NEDERLANDS TIJDSCHRIFT VOOR

MEDISCHE MICROBIOLOGIE

Supplement bij drieëntwintigste jaargang, april 2015

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

Papendal, 14 & 15 april 2015
Programma-overzicht
Abstracts
Auteursindex

23e jaargang . april 2015 . Supplement

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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:

Meeting secretariat

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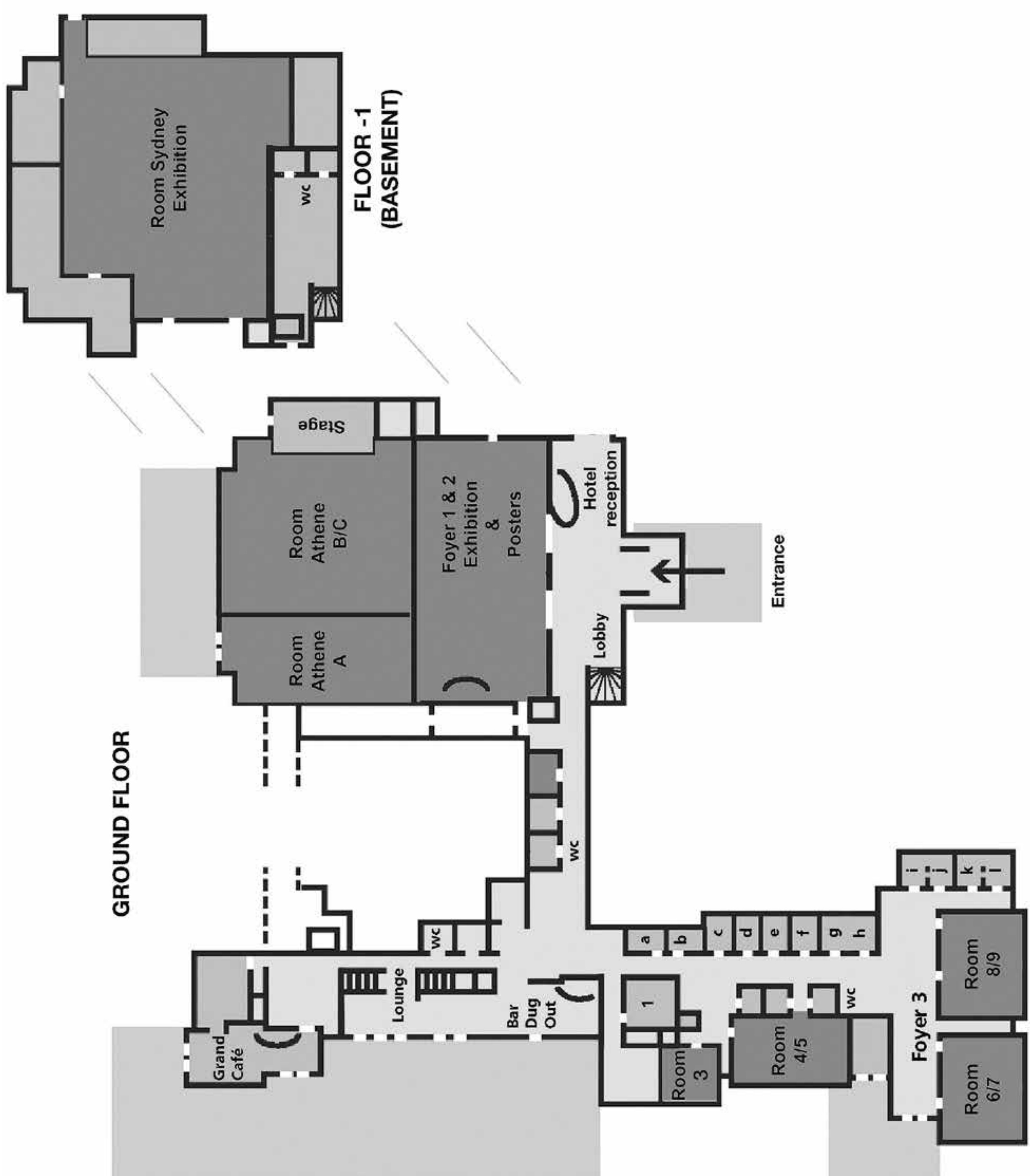
SCHEMATIC PROGRAMME

TUESDAY APRIL 14, 2015

	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 - 13:00		Leeuwenhoek Medal Winner & Award Ceremony					
13:00 - 14:00	Lunch						
14:00 - 15:30		Emerging viruses: clinical and diagnostic implications - Organised by the NWKV & WMDI	Staphylococcal pathogenesis	Challenging tools and best practices to educate the next generation microbiologists	Clinical microbiology 1	Microbial Interactions in anaerobic environments	Screening, engineering and production of novel antimicrobials
15:30 - 16:00	Coffee/tea			KNVM Business meeting			
16:00 - 17:30		Vaccines of the National Immunization Program: benefits and challenges	Pathogenesis 1	General microbiology	Clinical microbiomics	Microbial cell factories	Virology
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						

WEDNESDAY APRIL 15, 2015							
	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		Evidence based medicine for infection control: A curse in disguise?	Clinical microbiology 2	Bacterial spores in health & disease	Pathogenesis 2	Microbiota	Bachelor and Master (BAMA) Symposium
10:30 - 11:00	Coffee/tea						
11:00 - 12:30		Intestinal microbiota in health and disease	Clinical cases in medical microbiology: an interactive session. Organised by the WAMM, NVMy & NWKV	Peptidoglycan synthesis and recycling	Treatment of parasitic diseases	Broadly neutralizing antibodies in antibody treatment and vaccine development	Bachelor and Master (BAMA) Symposium
12:45 - 13:45				BBC-MMO business meeting			
14:00 - 15:30		Host pathogen inter-actions in bacterial meningitis	Microdebate: Dual-Use in microbiological research, consequences and pitfalls of new regulations – intitiated by KNVM board	Microbial systems ecology – Section microbial ecology	Public Health	Vancomycin resistant enterococci. How to survive in lab and in the hospital.	Epigenetics of host-pathogen interactions
15:30 - 16:00	Coffee/tea						
16:00 - 18.00		NVMM Business Meeting					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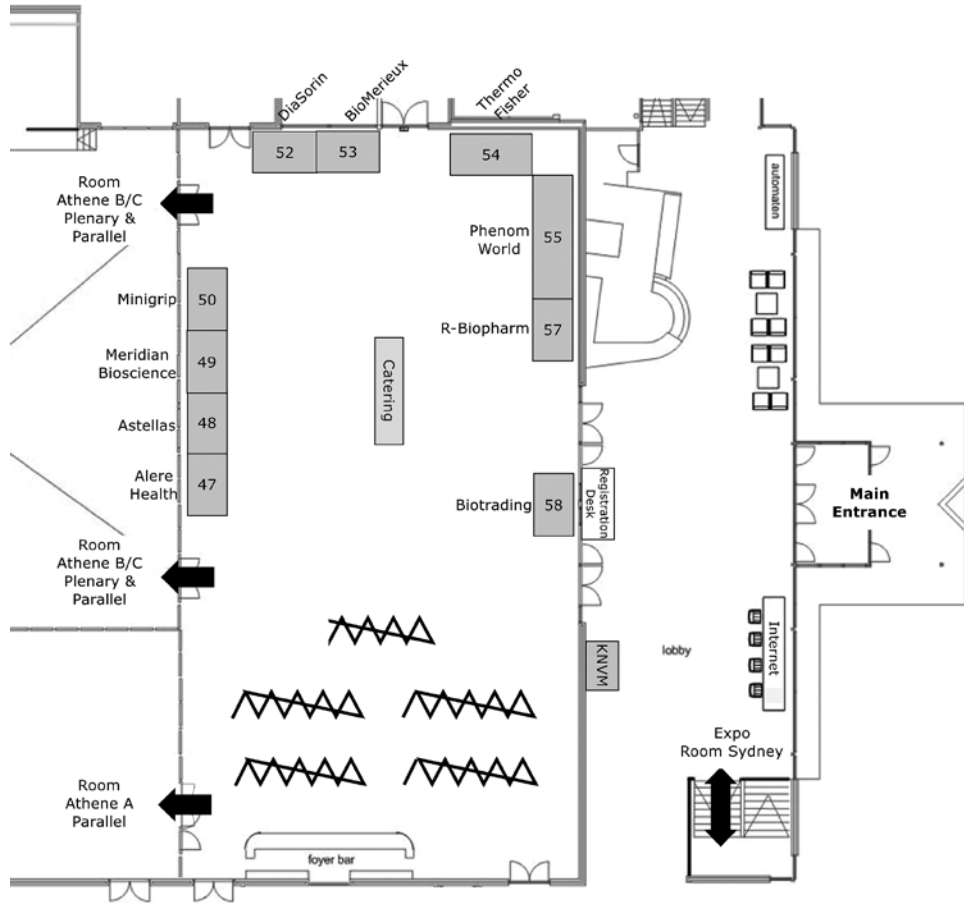
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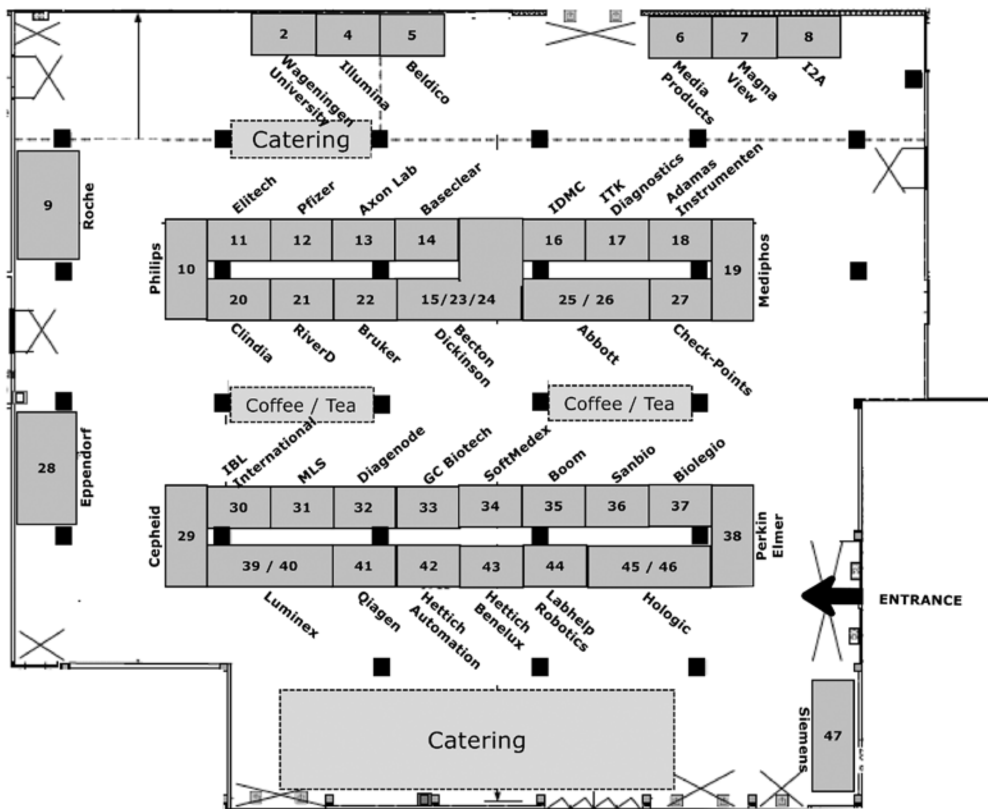
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MONDAY 13 APRIL 2015

Afternoon **National examination for medical microbiologists in training**

Restaurant

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

TUESDAY 14 APRIL 2015

09:00 - 09:30 Registration

09:30 - 11:00 PLENARY SESSION

Athene B/C *Chair: Menno de Jong*

09:30 - 10:15 **Microbe-host interactions in chronic intestinal inflammation – microbial dysbiosis versus pathobiont selection**
O001 Dirk Haller (Germany)

10:15 - 11:00 **Broadly neutralizing antiviral antibodies**
O002 Antonio Lanzavecchia (Switzerland)

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 **Leeuwenhoek Medal Winner**

12:15 - 13:00 **Award ceremony**

Kiemprijs
- Category Medical Microbiology: Guido Bastiaens - Efficacy and safety of the mosquitocidal drug Ivermectin to prevent malaria transmission after treatment: a double-blind, randomized, clinical trial
- Category General and Molecular Microbiology: Daan Swarts - DNA-guided DNA interference by a prokaryotic Argonaute

Westerdijk Award
- Category medical microbiology: Lilly Verhagen – Thesis 'Respiratory infections in Venezuelan children, interplay between host, pathogen and environment'
- Category general microbiology: Walter Bronkhorst – Thesis 'Small RNA-based antiviral defense in insects'

Van Leeuwenhoek Award
- Reindert Nijland - Staphylococcal alpha-phenol soluble modulins contribute to neutrophil lysis after phagocytosis

13:00 - 14:00 **Lunch**

14:00 - 15:30 PARALLEL SESSIONS

Athene B/C **Emerging viruses: clinical and diagnostic implications - Organised by the NWKV & WMDI**
Chairs: Annelies Riezebos-Brilman & Els Wessels

14:00 - 14:30 **Arboviruses**
O003 Jonas Schmidt-Chanasit (Germany)

14:30 - 15:00 **Laboratory preparedness 2014: lessons from the Ebola outbreak**
O004 Marion Koopmans

15:00 - 15:15 **Re-emergence of chikungunya virus**
O005 Jolanda Smit

15.15 - 15.30 **Polio-eradication: the final stage?**
O006 Ton van loon

Athene A **Staphylococcal pathogenesis**
Chair: Suzan Rooijakkers

14:00 - 14:30 **Host adaptation of S. aureus**
O007 Jose Penades (United Kingdom)

14:30 - 15:00 **Intravital imaging of S. aureus replication within Kupffer cells**
O008 Bas Surewaard

15:00 - 15:15 **Staphylococcus aureus protects its immune-evasion proteins from degradation by neutrophil serine proteases**
O009 Daphne Stapels

15.15 - 15.30 **LukMF' is the major leukotoxin of bovine S. aureus and targets neutrophils through CCR1**
O010 Manouk Vrieling

Room 3 **Challenging tools and best practices to educate the next generation microbiologists**
Chairs: Loek van Alphen & Annelies van Goor

14:00 - 14:30 **Taking a scientific approach for Science Education. The importance of defining what to learn and how to learn.**
O011 Peter van Beukelen

14:30 - 15:00 **Last comes First!**
O012 Els de Hullu

15:00 - 15:15 **Tricks and experiences with computer-driven practical courses in microbiology**
O013 Girbe Buist

15.15 - 15.30 **More than MOOCs**
O014 Fred Mulder

Room 4/5 **Clinical microbiology 1**
Chairs: Bart Vlaminckx & Suzan van Mens

14:00 - 14:15 **Development of novel Synthetic Antimicrobial Antibiofilm Peptides (SAAPs) to prevent biomaterial-associated infection**
O015 Martijn Riool

14:15 - 14:30 **Efficacy and safety of the mosquitocidal drug ivermectin to prevent malaria transmission after treatment: a double-blind, randomized, clinical trial**
O016 Guido Bastiaens

14:30 - 14:45 **Rapid detection of cyp51A-promoter based voriconazole resistance in *Aspergillus fumigatus* isolates in a high incidence population**
O017 Jori Fuhren

14:45 - 15:00 **A systematic review of the diagnostic accuracy of serological tests for Lyme Borreliosis**
O018 Hein Sprong

15:00 - 15:15 **Epidemiology, management and risk-adjusted mortality of ICU-acquired enterococcal bacteraemia: a prospective observational study**
O019 David Ong

15:15 - 15:30 **Evolution of colistin resistance in *Klebsiella pneumoniae***
O020 Axel Janssen

Room 6/7 **Microbial Interactions in anaerobic environments**
Session sponsored by: Soehngen Institute of Anaerobic Microbiology
Chairs: Mike Jetten & Diana Sousa

14:00 - 14:30 **Microbial interactions in the deep subsurface**
O021 Axel Schippers (Germany)

14:30 - 14:45 **Cooperation of anaerobic methane and ammonium oxidizing micro organisms**
O022 Karin Stultiens

14:45 - 15:00 **Interspecies electron transfer in fatty acid-degrading communities**
O023 Vicente Sedano Núñez

15:00 - 15:15 **The unknown diversity of anaerobic micro-organisms in the Black Sea**
O024 Laura Villanueva

15:15 - 15:30 **Enrichment of DNRA bacteria**
O025 Eveline van den Berg

Room 8/9 **Screening, engineering and production of novel antimicrobials**
Chairs: Oscar Kuipers & Gilles van Wezel

14:00 - 14:30 **Miniaturizing Fleming: Applying high-throughput directed evolution to ribosomal peptides?**
O026 Sven Panke (Switzerland)

14:30 - 14:45 **Introducing non-canonical amino acid residues in the antimicrobial peptide nisin**
O027 Maike Bartholomae

14:45 - 15:00 **Unravelling the mode of action of the lantibiotic Pep5**
O028 Sabine Oppedijk

15:00 - 15:15 **Antibacterial mechanism of action of chicken cathelicidin-2**
O029 Viktoria Schneider

15:15 - 15:30 **Antimicrobials from Streptomycetes**
O030 Changsheng Wu

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

Room 3

15:30 - 16:00 **KNVM Business meeting**

16:00 - 17:30 **PARALLEL SESSIONS**

Athene B/C **Vaccines of the National Immunization Program: benefits and challenges**
Chairs: Audrey King & Marjolein van Gent

16:00 - 16:30 **Global control of measles and rubella: do we need new vaccines or alternative vaccination routes?**
O031 Rik de Swart

16:30 - 16:45 **Immune evasion by variable expression of two vaccine components, pertactin and filamentous hemagglutinin, during colonization of *Bordetella pertussis* in the immunized murine model**
O032 Anne Zeddeman

16:30 - 16:45 **Strain surveillance after implementation of pneumococcal vaccination**
O033 Karin Elberse

17:00 - 17:15 **Mumps vaccine failure in adolescents**
O034 Sigrid Gouma

17:15 - 17:30 **13-valent pneumococcal conjugate vaccination response in patients after community acquired pneumonia**
O035 Gertjan Wagenvoort

Athene A **Pathogenesis 1**
Chairs: Pieter-Jan Haas & András Spaan

16:00 - 16:15 **GacA is essential for Group A Streptococcus and defines a new class of monomeric dRDP-4 dehydrorhamnose reductases**
O036 Samantha van der Beek

16:15 - 16:30 **The ESX-5 system of pathogenic Mycobacteria is involved in capsule integrity through its substrate PPE10**
O037 Louis Ates

16:30 - 16:45 **Genome-wide screening for genetic determinants involved in decreased susceptibility to the antiseptic chlorhexidine in the multidrug-resistant opportunistic pathogen *Enterococcus faecium***
O038 Ana Guzman

16:45 - 17:00 **The role of a type VII secretion chaperone in the specific substrate recognition in pathogenic mycobacteria**
O039 Trang Phan

17:00 - 17:15 **The role of antibodies in anti-fungal immunity against *Aspergillus fumigatus***
O040 Steven Braem

17:15 - 17:30 **Identification of a new staphylococcal myelo-peroxidase (MPO) inhibitor**
O041 Nienke de Jong

Room 3 **General microbiology**
Chair: Stanley Brul

16:00 - 16:15 **Physiology of *Saccharomyces cerevisiae* at near zero-growth rates: towards uncoupling metabolism from growth**
O042 Tim Vos

16:15 - 16:30 **Peptidoglycan present in Planctomycetes after all? Insights from an anammox bacterium**
O043 Muriël van Teeseling

16:30 - 16:45 **Sporulation temperature has limited effect on the spore proteome of *Bacillus weihenstephanensis***
O044 Sacha Stelder

16:45 - 17:00 **CRISPR/Cas9: a molecular Swiss army knife for simultaneous introduction of multiple genetic modifications in *Saccharomyces cerevisiae***
O045 Robert Mans

17:00 - 17:15 **Characterization of a Nitrospira species enriched under anaerobic, denitrifying conditions**
O046 Maartje van Kessel

17:15 - 17:30 **Quest for new antimicrobial drug targets: genome-wide screening of essential genes of the emerging zoonotic pathogen *Streptococcus suis* and validation of YycF/G Two-component System as antimicrobial drug target**
O047 Agnieszka Bem

Room 4/5 **Clinical microbiomics**
Chairs: Dries Budding & John Penders

16:00 - 16:30 **Probiotics in Very-Low-Birth-Weight Neonates in relation to late onset staphylococcal sepsis**
O048 Piotr Heczko (Poland)

16:30 - 17:00 **The microbiota in colorectal cancer: Cause or effect?**
O049 Annemarie Boleij

17:00 - 17:15 **Characterization of gut microbiota profiles by disease activity in patients with Crohn's disease**
O050 Danyta Tedjo

17:15 - 17:30 **Microbiota profiling in clinical diagnostics**
O051 Anat Eck

Room 6/7 **Microbial cell factories**
Chairs: Pascale Daran-Lapujade & Ruud Weusthuis

16:00 - 16:30 **Single-cell metabolite sensors as tools for strain and enzyme development**
O052 Michael Bott (Germany)

16:30 - 16:45 **Engineering precursor supply and free-energy conservation for anaerobic fermentative production of fuels and chemicals**
O053 Ton van Maris

16:45 - 17:00 **Comparative genome, transcriptome and metabolome analysis of the strain lineage of β-lactam producing strains of *Penicillium chrysogenum***
O054 Oleksandr Salo

17:00 - 17:15 **Metabolic engineering of *Escherichia coli* for itaconic acid production**
O055 Kiira Vuoristo

17:15 - 17:30 **Heterogeneity within the mycelium of the cell factory *Aspergillus niger***
O056 Pauline Krijgsheld

Room 8/9 **Virology**
Chairs: Steven van Beurden & Peter Rottier

16:00 - 16:15 **Reduced soluble CD14 in neonates hampers efficient activation of dendritic cells by Hepatitis B surface antigen**
O057 Nadine van Montfoort

16:15 - 16:30 **A novel iridovirus causes scale drop disease in Lates calcarifer (Asian seabass)**
O058 Ad de Groof

16:30 - 16:45 **H7N9 and H6N1 influenza A virus hemagglutinins engineered to bind human type receptors reveal a novel layer of specificity beyond the a2-6 linkage of sialic acid**
O059 Robert de Vries

16:45 - 17:00 **Novel insights in the tropism of avian viruses: glycan specificities of coronaviral attachment proteins**
O060 Hélène Verheije

17:00 - 17:15 **Two cases of chromosomal integration of human herpes virus 6 in patients with idiopathic cardiomyopathy**
O061 Fleur Koene

17:15 - 17:30 **Prevalence and incidence of HEV infection in the Netherlands: risk factors for donors and risk of plasma pool rejection**
O062 Boris Hogema

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 15 APRIL 2015

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

09:00 - 10:30 PARALLEL SESSIONS

Athene B/C **Evidence based medicine for infection control: A curse in disguise?**
Chair: Christina Vandenbroucke-Grauls

09:00 - 09:30 **Quality of care and evidence based guidelines: a view from the Federation of Medical Specialists in The Netherlands**
O065 Teus van Barneveld

09:30 - 10:00 **The limitations and adverse effects of Evidence Based Recommendations in guidelines for infection control**
O066 Jan Kluytmans

10:00 - 10:15 **Evidence based medicine for infection control: a curse in disguise? – The example of air quality in the OR. Disturbing the system: why laminar air flow may be ineffective in real life.**
O067 Jacobien Veenemans

10:15 - 10:30 **Evidence-based medicine – a curse in disguise? The example of timing of perioperative antimicrobial prophylaxis**
O068 Maaike van Mourik

Athene A **Clinical microbiology 2**
Chairs: Pieter-Jan Haas & Florine Frakking

09:00 - 09:15 **PCR based detection of *Tropheryma whipplei* carriership and strain variability in the Netherlands**
O069 Tim Severs

09:15 - 09:30 **Both host immune status and complement resistance of non-typeable *Haemophilus influenzae* contribute to its ability to cause sepsis**
O070 Jeroen Langereis

09:30 - 09:45 **Test of cure after treatment of anogenital *Neisseria gonorrhoeae* infection using nucleic acid amplification tests – a prospective cohort study**
O071 Carolien Wind

09:45 - 10:00 **Dynamics of the gut microbiota composition and resistome during prophylactic antibiotic therapy**
O072 Teresita de Jesus Bello Gonzalez

10:00 - 10:15 **The post-vaccine microevolution of invasive pneumococcal disease**
O073 Amelieke Cremers

10:15 - 10:30 **Effect of factor H controlled alternative pathway activity on nasal tissue colonization and severity of invasive pneumococcal disease in mice**
O074 Erika van der Maten

Room 3 **Bacterial spores in health & disease**
Chairs: Stanley Brul & Balkumar Marthi

09:00 - 09:30 **Towards new antimicrobials for *Clostridium difficile* infection**
O075 Frank Schuren

09:30 - 10:00 **Spore surface display and vaccine delivery**
O076 Ezio Ricca (Italy)

10:00 - 10:15 **Bacterial inner spore membrane proteomics**
O077 Chris de Koster & Stanley Brul

10:15 - 10:30 **Spore Challenges from the food industry**
O078 Jan Willem Sanders

Room 4/5 **Pathogenesis 2**
Chairs: Jetta Bijlsma & Susanna Commandeur

09:00 - 09:15 **Salmonella outer membrane vesicles displaying high densities of pneumococcal antigen at the surface offer protection against colonization**
O079 Kirsten Kuipers

09:15 - 09:30 **Active immunization with an octa-valent *S. aureus* antigen mixture in models of *S. aureus* bacteremia and skin infection in mice**
O080 Dennis Koedijk

09:30 - 09:45 **Lack of pAp phosphatase leads to mislocalized cell division proteins in *Streptococcus pneumoniae***
O081 Clement Gallay

09:45 - 10:00 **Mycobacterium marinum can cross the blood-brain barrier via different migration routes**
O082 Lisanne van Leeuwen

10:00 - 10:15 **Pseudomonas aeruginosa protease IV protects from MAC-dependent killing by primarily degrading C6**
O083 Evelien Berends

10:15 - 10:30 **Analysis of the molecular mechanism that is responsible for the induction of the antibiotic stress marker iniBAC in pathogenic mycobacteria**
O084 Maikel Boot

Room 6/7 **Microbiota**
Chair: Paul Savelkoul

09:00 - 09:15 **Insights into the degradation of dietary plant toxins by insect gut microbiota**
O085 Cornelia Welte

09:15 - 09:30 **Immunoglobulin A coating identifies colitogenic members of the microbiota in inflammatory bowel disease**
O086 Marcel de Zoete

09:30 - 09:45 **Gestational age influences intestinal microbiota development in preterm infants**
O087 Romy Zwittink

09:45 - 10:00 **Micelle PCR reduces artifact formation in 16S microbiota profiling**
O088 Ruud Jansen

10:00 - 10:15 **Challenges in ancient microbiome reconstruction using 16S rRNA gene amplification**
O089 Kirsten Ziesemer

10:15 - 10:30 ***Enterococcus faecium* genome dynamics during long-term patient gut colonization**
O090 Jery Baan

Room 8/9 **Bachelor and Master (BAMA) Symposium**
Chairs: Marie-Monique Immink en Martine Reij

09:00 - 09:15 **Response of sediment bacterial communities to polycyclic aromatic hydrocarbons, evidenced by the analysis of phylogenetic and functional biomarker genes**
BAMA-07 Ruud Kuin

09:15 - 09:30 **Synthesize lyso-phosphatidylserine from phosphatidylcholine and test it for activation of the Toll Like Receptor 2**
BAMA-02 Roos van Schuijlenburg

09:30 - 09:45 **Why gastro-esophageal reflux disease is related to otitis media: exploring the nasopharyngeal and middle ear microbiota in children with otitis media**
BAMA-03 Marjolein de Zeeuw

09:45 - 10:00 **Characterization of a novel bicomponent pore-forming toxin harboured by equine isolates of *Staphylococcus aureus***
BAMA-04 Glen van Wigcheren

10:00 - 10:30 **The Silver Winning iGEM-2014 project: BananaGuard**
BAMA-05 Rik van Rosmalen & Bob van Sluijs

10:30 - 11:00 Coffee/tea break

11:00 - 12:30 PARALLEL SESSIONS

Athene B/C Intestinal microbiota in health and disease
Chairs: Clara Belzer & Paul Savelkoul

11:00 - 11:30 **Microbiota in Americans and Africans; the impact of diets**
O091 Erwin Zoetendal

11:30 - 12:00 **Benefits of and access to locally produced functional fermented foods in Africa**
O092 Remco Kort

12:00 - 12:15 ***Akkermansia muciniphila*: microbial cross feeding at the mucosal surface**
O093 LooWee Chia

12:15 - 12:30 **Driver and passenger bacteria in colorectal cancer**
O094 Bas Dutilh

Athene A Clinical cases in medical microbiology: an interactive session
Organised by the WAMM, NVMy & NWKV
Chairs: Ed Kuijper, Jean-Luc Murk, Paul Verweij & Rolf Vreede

11:00 - 11:30 **Hypogammaglobulinemia and recurrent cellulitis**
O096 Marja Konstantinovski

11:30 - 12:00 **Necrotizing soft tissue infection caused by *Scedosporium apiospermum***
O097 Bram Lestrade

12:00 - 12:30 **Is it really chickenpox?**
O098 Jan Sinnige

Room 3 Peptidoglycan synthesis and recycling
Chairs: Tanneke den Blaauwen & Dirk-Jan Scheffers

11:00 - 11:30 **Peptidoglycan recycling – a major salvage pathway of bacteria and novel drug target**
O099 Christoph Mayer (Germany)

11:30 - 12:00 **Off the wall: from filamentous growth to primordial cells and back again**
O100 Dennis Claessen

12:00 - 12:15 **FRET-based assay to study protein-protein interactions in the periplasmic space of *Escherichia coli***
O101 Nils Meiresonne

12:15 - 12:30 **Effects of (l)antibiotics on *Bacillus subtilis* cell wall synthesis**
O102 Danae Morales Angeles

Room 4/5 Treatment of parasitic diseases
Chair: Tba

11:00 - 11:30 **Update on the prevention and treatment of malaria in travellers**
O103 Perry van Genderen

11:30 - 12:00 **Tba**
O104 Foekje Stelma

12:00 - 12:30 **Tba**
O105 tba

Room 6/7 Broadly neutralizing antibodies in antibody treatment and vaccine development
Chair: Peter Rottier & Ben van der Zeijst

11:00 - 11:30 **HIV-1 neutralizing antibodies induced by a native-like glycoprotein trimer**
O108 Rogier Sanders

11:30 - 12:00 **Broadly neutralizing antibodies in the fight against influenza**
O109 Ronald Vogels

12:00 - 12:15 **A RSV prefusion F-specific antibody that changed the RSV landscape**
O110 Tim Beaumont

12:15 - 12:30 **Neutralizing antibodies in HCV vaccine development**
O111 Sabrina Merat

Room 8/9 Bachelor and Master (BAMA) Symposium
Chairs: Marie-Monique Immink en Martine Reij

11:00 - 11:45 **Poster session/discussion**

11:45 - 12:00 **The outer surface capsule modulates binding of *Campylobacter jejuni* to Siglec-7-expressing cells**
BAMA-06 Sandra Franch-Arroyo

12:00 - 12:15 **Delia radicum resistance to the mustard oil bomb: Bacterial gut symbionts help to degrade isothiocyanate**
BAMA-01 Tijs van den Bosch

12:15 - 12:30 **Mycelium design**
BAMA-08 Freek Appels

12:30 - 14:00 Lunch

Room 3
12:45 - 13:45 BBC-MMO Business meeting

14:00 - 15:30 PARALLEL SESSIONS

Athene B/C Host pathogen interactions in bacterial meningitis
Chairs: Arie van der Ende & Astrid van der Sar

14:00 - 14:30 **The neurovascular unit in health and disease**
O112 Elga de Vries

14:30 - 15:00 **Pneumococcal meningitis**
O113 Diederik van de Beek

15:00 - 15:15 **Tuberculous meningitis; Histological lessons from South African patients**
O114 Martijn van der Kuip

15:15 - 15:30 **Host-pathogen interaction at the intestinal mucosa correlates with zoonotic potential of *Streptococcus***
O115 Laura Ferrando

Athene A **Microdebate: Dual-Use in microbiological research, consequences and pitfalls of new regulations – intitiated by KNVM board**
Chairs: Jetta Bijlsma & Pieter-Jan Haas

14:00 - 15:30
O116 **The consequences and impact of the new regulations for dual-use microbiological research in the Netherlands will be discussed with representatives from scientific and governmental authorities and the public present at the debate.**
Moderator: Hidde Boersma
Debaters: Koos van der Bruggen (KNAW), Ron Fouchier (Erasmus MC), Wim van Haren (Ministry)

Room 3 **Microbial systems ecology – Section microbial ecology**
Chair: Lenie Dijkshoorn & Guus Roeselers

14:00 - 14:30 **Microbial metabolism at the system level: network modelling and multi-omics integration for environmental and clinical microbiology**
O121 Marco Fondi (Italy)

14:30 - 15:00 **A novel microbial metabolism in marine sediments: electrogenic sulfur oxidation by cable bacteria**
O122 Jeanine Geelhoed

15:00 - 15:15 **Microbial ecology in health and disease: a machine learning approach**
O123 Evgeni Tsivtsivadze

15:15 - 15:30 **An *in vitro* characterization of the impact of prebiotics on gut microbiota from lean and obese donors**
O124 Marisol Morales

Room 4/5 **Public Health**
Chair: Daan Notermans

14:00 - 14:15 **Risk factors for the acquisition of OXA-48 producing Enterobacteriaceae in a hospital outbreak setting: a matched case-control study**
O125 Mirjam Dautzenberg

14:15 - 14:30 **Environmental contamination with *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* (KPC-KP) during an outbreak in The Nederlands**
O126 Veronica Weterings

14:30 - 14:45 **Effect of prevention of direct contact between pigs on spread of *Streptococcus suis* serotype 9 in a pig population**
O127 Niels Dekker

14:45 - 15:00 **Expansion of the population of successful methicillin-resistant *Staphylococcus pseudintermedius* lineages and the dissemination of antimicrobial resistant phenotypes**
O128 Birgitta Duim

15:00 - 15:15 **Presumed nosocomial transmission events of Livestock-associated MRSA are confirmed by high resolution typing**
O129 Thijs Bosch

15:15 - 15:30 **Comparative whole genome sequencing of zoonotic and invasive porcine *Streptoccoccus suis* provides clues to virulence and zoonotic potential**
O130 Niels Willemse

Room 6/7 **Vancomycin resistant enterococci. How to survive in lab and in the hospital.**
Chairs: Ellen Mascini & Ellen Stobbering

14:00 - 14:30 **VRE-typing**
O131 Rob Willems

14:30 - 14:45 **VRE-diagnostics; the NVMM guideline**
O132 Jan Sinnige

14:45 - 15:00 **VRE; impact and outbreakmanagement**
O133 Thijs Tersmette

15:00 - 15:15 **VRE risk management in the EMCRotterdam; it's possible to keep it small**
O134 Greet Vos

15:15 - 15:30 **VRE-tool kit**
O135 Annet Troelstra

Room 8/9 **Epigenetics of host-pathogen interactions**
Chair: Sarah Sengstake

14:00 - 14:30 **Epigenetics in Virology**
O136 Walter Doerfler (Germany)

14:30 - 15:00 **Epigenetic control of gene expression in African trypanosomes**
O137 Gloria Rudenko (United Kingdom)

15:00 - 15:15 **The resistance gene Ty-1 triggers an epigenetic antiviral defense against tomato yellow leaf curl virus**
O138 Richard Kormelink

15:15 - 15:30 **DNA methylation in *Mycobacterium tuberculosis* and consequences for growth in distinct host environments**
O139 Sarah Sengstake

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

Athene B/C

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

O001

Microbe-host interactions in chronic intestinal inflammation – microbial dysbiosis versus pathobiont selection

D. Haller
Technische Universität München, München, Germany

The increasing incidence of chronic disorders is considered to be the consequence of environmental and individual risk factors. Inflammatory processes are key mechanisms in the etiopathology of immune-mediated pathologies including inflammatory bowel diseases (IBD). Genome-wide association studies identified 163 susceptibility loci in IBD with substantial overlap between other immune disorders or infections providing clear evidence for a central role of intestinal bacteria in the pathogenesis of chronic inflammatory disorders. Genetic predispositions and inflammation of the host were shown to induce altered composition and metabolic activity of microbial communities, defined as dysbiosis and, as expected, IBD patients are characterized by dysbiotic changes in the gut microbial ecosystem. Despite the fact that a variety of susceptibility genes suggest a role for microbial triggers in the pathogenesis of the two major IBD phenotypes including Crohn's disease and ulcerative colitis the causal mechanisms of microbe-host interactions are unclear.
There is rising evidence that protective or deleterious effects of intestinal bacteria are strain- or species-specific, e.g. PrtP expression in *LactoBacillus casei*, the presence of polysaccharide A in *Bacteroides fragilis*, or gelatinase E production or the presence of cell wall-associated lipoproteins by colitogenic *Enterococcus faecalis*. Some specific pathobionts, e.g. *Bilophila wadsworthia* in IL-10$^{-/-}$ mice, have been selected from the commensal microbiota with the capability to transfer colitis into susceptible GF hosts. Due to the lack of germfree models for Crohn's disease, proof for causality of microbes or dysbiosis in the onset of ileitis is lacking. We showed that inflammation is associated with the development of dysbiosis and, most importantly, microbiota transfer experiments confirmed a causal relationship between microbial dysbiosis and disease initiation in a mouse model of Crohn's disease-like ileitis. Transmissive pathology was induced by a compositionally and functionally diverse microbiota, while single associations with a Crohn's disease-derived pathobiont was not successful to transfer disease. All our results point towards a community effect of the complex microbiota and loss of aggressive, or gain of protective mechanisms, rather than the selection of aggressive phylotypes as single agents causing Crohn's disease. Understanding the true nature of a dysbiotic and disease-conditioning microbiota seems of essential importance to judge the risk of relapse in IBD patients after therapeutic intervention or to achieve best possible clinical efficacy in fecal microbiota transplantation (FMT) trials.

O004

Laboratory preparedness 2014: lessons from the Ebola outbreak

M. Koopmans
Erasmus MC, Department of Viroscience, Rotterdam

In April 2014, the World Health Organization announced an outbreak of Ebola caused by a virus belonging to the Ebola Zaire species. The outbreak started in the Gueckedou region in Guinea, but had spread to the neighboring countries Liberia en Sierra Leone by the time the diagnosis Ebola was made. Reasons for this delayed recognition are the fact that Ebola outbreaks had never occurred in this part of Africa, and that the clinical presentation was mostly of a severe diarrheal disease syndrome, initially considered to be cholera. A detailed trace back investigation showed that cases had occurred already early December 2013, and as a consequence the outbreak was widespread by the time it was recognized. Factors contributing to further spread were the lack of a health infrastructure, lack of communication channels, high risk local practices (particularly burial rituals), and the porous national borders leading to uncontrolled mobility of patients, for instance to visit traditional healers across borders. A whole genome sequencing molecular study conducted using patient samples collected in July 2014 showed considerable diversity, but a clear common ancestry, consistent with ongoing person to person transmission rather than renewed introduction.
After initial optimism, by end June it became clear that the public health efforts to control the disease were failing, due to lack of local acceptance, but also insufficient resources to triage, treat, and trace patients. Some international spread through infected healthcare workers drew attention of the international community, and triggered widespread preparedness planning in healthcare facilities around the world. More slowly, international aid efforts were stepped up to help support the outbreak control activities in West Africa. Laboratory preparedness planning involved the validation of available diagnostic assays against the outbreak strain, and setting up methods for safe handling and transport of specimens nationally and internationally. Due to the size of the outbreak, for the first time intensive care specialists were brought into the field who criticized the lack of basic laboratory support for clinical centres. Although there is no consensus, specialists agreed on the need for basic clinical chemistry and hematological parameters, to monitor the

need for rehydration and electrolyte treatment. However, given that Ebola virus is a class 4 pathogen, it proved to be challenging to arrange such service in the field. The same applies for state of the art diagnostics, which are mostly based on molecular biological methods that were only marginally available in the three countries. As part of the international aid effort, along with treatment centres, mobile laboratories were deployed, three of which donated by the Dutch government. These were build, deployed and operated with help of over 100 volunteers from the (medical and veterinary) virology and clinical microbiology community in The Netherlands. Combined, this experience showed the potential for generic capacity building, with provision of general molecular diagnostic expertise coupled with expert knowledge of biosafety. This included collaboration across domains with combined teams of medical and veterinary laboratory experts. It also provided important insights in the need for improved capacity building, including among others the need for international coordination of efforts, for (much) cheaper diagnostic facilities tuned to the local situation, and the need for reagents not requiring a cold chain.

O005

Re-emergence of chikungunya virus

J.M. Smit

University Medical Center Groningen, Department of Medical Microbiology, Groningen

Chikungunya virus (CHIKV) is a major emerging pathogen which causes acute fever and chronic musculoskeletal pain in humans. CHIKV is an alphavirus and transmitted to humans via Aedes mosquitoes. Previously, CHIKV infection was seen predominantly seen in countries surrounding the Indian Ocean. In recent years the virus invaded into new areas including Europe, the Middle East and the Americas. In just one year, CHIKV has spread to 42 countries or territories within the Americas resulting in 1 million suspected cases of which more than 25,000 cases are laboratory confirmed. It is hypothesized that CHIKV will continue to spread rapidly through the Americas as the population is not immune to the infection. I will discuss the epidemiological, clinical, virological, and immunological aspects of CHIKV disease at the symposium.

O006

Polio-eradication: the final stage?

A.M. van Loon

WHO, RCC, Copenhagen, Denmark

Introduction: In May 1988, the World Health Assembly adopted a resolution for the eradication of poliomyelitis. The World Health Organisation (WHO) was charged with the development and execution of a polio eradication strategy. The aim was to interrupt transmission of wild poliovirus (wPV) by the year 2000 through enhanced vaccination and improved surveillance followed by certification of the absence of wPV circulation.

Status: Since 1988, progress has been formidable: the number of cases dropped from an estimated 300,000 in 1988 in over 125 countries to a few hunderd in a small number of countries. However, eradication is not yet achieved. The strategy – strenghtening routine vaccination supplemented with national/regional immunization days, and surveillance through reporting of acute flaccid paralysis in children < 15 yrs – was successful in most countries. Competing priorities, lack of commitment, resources and safety of vaccinators are the main reasons for the failure to adequately vaccinate in countries remaining endemic. Vaccine failure occurred in highly populated regions with poor sanitation (India), but deleting poliovirus type 2 (PV2) from the live, attenuated vaccine rapidly improved its effectiveness.

Since 2011, only three countries (Pakistan, Afghanistan and Nigeria) remain endemic, but outbreaks occurred in neighbouring countries with immunisation gaps (Somalia, Syria, Cameroon). 2013 saw 416 cases of polio, of which 61.5% occurred in non-endemic countries, whereas in 2014 most (94.5%) of 350 cases were reported from endemic countries (90% from Pakistan). All reported cases were due to wPV1. The other wPV's were lastly isolated in 1999 (wPV2) and November 2012 (wPV 3).

Thus, we may finally approach the endgame. However, whereas 2015 may see a polio-free Africa, Pakistan is a disaster with increasing numbers of cases and dwindling vaccination coverage.

To prevent spread from endemic countries, the Director-General of WHO declared on 5 May 2014 the international spread of wPV from endemic to non-endemic countries a Public Health Emergency of International Concern (PHEIC) under the International Health Regulations, but more stringent measures will probably be needed.

Europe has been free of endemic circulation since 1998, but importation occurred a number of times, with or without cases of poliomyelitis (a.o. Russia, Tajikistan). Most recently, extensive silent circulation of wPV1 for more than one year was detected through environmental surveillance in Israel; no cases were seen. Within Europe, the Netherlands is one of the countries with pockets of low vaccination coverage, and thus suseptibility to a polio-outbreak.

Once wPV transmission has been stopped, challenges remain. These include (1) stopping OPV use because of the risk of cases due to vaccine-derived PV's reverting to neurovirulence, (2) containment of all PV's, wild and vaccine, in laboratories and vaccine producing facilities and (3) certification of the absence of PV circulation worldwide.

Conclusion: We are closer than ever to polio eradication. This year's vaccination efforts, particularly in Pakistan, will be decisive for stopping wPV circulation, but this will probably not happen before 2016. Thereafter, high quality surveillance will still be needed for several years to provide the reassurance that poliomyelitis has truelly been eradicated.

O007
Host adaptation of *Staphylococcus aureus*

J.R. Penadés
University of Glasgow, Institute of Infection, Immunity and Inflammation, Glasgow, United Kingdom

Staphylococcus aureus is a major human pathogen which is also responsible for economically important infections of a variety of livestock including cows, sheep, goats, poulty and rabbits. In addition, some livestock-associated strains have the capacity to cause zoonotic infections of humans. Recent studies have demonstrated that livestock strains evolved from human-to-animal host jumps followed by host-adaptive evolution. However, the mechanistic basis for the host-adaptation of *S. aureus* is not well understood. On-going phylogenetic and functional studies have identified molecular correlates of host-adaptation including mobile genetic elements and chromosomal mutations which may underlie the capacity of *S. aureus* to infect different livestock species. In this talk we will discuss the recent advances about the genetic events leading to the host-specialization of livestock *S. aureus*, with special emphasis on the mechanistic basis for these events and examining their role in pathogenesis.

O008
Intravital imaging of *Staphylococcus aureus* replication within Kupffer cells

B.G.J. Surewaard, F.J. Zemp, J. Conly, R.M. Yates, P. Kubes
University of Calgary, Snyder institute of chronic disease, Calgary, Canada

The most important mechanism of the host for clearing *S. aureus* is phagocytosis, leading to subsequent intracellular killing of the pathogen. CA-MRSA strains are very efficient in circumventing this phagocyte mediated killing, with multiple studies demonstrating that *S. aureus* can withstand the attack of phagocytes and even kill these cells post-phagocytosis. However, thus far no studies have conclusively shown that this mechanism occurs *in vivo*; therefore, we used intravital microscopy to decipher whether *S. aureus* can survive and replicate inside liver Kupffer cells (KCs) in real time during staphylococcal infection. We hypothesize that this intracellular immune evasion is an important antibiotic resistant mechanism for *S. aureus*. KCs are strategically located in liver blood vessels, function as immune sentinels for the circulation and are essential in the rapid capture of bacteria from the bloodstream that occurs during sepsis. Intravenous infection of mice with fluorescent *S. aureus* results in rapid uptake and killing in 90% of KCs, but in about 10% overwhelming intracellular replication of *S. aureus* occurs, leading to KC lysis. The generation of reactive oxygen species (ROS) is crucial in controlling *S. aureus* post-phagocytosis, as we show that in NADPH oxidase-deficient mice, massive intracellular replication occurs in nearly all KCs. To test whether intracellular survival of *S. aureus* is an antibiotic evasion mechanism, we i.v. injected fluorescent-labeled vancomycin. Vancomycin binding to bacteria was only observed in the bloodstream whereas intracellular bacteria did not come in contact with the antibiotic. In addition, prophylactic vancomycin treatment resulted in efficient staphylococcal clearance from the liver whereas vancomycin treatment post-infection was ineffective. Collectively, we demonstrate that intracellular replication of *S. aureus* occurs *in vivo* within liver Kupffer cells, and that intracellular habitation protects the microorganism from being killed by vancomycin.

O009
Staphylococcus aureus protects its immune-evasion proteins from degradation by neutrophil serine proteases

D.A.C. Stapels, A. Kuipers, M. Von Koeckritz-Blickwede, M. Ruyken, A. Tromp, M.J. Horsburgh, C.J.C. de Haas, J.A.G. van Strijp, K.P.M. van Kessel, S.H.M. Rooijakkers
UMC Utrecht, Department of Medical Microbiology, Utrecht

Neutrophils store large quantities of neutrophil serine proteases (NSPs) that contribute to antibacterial immune defenses. Even though neutrophils are indispensable in fighting *Staphylococcus aureus* infections, the importance of these NSPs in anti-staphylococcal defense is yet unknown. We recently discovered that *S. aureus* produces three highly specific inhibitors for NSPs (the extracellular adherence proteins: Eap, EapH1 and EapH2), emphasizing the importance of NSPs in staphylococcal defense. In this study we demonstrate that NSPs can functionally inactivate secreted virulence factors of *S. aureus*. In return, *S. aureus* uses its Eap proteins to effectively protect other secreted proteins from NSP degradation. Specifically, we find that a large group of *S. aureus* immune-evasion proteins is vulnerable to proteolytic inactivation by neutrophil elastase and/or cathepsin G *in vitro*, as tested by immunoblotting and in functional assays. Interestingly, proteins with similar immune-escape functions appeared to have differential cleavage sensitivity towards NSPs. By using targeted *eap* mutants of *S. aureus*, we found that the secreted

virulence factors are also degraded *in vivo*. Eap-dependent protection against NSP cleavage was demonstrated both in complex bacterial supernatants *in vitro* and during an infection *in vivo*. These findings show that 1) NSPs target *S. aureus* virulence factors during infection and 2) that Eap can inhibit this degradation. The latter explains why this role of NSPs was masked in previous studies. Furthermore, our study indicates that therapeutic inactivation of Eap proteins can help to restore the natural host immune defenses against *S. aureus*.

