecal samples contained only non-*E.coli* ESBL-suspected isolates. Mean CFU/g faeces of suspected ESBL-producing *Enterobacteriaceae* of 96 faecal samples was 8,1 x 10^8. The average ratio of ESBL-producing *Enterobacteriaceae* was 2%. A first screening of ESBL genes by PCR and sequence analysis showed presence of $bla_{\text{ctx-m-1}}$ $bla_{\text{ctx-m-14}}$, $bla_{\text{ctx-m-15}}$, $bla_{\text{ctx-m-55}}$, $bla_{\text{shv-12}}$ and $bla_{\text{cmy-2}}$.
Conclusions: 1. ESBL-producing *Enterobacteriaceae* are abundant in dogs, though faecal shedding seems highly dynamic over time. 2. In most ESBL-positive dogs, high concentrations of ESBL-producing *Enterobacteriaceae* were detected.

P130
Fluoroquinolone resistant *Salmonella* Typhi and Paratyphi A in travelers

R.G. Bentvelsen, A.T. Bernards, J. Gooskens
LUMC, Dept. of Medical Microbiology, Leiden

Introduction: *Salmonella enterica* serotypes Typhi and Paratyphi A are endemic in developing countries and may cause travel-associated diarrhea and enteric fever with frequent bacteremia. Fluoroquinolones, macrolides and third-generation cephalosporins are the current standard choice in antibiotic therapy, although drug resistance is emerging in endemic areas. We aim to evaluate current antimicrobial resistance and clinical manifestations among travelers returning from endemic areas with invasive *S.* Typhi and *S.* Paratyphi A infections.
Methods: Foreign travelers with culture-proven typhoid and paratyphoid fever and presenting at Leiden University Medical Center (LUMC) between 2012 and 2013 were included. Medical records were evaluated for travel-history, antimicrobial therapy, clinical data and outcome. *Salmonellae* cultured from blood samples were identified by using antisera for the agglutination of specific Salmonella O, H and Vi antigens (Thermo Fisher Scientific – Remel Products). Identification was confirmed biochemically using API 20E strips (BioMrieux) and all strains were forwarded to the reference laboratory at the National Institute for Public Health and the Environment (RIVM) for further characterization and confirmation. Antimicrobial susceptibility testing including ESBL screening was performed by disk diffusion, and MICs were determined by Etest (BioMrieux) for amoxicillin, chloramphenicol, co-trimoxazole, nalidixic acid, ciprofloxacin, azithromycin and ceftriaxone. Quinolone resistance was defined as ciprofloxacin MIC > 0.06 mg/L (EUCAST guidelines 2014) by duplicate testing. For azithromycin clinical breakpoints were not available, EUCAST notes use in *S.*Typhi (MIC = 16 mg/L for wild type isolates).
Results: During the study period, we identified 1 *S.* Typhi and 3 *S.* Paratyphi A isolates among travelers (age range 19-62 years) returning from Southeast Asia and the Indian subcontinent. Ciprofloxacin was administered as routine empirical treatment in all 4 cases (n = 2 monotherapy, n = 2 combined therapy). Antimicrobial susceptibility testing revealed 3 quinolone resistant isolates and antimicrobial treatment was switched to ceftriaxon or co-trimoxazole. One case developed a poor clinical response to azithromycin and ciprofloxacin and two cases developed recurrent bacteremia, but fortunately all patients were eventually cured. Two *S.* Paratyphi A isolates were low-level ciprofloxacin resistant (MIC 1.0 - 1.5 mg/L) and *S.* Typhi was high-level resistant (MIC > 32 mg/L). One *S.* Paratyphi A isolate showed mixed quinolone susceptibility results (ciprofloxacin MIC 0.047 mg/L and 0.094 mg/L by duplicate testing) and was susceptible to nalidixic acid (MIC 4.0 mg/L). Azithromycin MICs were elevated in *S.* Paratyphi A (MIC > 16 mg/L), though these preliminary findings remain to be confirmed by broth microdilution method. All *Salmonellae* were susceptible to ceftriaxone and none were multidrug resistant or demonstrated phenotypic ESBLs.
Conclusion: Fluoroquinolone resistance and elevated azithromycin MICs were frequently observed in *S.* Typhi

and *S*. Paratyphi A isolated from travelers returning from Southeast Asia and the Indian subcontinent. Increasing trends in resistance to first-line antimicrobials indicate that ceftriaxone should be considered for routine empirical treatment and warrant continued nationwide antimicrobial resistance surveillance.

P131

Dutch guideline for preventing nosocomial transmission of highly resistant microorganisms (HRMO) in long term care facilities

E.P. Poot[1], A. Voss[2], Guideline Development Group
[1]*WIP/LUMC, Leiden,* [2]*Radboudumc/Canisius-Wilhelmina hospital, Dept. of Medical Microbiology, Nijmegen*