O010

LukMF' is the major leukotoxin of bovine *S. aureus* and targets neutrophils through CCR1

M. Vrieling[1], K.J. Koymans[1], D.A.C. Heesterbeek[1], P.C. Aerts[1], V.P.M.G. Rutten[2], C.J.C. de Haas[1], K.P.M. van Kessel[1], A.P. Koets[2], R. Nijland[1], J.A.G. van Strijp[1]
[1]UMC Utrecht, Department of Medical Microbiology, Utrecht, [2]Utrecht University, Faculty of Veterinary Medicine, Department of Infectious Diseases and Immunology, Utrecht

Introduction: *Staphylococcus aureus* (*S. aureus*) is a ubiquitous pathogen and a major cause of mastitis in dairy cattle. Bi-component pore forming toxins are secreted virulence factors of *S. aureus* capable of killing a broad range of leukocytes including phagocytes, key players in the host defence against *S. aureus*. LukMF' is a bi-component toxin and a virulence factor associated with bovine mastitis. However, its contribution to the pathophysiology of mastitis is not well understood. We set out to identify the molecular targets of LukMF' on bovine phagocytes and assess the importance of this toxin amongst other secreted toxins of bovine *S. aureus*.
Methods: All described bovine chemokine receptors were cloned and transiently expressed in 293T cells. Transfected cells were incubated with recombinant LukMF' and pore-formation was determined by measuring DAPI-fluorescence in a flowcytometer. The relative promoter activities of LukMF', LukAB, LukED, HlgAB and HlgCB were assessed in a representative German bovine mastitis field isolate (S1444) using GFP promotor reporter plasmids. LukM concentration in culture supernatants was determined by ELISA. Bovine neutrophils were treated with WT or ΔLukMF' S1444 culture supernatant and pore-formation was measured as described above. In addition, the effect of LukMF' secretion by *S. aureus* S1444 on bovine neutrophils was studied in a fibrin gel matrix, where gradients of secreted proteins can develop by diffusion through the gel.
Results: We show that LukMF' specifically lyses cells expressing CCR1 and to a lesser extend cells expressing CCR2 and CCR5. In contrast to human neutrophils, bovine neutrophils express significant cell surface levels of CCR1 and are thereby susceptible for LukMF' induced pore formation. During growth of a field isolate of bovine mastitis (S1444) in standard culture media and milk, the LukMF' promotor showed the highest activity as compared to the promotors of LukAB, LukED, HlgAB and HlgCB. High expression of LukM in culture supernatant was confirmed by ELISA. Culture supernatant of WT S1444 was highly toxic to bovine neutrophils, while the cytotoxicity of supernatant of ΔLukMF' S1444 was extremely reduced. In a fibrin gel matrix, LukMF' producing WT colonies eliminated adjacent bovine neutrophils up to a distance of 2 mm, while ΔLukMF' colonies failed to kill neutrophils in this setting.
Conclusions:
1: LukMF' targets bovine neutrophils in a CCR1-dependent manner.
2: LukMF' is the most potent and highly expressed leukotoxin of bovine *S. aureus*
3: Bovine *S. aureus* can eliminate neutrophils at distance through secretion of LukMF'.
4: Our data support the hypothesis that LukMF' is an important virulence factor in the pathophysiology of *S. aureus* mastitis.

O011

Taking a scientific approach for Science Education. The importance of defining what to learn and how to learn

P. van Beukelen
Utrecht University, Faculty of Veterinary Medicine, Utrecht

What is needed to foster learning in science education? Two aspects are of utmost importance when developing a curriculum or even a course in higher education.
First it is necessary to develop a clear understanding of what has to be learnt. Which competencies have to be developed and which programme outcomes and learning objectives are helpful in developing these competencies? Secondly it is helpful to achieve a clear understanding of how is learnt, what is fostering learning in a sustainable way. When aspects that foster learning are unravelled, an evidence-based education philosophy can be established.
Developing a framework of competencies can be done by studying literature on competency frameworks in comparable professions, and/or by research under the different stakeholders of the profession in question and the curriculum preparing for that profession. In veterinary medicine a competency framework was developed by qualitative research, using focus groups of young veterinarians out of the different sectors of the profession, and focus groups of animal owners. The draft competency framework was validated by a Delphi procedure with veterinary experts and other stakeholders. After defining the competency framework, it was validated in an inter-

national survey in ten countries. This research resulted in a competency framework, the VetPro, of 7 competency domains: Veterinary expertise, Scholarship, Health and welfare, Entrepreneurship, Communication, Collaboration, and Personal Development, and 18 underlying competencies. It is important to stress the fact that this framework is only working in an integrated way: none of the competency domains can work separately from the others. The VetPro is used to define and describe the program outcomes and learning objectives of the curriculum in more detail. This approach can be of interest for education in the broad field of microbiology as well.
Quite a lot of evidence has been found in recent years that student-centred active learning, in a social constructivist way, fosters learning, also in the long run, in comparison to more teacher-centred passive didactic concepts. Especially for the development of competencies like communication, collaboration, entrepreneurship and personal development, there is no other way than a didactic concept in which the learner is central. In the veterinary medicine curriculum an evidence-based education philosophy was developed, existing of eight statements, about topics as: importance of personal contact, importance of (inter-)active learning, own responsibility of the student for professional and academic development, coherent system of coaching, feedback and assessment, and alignment of the curriculum.
What to learn and how to learn comes together in the competency domain 'personal development'. Some examples will be given how to implement educational aspects of 'personal development' within a curriculum in higher education. Specific attention to the 'personal development' competency domain can increase the self-efficacy of students and young graduates, which is of utmost importance for an adequate transition into the professional world. Methods used in the curriculum to address 'personal development', like tutoring and peer feedback, can be used in lifelong learning educational programmes as well.

O012

Last comes first

A.E. De Hullu
Formerly Leiden University, ICLON, Leiden

Learning is the result of thinking, and learners learn best when they are challenged and motivated in a one on one teaching situation. But, in practice, educators are often confronted with rather less than optimal situations and have to deal with large and diverse groups of learners and to cover a wide range of material. How to solve this conundrum and keep it practical too? This issue has been given much thought by, among others, Fred Janssen, Leiden University. This talk aims to introduce you to two of his most practical and influential ideas. His 'whole task-first' principle challenges learners to actively construct knowledge in meaningful contexts. To find such contexts, the perspectives that he has developed for Biology education support educators. These twelve perspectives form a comprehensive overview of 'lenses' with which to view (micro-) biological objects and phenomena and develop challenging and interesting problems, which require thought. In fact, most student books end with such problems, and one way of solving the conundrum is moving such problems to the fore: last comes first.

O013

Tricks and experiences with computer-driven practical courses in microbiology

G. Buist
University Medical Center Groningen, Department of Medical Microbiology, Groningen

Practical courses on medical microbiology are used to give medical students a better understanding of the way in which infectious diseases are diagnosed. Students perform experiments to experience the importance of proper hand hygiene in order to understand the basis for the prevention of the transfer of microorganisms between patients via health care staff. They also acquire a thorough understanding of microorganisms and obtain some familiarity with practical microbiological diagnostics and the serological terminology. This has been done successfully for many years by using a printed practical manual and the hands-on explanation by student assistants. In 2014 the University Medical Center Groningen started with the new curriculum G2020 which amongst others has the aims to increase active learning and to stimulate academic development within a minimal number of contact hours. To realize these goals within the practical 'infectious diseases' we integrated links to video based instructions to show the students how to collect patient samples, use lab equipment, to understand the concept of specific methodologies, and how to interpret lab results. To prepare for the practical students had to watch the videos and during the practical students used tablets for viewing and reading of the instructions. Some of the experiments were based on patient case studies for which the students had to conduct diagnostic tests such as PCR or ELISA and they had to determine a diagnosis on basis of the experimental outcomes. The results of the experiments and answers on specific questions had to be completed in the survey platform SurveyMonkey. The outcomes of some of the experiments were discussed with the students during a response lecture and linked with the theory. Currently we are setting up a web based platform for a stepwise and video based explanation and instruction of the experiments.

O014

More than MOOCs

A.J. Mulder
NVAO, Policy Advisor, The Hague

How about a *Spitz* MOOC, a *Crowd Data* MOOC, or *Twin* MOOCs?
Millions of people around the globe are learning in hundreds of Massive Open Online Courses (MOOCs). Universities typically launch their MOOCs through online platforms based in the US (Coursera, EdX). Nearly two thirds of MOOCs worldwide are offered by universities in North America, with Europe providing around a quarter and the remaining four continents barely contributing.
Strikingly, the obvious question as to what constitutes a good MOOC remains largely unanswered. In my presentation, I will draw from emergent research on instructional quality of MOOCs and report on one author's typology of MOOCs that I find helpful (featuring *Spitz*, [3DOTS]).
Referring to the title of my talk: yes, there is much more than MOOCs, but presenting – and discussing – in terms of MOOCs is guaranteed to broaden into wider issues of online and blended provision and learning with relevance for "educating the next generation microbiologists".

O015

Development of novel Synthetic Antimicrobial Antibiofilm Peptides (SAAPs) to prevent biomaterial-associated infection

M. Riool[1], L. de Boer[1], P.H.S. Kwakman[1], A. de Breij[2], P.H. Nibbering[2], J.W. Drijfhout[2], S.A.J. Zaat[1]
[1]*AMC / UvA, Department of Medical Microbiology, Amsterdam,* [2]*LUMC, Department of Infectious Diseases, Leiden*

Biomaterial-associated infection (BAI) is a major cause of failure of indwelling medical devices. *Staphylococcus aureus* and coagulase-negative staphylococci are the most common causative agents of BAI. *Bacteria* can cause infection by either adhering to foreign bodies and subsequent biofilm formation or by colonizing the tissue surrounding these materials. Due to the combined presence of biomaterial and bacteria, the local immune response is compromised, leading to inability of host immune cells to kill phagocytosed bacteria. Direct treatment of these infections by antibiotics is difficult due to the localization of the bacteria, and their low metabolic state. Moreover, antibiotic resistance is an increasing problem. Therefore, alternative approaches to combat such infections are urgently needed.
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* and *in vivo* antimicrobial and antibiofilm activities using the antimicrobial peptides (AMPs) LL-37 and trombocidin-1 (TC-1) as a scaffold.
The newly developed peptides kill a wide spectrum of Gram-positive and -negative (antibiotic resistant) bacteria at concentrations ranging from 0.8 - 8 μM in PBS. In presence of 50% human plasma the bactericidal concentrations were 2 - 32-fold higher, depending on the peptide and tested strain. The SAAPs prevented biofilm formation of *Staphylococcus aureus* at concentrations of 3.2 - 12.8 μM. They also had potent anti-inflammatory activity: they inhibited production of IL-12 and IL-8 by cells in whole blood upon stimulation with lipopolysaccharide (LPS) and UV-killed *S. aureus*.
The different SAAPs were eluted from innovative coatings designed using pharmaceutically approved polymers and lipids (PolyPid), tailored to accommodate an initial high rate short term release in the first days, and subsequent zero-order kinetic release over approximately 30 days. The coatings applied on titanium implants reduced the numbers of *S. aureus* colonizing the implant after 1 day in the mouse subcutaneous biomaterial-associated infection model.
Thus, the promising characteristics and activity of the SAAPs and their controlled release coating, both developed in BALI, indicate a strong potential to prevent biomaterial-associated infection.

O016

Efficacy and safety of the mosquitocidal drug ivermectin to prevent malaria transmission after treatment: a double-blind, randomized, clinical trial

G.J.H. Bastiaens[1], A.L. Ouédraogo[2], A.B. Tiono[2], W.M. Guelbéogo[2], K.C. Kolylinski[3], A. Ouédraogo[2], A. Barry[2], E.C. Bougouma[2], I. Nebie[2], M.S. Ouattara[2], K.H.W. Lanke[1], L. Fleckenstein[4], R.W. Sauerwein[1], H.C. Slater[5], T.S. Churcher[5], S.B. Sirima[2], C. Drakeley[6], T. Bousema[1]
[1]*Radboudumc, Department of Medical Microbiology, Nijmegen,* [2]*Centre National de Recherche et de Formation sur le Paludisme, Department of Biomedical Sciences, Ouagadougou,* [3]*Walter Reed Army Institute of Research, Entomology Branch, Silver Spring,* [4]*The University of Iowa, College of Pharmacy, Iowa City,*[5]*Imperial College London, MRC Centre for Outbreak Analysis and Mo, London,* [6]*London School of Hygiene and Tropical Medicine, Department of Immunology and Infection, London, United Kingdom*

Introduction: Artemisinin combination therapy effectively clears asexual malaria parasites and immature gametocytes but does not prevent post-treatment malaria transmission. Ivermectin (IVM) may reduce malaria transmission by killing mosquitoes that take blood meals from IVM treated humans.
Methods: In this double-blind, placebo-controlled trial, 120 asymptomatic *Plasmodium falciparum* parasite carriers

were randomized to receive artemether-lumefantrine (AL) plus placebo or AL plus a single or repeated dose (200 μg/kg) of ivermectin (AL-IVM1 and AL-IVM2, respectively). Mosquito membrane feeding was performed 1, 3, and 7 days after initiation of treatment to determine *Anopheles gambiae* and *Anopheles funestus* survival and infection rates.
Results: The AL-IVM combination was well tolerated. IVM resulted in a 4- to 7-fold increased mortality in mosquitoes feeding 1 day after IVM ($p < 0.001$). Day 7 IVM plasma levels were positively associated with body mass index ($r = 0.57$, $p < 0.001$) and were higher in female participants ($p = 0.003$), for whom *An. gambiae* mosquito mortality was increased until 7 days after a single dose of IVM (hazard rate ratio, 1.34 [95% confidence interval, 1.07-1.69]; $p = 0.012$). Although we found no evidence that IVM reduced *Plasmodium* infection rates among surviving mosquitoes, the mosquitocidal effect of AL-IVM1 and AL-IVM2 resulted in 27% and 35% reductions, respectively, in estimated malaria transmission potential during the first week after initiation of treatment.
Discussion: We conclude that IVM can be safely given in combination with AL and can reduce the likelihood of malaria transmission by reducing the life span of feeding mosquitoes.

O017

Rapid detection of cyp51A-promoter based voriconazole resistance in *Aspergillus fumigatus* isolates in a high incidence population

J. Fuhren[1], T.T. Severs[1], W.S. Voskuil[1], C.H.E. Boel[1], P.J.A. Haas[1], J.F. Meis[2], J.G. Kusters[1]
[1]*UMC Utrecht, Department of Medical Microbiology, Utrecht,* [2]*Canisius Wilhelmina Hospital, Department of Medical Microbiology, Nijmegen*

Introduction: Each year *Aspergillus fumigatus* is responsible for at least 200.000 life-threatening invasive infections worldwide. Voriconazole is the standard of care in the treatment invasive aspergillosis. The increase in voriconazole resistance is complicating its choice for empirical therapy. Resistance is predominately based on mutations in the *cyp51A* gene and duplications in the promoter region (TR34 & TR46). Currently routine azole susceptibility is determined through microbroth dilution but this is laborious due to the need of subculturing and sporulation of the fungus, and can take up to two weeks. For adequate patient treatment a more rapid method is needed to routinely detect resistant isolates. The aim of this study was to develop a rapid molecular based method to pre-screen for azole resistance in *A. fumigatus*.
Methods: Phenotypic azole susceptibility profiles and *cyp51A* genotypes were determined in the 105 isolates of 105 consecutive high-risk patients from the hematology ward and ICU of the UMC Utrecht that were suspected to have an invasive *A. fumigatus* infection during 2011-2013. Additionally a probe-based multiplex qPCR was evaluated that enables rapid recognition of resistance-associated promoter duplications in *A. fumigatus* isolates. This qPCR specifically amplifies the promoter region of the *cyp51A* gene and uses three detection probes; a *cyp51A* gene control probe, and probes for the TR34 and TR46 voriconazole resistance associated promoter duplications.
Results: 24/105 (22.9%) of the patient isolates had an elevated voriconazole MIC. Sanger sequencing of the *cyp51A* gene showed that in 18/24 (75%) isolates the resistance were associated with mutations in the *cyp51A* ORF and had a tandem repeat duplication in the promoter region, identifying this as the main resistance mechanism. The other 6 isolates showed no duplications in the promoter region. The probe-based multiplex qPCR detected promoter duplications in 18 of the 18 isolates in which ORF mutations and promoter duplication were found through Sanger sequencing. None of the sensitive isolates nor the non-mutated voriconazole-resistant isolates were positive in the qPCR.
Conclusion: To our knowledge our results represent the highest incidence of azole resistance in *A. fumigatus* isolates in a clinical setting to date. The population used is well-defined and unbiased, thus accurately represents incidence of azole resistance in clinical *A. fumigatus* isolates. The high prevalence of azole resistance hampers the use of voriconazole as empirical treatment. The probe-based multiplex qPCR recognized all 18 *cyp51A*-promoter duplications in 105 *A. fumigatus* isolates and delivers a rapid method of identifying the majority of resistant isolates. By screening for the most dominant resistance mechanism the remaining resistance percentage drops to 6.9% (6/87) due to non-*cyp51A* based resistance mechanisms not detected by the qPCR.

O018

A systematic review of the diagnostic accuracy of serological tests for Lyme Borreliosis

H. Sprong, M.M.G. Leeflang, C.W. Ang, H. Berkhout, H. Bijlmer, A.H. Brandenburg, N. van Burgel, A. van Dam, R. Dessau, V. Fingerle, J.W.R. Hovius, B. Jaulhac, B. Meijer, W. van Pelt, J.F.P. Schellekens, R. Spijker, F.F. Stelma, G. Stanek, F. Verduyn-Lunel
National Institute of Public Health and Environment (RIVM), Laboratory for Zoonoses and Environmental Microbiology, Bilthoven

We systematically reviewed the accuracy of serological tests for the diagnosis of Lyme borreliosis in Europe. We searched EMBASE and contacted experts. Seventy-eight studies evaluating EIA or immunoblot (IB) against a reference standard of clinical criteria were included.

Quality was assessed using QUADAS-2 and we used a hierarchical meta-regression method. Summary estimates of sensitivity for EIA or IB: erythema migrans 0.50 (95% CI 0.40 to 0.61); neuroborreliosis 0.77 (95% CI 0.67 to 0.85); Lyme arthritis 0.96 (95% CI 0.89 to 0.98); acrodermatitis chronica atrophicans 0.97 (95% CI 0.94 to 0.99); unspecified Lyme borreliosis 0.73 (95% CI 0.53 to 0.87). Specificity was around 95% in studies with healthy controls, but around 80% in cross-sectional studies. Two-tiered algorithms or antibody indices outperformed neither EIA or IB. The usefulness of the serological tests for Lyme disease will depend on the prevalence and subsequent predictive values in the setting where the tests are being used.

O019

Epidemiology, management and risk-adjusted mortality of ICU-acquired enterococcal bacteraemia: a prospective observational study

D.S.Y. Ong, K. Safdari, P.M.C. Klein Klouwenberg, C. Spitoni, J.F. Frencken, E. Witteveen, J. Horn, M.J.M. Bonten, O.L. Cremer
UMC Utrecht, Department of Medical Microbiology, Utrecht

Introduction: The occurrence of enterococcal bacteraemia has been associated with case fatality rates ranging from 23 to 48% in critically ill patients, but it remains unknown to what extent death is caused by these infections. Therefore, we described the epidemiology, management and mortality of intensive care unit (ICU)-acquired bacteraemia caused by enterococci.

Methods: Between January 2011 and March 2013 we included consecutive patients with an ICU length of stay of more than two days in two tertiary care centres in the Netherlands. ICU-acquired bacteraemia was defined as a first positive blood culture occurring more than two days after ICU admission. We used competing risk survival regression and time-dependent adjustments by marginal structural modelling to estimate the attributable ICU mortality due to enterococcal bacteraemia from both the individual patient's as well as the population's perspective.

Results: Among 3080 admissions, 266 events of ICU-acquired bacteraemia occurred in 218 (7%) patients, of which 76 were caused by enterococci (incidence 3.0 per 1000 patient days at risk). Catheter removal in case of a suspected catheter related bloodstream infection due to enterococci was less aggressive and antibiotic treatment duration was shorter than advocated by international guidelines. Enterococcal bacteraemia was independently associated with increased ICU mortality (adjusted cause-specific hazard ratio (CSHR) 2.0, 95% confidence interval (CI) 1.3-3.2, adjusted subdistribution hazard ratio (SHR) 2.6, 95% CI 1.5-4.3). However, in the subgroup of catheter related bloodstream infections, enterococcal bacteraemia did not increase mortality when directly compared to coagulase-negative staphylococci (CoNS) bacteraemia (adjusted SHR 0.9, 95% CI 0.5-1.7). The population attributable fraction of ICU mortality was 4.9% by day 90.

Conclusions: ICU-acquired enterococcal bacteraemia events are associated with increased ICU mortality, but the attributable mortality caused by enterococci is comparable to that caused by CoNS infections.

O020

Evolution of colistin resistance in *Klebsiella pneumoniae*

W. van Schaik, A.B. Janssen, D.J. Doorduijn
UMC Utrecht, Department of Medical Microbiology, Utrecht

Introduction: Colistin (polymyxin E) is an amphipathic, cationic non-ribosomally synthesized decapeptide with a short fatty acid chain. It is currently used as an 'antibiotic of last resort' in the treatment of infections with multi-drug resistant Gram-negative bacteria. Colistin exerts its antimicrobial effect by destabilizing the outer membrane of Gram-negative bacteria through electrostatic interactions with negatively charged lipopolysaccharides (LPS). In this study, we aimed to describe the evolutionary trajectories by which *Klebsiella pneumoniae* can evolve resistance to colistin and to assess the effect of colistin resistance on virulence characteristics.

Methods: We randomly selected four colistin-susceptible *K. pneumoniae* strains from the diagnostic laboratory of our institute and evolved these *in vitro* to high-level resistance to colistin by culturing in the presence of increasing concentrations of the antibiotic. The mutations that occurred during the evolution of colistin resistance where identified by whole genome sequencing on the Illumina MiSeq platform. The isogenic colistin-susceptible and colistin-resistant strain pairs were also analyzed for their susceptibility to the human antimicrobial peptide LL-37.

Results: We found that susceptible *K. pneumoniae* can readily evolve high-level resistance to colistin (minimum inhibitory concentration (MIC) =128 μg/ml). Interestingly, we found that other Gram-negative nosocomial pathogens (*Acinetobacter baumannii*, *Escherchia coli*, and *Pseudomonas aeruginosa*) were slower to evolve resistance or did so to a lower MIC. Only in one *K. pneumoniae* strain, colistin resistance led to a loss of fitness, whereas the maximum growth rates of the other colistin susceptible strains were not significantly different from the parental colistin-susceptible strain. Colistin resistance in the four *K. pneumoniae* strains was associated with mutations in the genes encoding the PhoPQ two-component system, the LPS-assembly protein LptD and the regulator of LPS biosynthesis YciM. Notably, none of the four strains had identical mutations, indicating that there are multiple independent pathways that lead to colistin resistance in *K.*

pneumoniae. One of the colistin-resistant strains showed increased susceptibility to the human antimicrobial peptide LL-37, compared to the parental strain. In the other three strain pairs there was no difference in resistance to LL-37 between the colistin-susceptible parental strain and the strain that was evolved to colistin-resistance.

Conclusion: We show that colistin resistance in *K. pneumoniae* can evolve rapidly and without noticeable fitness costs. The multiple pathways that lead to colistin resistance in *K. pneumoniae* may have different effects on the virulence characteristics of this nosocomial pathogen. Currently, we study the susceptibility of the isogenic colistin-susceptible and -resistant strain pairs to the complement membrane attack complex and its virulence in a high-throughput model using the nematode *Caenorhabditis elegans*.

O021

Microbial interactions in the deep subsurface

A. Schippers

Federal Institute for Geosciences and Natural Resources (BGR), Sub-Department of Resource Geochemistry, Hannover, Germany

The Earth's deep biosphere includes a variety of subsurface habitats, such as mines and deep aquifer systems in the continental realm, and sediments and igneous rock in the marine realm. The presentation focuses on deeply-buried sediments considering the deep biosphere biomass, the predominant microorganisms and their diversity and activity, as well as implications for biogeochemistry. The marine deep biosphere has been explored at various ocean sites mainly within the framework of the Ocean Drilling Program (ODP) and the Integrated Ocean Drilling Program (IODP). Life was detected down to almost 2 km sediment depth so far (Ciobanu et al. 2014).

A fundamental question in deep biosphere research is its size or the biomass of living cells. The total number of prokaryotes (*Bacteria* and *Archaea*) in the subsurface sediments is decreasing with sediment depth and age mainly controlled by the content of organic carbon in the sediment as the microbial substrate (Parkes et al. 2000; D'Hondt et al. 2004). Kallmeyer et al. (2012) estimated the values for the global biomass in marine sediments being $2.9 \cdot 10^{29}$ cells, corresponding to 4.1 petagram (Pg) C and ~0.6% of Earth's total living biomass. The total microbial abundance in subseafloor sediments ($2.9 \cdot 10^{29}$ cells) is now similar to the estimates for the total number of prokaryotes in seawater ($1.2 \cdot 10^{29}$) and in soil ($2.6 \cdot 10^{29}$).

The biomass of the deep subsurface biosphere comprises the three domains of life *Archaea*, *Bacteria* and *Eukarya*, as well as spores and viruses. CARD-FISH (and FISH) has been applied for the quantification of living *Bacteria* and *Archaea* in deeply buried subsurface sediments. Another technique with high sensitivity is quantitative, real-time polymerase chain reaction (qPCR; Schippers et al. 2005). A quantification of particular microbial groups in deep subsurface sediments has been done by qPCR in several studies. Eukaryotic 18S rRNA genes were orders of magnitude less abundant than prokaryotic 16S rRNA genes. The ratio of *Archaea* versus *Bacteria* seems to be variable depending on the type of sediment (Breuker et al. 2013).

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O022

Cooperation of anaerobic methane and ammonium oxidizing microorganisms

C.L.M. Stultiens, H.J.M. Op den Camp, B. Kartal, M.S.M. Jetten

Radboud University, Department of Microbiology, Nijmegen

Anaerobic treatment of wastewater has many advantages over conventional wastewater treatment. For instance, methane gas is produced, which can be used as an energy source. However, especially in temperate climates, dissolved methane contributes to the greenhouse effect upon discharge of the effluent to surface waters. In addition, ammonium, another major end product of anaerobic digestion, must be removed via nitrification and denitrification, which require external input of oxygen and organic carbon, respectively. 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 without the need of external input of electron donors or acceptors. A stable coculture of n-damo and anammox bacteria was set up to study the cooperation of both groups of bacteria in more detail. The affinity of n-damo bacteria for nitrite was determined and the contributions of groups of microorganisms to the removal of methane and ammonium were examined. Another feasible combination could be the cooperation between the recently discovered nitrate-dependent methane oxidizing microorganisms and anammox bacteria. These archaea,

that were first enriched together with n-damo bacteria, perform anaerobic oxidation of methane (AOM) with nitrate as electron acceptor. Next to the dominant archaea in the enrichment, also anammox bacteria were present. Studies in our lab indicate that the AOM performing archaea, may also produce ammonium, making it ideal collaborators for anammox bacteria. Increased knowledge on the cocultures mentioned above, will help to implement these processes in wastewater treatment systems.

O023

Interspecies electron transfer in fatty acid-degrading communities

V.T. Sedano Núñez, J.A. Boeren, A.J.M. Stams, C.M. Plugge
Wageningen University, Laboratory of Microbiology, Wageningen

In the absence of inorganic electron acceptors anaerobic degradation of propionate, butyrate and long chain fatty acids is only possible if the products -acetate, hydrogen and formate- are kept in low concentrations. Consequently, interspecies hydrogen and formate transfer is a key process in methanogenic environments. Many syntrophic associations are obligatory; nevertheless several acetogenic bacteria possess the ability to perform sulfate respiration, which is the case for the propionate degrader *Syntrophobacter fumaroxidans*. Transcriptomic and proteomic studies in *S. fumaroxidans* have allowed us to identify energy conservation and electron transfer mechanisms used during syntrophic and sulfate dependent growth. The role of *S. fumaroxidans* as a syntroph has been studied, but its metabolic flexibility and adaptation to changing environments has never been assessed. Hypothesizing that *S. fumaroxidans* is a better syntroph than sulfate reducer perturbations in pure cultures and cocultures of *S. fumaroxidans* and *Methanospirillum hungatei* were provoked. The addition of sulfate in syntrophic cocultures triggered a metabolic shift in *S. fumaroxidans*. Sulfate started to be reduced and the methane production rate decreased by 40%. Moreover, the perturbation of the sulfidogenic axenic culture of *S. fumaroxidans* consisted in introducing a fraction of *M. hungatei* culture to the batch system. Despite the high activity and fraction of the syntrophic partner added to *S. fumaroxidans* the methane detected after perturbation was minimal (< 1 mM). Complementary trials showed inhibition of the methanogenic partner at sulfide concentrations present at the moment of the perturbation (above 5 mM). This could have hampered the metabolic shift of *S. fumaroxidans* towards syntrophy. Therefore an alternative syntrophic partner that could scavenge the H2 and/or formate from *S. fumaroxidans* while tolerating high levels of sulfide in the medium was attempted. *Desulfovibrio* sp. strain G11 was added as the syntrophic fraction in a latter perturbation. The experiment resulted in a coculture of *S. fumaroxidans* and *Desulfovibrio* G11. Quantitative PCR with specific primers for 16S rRNA genes of *S. fumaroxidans* and *Desulfovibrio* G11 showed growth of both microorganisms. However the question whether *S. fumaroxidans* switched its metabolism from sulfidogenesis to syntrophy still has to be answered. From comparative proteomic analysis of *S. fumaroxidans* growing on propionate with sulfate and in coculture with *M. hungatei* revealed that many proteins of the sulfidogenic pathway (DrsABC, AprAB, APS, Sat, etc.) were as well produced during syntrophic growth. Nevertheless the abundance ratio of a protein complex, identified as heterodisulfide reductase-like (Hdl), is remarkably not detected in the syntrophic proteome. Thus, the production of Hdl of *S. fumaroxidans* could be used as an indicator of whether the bacterium is undergoing a syntrophic or a sulfidogenic metabolism in the coculture with *Desulfovibrio* G11. Transcriptomic experiments of the gene cluster coding for such protein complex are currently being performed which might help to elucidate if the obtained association of *S. fumaroxidans* with *Desulfovibrio* G11 is of syntrophic or of parasitic character.

O024

The unknown diversity of anaerobic microorganisms in the Black Sea

L. Villanueva, J.S. Sinninghe Damste
Royal Netherlands Institute for Sea Research, Department of Marine Organic Biogeochemistry, 'T Horntje

The Black Sea is the largest permanently stratified anoxic basin in the world with a surface oxic layer separated by a gradual redoxcline from sulfide-rich anoxic waters. The presence of overlapping gradients of oxygen, sulfur and nitrogen compounds and the permanent anoxia in the deep water column makes this system an ideal model for ancient and modern anoxic environments. Different microbial metabolisms have been previously detected in this system but the diversity of these microbial groups across the water column and potential interactions between them have not been yet addressed. Here, we estimated the microbial diversity across the Black Sea water column in high resolution (15 depths from 50 to 2000 m depth) by means of 16S rRNA amplicon pyrosequencing and quantified the abundance of targeted microbial groups by quantitative PCR. Oxygen was quickly depleted in the water column in the upper 80 m, while sulfide concentrations increased steadily from 100 m reaching 100 µM at 2000 m depth. Certain microbial groups involved in the nitrogen and sulfur cycles occupied restricted niches in the water column, for example, the percentage of reads attributed to *Sulfurimonas* ranged from 7-24% from 105 to 170 m depth.

On the other hand, the uncultured group *SUP05* mediating nitrate reduction coupled to sulfide oxidation contributed with 7% of the total reads right below the redoxcline. The uncultured group SAR406, potentially involved in the dissimilatory polysulfide reduction and sulfur oxidation, was present below the redoxcline and represented more than 30% of the total reads between 500-1000 m depth. Dramatic changes in the dominant bacterial groups were observed between below the redoxcline (105-250 m) and deeper waters (500-2000 m) as the percentage of reads of *Sulfurimonas*, *Chromatiales*, *SUP05*, and *Bacteroidales* decreased, while sequences of bacterial groups closely related to anaerobic fermentative microorganisms increased (up to 27% between 1000-2000 m), suggesting the importance of these anaerobic groups in the carbon cycling in deep waters. These results support the existence of a complex diversity of microorganisms involved in the nitrogen, sulfur and carbon cycle despite of the apparently similar physicochemical conditions in the anoxic waters of the Black Sea.

O025

Enrichment of DNRA bacteria

E.M. van den Berg
TU Delft, Department of Biotechnology, Delft

Traditionally nitrate conversion in ecosystems is related to heterotrophic denitrification and nitrate assimilation. Dissimilatory nitrate reduction to ammonium (DNRA) is recognised but minimal studied. There are no pure cultures primarily enriched on this conversion, nor are there defined reactor of microcosm studies on this microbial conversion. In wastewater treatment DNRA would be an unwanted conversion, since the aim is to convert ammonium into nitrogen gas. In agricultural soils DNRA might be a desired activity since it immobilises nitrogen in the soil. A better understanding of the competition between DNRA and conventional denitrification is desired to better understand natural nitrogen cycling as well to better manage environmental and agricultural systems. We choose to use a chemostat based enrichment culture system for the study of the nitrate reduction processes, because the environmental conditions can be simulated reasonably well while the system is quantitatively defined in terms of carbon and nitrogen-turnover.

A conventional denitrifying culture was enriched from activated sludge on nitrate as electron acceptor and with acetate as growth and energy limiting substrate in a chemostat system. In order to select for DNRA-organisms we simulated nitrate limiting conditions. We were able to show that a dominant DNRA culture can be reproducibly obtained in a continuously operated enrichment system under electron acceptor limitation, with suitable dilution rate. The corresponding population is a highly enriched culture of a *Geobacter* species.

This system provides a new opening to study the DNRA process and its competition with denitrification and can be used to obtain systematic insight in the environmental conditions of DNRA occurrence. We demonstrate this in study of the effect of the COD/N mass ratio of the available substrates in the influent.

In our contribution we will describe the properties and composition of the enrichment culture as well as discuss the competition between the DNRA- and denitrification processes and the importance of COD/N ratio and dilution in this.

O026

Miniaturizing Fleming: Applying high-throughput directed evolution to ribosomal peptides

S. Schmitt[1], M. Held[1], M. Montalban[2], O.P. Kuipers[2], D. Peterhoff[3], R. Wagner[3], S. Panke[1]
[1]*ETH Zurich, Department of Biosystems Science and Engineering, Basel, Switzerland,* [2]*University of Groningen, Department of Molecular Genetics, Groningen,* [3]*University of Regensburg, Department of Molecular Microbiology and Gene Therapy Unit, Regensburg, Germany*

Synthetic biology brings with it a drastic increase in the scope of experimental operations, which it typically accommodated by a tendency to miniaturize and parallelize experimentation. While this is relatively straight forward for *in vitro* approaches such as DNA assembly, suitable approaches are less clear when interaction of living cells is required. We will discuss recent efforts of our laboratory to develop nanoliter-sized cultivation systems and their resulting applications to high-throughput screening strategies in the fields of vitamin production and engineering of novel antibiotics. For the latter application, we address ribosomally synthesized peptides, as these are easily amenable to directed evolution approaches, and here lantibiotics, such as nisin. Exploiting the considerable flexibility of the *Lactococcus lactis* posttranslational modification machinery to synthesize nisin variants, we miniaturized classic inhibition zone assays to nanoliter level and thus enable high-throughput approaches to the engineering of novel antibiotics.

O027

Introducing non-canonical amino acid residues in the antimicrobial peptide nisin

M.B. Bartholomae[1], O.P.K. Kuipers[1], N.B. Budisa[2], T.B. Baumann[2]
[1]*University of Groningen, Molecular Genetics, Groningen,* [2]*Technical University of Berlin, Institute for Chemistry, Berlin, Germany*

The increasing number of bacteria which are multiresistant to antibiotics leads to high costs in the healthcare sector, e.g. due to longer stays in the hospital, especially in intensive care units.[1] Therefore, new potential antibiotics are urgently needed and lantibiotics are promising candidates. Nisin is the best studied example, a short peptide from *Lactococcus lactis* which reveals high antimicrobial activity against Gram^{+} bacteria. Distinct serine and threonine residues of the prepeptide are dehydrated after translation by the dehydratase NisB to Dehydroalanine (Dha) or Dehydrobutyrine (Dhb), respectively. In a second step, the cyclase NisC catalyzes the formation of either lanthionine or methyllanthioninerings between Dha or Dhb and the associated cysteines. By the transporter NisT, the fully modified peptide is transported out of the cell and the leader peptide is cleaved off by the protease NisP to release active nisin.[2] To increase the variety of natural lantibiotics the incorporation of non-canonical amino acids (ncAAs) is a promising approach. One possibility to synthesize nisin variants containing ncAAs is the Stop codon suppression system.[3] Hereby, the Pyrrolysine tRNA synthetase and and its corresponding tRNA from *Methanosarcina mazei* (PylRS/tRNA$_{Pyl}$*Mm*) are utilized, because this tRNA synthetase recognizes the Amber stop codon TAG as a sense codon. Due to the low substrate specifity and high substrate tolerance of this enzyme, it can charge its tRNA with almost any possible amino acid variant which then can be incorporated into the growing polypeptide chain.[4] Since codon usage analysis in *L. lactis* revealed that TAG represents the least frequent stop codon, the normal translation of natural proteins should hardly be affected.[5] We successfully applied the PylRS/tRNA$_{Pyl}$*Mm* system to L. lactis. To analyze the activity of the tRNA synthetase, we utilized a GFP variant where N150 was replaced by a TAG. The full sized expressed GFP was detected, indicating the successful incorporation of the ncAA variant.

Afterwards, all sense codons of *nisA* were replaced by TAG codons and all resulting gene variants were coexpressed with PylRS/tRNA$_{Pyl}$*Mm* and the nisin modification enzymes NisBTC. As proof of principle experiments, the lysine variant N_{ε}-Boc-Lysine was utilized to replace S33 of nisin. The resulting nisin variant was purified and mass spectrometry analysis revealed effective incorporation of Boc-Lysine into nisin. The resulting peptide was still modified by NisBTC, so the activity of the enzymes was not hampered by expression of PylRS/tRNA$_{Pyl}$*Mm*. Antimicrobial activity tests revealed no influence of the ncAA on the basic activity of this nisin variant against a nisin sensitive *L. lactis* indicator strain. These proof of principle reactions finished the basic set-ups of the system and allow now the test of different ncAAs variants at all relevant positions of nisin.

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O029

Antibacterial mechanism of action of chicken cathelicidin-2

V.A.F. Schneider, G. Posthuma, H.P. Haagsman, E.J.A. Veldhuizen

Utrecht University, Faculty of Veterinary Medicine, Utrecht

The increased occurrence of antibiotic resistance and the continuously declining approval of new antibiotics represent a serious threat to public and veterinary health. Host defense peptides are considered to be an alternative to antibiotics with potential use in human and veterinary medicine. Chicken cathelicidin-2 (CATH-2), one of the four known cathelicidin-like host defense peptides in chickens, has immunomodulatory and direct killing activities. At micromolar concentrations (5-20 μM), CATH-2 kills both Gram-positive and Gram-negative bacteria. In this study the antimicrobial mechanism of action of CATH-2 was investigated using Gram-negative *E. coli* bacteria. Live-imaging with confocal fluorescence microscopy demonstrated that FITC-labelled CATH-2 mainly localized at the membrane of *E. coli* , with a preference for the bacterial septum of dividing cells. Upon binding, the bacterial membrane was readily permeabilized as was shown by propidium iodide influx into the cell. To further clarify the mechanism of bactericidal action of CATH-2, concentration and time effects of the peptide on *E. coli* cells were examined by transmission electron microscopy. Compared to untreated bacteria, CATH-2-treated *E. coli* showed dose-dependent morphological changes. At sub-MIC concentrations of CATH-2, intracellular granulation and wrinkled membranes were observed, while at MIC values and higher, membrane breakage and cell lysis occurred. CATH-2 caused these effects within 1-5 minutes after incubating with the bacteria. Next the binding of the peptide to LPS was studied, by using different *E. coli* strains with rough or smooth LPS. Our results showed that for *E. coli* with rough LPS (LPS missing the O-antigen) MIC values were 2-4 fold lower than MIC values of *E. coli* with smooth LPS (full-length LPS). In conclusion, our data show that the bacterial membrane is the initial target of CATH-2.

O030

Metabolomics tool facilitates new antibiotics discovery

C. Wu
Leiden University, Faculty of Science, Institute of Biology, Leiden

Metabolomics is a high throughput analytical technique used to globally measure low molecular weight metabolites, allowing simultaneous metabolic comparison of different biological samples and thus highlighting differentially produced compounds as potential biomarkers. Though microbes are renowned as prolific sources of antibiotics, the traditional approach for new anti-infectives discovery is time-consuming and labor-intensive. In this talk, the use of NMR- or MS-based metabolomics is proposed as an efficient approach to find antimicrobials in microbial single- or co-cultures.

O031

Global control of measles and rubella: Do we need new vaccines or alternative vaccination routes?

R.L. de Swart[1], R.S. van Binnendijk[2], M.P.G. Koopmans[1]
[1]*Erasmus MC, Department of Viroscience, Rotterdam,* [2]*National Institute of Public Health and the Environment, Center for Infectious Disease Research, Bilthoven*
Both labs are accredited by the World Health Organization as National Measles and Rubella Laboratories.

Measles remains an important cause of childhood morbidity and mortality. The disease is associated with immune suppression, and causes increased susceptibility to opportunistic infections. Rubella is a relatively benign childhood disease, but when experienced during pregnancy may cause severe birth defects referred to as congenital rubella syndrome. Both measles and rubella are diseases that can be eradicated: no animal reservoirs exist and live-attenuated measles and rubella virus vaccines are safe and effective. Based on the availability of multivalent vaccines, the World Health Organization (WHO) aims at combined global control of both diseases. At present all WHO regions have targeted measles and rubella for regional elimination, defined as the absence of endemic measles or rubella cases in a defined geographic area for a period of at least 12 months, in the presence of a well-performing surveillance system.[1] Therefore, national surveillance of measles and rubella remains of critical importance, and molecular epidemiology has become a major tool to distinguish between endemic transmission and virus importation.

It was long thought that new generation measles vaccines would be required to control this highly infectious disease. In particular, non-replicating temperature-stable vaccines were developed that allowed immunization of young infants in the presence of maternally derived antibodies. Several candidate vaccines showed promising results in preclinical animal studies, but none of these have been further developed in clinical trials.[2] In addition, existing live-attenuated vaccines, administered in a two-dose schedule, were successfully used to achieve regional elimination of measles and rubella in the Americas.[3] Therefore, current programs rely on the use of existing vaccines (www.measlesrubellainitiative.org). However, alternative routes of administration for measles and rubella vaccines are still under evaluation, including aerosol inhalation (either after nebulization or as a dry powder) and microneedles.[4,5] The respiratory route mimics the natural route of transmission, and helps to avoid problems associated with injection safety and contaminated waste disposal. In addition, the vaccine can be administered by minimally-trained personnel, which facilitates mass vaccination campaigns. We have compared different routes of measles vaccination in a non-human primate model, and assessed both virus tropism and immune responses. Live-attenuated MV strain Edmonston-Zagreb predominantly infected macrophages and dendritic cells, irrespective of vaccination route. However, respiratory vaccination required delivery to the lower respiratory tract for optimal induction of protective immune responses. Novel routes of administration of measles and rubella vaccines may contribute to increasing and sustaining vaccination coverage, and thus support global control programs.

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O032

Immune evasion by variable expression of two vaccine components, pertactin and filamentous hemagglutinin, during colonization of Bordetella pertussis in the immunized murine model

A. Zeddeman[1], K.E. Kok[1], M. van Gent[1], C.J. Heuvelman[1], H.G.J. van der Heide[1], F.J.H. van Opzeeland[2], S. van Selm[2], D.A. Diavatopoulos[2], F.R. Mooi[1]
[1]*RIVM (National Institute for Public Health and the Environment), IDS, Bilthoven,* [2]*Radboudumc, Laboratory of Pediatric Infectious Disease, Nijmegen*

Increasing evidence indicates that the replacement of whole cell vaccines by acellular vaccines (ACVs) has contributed to the resurgence of pertussis. Proposed explanations for this phenomenon are the more limited duration of protection conferred by ACVs, combined with adaptation of the causative agent of pertussis, *Bordetella pertussis*. The currently used ACVs contain one to five purified pertussis antigens, and a striking recent observation is the emergence of strains that do not produce one of these antigens, pertactin (Prn).

Here, we investigated another ACV component, filamentous hemagglutinin (FHA) and identified the genetic cause of non-expression of FHA in some clinical isolates. Also, we show that the FHA gene is subject to phase variation resulting in a mixed pathogen population of FHA+ and FHA- phase variants in mice. The balance between FHA+ and FHA- phase variants determines the amount of FHA produced by the population and, interestingly, depends on the colonization site, the immune status of the mouse and the Prn-phenotype. The FHA- phase variation was only observed in Prn-deficient strains and was most abundant in the lungs of ACV vaccinated mice. Phase variation of FHA was not observed in strains producing Prn or in strains colonizing the nasopharynx. Prn-deficient strains were more capable of colonizing ACV immunized mice, compared to Prn-producing strains. Our findings suggest that this may be the effect, not only of non-production of Prn, but also of the down-regulation of FHA.

These studies suggest that ACV induced immunity may be less effective against Prn-deficient strains, particularly in the lungs, also due to FHA phase variation. Most likely, this effect is greater in hosts with waning ACV immunity. Also, our findings suggests that FHA is more important for colonization of the upper respiratory tract than the lungs. Further, phase variation of FHA was related to Prn-deficiency, which suggests an interaction between these two adhesins. Above all, this research highlights the importance of including multiple antigens in pertussis vaccines. The genes of these antigens should preferably not contain regions known to be prone to phase variation. These lessons learned from pertussis should also be taken into consideration when developing vaccines for other pathogens.

O033

Strain surveillance after implementation of pneumococcal vaccination

K. Elberse[1], G. Wagenvoort[2], G. Pluister[1], H. de Melker[1], E. Sanders[1], A. van der Ende[3], M. Knol[1]

[1]RIVM, IDS-Bac, Bilthoven, [2]St. Antonius Hospital, Department of Medical Microbiology and Immunology, Nieuwegein, [3]AMC, Department of Medical Microbiology, Amsterdam

Streptococcus pneumoniae is a human pathogen and a major cause of mortality and morbidity worldwide. Nationwide vaccination of infants with conjugated polysaccharide vaccines has been implemented successfully in many countries. Facilitated by the high recombination rate of the pneumococcus, pressure of the vaccine may select for capsular variants. These may be variants that have altered capsular genes or these variants may have acquired complete capsular genes or fragments of capsular genes by horizontal gene transfer. Pneumococci may emerge that have altered capsular genes but retained their original genetic background, resulting in isolates with similar virulence but undermining vaccine pressure. To address this, temporal analysis of the pneumococcal population in the pre- and post PCV era are closely monitored using a range of typing methods. To date, the temporal analysis of the pneumococcal population in the pre- and post PCV era showed no large differences. However, certain serotypes showed an increased number of genotypes, for example serotypes 12F and 33F, indicating an increasing variety of isolates and potentially increasing virulence of these serotypes. Remarkably, a new genotype of serotype 12F was significantly more associated with meningitis as clinical outcome. Therefore, not only serotype, but also genotype may influence clinical outcome. More virulent lineages within emerging serotypes may fill the niche of the vaccine serotypes, impacting the overall clinical outcomes of pneumococcal disease. Exemplary is also serotype 3; genotyping of serotype 3 isolates revealed a lineage containing 15% of the serotype 3 isolates with a genetic composition compared to the most common serotype 3 lineage in the Netherlands. This new lineage causes relatively more pneumonia compared to the existing lineage, although not statistically significant. Serotype 3 is an important serotype to monitor, because of increasing incidences and no protection from currently used PCV10 in the Netherlands. Based on current findings, the effects of vaccination on the composition of pneumococcal population should be closely monitored, using both serotyping and genotyping methods, and results should be related to the results of clinical surveillance for full understanding of vaccine impact on the pneumococcal population.

O034

Mumps vaccine failure in adolescents

S. Gouma[1], H. ten Hulscher[1], T. Schurink-van 't Klooster[1], M. Koopmans[2], R. van Binnendijk[1]

[1]RIVM, Centre for Infectious Disease Control, Bilthoven, [2]Erasmus MC, Department of Viroscience, Rotterdam

Introduction: During recent years, various mumps outbreaks have occurred among fully vaccinated adolescents in the Netherlands, notably students. The

resurgence of mumps indicates that the mumps vaccine provides limited immune protection, but no good correlate of protection has been established so far. To study immunological mechanisms involved in protection against mumps virus infection and clinical mumps, we have analyzed pre-outbreak serum antibody levels of twice MMR vaccinated persons who have been infected with wild type mumps virus during the outbreak, and we have compared these results with pre-outbreak antibody levels in persons who have not been infected.

Methods: Pre-outbreak serum samples from 34 mumps vaccinated adolescents with a serologically confirmed mumps virus infection (11 symptomatic infections and 23 asymptomatic infections) were compared with sera obtained from 59 vaccinated age-matched controls with no clinical or laboratory indication for recent mumps virus infection. IgG concentrations in all samples were measured by the Luminex enzyme immunoassay using the Jeryl Lynn vaccine strain (genotype A) as antigen. Virus neutralizing antibody levels were measured against both the Jeryl Lynn vaccine strain and against genotype G wild type viruses isolated from mumps patients, because genotype G was the predominant mumps virus genotype during recent mumps outbreaks.

Results: Although the median pre-outbreak IgG concentration was lower for persons with a mumps virus infection than for persons without a mumps virus infection (99 RU/ml versus 193 RU/ml; $p = 0.013$), median pre-outbreak neutralizing antibody level against the Jeryl Lynn vaccine strain did not statistically significantly differ between persons with and without a mumps virus infection (74 ND50 versus 109 ND50; $p = 0.067$). However, the median neutralizing antibody level against the wild type genotype G strain was lower for persons with a mumps virus infection than for persons without a mumps virus infection (34 ND50 versus 63 ND50; $p = 0.007$). Furthermore, ROC analysis showed that the area under the curve (AUC) was larger for wild type strains (0.668) than for the Jeryl Lynn strain (0.615). The median pre-outbreak IgG concentration and neutralizing antibody level against the Jeryl Lynn strain and the wild type strains were similar in persons with symptomatic and asymptomatic mumps virus infections ($p = 0.751$, $p = 0.737$ and $p = 0.863$, respectively). No clear cutoff value could be determined as correlate of protection based on either IgG concentrations or neutralizing antibody concentrations.

Conclusion: Our data show that pre-outbreak neutralizing serum antibodies against mumps wild type genotype G strains are a better marker for protection than neutralizing serum antibodies against the Jeryl Lynn vaccine strain. However, a correlate of protection, based on specific antibody responses in serum alone, including virus neutralization, could not be established. Probably other factors, such as mucosal and cellular immunity, play a role in the protection against mumps virus infection. Furthermore, pathogenic differences between mumps virus wild type strains might enable a particular strain to evade these immune response mechanisms. These factors are currently investigated.

O035

13-valent pneumococcal conjugate vaccination response in patients after community acquired pneumonia

G.H.J. Wagenvoort, B.J. Vlaminckx, B. Meek, G.T. Rijkers, D.A. van Kessel, J.C. Grutters

St. Antonius Hospital, Department of Medical Microbiology and Immunology, Nieuwegein

Introduction: Community acquired pneumonia (CAP) is a major cause of morbidity, mortality. Furthermore, CAP patients are at high risk for readmission due to pneumonia. Since *S. pneumoniae* is the most common cause of CAP, pneumococcal vaccination post-CAP may prevent readmission and be a cost-effective strategy. Post-CAP vaccination with a 23-valent pneumococcal polysaccharide vaccine was unable to prevent CAP later in life as demonstrated by Ortqvist et al. (Lancet, 1998). It is therefore of interest to determine if post-CAP vaccination with a pneumococcal conjugate vaccine (PCV) will show efficacy. To test if a 13-valent conjugate vaccine (PCV13) is immunogenic in patients after pneumococcal CAP (pneumoCAP), we conducted the CAPolista trial (ClinicalTrials.gov Identifier: NCT02141009).

Methods: Informed consent was obtained from 59 CAP patients: 29 pneumoCAP patients diagnosed by conventional methods (culture and urine antigen testing), 4 pneumoCAP patients diagnosed by serology and 26 CAP patients with another known pathogen (cCAP). Serum samples were obtained before and 3-4 weeks after vaccination with PCV13. Specific IgG antibodies were measured using Luminex xMAP technology. Vaccine responses were categorized as *good* when post vaccination antibody concentration ≥1.3 μg/mL for ≥9/13 serotypes. Serotype specific response was defined *adequate* when concentration demonstrated either ≥4-fold increase against the previously invading serotype + concentration > 0.35 μg/mL or ≥1.3 μg/mL regardless of the fold increase.

Results: Baseline characteristics, including mean age and comorbidities, were similar (59 and 57 years, 27% and 31%, respectively). Comorbidities were defined as concomitant kidney disease, DM, neoplastic disease, congestive heart failure and COPD. In the pneumoCAP group there were significantly more females than in the cCAP group (67% versus 31%, $p < 0.01$).

Preliminary data show that in the pneumoCAP group, 91% had a good vaccine response, compared to 89% in the cCAP group ($p = 0.76$). Within the pneumoCAP group,

the infecting pneumococcal serotype was determined using quelling reaction in 7 patients of which 6 had a serotype included in PCV13. Of these, 4 failed to induce a serotype specific IgG response during infection, as previously described by van Mens et al. (Clinical and Vaccine Immunology, 2011). Upon vaccination all these patients elicited an adequate serotype specific response, including the pneumococcal serotype responsible for the pneumoCAP episode.
Conclusion: Our results show that in both CAP groups, PCV13 elicits an adequate antibody response. Furthermore, most patients earlier defined as hyporesponders during CAP, respond well to PCV13 vaccination. These results confirm the highly immunogenic properties of PCV13. In order to assess the clinical efficacy of conjugate vaccines in preventing re-admission with pneumonia and long-term mortality, prospective clinical trials should be conducted.

O036

GacA is essential for Group A Streptococcus and defines a new class of monomeric dTDP-4-dehydrorhamnose reductases

S.L. van der Beek[1], Y. Le Breton[2], A.T. Ferenbach[3], D.M.F. van Aalten[3], I. Navratilova[4], K.S. McIver[2], H.C. Dorfmueller[3,5], N.M. van Sorge[1]

[1]UMC Utrecht, Department of Medical Microbiology, Utrecht, [2]University of Maryland, Department of Cell Biology and Molecular Genetics, Maryland, [3]University of Dundee, Department of Molecular Microbiology, Dundee, United Kingdom, [4]University of Dundee, Department of Biological Chemistry and Drug Discovery, Dundee, United Kingdom, [5]Rutherford Appleton Laboratory, Didcot, United Kingdom

Background: *Streptococcus pyogenes*, also referred to as Group A *Streptococcus* (GAS), is a human-restricted Gram-positive pathogen that ranks in the top ten of infection-related causes of mortality worldwide. GAS expresses a cell wall polysaccharide, known as Group A Carbohydrate (GAC), which consists of a polyrhamnose backbone and alternating *N*-acetylglucosamine side chains. We recently identified the gene cluster responsible for GAC biosynthesis and demonstrated that the GlcNAc side chain contributes to virulence. Ongoing work aims to characterize the GAC biosynthesis pathway. The sugar nucleotide dTDP-rhamnose is a critical constituent of the GAC. The dTDP-L-rhamnose biosynthesis pathway is highly conserved in nature and requires four enzymatic steps. The final step is catalyzed by the dTDP-4-dehydrorhamnose reductase (RmlD) in an NAD(P)H-dependent manner. The first gene of the GAC gene cluster, gacA, is annotated as an RmlD enzyme. Previous structure elucidation of RmlD from *Salmonella enterica* demonstrated that Mg^{2+}-dependent dimerization is essential for enzymatic activity.
Objective: Identify the structure and function of the *gacA* gene product.
Methods: We generated recombinant GacA protein, which was used in biochemical assays and for structural analysis using X-ray crystallography. In addition, we analyzed a saturated transposon library of GAS by whole genome sequencing using HiSeq to screen for essential genes. To confirm the function of GacA in an intact bacterium, we heterologously expressed *gacA* in a *S. mutans rmlD* knockout and analyzed growth and phenotype by scanning electron microscopy.
Results: We demonstrate that GacA catalyzes the biosynthesis of dTDP-rhamnose in a novel Mg^{2+} independent monomeric manner. A high resolution crystal structure of GacA to 1.1Å confirms that key active site residues are conserved with other members within the RmlD family. In contrast, GacA lacks the Mg^{2+} binding motif that is required for dimerization in Salmonella RmlD. We identified *gacA* as an essential gene for GAS by analyzing a saturated random transposon library. We were able to confirm the enzymatic function of GacA through heterologous expression in a *S. mutans rmlD* knockout, which restored attenuated growth and aberrant cell division.
Conclusions: *gacA* is an essential rhamnose biosynthesis gene in GAS and defines a new class of monomeric RmlD enzymes. Our report provides a framework for future inhibitor screenings to identify novel inhibitors that interfere with GAC biosynthesis in GAS and dTDP-rhamnose biosynthesis through related RmlD enzymes in other human pathogens.

O037

The ESX-5 system of pathogenic Mycobacteria is involved in capsule integrity through its substrate PPE10

L.S. Ates[1], R. Ummels[1], A.D. van der Woude[1], J. Bestebroer[1], G. van Stempvoort[1], N.N. van der Wel[2], W.B. Bitter[1]

[1]VUmc Amsterdam, Department of Medical Microbiology and Infection Control, Amsterdam, [2]AMC, Van Leeuwenhoek Center for Advanced Microbiology, Amsterdam

Abstract: Tuberculosis causes more than a million deaths annually. The causative agent of tuberculosis, *Mycobacterium tuberculosis* can survive inside macrophages and is able to persist in this environment over long times. One essential factor in this process is it's distinctive membrane composition which, apart from the inner membrane, contains a hydrophobic outer membrane and a capsular layer, consisting of polysaccharides, lipids and proteins. Recently, it has been reported that this capsular layer contains proteins secreted by the TypeVII secretion system ESX-1.[1] ESX-1 substrates are known to be essential for the virulence of Mycobacteria, by facilitating the escape

of the bacterium from the phagosomal compartment. In this study we show that ESX-5, another TypeVII secretion system, is involved in maintaining the integrity of this capsule. We identified the ESX-5 substrate PPE10 to be the main factor responsible for this phenomenon.

Results: A transposon mutant in the gene *ppe10* was identified in the model organism *M. marinum* by screening for colonies that secreted more PE_PGRS proteins. Further analysis by immunoblot of this mutant showed that this mutant did not secrete higher absolute amounts of PE-PGRS proteins, but that these proteins were more loosely attached to the surface. Another surface localized protein, the ESX-1 substrate EspE, was also shown to be more loosely attached to the cell surface of both the ppe10::tn strain and an *esx5* deficient strain *EspG5::tn*, by immunoblot as well as immuno-EM. Since EspE is known to be a capsular component, we hypothesized that the capule formation of *ppe10::tn* and *espG5::tn* could be impaired. Indeed, an ESX-5 mutant of *M. tuberculosis* was shown to be impaired in capsule biogenesis by Cryo-EM.

The differential localization of capsular proteins, was shown to have an effect on the ESX-1 dependent membrane-disrupting potential of *M. marinum* by an hemolysis assay. The role of this phenomenon on the virulence of *M. marinum* was confirmed in *in vivo* experiments in zebrafish embryo's. Approximately 10-fold less cfu were recovered from zebrafish infected with either the *ppe10::tn* or *espG5::tn* strain compared to the wild-type- or *ppe10* complemented strains of *M. marinum*. These data show that the capsular localization of ESX-1 substrates is dependent on a functional ESX-5 system and that the ESX-5 substrate PPE10 is the main component responsible for this. Furthermore, the differential localization of ESX-1 substrates has implications for virulence that explain virulence defects observed in ESX-5 mutants in the past.

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O038

Genome-wide screening for genetic determinants involved in decreased susceptibility to the antiseptic chlorhexidine in the multidrug-resistant opportunistic pathogen *Enterococcus faecium*

A. Guzman, J. Wijngaarden, X. Zhang, M. Rogers, E. Majoor, M.J.M. Bonten, R.J.L. Willems, W. van Schaik

UMC Utrecht, Department of Medical Microbiology, Utrecht

Enterococcus faecium is a natural inhabitant of the gastrointestinal tract of humans. However over the last few decades it has emerged as one of the primary causes of nosocomial infections. Its ability to acquire resistance determinants to almost all antibiotics has severely limited treatment options. Although understanding the mechanisms of antibiotic resistance may lead to the discovery of novel drug targets and eventually to the development of novel therapeutics, it is equally important to prevent the spread of multidrug resistant pathogens (MDR) in the nosocomial environment. Compounds such as chlorhexidine (CHX), have been commonly and effectively used for this purpose in surgical scrubs, as washing/bathing antiseptics for patients and as surface disinfectants for decades. Worryingly, decreased susceptibilities in MDR pathogens to disinfectants are currently also being reported.

In this study, we used a high-density transposon mutant library constructed in E1162, a sequenced *E. faecium* clinical isolate, to perform a genome-wide screening for genetic determinants involved in decreased susceptibility to CHX. Screening of the transposon mutant library was done using M-TraM (Microarray-based Transposon Mapping), a technique that allows to track the presence of all mutants in the library by simultaneously mapping the transposon insertion sites using microarray hybridization. The use of M-TraM led to the identification of candidate genes that could be involved in loss of susceptibility to this antiseptic. Among the genes identified, we found a two-component system (2CS), composed of a sensor histidine kinase and its DNA-binding response regulator.

In order to validate the M-TraM results, we constructed markerless mutants in the genes encoding the response regulator and the histidine kinase. We have shown that interfering with individual components of this 2CS is sufficient to significantly extend the lag phase of *E. faecium* E1162 in the presence of CHX. *In trans* complementation of the individual mutations of this 2CS, were able to restored normal growth. Our results thereby confirms the role of this two-component system in the loss of susceptibility of *E. faecium* to chlorhexidine. The genes that are regulated by this 2CS are currently being identified and their role in chlorhexidine resistance will be investigated.

O039

The role of a type VII secretion chaperone in the specific substrate recognition in pathogenic mycobacteria

T.H. Phan[1], N. Korotkova[2], R. Ummels[3], C.C. Creekmore[2], T.J. Evans[2], K.V. Korotkov[2], W. Bitter[1,3], E.N.G. Houben[1,3]

[1]*VUmc, Department of Molecular Cell Biology, Amsterdam,* [2]*University of Kentucky, USA,* [3]*VUmc, Department of Medical Microbiology and Infection Control, Amsterdam*

Background: Type VII secretion (T7S) is employed by mycobacteria, including the important pathogen *Mycobacterium tuberculosis*, to export protein effectors across their highly unusual cell envelope. *M. tuberculosis* contains five paralogous T7S systems, ESX-1 to ESX-5,

each having its own role in viability and/or virulence. The mycobacteria-specific PE/PPE proteins are one group of proteins that are secreted via these secretion systems, of which at least some are secreted as heterodimers. We have previously shown that the cytosolic chaperone EspG specifically interacts with cognate PE/PPE dimers and is required for their successful secretion.

Objectives: Our aim is to understand the determinant factors for substrate-specific binding of EspG to T7S substrates.

Methods: The crystal structure of ESX-5 chaperone $EspG_5$ with the ESX-5 substrate pair PE25/PPE41 was determined and important residues for chaperone-binding of several PPE proteins were mutated. Additionally, EspG-binding domains of ESX-1 and ESX-5 substrates were exchanged.

Conclusions: The structure revealed that $EspG_5$ interacts with the elongated end of PPE41, a region containing several hydrophobic residues that are conserved in PPE substrates of different secretion systems. Mutating these residues of multiple ESX-5 PPE substrates and an ESX-1 dependent PPE protein abolished the interaction with the cognate chaperone and blocked their secretion by *Mycobacterium marinum*. Subsequently, when we exchanged the complete EspG-binding domain between an ESX-1 and ESX-5 PPE protein we were able to alter chaperone-binding specificity. Now, we are testing the effect of system specific chaperone binding on rerouting of ESX substrates. In summary, we have elucidated the molecular determinants for EspG-specific binding to PPE substrates and the key role of chaperone-binding in secretion.

O040

The role of antibodies in anti-fungal immunity against *Aspergillus fumigatus*

S.G.E. Braem[1], J.W. Hoepel[1], C.P.M. van Kessel[1], H. Wösten[2], J.A.G. van Strijp[1], P.J.A. Haas[1]

[1]*UMC Utrecht, Department of Medical Microbiology, Utrecht,* [2]*Utrecht University, Department of Microbiology, Utrecht*

Introduction: *Aspergillus fumigatus* is an important airborne fungal pathogen and a major cause of invasive fungal infections. Invasive aspergillosis is classically associated with specific risk factors, like neutropenia, cytotoxic chemotherapy, immune suppression and transplantation. The innate immune system plays a crucial role in the defense against *Aspergillus* invasion. Although resident leukocytes in the alveolar lung tissue are the first line of defense against invasive aspergillosis, additional recruitment of neutrophils is necessary for efficient clearing of *A. fumigatus* infections. Recently, we described complement activation on the fungal surface as a crucial process in effective opsonization, phagocytosis and killing by neutrophils of *A. fumigatus*. This complement activation was mainly initiated via the classical complement pathway. Since the classical complement pathway is initiated by binding of antibodies to the fungal surface, we studied the role of antibodies in the innate immune response against *A. fumigatus*.

Methods: To study antibody levels against *A. fumigatus*, sera were collected from healthy individuals and patients receiving cytotoxic chemotherapy or undergoing allogeneic stem cell transplantation. Swollen conidia were incubated with the different sera and bound antibodies were measured by flow cytometry. To investigate the difference in complement activation, uptake and killing of swollen conidia by neutrophils between sera containing low and high specific *Aspergillus* antibodies, C3b deposition was measured, neutrophil phagocytosis assays were performed and complement-dependent outgrowth assays were conducted, respectively.

Results: Sera from healthy individuals contain specific antibodies against *A. fumigatus* swollen conidia. Specific antibody levels differ between individuals. High antibody levels result in more C3b deposition on the fungal surface and more complement-dependent phagocytosis by neutrophils than low antibody levels. Also neutrophil killing is more effective in sera with high antibody levels. Phenotypes observed in sera with low antibody levels could be restored by the addition of isolated IgG from serum with a high amount of specific antibodies against *A. fumigatus*. Sera from patients contain a divers range of specific antibodies. Stratification of patient sera shows that there are differences in the amount of specific *Aspergillus* antibody levels between patients suffering from invasive aspergillosis and patients without *Aspergillus* infection.

Conclusion: Antibodies are highly important in effective opsonization, complement-dependent phagocytosis and killing by neutrophils of *A. fumigatus*. The amount of specific *Aspergillus* antibodies in serum against *A. fumigatus* seems to differ between patients with and without invasive aspergillosis.

O041

Identification of a new staphylococcal myeloperoxidase (MPO) inhibitor

N.W.M. de Jong, R. Nijland, C.J.C. de Haas, J.A.G. van Strijp, P.J.A. Haas

UMC Utrecht, Department of Medical Microbiology, Utrecht

Introduction: *Staphylococcus aureus* is highly adapted to its host and has evolved many strategies to resist opsonization and phagocytosis. However, even after uptake by neutrophils, *S. aureus* shows resistance to phagocytic killing. This indicates the existence of unknown intracellular acting evasion molecules.

Methods: To explore these unknown factors, we used secretome phage display to screen for yet unidentified staphylococcal proteins that specifically act inside the

phagolysosome. We coated degranulated neutrophilic content to select for specific binding to phages from our library, which contains 7.2 x 10^7 phages expressing all secreted *S. aureus* proteins.

Results: With this novel screening method we found Staphylococcal Peroxidase INhibitor (SPIN) a highly specific inhibitor of human MPO (myeloperoxidase). SPIN is a small and highly conserved protein of 8.4 kDa located on the pathogenicity island νSaα. SPIN binds human MPO with an affinity of 8.0 nM and inhibits its activity at 13.2 nM (EC50). MPO is a major constituent in azurophilic granules of neutrophils and catalyses the reaction of H_2O_2 to the powerful antimicrobial agent hypochlorous acid (HOCL). MPO is released into the phagosome upon phagocytosis of *S. aureus*, and inhibiting MPO would greatly benefit in survival of the pathogen after phagocytosis. By using GFP promoter reporter in USA300, we showed that SPIN is expressed during exponential growth phase but when inside the neutrophil phagosome this promoter is already on after one hour. The presence or absence of the gene (WT vs KO) results in a difference of fluorometric outgrowth rebound after phagocytosis.

Conclusions: In summary, (1) our data show that SPIN is a novel identified staphylococcal evasion molecule that potently inhibits MPO and thereby contributes to resistance to killing. SPIN binds to MPO with an affinity of 8.0nM (2). SPIN is present in all human staphylococcal isolates and is expressed especially when *S. aureus* is taken up by neutrophils (3).

O042

Physiology of *Saccharomyces cerevisiae* at near zero-growth rates: towards uncoupling metabolism from growth

T. Vos, X.D.V. Hakkaart, P. de la Torre, J.T. Pronk, P. Daran-Lapujade
TU Delft, Department of Biotechnology, Delft

A major challenge in the production of added-value compounds by microbes, such as the popular *Saccharomyces cerevisiae*, is to uncouple product from biomass formation. Uncoupling of growth from product formation requires cells to be in a metabolically active, non-dividing state. Such a metabolically active state can be achieved in glucose-limited retentostats (modified chemostats with full cell retention) leading to doubling times below 30 days. While demonstrated using *S. cerevisiae* under anaerobicity, many industrially relevant compounds require a net ATP input that only be achieved under aerobic environment. Aerobic industrial cultivation of *S. cerevisiae* is typically performed in sugar-limited fed-batch reactors which, due to technical constraints, have to be operated at low specific growth rates. We have designed retentostat setups that enable cultivation of fully respiring yeast at near-zero growth rates for prolonged periods of time. In aerobic retentostat, doubling times up to 200 days were achieved while culture viability remained high over 20 days. Yeast cells remained metabolically active and invested virtually all sugar in maintenance processes. The maintenance energy requirement (m_s) estimated from these aerobic retentostats was remarkably lower than that reported for anaerobic yeast cultures. As observed during anaerobic retentostats, yeast cells in aerobic retentostats displayed an extreme robustness towards heat-shock. The developed retentostat setup allows to characterize metabolically active *S. cerevisiae* under aerobic near-zero growth conditions, and provides an important step towards the uncoupling of metabolism from growth.

O043

Peptidoglycan present in Planctomycetes after all? Insights from an anammox bacterium

M.C.F. van Teeseling[1], R.J. Mesman[1], E. Kuru[2], A. Espaillat[3], F. Cava[3], Y.V. Brun[2], M.S. VanNieuwenhze[2], B. Kartal[1], L. van Niftrik[1]
[1]*Radboudumc, Department of Microbiology, Nijmegen,* [2]*Indiana University, Interdisciplinary Biochemistry Program, Bloomington, USA,* [3]*Umea University, Department of Molecular Biology and Lab, Umea, Sweden*

Planctomycetes are among the few bacteria proposed to lack peptidoglycan, a cell wall structure crucial for cell shape and integrity. Therefore, the planctomycetal cell envelope was considered to be neither Gram-positive nor Gram-negative. In addition to their exceptional cell envelope, their cell plan was found to have a unique degree of compartmentalization. Anaerobic ammonium-oxidizing (anammox) Planctomycetes are key players in the global nitrogen cycle that release fixed nitrogen back to the atmosphere as N2. We set out to investigate the proposed lack of peptidoglycan in an anammox Planctomycete in order to better understand the cell envelope of this intriguing bacterial phylum. To this end, we used complementary state-of-the art techniques such as cryo-transmission electron microscopy, peptidoglycan-specific probes and structured illumination microscopy and ultrasensitive UPLC-based muropeptide analysis. Our results suggest that Planctomycetes could best be understood as Gram-negative bacteria.

O044

Sporulation temperature has limited effect on the spore proteome of *Bacillus weihenstephanensis*

S.K. Stelder, H.L. Dekker, L.J. de Koning, C.G. de Koster, S. Brul
University of Amsterdam, Department of Molecular Biology and Microbial Food Safety, Amsterdam

Bacillus weihenstephanensis is a spore forming food pathogen known for its ability to sporulate, germinate, grow and produce toxins at lower temperatures than its close relatives. As such, it presents an increased risk to food safety where standard procedures rely on cold-inactivation of microbes. Properties of the infective form *Bacilli*, the spore, are known to vary with the conditions under which the spores were produced. For instance, lower sporulation temperature has been indicated to result in a lower wet heat resistance. This resistance is in part attributed to the spore coat layers, proteinaceous layers surrounding the core which contains the bacterial DNA, though no specific singular protein can be indicated to confer heat resistance suggesting an acquired trait of the total spore proteome. Analysis of the proteome of spores of *B. weihenstephanensis* strain WSBC 10204 produced at either 12°C or 30°C by mass spectrometry provided insight into variations in the protein content of the spore coat at different sporulation temperatures. Interestingly, only minor differences were observed, suggesting wet heat resistance does not depend on the identity of the proteins making up the coat layers. As wet heat resistance was indeed lower for the spores produced at lower temperatures, low temperature might instead affect the rate of spore maturation instead, a process where spore coat proteins are cross-linked after they have been deposited onto the spore.

O045

CRISPR/Cas9: a molecular Swiss army knife for simultaneous introduction of multiple genetic modifications in *Saccharomyces cerevisiae*

R. Mans, H.M. van Rossum, M. Wijsman, A. Backx, N.G. Kuijpers, M. van den Broek, P. Daran-Lapujade, J.T. Pronk, A.J.A. van Maris, J.M.G. Daran
TU Delft, Department of Industrial Microbiology, Delft

A variety of techniques for strain engineering in *Saccharomyces cerevisiae* have recently been developed. However, especially when multiple genetic manipulations are required, strain construction is still a time-consuming process. This study describes new CRISPR/Cas9-based approaches for easy, fast strain construction in yeast and explores their potential for simultaneous introduction of multiple genetic modifications. An open-source tool (http://yeastriction.tnw.tudelft.nl) is presented for identification of suitable Cas9 target sites in *S. cerevisiae* strains. A transformation strategy, using *in vivo* assembly of a guideRNA plasmid and subsequent genetic modification, was successfully implemented with high accuracies. An alternative strategy, using *in vitro* assembled plasmids containing 2 gRNAs was used to simultaneously introduce up to 6 genetic modifications in a single transformation step with high efficiencies. Where previous studies mainly focused on the use of CRISPR/Cas9 for gene inactivation, we demonstrate the versatility of CRISPR/Cas9-based engineering of yeast by achieving simultaneous integration of a multi-gene construct combined with gene deletion and the simultaneous introduction of 2 single-nucleotide mutations at different loci. Sets of standardized plasmids, as well as the web-based Yeastriction target-sequence identifier and primer-design tool, are made available to the yeast research community to facilitate fast, standardized and efficient application of the CRISPR/Cas9 system.

O046

Characterization of a *Nitrospira species* enriched under anaerobic, denitrifying conditions

M.A.H.J. van Kessel, S. Lücker, D.R. Speth, M.S.M. Jetten, H.J.M. op den Camp
Radboud University, Department of Microbiology, Nijmegen

Members of the genus *Nitrospira* are dominant in many natural habitats and of vital importance for wastewater treatment. They are chemolithoautotrophic organisms capable of growth with nitrite and CO2 as their sole energy and carbon source. It further has been demonstrated that some *Nitrospira* can utilize simple organic carbon compounds and molecular hydrogen as alternative substrates and can switch to nitrate reduction under oxygen limitation. However, this metabolism has been assumed to be a survival strategy and in most systems studied so far *Nitrospira* was outcompeted by denitrifying organisms when anoxic conditions prevailed. Here, a community of nitrogen cycle bacteria was enriched from the anaerobic compartment of a biofilter connected to a recirculating aquaculture system. The culture was fed with water from the aquaculture system, supplemented with ammonium, nitrite and nitrate. A stable enrichment culture was obtained which anaerobically converted ammonium, nitrite and nitrate into dinitrogen gas. The culture was dominated by anaerobic ammonium-oxidizing Brocadia species, a denitrifier (*Denitratisoma*), and a *Nitrospira* species. Anammox bacteria and *Nitrospira* co-aggregated, while the denitrifiers formed separate clusters. This culture shows that anaerobic ammonium oxidizers and nitrite oxidizers, who were considered to be mutually exclusive, can be grown as a stable co-culture. Furthermore, this shows that some *Nitrospira* species can be competitive under nitrate-reducing conditions. Thus, this study demonstrates another unexpected lifestyle for an organism believed to be only competitive under aerobic lithoautotrophic conditions. It further indicates that *Nitrospira* in combination with anammox can be of interest for anoxic wastewater treatment systems.

O047

Quest for new antimicrobial drug targets: genome-wide screening of essential genes of the emerging zoonotic pathogen *Streptococcus suis* and validation of YycF/G Two-component System as antimicrobial drug target

A.E. Bem[1], A.D.G. de Greeff[2], H.B. Bootsma[3], M.I. de Jonge[3], H.S. Smith[2], A.Z. Zomer[3], A.M. Marina[4], P.V.B. van Baarlen[1], J.M.W. Wells[1]

[1]Wageningen University, Sub-Department of Host-Microbe Interactomics, Wageningen, [2]Central Veterinary Institute, Lelystad, [3]Radboudumc, Department of Pediatric Infectious Diseases, Nijmegen, [4]Instituto de Biomedicina de Valencia-Consejo Superior de Investigaciones Cien, Valencia, Spain

Introduction: *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. Antibiotic resistance of *S. suis* clinical isolates has been reported worldwide. The aim of this study was to identify novel antimicrobial drug targets of *S. suis* and validate some of these targets.

Methods: In this study essential genes of *S. suis* S10 have been identified in a high-throughput genome-wide screen using a mariner transposon insertion mutant library of *S. suis* S10. We compared a list of *S. suis* S10 essential genes making use of known essential orthologs of *S. pneumoniae* TIGR4, *S. sanguinis* SK36 and *B. subtilis* 168. Several of the essential genes were selected as attractive drug targets based on these comparative studies. We made a conditional knock-out of, yycF/G to elucidate the regulatory network of this Two-component System (TCS) in *S. suis* S10 and to validate its essentiality.

Results: We identified 281 essential genes of *S. suis* S10 and investigated their predicted function. We found out that orthologs of 54 essential genes were shared within *S. pneumoniae* TIGR4, *S. sanguinis* SK36, *B. subtilis* 168 and *S. suis* S10. Only one TCS, *yycF/G*, was found to be essential in selected four bacteria. We show that *yycF/G* is essential in *S. suis* S10.

Conclusions: 1) Genome-wide screening of a transposon insertion mutant library of *S. suis* S10 revealed 281 essential genes. 2) Orthologous gene comparison of lists of essentials of four selected Gram-positive bacteria identified 54 shared essential genes. 3) *yycF/G* was the only essential Two-component System in four selected Gram-positive bacteria. 4) Studies using a conditional knock-out of *yycF/G* in *S. suis* S10 validated its essentiality and suggested it could be used as a drug target for novel antimicrobials.

O048

Probiotics in Very-Low-Birth-Weight Neonates in relation to late onset staphylococcal sepsis

M. Strus, A.Cichońska, G.Stefański, P.B. Heczko

Jagiellonian University Medical College Krakow, IBSS Biomed Inc. Krakow, Poland and the Polish Neonatal Infections Network, Krakow, Poland

Late onset sepsis (LOS) is a major contributor to neonatal morbidity and mortality in premature infants. Distortions in the establishment of normal gut microbiota and overgrowth of the potentially pathogenic bacteria that colonize the digestive tract might increase the risk of LOS via disruption of the mucosal barrier with resultant translocation. Coagulase-negative staphylococci (CONS) are the most prevalent microbes isolated from neonatal sepsis cases followed by Gram-negative rods. Thus, correction of the distortions in intestinal microbiota is a necessary first step to design novel approaches that might lead to early interventions to prevent LOS in neonates. To verify a hypothesis, that it is possible to induce beneficial alterations in gut microbiota of WLBW neonates by oral supplementation with probiotic bacteria, a multicenter, randomized, double blind placebo-controlled clinical study was performed on WLBW neonates. Altogether 182 patients were enrolled in 8 neonatal intensive care clinical centers in Poland. The neonates were given intragastrically either a mixture of *LactoBacillus rhamnosus* KL53A and *Bifidobacterium* breve PB04 twice a day or placebo. The supplementation started before first 48 hours of life and was continued up to 6 weeks of life or until discharge. Fecal samples of the neonates were collected at weekly intervals, kept frozen and transported to central laboratory where main groups of bacteria were enumerated. Blood samples were taken always when sepsis was suspected and checked for bacterial growth in local laboratories at the clinics. It appeared that supplementation of WLBW neonates with the tested mixture of *L. rhamnosus* and *B. breve* was related to presence of the increased numbers of lactobacili and bifidobacteria in their faeces for several weeks. On the other hand, numbers of the potential pathogens like as staphylococci and Gram-negative rods were significantly lower in faeces of the neonates supplemented with probiotics versus control ones. These alterations in gut microbiota were related to clinical effects. It was demonstrated that presence of the elevated numbers of *B. breve* was significantly and timely correlated with the significant reduction of the sepsis rates, predominantly caused by CONS. From a safety perspective, no probiotic bacteria were isolated from blood cultures. Beneficial clinical effects of the oral probiotic supplementation in neonates are reportedly related to more competent

gut-related immunity, and less pathogenic components of the gut microbiota. This is a first report, that supplementation of WLBW neonates with *L. rhamnosus* and *B. breve* may alter their gut microbiota and reduce rates of the staphylococcal sepsis.

O049

The microbiota in colorectal cancer: Cause or effect?

J.M. Boleij
Radboudumc, Departement of Pathology, Nijmegen

Our gut microbes are an extension of our body and can be seen as an additional "organ" that contains a wealth of functions that are critical for our health status. A range of studies increasingly underlines the role of the microbiota in colorectal cancer, giving rise to new potentials for CRC-treatment, diagnosis and prevention. Researchers worldwide have invested in this topic to increase our knowledge on the gut microbiota in the pathogenesis of CRC and translate these results to the clinic.

In a healthy colon the mucus layer and epithelial barrier protects the colon wall from direct bacterial-epithelial contact. However, during CRC progression, epithelial permeability increases and alteration in mucus glycosylation and expression patterns lead to a reduced mucosal barrier, leading to direct bacterial-epithelial contact. Changes in microbial organization and composition can be a direct consequence of the changing host-environment in CRC, or can be indirectly caused by the effects of individual pathogenic microbes on the tissue, e.g. by tissue damage and inflammation. Certain microbes might benefit from this tumor-environment. In recent years deep-sequencing of bacteria in CRC-tumors and corresponding normal tissue has provided the first evidence of microbial alterations in sporadic CRC. These differences are already apparent in the mucosa of tubular adenoma patients and further dysbiosis occurs in carcinomas. For example, several research groups independently found that *Fusobacterium nucleatum* was enriched in colorectal tumors compared to normal mucosa, and mostly absent in healthy controls. With *in vitro* and *in vivo* studies it was subsequently shown that the FadA adhesin of *F. nucleatum* binds to E-cadherin and stimulates carcinogenesis via the activation of β-catenin signalling.

In addition, several other (opportunistic) pathogens have been described that can have an impact on CRC progression by direct mutagenic effects on the epithelium via action of bacterial toxins, or indirectly via initiation of chronic inflammation and production of reactive oxygen species. One such pathogen is Enterotoxigenic *Bacteroides fragilis* (ETBF). ETBFproduces a 20 kDa metallaprotease (BFT) that acts directly on the wnt-signaling pathway. Due to cleavage of E-cadherin, β-catenin accumulates in the nucleus and results in increased cell proliferation. *In vivo* ETBF results in persistent colitis and leads to Th17 driven carcinogenesis. A recent study comparing *bft*-detection in CRC patients versus healthy controls indicated that the mucosa of CRC patients is more often bft-positive (90%) than the mucosa of healthy controls (50%) and thus might have a direct association with CRC development in humans as well.

Especially microbes that have a higher abundance in colorectal cancer patients, but are almost never present in healthy controls could be of value for the diagnosis of colorectal cancer. This approach was recently applied, where metagenomic classifiers of bacterial composition were compared to the hemoccult Fecal Occult Blood Test (FOBT) that is routinely used in screening for CRC in many countries. The accuracy of the metagenomic classifier was slightly better than that of the Hemoccult FOBT and combination of the two tests improved the accuracy of the Hemoccult FOBT. Furthermore, it was found that the discrimination of cancer patients and controls was mainly attributed to four bacterial species; two *Fusobacterium nucleatum* species, *Porphyromonas asaccharolytica* and *PeptoStreptococcus stomatis*. How the metagenomic classifier relates to the immunogenic FOBT that is currently being used for colorectal cancer screening in the Netherlands, has not been established yet. However, the results seem promising for the incorporation of microbial composition and function in diagnosis and disease monitoring of colorectal cancer. Future research needs to focus on less expensive and simpler approaches as both reduction in costs and analytic effort would be desirable for integration in routine clinical practice.

O050

Characterization of gut microbiota profiles by disease activity in patients with Crohn's disease

D.Tedjo[1,2], J. Penders[2], A. Smolinska[3,4], P. Savelkoul[2], A. Masclee[1], M. Pierik[1], C. Driessen[2], M. Lucchesi[2], D. Jonkers[1]
[1]*MUMC+, School for Nutrition and Translational Research in Metabolism (NUTRIM), Division Gastroenterology-Hepatology, Maastricht,* [2]*MUMC+, School for Nutrition and Translational Research in Metabolism (NUTRIM), Department of Medical Microbiology, Maastricht,* [3]*MUMC+, School for Nutrition and Translational Research in Metabolism (NUTRIM), Department of Toxicology, Maastricht,* [4]*Top Institute Food and Nutrition, Wageningen*

Crohn's disease (CD) is a chronic relapsing gastrointestinal disease characterized by mucosal inflammation of the gastrointestinal tract, which can lead to irreversible

intestinal damage. Monitoring of the mucosal inflammation is crucial to prevent complications and disease progression; however endoscopy of the gastrointestinal tract (the golden standard to asses mucosal inflammation) is an invasive and expensive procedure. Currently available noninvasive biochemical inflammatory markers and clinical activity scores do not correlate well with mucosal inflammation, especially not for patient with ileal disease. Previous studies demonstrated associations between the fecal microbiota composition and CD, implying that the fecal microbiota composition may provide information about the disease status. Studies comparing CD patient with active disease and CD patients in remission showed differences in specific taxa, but these findings could not always be reproduced or confirmed, possibly due to confounding factors such as medication use.
Large population-based patient cohorts are required to overcome heterogeneity between patients and control for potential confounding factors. Within the IBD South Limburg cohort, a population-based cohort of 2837 IBD patients, we examined the role of the gut microbiota in the course of this disease.
In a selected group of CD patients we analyzed the microbiota during remission and subsequent exacerbation using 16S amplicon pyrosequencing. By comparing within and between sample diversity measurements and unsupervised learning techniques, we demonstrated shifts in microbiota composition that were mainly patient-specific. Most pronounced were the marked increases in the relative abundance of *Bacteroides fragilis* in some patients, implying a role of this bacterium or its products (e.g. toxins) in disease progression. Moreover, analysis showed that differences in medication use, in particular thiopurines, strongly contributed to inter-individual differences in microbial diversity and community structures. Thiopurine use was associated with a marked decrease in the abundance of specific microbial taxa, including *Faecalibacterium prausnitzii*, as well as with an overall decreased species richness and diversity.
Although the before mentioned techniques are useful for further pathophysiological insight, they are not optimal for predicting disease course. Supervised machine learning techniques are designed to handle highly complex and sparse data sets, are able to develop predictive models and until recently, are rarely used in microbiota data analysis. In a subsequent study we used such techniques to analyze the fecal microbial composition of 194 samples of CD patients. This presentation reports on the role of the microbiota in disease course of CD and illustrates how supervised machine learning techniques can be applied to microbiota data to build a predictive model to identify specific fecal bacterial taxa to differentiate CD patients in remission from CD patients with active disease.

O051

Applications of intestinal microbiota profiling in clinical diagnostics

A. Eck
VUmc, Department of Medical Microbiology and Infection Control, Amsterdam

The human body is colonized by a vast number of microbes, collectively referred to as the human microbiota. A growing number of research initiatives focus on the link between the microbiota and human health and new insights are emerging in rapid succession. As disturbance of the microbiota–host relationship is associated with numerous diseases, such as chronic inflammatory digestive tract diseases, the human microbiota seems to have a promising potential for clinical diagnostics. However, to use microbiota composition as a diagnostic tool several challenges need to be overcome.
In most cases, independent of the technique used, human-associated microbial communities' data will be of a high-dimensional nature. This means that the number of variables measured, or number of bacterial taxa, exceeds the number of samples, or number of individuals, and traditional statistics approaches are not applicable. Additionally, high inter-individual variation, especially in the gut microbiota, makes it difficult to identify global disease biomarkers. Most of the studies that explore host - microbiota interactions are retrospective studies, while developing a (personalized) predictive diagnostic application requires a longitudinal observation and a prospective study design that will help to determine the risk factors for a disease. Finally, for a diagnostic test it is critical to generate the data in a highly standardized manner that will be applied in exactly the same way in every laboratory at all times.
With these guidelines in mind, we developed IS-pro: a reproducible, high-throughput PCR-based bacterial profiling method, easily implementable in a general clinical laboratory. IS-pro was specifically optimized and validated for the complex microbiota of the human intestinal tract. A major goal of our studies is the identification of important groups of microorganisms that vary according to physiological or disease states in the host, but the incidence of rare species and the large numbers of species observed make that goal difficult to obtain using traditional approaches. Fortunately, similar problems have been addressed by the machine learning community in other fields of study such as microarray analysis and text classification.
We apply existing classification approaches both for selecting discriminating subsets of species and for building prediction models that can accurately classify new samples. We also aim to exploit structures inherent

in microbial community data for the development of novel approaches. These techniques will enable us to develop IS-pro diagnostics applications based on microbial profiles for different disorders and as a prognostic tool.
We have recently shown that diverticulitis can be diagnosed based on intestinal microbiota analysis with relatively good accuracy. Other promising results include periodontitis diagnosis based on the characterization of the bacterial community in dental plaques, in vitro fertilization (IVF) success prediction based on the vaginal microbiome and inflammatory bowel disease (IBD) diagnosis and disease course prediction. IS-pro research is ongoing constantly, developing a unique knowledge base on microbiota and its responses to different perturbations. Because of the high level of standardization, all data from different studies can be combined to gain a broad overview of the human microbiota and to identify novel associations.

O052

Single-cell metabolite sensors as tools for strain and enzyme development

M. Bott
IBG-[1]: Biotechnology, Institute of Bio- and Geosciences, Forschungszentrum Juelich, Juelich, Germany

Transcriptional regulators usually function as sensors and regulators, as their activity is controlled by specific stimuli, such as metabolites, redox status, toxic compounds, or inorganic ions. We have exploited this property for the construction of biosensors, which are able to detect the concentrations of amino acids such as L-lysine or L-methionine in single bacterial cells and convert this information into a fluorescence output by using EYFP or other autofluorescent proteins as reporter (for a recent review see.[1] The response was linear in a certain concentration range.[2,3] The lysine sensor pSenLys, which is based on the transcriptional regulator LysG of *Corynebacterium glutamicum*, was used to isolate by fluorescence-activated cell sorting (FACS) single producer cells from a library of randomly genome-mutagenized cells of *C. glutamicum*. Sequencing and further characterization of these clones led to the identification of known, but also of novel mutations triggering lysine overproduction.[2]
As random mutagenesis often leads to hundreds of mutations in a single genome whose relevance for production is not obvious, a novel methodology termed RecFACS was developed, which combines biosensors, FACS, and recombineering. It allows to rapidly identify those point mutations that cause a "productive" phenotype, such as lysine overproduction, and can be used to screen hundreds of mutations, including those in essential genes, e.g. *murE*. It also allows to isolate and differentiate the productive variants of a site-specific saturation mutagenesis within the genome.[4]
Another highly useful application of the biosensors was demonstrated for altering the allosteric properties of key enzymes in biosynthetic pathways. Using plasmid-encoded mutant libraries of aspartate kinase (LysC), N-acetylglutamate kinase (ArgB), and ATP-phosphoribosyltransferase in cells carrying the pSenLys sensor, which also detects arginine and histidine, it was possible to rapidly isolate feedback-resistant variants of the corresponding enzymes, whose presence was sufficient to cause overproduction of lysine, arginine, and histidine.[5]
Besides sensors for amino acids, we also developed the pSenSox biosensor for measuring the NADPH/NADP+ ratio in *Escherichia coli* based on the transcriptional regulator SoxR and its soxS target promoter.[6,7] In proof-of-principle experiments with an NADPH-dependent alcohol dehydrogenase we showed that pSenSox can be used for HT-screening of NADPH-dependent enzymes, e.g. for variants with improved activity for a desired substrate.[7]

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O053

Engineering precursor supply and free-energy conservation for anaerobic fermentative production of fuels and chemicals

A.J.A. van Maris
TU Delft, Department of Biotechnology, Delft

Current biotechnological products with the lowest cost and highest yield are those produced as metabolic end-products of ATP-generating pathways under anaerobic conditions. This is for instance the case for two classical biotechno-

logical products: ethanol and lactic acid. Besides resulting in high yields, this eliminates the need for costly aeration and decreases cost of bioreactors.
Although many novel products are now produced with metabolically engineered microorganisms, only a few of these are produced under anaerobic conditions. Based on specific examples of yeast metabolic engineering, this presentation describes how engineering of redox metabolism, precursor supply and free-energy conservation can facilitate the anaerobic production of fuels and chemicals.

O054

Comparative genome, transcriptome and metabolome analysis of the strain lineage of β-lactam producing strains of *Penicillium chrysogenum*?

O.V. Salo[1], I. Ries[2], A.L. Bovenberg[3], J.M. Driessen[1]
[1]University of Groningen, Molecular Microbiology, Groningen, [2]Leiden University, Center for Drug Research, Leiden, [3]DSM Biotechnology Center

Penicillium chrysogenum is well known producer of penicillins. To reach the production capacities required for the modern industrial application, this strain joined the unprecedented classical strain improvement program (CSI) more than six decades ago. Recent post-genomic era reviled the neglected potential of this fungus to produce other bioactive compounds than β-lactams. More than 20 metabolites derived from recently discovered secondary metabolite pathways only partially demonstrate the diversity encoded by the secondary metabolite gene clusters with not yet described properties. However, despite the available information about genetics and biochemistry of lactam biosynthesis, little is known about the impact of CSI on other secondary metabolite pathways available in this fungus. In this work we perform a comparative analysis of β-lactam producing strains that have been subjected to CSI from genome, transcriptome and metabolome perspective. The results of our analysis indicate the mutagenic impact of CSI on eight secondary metabolite genes out of 31 (20 PKSs and 11 NRPs) present in the genome. Additionally, we discovered that key Velvet complex proteins (LeaA and VelA) involved in global regulation of secondary metabolism in filamentous fungi, have been repeatedly targeted by the mutagenesis during the improvement program. Finally, altering of the SM genes expression and dramatic changes of the secondary metabolite profiles between the subjected strains allowed us to identify the PKS gene cluster potentially involved sorbicillinoids biosynthesis in *P. chrysogenum*.

O055

Metabolic engineering of Escherichia coli for itaconic acid production

K.S Vuoristo[1], A.E. Mars[2], J. Vidal Sangra[1], J. Springer[2], G. Eggink[2], J.P.M. Sanders[2], R.A. Weusthuis[1]
[1]Wageningen University, Subdivision Bioprocess Engineering, Wageningen, [2]Wageningen University and Research center, Subdivision Biobased Products, Wageningen

Interest in sustainable development has led to efforts to replace petrochemical-based monomers with biomass-based ones. Itaconic acid is a C5-dicarboxylic organic acid, which can be polymerized at a high conversion rate, and as such, a building block for the chemical industry with many potential applications. Itaconic acid has traditionally been produced by *Aspergillus terreus*, but as the process has not reached cost-efficiency in large scale, also recombinant production hosts such as *Escherichia coli* have been investigated. In the work of Li et al. (2011), a key gene for itaconate biosynthesis, *cis*-aconitate decarboxylase (*cadA*) from *A. terreus* was successfully expressed in *E. coli*, but product titre remained low. The purpose of our work was to study the itaconic acid production mechanism and thus increase the efficiency of the pathway in *E. coli*. We started by optimizing cadA expression conditions and also improved the availability of the precursors citrate and cis-aconitate by overexpression of heterologous citrate synthase and aconitase. The itaconate titre was further increased by deleting the genes encoding phosphate acetyltransferase and lactate dehydrogenase. These deletions in *E. coli's* central metabolism resulted in the accumulation of pyruvate, which is a precursor for itaconate biosynthesis. As a result, itaconate production in aerobic bioreactor cultures was increased up to 690 mg/L. The maximum yield obtained was 0.09 mol itaconate/mol glucose. Strategies for a further strain improvement will also be presented.