Introduction: The Dutch Working party on Infection Prevention (WIP) develops national guidelines for healthcare facilities, including long term care facilities. The guidelines are considered as professional standards. Recently, a draft version of a new guideline on Highly Resistant Micro Organisms (HRMO) for long term care facilities was developed and published for public consultation in January 2014.
Methods: WIP-guidelines are developed following a standardized procedure, with a guideline development group (GDG) playing a central role in formulating the recommendations. Representatives of the Dutch Societies for Medical Microbiology, for Hygiene and Infection Prevention, for Elderly Care Physicians and Social Geriatricians, for Public Health Physicians and for Gerontology Clinical Nurse Specialists were included in the GDG. To develop the HRMO guideline for long term facilities the GDG used the hospital HRMO guideline (2012) as a starting point. If necessary, recommendations were adapted, for example when the feasibility of the recommendation or the homely environment in which elderly are taken care for was threatened.
Results: Due to the lack of isolation guidelines for long term care facilities the most important change was including a table with tailor-made recommendations for each HRMO. Recommendations on supplementary infection prevention measures were formulated on hand hygiene, personal protective equipment, shared or individual toilets/bathrooms, visitation of common, social-rooms, use of nursing materials, cleaning and disinfection, and transport of waste and linen. Compared to the hospital guideline the most important changes were: for each HRMO wearing a gown is recommended during direct patient care, no mask is recommended for *Acinetobacter species*, the recommendations for personal chamber use and visiting the living room are less restrictive, individual or shared use of sanitation are more specific for each HRMO and wearing a mask in case of *Streptococcus pneumonia* is restricted to the acute stage of pneumonia. Another important change is that the interval period of two negative cultures for finishing the supplementary measures is extended from 24 hours to one year for CPE *and Enterococcus faecium*, this is rather practice- than evidence-based. Finally, no specific recommendations were formulated in case of an outbreak.
Conclusion: This draft guideline publishes infection prevention measures for policy making staff in long term facilities in order to prevent the transmission of HRMO, for which is an urgent request considering recent outbreaks. How long patients with HRMO should be considered positive, whether a patient follow-up system for HRMO should be recommended and the feasibility and effectiveness of these measures are still unclear.

P132

Cell-free propagation of *Coxiella burnetii* does not affect its relative virulence

R. Kuley[1], H. Smith[1], H. Roest[1], D. Frangoulidis[2], M. Wells[3], M. Smits[4], A. Bossers[4]
[1]*Central Veterinary Institute, Dept. of Infection Biology, Lelystad,* [2]*Bundeswehr Institute of Microbiology,* [3]*Wageningen University,* [4]*Central Veterinary Institute*

Q fever is caused by the obligate intracellular bacterium *Coxiella burnetii (Cb)* and is primarily a zoonotic infection. In vitro growth of the bacterium is usually limited to viable eukaryotic host cells imposing experimental constraints for molecular studies, such as the identification and characterization of major virulence factors. Studies of pathogenicity might benefit from the recent development of an extracellular growth medium. However, it is crucial to investigate the consistency of the virulence phenotype of strains propagated by the two fundamentally different culturing systems. The aim of this study was to assess the relative virulence of *Cb* strains propagated in cell and cell-free based culturing systems.
In the present study we used an immune-competent mouse model to evaluate virulence-associated characteristics of several *Cb* strains cultured in cell-based and cell-free systems. We assessed the virulence of *these* strains based on 'splenomegaly' (increase in weight of spleen in relation to the body weight) and 'RT-PCR quantification of bacteria in the spleen' as the most important read-out parameters. The relative virulence of *Cb* strains is expressed as the 'Relative spleen weight per number of *Cb bacteria*' considering both splenomegaly and number of *Cb* in the spleen. The relative virulence showed strain-specific ($p < 0.05$) but similar virulence values ($p > 0.05$) for the same strain, irrespective the method of in vitro propagation.
Our results clearly demonstrate that the culture system does not significantly influence the relative virulence of

the tested strains. As LPS present on the Cb bacterial cell surface is the only virulent factor known and no significant differences between the two propagation systems were found, indicating that the LPS and possibly other proteins present on cell surface of the bacteria are not changed significantly conserving the infection potential of these strains. Gene expression and protein content measurements could be used to confirm this conclusion. This indicates that the cell-free culturing system could be useful for identification and characterization of virulence factors and development of effective subunit vaccines which would offer great potential in the control of Q fever outbreaks as well as limiting its transmission to humans.

P133

Organic acid (OAs) production from starch waste by rumen microorganisms

S. Palakawong Na Ayudthaya, A.H. van Gelder, A.H.P. van de Weijer, C.M. Plugge
Wageningen University, Laboratory of Microbiology, Wageningen

Organic waste is an important source to produce renewable biochemicals and partially replace the use of fossil fuels. Organic acids (OAs) and Volatile fatty acids (VFAs) currently originate from fossil fuels. However, VFAs/OAs can also be produced from waste by microbial fermentation. Starch waste can be converted into OAs or VFAs. The rumen is a powerful living anaerobic "bioreactor" that contains rumen microorganisms and efficiently ferments complex organic compounds. Therefore, in this research we used rumen fluid as biocatalysts for efficient OAs production from starch waste. We investigated the rumen microbial diversity during organic acids production, using two different sources of rumen fluids. One was obtained from fistulated Holstein cows in Thailand and the other from fistulated Holstein cows from the Netherlands.
A dished bioreactor with 0.8l working volume with anaerobic medium with 7% (w/v) starch waste was used. We mimicked the fermentation conditions as in cow rumen by applying a temperature of 39°C, CO_2 and N_2 headspace and controlling the pH at 7 with 1M $NaHCO_3$. The production of OAs was quantified over 10 days by high-pressure liquid chromatography (HPLC); the microorganisms were quantified by MPN counts using reinforced clostridial medium (RCM) and the microbial diversity was determined by denaturing gradient gel electrophoresis (DGGE). The results showed that lactate (up to 250 mM) was mainly produced by rumen microorganisms from both sources (Thailand/ Netherlands) in the first 3 days of fermentation. Later, acetate (200 mM), propionate (146 mM), butyrate (85 mM) and others were produced and lactate disappeared. Lactic acid bacteria (*Streptococcus, Lactobacillus, Enterococcus* and *Clostridium* spp.) have been found based on the MPN technique through the whole fermentation process. Microbial communities from both inocula during starch waste fermentation changed over time, as was revealed by the 16S rRNA DGGE analysis.
In summary, starch waste is suitable as alternative source for organic acids production using rumen fluid as an inoculum. Microbial diversity changed during the starch waste fermentation process.