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O056

Heterogeneity within the mycelium of the cell factory *Aspergillus niger*

P. Krijgsheld, F. Wang, H.A.B. Wösten
Utrecht University, Department of Microbiology, Utrecht

Colonies of *Aspergillus* niger are characterized by zonal heterogeneity in growth, sporulation, gene expression and secretion. Wild-type colonies of *Aspergillus* niger grow and secrete enzymes at their periphery and in the central

zone but not in the intermediate zone. In the latter zone, secretion is suppressed by the sporulation pathway. For instance, inactivation of the sporulation gene *flbA* results in colonies that secrete proteins throughout the mycelium. The heterogeneity in growth, secretion and development in wild-type colonies implies that RNA and proteins should not be isolated from whole cultures to prevent that accumulation is levelled out by averaging zones with different activities. Indeed, inactivation of the sporulation gene *flb*A of *A. niger* does not impact expression of the sporulation gene *brlA* when RNA is extracted from whole colonies grown between porous membranes. Yet, there is an effect on expression of this master regulator of asexual development when different zones of wild-type and Δ*flbA* cultures are compared. Transcripts of *brlA* are absent in all zones of wild type *A. niger* colonies and in the intermediate and outer zones the Δ*flbA* colonies. In contrast, *brlA* is expressed in the center of Δ*flbA* colonies. This indicates that *FlbA* is part of the *brlA* regulatory network in the central zone of *A. niger* colonies but not in the middle and outer zones. Similarly, by analyzing expression of amylolytic genes in zones of colonies, we were able to deduce that an additional three transcription factors should be involved in regulation of starch degradation. This effect was masked by isolating RNA from whole colonies.

O057

Reduced soluble CD14 in neonates hampers efficient activation of dendritic cells by Hepatitis B surface antigen

N. van Montfoort[1], E. van der Aa[1], A. van den Bosch[1], H. Brouwers[1], T. Vanwolleghem[1], H. Javanbakht[2], A.M. Woltman[1]

[1]*Erasmus MC, Department of Gastroenterology and Hepatology, Rotterdam,* [2]*Infectious Diseases Discovery & Translational Area, Roche Innovation Center Basel, Switzerland*

Whereas 95% of adults clear infection with Hepatitis B virus (HBV) by mounting effective HBV-specific immunity, 95% of infected neonates develop chronic infection. The underlying mechanism is not known. We previously demonstrated that BDCA1+ mDC interact with HBV surface antigen (HBsAg) in chronic HBV patients. The purpose of this study was to examine the interaction of BDCA1+ mDC with HBsAg *in vitro*, investigate the receptors involved and compare the response between adults and neonates.

Exposure of peripheral blood-derived BDCA1+ mDC to HBsAg resulted in dose-dependent DC maturation, cytokine production and enhanced capacity to activate antigen-specific CTL. By using CD14 neutralizing antibodies, we identified a crucial role for CD14 in HBsAg-mediated maturation of DC, which was independent of CD14 expression on a small sub-population of mDC. HBsAg-mediated DC maturation was completely absent in serum-free cultures, however, could be restored when supplemented with FCS and plasma obtained from healthy adults, that both contain soluble CD14 (sCD14), indicating a potential role for sCD14 in HBsAg-mediated DC maturation. Notably, we showed that sCD14 directly interacts with HBsAg and that sCD14-HBsAg complexes can be detected in the serum of HBV-infected patients, but not healthy controls. Since the amount of sCD14-HBsAg complexes was correlated with the viral load and the concentration of HBsAg in the serum of patients, the clinical significance of sCD14 and sCD14-HBsAg complexes in chronic HBV patients needs to be further examined. Although neonatal mDC responded to HBsAg in the presence of FCS, neonatal cord blood contained significantly less sCD14 than adult plasma, which correlated with significantly reduced HBsAg-mediated mDC maturation.

We conclude that sCD14 is a pattern-recognition receptor for HBV. Furthermore, our findings suggest that reduced sCD14 in neonates is associated with a poor immune activation after perinatal HBV infection and may explain why perinatal HBV acquisition frequently results in persistent infection.

O058

A novel iridovirus causes scale drop disease in *Lates calcarifer* (Asian seabass)

A. de Groof[1], L. Guelen[1], M. Deijs[2], Y. van der Wal[1], M. Miyata[3,5], K.S. Ng[3], L. van Grinsven[1], B. Simmelink[1], Y. Biermann[1], L. Grisez[1], J. van Lent[4], A. de Ronde[2], S.F. Chang[3], C. Schrier[1], L. van der Hoek[2]

[1]*MSD Animal Health / Intervet International bv., Department Discovery & Technology, Boxmeer,* [2]*University of Amsterdam, AMC, Laboratory of Experimental Virology, Department of Medical Microbiology, Center for Infection and Immunity Amsterdam (CINIMA), Amsterdam,* [3]*MSD Animal Health Innovation Pte Ltd, Singapore,* [4]*Wageningen University, Laboratory of Virology and Wageningen Electron Microscopy Centre, Wageningen,* [5]*Technology Development, School of Applied Science, Temasek Polytechnic, Singapore*

From 1992 onwards, outbreaks of a previously unknown illness have been reported in Asian seabass (*Lates calcarifer*) kept in maricultures in Southeast Asia. The most striking symptom of this emerging disease is the loss of scales. It was referred to as scale drop syndrome, but the etiology remained enigmatic. By using a next-generation virus discovery technique, VIDSCA-454, viral sequences were detected in serum of diseased fish which appeared genetically distinct from known viruses. The near complete genome sequence was determined, which shows a unique

genome organization. Based on homology of a series of putatively encoded proteins, the virus is a novel member of the megalocytiviruses of the iridoviridae family. The virus was isolated and propagated in cell culture, where it caused a cytopathogenic effect in infected Asian seabass kidney and brain cells. Electron microscopy revealed icosahedral virions of about 140 nm in diameter and the presence of an inner intermediate lipid membrane within the capsid structure. *In vitro* cultured virus induced scale drop syndrome in Asian seabass *in vivo* and the virus could be reisolated from these infected fish. These findings imply that the virus is the causative agent for the scale drop syndrome, as each of Koch's postulates is fulfilled. We have named the virus Scale Drop Disease Virus. Vaccines prepared from BEI- and formalin inactivated virus, as well as from *E. coli* produced major capsid protein provide efficacious protection against scale drop disease.

O059

H7N9 and H6N1 influenza A virus hemagglutinins engineered to bind human type receptors reveal a novel layer of specificity beyond the a2-6 linkage of sialic acid

R.P. de Vries[1], W. Peng[2], R. McBride[2], J.C. Paulson[2]
[1]*Utrecht University, Department of Medicinal Chemistry a,d Chemical Biology, Utrecht,* [2]*The Scripps Research Institute, Cell and Molecular Biology, La Jolla, CA, USA*

Influenza A virus is a serious pathogen in livestock and humans. The virus resides in the wild waterfowl population but is able to cross the species barrier to various mammals. One part of this barrier is the recognition of sialic acid receptors. Avian viruses prefer binding to sialic acid with an a2-3 linkage to the penultimate galactose (avian type), whereas human viruses bind to sialic acid with an a2-6 linkage (human type). All previous human pandemics were of avian or swine origin and were accompanied by amino acid mutations in HA that shifted binding from avian to human type. With the continuous threat of avian viruses, H5N1, H6N1, H7N9 and H10N8, infecting humans that if adapted to bind human type receptors, could spark another pandemic, it is vital to know which amino acid changes are required in these subtypes for this specificity shift. Several assays have been developed to assess receptor-binding specificity of IAVs, all have some drawbacks such as being only qualitative, using only terminal parts of more complex glycans, but most importantly lacking the representation of biological relevant glycans found in the human lung. Recently it has been reported that the glycome of the human respiratory tissues contain highly complex N-linked glycans containing multiple Galb1-4GlcNAc (LacNAc) repeats on their antennae. To represent these glycans on our glycan-arrays we created a series of bi- and tri-antennary N-linked glycans with 2 to 5 LacNAc repeats by chemo-enzymatic synthesis. These structures were sialylated with either a2-3 or a2-6 linked sialic acid and printed on a glycan-array and then probed with human and avian recombinant hemagglutinins (HA). Although the paradigm of avian viruses binding to a2-3 and human viruses binding to a2-6 linked sialic acids was maintained. As shown for human H7N9 and H6N1 viruses not yet adapted to human type receptors, bound to avian type receptors. However when selective mutations were introduced in the conserved binding pocket, we were able to create H7 and H6 HAs that specifically bound to human type receptors. Inspection of the glycans bound revealed another layer of specificity towards complex N-linked glycans bearing 3 to 5 LacNAc repeats. These glycans were very similar to those bound by the human 2009 pandemic Cal/04/09 HA. Cal/04/09 did not bound to any glycan array before, as previous arrays did not contain these highly complex structures, but perhaps more importantly, Cal/04/09 transmits highly efficient by respiratory droplets between ferrets and human. We hypothesize that the arms of these N-linked glycan can bind to two monomers within the HA trimer, furthermore we are currently exploring the biological consequences of binding these highly complex structures by engineered H7N9 and H6N1 viruses.

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O060

Novel insights in the tropism of avian viruses: glycan specificities of coronaviral attachment proteins

M.H. Verheije[1], I.N. Ambepitiya Wickramasinghe[1], R.P. de Vries[2], A. Grone[1]
[1]*Utrecht University, Faculty of Veterinary Medicine, Department of Pathobiology, Utrecht,* [2]*Utrecht Institute for Pharmaceutical Sciences, Department of Medicinal Chemistry, Utrecht*

Coronaviruses (CoVs) are pathogens of both veterinary and human importance. Understanding their tropism is critical not only to prevent infections in their native host, but also to recognize the potential risk of these viruses jumping the species barrier. Interestingly, some coronaviruses of veterinary importance, including the chicken infectious bronchitis virus (IBV), use, like influenza virus, glycans as means of attachment to susceptible cells and subsequent entry. For IBV it is known that a2,3-linked sialic acid is required as a host surface factor to infect chicken cells. The glycan specificity of other avian coronaviruses, if any, is however yet unknown. In order to gain insights into the host and tissue tropism of avian coronaviruses, we first developed a unique avian tissue

microarray (TMA), comprised of tissues of 10 different bird species from the orders *Anseriformes* and *Galliformes*. Recombinantly expressed viral attachment proteins were used to profile the binding pReferences of S1 proteins of six different avian coronaviruses using protein histochemistry. While S1 of chicken, pigeon and partridge CoVs attached to the respiratory tract of the respective hosts, the S1 of turkey, quail, and guineafowl CoVs predominantly bound to intestinal tissues of their hosts. This binding is in accordance with the reported pathogenicity of these viruses *in vivo*. Interestingly, the binding of respiratory pathogens required the presence of sialic acids on the tissues, while turkey, quail and guineafowl CoV S1 could still bind to desialylated tissues. To define the glycan specificity of these S1s, glycan arrays were performed, showing that chicken CoV S1 recognized in particular a specific a2,3-linked disialoside. Blocking studies on the TMA revealed that IBV CoV S1 preferred binding to a2-3 sialic acid subtype I lactosamines. Strikingly, the binding of pigeon and partridge CoV S1 to tissues could not be blocked by this a2-3 sialylated lactosamine, indicating that these viral attachment proteins have a yet unknown preference for other sialosides. Furthermore, the glycan array revealed that turkey and quail CoV recognize a novel viral attachment factor. More specifically, binding was observed to galactose-N-acetylglucosamine repeats (LacNAc), which could also block the binding of these S1 proteins to tissues. Finally, we studied the distribution of these glycans across species and tissues on the TMAs by lectin histochemistry. Our data revealed that both glycan subtypes are expressed on respiratory as well as intestinal tissues of various avian species, although to different extend. The presence of attachment factors for these avian coronaviruses was further confirmed by protein histochemistry using our unique spike proteins. Taken together, our data indicate that the initial requirement of a virus to infect a novel host can be fulfilled. Whether avian coronaviruses truly have a broader species tropism than previously believed remains to be seen.

O061

Two cases of chromosomal integration of human herpes virus 6 in patients with idiopathic cardiomyopathy

F.M.H.P.A Koene[1], M. Haeseker[1], M. Hazebroek[1], A. Brink[2], I. van Loo[1]

[1]*MUMC, Department of Medical Microbiology, Maastricht,* [2]*PathoFinder, Maastricht*

Introduction: Chromosomally integrated human herpes virus 6 (ciHHV6) is a condition found in less than 1% of the healthy population. The complete genome of HHV6 is integrated into the host germ line genome and is inherited in a Mendelian manner. We present two cases with heart disease and a high viral load of HHV6.

Case description: Case 1: a 22 year old woman was referred from a peripheral hospital with left ventricle hypertrophy. The left ventricle function was 62%. There were no abnormalities seen with echocardiography. In the infectious diseases work-up a high viral load of HHV6 was detected, increasing in a 9 month period from $2^{*}10^4$ to $8^{*}10^5$ copies/ml.

Case 2: a 56 year old man was admitted to the hospital after an out of hospital cardiac arrest. Electrocardiogram showed a complete left bundle block. An additional cardiac magnetic resonance imaging showed a mid-myocardial septal late enhancement, which could be an indication for myocarditis. Four months after the cardiac event during follow up a left ventricle dysfunction (40%) was noted with normal left ventricle dimensions. In the infectious diseases work-up a high plasma load of HHV6 was found ($3^{*}10^4$copies/mL).

In both cases the question arose whether the presence of HHV6 was associated with acute infection, reactivation or ciHHV6. To differentiate ciHHV6 from acute infection, whole blood and hair follicle samples were analysed. In the index patients we found an HHV6 load in the whole blood samples of $8^{*}10^8$ and $4^{*}10^7$ copies/mL respectively and a cycle threshold of 29 and 26 respectively in the hair follicles, confirming both patients had ciHHV6.

The family members of the first index patient were tested for ciHHV6. The sister and the father of the index patient and 4 out of 5 sisters of the father were ciHHV6 positive. The brother of the father was ciHHV6 negative. However, the father, as well as the brother of the father, suffered from cardiomyopathy. The other ciHHV6 positive family members had no heart disease. In this family we determined by fluorescence in situ hybridization(FISH) that HHV6A genome was integrated in chromosome 17. The family members of the second index patient have yet to be tested and site of integration has to be determined.

Discussion: These cases show that ciHHV6 can be misdiagnosed as an acute infection of HHV6. However, ciHHV6 is easily diagnosed when hair follicle and whole blood samples are tested. Whether ciHHV6 has a significant role in viral myocarditis or cardiomyopathy is unknown. It is possible the viral genome could be triggered to replication or there might be a constant low level replication of the virus causing cell damage. To our knowledge there is only one study that has shown HHV6A integration in the telomeres of chromosomes during latency and is capable of producing virions. To find out whether ciHHV6 plays a role in cardiomyopathy, further research is necessary in these families, determining site of integration, active replication of HHV6 and response to therapy.

O062

Prevalence and incidence of HEV infection in the Netherlands: risk factors for donors and risk of plasma pool rejection

B.M. Hogema[1], E. Slot[2], K. van den Hurk[2], M. Molier[2], H.L. Zaaijer[2]

[1]*Sanquin Blood Supply Foundation, Blood-borne infections, Amsterdam*

The incidence of hepatitis E virus (HEV) infection in the Netherlands is very high. We previously estimated 1.05% of healthy blood donors are infected each year. The source and transmission route(s) of HEV are enigmatic, although pigs are the most likely major reservoir. To clarify the relation between meat consumption and HEV infection, we compared anti-HEV IgG seroprevalence between vegetarian and randomly selected donors. The age-adjusted seroprevalence was 24.0 and 12.4% respectively, and the seroprevalence was strongly age-dependent for both groups.

The incidence of HEV infection among blood donors was estimated by screening plasma donations used for production of solvent-detergent treated plasma (Omniplasma). Screening in pools of 96 donations started in January 2013. Screening 50,362 donations resulted in identification of 35 confirmed HEV-RNA positive donations from 33 donors. Donations made up to 60 days before and after a positive donation were not used for production and, if possible, were tested for presence of HEV-RNA. 27 of 72 donations could be tested, and 14 were HEV-RNA positive. The low viral loads were consistent with lack of detection during the screening in pools. Testing donations from donors with proven HEV infection enables us to more accurately estimate the viremic period and the number of positive donations. With these data, the risk that an SD plasma pool is rejected due to detection of HEV-RNA can be calculated. We estimate that 1 in 696 donations contains HEV-RNA, and that the viremic period is 69 days.

O066

The limitations and adverse effects of Evidence Based Recommendations in guidelines for infection control

J.A.J.W. Kluytmans

Amphia Hospital, LMI, Breda

Infection control in The Netherlands has been extremely successful. Especially, for the control of antibiotic resistance including a wide array of different resistance mechanisms and microbial species. The guidelines that are being used in infection control are largely based on expert opinion with few recommendations that are backed up by undisputable scientific evidence.

The reasons for this are manifold. First there is a lack of commercial interest for most of the preventive measures that limits the financial resources that are available to perform studies of the highest scientific quality. Second, many of the interventions rely on a multifaceted approach and the individual components of the so-called prevention bundles have not resulted in a significant reduction of the infection rate or have not been studied. Third, many of the preventive measures deal with behavior and/or compliance. These items are difficult to study in a randomized controlled design. Especially when behavior is changed during a prolonged period, a randomized controlled trial is not a suitable study design. Even cluster-randomized trials cannot always be used because a center that starts with a positive change of behavior cannot change back to the initial level once the behavior has changed.

As an example, I propose the Dutch MRSA control guideline based on the so-called "Search and Destroy Strategy (SDS)". This strategy was developed and used in the 1980's when the first cases of MRSA were detected coming form foreign hospitals. The SDS was based on the knowledge of the sources and transmission routes of *Staphylococcus aureus*, which had been studied extensively in the 1950's and 1960's.

Based on these microbiological observations, control measures were defined and incorporated in the National Guideline of the WIP. The recommendations today are essentially identical to the initial guideline in 1985 and the SDS is still a highly successful strategy. Many studies have been performed, trying to define the most effective control measures but the results have been contradictory. Other countries have always failed to control MRSA and report high rates of invasive MRSA infections, which are still rare in The Netherlands (<40 cases of bacteremia per year in the entire country in 2009). Some studies have demonstrated that it is possible to reduce high rates using hand hygiene or other basic hygienic control measures. But the resulting infection rates were still much higher than what is nowadays found in The Netherlands. MOst foreign hospitals have more cases of MRSA bacteremia per year than all Dutch hospitals together.

There are many other examples of recommendations that are not based on the highest level of scientific evidence that are associated with low rates of transmission or nosocomial infections.

The situation as described above should not be regarded as a motivation to stop performing well-designed trials to define the optimal infection control measures. These trials are important and deserve more funding as it helps us to preserve patient safety and often result in cost-effective recommendations. However, the recommendations should also be based on other kind of information, like micro-biological experiments and knowledge on the sources and

behavior of microorganisms. Also new trial designs that are suitable to study multifaceted interventions and the effects of behavioral changes should be considered more seriously in the current guidelines. This requires a high level of expertise on the combined aspects of microbiology, epidemiology, healthcare and infection control.

O067

Evidence based medicine for infection control: a curse in disguise? - The example of air quality in the OR. Disturbing the system: why laminar air flow may be ineffective in real life

J. Veenemans
Wageningen UR, Wageningen

Unidirectional flow systems, also known as laminar air flow systems, are used to guarantee optimal air quality in the operating room and are widely considered to be an essential element of strategies to prevent surgical site infections. Several international guidelines recommend the use of unidirectional flow (UDF) during the implantation of prosthetic devices, and the vast majority of Dutch hospitals currently have operating rooms that use this system. A recent update of the Dutch guideline for air quality control in operating rooms expresses 'a strong preference' to perform prosthetic joint surgery in operation rooms equipped with a UDF.

Although the advantage of UDF supplying ultra-clean air to the operating site seems theoretically obvious, the evidence in its favour is very limited and inconclusive: the only randomised trial comparing ultra-clean air systems to conventional ventilation showed a 60% reduction in the risk of prosthetic joint infection, but this study was conducted more than 30 years ago, and had several methodological limitations. Notably, the observed reduction in the incidence of infections due to ultra-clean air was largely restricted to patients not receiving pre-operative antibiotic prophylaxis. A meta-analysis of studies published between 2000 and 2011, and including approximately 200,000 procedures, shows that compared to conventional ventilation systems, UDF was in fact associated with *increased* infection rates following knee and hip prosthesis surgery (RR 1.36 [95% CI 1.06-1.74] and 1.71 [1.21-2.41], respectively). Although this meta-analysis is based on observational studies with inherent methodological limitations, these results certainly question whether the recommendations in current guidelines to implement the very costly unidirectional flow system are justified.

While UDF systems may work effectively at rest, the stability of the air flow in real-life circumstances will be compromised by multiple factors such as the presence of operating equipment (e.g. lights), movement of operating personnel in the air flow, door openings and forced-air warming blankets. The presence of these elements can in fact result in a higher level of air turbulence than that would have been present using conventional ventilation, providing a logical explanation for the contradicting findings outlined above. The role of several of these disturbing factors will be illustrated during this presentation.

O068

Evidence-based medicine – a curse in disguise? The example of timing of perioperative antimicrobial prophylaxis

M.S.M. van Mourik, M.J.M. Bonten
UMC Utrecht, Department of Medical Microbiology, Utrecht

Introduction: Antimicrobial prophylaxis surrounding surgical procedures is broadly accepted as an effective and necessary measure to prevent the development of surgical site infections (SSI), in particular for procedures associated with increased risk of infection or severe consequences of SSI. Despite this broad acceptance, recently developed draft guidelines have not formulated an explicit recommendation regarding the timing and redosing of antimicrobial prophylaxis due to a lack of high-quality evidence derived from randomized clinical trials. We aimed to provide an overview of the existing literature regarding timing and redosing of perioperative antimicrobial prophylaxis, identify gaps in knowledge and define elements necessary to answer existing questions.

Methods: Review of the published literature, focused on identifying studies applicable to currently accepted 'best practice'. Articles were identified through MEDLINE and cross-referencing. Available studies were evaluated regarding methodological quality and applicability.

Results: Randomized clinical trials focusing specifically on the issue of timing of antimicrobial prophylaxis were scarce. However, several observational cohort studies in different patient populations comparing different timing regimens were identified. These studies, however, are not always applicable to current 'best practices' as they compare different antimicrobial regimens, consider prolonged antimicrobial prophylaxis or assess various timing definitions. Given the observational nature of most studies, (residual) confounding cannot be excluded as – in general – protocol violations may be due to either the patient's underlying severity of disease or (emergency) scheduling of operations. Overall, however, there is evidence that antimicrobial prophylaxis is associated with an optimal time window to prevent SSI and redosing in prolonged procedures is desirable. In order to obtain more detailed and robust information, a carefully designed clinical trial may provide some answers. Important characteristics of such a trial include a design adapted to the limited degree of clinical equipoise (e.g. a stepped wedge

design), clear definitions and rigorous surveillance of SSI, the absence of simultaneous other interventions, and standardization infection prevention measures with accepted best-practices regarding duration of prophylaxis, *Staphylococcus aureus* decolonization, chlorhexidine washing, etc... Given the low incidence of SSI after (most) surgical procedures, the feasibility of such a trial may be hampered by the required sample size. Alternatively, we may choose to accept evidence from high-quality observational studies with sophisticated control for confounding, perhaps using propensity scores or instrumental variables. When translating evidence to policy, special consideration must be given to 1) whether guidelines should be restricted to randomized controlled trials, possibly leading to unnecessary vagueness in formulated recommendations and 2) whether process measures should be derived from circumstantial evidence regarding their association with clinical patient outcomes.
Conclusion: Perioperative antimicrobial prophylaxis has benefit in prevention of surgical site infection. Evidence for optimal timing and redosing of prophylaxis, however, is fragmented and often derived from observational studies, thus hampering the formulation of unequivocal evidence-based recommendations. Design of future studies could address gaps in knowledge by optimizing control for confounding and ensuring applicability of results to current 'best practices'.

O069

PCR based detection of *Tropheryma whipplei* carriership and strain variability in the Netherlands

T.T. Severs, R.A.V. Dolmans, S. van Marm, C.H.E. Boel, J.G. Kusters
UMC Utrecht, Department of Medical Microbiology, Utrecht

Introduction: Whipple's disease is a rare, potentially fatal multisystemic disorder resulting from infection with the rod-shaped, gram-positive bacterium *Tropheryma whipplei*. *T. whipplei* cannot be cultured on conventional media and only few laboratories are routinely screening for *T. whipplei* infections. Asymptomatic carriership is a precondition of Whipple's disease that is thought to be relatively common, but reliable data on the incidence of asymptomatic carriership of *T. whipplei* in the general population are sparse. The aim of our study was to investigate the incidence of asymptomatic *T. whipplei* carriership and to establish data on strain variability in the Dutch population.
Methods: 364 fecal samples from Dutch primary care patients with diarrhea, collected between July and November 2012 were included. The samples were tested for presence of *T. whipplei* DNA using three different PCR's: PCR 1, a probe-based PCR (53.3), developed by Raoult et al.; PCR 2, a PCR developed by Reischl et al., targeting the *rpoB*-gene; and PCR 3, a newly developed VNTR PCR (this study). To solve putative discrepancies between these PCR reactions the amplicons from the positive PCR reactions were sequenced by routine Sanger sequencing and the identity of the amplicon was established with a BLAST analysis against the NCBI database. Sequencing of the amplicon of PCR 3 was also used to establish the number of repeats of the VNTR sequence.
Results: The number of positive samples varied significantly between the three PCR reactions with 46/364 (12.6%), 23/364 (6.3%), 21/364 (5.8%) positives for PCR 1, 2 and 3 respectively.Only 19/364 (5.2%) samples were found positive in all three PCR tests. Sequencing data of the discrepant samples revealed that none of the 27 discrepant samples of PCR 1 contained sequences that were of *T. whipplei* origin. Also 3/4 discrepant samples for PCR 2 did not contain *T. whipplei* derived PCR products, while the PCR product of the 4th discrepant sample was *T. whipplei* specific.For PCR 3 sequencing of the two discrepant samples revealed one sample was truly positive (shared with PCR2) and the other was false positive. This suggests a true prevalence of 20/364 (5.5 %) asymptomatic carriers in the Dutch population. The number of VNTR repeats varied between 2 and 7 repeats and there was no single predominant type present.
Conclusion: To our knowledge this is the first report describing the prevalence of *T. whipplei* in a restricted number of fecal samples from the Netherlands. While PCR 2 and 3 seem to provide a reasonable accurate estimate of the incidence of *T. whipplei* PCR 1 seems less precise. The observed variation in the number of VNTR repeats suggests that there is considerable variation between circulating *T. whipplei* strains in the Netherlands. As the positivity rate is low (5.5%) the number of samples needs to be expanded in order to allow for a more solid conclusion.

O070

Both host immune status and complement resistance of non-typeable *Haemophilus influenzae* contribute to its ability to cause sepsis

J.D. Langereis[1], C. Puig[2], S. Marti[2], C. Ardanuy[2], J. Liñares[2], M.I. de Jonge[1]
[1]*Radboudumc, Laboratory of Pediatric Infectious Diseases, Department of Pediatrics, Nijmegen,* [2]*Hospital Universitari Bellvitge, IDIBELL-Universitat de Barcelona, Department of Microbiology, Barcelona, Spain*

Background: *Haemophilus influenzae* are bacteria that form part of our oropharyngeal microbiota where they reside without causing disease symptoms. However, these bacteria have also been associated with a wide variety of inflammatory diseases such as otitis media, pneumonia and sepsis. The introduction of the *H. influenzae* serotype

b (Hib) vaccine has decreased the number of sepsis cases caused by Hib drastically. Other *H. influenzae* serotypes, and non-typeable *H. influenzae* (NTHi) are generally associated with mild inflammatory diseases, however, since the introduction of the Hib vaccine, the numbers of sepsis cases by NTHi increased substantially. In this study we determined the contribution of the patient's immune status and the bacterial complement resistance in the ability for NTHi to cause sepsis.

Methods: Sixty oropharyngeal NTHi strains were isolated from oropharyngeal swabs taken from children between 1 and 5 years in a prospective point-prevalence study (2004-2005) conducted in Oviedo, Spain. The study was approved by the Ethics Committee from the Hospital Universitario Central in Asturias. Fifty-four blood NTHi isolates were collected in a six-year laboratory-based study (2008-2013) conducted at the Bellvitge University Hospital in the south of Barcelona, Spain. This study was approved by the Clinical Research Ethics Committee from the Bellvitge University Hospital (PR223/14).

NTHi was identified by mass spectrometry using MALDI-TOF Biotyper version 3.0. Differentiation between *H. influenzae* and *H. haemolyticus* was performed by PCR amplification of *fucK*, *iga* and *lgtC* genes. Capsular serotype was determined by PCR.

The level of complement resistance of the NTHi isolates was determined with 10% pooled normal human serum (NHS) or 10% heat-inactivated (HI)-NHS. NTHi was collected from overnight plates and diluted in Hanks' buffered salt solution (HBSS) without phenol red containing Ca^{2+} and Mg^{2+} plus 0.1% gelatin to 20.000 CFU/mL. Fifty µL bacterial culture was mixed with 50 µL 20% NHS or 20% HI-NHS diluted in HBSS without phenol red containing Ca^{2+} and Mg^{2+} plus 0.1% gelatin and incubated 1h at 37°C. Survival was determined by dividing the colony-forming unit (CFU) counts in NHS with the CFU count in HI-NHS. All experiments were conducted with the same batch NHS obtained from GTI diagnostics.

Results: Survival of NTHi blood isolates was significantly higher (20.8%) compared to oropharyngeal NTHi isolates (12.6%) ($p = 0.026$), indicating that complement-resistant NTHi strains were more often found in blood compared to the oropharynx. However, survival of NTHi isolated from patients with blood cancers (5.8%) and patients with neutropenia (5.2%) was significantly lower compared to oropharyngeal NTHi isolates, indicating that these patients were highly susceptible for sepsis caused by NTHi, even by strains sensitive to complement-mediated killing.

Analysis of complement resistant NTHi strains showed increased lipooligosaccharide size that decreased IgM binding and C3 opsonization.

Conclusion: Even though NTHi is usually linked to mild inflammatory diseases, both host immune status and bacterial complement resistance are important determinants of invasive disease, such as sepsis, caused by this bacterium. Therefore, NTHi should be considered as a potential invasive pathogen.

O071

Test of cure after treatment of anogenital *Neisseria gonorrhoeae* infection using nucleic acid amplification tests – a prospective cohort study

C.M. Wind[1,2], M.F. Schim van der Loeff[3,4], M. Unemo[5], R. Schuurman[6], A.P. van Dam[7,8], H.J.C. de Vries[1,2,4]

[1]STI Outpatient Clinic, Cluster of Infectious Diseases, Public Health Service Amsterdam, Amsterdam, [2]AMC, Department of Dermatology, Amsterdam, [3]Cluster of Infectious Diseases, Public Health Service Amsterdam, Amsterdam, [4]AMC, Center for Infection and Immunity Amsterdam (CINIMA), Amsterdam, [5]WHO Collaborating Centre for Gonorrhoea & Other STIs, National Reference Laboratory for Pathogenic Neisseria, Department of Laboratory Medicine, Microbiology, Örebro University Hospital, Örebro, Sweden, [6]UMC Utrecht, Department of Medical Microbiology, Utrecht, [7]Public Health Laboratory, Cluster of Infectious Diseases, Public Health Service Amsterdam, Amsterdam, [8]Onze Lieve Vrouwe Gasthuis General Hospital, Department of Medical Microbiology, Amsterdam

Introduction: Treatment failure due to antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* (Ng) is an emerging problem. Previously, Ng diagnostics and tests of cure (TOC) have relied on bacterial culture only. Current guidelines recommend the use of nucleic acid amplification tests (NAATs) to diagnose Ng. As a result, NAATs have rapidly replaced Ng culture in many settings. However, documented evidence for the timing of TOC using NAATs after treatment of gonorrhoea is lacking. We aimed to assess the appropriate timing of a TOC when using modern Ng NAATs.

Methods: In this prospective cohort study we included patients visiting the STI Clinic Amsterdam from March 2014 (ongoing) who were diagnosed with anogenital Ng, using NAAT (APTIMA Combo 2 assay [AC2], Hologic). One anatomical location was included per patient. Patients were treated with a single 500 mg intramuscular dose of ceftriaxone. Swabs and urine for an RNA-based NAAT (AC2) and a DNA-based NAAT (Cobas 4800 NG/CT assay [C4800], Roche), as well as samples for conventional Ng culture were collected prior to treatment. During 28 days following treatment patients collected self-samples on a daily basis for both NAATs, and recorded sexual contact and use of medication in a diary. After 28 days patients visited the STI clinic again to hand in their samples and diary. At this visit samples for an end-of-study direct culture and NAATs were collected. All NAAT samples were analyzed with AC2 and C4800 assays. Clearance was

defined as three consecutive negative results. A blip was defined as a positive test result after clearance. Re-infection was defined as the occurrence of three or more positive test results after initial clearance, whereby positivity was observed for at least one day in both the DNA and the RNA-based test.
Results: The preliminary data of 57 patients showed a median number of self-collected samples of 27. Anatomical locations were distributed evenly (urine: 20, vagina: 16, rectum: 21). Twenty-one (37%) patients had a *Chlamydia trachomatis* co-infection. All patients cleared the Ng infection during the study. The median time to clearance was 2 days for both NAATs. The time to clearance in 100% of the patients was 7 days for the AC2 and 15 days for the C4800. However, blips occured in 6 (AC2) and 9 (C4800) patients respectively. A re-infection was observed in one patient.
Conclusions: Median time to clearance with either a DNA- or an RNA-based NAAT method was 2 days after treatment of anogenital gonorrhoea.
Clearance rate of 100% was reached 7 days after treatment using an RNA-based NAAT, and 15 days after treatment using a DNA-based NAAT.
Isolated positive test results after clearance (blips) occurred in 11% (RNA-NAAT) and 16% (DNA-NAAT) of patients.

O072

Dynamics of the gut microbiota composition and resistome during prophylactic antibiotic therapy

T.D.J. Bello Gonzalez[1], E. Buelow[2], J. Bayjanov[2], W.A.A. de Steenhuijsen Piters[2], R.J.L. Willems[2], M.J.M. Bonten[2], M.W.J. van Passel[3], H. Smidt[1], W. van Schaik[2]
[1]*Wageningen University, Department of Microbiology, Wageningen,* [2]*UMC, Utrecht,* [3]*National Institute for Public Health and Environment, Bilthoven*

Introduction: Nosocomial infections in intensive care units (ICUs) are a frequent cause of patient morbidity and mortality. Several studies have shown that prophylactic antibiotic therapy such as Selective Digestive Decontamination (SDD) and Selective Oropharyngeal Decontamination (SOD) can improve patient survival during ICU hospitalization by preventing infections caused by opportunistic pathogens originating from the patient's own microbiota. However, the impact of prophylactic antibiotic therapies during ICU and prolonged hospital stay in critically ill patients on the composition of the gut microbiota and its reservoir of antibiotic resistance genes (the resistome) has not yet been studied in detail.
Methods: We evaluated the dynamics of the gut microbiota and gut resistome in 13 ICU patients (11 patients receiving SDD, two patients receiving SOD) and 10 healthy adults. By implementing, using 16S ribosomal RNA gene-targeted array-based phylogenetic fingerprinting and nanolitre-scale quantitative PCRs targeting a broad range of 84 different antibiotic resistance genes (ARGs), the gut microbiota composition and the resistome in critically ill patients and healthy adults was monitored.
Results: The microbiota composition of ICU patients is distinct from the microbiota of healthy adults and is characterized by changes in the abundance of the phyla Bacteroidetes and Firmicutes. At deeper taxonomic level, focus in the group of bacteria targeting by these antibiotic prophylactic therapies (*Staphylococcus, Enterococcus* and Proteobacteria); more pronounced differences of bacterial populations were observed between ICU patients and healthy adults. We observed that the levels of 13 ARGs, including genes that confer resistance to β-lactams and aminoglycosides, change significantly in the resistome of ICU patients compared to healthy adults.
Conclusions: Prolonged ICU hospitalization leads to a profound disruption of the gut microbiota and selects for β-lactam and aminoglycoside resistance genes, which may be associated with the use of these classes of antibiotics in SOD and SDD during ICU hospitalization.

O073

The post-vaccine microevolution of invasive pneumococcal disease

A.J.H. Cremers[1], F.M. Mobegi[1], M.I. de Jonge[1], S.A.F.T. van Hijum[1], J.F. Meis[2], P.W.M. Hermans[1], J.G. Ferwerda[1], S.D. Bentley[3], A.L. Zomer[1]
[1]*Radboudumc, Department of Pediatrics, Nijmegen,* [2]*Canisius-Wilhelmina Hospital, Department of Medical Microbiology and Infectious Diseases, Nijmegen,* [3]*Wellcome Trust Sanger Institute, Pathogen Genomics group, Hinxton Cambridge, United Kingdom*

Introduction: After the introduction of pediatric pneumococcal vaccination, rates of invasive pneumococcal disease (IPD) have decreased. However, the genetic background of the pediatric pneumococcal carriage population did not change dramatically in the US. We investigated whether vaccination has influenced population genomics in IPD in the Netherlands.
Methods: We serotyped and sequenced 350 strains of *S. pneumoniae* isolated between 2001-2011 from adult IPD patients in Nijmegen. After genome assembly, mapping, annotation and OG (orthologous group) assignment, a core genome was established. A phylogenetic tree deduced from the core genome's superalignment revealed tight clustering of isolates per serotype. Capsular switches were inferred from phylogeny in the core genome, and diversity was determined by phylogenetic distances between the accessory genomes.

Results: Upon the introduction of the 7-valent pneumococcal conjugated vaccine (PCV7) in 2006 a gradual decrease in vaccine serotypes and serotype replacement were observed, although capsular switches remained rare. The diversity of the accessory genome dropped shortly after the introduction of PCV7 ($p < 0.0001$). Genes that contributed to a re-expansion of diversity afterwards were comparable to those pre PCV7, although few genes dispersed from their prevalence in the original gene pool.
Conclusion: Despite serotype replacement in pneumococcal disease after pediatric vaccination with PCV7 we observed a temporary bottleneck in gene diversity, which re-expanded mainly by genes already present in the original gene pool. These observations show similar dynamics in pneumococcal population restructuring, both in asymptomatic carriage and IPD. We suggest the use of whole genome sequencing for surveillance of pneumococcal population dynamics that could give a projectile on the course of disease, facilitating effective prevention and management of IPD.

O074

Effect of factor H controlled alternative pathway activity on nasal tissue colonization and severity of invasive pneumococcal disease in mice

E. van der Maten, D. Westra, S. van Selm, J.D. Langereis, H.J. Bootsma, F.J.H. van Opzeeland, P.W.M. Hermans, R. de Groot, M.M. Ruseva, M.C. Pickering, L.P. van den Heuvel, N.C. van de Kar, M.I. de Jonge, M. van der Flier
Radboudumc, Laboratory of Pediatric Infectious Diseases, Nijmegen

Background: *Streptococcus pneumoniae* is one of the major human bacterial pathogens causing life-threatening infections. The complement system is an essential part of the innate host-defense against pathogens. Complement C3 opsonization plays a vital role in opsonophagocytic killing of Gram-positive bacteria. The alternative complement pathway amplification loop plays a crucial role in amplifying the initial activation of the classical and/or lectin complement pathways. We studied the effect of reduced or enhanced alternative pathway activity as a consequence of higher or lower complement factor H (FH) levels on susceptibility and severity of infection with the Gram-positive microorganism *S. pneumoniae*.
Methods: In experiment I, female C57BL/6 wild-type (FH +/+) mice were intravenously infected with a pneumococcal serotype 4 strain (TIGR4) followed by injection with purified human FH (n = 10). At 21 hours, blood bacterial titers and serum IL-6 and MIP-2 levels were determined. In experiment II, male C57BL/6 FH +/+ and FH +/- mice were infected intravenously with the pneumococcal TIGR4 strain (n = 10). FH +/- mice showed partial C3 deficiency due to increased complement consumption. At 19 hours blood bacterial titers and serum IL-6 and MIP-2 levels were determined. In experiment III, male C57BL/6 FH +/+ and FH +/- mice were infected intranasally with TIGR4 (n = 5). Nasal mucosal and tissue colonization were assessed by quantification of pneumococcal colony forming units in nasal wash and homogenized nasal tissue. The effect of serum FH levels on pneumococcal C3 opsonisation was assayed *in vitro*: bacteria were incubated in sera of mice with increased or decreased FH levels. Pneumococcal C3 opsonisation was measured by flow cytometry.
Results: In experiment I, increased FH levels resulted in significantly increased pneumococcal titers and significantly increased IL-6 and MIP-2 levels compared with the control mice. Excess FH resulted in reduced pneumococcal C3 opsonisation by alternative pathway activation. In experiment II, reduced FH levels in FH +/- mice resulted in significantly decreased pneumococcal titers and significantly decreased IL-6 and MIP-2 levels compared with FH +/+ mice. *Bacteria* incubated in sera of FH +/- deficient mice showed significantly enhanced C3 opsonisation by alternative pathway activation compared with FH +/+ sera. In experiment III, pneumococcal titers in nasal tissue were significantly decreased in FH +/- mice compared to FH +/+ mice.
Conclusions: In conclusion, (1) high FH levels, in mice treated with exogenously applied FH, leads to more severe pneumococcal disease and impaired pneumococcal clearance as a consequence of reduced pneumococcal C3 opsonization by the alternative pathway. (2) Decreased FH levels in FH +/- mice, enhances pneumococcal C3 opsonisation by increased alternative pathway activity resulting in improved pneumococcal clearance, despite the partial C3 deficiency. (3) Decreased FH levels in FH+/- mice enhances pneumococcal clearance from nasal tissue and thus reduces pneumococcal colonization. Altogether, these findings support the hypothesis that an individual's alternative pathway activity is associated with the susceptibility to and severity of invasive pneumococcal disease.

O075

Towards new antimicrobials for Clostridium difficile infection

F.H.J. Schuren, R.C. Montijn
TNO, Microbiology & Systems Biology, Zeist

Clostridium difficile associated diarrhea (CDAD, also called CDI for *C. difficile* infection) is a worldwide emerging health problem. The highest incidence of this disease occurs in elderly people often following an antibiotic treatment. Especially the high recurrence rate (up to 20%) after treating a first episode of this disease leads to the associated high healthcare costs and increased mortality. The spores

which this *Clostridium* species forms most likely play an important role in this recurrence: antibiotics for which the vegetative cells of this organism are sensitive cannot prevent this recurrence. Existing treatment options encompass the "old"antibiotics metronidazole and vancomycin and the recently introduced fidaxomicin, but none of these is active against spores. The strong increase in CDI and the unsatisfying efficacy of existing treatment options has led to a surge of novel treatment options which are currently under development. These include antibodies targeting toxins produced by *C. difficile*, probiotics and Fecal Material Transplant (FMT), based on replacing the patient's intestinal microbiota with that of a healthy donor. The high efficacy of FMT clearly indicates the importance of the environment: a changed intestinal microbiota after antibiotic treatment in which *C. difficile* can thrive and which the body apparently in many cases cannot restore. However, an abrupt change of this environment by FMT lead to complete restoration. The success of FMT opens up the possibility of other treatment options for CDI, not necessarily directly combating *C. difficile*, but also by modulating the microbiota environment. Another currently non-existing treatment option would be compounds which target the bacterial spore, e.g. by preventing spore germination. Since *C. difficile* spore germination depends on the presence of the germination inducer taurocholate, a metabolite of bile acids, a clear link between human intestinal physiology and metabolism and potential outgrowth of *C. difficile* appears to exist, which may also allow for novel treatment options. In the presentation developments which may lead to new antimicrobials against *C. difficile* will be discussed in more detail.

O076

Spore surface display and vaccine delivery

R. Isticato, L. Baccigalupi, E. Ricca
Federico II University, Department of Biology, Naples, Italy

Bacterial spores are peculiar cell forms differentiated by members of the *Clostridium* and *Bacillus* genera in response to nutrient starvation and to other unfavourable conditions. Once produced, spores are metabolically quiescent, highly resistant and able to persist indefinitely in conditions that would be lethal for normal cells. However, when spores are in environmental conditions favourable for cell growth, they germinate, originating cells able to grow and, eventually, to sporulate again. The peculiar structure and the resistance/stability properties of the bacterial spore has fostered its use as a biotechnological tool for a variety of potential applications. Spore formers have been widely used as hosts for the industrial production of enzymes and antibiotics, while spores have found applications as natural insecticides, biodosimeters, and health promoters in commercial products (probiotics) for human and animal use.[1] More recently, spores of the non-pathogenic species *Bacillus subtilis* have been exploited as possible vectors for the delivery of drugs, antigens and enzymes.[2] *B. subtilis* proteins localized on the spore surface have been used as carriers to fuse and anchor heterologous proteins. This recombinant approach has been used over the years to display several different antigens with various spore surface proteins as carriers.[1,2] In some cases the recombinant spores have been assessed as mucosal vaccines in animal models and proved able to induce specific and protective immune responses.[1,2] A non-recombinant approach, based on the spontaneous adsorption of molecules on the negatively charged spore, has been also developed and used to display enzymes and antigens.[2] The safety record of *B. subtilis* and the efficiency of the spore-based display system propose the spore as an attractive new tool for the development of next-generation mucosal vaccines. An overview on the use of spores to display antigens and enzymes will be presented.

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O077

Bacterial inner spore membrane proteomics

C.G. de Koster, L. Zhen, R. de Boer, W. Abhyankar, L.J. de Koning, S. Brul
Swammerdam Institute for Life Sciences, Mass Spectrometry of Biomacromolecules, Amsterdam

Bacterial spores cause major problems in the food industry due to their ubiquitous occurrence and their intrinsic high stress resistance characteristics. In addition some spore-formers are highly toxigenic such as *Clostridium botulinum* and, though to a lesser extent, *Bacillus cereus*. The anaerobic endospore former *Clostridium difficile* is an important human pathogen and one where the primary cause of disease is due to the transmission of spores. The germination of *Bacillus* and *Clostridium* spores in a favorable environment is usually triggered by germinants such as amino acids, purine derivatives, carbohydrates or alkali ions. Germination in response to nutrients is mediated by receptors that reside in the inner spore membrane. Important characteristics that can control transport of germinants to the inner membrane are protein identity, quantity, spatial distribution over the exosporium, spore coat and cortex. In *Bacillus subtilis* the germinant receptor is composed out of 3 proteins, A, B and C, encoded by a tricistronic operon. Three homologues gene clusters gerA, gerB and gerK code for germinant receptor proteins. Subunits A and B are integral membrane proteins, whereas C is a peripheral membrane protein. In *Clostridium* species germinant receptor binding is less well understood. Bioinformatics analysis of sequenced *Clostridium* species

revealed lower numbers of genes encoding germinant receptors than *Bacillus subtilis*, while in addition many incomplete operons were found. Genome comparison of sequenced *Bacillus* and *Clostridium* species indicates that GerA, GerB and GerK family homologues encoding the known germinant receptor units are absent in the genome of *Clostridium beijerinckii* and *Clostridium difficile*. Most of the proteins involved in spore germination such as the germinant receptors are present in or adjacent to the inner spore membrane. In *Bacillus subtilis* the inner membrane germinant receptor proteins are organized in a cluster denoted as the germinosome. Germinant receptor binding results in commitment of a spore to continue through germination. Commitment is associated with a change in permeability of the inner membrane such that H^+. Na^+ and K^+ are released followed by the release of the core Calcium dipicolinic acid (CaDPA) content. While germinant receptors have been identified in various *Bacilli* and *Clostridia* the mapping of proteins that constitute downstream signaling routes leading to opening of the CaDPA channels followed by degradation of cortex and spore coat is far from complete. How a germinant receptor activated by germinant binding could form a CaDPA channel is also unclear. A possibility is that germinants signal other hitherto unknown germinosome proteins.

Understanding of spore germination demands further experimentation to unravel the identity of important proteins in the cascade of molecular events that leads finally to outgrowth of spores. We have unrevealed the identity of many *Bacillus subtilis* and *Clostridium difficile* coat and exosporium proteins. To assess the identity of inner membrane proteins involved in *Bacillus subtilis* and *Clostridium difficile* germination we developed a mass spectrometry based proteomics method. We first isolated and analyzed the inner spore membrane fraction of *Bacillus subtilis*. To get access to the inner membrane protein complexes we removed the outer spore layers, id est coat and cortex. Next, we isolated the *Bacillus* inner spore membrane protein complement through differential ultracentrifugation. We identified various integral inner membrane proteins using a combination of liquid chromatography and Fourier transform ion cyclotron resonance mass spectrometry. We detected known members of the Ger family of germinant receptor proteins, the CaDPA channel proteins SpoVAC and SpoVAF, novel transporters, putative transporters and many uncharacterized proteins with a predicted trans membrane helix. Interestingly, we also detected membrane associated proteins such as the cortex-lytic enzyme SleB.

O078

Spore Challenges from the food industry

J.W. Sanders, R. Kemperman, A. van Zuijlen, B. Marthi
Unilever R&D, Vlaardingen

In the production of safe and stable foods bacterial spores are one of the key spoilage targets. They differ from other bacterial contaminants in the sense that spores can resist severe preservation methods such as sterilization and single cells are sufficient to spoil a food pack. Bacterial spores can be found on various food raw materials, in particular spices. For the future development of milder, less processed and less preserved foods it is essential to better understand the properties of the potential spoilage microflora. This will help to develop more specific identification methods, to evaluate the efficacy of novel antimicrobials in relation to the biodiversity and heterogeneity of possible contaminants and to identify targets for future preservation strategies. Examples of the above are:

- A strain specific (PCR based) detection method for the thermophilic spore former *Bacillus coagulans* was developed to find the root cause of a repeated contamination of a pasteurized acid sauce. This showed that only analysis of multiple variable regions could confirm the independency of different isolates. Therefore, multi locus sequence typing (MLST) is preferred for identification of isolates at the strain level.
- As the source and history of bacterial spore contaminants in food are never clear it is essential to consider both the biodiversity and spore-to-spore heterogeneity of spoilage agents. Single cell live imaging was used to show the delay of germination in a *B. subtilis* spore population and reduced growth speed of the resulting vegetative cells by tea components. Therefore, single cell live imaging is a valuable tool to evaluate potential novel preservatives.
- Heat inactivation studies on a multitude of *B.subtilis* strains (both lab strains and food isolates) identified two groups, with a 100-fold difference in spore heat resistance. A genome wide association study identified the genetic basis for sterilization resistance.

The future challenges for preservation research are the quantification of heterogeneity across strains and species, as numerical input for predictive modeling of spoilage. Integration of the available mechanistic knowledge on germination and preservation resistance will lead to the definition of validated markers for the characterization of novel isolates and the definition of targets for new preservation strategies.

O079

Salmonella outer membrane vesicles displaying high densities of pneumococcal antigen at the surface offer protection against colonization

K. Kuipers[1], M.H.D. Daleke-Schermerhorn[2], W.S.P. Jong[2], C.M. ten Hagen-Jongman[3], F. van Opzeeland[1], E. Simonetti[1], C. van der Gaast[1], A. Zomer[4], J. Luirink[2], M.I. de Jonge[1]

[1]Radboudumc, Department of Pediatrics and Laboratory of Medical Immunology, Nijmegen, [2]Abera Bioscience, Stockholm, Sweden, [3]VU University, Amsterdam, [4]Utrecht University, Department of Infectious Diseases and Immunology, Utrecht

Introduction: Bacterial outer membrane vesicles (OMVs) are attractive vaccine formulations because they combine the best of two worlds: (i) they have intrinsic immunostimulatory properties, like their parental cells but (ii) are non-living particles, and hence safer to use. In principle, heterologous antigens incorporated into OMVs will elicit specific immune responses, especially if presented at the vesicle surface and thus optimally exposed to the immune system. Recently, we developed an autotransporter Hbp platform, designed to efficiently and simultaneously display multiple antigens at the surface of bacterial OMVs. In this study, we explored the feasibility of our platform for vaccine development and present two approaches for a broadly protective pneumococcal vaccine.

Methods: Fragments of two *Streptococcus pneumoniae* proteins, PspA and pneumolysin, were surface displayed on Salmonella OMVs. Mice received three intranasal immunizations with OMVs with a two-week interval. Intranasal challenge with *S. pneumoniae* was performed three weeks after the final immunization and mice were sacrificed three days post-infection. Nasal tissue was isolated to determine bacterial loads and local IL-17A responses. PspA-specific IgG was measured in both nasal tissue and sera. Whole cell ELISAs with pneumococcal clinical isolates are being performed to determine the cross-reactivity of antibodies raised against the N-terminal part of PspA (a1a2).

Results: The current study showcases for the first time that intranasally administered *Salmonella* OMVs displaying high levels of antigens at the surface induced strong protection in a murine model of pneumococcal colonization, without the need for a mucosal adjuvant. Reduction in bacterial recovery from the nasal cavity correlated with local production of antigen-specific IL-17A. Furthermore, the protective efficacy, the production of antigen-specific IL-17A, and local and systemic IgGs, were all improved at a higher concentration of the displayed antigen. As only the a1a2 part of PspA elicited strong protection, we investigated which region(s) in a1a2 could mediate cross-protection between pneumococcal strains using a pneumococcal strain collection of 350 clinical isolates. Preliminary data indicate that cross-reaction between pneumococcal strains is mediated by two specific regions of a1a2, which remarkably, are variants present in almost all clinical isolates. As PspA is highly variable among serotypes, the percentage coverage of pneumococcal strains could be increased by combining the cross-reactive regions from different sequences for surface display on Salmonella OMVs. Another approach to increase coverage of a pneumococcal vaccine is to add additional antigens. We successfully displayed four conserved proteins, essential for pneumococcal infection, and we will test their efficacy *in vivo*.

Conclusion: Here we demonstrate for the first time that intranasally administered OMVs decorated with heterologous antigens at the surface 1. induce strong protection 2. which correlates with local IL-17A production. 3. This discovery highlights the importance of an efficient antigen expression system for development of recombinant OMV-based vaccines. In conclusion, our findings demonstrate the suitability of the Hbp platform for development of a new generation of OMV vaccines, and illustrate the potential of using this approach to develop a broadly protective mucosal pneumococcal vaccine.

O080

Active immunization with an octa-valent *S. aureus* antigen mixture in models of *S. aureus* bacteremia and skin infection in mice

D. Koedijk[1], S. van den Berg[2], J.W. Back[3], J. Neef[3], A. Dreisbach[3], J.M. van Dijl[3], I.A.J.M. Bakker-Woudenberg[3], G. Buist[3]

[1]UMCG, MMB, Groningen, [2]Erasmus MC, Department of Medical Microbiology and Infectious Diseases, Rotterdam

Proteomic studies with different *Staphylococcus aureus* isolates have shown that the cell surface-exposed and secreted proteins IsaA, LytM, Nuc, the propeptide of Atl (pro-Atl) and four phenol-soluble modulins a (PSMa) are invariantly produced by this pathogen. Therefore the present study was aimed at investigating whether these proteins can be used for active immunization against *S. aureus* infection in mouse models of bacteremia and skin infection. To this end, recombinant His-tagged fusions of IsaA, LytM, Nuc and pro-Atl were isolated from *Lactococcus lactis* or *Escherichia coli*, while the PSMa1-4 peptides were chemically synthesized. Importantly, patients colonized by *S. aureus* showed significant immunoglobulin G (IgG) responses against all eight antigens. BALB/cBYJ mice were immunized subcutaneously with a mixture of the antigens at day one (5 μg each), and boosted twice (25 μg of each antigen) with 28 days interval. This resulted in high IgG responses against all antigens although the response against pro-Atl was around one log lower compared to the other antigens. Compared to placebo-immunized mice, immunization with the octa-valent antigen mixture did not reduce the *S. aureus* isolate P load in blood, lungs, spleen, liver, and kidneys in a bacteremia model in which the animals were challenged for 14 days with a primary load of 3×10^5cfu. Discomfort scores and animal survival rates over 14 days did not differ

between immunized mice and placebo-immunized mice upon bacteremia with *S. aureus*USA300 (6 x 10^{5}cfu). In addition, this immunization did not reduce the *S. aureus* isolate P load in mice with skin infection. These results show that the target antigens are immunogenic in both humans and mice, but in the used animal models do not result in protection against *S. aureus* infection.

O081

Lack of pAp phosphatase leads to mislocalized cell division proteins in *Streptococcus pneumoniae*

C.G. Gallay[1], K. Kuipers[2], M. Rohde[3], V. Martínek[4], S. van der Beek[5], H. Venselaar[6], A. Zomer[7], H. Bootsma[8], M.I. de Jonge[2], J.W. Veening[1]

[1]University of Groningen, Department of Molecular Genetics, Groningen, [2]Radboudumc, Laboratory of Pediatric Infectious Diseases, Nijmegen, [3]Helmholtz Centre for Infection Research, Department of Medical Microbiology, Braunschweig, Germany, [4]Charles University, Faculty of Science, Department of Biochemistry, Prague, Czech Republic, [5]UMC Utrecht, Department of Medical Microbiology, Utrecht, [6]Radboudumc, CMBI, Nijmegen, [7]Utrecht University, Faculty of Veterinary Medicine, Department of Infectious Diseases and Immunology, Utrecht, Netherlands, [8]Centre for Infectious Disease Control, Bilthoven

Introduction: The Gram-positive bacterium *Streptococcus pneumoniae* is a major human pathogen responsible for severe diseases such as pneumonia or septicemia. With the emergence of antibiotic resistance and because all the strains are not covered by the vaccination programs, an interesting approach would be to target conserved pneumococcal proteins. Using genomic array footprinting, it was shown that lack of the protein SP1298 (Pde2), belonging to the DHH subfamily I, reduces pneumococcal virulence at various stages of disease. In the current study, we provide insight into the protein functionality and explain the reduced virulence of the inactivated SP1298 mutant.
Methods: A strain lacking *sp1298* was constructed in *Streptococcus pneumoniae* Tigr4. Conversion of 3'-phosphoadenosine-5'-phosphate (pAp) into adenosine 5'-phosphate (AMP) by purified recombinant SP1298 was analyzed using HPLC. Morphological observations were performed by scanning and transmission electron microscopy and by fluorescence microscopy using a membrane dye. Cell integrity was tested by ethanol, Triton X-100 and lysozyme assays. In order to study the divisome dynamics in absence of SP1298, division proteins DivIVA and FtsA were fused to the green fluorescent protein (GFP) to assess their localization by time-lapse microscopy.
Results: Based on sequence homology, SP1298 is predicted to be a pAp phosphatase, converting pAp into AMP and inorganic phosphate (Pi), which was confirmed in multiple enzymatic assays. It is known that pAp inhibits the ACP synthase (AcpS), responsible for lipid precursors production. Taking this into account, absence of SP1298 may limit the lipid precursors formation and thus disturb cell integrity. Morphological observations showed that the strain lacking SP1298 presents an aberrant morphology, illustrated by shorter chain formation, compared to the wild-type strain and exhibits a striking 'pointy' phenotype. We also showed that the mutant strain is delayed in growth and more sensible to organic solvent and detergent, consistent with cell envelope defects. To investigate the underlying mechanism responsible for this phenotype, we localized key proteins involved in cell division, e.g. DivIVA and FtsA. Surprisingly, division proteins are completely mislocalized in absence of SP1298, which could be indirectly caused by a disrupted membrane homeostasis.
Conclusion: Inactivation of SP1298, leading to the accumulation of pAp in the cell, results in severe morphological defects and a higher sensitivity for external stress. These observations are thus consistent with a disruption in the cell integrity of the bacterium. Furthermore, our recent data suggest that this protein is involved in division site selection in *S. pneumoniae*. Taken together, these results may help to develop new potential inhibitors to treat pneumococcal infections and to design future pneumococcal vaccines.

O082

Mycobacterium marinum can cross the blood-brain barrier via different migration routes

L.M. van Leeuwen

VUmc, Department of Medical Microbiology and Infection Control, Amsterdam

Tuberculous meningitis (TBM) is a major disease burden in children in TB endemic countries. To further understanding of early pathogenesis and possibly improve early diagnosis and treatment, we use our recently adapted zebrafish – *Mycobacterium marinum* infection model for TBM to study early granuloma formation in brain and meninges of zebrafish embryos. Here, we dissected the migration mechanism employed by *M. marinum* to cross the blood-brain barrier (BBB) and cause infection of the central nervous system. To study the role of macrophages in this process, we depleted the macrophage pool in zebrafish embryos with pu.1 morpholinos and clodronate filled liposomes. Embryos lacking phagocytic cells showed an increased overall infection rate and a higher bacterial load in the head at 5 days post infection in comparison to the control group. Detailed analysis showed that in the presence of macrophages, *M. marinum* leaves the bloodstream and forms early granulomas consisting of

mycobacteria and L-plastin positive phagocytes in brain tissue. Virtually all *M. marinum* inside and outside the blood vessels were present in phagocytic cells, suggesting that mycobacteria use macrophages as a Trojan horse to cross the BBB. As expected, we did not observe granuloma structures after macrophage depletion. However, we still found extravasated mycobacteria in the brain parenchyma. Most of these mycobacteria were found in close vicinity of blood vessels, following the blood vessel shape in a longitudinal manner. In conclusion, we observed that *M. marinum* preferably use macrophages as Trojan Horses to cross the BBB. However, in the absence of macrophages *M. marinum* can adept and seems capable of using additional migration routes.

O083

Pseudomonas aeruginosa **protease IV protects from MAC-dependent killing by primarily degrading C6**

E.T.M. Berends[1], L. Nijland[1,2], W.R. Miellet[1], D.A.C. Heesterbeek[1], M. Ruyken[1], S.H.M. Rooijakkers[1], B.W. Bardoel[1,3]

[1]UMC Utrecht, Department of Medical Microbiology, Utrecht, [2]Utrecht University, Faculty of Science, Department of Chemistry, Membrane Biochemistry and Biophysics, Utrecht, [3]Max Planck Institute for Infection Biology, Department of Cellular Microbiology, Berlin, Germany

Pseudomonas aeruginosa is a Gram-negative opportunistic human pathogen that causes severe infections in cystic fibrosis and immunocompromised patients. The complement system is an essential part of the innate immune system that protects against bacterial infections. Upon recognition of invading microbes it rapidly attracts phagocytes to the site of infection and opsonizes bacteria for phagocytosis. In the terminal pathway, formation of the Membrane Attack Complex (MAC, or C5b-9) directly eliminates Gram-negative bacteria. However, many bacterial pathogens evolved strategies to evade complement functions and enhance survival in the host.

Here, we identified that protease IV (PIV) from *P. aeruginosa* efficiently interferes with MAC functioning in the terminal complement pathway. We found that protease IV cleaves multiple complement components in serum including C2, Factor B, C4, C5, C6 and C7. However, C6 was degraded most efficiently by PIV at physiologically relevant concentrations. By cleaving C6 and C7, PIV efficiently blocks MAC effector functions. When comparing PIV to *Pseudomonas* elastase and alkaline protease, two other secreted pseudomonal proteases, we found that PIV most effectively prevents MAC-mediated killing of *P. aeruginosa*. Finally, PIV was able to protect a clinically relevant *P. aeruginosa* strain from the serum bactericidal activity.

Taken together, we identified protease IV as an inhibitor of the terminal complement pathway by degrading C6 and C7. PIV is the predominant protease secreted by *P. aeruginosa* in evasion of the MAC thereby protecting *Pseudomonas* strains against complement-mediated killing. The insights on secreted immune modulators by *P. aeruginosa* will increase our understanding of pathogenicity and provide new avenues for the development of treatments.

O084

Analysis of the molecular mechanism that is responsible for the induction of the antibiotic stress marker iniBAC in pathogenic mycobacteria

M. Boot[1], A.M. Abdallah[2], M. Sparrius[1], A.M. van der Sar[1], W. Bitter[1]

[1]VUmc, Department of Medical Microbiology and Infection Control, Amsterdam, [2]King Abdullah University of Science and Technology, Pathogen Genomics, Thuwal, Kingdom of Saoudi Arabia

Mycobacterium tuberculosis is the causative agent of tuberculosis (TB), an infectious disease that kills over 1.5 million people annually. Treatment of the disease consists of a combination of antimycobacterial drugs: ethambutol, isoniazid, rifampin and pyrazinamide. 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. One way to discover new antibiotics is to analyze the stress responses that bacteria encounter during treatment with sublethal concentrations of antibiotics. Insights into proteins that are involved in stress responses can subsequently lead to the discovery of relevant novel drug targets.

Previously, we examined which genes in *Mycobacterium tuberculosis* were active upon treatment with sub-lethal concentration of the four first-line antibiotics. RNA sequencing led to the identification of genes that were specifically up-regulated during antibiotic treatment *in vitro*. The *iniBAC* operon was specifically and highly induced after treatment with two different antibiotics that target the cell envelope, <ie. isoniazid (INH) and ethambutol (EMB). Subsequently, we cloned the iniBAC promoter in front of a fluorescent reporter. To determine which conditions resulted in the upregulation of the *iniBAC* operon, we performed random transposon mutagenesis on *Mycobacterium marinum* cells containing this indicator plasmid and identified colonies with increased expression. Analysis of the mutants revealed multiple hits in the vitamin B12 biosynthesis pathway and in the methyl-malonyl-CoA mutase proteins mutAB. MutAB is a key enzyme that plays a role in the degradation of proprionate to synthesize important precursors for

lipid biosynthesis and furthermore this enzyme is also vitamin B12 dependent. We propose that a mutation in *mutAB* leads to the build-up of toxic intermediates that are formed upon the degradation of proprionate and that the *iniBAC* operon is expressed to cope with these otherwise bactericidal molecules. *In silico* analysis of *iniA* and *iniC* shows that these genes contain the GTP-ase domains of dynamin-like proteins, possibly indicating vesiculation as a mechanism to shield the bacterium from toxic intermediates. Interestingly, *M. tuberculosis* has a disrupted vitamin B12 biosynthesis pathway and therefore probably grows, under commonly used laboratory conditions, under stress-inducing conditions. Inside the host *M. tuberculosis* incorporates vitamin B12 from the environment. Currently, we are determining the exact function of IniBAC in cell physiology by determining its location and effects of knockout mutations.

O085

Insights into the degradation of dietary plant toxins by insect gut microbiota

C.U. Welte, R. de Graaf, T. van den Bosch, J. Simon, H. op den Camp, M. Jetten

Radboud University, Department 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 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 of *D. radicum* contain enzymes that break down the isothiocyanates and are thus crucial for survival and phytopathogenicity.

We substantiated this hypothesis by isolating several 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. When we isolated and sequenced plasmid DNA of those strains it became apparent that they shared several genes which are prime candidates to encode enzyme systems that break down toxins. One of the interesting shared genes encoded a hydrolase of the beta lactamase family that may be involved in the breakdown of isothiocyanates. This protein (SaxA) was produced in *Escherichia coli*, purified and subjected to enzyme activity assays where it catalysed the breakdown of 2-phenylethyl- and allyl-isothiocyanate.

When we compared the *saxA* gene sequence to the metagenome of the *D. radicum* microbial gut community, it became apparent that on average about 5 % of the community members contained one copy of the hydrolase SaxA which matches well with the number of 8 % of 16S rRNA reads of the metagenome that mapped best to the microbial strains that showed to break down 2-phenylethyl isothiocyanate.

Taken together, the results of this study demonstrate for the first time that microorganisms are important for the breakdown of isothiocyanates in 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 utilizing the plasmid-encoded hydrolase SaxA.

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O086

Immunoglobulin A coating identifies colitogenic members of the microbiota in inflammatory bowel disease

M.R. de Zoete, N.W. Palm, T.W. Cullen, J.H. Cho, A.W. Goodman, R.A. Flavell

Yale University, Immunobiology, New Haven, USA

Specific members of the intestinal microbiota dramatically affect inflammatory bowel disease (IBD) in mice. In humans, however, identifying bacteria that preferentially affect disease susceptibility and severity remains a major challenge. Here, we used flow cytometry-based bacterial cell sorting and 16S sequencing to characterize taxa-specific coating of the intestinal microbiota with immunoglobulin A (IgA-SEQ) and show that high IgA-coating uniquely identifies colitogenic intestinal bacteria in a mouse model of microbiota-driven colitis. We then used IgA-SEQ and extensive anaerobic culturing of fecal bacteria from IBD patients to create personalized disease-associated gut microbiota culture collections with pre-defined levels of IgA coating. Using these collections, we found that intestinal bacteria selected on the basis of high coating with IgA conferred dramatic susceptibility to colitis in germ-free mice. Thus, our studies suggest that IgA-coating identifies inflammatory commensals that preferentially drive intestinal disease. Targeted elimination of such bacteria may reduce, reverse, or even prevent disease development.

O087

Gestational age influences intestinal microbiota development in preterm infants

R.D. Zwittink[1], D. van Zoeren-Grobben[2], R. Martin[3], R.A. van Lingen[2], L.J. Groot Jebbink[2], I.B. Renes[3], R.M. van Elburg[3], C. Belzer[1], J. Knol[1,3]

[1]*Wageningen University, Department of Microbiology, Wageningen,* [2]*Isala Klinieken, Paediatrics/Neonatology, Zwolle,* [3]*Danone Nutricia Research, Gut Biology and Microbiology, Utrecht*

Introduction: Early life microbiota development is fundamental for health in later life by affecting development of the gastrointestinal tract and immune system through host-microbe interactions. In early life, the intestinal microbiota is dynamic with increased susceptibility to host- and environmental factors. Preterm birth is associated with organ immaturity, hospitalisation, antibiotic treatment and formula feeding, which may impact the development of the intestinal microbiota in preterm infants. Our aim is to study the establishment and functionality of the intestinal microbiota of preterm infants born at varying gestational ages (GA).

Methods: Faecal samples from five extremely preterm (EP, 25-27 weeks GA) and five very preterm (VP, 30 weeks GA) infants were collected during the first six postnatal weeks. Faecal microbiota composition was investigated by 454 pyrosequencing of the 16S rRNA gene. To functionally characterise the intestinal microbiota, we studied the faecal metaproteome by LC-MS/MS.

Results: A temporal pattern in microbiota development is observed in all preterm infants, during which a highly diverse microbiota composition of meconium develops towards a *Bifidobacterium* dominated microbiota at postnatal weeks 3-6. At this time, *Bifidobacterium* spp. are significantly more abundant in VP than in EP infants ($p < 0.05$), indicating delayed colonisation with *Bifidobacterium spp.* in EP infants. Redundancy analysis reveals a different microbiota composition between VP and EP infants at postnatal weeks 3-6. Gestational age explains 23.4% of the variation ($p = 0.002$) and separation is mainly driven by *Bifidobacterium* spp. and *Streptococcus spp.* relating to VP and EP infants respectively. Faecal protein profiles of all preterm infants demonstrate a dominance of human proteins during the first six postnatal weeks. However, a rapid increase in microbial proteins is observed over time in all VP infants but not in all EP infants. In VP infants, proteins derived from *Bifidobacterium spp.* are most abundant, covering 68.5±10.8% of the total identified microbial proteins at postnatal weeks 3-6. Functionally, these proteins are mainly involved in milk fermentation. In EP infants, *Bifidobacterium* derived proteins are low at postnatal weeks 3-6 (4.8±5.3%) and fermentation processes are covered by facultative anaerobic *Streptococcus, Enterococcus* and *Klebsiella* species.

Conclusion: Intestinal microbiota development is different between infants born extremely preterm and very preterm. The transition towards a *Bifidobacterium* dominating state is delayed in EP infants compared to VP infants indicating that the degree of prematurity plays an important role in development and activity of the intestinal microbiota.

O088

Micelle PCR reduces artifact formation in 16S microbiota profiling

S.A. Boers[1], J.P. Hays[1], R. Jansen[2]

[1]*Erasmus MC, Department of Medical Microbiology and Infectious Diseases, Rotterdam,* [2]*Regional Laboratory of Public Health, Molecular Biology, Haarlem*

Introduction: The cornerstone of microbiota profiling is the sequencing of 16S rRNA PCR amplicons with next generation sequencing (NGS). The main disadvantage of this approach is the formation of PCR amplification artifacts, such as chimeric sequences that can lead to incorrect taxonomic identification and overestimated microbial diversity. Although chimeric sequences can be filtered out with specialized software after the PCR and NGS, the generation of chimeric products can still seriously reduce the amount of useful information obtained in a single sequencing run. Here we introduce a micelle based amplification strategy that greatly reduces artifact production during PCR amplification and subsequent NGS sequencing. Micelle PCR is a single-molecule clonal amplification method in which template DNA molecules are separated into a large number of physically distinct reaction compartments using a water in oil emulsion.

Methods: Universal 357F and 936R primers were used to amplify the 16S rRNA V3-V5 region from a synthetic microbial community containing equimolar 16S rRNA operon counts derived from 20 different bacterial species. Both micelle PCR and traditional PCR methods were used. Identical protocols were utilized for determining the microbiota for low-concentration DNA (nose swabs), high-concentration DNA (feces) and high-concentration DNA / hyper diverse (soil) samples. Amplicons were sequenced using 454 sequencing (GS Junior, Roche) employing two subsequent PCRs that is known to generate high percentages of chimeric sequences. The degree of chimera formation was determined using UCHIME. Microbiota profiles were determined by clustering operational taxonomic units (OTUs) with 97% similarity (MOTHUR).

Results: Micelle PCR generated 1.5% chimeric sequences and 20 OTUs in the synthetic community, compared to 56.9% chimeras and 70 OTUs using traditional PCR NGS sequencing. Chimeric products not recognized as actual chimeras were the cause of this overestimation, as most of the 70 OTUs were only found once. In addition, micelle PCR data exhibit an average 0.85-fold difference from the expected percentage in the synthetic community, with a maximum overestimation of 1.83-fold and a maximum underestimation of 0.20-fold. On the other hand, traditional PCR data showed an average 0.63-fold difference from expected percentage with an overestimated maximum of 2.48-fold and underestimated maximum of

0.03-fold. Similar results were obtained for nose swabs, feces and soil samples.

Conclusions: Micelle PCR drastically reduces chimera formation without the reliance on complex computational methods, resulting in improved microbial diversity estimates. In addition, micelle PCR prevents PCR competition resulting in highly reliable quantitative microbiota profiles.

O089

Challenges in ancient microbiome reconstruction using 16S rRNA gene amplification

K.A. Ziesemer[1], A.E. Mann[2], M.L.P. Hoogland[1], H. Schroeder[3], K. Sankaranarayanan[2], C.G. Warinner[2]

[1]Leiden University, Department of Archaeology, Leiden, [2]University of Oklahoma, Department of Anthropology, Norman, Ok, USA, [3]University of Copenhagen, Center for Geogenetics, Copenhagen, Denmark

Dental calculus, formed by periodic mineralization of dental plaque, is an ideal biomolecular reservoir as we seek to understand the ancient oral microbiome. To date, characterization of the ancient oral microbiome, as well as the ancient gut microbiome (i.e., coprolites), has primarily been accomplished through a phylotyping approach involving targeted amplification and sequencing of variable regions in the 16S rRNA gene. Specifically, the V3 region (*E coli* 341-534) of this gene, with a short amplification length and good taxonomic resolution, has been identified through *In silico* and *in vitro* analyses as an excellent candidate for ancient DNA amplification and community reconstruction. Nevertheless, in practice this phylotyping approach often results in unusual taxonomic frequency data. In this study, we use targeted (amplicon) and non-targeted (shotgun metagenomics) sequencing methods on four archaeological dental calculus samples to better understand these discrepancies. The four samples were chosen from diverse geographic and temporal contexts: Middenbeemster, Netherlands (159 BP); Guadeloupe, Caribbean (700 BP); Samdzong, Nepal (1900 BP); and Camino del Molino, Spain (4000 BP). Through comparisons of microbial taxonomic counts from paired amplicon and shotgun sequencing datasets, we show preferential amplification of archaea and the candidate bacterial phylum TM7 and underamplification of Spirochaetes and many important bacterial genera (e.g., *Streptococcus*) in amplicon datasets. Through informatics analysis, we demonstrate that extensive length polymorphisms in the V3 region are a consistent and major cause of amplification dropout and taxonomic bias in ancient microbiome reconstructions based on amplicon sequencing. We conclude that systematic amplification bias confounds attempts to accurately reconstruct microbiome taxonomic profiles using 16S rRNA V3 amplicon data.

O090

Enterococcus faecium genome dynamics during long-term patient gut colonization

J. Baan[1], J. Bayjanov[1], M. Watson[2], R.J.L. Willems[1], W. van Schaik[1]

[1]UMC Utrecht, Department of Medical Microbiology, Utrecht, [2]University of Edinburgh, Edinburgh Genomics, The Roslin Institute, Edinburgh, Scotland

Since the mid-1980s *Enterococcus faecium*, normally a harmless gut commensal, has emerged as an important nosocomial pathogen that has acquired multiple antibiotic resistances. Patients can be colonized with multidrug-resistant *E. faecium* during hospitalization. These bacteria can remain present in the intestinal tract for a long period of time after hospital discharge. Our aim is to analyze the genome dynamics of multi drug-resistant *E. faecium* isolates that colonized the gut of patients over a large timespan. The genomes of 96 isolates obtained over 8 years from 5 different patients were sequenced. We generated a core genome-based phylogenetic tree with these genomes, together with 70 previously published *E. faecium* genomes of diverse origins. Furthermore, we investigated highly similar subsets of patient isolates to describe micro-evolution in closely related strains. Whereas some patients were colonized for almost 2 years with a clonal population of *E. faecium*, other patients were colonized by a diverse population of *E. faecium* strains. Almost all isolates could be assigned to a hospital-associated sub-population of *E. faecium*. By studying the pan-genome of the strains in this patient isolate dataset, we identified gene gain and gene loss events. We also found evidence for recombination events, and for transmission of *E. faecium* strains between patients during hospitalization. This study highlights the different mechanisms that contribute to the genomic flexibility of clinical *E. faecium* strains. This may be a crucial factor in the ability of *E. faecium* to rapidly adapt to new ecological niches.

O091

Microbiota in Americans and Africans; the impact of diets

E.G. Zoetendal

Wageningen University, Laboratory of Microbiology, Wageningen

From a microbiological perspective, the past decades have been characterised by an explosive increase in studies focusing on the intestinal microbiota in health and disease, largely facilitated by rapid developments and the application of high-throughput culture-independent technologies, notably those using 16S ribosomal RNA or its encoding gene as a marker for bacterial identification. These studies have demonstrated that each individual has

a unique microbiota composition, with higher similarity within monozygotic twin pairs than random individuals. Despite this individual uniqueness, it is remarkable that the microbiota composition can be drastically different between human populations that are living at different continents, which was especially observed when populations from rural areas in Africa and other parts of the world are compared to those living in the Western world. Although diet- and lifestyle differences have been suggested to have a major impact on these differences in microbiota composition, it is not clear what the impact of these and other factors are as studies are generally based on baseline comparisons. Hence, it is evident that there is a need for dedicated dietary intervention studies in humans to differentiate between correlation and causality.

This seminar will provide an overview of comparative studies between microbiotas of African and Western populations in relation to their diets and lifestyle. Special attention will be given to a unique study in which the microbiota was compared when diets between rural African and African American populations were reciprocally exchanged.

O092

Benefits of and access to locally produced functional fermented foods in Africa

R. Kort[1,2,3,4] and W. Sybesma[1]

[1]*Yoba for Life foundation, Amsterdam,* [2]*TNO Microbiology and Systems Biology, Zeist,* [3]*VU University, Department of Molecular Cell Physiology, Amsterdam,* [4]*Micropia, Natura Artis Magistra, Amsterdam*

Diarrheal diseases and associated malnutrition remain a leading cause of mortality and morbidity of children in Sub-Saharan Africa.[1] The production and consumption of functional fermented foods with probiotics could be one avenue by which the health of the children can be improved. The process of fermentation contributes to food preservation and minimization of postharvest losses. In addition, fermentation can alleviate malnutrition by enrichment of food with vitamins, detoxification of raw materials and improvement of the bio-availability of nutrients.[3,10]

The effects of probiotics for the treatment of acute diarrhea have been documented in a 2010 Cochrane systematic review including 63 studies, which reports on a significant reduction in the mean duration of diarrhea and stool frequency already on the second day of treatment.[2] To our current understanding, the mechanisms of action of probiotics include the exclusion or inhibition of pathogens, either direct or through interactions with the commensal microbiota, the capacity to enhance the epithelial barrier function and the modulation of host immune responses.

Among the many currently marketed probiotics, *Lactobacillus rhamnosus* GG is one of the most widely studied and consumed. This strain has been selected as a probiotic strain, because of its resistance to acid and bile, relatively high growth rates and adhesion capacity to the intestinal epithelial layer.[4] A recent meta-analysis of fifteen RCTs (2963 participants) shows that *L. rhamnosus* GG significantly reduced the duration of diarrhea compared with placebo or no treatment when used at a daily dose of $\geq 10^{10}$ CFU.[9]

At present, probiotic products are mainly available in the western world (including Japan and Oceania) and not readily available or affordable to the majority of people in African countries. In order to generate access to probiotic strains in Africa, we have proposed the use of generic probiotics, for those strains for which the patent has expired.[8] In this context we refer to *L. rhamnosus* yoba 2012, which is the generic variant of *L. rhamnosus* GG.[4] After isolation, sequencing and comparison of the genome of the generic strain to that of the reported genome of *L. rhamnosus* GG ATCC5103,[8] we developed a stable dried bacterial starter culture that enables the fermentation of diverse foods, including milk and cereals, into functional fermented products.

We have shown that this starter culture has a high capacity to ferment milk and other ingredients,[7] which are part of the daily diet in Africa. As the *L. rhamnosus* yoba bacterium multiplies efficiently in milk and other ingredients under the condition used, the fermented product facilitates an intake of more than 10^{10} of probiotic bacteria per portion (*e.g.* 250 ml of fermented milk). The specially designed starter culture is suitable for long-term storage at ambient temperatures, which makes it convenient for use in rural areas in in Africa, without the need for a cooled distribution chain.

The introduction of the starter culture was accompanied by a social business model to allow farmers to profit from their sales by the controlled production of a healthy fermented milk.[6] The initiative is rapidly growing in east-Africa, including Uganda, Tanzania and Kenya. At present in Uganda, 30 dairy cooperations and small dairy enterprises produce more than 3,000 liters probiotic fermented milk per week, reaching at least 10,000 beneficiaries.

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O093

Akkermansia muciniphila: microbial cross feeding at the mucosal surface

L.W. Chia[1], S. Aalvink[1], H. Wopereis[1,2], J. Knol[1,2], W.M. de Vos[1,3], C. Belzer[1]
[1]*Wageningen University, Laboratory of Microbiology, Wageningen,* [2]*Nutricia Research, Utrecht,* [3]*University of Helsinki, Department of Veterinary Biosciences Department of Bacteriology & Immunology, Finland*

Despite the dynamic conditions in human gastrointestinal tract, the microbial population is found to be a stable and niche-specific ecosystem. This is maintained by the complex interplay between diet, host, and microbiota; in which microbial species can obtain energy from incoming food and host derived components, as well as microbial cross-feeding. As an example, bio-active molecules, such as short chain fatty acid (SCFA), produced by microbial fermentation can be used as substrate or growth factor among microbial entities. This study aims to elucidate the syntrophy relationship between key species at intestinal mucosal surface; specifically between the mucin-degrading *Akkermansia muciniphila* and the butyrate-producing bacteria *Anaerostipes caccae, Faecalibacterium prausnitzii* and *Eubacterium hallii*. Butyrate producers were tested for the ability to utilize mucin-derived metabolites, followed by co-culture experiment with *A. muciniphila*. Growth (optical density), metabolites (HPLC) and bacterial counts (q-PCR and FISH) were monitored. The results showed that *A. caccae, F. prausnitzii*, and *E. hallii* are not able to grow on mucin; but some of the mucin-derived sugars can be utilized by the micro-organisms with butyrate, lactate, and formate as the main metabolites. In addition, acetate promotes the growth and butyrate production of *A. caccae*. Furthermore, co-culture in basal media with mucin as sole carbon and nitrogen source supported the growth of both mucin-degrader and butyrate producers, with the abundance of 10^8 and 10^6 respectively. Succinate, acetate, 1,2-propanediol, propionate, and butyrate were produced. Interestingly, the co-culture of *A. muciniphila* and *E. hallii* produced relatively high amounts of propionate, indicating mutual benefits.
In conclusion, we demonstrated cross-feeding interaction between mucin degrader and butyrate producers, in which *A. muciniphila* degrades mucin into simple sugars and mainly acetate and propionate. Subsequently, the mucin-derived metabolites promote the growth of butyrate-producing bacteria. We hypothesize that at the mucosal surface many of these trophic relationship are taking place. Unravelling these will give insight into the mucus-associated microbiota and its potential health benefits for the human host.

O099

Peptidoglycan recycling – A major salvage pathway of bacteria and novel drug target

J. Gisin, A. Schneider, A. Duckworth, B. Nägele, S. Unsleber, I. Hinderberger, M. Borisova, C. Mayer
University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine Tübingen (IMIT), Department of Microbiology and Biotechnology, Tübingen, Germany

Bacteria recycle as much as half of the peptidoglycan (PGN) of their cell wall within one generation. Peptidoglycan (or cell wall) recycling has attracted much attention since it controls the expression of chromosomal-encoded AmpC-beta-lactamases (cephalo-sporinases) in pathogenic bacteria such as *Pseudomonas aeruginosa*. However, little is known about this pathway apart from the Gram-negative model organism *Escherichia coli*.[1] In this organism the N-acetylmuramic acid (MurNAc) 6-phosphate etherase MurQ was shown to be required for recovery of the MurNAc content of its PGN and, in addition, for growth on MurNAc as the sole source of carbon.[2,3] Intriguingly, however, many Gram-negative bacteria, e.g. all pseudomonades lack MurQ. Therefore, we hypothesized that an alternative recycling route is present in these organisms. We discovered a pathway that channels cell wall sugars directly to peptidoglycan biosynthesis[4] and further showed that interference with this pathway in *P. aeruginosa* results in an up to eight fold increased susceptibility to the antibiotic fosfomycin.[5] The novel PGN recycling route comprises an anomeric N-acetylglucosamine (GlcNAc)/MurNAc kinase (AmgK) and a MurNAc-α-1-phosphate uridylyl transferase (MurU) and bypasses the *de-novo* biosynthesis of UDP-MurNAc, the first committed PGN precursor, thereby contributing to intrinsic fosfomycin resistance. Thus, inhibition of PGN recycling can be applied as a new strategy for combinatory therapy against multidrug-resistant *P. aeruginosa*

strains. The novel recycling pathway is conserved within Gram-negative bacteria, excluding enterobacteria, but absent in Gram-positives.

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O100

Off the wall: from filamentous growth to primordial cells and back again

D. Claessen, K. Ramijan, M. Petrus, A. van der Meij, J. Willemse

Leiden University, Faculty of Science, Institute of Biology, Leiden

Streptomycetes are filamentous bacteria that grow by apical tip extension. This process is orchestrated by the tropomyosin-like protein DivIVA, which is present at hyphal tips. DivIVA interacts with various proteins, among which the cellulose synthase-like protein CslA. This protein synthesizes a β-(1,4)-glycan, which is thought to protect growing apices that are continuously being remodeled. To obtain further insight in the role of DivIVA and CslA in polar growth and morphogenesis, we have recently generated so-called *Streptomyces* L-forms that can grow without peptidoglycan. As a consequence, such cells are round and lack any obvious form of polarity. L-form cells have recently been suggested to resemble primordial cell, based on the observation that their growth and proliferation do not require the canonical cytoskeletal or cell division proteins. Instead, their proliferation can merely be explained by physical processes. However, our work on *Streptomyces* L-forms suggests that these cells require glycans, such as those formed by CslA, for their growth. Such glycans might have served for protection of early life forms, before the modern cell wall was invented. We have recently isolated an L-form mutant strain, which readily switches back and forth between mycelial and L-form growth. This mutant with the capability to re-synthesize peptidoglycan is crucial to understand which genes play an essential role in proliferation of L-forms, but also to unravel the mechanism underlying filamentous growth.

O101

FRET-based assay to study protein-protein interactions in the periplasmic space of *Escherichia coli*

N.Y. Meiresonne, P. Puri, T. den Blaauwen

University of Amsterdam, Department of Bacterial Cell Biology, Amsterdam

In Gram-negative bacteria many of the interacting protein domains of the cell division machinery as well as of major multidrug efflux pumps reside in the periplasm. So far, no *in vivo* assays are available to detect and measure these interactions. Here we present a Fluorescence Resonance Energy Transfer (FRET)-based method for studying protein-protein interactions in the *E. coli* periplasm. Periplasmic FRET experiments are challenging as protein folding and chromophore maturation are often hampered by the oxidative environment of the periplasm and translocating unfolded fluorescent proteins by the Sec translocon can be problematic. We could show that mNeonGreen is not only translocated to the periplasm but is also able to fluoresce in this oxidative environment. Expressing mNeonGreen in tandem with mCherry using a periplasmic localization signal resulted in high efficiency periplasmic FRET. mNeonGreen and mCherry were then used as FRET pair to study the *in vivo* oligomerization of the D-D-carboxypeptidase Penicillin-Binding Protein 5 (PBP5) as well as interactions between subunits of the AcrA/AcrB/TolC multidrug efflux pump. Fluorescence Lifetime Imaging Microscopy (FLIM) confirmed the FRET efficiencies of the periplasmic tandem and of PBP5 oligomerization. We conclude that periplasmic FRET using mNeonGreen and mCherry can be efficiently employed to investigate protein-protein interactions in the periplasmic space *in vivo*. Thus, our method constitutes a promising tool for studying the bacterial cell division complex as well as multidrug efflux pumps. It may also prove valuable in screening antibacterial compounds interfering with these structures.

O102

Effects of (l)antibiotics on *Bacillus subtilis* cell wall synthesis

D. Morales Angeles

University of Groningen, Department of Molecular Microbiology, Groningen

The cell wall is a giant macromolecule that surrounds the cytoplasmic membrane of bacteria. Peptidoglycan (PG) is the main component of the cell wall. The incorporation of PG into the cell wall is coordinated by two different machineries, the 'divisome' at the site of division, and the 'elongasome' along the lateral wall. We used various antibiotics to block different steps of PG synthesis in *Bacillus subtilis*. Special attention was given to the

action of lantibiotics, antimicrobial peptides that bind LipidII, the precursor molecule for PG. Fluorescent dyes were used to test the presence/absence and localization of LipidII and new PG after blocking PG synthesis. GFP-fusions were used to investigate the effects of cell wall synthesis inhibition on the localization of PBPs (Penicillin Binding Proteins). In addition, we studied the effects of (l)antibiotics on membrane integrity. In this talk, I will provide an overview of our findings

O108

HIV neutralizing antibodies induced by native-like envelope trimers

W. Sanders
AMC, Department of Medical Microbiology, Amsterdam

Inducing HIV-1 neutralizing antibodies against neutralization-resistant (Tier-2) virus strains has been a challenge. Our working hypothesis has been that stable native-like envelope trimers should induce neutralizing antibodies (NAbs). We show here that such trimers based on the pediatric founder virus BG505 (BG505 SOSIP.664 gp140), consistently induced neutralizing antibodies (NAbs) against the autologous, neutralization-resistant (Tier-2) BG505.T332N virus at high titers in rabbits and macaques. Cross-reactive NAbs against more sensitive (Tier-1) viruses were also induced. The Tier-1 and the autologous Tier-2 NAb responses were uncorrelated, implying that different pathways and B-cell subsets are involved. Tier-1 NAbs were depleted by linear V3 peptides, while Tier-2 NAbs recognized several conformational epitopes that differed between animals, that sometimes involved glycosylation sites and that were similar to some broadly active NAb epitopes. We have designed amino acid substitutions that further stabilize and antigenically improve BG505 SOSIP.664 trimers. The same substitutions also allowed the generation of stable native-like trimers based on virus isolates from clades B and C, including a clade B isolate from an elite neutralizer that developed broad neutralization within 9 months post seroconversion. Finally, we designed a stable native-like trimer that is able to interact with the germline versions of diverse bNAbs. These rationally designed trimers represent suitable starting points for lineage and/or polyvalent vaccines aimed at inducing NAbs able to counter diverse Tier-2 isolates.

O109

Broadly neutralizing antibodies in the fight against influenza

R. Vogels[1], T. Kwaks[1], B. Brandenburg[1], R.H.E. Friesen[2], J. Goudsmit[3]
[1]Janssen R&D Infectious Disease and Vaccines, Viral Vaccines, Leiden, [2]Biotechnology Center of Excellence, Janssen R&D, Spring House, Pa, [3]Janssen Prevention Center, Janssen R&D, Leiden

Influenza viruses have significant impact on public health and especially among high risk groups infection can result in hospitalization and death. Worldwide, the annual influenza epidemics cause about 3-5 million cases of severe illness and about 250,000 to 500,000 deaths (WHO fact sheet #211, March 2014). In addition, influenza virus poses a serious global threat due to the risk of a pandemic caused by a virus strain (in part) derived from an avian influenza virus by direct interspecies transmission or through exchange of genetic material between avian and human viruses. The hemagglutinin (HA) surface protein is the major antigenic protein of the influenza virus and required for entry of host cells. The HA envelope protein is a trimer with a membrane-distal highly variable head region that contains the receptor binding site and a much more conserved membrane-proximal stem region. In response to natural infection or following vaccination with influenza vaccines, serotype-specific neutralizing antibodies are generated against HA, preventing the interaction between the virus and the host cells and therefore internalization. Despite the massive effort to keep pace with the changing HA sequences and updating of the annual vaccines with the three seasonal virus strains, the vaccine efficacy is limited partly due to incidental mismatch of the vaccine sequence and the circulating influenza variant and partly due to the inefficiency of the human immune system (especially in elderly and immune compromised individuals) to mount a long-lasting protective response. For that reason, better treatment options and an improved vaccine able to induce broadly neutralizing antibodies (bnAbs) are long-sought solutions. Crucell/Janssen as well as other research groups succeeded in isolating human antibodies that neutralize many virus strains within or across influenza A group I and/or group 2, and influenza B. These findings proved that the virus has conserved epitopes that are sensitive to bnAbs raising hope for the design of a universal vaccine (reviewed in Ekiert&Wilson, 2012; Curr Opinion in Virology 2, p134). Importantly, in pre-clinical small animal models these bnAbs proved protective against a lethal dose of influenza virus both in prophylactic as well as therapeutic applications suggesting the monoclonal antibodies can be used as treatment modality. Detailed characterization and crystallography of HA-Fab complexes revealed the molecular binding sites and the mechanism of action (MOA) of the bnAbs. Most of these bnAbs were shown to bind to the stem region of HA which is much more conserved compared to the variable head region. Binding of the bnAbs to conformational epitopes in the stem region was shown to inhibit the fusion process between the viral membrane and the endosomal membrane thereby

abrogating infection. Some bnAbs were shown to inhibit virus replication through inhibition of cleavage of HA, a step necessary to enable the fusion process, or by inhibiting the egress of the newly formed viral particles (Brandenburg et al., 2013; PlosOne 8(12)). The detailed insight in the molecular interaction of the Abs and HA and the resulting inhibition of pivotal processes mediated by HA, shows nicely the structure-function relation of the HA molecule and the versatility of antibodies to intervene. In addition to the direct neutralizing effects, bnAbs were also shown to mediate interactions with Fc-receptors on immune cells *in vitro* and in pre-clinical animal models. Such indirect MOA can mobilize e.g. macrophages and NK cells to clear viruses and virus-infected cells, and are effective even in the absence of direct neutralizing activities.
The knowledge on the conserved epitopes and interaction of the mAbs will be very useful for the design of therapeutic and prophylactic drugs as well as for the design of antigens that elicit similar broadly acting antibodies following vaccination.

O110

Clinical utility of broadly reacting highly neutralizing antibody against RSV

T. Beaumont
AIMM Therapeutics, Infectious disease, Amsterdam

In the past couple of years, AIMM Therapeutics has established a technology, called AIMSelect™ that allows for the isolation of human monoclonal B cell lines that express the immunoglobulin on the cell membrane and secrete antibodies in the culture supernatant. The *in vitro* life span of the cells is enhanced by introduction of two genes, BCL-6 and BCL-xL, which inhibit terminal differentiation and promote survival, respectively. Monoclonal antibodies can be selected by screening supernatants of B cell clones for binding to the desired target or for functional activities. Palivizumab, a humanized monoclonal antibody directed against the RSV Fusion (F) protein, is approved and highly effective as passive immunoprophylaxis to prevent RSV disease in infants at highest risk; however, a significant unmet medical need remains for RSV prevention in full-term otherwise healthy infants. We have generated a panel of human monoclonal antibodies against human RSV A and B that were discovered by screening for their capacity to inhibit viral infection. D25, the most potent antibody, is currently being developed for the prevention of RSV disease in all infants, which is currently in Phase I/IIa clinical trial in premature infants. Studies of D25 in complex with the F protein showed that the epitope consisted of a quaternary, prefusion F specific structure. Solving the metastable prefusion form of the F protein elucidated the working mechanism of the protein and stimulated developments in RSV vaccine design.