P134

Degradation of fibre related components from rumen fibrous biomass in dairy cows

J.D. Vaidya, C. Ye, B. van den Bogert, A.J.M. Stams, H. Smidt, C.M. Plugge
Wageningen University, Dept. of Microbiology, Wageningen

Ruminant animals gain most of their energy from plant fibres, which are degraded by complex microbiota in the rumen. Microbial degradation of the fibres yields carbohydrate monomers that are in turn fermented to short chain fatty acids (SCFAs). Methane (CH_4), a potent greenhouse gas, is also produced, but has no nutritional value to the ruminant and is eructated into the environment. In the past, rumen microbiologists focused on elucidating the attachment of bacteria to feed particles and their role in initial fibre degradation. Nonetheless, many fibre associated microbes remain uncultured and unidentified. To further our understanding of microbial degradation of fibres in the rumen, we enriched and analysed the microbiota from the rumen of dairy cows for communities dedicated to the degradation of plant derived fibres.
Two fistulised Holstein Frisian cows were fed either 100% grass silage or 100% maize silage. The cows were allowed to adapt to their diet for 17 days after which fibrous content from the rumen was collected through the fistula and stored in anaerobic weck pots. Samples were transported to the laboratory, and kept at -80C in a 25% anaerobic glycerol solution. Prior to cultivation, the maize and grass derived fibrous content was washed with anaerobic PBS and inoculated (0.4%, w/v) in serum bottles with growth medium or growth medium supplemented with different fibre related components (1 g/L): glucose, lignin, xylose, cellulose, cellobiose, pectin, amylopectin, and xylan in duplicates (2x). Enrichments were incubated at 40C for 14 days during which samples were obtained at day 0, 3, 7, 11, and 14 that were analysed using HPLC and GC to measure SCFAs, hydrogen (H_2), and CH_4 concentrations.
Enrichments supplemented with glucose, cellobiose, pectin, amylopectin, and xylan showed highest SCFAs concentrations, in particular acetate, propionate, and butyrate. Bottles inoculated with maize derived fibrous content showed relatively high propionate levels at day 0,

which was most likely due to the initial concentrations present in the inoculum. SCFA concentrations were low for enrichments supplemented with lignin and were comparable to those for cultures inoculated with only grass or maize derived content from rumen. CH_4 measurements on day 7 were highest in enrichments with grass derived fibres supplemented with pectin and amylopectin, whereas CH_4 concentrations were highest for maize silage enrichments supplemented with pectin. Enrichments with only the growth medium showed similar CH_4 production for grass and maize derived fibres.
In conclusion, we found that 1) Acetate, propionate, and butyrate were the main SCFAs detected in all enrichments, 2) lignin as well as grass and maize derived fibres showed least degradability within 14 days of the enrichment, 3) glucose, cellobiose, amylopectin, xylan, and pectin were relatively easy to degrade by the rumen microbiota, and 4) CH_4 production is dependent on fibre degradability and product formation.

P135

Carriage of *Streptococcus pneumoniae* in saliva of dutch primary school children

A.L. Wyllie, M.H.B. Schellens, M.J.N. Chu, J. van Engelsdorp Gastelaars, D. Bogaert, E.A.M. Sanders, K. Trzcinski
Wilhelmina's Children Hospital, University Medical Centre Utrecht, Dept. of Pediatric Immunology and Infectious Diseases, Utrecht

Background and aims: Saliva has a history as being one of the most sensitive methods for *Streptococcus pneumoniae* carriage detection (Heffron, 1939). Here, we applied molecular diagnostic methods to study pneumococcal carriage in saliva from schoolchildren.
Methods: Saliva was collected from 50 students (aged 5 to 10 years) of a rural school near Utrecht, transported to the lab on wet ice, cultured and the remaining volume stored frozen. Cultures were inspected for *S. pneumoniae* colonies and then all bacterial growth was harvested. DNA extracted from raw and culture-enriched samples was tested for the presence of *S. pneumoniae* specific genes *lytA* and *piaA* using quantitative-PCR (qPCR) and considered positive when both genes had C*T* values below 40. Sample serotype composition was determined in DNA extracted from culture-enriched saliva samples using qPCR (Azzari, 2010) and by analysing sequences generated by conventional PCR (Carvalho, 2010; Carvalho 2013).
Results: Two children (4%) were culture-positive for *S. pneumoniae*. Thirty-two (64%) children were qPCR-positive for *S. pneumoniae* in raw saliva and 44 (88%) in culture-enriched samples. Using molecular methods to determine sample serotype composition, we detected 83 pneumococcal strains of 22 serotypes in 40/50 samples from carriers, with 26 carriers (59% of 44) positive for 2 to 6 serotypes.
Conclusion: Conventional culture detection of *S. pneumoniae* in saliva is extremely difficult due to saliva's polymicrobial nature. These limitations were addressed by combining culture-enrichment and sensitive molecular methods, resulting in more than ten-fold higher rates of pneumococcal carriage and high rates of co-colonisation detected in schoolchildren.