O112

The neurovascular unit in health and disease

H.E. de Vries
VUmc, Department of Molecular Cell Biology and Immunology, Amsterdam

Optimal brain functioning is closely controlled by the specialized brain microvasculature which forms a physical and molecular barrier, the so called blood brain barrier (BBB), thereby protecting the brain. The specialized endothelial cells in the brain capillaries keep unwanted compounds out of the brain by forming the blood-brain barrier (BBB). The barrier is not a rigid structure but it actively regulates the regional homeostasis of the brain. Surrounding cell types dynamically regulate the barrier function, thereby forming the neurovascular unit. Particularly astrocytes are crucial in the induction and maintenance of the specialized phenotype of the brain endothelium.A number of neurological diseases are marked by the occurrence of inflammatory and oxidative-stress related events in the vasculature of the central nervous system (CNS). Pathological alterations in the integrity and function of the brain vasculature and its surrounding cell types are observed in a wide range of neurological diseases such as multiple sclerosis, stroke, brain cancer, and vascular dementia.
In multiple sclerosis (MS), a chronic inflammatory disease of the CNS, pathological hallmarks are the dysfunction of the blood-brain barrier (BBB) a, cellular infiltration, macrophage infiltration, demyelination, oligodendrocyte damage, gliosis and axonal damage and loss. In MS, leukocytes gain access to the CNS and cause massive tissue damage. Our studies have indicated that, already in early phases of MS, the function of the brain vasculature is impaired in a way that suggests that vascular pathology and subsequent leukocyte infiltration may pave the way for neurodegeneration in MS. Therefore, improving or repairing BBB function is a promising target to limit the process of neuro-inflammation The research focus of the De Vries group is therefore on understanding how alterations at the neurovascular unit underlie neuroinflammatory and neurodegenerative conditions to find novel ways to counteract these detrimental processes; topics that will be discussed within the presentation.

O114

***Tuberculous meningitis*; Histological lessons from South African patients**

S.D. Zaharie[1], C.S. van Dam[2], S.L. Roest[2], S.L. van Elsland[3], R. van Toorn[3], R. Solomons[3], M. van der Kuip[2], A.M. van Furth[2]
Stellenbosch University, [1]Department of Anatomical Pathology and [3]Department of Pediatrics/Child Health, Faculty of

Medicine/Health Sciences, Cape Town, South Africa; [2]VUmc, Department of Pediatric Infectious Diseases/Immunology, Amsterdam

Introduction: Tuberculous meningitis (TBM) is the most severe complication of pulmonary tuberculosis. Traditionally, its pathogenesis is described as the transpial rupture of a parenchymal granuloma with subsequent mycobacterial spill in the subarachnoidal space (*Rich focus hypothesis*). From a clinical point of view, magnetic resonance imaging allows differentiation between different central nervous system (CNS) granuloma, with specific granuloma types being indicative for a poor outcome of disease.

Aim: To describe different granuloma types and their CNS distribution in deceased TBM patients.

Patients and methods: Post-mortem brain specimens were stained with hematoxylin-eosin, Ziehl-Neelsen reagents and reticulin-silver in a historical (1975-2012) cohort of 90 children and adults (56% advanced stage (III) disease on admission).

Results: Granuloma were mainly located in the leptomeninges (87% vs 49% in parenchyma), with a combination of these two locations in 75%. We observed granuloma that were non-necrotizing (54%), necrotizing/reticulin positive (*gumma*; 22%) and necrotizing/reticulin negative (*pseudo-abscess*; 85%). Often a mix of granuloma were observed (57%). In 32% of patients, exclusively *pseudo-abscess* type granuloma were observed.

Conclusion: Patients presenting with advanced stage TBM who eventually die, show a predominance of necrotizing/reticulin negative granuloma or *pseudo abscess*, mainly and often solely (20%) occurring in the leptomeninges. Direct meningeal involvement without a primary parenchymal focus provides an alternative for the traditional *Rich focus hypothesis*. A tailored approach of the *pseudo-abcess* type granuloma might provide further insight in TBM pathogenesis and its (often detrimental) clinical outcome.

O115

Host-pathogen interaction at the intestinal mucosa correlates with zoonotic potential of *Streptococcus suis*

M.L. Ferrando[1], N. Willemse[1], W.J. van Rooijen[1], A. de Greeff[2], N. Stockhofe-Zurwieden[2], J. Nielsen[3], A. Heuvelink[4], Y. Pannekoek[1], H. Smith[2], A. van der Ende[2], C. Schultsz[1]

[1]AMC, Department of Medical Microbiology, Amsterdam, [2]Central Veterinary Institute, Lelystad, [3]National Veterinary Institute, Lindholm, Sweden, [4]GD Animal Health, Deventer

Streptococcus suis (SS) is an emerging zoonotic pathogen, which can cause severe disease including meningitis and septic shock in humans and pigs. *S. suis* serotype 2 (SS2) of MLST clonal complex 1 (CC1) is responsible for >90% of reported human cases. In The Netherlands, SS2 belonging to clonal complex 20 (CC20), also contributes to human disease. In contrast, human cases with *S. suis* serotype 9 (SS9), part of clonal complex 16 (CC16), the main cause of porcine SS infections in Northern Europe, were never reported. The consumption of pork meat, potentially contaminated with SS2, was identified as a risk factor for SS2 human meningitis, indicating that the gastro-intestinal tract (GIT) may serve as entry site of SS zoonotic infection. We aimed to provide experimental evidence using *in vivo* and *in vitro* models to show that *S. suis* is able to translocate across the porcine and human intestinal barrier. To better understand the zoonotic potential of *S. suis*, we compared the interaction of *S. suis* strains with different serotypes/genotypes with the host Intestinal Epithelial Cells (IEC). In addition, we evaluated the role of potential virulence factors in SS-host intestinal mucosa interaction. In particular, the surface Streptococcal adhesin P (SadP), which binds the Galα1-4Gal epitope present in the globotriaosylceramide (Gb3/CD77) host cell-receptor, was studied for its role in adhesion of zoonotic and non-zoonotic SS strains to the host-intestinal cells.

Methods: SS was orally administered into the stomach of piglets to study gastro-intestinal translocation *in vivo*. We compared *in vitro* interaction (adhesion/translocation) of invasive SS strains, isolated from patients and piglets and isogenic un-encapsulated mutant strains, with human and porcine IEC. The transcriptional expression of the most characterized virulence-related genes were measured by quantitative PCR during the SS2 and SS9 interaction with the IEC. SadP ko-mutants were generated and tested for their adhesion capacity to IEC.

Results: 24-48 hours after ingestion of SS2, two out of 16 piglets showed clinical symptoms compatible with SS infection. SS2 was detected in intestinal mesenteric lymph nodes of 40% of challenged piglets indicating SS intestinal barrier translocation.

SS2 strains showed significantly higher adhesion to human IEC compared to invasive strains with other serotypes. In contrast, invasive SS9 strains showed significantly higher adhesion to porcine IEC. Translocation across human IEC, which occurred predominately via a para-cellular route, was significantly associated with CC1, the predominant zoonotic genotype. Adhesion and translocation were dependent of capsular polysaccharide (CPS) production. During the SS2 interaction with IEC, *cps* genes were downregulated, whilst proteins involved in adhesion to host cell-receptors and extracellular matrix were upregulated. In particular, the expression of SadP was significantly increased in presence of human IEC. Sequence analysis of SadP of 111 invasive strains isolated from humans and pigs, revealed substantial variation across different serotypes/genotypes at amino acid (aa) level and the SadP

aa sequences clustered according to SS clonal complexes. *In vitro* analysis of SadP deficient mutants indicated that only SadP types associated with SS2 of CC1, contribute to the binding capacity of SS to human and porcine IEC.
Conclusions: SS2 should be considered a food-borne pathogen. SS interaction with human and pig IEC correlates with SS serotype and genotype. In addition to differences in capsule structure, differences in adhesin structure or in receptor affinity may explain the observed differences in adhesion of strains of SS2 and SS9 to human and porcine IEC, contributing to the zoonotic potential of SS2. The recognition of the porcine GIT as an infection site may allow the identification of novel tools for better intervention of SS2 infection in pigs and as zoonotic disease.

O116

Microdebate: Dual-Use in microbiological research, consequences and pitfalls of new regulations

KNVM board

To facilitate discussions on current dilemmas in microbiology the KNVM together with its scientific committee organizes its first annual debate during the scientific spring meeting. This Microdebate addresses a current topic concerning both science and society. The first Microdebate will focus on bio-security and Dual-Use of microbiological research.
In microbiology and life-sciences in general, dual-use research "encompasses biological research with legitimate scientific purpose, the results of which may be misused to pose a biologic threat to public health and/or national security." The overall biological technological advances especially in sequencing, synthetic biology, informatics and automation has increased the overall scientific insight and has opened up unforeseen/novel avenues to manipulate microorganisms. This rapid development of technology changes the microbiological landscape in an unpredictable way. Computational biology, systems biology, nanotechnology, and synthetic biology are in the forefront of new microbiological research. Discoveries and innovations happening today will lead to new applications and discoveries in the near future in unpredictable ways. In addition microbiological research occurs in an increasing international, open access and competitive scientific environment.
The dual-use research dilemma in the life sciences refers to the problem of producing and publishing research that is directed toward or intended to improve public health, animal health, or agricultural productivity, but that in the hands of a rogue state, terrorist group, or individual, could be used to impair public health. Although the use of biological agents for harm has been around for centuries, the collaborative nature in life sciences and current rapid advances and the relatively 'low tech' availability of materials and equipment to propagate a biological attack makes protecting information and materials from abuse much more challenging. The unpredictable nature of scientific advances makes effectively identifying research of concern extremely difficult and defining regulations nearly impossible. An update in the near future of current national regulations to meet European standards is expected in the Netherlands.
Representatives from scientific and governmental authorities are invited to participate in the Microdebate and to share their point of view on new regulations regarding Dual-Use research and bio-security. The consequences and impact of the new regulations for microbiological research in the Netherlands will be discussed with the representatives from scientific and governmental authorities and the public present at the debate.

O121

Metabolic modeling and multi-omics integration: new opportunities for environmental and clinical microbiology

M. Fondi
University of Florence, ComBo (Florence Computational Biology group), Florence, Italy

The ease at which genomes are currently sequenced has assigned to genomics one of the first steps in microbial systems biology. Regardless of the technique used, assembly and annotation typically follow genome sequencing and return an almost complete picture of the genetic reservoir of a given microorganism. One of the most important drawbacks associated with the booming of genomics resides in the possibility to (almost) automatically derive the potential metabolic landscape of a strain, given its genome. This is of great importance since bacteria continuously provide industry with novel products/processes based on the use of their metabolism and numerous efforts are being undertaken to deliver new usable substances of microbial origin to the marketplace, including pharmaceuticals, biofuels and bioactive compounds in general.
Metabolic modelling refers to a large plethora of *In silico* approaches that can be adopted to quantitatively simulate chemical reactions fluxes within the cell, including metabolic adjustments in response to external perturbations. In recent years the application of such computational technique to in depth investigate microbial metabolism has spread tremendously in microbiological research; indeed, genome scale models have revealed powerful tools to study a vast array of biological systems and applications in industrial and medical biotechnology, including biofuel generation, food production, and drug development. Well designed metabolic models can help predict the

system-wide effect of genetic and environmental perturbations on an organism, and hence drive metabolic engineering experiments.
An even more realistic picture of the metabolic traits of a given organism can be obtained by exploiting high-throughput data from innovative technologies such as transcriptomics, fluxomics, proteomics. Such diverse data types can also be mapped over metabolic models and, in this way, specific functional states derived. By exploiting gene expression data, for example, genome scale metabolic networks can be turned into condition specific models in which only those reactions corresponding to "active" genes will be present and active. Such implementations have been shown to provide detailed pictures of the actual metabolic state of a given cell leading to a deeper understanding of the basic cellular functioning and of the possible consequences of environmental perturbations (e.g. gene knock-outs, growth medium manipulation).
This talk will illustrate these concepts together with the possibilities they open up in current microbiological research, ranging from clinical to environmental areas. Finally, a specific study-case involving the system-level metabolic adaptation of the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125 to cold shock will be presented and discussed.

O122

A novel microbial metabolism in marine sediments: electrogenic sulfur oxidation by cable bacteria

J.S. Geelhoed[1], S. van de Velde[2], L. Burdorf[1], F. Meysman[1,2]
[1]*Royal Netherlands Institute for Sea Research, Department of Ecosystem Studies, Yerseke,* [2]*VUB, Department of Analytical, Environmental and Geo-chemistry, Brussels, Belgium*

Recently, a novel microbial mechanism of sulfur oxidation mediated by filamentous bacteria has been described for marine sediments. These so-called cable bacteria grow from the oxic sediment surface into the sulfidic layer of the sediment down to a depth of several centimeters. The oxidation of sulfide in deeper sulfidic sediment layers is coupled to oxygen reduction at the sediment surface, and in this process, electrons are transported from cell to cell along the length of the bacterial filament. Due to the spatial separation of redox processes, proton production and consumption also takes place in distinct layers, and a characteristic pH profile develops in the sediment. Marine sediments from different sites in The Netherlands and Belgium were examined for the presence and activity of cable bacteria. Microsensor depth profiles of oxygen, sulfide and pH can be used to detect sediments with strong metabolic activity of cable bacteria. We characterized the phylogenetic diversity of cable bacteria from these electro-active sediments, and used fluorescent in situ hybridisation and quantitative pcr to estimate their abundance. For a North Sea site where cable bacteria dominate the sediment geochemistry, the microbial community was studied using shotgun metagenomics. Metagenomic analysis was performed for different sediment depths: the oxic surface sediment, the suboxic zone where neither oxygen nor sulfide are present, and the deeper sulfidic zone. The results provide insight in the microbial community composition and functioning, and possible interactions between cable bacteria and other taxonomic groups.

O124

An *in vitro* characterization of the impact of prebiotics on gut microbiota from lean and obese donors

M. Aguirre[1], F.J. Troost[1], D.M.A.E. Jonkers[1], G. Roeselers[2], K. Venema[3]
[1]*Maastricht University, Department of Human Biology, Maastricht,* [2]*TNO, Department of Microbiology and Systems Biology, Zeist,* [3]*Beneficial Microbes, Consultancy, Wageningen*

The adoption of a modern/Western type lifestyle, characterized by a high consumption of energy-dense foods and reduced physical activity, has been accompanied by the growth of obesity in developed and industrialized countries. Still, the long-held belief of considering obesity as mainly associated with an imbalance in energy consumed when compared to expenditure, seems to be incomplete given the recent mechanisms proposed underlying obesity. Namely, growing evidence suggests a less simplistic event which involves a combination of factors including: environment, genetics, diet and lifestyle, adipose tissue and systemic inflammation. Moreover, the gut microbiota has been proposed as an environmental factor that plays a crucial role in obesity.
Obesity could lead to an important number of metabolic diseases that lead to increased morbidity and mortality which implies, besides a detrimental quality of life, high health costs. Therefore, a better understanding of the interaction of diet, microbiota and host are fundamental in recommending lifestyle and therapeutic approaches to tackle obesity in humans.
In vitro studies offer the great opportunity of examine microbe-microbe and microbe-substrate interactions in depth by carefully controlling all variables and avoiding host derived interactions. Hence, *in vitro* models closely mimicking the microbial metabolism in the human intestine can be used to get further insight in the complex mechanistic processes mediated by the gut microbiota.
In our most recent study we evaluate the impact of prebiotics on gut microbiota composition and activity using an *in vitro* model of the proximal colon: TIM-2. Such study adds to knowledge by suggesting that not all substrates are fermented in an identical manner by the gut microbiota, as

clearly shown by the different measurements of SCFA and BCFA observed in lean and obese microbiota pointing the possible implications in energy extraction if similar effects happen *in vivo*.

O125

Risk factors for the acquisition of OXA-48 producing Enterobacteriaceae in a hospital outbreak setting: a matched case-control study

M.J.D. Dautzenberg[1], J.M. Ossewaarde[2], M.J.M. Bonten[1]
[1]*UMC Utrecht, Julius Center for Health Sciences and Primary Care, Utrecht,* [2]*Maasstad Hospital, Department of Medical Microbiology, Rotterdam*

Introduction: In the context of a large outbreak of OXA-48 positive Enterobacteriaceae (OXA-E) in a Dutch hospital [Euro Surveill. 2014;19(9):pii=20723] we aimed to determine risk factors for acquisition of OXA-E using a matched case-control study design.
Methods: All patients included in this study were admitted to the Maasstad Hospital during the OXA-E outbreak period (July 2009 to July 2011). During this outbreak 72,147 patients were screened according to risk group classification based on assumed risk of acquisition of OXA-E using high-throughput PCR methods. Cases are defined as patients that had been hospitalized during the outbreak period and were detected as OXA-E carrier. Controls had also been hospitalized during the outbreak period, but had negative results in OXA-E screening tests. For each case three controls were selected.
To adjust for colonization pressure, controls were matched to cases based on hospital ward of culture obtainment, date of culture (+/- 1 week), and length of stay in the hospital during the outbreak period (LOS). Cases detected in post-discharge screening were matched similarly on hospital ward and date of last admission before culture obtainment, and LOS.
Potential risk factors were collected from the hospital information system and included age, gender, hospital admission and discharge dates, ward of admission, in-hospital antibiotic treatment (penicillins, cephalosporins, carbapenems, fluoroquinolones and other antibiotics) and Charlson Comorbidity Index (CCI). Data analysis was performed using multivariable conditional logistic regression.
Results: In total, 73 cases were detected, and 211 matched controls were included. Two potential cases were excluded, as no controls were available, and for one case only one control was available. Cases were 60.6% male, with a median age of 70.3 years (IQR 61.0-78.1), and a median CCI of 2 (IQR 1-3). Controls were 44.1% male, with a median age of 65.5 years (IQR 48.0-78.9), and a median CCI of 1 (IQR 0-2). Use of antibiotics before culture obtainment during the outbreak period was 69.0 and 52.5% for penicillins, 18.3 and 32.2% for cephalosporins, 2.8 and 4.3% for carbapenems, 47.9 and 28.9% for fluoroquinolones and 23.9 and 24.6% for other antibiotics, for cases and controls, respectively. Median LOS was 18.5 days (IQR 6.4-38.6) for cases and 11.2 days (IQR 5.1-26.6) for controls.
In the multivariable conditional logistic regression model, previous use of fluoroquinolones during the outbreak period (OR 2.18, 95% CI 1.05-4.55) was a risk factor for acquisition of OXA-E, and previous use of cephalosporins was protective (OR 0.35, 95% CI 0.15-0.79). Gender, age, CCI, previous use of carbapenems, beta-lactams or other antibiotics were not significantly associated with acquisition of OXA-E.
Conclusion: This hypothesis generating study suggests that use of fluoroquinolones was a risk factor for acquisition of OXA-E during a large hospital outbreak of OXA-E, and that use of cephalosporins was protective. These findings may be used to identify high-risk patients for screening and isolation in other OXA-E outbreak settings, but should – preferably – be validated in future studies.

O126

Environmental contamination with *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* (KPC-KP) during an outbreak in The Nederlands

V.A.T.C. Weterings[1], E. Thewessen[2], J. Kluytmans[1,3], J. Veenemans[1]
[1]*Amphia Hospital, Laboratory for Microbiology and Infection Prevention, Breda,* [2]*De Riethorst Stromenland, Geertruidenberg,* [3]*UMC Utrecht, Julius Centre, Utrecht*

Introduction: Between July and December 2013, an outbreak of a pan-resistant KPC-KPoccurred in a hospital and a nursing home in The Netherlands, with transmission occurring in both institutions despite implementation of contact precautions following national guidelines for the control of MDRO. Six patients were found positive for KPC-KP. One transmission occurred in the nursing home after all KPC carriers were isolated in a separate ward with dedicated staff. Further dissemination was eventually stopped by cohorting all KPC-carrying patients in a separate facility. Here, we describe the results of the environmental screening cultures collected during the outbreak at all locations.
Methods: To assess the extent of environmental shedding by the index patient during patient care, solid agar plates (n = 9) were placed in the hall, anteroom, next to the bed, on the windowsill and on the bathroom sink. The agar plates were opened for 1.5h, when the index patient received physiotherapy and tracheostomy care. This included suction of secretions and rinsing of the inner cannula of the trachea cannula in the bathroom.
In the nursing home, a range of high touch surfaces (e.g. night cabin, doorknob) and equipment (e.g. glucose

meter, patient lift) were sampled using 10x10cm sterile gauzes moistened with sterile saline. In the separate location, environmental cultures (gauzes) were taken nine times in ten weeks from predefined items (n = 25). Gauzes were placed in 10mL selective broth (TSB-VC) that was inoculated after overnight growth at 35-37°C on two selective media (Colorex KPC, CHROmagar, BioTrading Benelux, the Netherlands; EbSA plate, Cepheid Benelux, Belgium) and again grown overnight at 35-37°C. Identification and susceptibility testing was performed for all oxidase negative isolates.

Results: In the hospital, KPC-KPwas detected in the room of the index patient on three of nine sedimentation agar plates (2x next to the bed and on the sink). In the nursing home, KPC-KP was identified in 4/24 environmental samples (2x night cabins, doorknob and glucose meter). Notably, the glucose meter was shared by KPC-carriers and non-carriers. In the separate location, a high level of environmental contamination of KPC-producing Gram-negative bacteria (32/134 samples) was found in patient rooms at the two patient lifts, and less frequently in communal areas. In addition, KPC-producing *E.aerogenes* (2x patient rooms, kitchen worktop) and KPC-producing *A. baumanii* complex(glucose meter) were detected.

Conclusion: We found extensive environmental contamination in all settings. Our findings add to growing evidence that *Klebsiella* spp. are found in the environment more frequently than other coliforms, and show that the environment can act as important reservoir for transmission during KPC-KP outbreaks. The ability to contaminate and persist in the environment may explain its high transmission potential, and must be taken into account in environmental cleaning procedures. Lastly, air sedimentation cultures showed environmental shedding of the outbreak strain in the (dry) patient environment, indicating that healthcare workers and environment may be contaminated by indirect contact. To what extent the latter contributes to transmission is yet unclear, but it is certainly an aspect that is not taken into considerations in many infection control guidelines.

O127

Effect of prevention of direct contact between pigs on spread of *Streptococcus suis* serotype 9 in a pig population

C.N.T. Dekker, A. Bouma, A.J.J.M. Daemen, D. Klinkenberg, L.A.M.G. van Leengoed, J.A. Wagenaar, J.A. Stegeman

Utrecht University, Faculty of Veterinary Medicine, Infectious Diseases & Immunology, Utrecht

Introduction: The spread of an infectious agent in a population can be reduced by interfering in the infectiousness or susceptibility of individuals, and/or in their contact structure such as to prevent direct (unprotected) contact between individuals that are present in the same room. The aim of our study was to quantify in an experimental setting with the pig as host and *Streptococcus (S.) suis* as infectious agent the effect of prevention of direct contact between infectious and susceptible individuals on transmission.

Methods: In three replicate experiments, *S. suis*-free pigs were housed in boxes either in pairs (25 pairs in total) or alone (15 pigs in total). The distance between boxes (i.e. 80 - 100 cm) was sufficient to prevent direct contact between pigs in different boxes. At 7 weeks of age, one pig of each pair was inoculated in the nose with 5 mL saline containing 1 x 10^9 colony forming units *S. suis* serotype 9. The other pigs were exposed to *S. suis* by either direct (pairs) or indirect contact (individually housed pigs). Colonization with *S. suis* was monitored by collecting tonsillar brush and saliva swab samples regularly from all pigs for 4 weeks post inoculation, and culturing them on selective media. The parameter estimates derived from these experiments were used in a mathematical simulation model to study the effect of separation of pigs in a conventional farm.

Results: All inoculated pigs became infected, and their pen mates became colonized within 2 days. Thirteen indirectly exposed pigs became positive within 7-25 days after exposure. The rate of transmission between pen mates, i.e. direct transmission, was estimated to be 3.58 (95%CI: 2.29-5.60) secondary cases per infected pig in a susceptible population per day on average. The rate of transmission between the pairs and the in individually housed pigs, i.e. indirect transmission, increased in time, depending on the cumulative number of days pigs in that same room tested positive for the presence of *S. suis*. The resulting parameter estimate was 0.001 (95%CI: 0.0006-0.0017) new infections per pig per day for each day that an infected pig was tested positive for *S. suis*. The average transmission rate during the experimental period was approximately 36 times lower for indirectly compared to directly exposed pigs. In the simulation exercise using parameter estimates and conventional farm settings, all pigs were *S. suis* positive within 14 days after introduction of one infected pig in a room, regardless of whether the litters were kept separated or mixed in groups.

Conclusion: 1. Based on the data collected with the animal model we conclude that prevention of direct contact reduces the rate at which susceptible pigs become colonized with *S. suis*.

2. Simulation studies using these parameters showed, however, that such intervention measure would not limit *S. suis* serotype 9 spread in a commercial pig farm to a relevant extent.

O128

Expansion of the population of successful methicillin-resistant *Staphylococcus pseudintermedius* lineages and the dissemination of antimicrobial resistant phenotypes

B. Duim, K.M.H.W. Verstappen, E.M. Broens, J. Hordijk, A.L. Zomer, M. Mirlin, A. Timmerman, J.A. Wagenaar
Utrecht University, Faculty of Veterinary Medicine, Infectious Diseases and Immunology, Utrecht

Introduction: Methicillin-resistant *S. pseudintermedius* (MRSP), often multi-drug resistant (MDR), emerged recently and is of concern for both animal health, regarding treatment failure and public health for being a reservoir of resistance genes and the zoonotic potential. MRSP is a major canine opportunistic pathogen that typically causes skin and wound infections. Knowledge of the presence and distribution of specific MRSP lineages over time, their antimicrobial resistance phenotypes and association with certain clinical conditions may help to understand the emergence and spread of MRSP among dogs. In our study we investigated the molecular epidemiology of MRSP lineages in the Netherlands over a 20 years period; i) including the MRSP/MSSP ratio and association with clinical disorder; ii) and the dynamics of the antimicrobial resistance phenotypes.
Methods: From the database of the Veterinary Microbiological Diagnostic Centre (VMDC) of Utrecht University, all phenotypic resistances and clinical data of *S. pseudintermedius* isolates between 1993 and 2014 were collected. We performed Multi Locus Sequence Typing (MLST) of 478 MRSP isolates and determined the population structure of the Dutch isolates with eBURST and compared it with the population based on the global MLST database.
Results: MRSP was first isolated in 2004. The annual ratio of the number of isolated MRSP to methicillin-susceptible *S. pseudintermedius* (MSSP) in dog increased after the first isolation from 0.9% to 8%. The majority of isolates were associated with pyoderma (MRSP 44%/MSSP 19%), wound infections (MRSP 20%/MSSP 9%) and otitis externa (MRSP 19%/MSSP 37%). We identified 39 sequence types (ST) belonging to 4 clonal complexes (CC) and 15 singletons. CC71 was, as in other countries the dominant lineage, but the population expanded with CC258, CC45 and unlinked isolates. The MDR phenotypes were highly diverse. Over time a shift towards more susceptible phenotypes was observed. This is caused by the presence of CC71 and CC45 isolates, which are occasionally less resistant, and the emerging CC258, a generally less resistant lineage.
Conclusion: Our data indicate diversification of the MRSP population in ten years and dynamics in the antimicrobial resistances. CC71 MRSP in the dog population emerged since 2004 and has partly been replaced by other lineages.

O129

Presumed nosocomial transmission events of Livestock-associated MRSA are confirmed by high resolution typing

T. Bosch[1], F. Landman[1], M. van Rijen[2], A. Haenen[1], J. Kluytmans[2], L.M. Schouls[1]
[1]*RIVM, Centre for Infectious Diseases Research, Diagnostics and Screening, Houten,* [2]*Amphia Hospital, Breda*

Introduction: In 2003, a novel MRSA variant associated with pigs and denominated LA-MRSA, emerged in the Netherlands. Currently, around 30% of all MRSA isolates submitted for typing in the Dutch MRSA surveillance program are LA-MRSA. One of the controversies regarding LA-MRSA is its ability to transmit between humans. Different studies have shown that transmission of LA-MRSA between humans seems to occur less frequently compared to other MRSA variants, particularly in the nosocomial setting. However, in most studies low-resolution spa-typing or PFGE was used, making it uncertain if transmissions of LA-MRSA actually occurred. Here, we investigated presumed nosocomial transmission events of LA-MRSA using the high-resolution typing technique whole genome mapping (WGM).
Methods: A total of 86 LA-MRSA isolates collected between 2008-2012 were suspected to be involved in 49 nosocomial transmission events based on available questionnaires of 9698 *S. aureus* isolates submitted for typing in the Dutch MRSA surveillance program. In total, 27 presumed nosocomial transmission events involved LA-MRSA isolates (n = 61) that were indistinguishable with current molecular typing techniques, spa-typing and MLVA. Of the presumed nosocomial transmissions, 23 concerned a single transmission event, while in four cases multiple events were linked to the index case. In addition, four presumed transmission events (n = 8), originating from a study on community-acquired MRSA in 17 hospitals, were also included in this study. WGM of the 69 LA-MRSA isolates was performed as described before.
Results: Based on previously established cut-off values for WGM of LA-MRSA we assessed that transmission had likely occurred in 22 of the 31 (71%) presumed events (similarities between WGMs per event ranging from 98%-100%). In five of the other events, the WGMs of the isolates were highly related with similarities between the maps ranging from 95%-98%. In the remaining four events, the WGMs differed considerably (similarities between maps of the events ranged from 71%-94%), making transmission unlikely.
There was a considerable degree of genotypic diversity between the isolates (n = 69) of the 31 different transmission events. The similarity between the maps ranged from 83%-100%, indicating that multiple strains were involved in transmissions. In contrast, only five different spa-types were found in the selection, with 52 isolates belonging to a single spa-type t011.

Conclusion: The majority of transmission events (71%), based on classical low resolution typing methods, were confirmed using WGM. In 4 of 31 cases (13%) transmission was considered unlikely using WGM. Furthermore, this study shows that transmission of LA-MRSA occurs with a diverse array of LA-MRSA isolates, indicating that transmissibility is not restricted to a particular LA-MRSA clone or variant.

O130

Comparative whole genome sequencing of zoonotic and invasive porcine *Streptoccoccus suis* provides clues to virulence and zoonotic potential

N. Willemse[1], K.J. Howell[2], Y. Pannekoek[1], A. van der Ende[1], C. Schultsz[1]
[1]*AMC, Department of Medical Microbiology, Amsterdam,* [2]*Cambridge University, Veterinary Medicine, Cambridge, United Kingdom*

Introduction: *Streptococcus suis* is a zoonotic swine pathogen and a major public health concern in Southeast Asia and China. In the Netherlands, where human infection is mainly an occupational hazard, zoonotic *S. suis* infections are caused by serotype 2 (SS2), MLST-clonal complex (CC) 1 and CC20 strains, whilst invasive porcine infections are most often caused by SS9 CC16 strains. CC1 strains are responsible for the vast majority of zoonotic infections worldwide, but CC20 strains have only been reported from the Netherlands. By means of comparative whole genome sequencing of invasive *S. suis* strains, we aim to identify the genetic basis of zoonotic potential and virulence.
Methods: We sequenced 24 strains (CC1 and CC20) from patients and 74 invasive porcine strains (CC1, CC16, CC20, CC13, CC27, CC28 and singletons) within the same time frame in the Netherlands. Whole genome sequences from 16 reference strains were downloaded from NCBI and included in the analysis. Strains were sequenced using MiSeq sequencing, assembled using SPAdes, annotated using Prokka and gene content was analyzed with OrthoMCL. Among other software, phylogenetics was performed using Gubbins, molecular dating was done using BEAST and gene differences were identified with a Discriminant Analysis of Principle Components (DAPC),
Results: The genome size of the draft assemblies ranged from 1.97 Mb to 2.41 Mb. Including 16 reference genomes, the *S. suis* core genome comprised of 1015 genes. A Bayesian Analysis of Population Structure (BAPS) based on the core genome alignment showed that the dataset clustered into 7 groups, representing the major CCs. Phylogeny using Maximum Likelihood based on the core genome SNPs showed that CC16 and CC20 strains were genetically closely related and clustered away from the other draft genomes and all References strains. However, gene content of CC16 isolates was significantly larger than that of CC20, as well as that of CC1. In addition, the CC16 isolates expressed serotype 9 as opposed to the CC20 and CC1 isolates which expressed serotype 2. Molecular dating indicated that the CC16 and CC20 diverged around 140 years ago. In addition, we identified the SalK/SalR two-component signal transduction system (TCSTS) in some CC20 strains. This TCSTS is involved in virulence and was identified in Chinese outbreak strains, but has not been found in European strains before. CC20 strains also contained a unique prophage encoding type 1 restriction-modification genes, which could explain the smaller genome sizes compared to the CC16 strains.
Conclusion: 1) *S. suis* strains with zoonotic potential contain less genes compared to invasive porcine strains. 2) CC20 and CC16 isolates are genetically closely related but differ in their gene content and serotype, which implies a loss/gain of genes and a capsule switch between the clonal complexes. 3) We postulate that the separation of CC16 and CC20 strains occurred late 19th century. 4) Finally, we identified genes unique to the zoonotic CC20 strains that may contribute to their evolution and their zoonotic potential.

O131

VRE-typing

R.J.L. Willems, M. de Been, J. Top
UMC Utrecht, Department of Medical Microbiology, Utrecht

During the 1990s vancomycin-resistant *Enterococcus faecium* (VRE) have disseminated rapidly throughout hospitals in the US. The last ten years increasing numbers of VRE have also been found in hospitals in various European countries. In both the US and Europe the emergence of VRE was preceded by a rise in ampicillin-resistant *E. faecium* (ARE). The phenotypic linkage between ampicillin and vancomycin resistance reflects sequential and independent acquisition of these resistance genes by hospital -associated clones. The last three years an increasing number of hospitals in the Netherlands reported clinical infections with VRE and an increased prevalence of VRE in screening cultures.
Typing of VRE to infer their clonal relatedness is important to detect uncontrolled hospital transmission. Different typing methods have been introduced for this of which Pulsed-Field Gel Electrophoresis (PFGE) have been considered for long the gold standard. However, PFGE, that index rapidly evolving variation, may give misleading results for epidemiological typing in recombining bacterial genera as the *Enterococcus*. The observation that *E. faecium*

isolates belonging to the same clone may differ in up to 7 PFGE bands suggests that PFGE might be too discriminatory to study the epidemiology of VRE. Several hospitals use an-in house Amplified Fragment Length Polymorhism (AFLP)-based methods that seem to perform reasonably well. In fact, AFLP provided the first insights into the population structure of *E. faecium* by identifying that isolates from hospitalized-patients, healthy humans and animals grouped into different clusters. Since AFLP combines the analysis of small conserved and variable DNA regions distributed over the whole genome, this technique was able to identify these clusters of related isolates that were not detected by PFGE. The disadvantage of AFLP is that it the reproducibility is suboptimal, which makes it less suitable for library typing <ie. studying the more long-term or regional epidemiology of VRE. The same is true for multiple-locus variable-number tandem repeat analysis (MLVA). Furthermore, studies have shown that the current MLVA typing scheme for typing *E. faecium* is less discriminatory than PFGE and Multilocus Sequence Typing (MLST).
More in depth analysis of the evolutionary relatedness of *E. faecium* genotypes on a population level came from MLST. This demonstrated that hospital-associated isolates form a polyclonal *E. faecium* subpopulation, genetically and evolutionary distinct from non-hospital human and animal isolates. MLST has also been used to study the countrywide epidemiology of VRE in Dutch hospitals. MLST of 487 VRE collected from 36 Dutch hospitals revealed a total of 30 different Sequence Types (STs), suggesting that at least 30 VRE clones are circulating in Dutch hospitals. The increase of VRE in Dutch hospitals can therefore not be attributed to spread of a single clone. On the other hand, 15 STs were found in more than one hospital, suggesting that also clonal transmission between hospitals may have contributed to this epidemic rise. Frequent found STs include ST117 (20 hospitals), ST203 (15 hospitals), ST18 (12 hospitals), and ST78 (6 hospitals). Whether MLST has enough discriminatory power to study the local hospital epidemiology of VRE is questionable.
Since costs of whole-genome sequencing (WGS) has decreased and bench top sequencing machines enable fast turnaround, WGS holds great promise for enhancing resolution in molecular epidemiological studies. Currently, the UMC Utrecht is developing a core genome (cg) MLST scheme for typing VRE (*E. faecium*) at the highest possible resolution. This *E. faecium* cgMLST scheme will index allelic variation of 1423 genes instead of the 7 genes in traditional MLST. This scheme will become available coming months but preliminary analysis already indicate that this scheme is able to identify VRE outbreaks and reliably discriminates epidemiologically linked from non-linked isolates.

O132

VRE-diagnostics; the NVMM guideline

J.C. Sinnige[1], R.J.L. Willems[1], G.J.H.M. Ruijs[2], E. Mascini[3], J.P. Arends[4], A. Troelstra[1]
[1]UMC Utrecht, Department of Medical Microbiology, Utrecht, [2]Isala, Medical Microbiology, Zwolle, [3]Rijnstate Hospital, Department of Medical Microbiology, Arnhem, [4]UMCG, Department of Medical Microbiology, Groningen

Objectives: The last three years an increased incidence of vancomycin resistant *Enterococcus* (VRE) was observed in Dutch hospitals. The emergence and dissemination of resistance to vancomycin in enterococci can lead to clinical isolates resistant to most antibiotics. Although enterococci are not highly pathogenic, the incidence of vancomycin resistance among clinical isolates is steadily increasing, and such isolates have become important as nosocomial pathogens and constitute an important reservoir of antibiotic resistance genes. 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. The search was restricted to published English articles. Relevant results of unpublished studies providing sufficient data were also included.
Results: The recommended strategy for the detection of vancomycin resistance in *E. faecium* is a two-step procedure, and consists of a screening step followed by a genotypic confirmation step. The screening step is based on the reduced susceptibility of VRE to vancomycin. The genotypic confirmation step is based on the detection of the *vanA* or *vanB* gene. A set of five specimens from different days should be used for the targeted screening for VRE carriage, provided that broth enrichment is used. The total number of cultures can be adapted after analysing the local epidemiology.
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.

O135

VRE-tool kit

A. Troelstra, C.A.M. Graaf-Miltenburg
UMC Utrecht, Department of Medical Microbiology and Infection Prevention, Utrecht

Vancomycin resistant *Enterococcus faecium* (VRE) has proven to be able to spread easily among patients on clinical wards. During the last couple of years, several Dutch hospitals have encountered outbreaks. Numbers of colonized patients that were detected, varied from a few to several hundreds of patients. It has become clear that much effort is needed to control a VRE-outbreak and that support from and strong collaboration with health care workers from all related disciplines, facilitating services and hospital board is vital.

During springtime 2014, medical microbiologists and infection control practitioners that experienced VRE-outbreaks in their hospitals, have met several times to discuss their findings and to share their pragmatic approaches.

A practical approach has been proposed and committed to paper and will be shared during this presentation; in example directions are shared on how to deal with a single finding of VRE, what actions are needed if more cases are detected, how to deal with contact tracing and screening, how to perform follow up, is it advantageous to perform VRE-prevalence screening on a regular basis and how to appoint the date that the outbreak is controlled.

O136

Epigenetic mechanisms in virology

W. Doerfler[1], [2], S. Weber[1]

[1]*Erlangen University Medical School, Institute for Virology, Erlangen, Germany,* [2]*University of Cologne, Institute of Genetics, Köln, Germany*

Epigenetic mechanisms have been increasingly recognized of playing an important role also in Virology. Two forthcoming volumes in the *Springer Verlag* series *Epigenetics and Human Health* document this development.[1,2] Starting in the late 1970's, our laboratory has been contributing to the concept of epigenetic gene regulation, particularly of integrated adenoviral DNA.[3] We have also shown that the insertion of foreign (viral) DNA can fundamentally alter the epigenetic profiles of cells, their methylation and transcription patterns.[4,5] In this report, we will address the following aspects of our current research program:

I The profiles of CpG methylation in HIV-1 proviral genomes in cells from HIV-1 infected individuals have been investigated at different stages of viral exposure.[6]

II [§] Among 28,869 genes in human cells, which carry a 5.6 kbp bacterial plasmid as foreign DNA insert, a substantial number of genes were differentially expressed when compared to non-transgenomic cells. The highest up-regulations were found in snoRNA genes. By using Illumina 450K Infinium arrays, a comparison of the methylation levels at 480,000 CpG sites between four non-transgenomic and five transgenomic cell clones revealed differential methylation in 3,791 CpG's (0.8%).[7] These findings might have far-reaching general consequences for the evaluation of experiments with transgenomic cells.

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§ Collaboration with A. Hofmann and P. Hoffmann, Institute of Human Genetics, Bonn University.

O137

The epigenetic control of gene expression in African trypanosomes

L. Kerry, S. Ridewood, C.-P. Ooi, J. Wood, B. Hall, J. Cheung, G. Rudenko

Imperial College London, Department of Life Sciences, The Sir Alexander Fleming Building, London, United Kingdom

The African trypanosome *Trypanosoma brucei* is a unicellular eukaryote, which causes African Sleeping Sickness in SubSaharan Africa. These parasites are best known for their highly sophisticated strategy of switching a Variant Surface Glycoprotein (VSG) coat during the course of a chronic infection. A single parasite has many hundreds of VSG genes, but transcribes one at a time in a stringently mono-allelic fashion from one of about 15 VSG expression site transcription units located at chromosome ends.

Trypanosomes have branched off from other eukaryotes relatively early. As a consequence, they have unusual features regarding the organisation of their genome and the way that they regulate their genes. The trypanosome genome is organised as very extensive polycistronic (multiple-gene containing) transcription units which are constitutively transcribed by RNA polymerase II. There is no transcriptional regulation of Pol II in trypanosomes. Gene dosage is mediated post-transcriptionally, and

primarily through RNA stability. The exception to this are the VSG expression sites, which are transcribed by RNA Pol I, which normally exclusively transcribes rDNA.
Our group is trying to understand the role of chromatin in an organism which has no regulated Pol II transcription. In addition, we are investigating the role of chromatin remodelling in control of the Pol I transcribed VSG expression sites. We have shown that active VSG expression sites are highly depleted of nucleosomes. In addition, we have identified a number of chromatin remodelers and histone chaperones which are key for VSG expression site control (including the ISWI and FACT complexes). Knowledge about chromatin and epigenetic modifications in these organisms is allowing us to investigate the role of chemical inhibitors in interfering with these processes. Hopefully these will eventually allow us to tackle this slippery parasite effectively in the field.

O138

The resistance gene *Ty-1* triggers an epigenetic antiviral defense against Tomato yellow leaf curl virus

R. Kormelink[1], P. Butterbach[1], M.G. Verlaan[1], A. Dullemans[2], D. Lohuis[1], R.G.F. Visser[1], Y. Bai[1]
[1]*Wageningen University, Laboratory of Virology, Wageningen,* [2]*Wageningen University & Research Centre, Biointeractions and Plant Health, Wageningen*

RNA interference (RNAi or RNA silencing) plays a major role in host development and gene regulation but also acts as a antiviral defense mechanism in plants and insects. The latter has just recently been proven in mammals as well.[1,2] However, there is one main difference between the antiviral RNAi defense against viruses in plants and those in insects and mammals, which is the requirement of an RNAi amplification cycle in plants. This cycle involves the production of dsRNA by host RNA-dependent RNA polymerases (RDRs) from aberrant or cleaved viral RNA in a siRNA-dependent or independent manner and leads to the generation of secondary siRNAs. Without this amplification, plants are not able to mount a strong RNAi response against a viral invasion. In Arabidopsis six RDRs have been identified (RDR1 to -6), of which RDR1, 2 and 6 are well known and characterized to play a role in the RNAi amplification cycle but for RDR 3, -4 and -5, no functions have been described so far.
Recently we have cloned one of the first dominant resistance (*R*) genes against *Tomato yellow leaf curl virus* (TYLCV), namely *Ty-1* and this gene codes for an RDR with sequence similarity to the Arabidopsis RDR3, -4 and -5 type of genes.[3] Instead of the classical *R* gene-mediated hypersensitive response, the *Ty-1* gene exhibits more of a tolerance phenotype and the involvement of an RDR in geminivirus resistance has unveiled a new class of resistance genes. Considering the role of RDR1, -2, and -6 in RNAi, a role for RDR3, -4, and -5, and thus of *Ty-1* in the amplification of the siRNA signal is tempting. To test this hypothesis, *Ty-1* lines were challenged with TYLCV and the amount of siRNAs and their distribution on the viral genome analysed in comparison to susceptible tomato Moneymaker. In addition, methylation of the TYLCV CP (V1) promoter region was analysed in viral genomic DNA collected from *Ty-1* in comparison to Moneymaker. Results from those experiments will be presented and altogether indicate that *Ty-1* confers resistance against TYLCV through enhanced transcriptional gene silencing (TGS). Interestingly, *Cucumber mosaic virus* (CMV), a distinct RNA plant virus that normally is targeted by post-transcriptional gene silencing (PTGS) and not by TGS, compromised this resistance. The durability of *Ty-1*-mediated resistance against geminiviruses will be discussed in light of its role in the RNAi pathway and the regular occurrence of mixed viral infections in open field cultivations.

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O139

DNA methylation in *Mycobacterium tuberculosis* and consequences for growth in distinct host environments

S. Sengstake
Royal Tropical Institute (KIT), KIT Biomedical Research, Amsterdam

Tuberculosis is a global disease which is transmitted by the airborne spread of *Mycobacterium tuberculosis* (MTB) the causative agent of tuberculosis. MTB enters the host by infecting alveolar macrophages that protect the airway epithelia of the lungs. Once inside macrophages the bacterium survives by blocking fusion of the phagosomes with lysosomes and thereby evades elimination by the macrophage. However, the presence of MTB infected macrophages elicits an inflammatory response leading to the characteristic lung lesions called granuloma. These granulomas are large structures formed by multiple cell types of the immune system containing different microenvironments. Mycobacteria are present in all these microenvironments.

Nutrient deprivation, low pH and oxygen limitation within a granuloma are known to control but not eliminate MTB. Further MTB establishes a dormant state permitting survival in the host for decades. How do mycobacteria survive and adapt to these conditions over decades? The morphological and developmental changes in the pathogens in response to the changing host environment or exposure to antimicrobials are summarised as pathogen plasticity. These activities are tightly controlled by alterations in pathogen gene expression via epigenetics. In the prokaryotic world, DNA methylation and histone posttranslational modifications are the best characterised epigenetic modifications with the effectors and targets both on the host and pathogen site. What role does epigenetic regulation play in the processes controlling adaption to microenvironments in MTB? Regarding MTB there is relatively little known about the contribution of epigenetics to the regulation of pathogen metabolism in distinct host environments.

Shell and Wong have pioneered the investigation of the functional effects of DNA methylation in MTB. Methylation of mycobacterial DNA was shown to influence bacterial survival during oxygen limited conditions.[1] Interestingly, the relevant DNA methylases differ depending on the mycobacterial families. Posttranscriptional modification of a guanosine in the bacterial 16S rRNA, was found to ensure translational fidelity.[2] The absence of a methylated guanosine in the 16S rRNA allows mistranslation to a certain extent but also induces low level resistance to an antimicrobial drug that interferes with the bacterial ribosome. Further research is required to elucidate the role of epigenetics in the regulation of phenotypic variability during the course of an MTB infection.

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P001

Response of polycyclic aromatic hydrocarbon degrading bacterial populations from coastal sediments of patagonia to hydrocarbon exposure

R.V.J. Kuin

University of Amsterdam, Department of Biological Sciences, Amsterdam

This research focused on the capabilities of bacterial communities from the marine environment to respond to hydrocarbon exposure, especially Polycyclic Aromatic Hydrocarbons (PAHs), by means of the selection of specific populations associated with their biodegradation. Coastal intertidal sediments from three sites with different environmental characteristics and hydrocarbon exposure history were chosen: Playa Fracasso, nonpolluted, Chubut; Caleta Córdova, polluted, Chubut; and Bahía Ushuaia, polluted, Tierra del Fuego. For each, an experimental setting of sediment-seawater slurries was built, with four conditions: no external hydrocarbon addition, oil (0,4% v/v), phenanthrene (0.2% w/v), and pyrene addition (0.2% w/v). After 20 days of incubation at 15°C, total DNA was extracted and analysed by quantitative real-time PCR (qPCR) of genes coding for aromatic-ring-hydroxylating dioxygenase (ARHD). This enzyme catalyzes the first step of PAHs metabolic pathway in bacteria. Three ARHD genes were tested: *phnA1* (described in *Cycloclasticus* spp., obligate oil-degrading marine bacteria), and two novel variants from yet-uncultured microorganisms, described for the first time at these sites (D and T variants). The effect of hydrocarbon addition in the amount of ARHD copies in the extracted DNA was evaluated in each case. In general, dioxygenase genes increased their abundances after hydrocarbon addition. The strongest response was observed for the *phnA1* gene, reaching more than 10^7 copies per microgram of DNA in some conditions. The T variant was found in high abundance and also showed a response to PAHs in both polluted sites, while the D variant showed a response only in sediments from the subantarctic site. The response of the sediments of the non-polluted site was weak, suggesting that they might be more vulnerable to the pollution with PAHs due to lack of adaptation of the community to the presence of pollutants. Our findings allowed us to identify key bacterial populations associated with PAH biodegradation in coastal sediments of Patagonia, and showed that both the type of environment and the hydrocarbon exposure history can influence the selection of specific populations at each site.