P136

High rates of *Streptococcus pneumoniae* carriage in saliva of elderly detected using molecular methods

A.L. Wyllie[1], C.L. Krone[1], J. van Beek[2], N.Y. Rots[2], A. Oja[1], M.J.N. Chu[1], J.P. Bruin[3], D. Bogaert[1], E.A.M. Sanders[1], K. Trzcinski[1]
[1]*Wilhelmina's Children Hospital, University Medical Centre Utrecht, Dept. of Pediatric Immunology and Infectious Diseases, Utrecht,* [2]*National Institute for Public Health and the Environment (RIVM), Centre for Immunology of Infectious Dis, Bilthoven,* [3]*Regional Laboratory of Public Health, Dept. of Diagnostic Microbiology, Haarlem*

Background: Pneumococcal disease disproportionally affects the very young and the elderly. Unlike in children, pneumococcal colonization in the elderly is rarely detected by conventional culture of nasopharyngeal swabs. Here, we tested nasopharyngeal and saliva samples for pneumococcal carriage in the elderly using molecular methods.
Methods: Trans-nasal nasopharyngeal, trans-oral nasopharyngeal and saliva samples were obtained from 135 persons aged 60-89 during an episode of influenza-like-illness (ILI) and during recovery, 6-8 weeks later. All samples (n = 272 per type) were tested for *S. pneumoniae* by conventional culture. Following, all visible plate growth (culture-enriched samples) as well as raw saliva were further processed for DNA extraction. Pneumococcal presence was detected by quantitative-PCR (qPCR) targeting two *S. pneumoniae*-specific genes, *lytA* and *piaA*.
Results: *S. pneumoniae* was cultured from 6 (2%) of 272 trans-nasal, 10 (4%) trans-oral and 6 (2%) saliva samples from 14 of 135 elderly. Ten (4%) culture-enriched trans-nasal, 27 (10%) culture-enriched trans-oral and 80 (29%) culture-enriched saliva samples were positive for *S. pneumoniae* by qPCR. The sensitivity of carriage detection was highest from qPCR of culture-enriched saliva samples (80 of 97, 82%), followed by raw saliva samples (44 or 45%) and culture-enriched trans-nasal and trans-oral samples combined (32 or 33%). In total, 65 of 135 (48%) individuals were positive for *S. pneumoniae* at least once; 52 (39%) during an ILI, 45 (33%) after recovery and 32 (24%) at both sampling events.

Conclusions: Using culture-enriched saliva for the molecular detection of *S. pneumoniae* greatly increases the sensitivity of pneumococcal carriage detection in the elderly.

P137

Carriage of *Streptococcus pneumoniae* in 24-month-old children detected using molecular methods

A.L. Wyllie[1], J. van Engelsdorp Gastelaars[1], M.J.N. Chu[1], N.Y. Rots[2], A.J. Wijmenga-Monsuur[2], J.P. Bruin[3], D. Bogaert[1], K. Trzcinski[1], E.A.M. Sanders[1]

[1]*Wilhelmina's Children Hospital, University Medical Centre Utrecht, Dept. of Pediatric Immunology and Infectious Diseases, Utrecht,* [2]*National Institute for Public Health and the Environment (RIVM), Bilthoven,* [3]*Regional Laboratory of Public Health, Dept. of Diagnostic Microbiology, Haarlem*

Background and aims: The gold standard for *S. pneumoniae* carriage detection is the conventional culture of a nasopharyngeal swab. Saliva however, has a history of being one of the most sensitive methods in surveillances on pneumococcal colonisation (Heffron, 1939). Here, we compared the sensitivity of nasopharyngeal swabs and saliva samples from PCV7-vaccinated 24-month-old children tested for *S. pneumoniae* and nasopharyngeal serotype carriage using conventional and molecular diagnostic methods.

Methods: Nasopharyngeal and saliva samples were simultaneously collected from 289 asymptomatic 24-month-old children, cultured and pneumococcal strains were serotyped by Quellung. DNA extracted from harvests of all bacterial growth on pneumococcus-selective medium was tested for *S. pneumoniae* and serotypespresent using quantitative-PCR (qPCR) targeting species-specific genes *lytA* (Carvalho, 2007) and *piaA* and sequences specific for subset of serotypes (Azzari, 2010).

Results: Altogether 240 (85%) of 289 24-month-old children were identified as carriers by any method. Molecular detection of *S. pneumoniae* in culture-enriched nasopharyngeal samples had highest sensitivity (73%) followed by qPCR of cultured saliva (60%) and conventional culture of nasopharyngeal swabs (57%). Isolation of live pneumococci from saliva generally failed due to abundant polymicrobial growth. For the subset of serotypes targeted by qPCR (1, 3, 6A/B/C/D, 7A/F, 8, 9A/V, 10A 14, 15C/D, 19A, 20, 22AF, 23F, 33A/F and 38) the number qPCR-positive results increased 2.2 when compared to culture results for nasopharyngeal samples alone.

Conclusion: Application of molecular methods to detect *S. pneumoniae* in culture-enriched samples and testing saliva along with nasopharyngeal swabs significantly increased the number of carriers and strains detected.

P138

Saliva and nasopharyngeal carriage of *Streptococcus pneumoniae* in parents of 24-month-old children

A.L. Wyllie[1], L.W. Rümke[1], M.J.N. Chu[1], N.Y. Rots[2], A.J. Wijmenga-Monsuur[2], J.P. Bruin[3], D. Bogaert[1], E.A.M. Sanders[1], K. Trzcinski[1]

[1]*Wilhelmina's Children Hospital, University Medical Centre Utrecht, Dept. of Pediatric Immunology and Infectious Diseases, Utrecht,* [2]*National Institute for Public Health and the Environment (RIVM), Bilthoven,* [3]*Regional Laboratory of Public Health, Dept. of Diagnostic Microbiology, Haarlem*

Background: Although historical records show relatively high rates of approximately 50% pneumococcal carriage in the saliva of adults (Heffron, 1939), contemporary surveillances on adult nasopharyngeal carriage report much lower rates of colonisation. Here, we compared the sensitivity of conventional culture and molecular methods for detecting pneumococcal carriage in nasopharyngeal and saliva samples in young-middle aged adults.

Methods: Trans-nasal nasopharyngeal, trans-oral nasopharyngeal and saliva samples were simultaneously collected from 299 parents of asymptomatic 24-month-old children. All samples were analysed using the standard conventional culture approach. All trans-oral and saliva samples were tested in quantitative-PCR (qPCR) targeting pneumococcal genes *lytA* and *piaA* in DNA extracted from samples culture-enriched for *S. pneumoniae*. Since we have previously shown (Trzcinski 2013, Krone ISPPD9) that molecular methods do not significantly improve detection of *S. pneumoniae* in trans-nasal samples from adults they were not tested with qPCR.

Results: By conventional culture, 29 (10%) adults were identified as carriers, with live pneumococci isolated from 26 (9%) trans-nasal and 4 (1%) trans-oral sample cultures. Isolation of live *S. pneumoniae* from saliva was virtually impossible due to abundant polymicrobial culture growth. By qPCR, 89 (30%) trans-oral and 84 (28%) saliva samples were pneumococcus-positive. Altogether, 137 of 299 (46%) adults were positive for *S. pneumoniae* by any method and 130 (95%) of all 137 carriers were detected by qPCR alone.

Conclusions: Molecular detection of *S. pneumoniae* in culture-enriched trans-oral and saliva samples were the most sensitive methods in this study, together equally contributing to the overall > 45% detected pneumococcal carriage rate.

P139

A metagenomic study of a drinking water supply reservoir in Central Indiana

S. van Hove[1], C. Teiling[1], I. Chorny[1], R. Schmieder[1], B. Steffy[1], N. Clercin[2], M. Stouder[2], M. Gray[3]

[1]*Illumina, Dept. of Regional Marketing, Chesterford, United Kingdom* [2]*IUPUI, Center for Earth and Environmental Science, Indianapolis, USA* [3]*Citizens Water, Indianapolis,USA*

Center for Earth and Environmental Science at IUPUI in partnership with the local water company, monitors and documents sampling of the Water Supply on a regular basis in the Eagle Creek Reservoir. As it is only a seasonal blooming of algae that is monitored, capturing the variation in genome signature might give an insight on the symbiosis of the blue-algae and its environment.
In this study, samples from the Eagle Creek reservoir were assessed in a metagenomic approach to understand the differences in taxonomic affiliation and functional occurrences of microbial communities. The samples were collected from April to October 2013 at different depths: near the surface, at 3 meters, at 6 meters and near the bottom of the reservoir. All environmental samples were sequenced using the NextSeq 500 Benchtop Instrument and the Hiseq 2000. Here, we present preliminary data of a comparison with the HiSeq 2000 instrument and a correlation between the meta-data collected and the metagenomes. This study provides new insights into potential biological processes associated with blooming, depth, and seasonal differences in fresh water.

BAMA-P01

Capsule of *S. pneumoniae* protects the bacteria from neutrophil-mediated phagocytosis

R.R. Roosenhoff, R. Nijland, J.A.G. van Strijp
UMC Utrecht, Dept. of Medical Microbiology, Utrecht

Introduction: Neutrophils are crucial for the defense of the immune system against invading microorganism. Additionally, they are the most abundant cells of the white blood cell population. In contrast to other phagocytes, neutrophils can effectively kill Gram-positive bacteria such as *Streptococci* and *Staphylococci*. Nevertheless, these bacteria have evolved mechanism to evade neutrophil mediated killing. *Staphylococcus aureus* for instance secretes various virulence factors such as staphyloxanthin thatcan deactivate the ROS process in neutrophils. *Streptococcus pneumoniae* on the contrary, does not only produce virulence factors, but the bacterium is surrounded by a capsule that protects the pathogen from phagocytosis. There are limited data available on the precise mechanism by which the capsule prevents the phagocytosis. The aim of this research was to evaluate the preferential activity of the neutrophils for *S. aureus* and (un)encapsulated *S. pneumonia* strains. Additionally, this is the first study that used time lapse microscopy to visualize the preference activity of neutrophils.