P002

Discontinuation of fluoroquinolone prophylaxis in neutropenic hematology patients; a retrospective analysis of clinical and microbiological impact

M.P.M. van der Linden[1], E.C. van der Hout[2], E.H. Wiltink[3], M. Sohne[1], M. Tersmette[1]

St. Antonius Ziekenhuis, Departments of [1]Medical Microbiology and Immunology, [2]Hematology, [3]Pharmacy and Pharmacology, Nieuwegein

Background: Treatment of patients with acute myeloid leukemia, high grade lymphoma and multiple myeloma with chemotherapy induces profound neutropenia and leaves patients severely immunocompromised. To prevent infectious complications, fluoroquinolone (FQ) prophylaxis has become common practice. However, its effectiveness has been debated, also because of increasing resistance

to FQ in Enterobacteriaceae. We studied the effects of the discontinuation of FQ prophylaxis in these patients.

Methods: In total 356 neutropenic episodes from 283 hematology patients diagnosed with acute myeloid leukemia (AML), high grade lymphoma (HL) and multiple myeloma (MM) over the period 2007-2013 were studied retrospectively. Three patient groups were compared: a ciprofloxacin prophylaxis group (289 episodes), a contemporary control group not receiving ciprofloxacin prophylaxis because of carriage of FQ-resistant Enterobacteriaceae (34 episodes) (January 2007- March 2013), and a group of patients included after complete discontinuation of ciprofloxacin prophylaxis (33 episodes) (March 2013-January 2014). Primary endpoints were 30 and 100 day mortality. Secondary outcome measures were duration of neutropenia, incidence of febrile neutropenia, total duration of hospital admission, number of (infection-related) admissions to the intensive care unit (ICU) and duration of the ICU-admission. Number of episodes with bacteraemia and the duration of antimicrobial therapy were assessed. Furthermore, the composition of blood and stool culture isolates and their resistance patterns were studied.

Results: A total of 5515 neutropenic patient days were recorded. Although we observed a significant increase in the occurrence of febrile neutropenia (76.5% and 93.9% vs. 65.4%; p=0.002) in both cohorts without ciprofloxacin prophylaxis, no significant differences were observed in mortality, duration of neutropenia, length of hospital stay, overall and infection-related ICU admissions, total time of stay at the ICU or the amount of ICU days/100 bed days between the three groups. Without ciprofloxacin prophylaxis, therapeutic use of antibiotics in DDD/100 bed days increased from 26.98 to 42.20 and 37.39. Total antibiotic consumption (prophylaxis and therapy combined) was higher in the prophylaxis group (65.84 vs. 42.20 and 37.39 DDD/100 bed days). No significant difference was observed in the occurrence of bacteraemia, but bacterial isolates were more often Gram-negative and less often ciprofloxacin resistant and ESBL-positive. Stool cultures results showed a similar trend.

Conclusions: Our results indicate that discontinuing ciprofloxacin prophylaxis is warranted since it does not lead to higher mortality or increased morbidity. Furthermore; beneficial effects on the use of antibiotics and prevalence of antibiotic resistance were observed. To substantiate these findings, further research is needed.

P003

Rapid detection of MRSA directly from Eswabs using the BD MAX system

I.O.M. op den Buijs, R.T.J.M. Roymans, I.C. Briels, L. van Dommelen, J.H.B. van de Bovenkamp

Stichting PAMM, Laboratory for Medical Microbiology, Veldhoven

Objectives: Meticillin-resistant *Staphylococcus aureus* (MRSA) prevalence is high in many European countries, but remains low in The Netherlands due to its 'Search and Destroy' policy. With the new Staph SR kit on the BD MAX system, MRSA results can be obtained within 2 hours and can therefore be helpful in performing isolation policy. The StaphSR assay distinguishes between MRSA and *S.aureus* by detection of the OrfX junction, present in MRSA, as well as the nuc gene, present in *S.aureus*. Furthermore, this assay detects both MecA and MecC genes. This study describes the evaluation of the BD MAX StaphSR assay for detection of MRSA directly from pooled Eswabs as a MRSA screening method, in comparison to enriched culture with subsequent incubation on a chromogenic agar from Eswab from individual patient sites.

Methods: Clinical samples from different bodysites (e.g. throat, nasal and perineum collected with Eswab) were collected from patients at risk for MRSA colonisation. A pool was made by combining 100µl of Eswab medium from the different body sites. From this pool, 200µl was transferred into a BD Sample Buffer Tube for molecular testing. For culture, the Eswabs were individually transferred into a Trypticase Soy Broth (TSB) and subsequently incubated (18-24 hr., 35°C). After incubation, 10 µl of the TSB was used to inoculate a ChromID MRSA agar (bioMérieux). Suspected colonies were determinedusing MALDI-TOF analysis (Bruker) and susceptibility testing was performed using VITEK2 (bioMérieux). MRSA strains were confirmed by in-house MRSA PCR. For discrepancy analysis, samples were retested with the Xpert SA Nasal Complete (Cepheid). True positivity was defined as a patient who tested positive with either culture, or Staph SR assay confirmed by the Xpert SA assay.

Results: Out of 200 eligible patients, 11 patients were culture positive in at least one out of three body sites. The StaphSR assay, detected 9 culture positive patients using pooled samples. With the StaphSR assay two additional (culture negative) MRSA positive patients were found. These two patients were confirmed MRSA positive by the Xpert SA and are considered as true positives. Sensitivity, specificity, positive and negative predictive values were 84.6%, 100%, 100% and 98.9% respectively when compared to our gold standard.

Conclusion: The BD MAX StaphSR assay is a promising method for rapid screening MRSA directly from pooled Eswabs. The system provides a walk-away workflow that makes it easy to integrate into any molecular microbiological laboratory.

P004

Limited value of the signal-to-cutoff ratio of the ARCHITECT HIV Ag/Ab Combo assay to discriminate primary from established infection

M. Hoogewerf, C.W. Ang

VUmc, Department of Medical Microbiology and Infection Control, Amsterdam

Introduction: A recent publication suggests that the magnitude of the Architect test S/CO value may be useful for determining the likelihood that a S/CO test result may represent acute or established HIV infection).[1] In primary HIV infections results of S/CO ratios were often = 100. We wanted to evaluate the usefulness of the S/CO ratio for discrimination of primary from established HIV infection in our population.

Methods: We retrospectively analyzed the results of the Architect HIV Ag/Ab Combo assays that have been performed on the Architect i1000SR (Abbott) in the VU Medical Center, Amsterdam, from November 2011-December 2014. Patients with a S/CO ratio = 1.0-= 100 were selected and categorized as having a primary or established infection according to the opinion of the treating physician. This was based on the combination of findings at history, physical examination, laboratory results and previous (negative) HIV tests.

Results: In a period of 3 years 9301 HIV Ag/Ab Combo assays had been performed. 139/9301 (1,5%) yielded a positive result (S/CO ratio = 1.0). 41/139 (29,5%) positive samples showed a S/CO ratio = 1.0-= 100. 11 of these 41 (26,8%) samples tested negative in the western blot. 1 Sample was retested in the Architect and the second S/CO ratio was 0.17, the sample was considered as negative. These 12 patients were mainly tested for screening purposes, did not have risk factors for acquiring HIV infection and were therefore considered false positive in the Architect. The S/CO ratio of these 12 samples varied from 1.07-6.55. From the other 29 samples with a S/CO ratio = 1.0-= 100 15 patients were considered to have an established HIV infection (S/CO ratio range 7.7-75.62), 4 patients were suspected for a primary HIV infection (range 9.57- 93.33) and in 10 patients the time of primary infection could not be deduced (range 9.57-59.28).

Conclusion: In only a minority of patients with a low S/CO ratio in the Architect HIV Ag/Ab Combo assay there were indications for a primary HIV infection. At least half of the patients was found to have an established HIV infection. In contrast to a previous publication, we conclude that the S/CO ratio of the Architect HIV Ag/Ab Combo assay is unreliable in discriminating a primary from an established HIV infection.

Reference

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P005

Group A *Streptococcus* targets specific C-type lectin receptors through LPxTG-linked proteins

R. van Dalen[1], P.J.M. Brouwer[1], J.A.G. van Strijp[1], B. Lepenies[2,3], N.M. van Sorge[1]

[1]*UMC Utrecht, Department of Medical Microbiology, Utrecht,* [2]*Max Planck Institute of Colloids and Interfaces, Department of Biomolecular Systems, Potsdam, Germany,* [3]*Freie Universität Berlin, Institute of Chemistry and Biochemistry, Department of Biology, Chemistry and Pharmacy, Berlin, Germany*

Introduction: Group A *Streptococcus* (GAS) infections are a major burden to human health. Infections range from mild disease such as 'strep-throat' to severe life-threatening diseases such as rheumatic fever and necrotizing fasciitis, affecting approximately 700 million people annually. A unique structure of the GAS cell wall is the Group A Carbohydrate (GAC), a peptidoglycan-linked polysaccharide consisting of a polyrhamnose backbone and immunodominant *N*-acetylglucosamine (GlcNAc) side chains. The GlcNAc side chain was shown to be an important virulence epitope of GAS,[1] however, the molecular mechanism remains to be unravelled. Host C-type lectin receptors (CLRs) are pattern-recognition receptors that are specialised in carbohydrate recognition and as such likely interaction partners for the GlcNAc epitope of GAC. We hypothesised that GAS modulates host immune responses by targeting specific CLRs through the GAC.

Methods: A panel of 14 CLR-human IgG Fc fusion constructs as well as Streptactin-tagged and FITC-labelled human and mouse Langerin constructs were screened for interaction with GAS 5448 wild-type (WT) by flow cytometry and immunofluorescence microscopy. Interacting lectins were screened in concentration-dependent binding curves on 5448 WT bacteria. Also, to check whether the GAS ligand was conserved, we assessed binding of CLRs to different GAS serotypes. To identify the interacting GAS ligand, we performed lectin blotting experiments using GAS 5448 whole cell lysates and screened specific GAS mutant strains for loss of binding.

Results: Murine Clec2, Mincle, Dectin-1, SignR1, DC-SIGN, langerin and human langerin bound to WT GAS 5448 in a concentration-dependent manner. Binding was not affected by changes in the GAC since binding was similar to the gacI mutant, which specifically lacks the GlcNaC GAC side chains.[1] However, binding was completely abrogated in sortase A-mutant GAS, which lacks 15 LPxTG-linked proteins on the cell wall. GAS mutants lacking individual LPxTG-linked proteins revealed that these CLRs do not interact with pili, sclA, CepA, epf, Spy_0843 or Spy_1494. Lectin blots indicated that the streptococcal ligand for Mincle is approximately 23kDa, which matches the LPxTG-linked protein Grab.

Conclusions: We have taken the first steps in the identification of the molecular interaction of GAS with the host's CLR repertoire. Future experiments aim to identify the exact GAS ligands for host CLRs and to verify these inter-

actions on transfected cell lines and with human primary cells to unravel the consequences for pathogenesis.

Reference

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P006

The search for a discriminating molecular marker: a comparison of genomes of entero-invasive *Escherichia coli* and *Shigella species*

M.J.C. van den Beld[1,2], K. Zhou[2], A.M.D. Kooistra-Smid[2,3], A.W. Friedrich[2], F.A.G. Reubsaet[1], J.W.A. Rossen[2]

[1]RIVM, Centre for Infectious Disease Research, Diagnostics and Perinatal Screening, Bilthoven, [2]University Medical Center Groningen, Department of Medical Microbiology, Groningen, [3]Certe Laboratory for Infectious Diseases, Groningen

Introduction: *Shigella* species and *Escherichia coli* are genetically highly related and share a large part of their genomes. Entero-invasive *E. coli* (EIEC) is a pathogenic *E. coli* and shares the invasive nature with *Shigella* species. Both harbor a large invasion plasmid (pINV) and multiple copies of the *ipaH*-gene, which is frequently used as a molecular target in diagnostic tests to detect *Shigella* in fecal samples. No literature about the comparison of *Shigella* and EIEC genomes is available, but Multi Locus Sequence Typing (MLST) indicates that EIEC is more related to *Shigella* than to other *E. coli*. In The Netherlands infections with *Shigella spp.* are notifiable, whilst infections with EIEC are not. Subsequently, it is important to be able to discriminate between both species on a molecular level. To reveal a unique signature in the genome that is usable to develop a discriminating diagnostic test for EIEC and *Shigella*, sixteen EIEC genomes were sequenced and compared to publically available *Shigella* genomes.

Methods: Sixteen EIEC genomes were sequenced with an Illumina Miseq and de novo assembled using CLC Genomic Workbench. An ad hoc scheme for MLST+ analysis was made using Seqsphere (Ridom). This scheme was acquired by collecting the shared genes of the sequenced EIEC genomes and the reference *E. coli* 53638 genome (accession number: NZ_AAKB02000001.1). The genes in this core genome were compared to analogous genes in eight *Shigella* genomes, downloaded from Genbank, in order to find differences between the two species. This analysis was also performed the other way around, with an ad hoc MLST+ scheme consisting of the shared genes of the eight downloaded *Shigella* genomes, with *S. flexneri* 2a 301 as reference. The genes in this core genome were compared with the sequenced EIEC genomes. Furthermore, a comparison of all regions of the EIEC genomes with the *Shigella* genomes was made, by using Blast Ring Image Generator (BRIG) v0.95. Fragments present in one species, but absent in the other, were selected.

Results: In the MLST+ analysis using the EIEC ad hoc scheme, the *Shigella* species and EIEC strains were separately clustered in a minimum spanning tree (MST). By using the core genome of *Shigella*, no discriminating differences in genes of *Shigella* and EIEC were found. Comparing the core genome of EIEC with the *Shigella* genomes, differences in the *ecnB* and *YchN* genes were found. However, these differences are also present in other non-invasive *E. coli* strains and cannot be used as EIEC-specific molecular markers for detection in fecal samples. Using BRIG, no fragments present in *Shigella*, but absent in EIEC were found. Although fragments present in EIEC and absent in *Shigella* were found, these fragments are not present in all EIEC strains.

Conclusion: This genome comparison did not reveal a single molecular marker that can be used for differentiation of EIEC and *Shigella* in fecal samples. More research is needed to develop a discriminating molecular test, based on multiple genes, for direct use on patient material.

P007

Trichomonas vaginalis and *Mycoplasma genitalium:* age-specific prevalence and disease burden in men attending a Sexually Transmitted Infections clinic in Amsterdam, The Netherlands

S.M. Bruisten, C. van der Veer, M.S. van Rooijen, H. de Vries, M. Himschoot

GGD Amsterdam, Public health laboratory, Amsterdam

Background: Men are not routinely tested for *Trichomonas vaginalis* (TV) and *Mycoplasma genitalium* (MG) in the Netherlands and therefore very few studies have looked into their prevalence and/or role in urogenital complaints in the Dutch male population.

Objective: To describe the age-specific prevalence and disease burden of TV and MG, and their co-occurrence with *Chlamydia trachomatis* (CT), in men attending the Sexually Transmitted Infection (STI) clinic in Amsterdam, the Netherlands.

Methods: We prospectively collected urine samples and clinical data from 526 heterosexual men and 678 men who have sex with men (MSM) who attended the STI clinic in 2014. To rule out the disease burden of *Neisseria gonorrhea* (NG), we excluded confirmed gonococcal cases. To investigate age as a risk factor, we oversampled older men to ensure a study population with an equal age distribution. Urine samples were assessed for TV and MG infection using TMA and PCR as molecular tests.

Results: The overall prevalence for TV was 0.5% (6/1,204). For MG this was 3.1% (37/1,204).For TV infection 4 out of

6 positive men were older than 40 years. No TV positivity was found in the MSM group. No age trend was observed for MG, nor were there any significant differences in MG positivity rates between heterosexual men and MSM. Although our study population had a positivity rate of 5.9% for CT (72/1,204), only one TV/CTand one MG/CT co-infection were observed. In addition we found one TV/MG co-infection. Of the 135 men (11.2%) who reported urogenital symptoms (burning sensation and/or urethral discharge) only 1 (0.7%) had a TV/MG co-infection and only 7 (5.2%) were infected with MG. In contrast, in 24 (17.8%) of men the reported symptoms correlated with a CT infection.

Conclusion: TV infectionis very rare in heterosexual men and is non-prevalent in MSM in Amsterdam, the Netherlands. Our results support previous findings that TV prevalence increases with age. MGis common in men in Amsterdam, but – just like TV infection – remains mostly asymptomatic. While the outcome of this study does not encourage the general testing for TV or MG in men, it should raise the awareness that urogenital symptoms that are not explained by NG or CT infection, could be explained by an MG infection.

P008

Prevalence of different human rhinovirus species in hospitalized and non-hospitalized children in The Netherlands

A.H.L. Bruning, X.V. Thomas, L. van der Linden, J.G. Wildenbeest, R.P. Minnaar, P.J. Sterk, M.P. van der Schee, K.C. Wolthers, D. Pajkrt

AMC, Department of Pediatric Infectious Diseases and Immunology, Amsterdam

Introduction: Rhinovirus (RV) infections are frequently occurring respiratory infections in young children. Clinical symptoms can range from asymptomatic infection and mild 'common cold' complaints to severe life-threatening lower respiratory tract infections. RV are classified into three species: A, B and C. The clinical significance of the various RV species in children is unclear. Some studies suggest that RV-C might cause more severe disease than the other RV-species and might be associated with childhood wheezing, but data are limited. The aim of this study is to evaluate the prevalence of various RV species and association with clinical symptoms in RV infected non-hospitalized as compared to RV infected hospitalized children.

Methods: Non-hospitalized children with mild or asymptomatic RV infection were selected from the EUROPA-trial, a prospective cohort study in the Netherlands, focusing on prediction of early signs of asthma. RV positive nasopharyngeal samples (NPS) from children admitted to the AMC, Amsterdam, because of respiratory symptoms were retrospectively identified. To create comparable groups, we selected RV-samples from hospitalized children born between May 2008 and May 2010 of whom samples were collected from November 2009 till December 2012 as are the samples from the EUROPA-trial. All RV-positive NPS from these two cohorts, detected using quantitative real time – polymerase chain reaction (q-RT-PCR) were genotyped by sequencing the VP4/VP2 region followed by phylogenetic analysis.

Results: A total of 121 RV-positive NPS from 114 children (49 hospitalized, 65 non-hospitalized) were analyzed. The most prevalent RV-species in both study groups was RV-A (n = 67) with a prevalence of 48.1% in the hospitalized and 60.9% in the non-hospitalized population. Hospitalized children tended to be younger (median 0.8 years, IQR 0.5 - 1.8 years) than non-hospitalized children (median 1.5 years, IQR 1.0 - 2.2 years) (p = 0.001). There was no significant difference between groups regarding gender. The most prevalent type in both populations was RV-A78. An infection with RV-B was the least identified in only 12 patients (9.9%). RV-C seems to be more present in hospitalized children, with a prevalence of 40.4% (n = 21) versus 30.4% (n = 21). The percentage of children admitted to the hospital within all RV-C infected children did not significantly differ from the percentage of children admitted to the hospital within all RV-A infected children (50% versus 37.3%). Subgroup analysis of severely ill hospitalized children admitted to the intensive care unit revealed that 53.8% (n = 14) of RV-infected children were infected with RV-A and 34.6% (n = 9) with RV-C. Mechanical ventilation due to respiratory insufficiency was needed in 77.8% of the RV-C infected children and 64.2% of the RV-A infected children.

Conclusion: In our study, RV-A was the most dominant species in both hospitalized and non-hospitalized RV infected children. Infection with RV-B was only sporadically detected. A higher detection rate of RV-C was seen among hospitalized children as compared to non-hospitalized children. However, in severely ill children admitted to the ICU the prevalence of RV-A and RV-C was comparable, suggesting that RV-A and RV-C are equally capable of inducing severe disease in young hospitalized children.

P009

Evaluation of an Antigen Detection Point-of-Care test for RSV and Influenza in a pediatric hospitalized population in The Netherlands

A.H.L. Bruning, K. van Dijk, H.W.M. van Eijk, G. Koen, J.B.M. van Woensel, F.H. Kruisinga, D. Pajkrt, K.C. Wolthers

AMC, Department of Pediatric Infectious Diseases and Immunology, Amsterdam

Introduction: Acute respiratory infections are a leading cause of childhood mortality and morbidity worldwide and rapid diagnosis of these infections improves patient management. Rapid antigen detection tests or 'point-of-care tests' (POCT) are a promising alternative for the more expensive and time-consuming polymerase chain reaction (PCR). The Sofia Fluorescent Immunoassay Analyzer (FIA) (Quidel, USA) is a rapid fluorescence-based lateral flow immunoassay in which samples are analyzed by a compact instrument (Sofia Analyzer) allowing detection within 15 minutes.
Aim: In this study we evaluated the diagnostic performance of the Sofia RSV FIA and Sofia Influenza A+B FIA compared to routinely used polymerase chain reaction (PCR); Additionally, the practical implementation of Sofia FIA and forthcoming clinical consequences of Sofia FIA testing was evaluated.
Methods: Children presenting with respiratory symptoms and admitted to either the Pediatric Intensive Care Unit or the Infant Ward of the Emma Children's Hospital, AMC, Amsterdam, underwent a combined nasal and throat swab. Nasopharyngeal swabs (n = 66) were tested for respiratory syncytial virus (RSV) and influenza A and B using the Sofia FIA. In addition, a multiplex PCR was performed on the same material. After diagnosis, pediatric residents and laboratory staff were asked to fill in a questionnaire to evaluate the practical benefits and limitations of the Sofia FIA.
Results: Compared to PCR, Sofia had a sensitivity of 75% and a specificity of 97.5% for RSV. For Influenza A, Sofia had a sensitivity of 66.7% and a specificity of 96.6%. Sensitivity of Sofia for Influenza B was 40% and specificity 89.7%. Six samples revealed false positive results using the Sofia FIA. Pediatric residents completed a total of 30 questionnaires. In eight cases (26,6%) the result of the test had consequences on isolation measures. In one case (3.3%) the duration of administering antibiotics was reduced. According to the pediatric residents, the Sofia test was time-consuming and difficult to perform. However, the availability of a POCT was highly appreciated.
Conclusion: The POCT Sofia FIA may be a potentially promising alternative as a simple, potentially time-saving and cheaper method for respiratory viral testing. However, test performances must improve, especially regarding sensitivity and rate of false positive results. During the implementation period of a POCT it is strongly advised to provide sufficient training moments for both laboratory staff as well as all other personnel involved to prevent avoidable problems with performing and interpreting tests. As yet, confirmation of negative POCT Sofia FIA-results by more sensitive tests (PCR) is recommended and awareness for false-positive test results is necessary.

P010

Seroprevalence of hepatitis E virus in men who have sex with men and in intravenous drug users in relation to HIV-1 infection in the Amsterdam Cohort Studies

S.M. Bruisten[1], J. van Neer[1], M. Bakker[2], N. Kootstra[2], S. Sadik[1]
[1]*GGD Amsterdam, Public Health Laboratory, Amsterdam,* [2]*AMC, Department of Clinical Virology, Amsterdam*

Background: Hepatitis E virus (HEV) is transmitted via the fecal-oral route. Hepatitis E infection is usually mild or without symptoms but it may infrequently lead to severe disease and complications, especially in chronically infected patients with immune-suppression, or in pregnant women. HEV infections in the Netherlands occur mostly sporadic and seroprevalence increase with age. Since transmission relies on bad hygienic conditions it is feasible that anal oral sex, which is practiced frequently by men who have sex with men (MSM) leads to an increased HEV seroprevalence relative to the blood bank population. Also sharing contaminated needles might lead to an increased HEV seroprevalence in intravenous drug users (IDU). Therefor we studied samples from the Amsterdam Cohort Studies (ACS) from both MSM and IDU risk groups, being either HIV-1 positive or negative.
Objective: To study the seroprevalence of HEV among MSM and IDU participants of the ACS in relation to HIV-1 and age.
Methods: Blood samples were retrospectively included from 200 MSM and 200 IDU participants from the ACS. Of these groups half were HIV-1 positive (n = 100 per group). Equal numbers of sera were selected to represent different age categories from 20 to 50 years. The HEV IgG and HEV IgM seroprevalence was determined using Wantai kits which are known to have the highest sensitivity and a very good specificity.
Results: The HEV IgG seroprevalence was shown to be 22% for the total of 400 tested samples. Also the same 22% were found for each ACS risk group, the MSM and IDU, and this was independent of HIV-status. This seroprevalence was quite comparable with the 27% IgG seroprevalence found in Dutch blood donors (Slot et al, Eurosurveillance, 2013). The seroprevalence increased with age category in the MSM group but not as clearly in the IDU group.The HEV IgM seroprevalence was 0%. Only one IDU sample was found to be IgM positive but after repeating the test it was negative. Also the HEV PCR was negative for this sample. Thus no acute HEV sera were identified in this ACS study.
Conclusion: The HEV seroprevalence was not increased in men who have sex with men nor in intravenous drug users, irrespective of HIV-1 infection.

P011

Long-term survival of *Enterococcus faecium* outside the human host

V. de Maat, W. van Schaik
UMC Utrecht, Department of Medical Microbiology, Utrecht

Background: *Enterococcus faecium* is a Gram-positive commensal of the gut of human and animals. However, in the last few decades it has acquired resistance to several antibiotics and has become an important cause of nosocomial infections. *E. faecium* can survive outside the human host for an extended period of time, which increases its ability to spread throughout the hospital and cause outbreaks.
Objectives: Our goal is to uncover the mechanisms used by *E. faecium* to survive outside the human host.
Methods: Using prolonged batch culture in brain heart infusion broth (BHI) or incubation in nutrient-free phosphate buffered saline (PBS) we determined the capacity of two clinical *E. faecium* strains (E745 and Aus0004) to survive at 20°C and 37°C.
Conclusion: We show that temperature has a critical role in the long-term survival of *E. faecium*. At 37°C viable counts in PBS drop by 90% after 2 - 3 days while at 200C the viable counts remain stable for 5 days. During prolonged culture in BHI, viable counts decrease by 90% after 7 days, but at 200C viable counts remain stable for 10 days. Our finding suggest that *E. faecium* has specific mechanisms to survive outside the human host for a prolonged period of time. Currently, high-throughput functional genomic experiments are being performed to identify the mechanisms which contribute to survival of *E. faecium* in nutrient-poor conditions at ambient temperature.

P012

Distinct SagA from hospital-associated clade A1 *Enterococcus faecium* strains contributes to biofilm formation

F.L. Paganelli[1], M. de Been[1], J.C. Braat[1], T. Hoogenboezem[2], C. Vink[2], J. Bayjanov[1], M.R.C. Roger[1], J. Huebner[3], M.J.M. Bonten[1], R.J.L. Willems[1], H.L. Leavis[1]
[1]UMC Utrecht, Department of Medical Microbiology, Utrecht, [2]Erasmus MC-Sophia Children's Hospital, Pediatric Infectious Diseases and Immuni, Rotterdam, [3]Hauner Children´s Hospital Ludwigs-Maximilian Universität München, Division of Pediatric Infectious Dise, MÜNchen, Germany

Enterococcus faecium is an important nosocomial pathogen causing biofilm-mediated infections. Elucidating *E. faecium* biofilm pathogenesis is pivotal for development of new strategies to treat these infections. In several bacteria extracellular DNA (eDNA) and proteins act as matrix components contributing to biofilm development. In this study, we investigated biofilm formation capacity and the role of eDNA and secreted proteins in 83 *E. faecium* strains with different phylogenetic origin that clustered in clade A1 and clade B. Although there was no significant difference in biofilm formation between *E. faecium* strains from both clades, addition of DNase I or proteinase K to biofilms demonstrated that eDNA is essential for biofilm formation in most *E. faecium* strains, whereas proteolysis primarily impacted on biofilms of clade A1 *E. faecium* strains. Secreted antigen A (SagA) was the most abundant protein in biofilms from clade A1 and B *E. faecium* strains, although localization differed between the two groups. sagA is present in all sequenced *E. faecium* strains, with a consistent difference in the repeat region between the clades, which correlated with proteinase K susceptibility in biofilms. This indicates an association between the SagA repeat profile and the localization and contribution of SagA in *E. faecium* biofilms.

P013

Spa 'non-typeable' *Staphylococcus aureus* isolates: evaluation of alternative primers

R.H.C.A. Deurenberg[1], A. Mellmann[2], C. Glasner[1], W. Baas[1], R. Köck[2], H. Grundmann[1], J.W.A. Rossen[1], A.W. Friedrich[1]
[1]University Medical Center Groningen, Department of Medical Microbiology, Groningen, [2]University Hospital Münster, Institute of Hygiene, Münster, Germany

Introduction: Typing of the *S. aureus* protein A (*spa*) repeat region is a popular sequenced-based method for characterising *S. aureus* isolates. SeqNet.org continuously performs studies to improve spa typing performance. Currently, up to 2 % of *S. aureus* isolates are non-typeable using standard primers spa-1113f and spa-1514r. This may be due to mutations or deletions in the primer attachment sites, or (partly) deletion of the *spa* locus. Here, we describe the evaluation of alternative primers to characterise *spa* 'non-typeable' *S. aureus* isolates.
Methods: Twenty-seven *S. aureus* isolates (12 MSSA and 15 MRSA) from various European countries (Belgium (1 MSSA/1 MRSA), France (1 MSSA/1 MRSA), Germany (12 MRSA), Ireland (5 MSSA/1 MRSA), Latvia (1 MSSA) and Slovenia (4 MSSA)), which were non-typeable using standard primers, were analysed. After species confirmation by PCR for the *S. aureus* specific *nuc* gene, isolates were subjected to spa typing using combinations of four alternative primers: spa_239f (5´-ACTAGGTGTAGGTATTGCATCTGT-3´), spa_1717r (5´-TCCAGCTAATAACGCTGCACCTAA-3´), spa_1084f (5´-ACAACGTAACGGCTTCATCC-3´) and spa_1618r (5´-TTAGCATCTGCATGGTTTGC-3´). After sequencing, the results were analysed using Ridom StaphType software.

Using the based upon repeat pattern (BURP) algorithm and the Ridom SpaServer content, the multilocus sequence typing (MLST) clonal complexes (CC) were inferred.

Results: Twenty-two of the 27 isolates (82%) could be *spa* typed using the alternative primers. Three of these 22 had a new 27 bp *spa* repeat (AAAGAAGACAACAACAAAAAGCCTAGC) and two had a new 25 bp *spa* repeat (AAAGAAGACGGGCAACAAGCCTGGT). One isolate carried an indel consisting of a single thymine residue between two *spa* repeats. The 22 typeable isolates could be spa typed using primer combinations spa_1084f/spa_1618r (n = 13), spa-1113f/spa_1717r (n = 4), spa_239f/spa_1514r (n = 2), spa_1084f/spa_1717r (n = 2) and spa_239f/spa_1717r (n = 1), respectively. The isolates were associated with MLST CC5 (t014/t077; n = 2), CC22 (t005/t032; n = 2), CC30 (t012/t122; n = 2), CC1 (t127; n = 2), CC45 (t026; n = 1), CC7/15 (t2616; n = 1), CC97 (t365; n = 1) and CC121 (t159; n = 1). Four isolates *spa* typed as t10097, t10991, t10992 and t13365 could not be assigned to a CC. Five MRSA isolates were spa non-typeable using standard or alternative primers.

Conclusion: When encountering *spa* non-typeable *S. aureus* isolates using standard primers, alternative primers, i.e. spa_1084f and spa_1618r, can be used to characterise the *spa* types for the majority of such isolates.

As *spa* non-typeable isolates were from different CCs, we found no correlation between the genetic background and *spa* non-typeability.

Around sixty more isolates are currently being analysed using the alternative primers.

Next generation sequencing will be performed on all non-typeable isolates to elucidate the cause of non-typeability with alternative and/or conventional primers.

P014

Determination of Non-tuberculous mycobacteria with Matrix Assisted Laser Desorption Ionisation – Time Of Flight Mass Spectrometry

B. Mulder, M. van den Boomgaard, R. Leussenkamp-Hummelink, N. Koehorst, M. de Boer, A. van der Zanden

LabMicTA, Microbiology, Hengelo

Non tuberculous mycobacteria (NTM) differ in their capacity to cause clinical disease. Therefore quick and accurate identification is important. Current determination is based on biochemical phenotyping and DNA sequencing and is time-consuming and expensive. We investigated the determination of NTM with Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) on accuracy, speed and cost.

Retrospectively, 122 isolates of 27 different species were determinated with MALDI-TOF MS. Results of the sequencing analysis were considered as gold standard.

MALDI-TOF MS analysis correctly identified 92% of the isolates on liquid media and 91% of the isolates on solid media. Of those 75% (liquid media) and 67% (solid media) had a score >2,000, indicating secure species identification. This combined with the low cost of €47,12 (MALDI-TOF MS) versus €376,54 (sequencing)) and quick results (daily versus weekly) makes MALDI-TOF MS a promising first identification method for NTM.

P015

Urogenital *Chlamydia trachomatis* strain types in relation to ethnicity and urogenital symptoms in a young screening population in Amsterdam, the Netherlands

S.M. Bruisten[1], B. Versteeg[1], M. Himschoot[1], I. van den Broek[2], R. Bom[1], A. Speksnijder[3], M. Schim van der Loeff[1]

[1]*GGD Amsterdam, Public Health Laboratory, Amsterdam,* [2]*RIVM, Epidemiology and surveillance unit, Bilthoven,* [3]*Naturalis biodiversity center, Research and education, Leiden*

Introduction: *Chlamydia trachomatis* is the most common bacterial sexually transmitted infection (STI) worldwide, and often causes asymptomatic infections. If not properly treated, these may result in severe complications including epididymitis in men, and pelvic inflammatory disease in women leading to ectopic pregnancy and tubal factor infertility.

Strain typing is important to understand the epidemiology of *C. trachomatis* and could provide more insight in the relationship of *C. trachomatis* strain types with ethnicity and with clinical symptoms. Previous studies, however, found conflicting results regarding associations between urogenital *Chlamydia trachomatis* infections and ethnicity or urogenital symptoms, using *ompA* based genotyping or high resolution multilocus sequence typing (MLST), but these were all performed in at risk populations. Therefore, we applied high resolution MLST on a sample of individuals from a selected young urban screening population to assess the relationship of *C. trachomatis* strain types with ethnicity and self-reported urogenital symptoms. Also, demographic and sexual risk behaviour characteristics of the identified clusters were analysed.

Methods: We selected *C. trachomatis* positive samples from the Dutch Chlamydia Screening Implementation study (CSI) among young individuals in Amsterdam, the Netherlands. Data on demographic characteristics was obtained from municipal registries. Data on sexual risk behaviour, and urogenital symptoms was obtained through a voluntary questionnaire. All samples were typed using high resolution MLST. Clusters were assigned using

minimum spanning trees and were combined with epidemiological data of the participants for subsequent analysis.
Results: We obtained full MLST data for *C. trachomatis* positive samples from 439 participants and detected 9 *ompA* genovars. MLST analysis identified 175 sequence types and 6 large clusters; in one cluster, participants with Surinamese/Antillean ethnicity were over-represented (58.8%) and this cluster predominantly consisted of genovar I. In addition, we also found one cluster in which participants with Dutch ethnicity were over-represented (90.0%) and that this cluster solely consisted of genovar G. A subgroup of 342 participants (77.9%) also completed a voluntary online questionnaire, providing information on urogenital symptoms. However, no association between *C. trachomatis* clusters and urogenital symptoms could be observed.
Conclusions: We found an association between urogenital *C. trachomatis* clusters and ethnicity among young screening participants in Amsterdam, the Netherlands. No association could be observed between *C. trachomatis* clusters and self-reported urogenital symptoms.

P016

Cluster analysis of *Chlamydia trachomatis* strains using two multilocus sequence typing schemes shows differences in discrimination of MSM strains versus those of heterosexuals

S.M. Bruisten[1], B. Versteeg[1], A. van der Ende[2], Y. Pannekoek[2]
[1]*GGD Amsterdam, Public Health Laboratory, Amsterdam,* [2]*AMC, Amsterdam*

Introduction: *Chlamydia trachomatis* remains the most common bacterial sexually transmitted infection worldwide. Despite testing, treatment, partner notification and counseling, huge public health efforts have not been able to control *C. trachomatis* infections. Therefore, to gain more insight in the epidemiology and transmission of *C. trachomatis*, multilocus sequence typing (MLST) schemes have been developed. However, there is no consensus regarding the use of an MLST scheme for epidemiological studies. Therefore, the objective of this study was to investigate the clustering of *C. trachomatis* strains using two MLST schemes with differing discriminatory capacities.
Methods: A retrospective analysis was performed using routinely collected data and samples from *C. trachomatis* infected men who have sex with men (MSM) and heterosexual women visiting the STI outpatient clinic of the PHS, Amsterdam. All selected samples were typed using two existing MLST schemes. One MLST scheme was based on 6 highly variable targets in the chlamydial genome, also referred to as high resolution MLST (hr-MLST-6) and the other MLST scheme was based on 7 housekeeping genes (MLST-7). For this study, the existing MLST-7 scheme was modified to a nested PCR enabling to test clinical samples. Sequences obtained from both MLST schemes were analyzed for cluster analysis and phylogenetic relationships.
Results: Full profiles were obtained for 175 samples; 84 (48%) were from MSM and 91 (52%) from heterosexuals. We detected 32 sequence types (STs) using the MLST-7scheme and 78 STs using the HR-MLST-6scheme. Cluster analyses using hr-MLST-6data showed segregation between isolates from MSM and heterosexuals. However, cluster analyses using the MLST-7 data did not show this segregation between isolates from MSM and heterosexuals.
Conclusions: The MLST-7 scheme was not able to discern closely related *C. trachomatis* strains over a small time frame confirming that it is more suitable for evolutionary studies. For short-term cluster analysis the HR-MLST-6scheme is considered more suitable to study the epidemiology and transmission of *C. trachomatis* in various populations.

P017

Molecular characteristics of extended-spectrum cephalosporin-resistant Enterobacteriaceae from humans in the community differ from those in broilers

E. van Duijkeren, L. Schouls, M.G. van Santen, A. Florijn, S.C. de Greeff, A.H.A.M. van Hoek
RIVM, CZO, Bilthoven

Introduction: Extended-spectrum-β-lactamase (ESBL)/AmpC producing Enterobacteriaceae have been found among humans worldwide. Most large-scale studies in humans, however, report data of patients or travelers and/or focus on ESBL-producing bacteria and/or certain bacterial species only (e.g. *Escherichia coli* or *Klebsiella pneumoniae*). Consequently, data on the prevalence of fecal carriage of ESBL/AmpC/carbapenemase producing Enterobacteriaceae in healthy humans in the community are scarce. Initially ESBL/AmpC-producing organisms were associated with hospitals and institutional care in humans, but they are now increasingly found in the community and in food-producing animals. A connection between ESBL/AmpC-producing bacteria in food animals and humans has been suggested. ESBL/AmpC-producing Enterobacteriaceae have frequently been reported in broilers and therefore they have been considered as a reservoir for ESBL/AmpC-encoding resistance genes with the potential to transmit to humans. The objective of the present study was to investigate the molecular characteristics of extended-spectrum cephalosporin (ESC)-resistant Enterobacteriaceae collected during a cross-sectional study examining the prevalence of faecal carriage of extended-spectrum β-lactamase-producing Enterobacteriaceae in a population sample of humans living in areas with high or low broiler density.

Methods: Extended-spectrum cephalosporin-resistant Enterobacteriaceae were identified by combination disc-diffusion test. The presence and composition of ESBL/AmpC/carbapenemase encoding genes were assessed by PCR and sequencing. For *Escherichia coli*, phylogenetic groups and MLST were determined.

Results: 175 ESC-resistant Enterobacteriaceae were cultured from 165 of 1,033 sampled persons, yielding a prevalence of 15.9 %. No carbapenemase genes were identified. Most isolates were *Escherichia coli* followed by *Citrobacter freundii* and *Enterobacter cloacae*. The predominant ESBL genes were $bla_{CTX\text{-}M\text{-}1}$ (n = 17), $bla_{CTX\text{-}M\text{-}15}$ (n = 16) and $bla_{CTX\text{-}M\text{-}14}$ (n = 9) but other allelic variants were also identified as well $bla_{SHV\text{-}12}$ and $bla_{TEM\text{-}52}$. The most common AmpC genes were $bla_{CMY\text{-}2}$ and $bla_{CMY\text{-}48\text{-}(like)}$. A large variety of *E. coli* genotypes was found, ST10 and ST131 being most common.

Conclusions: ESBL/AmpC genes in Enterobacteriaceae obtained from humans in the the community resembled those found in isolates from patients in Dutch hospitals, indicating that healthy humans act as a reservoir for transmission of these determinants to vulnerable people. The most prevalent ESBL/AmpC genes in isolates from humans on broiler farms as well as broilers are $bla_{CMY\text{-}2}$, $bla_{CTX\text{-}M\text{-}1}$ and $bla_{SHV\text{-}12}$, followed by $bla_{TEM\text{-}52}$, while $bla_{CTX\text{-}M\text{-}15}$ is not found. In contrast, in the present study, $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}15}$ and $bla_{CTX\text{-}M\text{-}14}$ were among the most prevalent ESBL-genes identified and *E. coli* isolates carrying $bla_{CMY\text{-}2}$, $bla_{SHV\text{-}12}$ and $bla_{TEM\text{-}52}$ were only found sporadically. The ESBL gene $bla_{CTX\text{-}M\text{-}1}$ has been discovered in humans in the community, patients, broiler farmers and broilers and it has been postulated that humans acquire this ESBL gene through contaminated poultry, because Dutch chicken meat has been shown to contain *E. coli* strains containing this resistance determinant. However, $bla_{CTX\text{-}M\text{-}1}$ is present in many different potential reservoirs, including cattle, companion animals, horses and pigs, and therefore conclusions regarding its origin cannot be drawn. In conclusion, the molecular characteristics of *E. coli* isolates differed from those of broilers suggesting that broilers are not an important source of ESBL/AmpC genes for humans living in the community.

P018

Double plasmodium infections in two travelers from Kenya

I.G. Manders[1], C.E. Delsing[1], G.J. Kootstra[1], B. Mulder[2]
[1]*Medisch Spectrum Twente, Department of Infectious Diseases, Enschede,* [2]*LabMicTA, Microbiology, Hengelo*

Despite increased travel to malaria endemic areas, reported cases of malaria in The Netherlands have decreased from 535 patients in 2000 to 164 in 2013. Patients mainly present with *Plasmodium falciparum* (74.8%) and less frequently with *P. vivax* (15.4%), *P. ovale* (7.3%) and *P. malariae* (2.5%) infections. Among travelers double malaria infections within one patient are rarely seen and therefore easily missed. We describe a Dutch couple who initially presented with *P. falciparum*, for which they were adequately treated. However, both showed relapse based on *P. ovale*, for which additional therapy is required. Diagnostic pitfalls include that 3 bands in a rapid malaria tests do not distinguish between the presence of *P. falciparum* only or a mixed infection and young trophozoites in thin and thick blood smears are identical in *P. falciparum* and *P. ovale* parasites. Double malaria infections should be considered in patients with recurrent fever after *P. falciparum* treatment, which is inadequate for *P. ovale* and *P. vivax* hypnozoites.

P019

Complicated *Pseudomonas aeruginosa* meningitis after visiting a swimming pool

B. Mulder[1], D.G. Knapen[2], F. de Sousa[2], T.H.F. Veneman[2]
[1]*LabMicTA, Microbiology, Hengelo,* [2]*ZGT Almelo, Intensive Care and Internal Medicine, Almelo*

Case: A previously healthy 75 year-old male was admitted to the Emergency Department with fever, vomiting and confusion since 8 hours, which started shortly after a visit to a public swimming-pool. Physical examination revealed a lethargic patient with evident nuchal rigidity. His vital signs were normal, his body temperature 39°C. Laboratory investigation showed an elevated C-reactive protein of 324mg/l and 18x10^{9} leucocytes/l, without other abnormalities. Analysis of cerebrospinal fluid (CSF) revealed a glucose concentration of 3.2mmol/l and 126.7 10^{6} leucocytes/l (93% PMNL). After taking blood and CSF cultures, treatment with ceftriaxone and amoxicillin was started according to the Dutch meningitis-guidelines. Aciclovir was started since a viral origin could not be excluded. CSF and blood cultures yielded the bacterium *Pseudomonas aeruginosa*, not sensitive to ceftriaxone nor amoxicillin. Aciclovir and ceftriaxone were replaced by ceftazidime after which the patient clinically improved. However, a secondary endopthalmitis caused complete blindness of his right eye. Enucleation will be necessary.

Conclusion: Community-acquired spontaneous meningitis caused by *P. aeruginosa* is rare but has a very high mortality rate. Swift recognition and treatment is of paramount importance. However, treatment according to Dutch meningitis-guidelines does not cover infection with *P. aeruginosa*, causing an important treatment-delay. Patients with a history of contact with service water, hot tubs, whirpools or swimmingpools should receive a different empiric antibiotic therapy. We suggest a regimen containing ceftazidim 3x2000mg instead of ceftriaxone as empiric treatment until culture results are available.

P020

Gentamicin is frequently underdosed in patients with sepsis in the emergency department

M. Cobussen[1], V. Hira[1], J.M.L. de Kort[2], D. Posthouwer[1], P.M. Stassen[1], M.B. Haeseker[1]

[1]*MUMC+, Department of Medical Microbiology, Maastricht,* [2]*Dr. Horacio E. Oduber Hospitaal, Department of Internal Medicine, Oranjestad*

Background: Gentamicin, in combination with a beta-lactam antibiotic, is frequently used as empirical treatment of sepsis in the emergency department (ED). In our hospital, the dose of gentamicin is 5 mg/kg given once. Because gentamicin is a concentration dependent bactericidal antibiotic, the gentamicin peak concentration should be sufficiently high to achieve an optimal effect. In this study, we investigated underdosing of gentamicin in patients with sepsis in the ED and differences in outcome between underdosed and adequately dosed patients.

Methods: Body weight, length, dose of gentamicin, and clinical characteristics of patients who visited our ED from April 2011 until April 2012 and who received gentamicin at the ED were retrospectively retrieved from electronic patient files. Underdosing of gentamicin was defined as a dose <4.5mg/kg andwas based on actual body weight. For obese patients (BMI >30), adjusted body weight was calculated.

Results: We included 173 patients with sepsis who received gentamicin. Thirty four (19.6%) patients were underdosed. The mean gentamicin dose was 3.8 ± 0.5 mg/kg in the underdosed group. Mortality within 28 days showed a trend in underdosed patients, compared to adequately dosed patients (20.6% vs. 11.5%, p = 0.17). Underdosed patients were more often admitted to the intensive care unit (ICU) directly from the ED (20.6% vs. 7.2%, p <0.05). There was no difference in severity of sepsis in both groups.

Conclusion: Gentamicin is underdosed in 19.6% of the patients with sepsis in our ED. In underdosed patients with sepsis, it is likely that target peak concentrations are not reached, which could negatively influence clinical outcome of infection. Adequate dosing of gentamicin might decrease mortality and improve clinical outcome. Standardized weighing and strategies aimed at correct dosing might decrease underdosing of gentamicin.

P021

Rapid and adequate confirmation of carbapenemase production with Cepheid Xpert Carba-R assay

M.G. Smith[1], B. Diederen[1], J. Scharringa[2], M. Leversteijn-Van Hall[3], A. Fluit[2], J. Cohen Stuart[1]

[1]*Medical Center Alkmaar, Department of Microbiology, Alkmaar,* [2]*UMC Utrecht, Department of Microbiology, Utrecht,* [3]*Bronovo Hospital, Department of Microbiology and Infection Control, Den Haag*

Introduction: Carbapenemase-producing Enterobacteriaceae are an emerging problem worldwide. Rapid and accurate detection of carbapenemase-producing strains is pivotal for adequate antibiotic therapy and infection control. The Cepheid Xpert Carba-R assay detects and identifies the most prevalent carbapenemases (KPC, VIM, IMP, NDM and OXA-48), using automated real-time polymerase chain reaction (PCR).

Methods and study aim: The test performance of the Xpert Carba-R was evaluated with 131 well-characterized non-repeat Enterobacteriaceae isolates, screened positive for carbapenemase production (meropenem MIC > 0,25 mg/L). The isolate collection contained 95 carbapenemase producing isolates (35 KPC-2 or KPC-3, 20 VIM-1, 4 KPC-2 plus VIM-1, 4 NDM, 4 IMP, and 28 OXA-48) and 36 negative control isolates producing ESBL, AmpC or GIM beta-lactamases. PCR and sequencing of beta-lactamase genes was used as reference test.

Results: The sensitivity of the Xpert Carba-R was 97% (92/95), with a 97% (35/36) specificity. The three negative isolates and the single false positive isolate remained discrepant after the Xpert Carba-R assay was repeated. The time to result was approximately 55 minutes with a hands-on time of only 2 minutes per isolate.

Conclusion: The Xpert Carba-R assay is a rapid and accurate instrument for the detection of the bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM} and bla_{OXA-48} genes.