Methods and results: To measure phagocytosis, the neutrophils were incubated with different strains of *S. aureus* and *S. pneumonaie* which expressed different fluorescent proteins. Two *S. aureus* strains (MW2 and Newman) expressing cyan fluorescent protein (CFP) and green fluorescent protein (GFP) were evaluated. Furthermore, two *S. pneumoniae* strains expressed red fluorescent protein (DsRed) or GFP and the two fjcap strains also expressed DsRed or GFP. The phagocytosis of neutrophils was visualized with confocal microscopy. The cells and bacteria were monitored for CFP, DsRed, GFP and bright field every 7 min at four positions in each well, to create a time-lapse movie of the interaction between the neutrophils and the bacteria strains. As expected, the *S. aureus* strains and the unencapsulated mutants *S. pneumoniae* were effortlessly internalized by neutrophils. However, astonishingly the movie illustrated that despite direct and repeated contact between neutrophils and encapsulated *S. pneumoniae* no phagocytosis occurred.
The events that were observed with the time lapse movie were quantified, by measuring phagocytosis by flow cytometry. The results of the flow cytometry were in accordance to the microscopy, because 95.3% of the neutrophils had internalized *S. aureus* MW2 and 78.2% ingested *S. pneumonia* ?cap. Additionally, only 6.4% of the neutrophils ingested the encapsulated *S. pneumonia* strain.
Conclusion: The study demonstrated that neutrophils have preference for bacteria that are not encased with a capsule structure. Solely, *S. aureus* and *S. pneumoniae* fjcap were internalized when mixed together with encapsulated *S. pneumoniae*. In conclusion, the encapsulated bacteria have an evolved mechanism to inhibit phagocytosis.

BAMA-P02

Expanding the database of IS-pro

S.R. Jeleniewski
Hogeschool Utrecht, Dept. of Life Sciences and Chemistry, Leusden

IS-pro, which stands for Interspace profiling, is a new DNA fingerprinting technique. Phylum specific, fluorescent labeled PCR primers are used to amplify the 16S-23S rDNA interspace regions. The 16S-23S rDNA interspace region length is species specific, and the fluorescent color of amplified fragment is phylum specific. The DNA fragments are separated by length with capillary gel electrophoresis, which results in a profile of colored peaks. These profiles can be linked to a database, which at the moment contains a limited number of species. To use IS-pro for clinical diagnostics, the database needs to be expanded. The database will be expanded by two approaches. The first approach is to select around 150 pathogenic bacteria, and from each species 5 different

strains are evaluated with IS-pro. From this, we know which peaks belong to the selected species. With the second approach, Illumina sequencing will be used to find out which bacteria are present in clinical samples, even when nothing is cultured. When all profiles can be sufficiently translated into the corresponding bacterial species, IS-pro can be implemented in clinical routine.

BAMA-P03

Bacterial modulation of coagulation pathways

T. Sentner, A. Kuipers, S.H.M. Rooijakkers
UMC Utrecht, Dept. of Medical Microbiology, Utrecht

The coagulation system plays several roles in the host defense against bacterial infections: coagulation supports innate defenses by entrapment and killing of invading bacteria inside fibrin clots or via the formation of small antibacterial and pro-inflammatory peptides. The coagulation cascade can be activated via different pathways that converge in a common pathway for clot formation. The intrinsic pathway of coagulation has been known to be initiated on the surface of bacteria and is able to further activate the common pathway of coagulation. In the last decade it has become clear that pathogenic bacteria have evolved mechanisms to avoid immune clearance. *Staphylococcus aureus* secretes a number of proteins that block critical steps in the inflammatory response, including neutrophil and complement inhibitors. Therefore, we set out to identify bacterial proteins that enable bacteria to modulate these pathways of coagulation. We mainly focused on two critical enzymes within these pathways: kallikrein, which is part of the intrinsic pathway, and thrombin, which is important in the final step of the formation of a fibrin clot. With purified proteins we set up functional coagulation assays to determine the activity of these enzymes. By the addition of both recombinant proteins and culture supernatants of pathogenic bacteria we try to identify modulators of coagulation.

BAMA-P04

Determination of transferability and molecular characterization of Extended Spectrum β-Lactamase (ESBL) producing *Escherichia coli* isolated form horses

C.J.A. Peters, E. Broens, B. Duim, J.A. Wagenaar, J. Hordijk
UMC Utrecht, Dept. of Infectious Diseases and Immunology, Utrecht

Introduction: The increasing prevalence of extended-spectrum beta-lactamases (ESBL) is a growing problem worldwide, not only in human or animal clinical samples but also in food-producing and companion animals. So far little is known about the presence of ESBL / AmpC-producing bacteria in horses in the Netherlands. In 2007-2009 8% of the *Enterobacteriaceae* derived from clinical samples from horses displayed inhibition zones = 25 mm for ceftiofur or cefquinome. (Dierikx et al, 2012). And a pilot prevalence study performed in 2012 showed a prevalence of 23% cefotaxime-resistant *Enterobacteriaceae* in horses without gastro-intestinal complaints (Leendertse *et al*., 2013 Poster 084, NVMM & KNVM voorjaarsvergadering 2013). The aim of this study was to perform a molecular characterization of these ESBL-producing *Escherichia coli* from above mentioned study and to investigate the transferability of ESBL genes by conjugation.

Methods: A total of 15 ESBL-producing *E.coli* were included. For all isolates ESBL genes, *E. coli* MLST type and plasmid incompatibility groups were determined using PCR and sequence analysis. For plasmid detection PBRT (PCR Based Replicon Typing) was used.

For conjugation three different methods were used, two of which included liquid mating and one included filter mating. The isolated donor strain and a recipient strain (*E. coli* K12 E3110) were mixed in LB-broth. For filter mating this suspension was subsequently inoculated on a Millipore filter. After incubation the conjugation mixes were plated onto LB-agar supplemented with cefotaxime, rifampicin or both to segregate the transconjugants from donor and recipient.

Results: Fourteen of the isolated strains contained ESBL type CTX-M-1 and one strain contained ESBL type SHV-12. Seven CTX-M-1 harboring strains contained an additional TEM-1b gene. Two different plasmid types were found. Seven strains harbored an IncHI1 plasmid, two strains contained an IncY plasmid and two strains contained both plasmids. In four isolates no plasmid could be determined by PBRT. In addition to the ESBL type and plasmid we also determined the sequence type of the *E. coli*. ST1250 was most common, in five strains. ST4164 and ST4165 were both found two times. Furthermore, ST10, 1277, 1462, 1586, 1784 and 4163 were all found once.

Conjugation failed in all three methods.

Conclusion: Although all fifteen *E. coli* strains were different, only little variation in ESBL type, plasmid and sequence type was found. It looks like the CTX-M-1 gene is dominant in horses, but because of the small number of strains used in this study no conclusion can be drawn regarding the epidemiology.

Conjugation using the three methods failed. Whether inappropriate methods were used or that the isolates were incompetent remains unclear and is topic of current investigation.

BAMA-P05

Molecular epidemiology of canine methicillin-resistant *Staphylococcus pseudintermedius* in the Netherlands

M. Spaninks[1], K.M. Verstappen[2], J. Hordijk[2], A.J. Timmerman[2], E.M. Broens[2], J.A. Wagenaar[2], B. Duim[1]
[1]*Utrecht University, Faculty of Veterinary Medicine, Infectious Diseases and Immunology, Utrecht,* [2]*Utrecht University, Dept. of Infectious Diseases and Immunology, Utrecht*

Introduction: *Staphylococcus pseudintermedius* is a commensal and a pathogen in dogs and cats. Zoonotic transmission seems to be rare. At the Veterinary Microbiological Diagnostic Centre (VMDC) of Utrecht University, methicillin-resistant *S. pseudintermedius* (MRSP) strains have been isolated from canine infections increasingly since 2006 with a stable proportion of approximately 10% MRSP among the total number of *S. pseudintermedius* over the last 7 years. MRSP isolates tend to be resistant to almost all classes of antimicrobials. Interestingly, from 2011 a slight shift towards more susceptible MRSP strains has been observed. The goal of this study was to determine whether strains from MLST clone ST71, the dominant clone in the Netherlands until 2011, is still present and is losing resistances or that other less resistant clones are emerging.
Methods: From the database of the VMDC all first isolates of confirmed canine MRSP patients isolated between January 2007 and December 2013 were selected (n = 446). The strains were confirmed as MRSP with *pta* PCR-RFLP and *mec*A and *mec*ALGA251 PCR and were subsequently typed by Multi locus Sequence Typing (MLST). By using MLST based on 7 genes, that enabled comparison of types in the PubMLST database, the types were compared with the trends in clonal distribution of MRSP in other European countries.
Results: MLST identified 15 known sequence types (ST) and 36 new types, which are currently being assigned. The known types were present in other European countries. From 2007 -2013 the dominant types were ST71, ST258, and ST45. The ST71 proportion gradually decreased from 87% in 2007 to 44% in 2013. ST45 belonged to 24% of the strains in 2009. In 2007 a single strain belonged to ST258 and belonged to 13% of the strains in 2013. ST261 was detected for the first time in 2008 and increased to 11% in 2013. In 2010 ST265 was detected for the first time and belonged to 6% of the strains in 2013.
Conclusions: This study shows that ST71 is still the dominant sequence type. However the diversity in sequence types increased during the study period and suggests that other types are emerging. Whether a change in susceptibility is associated with the emerging sequence types is currently under investigation and will be presented at the meeting.

BAMA-P06

Design and validation of a typing method for the molecular epidemiology of *Trichomonas vaginalis*

F. Tiemersma, M. Himschoot, D. Jonker, S. Bruisten
Public Health Laboratory, Public Health Service of Amsterdam, Amsterdam

Aim: *Trichomonas vaginalis* (TV) is a parasite that infects around 250 million people worldwide. Trichomoniasis is a sexually transmitted infection. Typing is an important tool to elucidate the transmission from person to person. We aimed to design and validate a typing method for molecular epidemiology of TV.
Approach: We started with a Multilocus Sequence Typing (MLST) method described in the literature. TV samples were obtained as positive control by culturing samples.
Results: Tested primers were not sensitive nor specific, producing many bands. A nested PCR was set up to ensure a higher specificity. We optimised PCR conditions. Control samples were amplified and sequenced. Sequences from control samples were analysed using two programs: BioNumerics and Mega. These programs can be used to perform cluster analysis.
Conclusion: Typing of TV using MLST is feasible. Positive TV samples from patients can also be amplified and sequenced.

BAMA-P07

Multiplex PCR test for the detection of West-Nile virus, Dengue virus and Chikungunya virus

A. van den Brom
Sanquin Blood Supply, Utrecht

Aim: To develop an ARBO-PCR for the simultaneous detection of West-Nile virus, Dengue virus and Chikungunya virus in blood donations. These viruses can be transmitted to humans by tropical mosquitoes.
Approach: Primers and probes were selected for West-Nile virus, Dengue virus and Chikungunya virus in highly conserved regions of their genomes. The PCR conditions are optimized. Subsequently the primers and probes will be combined into 1 multiplex PCR assay (ARBO-PCR).
Results: The West-Nile virus PCR is optimized. We tested different PCR Master Mixes, primer-and probe concentrations and PCR profiles. WNV panels containing lineage 1 and lineage 2 isolates were tested. The WNV PCR detects both lineages. The sensitivity of the assay is comparable to commercial assays for WNV. In addition, the specificity of the assay was addressed by testing 150 negative samples. Currently, the selected primers and probes for Chikungunya virus and Dengue virus are evaluated to determine the optimal PCR conditions.

Conclusion: We developed a sensitive WNV PCR detecting the most important lineages. This PCR will be multiplexed with the PCR assays for Chikungunya virus and Dengue virus.

BAMA-P08

Differences in quantitation of antibody titers due to alterations of pneumococcal polysaccharides as a result of chemical conjugation to Luminex beads

D. Sybrandi, B. Meek, B. de Jong
St Antonius Hospital, Dept. of Medical Microbiology and Immunology, Nieuwegein

Aim: To examine whether antibody-epitope composition of polysaccharides purified from *Streptococcus pneumoniae* is altered due to the use of different conjugation methods to Luminex beads.
Approach: Polysaccharide 4 (PPS 4) was conjugated to Luminex beads using 2 commonly used **Methods:** PLL-NHS and DMTMM. Both beads were evaluated using a set of selected pre- post-immune sera from humans. Intra- and interassay coefficients of variation (CV) were calculated, using cut-offs for acceptance of 15% and 20%, resp.
Results: Over a series of experiments we found that quantitation of anti-PPS4 antibody titers using each bead was not comparable; interassay CV > 20%. Furthermore, differences were consistent in time and the intra-assay CV for each conjugation method was acceptable.
Conclusion: Each conjugation method differently affects the polysaccharide, probably altering the number of antibody-epitopes. Importantly, differences in calculated titers do not result in different interpretations of titers. Current research focuses on whether differences in epitopes are quantitative or a qualitative.

BAMA-P09 - Abstract not available for publication

Bacteriological quality of seawater along the south coast of Curacao

T. Jonkers
Hogeschool Leiden, Leiden

BAMA-P010

The role of complement and IgG on the uptake of *Staphylococcus aureus* by neutrophils

T.A.D. Juliet
University Medical Center Utrecht, Dept. of Medical Microbiology, Utrecht

Introduction: Methicillin Resistant *Staphyloccocus aureus* (MRSA) is a gram-positive bacterium that is resistant to many antibiotics. This bacterium causes the majority of clinically based infections. Besides clinical infections MRSA also causes infections in the community. For this reason this bacterium is of great interest to study in order to understand its mechanism and thereafter design a product and/or technique to combat this bacterium or in either case come up with a way that it will no longer be able to infect humans. Mechanisms that need to be better understood is the phagocytosis of the bacteria i.e. the uptake of the bacteria, actual killing of the bacteria and immune evasion strategies that the bacteria uses to deviate from these. Neutrophils are mainly responsible for the clearance of the bacteria through phagocytosis; this is done with the help of IgG and the complement system. Phagocytosis is mainly induced by the complement system.
Methods: To prove that the complement system plays an important role in phagocytosis of the bacteria different experiments were done. A phagocytosis assay was done by using GFP-labeled *S. aureus* and freshly isolated human neutrophils and mixed with human pooled serum, complement inactivated human pooled serum, purified IgG, purified anti-*S.aureus* specific IgG, serum deficient in one complement factor, and serum without IgG to check for the role that each component of the complement play in this case; this was done by using flow cytometry. By using flow cytometry and Western Blot, C3 and IgG deposition on *S. aureus* was also measured.
Results: *S. aureus* is phagocytized in a serum concentration dependent manner. In high serum concentrations all neutrophils take up bacteria. This percentage decreases along with the serum concentration. The amount of bacteria taken up per neutrophils is also dependent of the serum concentration. In the case of complement-inactivated serum, the percentage of neutrophils that have taken up bacteria is reduced with almost 40%. There is almost no phagocytosis when no complement is present in comparison with normal HPS.
In a Western Blot it could be seen that for IgG and C3b deposition on *S. aureus*, the amount of C3b and IgG deposited on the bacteria is lower when the concentration of the serum is reduced. When HPS without IgG is added the amount of C3b deposition is reduced in comparison with normal HPS. When IgG is added to HPS without IgG, C3b deposition is restored to normal serum levels. This shows that IgG plays an important role for the deposition of C3b on the bacteria.
Conclusion: Complement and IgG play an important role in phagocytosis of *S. aureus* by neutrophils. Complement makes the phagocytosis process much more efficient. Although IgG does not play a direct role in opsonization when enough complement is activated. High concentrations of complement block detection antibodies from binding to IgG. This could be better seen with the use of Western Blot. Phagocytosis could not be stopped with a FcgR blocker. The exact mechanism needs to be further studied.

Notes

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