P023

MLVA is superior to spa-typing and sufficient to characterize MRSA for surveillance purposes

T. Bosch, G.N. Pluister, M. van Luit, F. Landman, M. van Santen-Verheuvel, C. Schot, S. Witteveen, W.K. van der Zwaluw, M.E.O.C. Heck, L.M. Schouls

RIVM, Centre for Infectious Disease Research, Diagnostics and Perinatal Screening, Houten

Introduction: In the Netherlands, a low MRSA prevalence country, MRSA surveillance has started in 1989 at the National Institute for Public Health and the Environment (RIVM). Since 2008, all *S. aureus* isolates for the Dutch MRSA surveillance have been typed by Staphylococcal protein A (*spa*-) typing and multiple-locus variable number of tandem repeat analysis (MLVA). In this study, we assessed which approach is best to type MRSA for surveillance purposes, *spa*-typing or MLVA or a combination of both methods.

Material & methods: All surveillance MRSA isolates received from 2008 until 2013 with complete *spa*- and MLVA-profiles (n = 20,771) were used for comparative analyses. Isolates were grouped as MRSA (n = 12,728) and livestock-associated (LA)-MRSA (n = 8,043), where LA-MRSA were defined as isolates belonging to MLVA

complex 398 (MC398). To assess the discriminatory power and the congruence of the typing methods, Simpson's index of diversity and the Wallace's coefficient were calculated.

Results: The number of MLVA-types (MTs) present (n = 1592) among MRSA isolates was nearly twice as high as the number of *spa*-types (n = 788). Among LA-MRSA isolates, 110 different *spa*-types and 136 MTs were found.

When sorted by type-frequency, 70% of the MRSA isolates represented 37 *spa*-types, while 139 MTs were found in these isolates. No significant difference was seen when LA-MRSA types were plotted and 70% of the isolates only represented two MTs and a single spa-type.

Diversity of the MLVA among the top 10 *spa*-types of MRSA isolates was high, with a DI of 0.96. In contrast, *spa*-diversity among the top 10 MLVA-types of MRSA isolates was considerably lower, with a DI of 0.83. In total, the top 10 *spa*-types (n = 5,892) yielded 394 different MTs while the top 10 MTs (n = 3,362) comprised 45 different *spa*-types.

Congruence analyses showed that the probability that two MRSA isolates with the same *spa*-type also have the same MT was low (Wallace's coefficient 0.27). However, two MRSA isolates with the same MT will also show the same *spa*-type in the vast majority of cases (Wallace's coefficient 0.90). Wallace coefficients of *spa*-typing and MLVA for LA-MRSA isolates showed relatively high values for both methods (0.87 and 0.96), indicating virtually complete congruence.

Conclusion: Based on the limited discriminatory power of *spa*-typing and the fact that performing both methods does not increase resolution we conclude that MLVA alone will suffice to characterize MRSA isolates for surveillance purposes.

P024

Presence of rhinovirus viremia in adult patients with high viral load in bronchoalveolar lavages

A.L. Klink, A.C.M. Kroes, E.C.J. Claas, J.J.C. de Vries
LUMC, Department of Medical Microbiology, Leiden

Introduction: Rhinovirus is a major cause of common cold and besides relatively mild upper respiratory tract infections rhinovirus can cause severe lower respiratory tract infections in immunocompromised patients, elderly patients and children.

Several studies have demonstrated rhinovirus in the blood of children, which was associated with asthma, higher nasopharyngeal loads and worse clinical signs and symptoms.

For this reason, it is important to further explore the significance of dissemination of the virus to the bloodstream (viremia) as a marker of severe disease.

The objective of this study was to determine whether rhinovirus viremia is present in adult patients and if this is associated with worse clinical symptoms/ outcome.

Methods: Rhinovirus positive bronchoalveolar lavages (BAL), Ct values < 25, tested between 2008 and June 2014 were selected and plasma and or serum samples of the patients were tested for the presence of rhinovirus RNA. All samples available (with a maximum of one per day), up to 4 weeks before and 4 weeks after BAL sampling date, were included. RNA was extracted using the Magna Pure LC, with Total Nucleic Acid Isolation Kit Large Volume. Rhinovirus RNA amplification was performed in duplicate with an in-house real-time polymerase chain reaction (amplifying 142-bp fragment of the 5'-UTR region using primers, probes and conditions previously described by Loens et al).

From patient files, if accessible, risk factors, symptoms, diagnosis, chest radiography, microbiological data and disease outcome were obtained.

Results: In the 6,5 years 37 patients had a BAL with rhinovirus Ct <25, and of 28 patients blood was available (89 samples). Four patients (15%) tested rhinovirus positive in their blood, Ct 34 - 42. Of these patients the median age was 60 years. Two patients were known smokers and one patient had lung fibrosis. Three patients received stem cell transplantation, and one had a kidney transplant.

Overall 15 of the 28 patients (54%) were stem cell transplantation patients and 6 (21%) solid organ transplantation patients. No significant difference could be found in risk factors, symptoms, radiological findings or co-pathogens. However patients with rhinovirus viremia had significant higher mortality, 100% versus 25%, p = 0,004 (chi-square).

Conclusion: Rhinovirus viremia does occur in adult patients with rhinovirus infection. Rhinovirus viremia could only be detected in a small number of patients, although a high risk group of immunocompromised patients with high rhinovirus load in BAL was tested. In this study rhinovirus viremia is a relevant prognostic factor as it is associated with higher mortality. Whether rhinovirus viremia plays a causative role in this poor prognosis or is the result of end stage disease needs to be determined.

P025

Phylogenetic diversity and antibiotic resistance in bacteria associated with three Mediterranean sponges

D. Versluis[1], D. Sipkema[1], H. Hauke Smidt[1], N. Mohamad[1], K. McPherson[1], M.W.J. van Passel[1,2]
[1]*Wageningen University, Laboratory of Microbiology, Wageningen,* [2]*Centre for Zoonoses and Environmental Microbiology, National Institute of Public Health and the Environment, Bilthoven*

Introduction: Sponges often harbour a dense and diverse microbial community that co-exists with the host in a mutualistic relationship. Thus far, little is known regarding the metabolic functions and possible host-microbe interactions of sponge-associated bacteria. Pure cultures of sponge bacteria are desirable in order to study their ecological roles and pharmaceutical potential. However, there is a large discrepancy between the cultivable bacterial fraction, which is predominantly comprised of *Proteobacteria* and *Actinobacteria*, and the community in its natural environment. Here, we study the cultivable fraction of bacteria from three Mediterranean sponges on different media that are either supplemented, or not supplemented, with antibiotics. In addition, we investigate if sponge-associated bacteria can potentially serve as a reservoir for resistance determinants that can be acquired by pathogenic bacteria.

Methods: Bacterial diversity in samples from *A. aerophoba*, *P. ficiformis* and *C. candelabrum* was analysed. The cultivable bacterial fraction from these sponges was studied on five different media in the form of 60 communities scraped from plates without antibiotics, as well as of individual isolates that were grown on these media supplemented with either one or two of 14 different antibiotics. The cultivation media were 2 variations of Mueller Hinton agar, 2 variations of Marine agar, and Mucin agar. Samples were inoculated directly on agar, and on filter membranes on top of each medium. (Combinations of) the following 14 antibiotics were used: polymyxin B, linezolid, daptomycin, vancomycin, penicillin, erythromycin, ciprofloxacin, tetracycline, chloramphenicol, sulfamethoxazole, trimethoprim, lincomycin, kanamycin and rifampycin. We applied bar-coded 16S rRNA gene amplicon sequencing for species identification.

Results: The bacterial community of the three sponge species was comprised of Proteobacteria, *Nitrospirae*, *Chloroflexi, Bacteroidetes, Actinobacteria* and *Acidobacteria* (>0.1 % relative abundance). Growth on all five different agar media in the absence of antibiotics was dominated by *Proteobacteria* (>50 % relative abundance), almost all of which was due to the high abundance of *Pseudovibrio spp*. Fifteen OTUs were detected for which the representative read had less than 90 % nucleotide identity when compared to the closest type strain. A library of over 600 isolates was obtained from media supplemented with antibiotics, and those strains were classified to be *Proteobacteria, Bacteroidetes, Actinobacteria* and *Firmicutes*. Resistance profiling of 31 individual isolates showed resistance against all antibiotics that were used in the resistance screen, except for cefotaxime and rifampicin. Three strains belonging to the family of *Flavobactericeae* were isolated that constitute new species.

Conclusion: We conclude that implementation of straightforward cultivation methods yields novel species, which opens the way for directed investigation of their metabolic potential. Nevertheless, the isolation novel bacteria is hampered by accelerated growth of a few strains (in this case *Pseudovibrio sp.*). We find that both the application of antibiotics, and decreasing the medium nutrient content, increases the relative abundance of novel species, thereby increasing the chance to obtain them in pure culture. Bacterial isolates were found to be resistant to a range of antibiotics. Functional studies and genome analyses are currently being performed to investigate the presence of resistance genes.

P026

Metronidazole addition to a selective medium increases the sensitivity of the isolation of Neisseria gonorrhoeae in pharyngeal cultures

R.O.P. Draga[1], I. Linde[2], A.P. van Dam[3]
[1]*AMC, Department of Medical Microbiology, Amsterdam,* [2]*Amsterdam Health Service, Public Health Laboratory, Amsterdam,* [3]*Onze Lieve Vrouwe Gasthuis General Hospital, Department of Medical Microbiology, Amsterdam*

Introduction: Gonorrhea, caused by *Neisseria gonorrhoeae*, is the second most prevalent bacterial sexually transmitted infection worldwide. Several selective media for the isolation of *N. gonorrhoeae* have been developed such as the Thayer-Martin (MTM), Martin-Lewis (ML) and the New York City medium (NYC) containing the antimicrobials vancomycin, colistin, trimethoprim and different antifungal agents. Our routinely used medium is the GC-Lect medium (Becton Dickinson Microbiology Systems [BDMS], Cockeysville, Md.) containing vancomycin (2 mg/l), lincomycin (1 mg/l), colistin, trimethoprim and amphotericin B. The medium has shown to prevent the growth of normal flora, especially yeasts, gram-positive cocci and *Capnocytophaga* species. However, in 2013, at the Amsterdam sexually transmitted infections (STI) clinic (the Netherlands), a total of 746 pharyngeal swabs were taken for the diagnosis of gonorrhea on GC-Lect media of which 245 (33%) were positive for *Capnocytophaga* species with spreading on the plate, due to the sliding motility of *Capnocytophaga*, obscuring the possible presence of *N. gonorrhoeae* colonies. In this study we tested a new in-house produced selective medium to prevent the growth of *Capnocytophaga* species in pharyngeal cultures without impeding the growth of *N. gonorrhoeae*.

Methods: Susceptibility testing of an oral *Capnocytophaga* strain was done with disc diffusion and subsequently by e-tests. An in-house produced selective medium (GCXP) consists of GC-agar base, a similar agar base, hemoglobin powder (OXOID) and Vitox enrichment (OXOID) and LCAT supplement, containing lincomycin, colistin, amphotericin B and trimethoprim. We compared this medium to GC-lect and to a newly developed medium GCXP containing additionally vancomycin (2 mg/l) and metronidazole (2

mg/l) (GCXP-MV). For this, 15 different *Capnocytophaga* and 6 *N. gonorrhoeae* strains were streaked in a 0.5 McFarland concentration (8log CFU/ mL) with a cotton swab onto these media. Finally, tonsil swabs, taken in 94 patients suspected of pharyngeal gonococcal infection were streaked onto the GC-Lect selective medium and GCXP-MV medium.

Results: Out of 19 antibiotics tested, metronidazole and vancomycin appeared to be helpful antibiotics to inhibit the growth of *Capnocytophaga* without inhibiting the growth of *N. gonorrhoeae*. 7/15 *Capnocytophaga* strains showed susceptibility to vancomycin, 9/15 strains showed susceptibility to metronidazole and only 1/15 strains was high-level resistant to both antibiotics. The GCXP allowed growth, but inhibited the surface spread of 12 out of 15 *Capnocytophaga* species. On the GCXP-MV medium growth of 14 out of 15 *Capnocytophaga* strains was inhibited, although from four strains some small colonies could still be discerned. The growth of the 6 *N. gonorrhoeae* strains on the GCXP-MV medium was not inhibited in comparison to the GC-Lect medium. *N. gonorrhoeae* could be cultured from 11/106 patients when GCXP-MV medium was used, whereas from GC-Lect medium only 5 cultures were positive. On the GCXP-MV medium swarming *Capnocytophaga* grew from only 2 patients, whereas these could be seen on 68/106 cultures on GC-lect-plates, of which 14 were completely overgrown.

Conclusions: The GXCP-MV medium containing vancomycin and metronidazole effectively inhibits the growth of *Capnocytophaga* species and improves the diagnosis of pharyngeal gonococcal infection.

P027

Detection of carbapenemase producing Enterobacteriaceae using a low-cost, easy to perform phenotypic test

D.J. Hetem, Y.S. Monkou, S.P. van Mens, J. Scharringa, A.C. Fluit, R.J. Rentenaar

[1]*UMC Utrecht, Department of Medical Microbiology, Utrecht*

Introduction: The emergence of carbapenemase producing Enterobacteriaceae is threatening the effectiveness of last resort antibiotics. Identifying these carbapenemase producing Enterobacteriaceae is important for administering adequate antibiotic treatment and installation of adequate isolation precautions. Here we used a phenotypic test for the detection of carbapenemase producing Enterobacteriaceae, developed by the National Institute for Public Health and the Environment (RIVM), based on the *in vitro* hydrolysis of meropenem by a bacterial lysate. In this study, we evaluate the sensitivity and specificity of this test using carbapenemase and non-carbapenemase producers of various Enterobacteriaceae.

Methods: We used 128 well-characterized non-repeat Enterobacteriaceae isolates suspected of carbapenemase production according to the Dutch guideline on detection of highly resistant micro-organisms, (typically with confirmed meropenem MICs = 0.5 mg/L), including 99 carbapenemase producers (36 KPC, one GES, 31 MBL, four KPC plus VIM, 25 OXA-48, two OXA-162), and 29 ESBL and/or AmpC-producing isolates. Beta-lactamase genes had previously been characterized by PCR and sequencing. Isolated colonies (1/3 - 1/2 of a calibrated 10 µl loop) were suspended in 200µL B-PER II protein extraction buffer (Thermo Fisher Scientific), to which a meropenem paper disk (10µg/disk, Oxoid) was added and incubated for 2 hours at 35-37°C. The meropenem disks were placed on a Mueller-Hinton agar on which a fully susceptible *Escherichia coli* (ATCC 25922) strain was inoculated, similar to the standard disk diffusion protocol, and incubated at 35-37°C. Bacterial growth of the susceptible *E. coli* after 18 h. incubation up till the paper disk was regarded as indicative of the presence of carbapenemases. Presence of a growth inhibition zone was regarded as negative. Test results were interpreted by two technicians, blinded for microorganism identity and susceptibility test results.

Results: Of the 99 bacteria harbouring carbapenemase genes, 94 and 93 were correctly identified as carbapenemase producers by the two lab technicians respectively. Five false negatives were seen by both lab technicians; three *Klebsiella pneumoniae* isolates with OXA-48 genes and two Proteus mirabilis with CMY-16 and VIM-1 genes. One *K. pneumoniae* OXA-162 was misclassified by only one of the technicians. No false positives were seen in the bacteria harbouring ESBL and/or AmpC, resulting in a 94-95% sensitivity and a 100% specificity.

Conclusion: This easy to perform phenotypic assay provides a cheap, sensitive and highly specific method for detection of carbapenemase producing Enterobacteriaceae. Nevertheless, some false negative test results may occur in *K. pneumoniae* with OXA-48-like enzymes or *P. mirabilis* harbouring VIM-1.

P028

Methicillin resistant *Staphylococcus aureus* in dental students

J.J. de Soet, M.A. Hoogenkamp, C.M.C. Volgenant

ACTA, Preventive Dentistry, Amsterdam

Introduction: Methicillin resistant *Staphylococcus aureus* (MRSA) is a pathogen, which is often found in relation to health care settings. Studies in the United States and Mexico have shown that the presence of MRSA in individuals working in a dental clinic is significantly higher than in the general population. This may be due to the nature of the work in the dental clinic; e.g. spreading of bacteria due to aerosol formation during dental treatment.

To develop appropriate regulations in oral health care in relationship to MRSA containment, it is important to get more insight into the prevalence of MRSA among oral health care workers. Therefore this study aimed to investigate the prevalence of MRSA on hand, nose and in the mouth of dental students in relationship to the time they have been treating patients in 4 different dental schools in Europe: Gothenburg, Rome, Thessaloniki and Amsterdam.

Methods: The study protocol was approved by the medical ethics review committee of the VU University Medical Centre and written informed consent was obtained from all participating students. Samples were taken from the nose (both anterior nares), the dorsum of the tongue and the hand (between ring- and little-finger) and incubated aerobically for 24 hours in Tryptic Soy Broth (TSB) at 37°C. Subsequently, aliquots of the samples were transferred to Manitol-Salt Agar and incubated aerobically for 24 hours at 37°C. Colonies with a yellow halo on the agar plate were picked and each isolate was subcultured in TSB containing various concentrations of oxacillin). Isolates exhibiting growth at the highest oxacillin concentration (4mg/ml) were checked for identity by a coagulase test.

Results: In total 531 dental students were sampled. The mean age of the group was 23.9 years and 63% was female. In Gothenburg no MRSA was found. In Rome five (3.2%) students were tested positive, in Thessaloniki 3 students (1,5 %) was tested positive and in Amsterdam five (5.5%) students were tested positive for MRSA in at least one of the samples. No statistical significant relationship between the age of the students, their gender, the number of years in training (with patient contact) or academic study years was found.

Discussion: MRSA has been found to be transmitted easily from patient to oral health care workers in several studies outside of Europe. Our study suggests that the transmission of MRSA may be lower than anticipated, possibly due to strict regulations in hygiene measures in Europe. A prevalence of 3-6% for MRSA is not alarming and does not support the introduction of obligatory MRSA tests in dentistry. If the MRSA prevalence in the population increases, regular MRSA testing for oral healthcare workers may be required in the future.

P029

Importance of methane for *Sphagnum*-associated microbial nitrogen fixation in peatlands

M.A.R. Kox[1], S.L. Aalto[2], T. Penttilä[3], M. Tiirola[2], M.S.M. Jetten[1], K.E. Ettwig[1]

[1]Radboud University, Department of Microbiology, Nijmegen, [2]University of Jyväskylä, Biological and Environment, JyvÄSkylÄ, Finland, [3]The Finnish Forest Research Institute, Vantaa, Finland

Pristine *Sphagnum*-dominated bogs are N-limited and depend on N_2 fixation by diazotrophs or atmospheric N-deposition for their N supply. *Sphagnum* mosses harbor a very diverse microbiome including numerous methanotrophs that contribute to the carbon supply of the mosses. It has been hypothesized that methane might also stimulate *Sphagnum*-associated N_2 fixation during early stages of peat development, thereby increasing *Sphagnum* growth and N content, although other studies did not see this effect.

The objectives are to elucidate whether methane indeed does stimulate N_2 fixation in *Sphagnum* mosses from ombro-, oligo- and mesotrophic field sites and to test whether this is affected by oxygen level. Furthermore, activity measurements were related to identity of the different diazotrophs including methanotrophs by analysis of total and diazotrophic microbial communities.

N_2 fixation and CH_4 oxidation activity were studied by incubating mosses with $^{15}N_2$, $^{15}N_2$ + $^{13}CH_4$ or no additions. Furthermore, different oxygen regimes (aerobic, micro-aerobic (either N_2-He atmosphere or submerged) were applied. DNA and RNA are to be extracted and 16S rRNA and nitrogenase (nifH) genes quantified and analyzed by qPCR and high-throughput sequencing of amplicons.

The isotope tracer-studies showed that only in oligotrophic sites the diazotrophic communities associated with *Sphagnum* reacted positively to methane addition and microaerobic conditions. For mosses from ombrotrophic and mesotrophic sites, diazotrophic activity was neither affected by methane addition, nor by reduced oxygen concentration. Combined, these results indicate that methane dependent nitrogen fixation may only be important under certain environmental conditions in *Sphagnum*-dominated peatlands. Conditions proven to be important for methanotrophy (pH, water level) and diazotrophic community composition are likely to affect diazotrophy as well, and will be subject of future studies.

P030

Humanized zebrafish as an improved *Staphylococcus aureus* infection model

M. van Gent[1], C.J.C. de Haas[1], J. den Hertog[2], J.A.G. Strijp[1]

[1]UMC Utrecht, Department of Medical Microbiology, Utrecht, [2]Hubrecht Institute, Department of Molecular Developmental Zoology, Utrecht

Multi-resistant Staphylococci (MRSA) pose an ever-increasing health threat to the human population. The lack of a proper *in vivo* animal model reliably mimicking human infections has prevented us from gaining deeper insight into the pathophysiology of this important bacterium and the development of alternative treatment strategies to cope with antibiotic resistance

(e.g. vaccination). The main reason for the host restriction of *S. aureus* is the strict human specificity of at least fifteen virulence factors expressed by this bacterium (e.g. adhesion molecules, toxins, and immune evasion molecules). We aimed to relieve the limitation of host restriction by developing an improved, humanized zebrafish infection model that incorporates the contribution of human-specific virulence factors.
The human C5a receptor senses the chemokine C5a that is released upon activation of the complement system and induces neutrophil activation and migration towards the site of infection. Three secreted *S. aureus* virulence factors target this receptor: Chemotaxis inhibitory protein of *S. aureus* (CHIPS) competes with ligand binding, while the bacterial pore-forming toxins Panton-Valentine Leukocidin and ?-hemolysin bind the C5a receptor to cause cell lysis. Harnessing our molecular understanding of the interactions between this receptor, its ligand C5a, and the bacterial evasion molecules, we designed a 'humanized' zebrafish C5a receptor that maintained responsiveness to its ligand, zebrafish C5a, while gaining sensitivity to the inhibitory activity of bacterial CHIPS. The required adaptations were introduced into the zebrafish genome using a modified CRISPR/Cas9 system. The constructed humanized zebrafish showed diminished neutrophil chemotaxis in the presence of CHIPS and are currently examined in infection models with various *S. aureus* strains and mutants.
Current efforts focus on extending this strategy to additional receptors targeted by human-specific *S. aureus* virulence factors. Relieving the limitations of host restriction in this improved infection model will be instrumental in studying the pathogenesis of *S. aureus* infection and the development of novel ways to combat the increasingly prevalent multi-resistant staphylococcal infections.

P031

Simultanious detection of human Norovirus and human Sapovirus in fecal samples by real-time PCR

H.F.M. Willemse-Erix, M.H.A. Hermans, C.J.J. Huijsmans
Jeroen Bosch Hospital, Department of Molecular Diagnostics, Den Bosch

Introduction: Human norovirus (NoV) and human Sapovirus (SaV) are recognized as a major cause of acute viral gastoenteritis. Usually, the disease progression is mild and self-limiting, but for young children and the elderly this kind of infection can be severe. Since these pathogens are highly contagious, they are also associated with outbreaks in child daycare and healthcare institutions. In that case, good and rapid diagnostics are necessary.
At this moment, most Laboratories use real-time PCR for the detection of these virusses. In this study we described the development of a reverse transcription PCR (RT-PCR) for the simultanious detection of NoV and SaV.
Methods: Two multiplex PCR tests were constructed. The first multiplex contains primers and probes for the detection of norovirus genotype 1 and sapovirus while the second multiplex detects norovirus genotype 2 and an internal control.
RNA extraction from fecal samples was performed using the EasyMag system. Amplification of targets was performed using the ABI 7500 FAST (Applied Biosystems). The PCR program consisted of 45 two step cycli with annealingtemperature of 60°C.
Two collections of well-known samples were used to evaluate the tests.
Results: The reproducibility and linearity of the two multiplex assays was according to expectation with a variance coefficient below 5%. For the two validation panels the outcome and ct-values of the assays were in concordance with previously found results and results from reference laboratories.
Furthermore, a ready-to-use mastermix including primers, probes and Taq polymerase was developed. This mastermix is stable for at least one week when stored at 4°C and can be frozen and thawed several times without loss of sensitivity. At this moment, the assay is used in routine diagnostics to establish usability and prevalence of both targets.
Conclusion: It can be stated that, for our hospital, the development of the noro-/sapovirus multiplex real-time RT-PCR assay has led to an improvement in the diagnostics of viral gastro-enteritis. The current detection of NoV is simplified and the detection of SaV is added without extra handling or costs.

P032

Validation of the GBS GeneXpert assay in a Dutch Tertiary center

A.L.M. van den Berg, L.N. van Belzen, A.C.C. van Oppen, P.G. Scheffer, J.G. Kusters, T. van der Bruggen
UMC Utrecht, Department of Medical Microbiology, Utrecht

Introduction: Group B Streptococci (GBS) are the most frequent cause of early-onset neonatal sepsis. In some countries, all pregnant women are routinely screened for GBS and when positive, treated with intrapartum antibiotic prophylaxis (IAP). While this strategy reduces the incidence of neonatal GBS sepsis, the disadvantage is overconsumption of antibiotics, promoting antimicrobial resistance, and medicalization of childbirth. In The Netherlands a risk-based strategy is advocated. Women with risk factors, such as premature rupture of membranes or prolonged rupture of membranes during term pregnancy, are screened for GBS by culture. When positive, these women receive IAP. However, the status of

GBS presence is often unknown and culture usually takes too long, because IAP must be started at least 4 hours before delivery. A rapid test (such as the GBS GeneXpert) could be of benefit provided that the test has a high sensitivity and specificity. The aim of this study was to validate the GBS GeneXpert assay.

Methods: In this prospective study (from September 2014 to March 2015), a total of 200 women with premature labor (< 37 wk) or premature rupture of membranes during term pregnancy will be included after informed consent. Vaginal-rectal swabs were collected for the GBS GeneXpert assay (Cepheid) and culture. During working hours the material was sent to the microbiology laboratory immediately, followed by notification of the laboratory. Outside working hours, the materials were sent to the laboratory without notification and the samples were processed the next day as early as possible. In the laboratory, PCR was carried out according to the manufacturer's instructions. Culture was performed with NGM agar and blood agar, (both from regular swab and PCR swab) with and without an enrichment broth (LIM). The performance of the GBS GeneXpert PCR was determined, using culture (any culture positive) as gold standard. Time to the test result in relation to the moment of delivery was monitored. A positive PCR result was followed by IAP.

Results and **Conclusions:** By December 2014, a total of 105 patients has been included. Interim analysis showed that GBS carrier state was 26% in this population. Compared to culture (any culture positive), the GBS GeneXpert assay had a sensitivity, specificity, NPV and PPV of 96.3%, 98.7%, 98.7% and 96.3, respectively. 70% of GBS GeneXpert results were available 4 hours or more before delivery. Test results that were too late to allow for effective IAP, were mostly the result of sampling outside laboratory working hours. Based on these results, the GBS GeneXpert assay is a highly accurate method to detect rectovaginal colonization with GBS. The method is rapid and provides a timely result in about 70% of pregnant women with risk factors, but practical issues, such as transport delay and sampling outside laboratory working hours, cause delay in the rest of cases. The pros and cons of different implementation strategies (e.g. point-of care-testing, 24/7 laboratories) must be further evaluated.

P033

Can gene regulation optimise fluxes through metabolic networks?

J.C. Hendriks, R. Planque, F.J. Bruggeman, J. Hulshof
VU, Systems Biology, Amsterdam

Microorganisms are confronted regularly with changing conditions. They use gene expression regulation to adapt concentrations of metabolic enzymes. One perspective on this behaviour comes from evolutionary theory: in order to persist, microorganisms have to sustain a high fitness to prevent being outcompeted by competitors. Accordingly, the objective of gene regulation is to adjust enzyme concentrations in such a way that metabolic fitness is maximised. Whether maximisation of fitness is attainable by way of gene regulation is however not at all understood. Perhaps such complex gene regulation is required that this cannot be considered feasible. Here I present a mathematical theory designed to answer whether gene regulation is capable of maximising the fitness of a metabolic network. The theory makes a number of predictions, two with direct biological relevance are: i) optimisation of metabolism is possible with elementary, biochemical mechanisms for gene regulation, and ii) the number of metabolites that function as inputs for gene regulation equals the number of parameters metabolism is optimised to. The theory shows for the first time that the basic mechanisms of gene regulation, by way of metabolite-binding transcription factors, is enormously powerful in steering metabolism to optimal states. These insights are of great help in synthetic biology and biotechnology applications where we aim to redesign microorganisms for particular objectives.

P034

The effect of sampling and storage on the fecal microbiota composition in healthy and diseased subjects

D.I. Tedjo, D.M.A.E. Jonkers, P.H. Savelkoul, A.A. Masclee, N. van Best, M.J. Pierik, J. Penders
MUMC+, School for Nutrition and Translational Research in Metabolism (NUTRIM), Department of Medical Microbiology, Maastricht

Introduction: Many large-scale cohort studies are currently being designed to study the influence of the human microbiome in health and disease. Adequate sampling strategies are required (in such studies) to limit bias due to shifts in microbial communities during sampling and storage. The aim of this study is to examine the impact of different sampling and storage conditions on the stability of fecal microbial communities.

Methods: Fecal samples from 10 healthy controls, 10 irritable bowel syndrome and 8 inflammatory bowel disease patients were aliquoted immediately after defection and stored at -80°C, -20°C for 1 week (1wk -20°C), +4°C for 24 hrs (24h +4°C) or room temperature for 24 hrs (24h RT). Furthermore, a fecal swab (FS) was collected and stored for 48-72 hours at RT. We used pyrosequencing of the 16S gene to investigate the stability of microbial communities.

Results: Comparisons between -80°C samples and 1wk -20°C, 24h +4°C or 24h RT samples, showed no significant difference in a-diversity. FS showed a significant higher

a-diversity compared to -80°C samples. UPGMA clustering and principal coordinate analyses showed the samples clustered significantly by test subject ($p < 0.001$ for unweighted Unifrac, weighted Unifrac and Bray-Curtis), but not by storage method. Bray-Curtis dissimilarity and (un)weighted Unifrac showed a significant ($p < 0.05$) higher distance between FS and -80°C samples versus the other methods and -80°C samples.
No significant change in the relative abundance of extreme oxygen species *Ruminococcus*, *Faecalibacterium* and *Roseburia* was observed between -80°C and 1wk -20°C, 24h +4°C or 24h RT. The relative abundance in *Ruminococcus* ($p < 0.05$, $\Delta_{median} = 1.2 \cdot 10^{-2}$%) and Enterobacteriaceae ($p < 0.05$, $\Delta_{median} = 8.4 \cdot 10^{-4}$%) was significantly higher in the FS compared to the -80°C samples.
Conclusions: In this study we demonstrated storage up to 24 hrs at room temperature did not significantly alter the fecal microbial community structure as compared to direct freezing of feces from both healthy subjects and patients with gastrointestinal disorders. When using fecal swabs DNA isolation should be optimized to ensure optimal preservation of microbial community structures.

P035

Optimization of 16S rRNA sequencing on patient materials

G.W.J. Verberne, B. van Rotterdam, D. Willemse, M. Hermans
Avans University, Forensic Laboratory Science, Breda

Introduction: There are many ways to identify bacteria. One of those methods is 16S rRNA sequencing. This gene has conserved regions and hyper variable regions. The nucleotides in the hyper variable regions, are different for each bacterial species. Because the gene has a small base pare length (± 1542 bp), the conserved regions haven't changed in many centuries. Furthermore every bacterium has this gene, which means that it is useful for detection of bacteria.
In the current protocol, PCR was performed using a annealing temperature of 53 °C. This resulted in aspecific bands, which makes it's impossible to obtain a interpretable sequence. In this study the PCR was optimized and evaluated on previously tested samples
Materials: Cultured *E. coli* and *S. aureus* were used for the optimization. A PCR gradient was performed using an annealing temperature ranging from 58°C - 68°C. After the annealing temperature was determent, the number of cycles was tested. The influence of numbers of cycles was tested at 20, 30, 35, 40, 45 and 50 cycles.
The adjusted protocol was used on bacterial strains and DNA isolated from patient materials. Results obtained where compared to previous results.
Results: The most optimal annealing temperature was found to be 60°C. At this temperature no aspecific bands were found. Further increasement of the temperature resulted in loss of detection. The samples that were tested on 20 cycles gave no amplification band. When the PCR amplification cycles were increased the negative control became positive. This was first shown when samples where amplified with 35 cycles. Which concluded that 30 cycles would be enough to perform a PCR for 16S rRNA sequencing.
Patient samples were tested using the adjustments in annealing temperature and number of cycles. Ten direct patient samples were tested. They were compared with the same samples performed at an annealing temperature of 53°C. The main difference was, that the sample that were amplified at 60°C didn't reveal any aspecific bands. Seven out of ten samples were used for sequencing. Identification was the same as previously tested.
Furthermore, thirty three bacterial cultured strains were tested. This resulted in a amplification band of all samples at the same height with no aspecific bands.
Conclusion: By altering the temperature to 60°C the specificity of the test increased. The tests also showed that 30 cycles was enough to perform a 16S rRNA sequencing test. A protocol was developed for samples like cultured blood, CSF and pus.

P036

Whole genome mapping as a novel high-resolution typing tool for *Legionella pneumophila*

F. Landman[1], S. Euser[2], J. Den Boer[2], E. Ijzerman[2], J. Bruin[2], L.M. Schouls[1], T. Bosch[1]
[1]*RIVM, IDS/BSR, Bilthoven,* [2]*Streeklab Haarlem*

Introduction: *Legionella pneumophila* is the causative agent for Legionnaires' disease and is responsible for two large outbreaks in the Netherlands. The current typing method for *Legionella* is sequence-based typing (SBT) in which seven gene fragments are sequenced. However, several studies have described situations in which SBT did not provide the sufficient discriminatory power needed to distinguish between outbreak-related *Legionella* isolates and non-outbreak isolates. Recently, a novel typing technique named whole genome mapping (WGM) was used as a successful typing tool for discriminating MRSA isolates belonging to the homogeneous livestock-associated clade. In this study, we assessed and validated the capability of whole genome mapping to differentiate *L. pneumophila* isolates. For this purpose, we used epidemiologically related and non-related *Legionella* isolates.
Methods: In total, 51 *Legionella* isolates were used in this study to create 57 whole genome maps (WGMs) as

described before. Two *L. pneumophila* isolates were used for reproducibility and stability experiments, and two isolates were selected for comparison of WGMs created in our laboratory with *in-silico* maps. The discriminatory power of WGM for *L. pneumophila* and the capability to identify transmission events was studied using 32 isolates obtained during two well-documented outbreaks in the Netherlands (Bovenkarspel (n = 25) and Amsterdam (n = 7)) and 16 epidemiologically unrelated *L. pneumophila* isolates. All isolates used in this study came from pre-existing collections and were also characterized by SBT.

Results: The WGMs, created using the same DNA on three different occasions, and the WGMs of an isolate that was sub-cultured for 30 consecutive days were indistinguishable (similarity between WGMs >99%), demonstrating high reproducibility and stability. A comparison between the real WGMs of two isolates their *in-silico* counterparts obtained from their whole genome sequences, showed similarities of 98.4% and 99.2%.

Within the Bovenkarspel outbreak, 24 of the 25 isolates showed a similarity of >99%. The last isolate differed from the other isolates mainly because of two deletions of 54kb and 30kb and yielded a similarity of 95%. All WGMs from the Amsterdam outbreak were indistinguishable (100% similarity). The WGMs of both outbreaks were clearly distinct with only a 56% similarity between the two clusters. Comparison of the WGMs of 16 epidemiologically unrelated isolates revealed considerable diversity, with similarities ranging from 43% to 77%. The WGMs of two unrelated isolates showed a >98% similarity with Bovenkarspel isolates and these strains were also indistinguishable with SBT.

Conclusion: WGM is an excellent reproducible and robust technique for typing *Legionella* strains. WGM was capable of confirming two well-documented outbreaks and revealed a clear distinction between both outbreaks and most non-related strains. The fact that two unrelated strains were regarded as highly related to the Bovenkarspel outbreak shows that epidemiological analyses together with genotypic comparisons between isolates is essential for accurate strain typing.

P037

MALDI-TOF MS for identification of Francisella tularensis to subspecies level in a clinical setting: Biomarkers or in-house Francisella library?

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-Bakker, F.A.G. Reubsaet

RIVM, IDS, Bilthoven

Introduction: Since October 2011, *Francisella tularensis*, the bacterium causing tularemia, is (re)emerging in multiple regions of the Netherlands. Three human cases were confirmed in 2014, one in the province of Zeeland and two in the province of Groningen. Infected hares, a natural reservoir of *F. tularensis*, were found in the province of Limburg, the province of Zeeland and the province of Utrecht. Tularemia displays a wide spectrum of symptoms including ulceroglandular or oropharyngeal infection, pneumonia and sepsis. Due to the low inoculation dose and the possibility to infect via an intact skin, *F. tularensis* is a serious threat for infections acquired in the laboratory. Virulence of *F. tularensis* depends on the subspecies. *F. tularensis* subspecies tularensis is highly pathogenic and should be handled under biosafety level 3 conditions. *F. tularensis* subspecies holarctica displays lower virulence and can as such be handled under biosafety level 2 conditions. *F. tularensis* subspecies novicida and *F. tularensis* subspecies mediasiatica are hardly virulent.

Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) is increasingly used in clinical laboratories for the identification of bacteria. However, *F. tularensis* is absent in the standard libraries of the most applied MALDI-TOF MS systems, the Vitek MS and the Bruker MALDI Biotyper System. The additional Bruker MALDI Biotyper Security library contains *F. tularensis* without subspecies differentiation. We have explored the development of an in-house *Francisella* library and the use of specific biomarkers for safe, fast and accurate determination of *F. tularensis* to subspecies level in a clinical laboratory setting.

Methods: Twenty-eight well characterized strains that are genetically or phenotypically related to *F. tularensis* were used in this study. After formic acid extraction, spectra were generated of all relevant *Francisella* species and all *F. tularensis* subspecies. The software of the MALDI Biotyper System was used to generate Main Spectra (MSPs) and create a *Francisella* library. All spectra of those strains were imported in BioNumerics for biomarker analysis.

Results: Our in-house *Francisella* library can determine the *F. tularensis* subspecies and the other *Francisella* species successfully with log scores between 2.3 and 2.8. Spectra generated in other laboratory can also be identified using this library. In addition, we determined one or more specific biomarkers for each (sub)species. When the genus *Francisella* is identified by the MALDI Biotyper Security Library or otherwise, for example 16S rDNA sequencing, those biomarkers can be used to differentiate between the *F. tularensis* and the other *Francisella* species. Biomarkers to differentiate *F. tularensis* subspecies were also identified.

Conclusion: MALDI-TOF MS, both using biomarkers or an in-house *Francisella* library, can successfully be applied in a clinical laboratory setting as a fast and specific method to determine *F. tularensis* subspecies.

P038

Genomic epidemiology of multiple *Acinetobacter baumannii* outbreaks in a veterinary intensive care unit

A.L. Zomer[1], B. Duim[1], E. Broens[1], J.W. Rossen[2], J. Robben[1], M. Leendertse[3], J. Wagenaar[1], J. Hordijk[1]
[1]*Utrecht University, Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht,* [2]*University Medical Center Groningen, Department of Medical Microbiology, Groningen,* [3]*AMC, Amsterdam*

Introduction: *Acinetobacter baumannii* is an emerging pathogen primarily found in health-care associated settings. Due to its potential to acquire and control a large reservoir of antimicrobial resistance genes and its ability to survive for extended periods in the environment, outbreaks are a threat and challenge for health care settings. Molecular epidemiology of *A. baumannii* is difficult because existing typing methods often lack the resolution to identify transmission chains in repeated outbreaks in the same setting. Phenotypic tests on antibiotic susceptibility are less informative because of the ease *A. baumanni* acquires and regulates an antimicrobial resistance repertoire. As an alternative, whole genome sequencing (WGS) forms an attractive method to investigate whether repeated outbreaks are independent events or are linked. Here, we investigated two outbreaks of *A. baumannii* in the companion animal intensive care unit (caICU) at Utrecht University which infected 4 dogs in 2012 and 3 dogs in 2014.

Methods: Multi Locus Sequence Typing (MLST) was performed for both outbreaks. Additionally, all outbreak strains, three European Control strains (EC-I, -II and -III) and four environmental strains were sequenced on an Illumina Miseq platform. Genomes were assembled using SPAdes, horizontal gene transfer regions were detected using ClonalFrame, time-measured phylogeny was reconstructed using BEAST and results were visualized using iTOL.

Results: Multi Locus Sequence Typing (MLST) of the isolates revealed that all animal isolates from both outbreaks in the caICU belong to sequence type 2. Isolates from the outbreaks had similar resistance phenotypes, suggesting that the outbreak events are linked. Time-measured phylogenetic reconstruction on WGS data of the clinical isolates, reference isolates and public genome sequences revealed, however, that the outbreaks are two independent events and that isolates from both outbreaks diverged at least 20 years before.

Conclusion: Our study shows the benefit of WGS in outbreak management in a clinical setting for pathogens for which traditional molecular typing methods lack resolution.

P039

The Staphylococcal toxins HlgAB and LukED are major hemolysins by specifically employing the duffy antigen receptor of chemokines

A.N. Spaan[1], T. Reyes-Robles[2], P. Yoong[2], M. Vrieling[1], C. Badiou[3], G. Lina[3], F. Vandenesch[3], Y. Colin Aronovicz[4], C.J.C. de Haas[1], K.P.M. van Kessel[1], C. Le Van Kim[4], V.J. Torres[2], T. Henry[3], J.A.G. van Strijp[1]
[1]*UMC Utrecht, Department of Medical Microbiology, Utrecht,* [2]*New York University School of Medicine, Department of Microbiology, New York, USA,* [3]*Université de Lyon, CIRI, International Center for Infectiology, Lyon, France,* [4]*Université Paris Diderot, Institut National de la Transfusion Sang, Paris, France*

The availability of iron is indispensible for infection with *Staphylococcus aureus*. Heme iron, concealed in erythrocytes, is the preferred source of iron for *S. aureus*. Experimentally, little is known about the actual mechanisms by which *S. aureus* releases heme iron from erythrocytes. Here, we show that the staphylococcal leukocidins γ-hemolysin AB (HlgAB) and LukED have potent hemolytic activity towards human erythrocytes. The hemolytic activity of both toxins is exclusively mediated by expression of the erythroid receptor Duffy Antigen Receptor of Chemokines (DARC). HlgAB and LukED employ the receptor in a differential manner. Using erythrocytes of DARC-negative individuals, we demonstrate that hemolysis provoked during bacterial growth is strictly facilitated by expression of the leukocidins HlgAB and LukED and their receptor DARC. These results suggest a central role of HlgAB and LukED in staphylococcal pathogenesis.

P040

Inactivation method for identification of highly pathogenic Gram-negative bacteria by matrix-assisted laser desorption ionization-time of flight mass spectrometry

A.C.A. Hendriks, M.C. van den Beld, A.S.D. Harpal, M.C. de Vries, F.A.G. Reubsaet
RIVM, IDS-BPD, Bilthoven

Introduction: *Brucella* species, *Burkholderia mallei, Burkholderia pseudomallei, Francisella tularensis,* and *Yersinia pestis* are occasionally found in Dutch patients, usually after travel abroad. Because these are all highly pathogenic bacteria, there is need for a rapid and reliable identification method. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is a frequently used technique for rapid identification of microorganisms, including bacteria which should be handled under biosafety level 3 conditions. In routine

settings, the prepared bacteria must be made inviable before performing MALDI-TOF MS analysis.In this study, Bruker's two standard sample preparation methods were tested to establish if these methods are reliable and safe methods for the identification of highly pathogenic Gram-negative bacteria.

Methods: In this study 42 strains of *Brucella* species, 10 *Burkholderia mallei*, 22 *Burkholderia pseudomallei*, 18 *Francisella tularensis*, 18 *Yersinia pestis*, and less pathogenic genetically related strains of aforementioned species were included. All the strains were tested by two of Bruker's standard methods: the formic acid extraction and the direct transfer method.The viability of the strains treated with the direct transfer method was tested by swapping spots of the MALDI target after adding HCCA matrix. Inactivation of the bacteria by the formic acid extraction was tested after adding ethanol absolute to the bacterial suspension.The swabs and suspensions were checked for growth on Columbia sheep blood agar or Chocolate agar supplemented with vitox, depending on the required growth conditions. Viability was checked after a minimum of 48 hours incubation, before the MALDI target and bacterial suspensions left the biosafety level 3 facility. The less pathogenic species were used to test adverse scenarios, for example too much bacterial culture or insufficient vortexing, which can possibly cause an inadequate inactivation.

Results: *Brucella* species, *Francisella tularensis*, and *Yersinia pestis* were unable to grow after both Bruker sample preparation methods. Onlyfour different *Burkholderia mallei* strains were viable, two while using the direct transfer method and two using the extraction method. Growth of the less pathogenic bacteria was found only by not mixing or by using too much bacterial culture (a full 10 μl inoculation loop).

Discussion and conclusion: Only four of the 110 strains, tested with two Bruker methods, did grow after swapping. Using the direct transfer method, applying too much bacteria on the MALDI target can explain the viable cells of two *Burkholderia mallei* strains. Adding HCCA matrix again inactivated the last viable cells. Too much colonies in the suspension or insufficient vortexing could have caused growth of the other two *Burkholderia mallei* strains, using the extraction method. New bacterial suspensions were made and these extracts were not viable.

In conclusion, Bruker's two standard methods can safely be used for identification of highly pathogenic bacteria by MALDI-TOF MS. It is recommended to include growth controls, to use a small amount of bacterial culture and to verify the covering of the HCCA matrix on the MALDI target.

P041

A thermo-regulated proline-rich surface protein of *Enterococcus faecium* binds to fibrinogen, fibronectin and platelets and contributes to biofilm formation

A. Guzman[1], R.T. Urbanus[1], F.L. Paganelli[1], X. Zhang[1], D. Bierschenk[1], A. Koekman[1], M. van Luit-Asbroekan[1], J.P. Ouwerkerk[1], M. Pape[1], D. Wobser[2], J. Huebner[3], A.P.A. Hendrickx[1], T.C. van Holten[1], M. Roest[1], M.J.M. Bonten[1], R.J.L. Willems[1], W. van Schaik[1]

[1]*UMC Utrecht, Department of Medical Microbiology, Utrecht,* [2]*University Medical Center Freiburg, Freiburg, Germany,* [3]*Hauner Children´s Hospital Ludwigs-Maximilian, München, Germany*

Enterococcus faecium is a commensal of the mammalian gastrointestinal tract but in the last decades, ithas become an important nosocomial pathogen, causing infections that are difficult to treat due to the organism's intrinsic and acquired antibiotic resistance and ability to form biofilms. However, little is known about the mechanisms of *E. faecium* involved in colonization and infection of the mammalian host.

We hypothesized that genes involved in colonization or infection might exhibit temperature-regulated control of expression, and we therefore performed a transcriptome analysis of *E. faecium* E1162 during mid-exponential growth at 25°C and 37°C. Thirty-three genes showed significantly higher expression at 37°C than at 25°C. One of the most highly upregulated genes (4.4-fold; 37°C versus 25°C) encodes a 48-kDa surface protein with an LPxTG-type anchor. The N-terminal domain is unique to *E. faecium* and closely related enterococci (*E. hirae* and *E. durans*). The C-terminal domain contains multiple proline-rich repeat regions and we consequently named this protein PrpA for proline-rich protein A.

Confocal and electron microscopy revealed that PrpA is found exclusively at the poles of the cells. We also showed by flow cytometry, that surface-exposed levels of PrpA are highest in exponentially growing cells at 37°C compared to stationary phase cultures or when grown at 25°C. The thermo-regulated production of PrpA on the surface of E1162 was also observed in other *E. faecium* strains. Furthermore, specific antibodies against PrpA were observed in patients that have previously suffered from an *E. faecium* bacteremia, indicating that PrpA is immunogenic. The heterologously expressed and purified N-terminal domain of PrpA was able to bind to fibrinogen, fibronectin and platelets. In addition, a *prpA* deletion mutant is defective in the early stages of biofilm formation compared to the wild type strain. Our data indicate that PrpA may contribute to pathogenesis of *E. faecium*-mediated infections.

P043

Development of tailored antimicrobial treatment regimens and novel host- pathogen insights for respiratory tract infections and sepsis. (TAILORED-Treatment)

J. Hays
Erasmus MC, MMIZ, Rotterdam, TAILORED-Treatment Consortium

Introduction: Despite their immense contribution to global healthcare, antibiotics are currently recognized as the most misused drugs in the world with global overuse estimated at 40%-70%. Antibiotic misuse often causes preventable adverse events that impact on patient care. Importantly, misuse also leads to the emergence of antibiotic resistance, one of the major threats to global health today. Unfortunately, current diagnostic tools for facilitating the appropriate use of antibiotics are often inadequate as antibiotic consumption and the spread of antibiotic resistance are continually increasing.
'TAILORED-Treatment' (www.tailored-treatment.eu) is an EU FP7 funded project consortium comprising 7 European partners. Our main goal is to establish a broad-based strategy (not limited to a particular antibiotic group) that can be implemented on a broad scale to increase the effectiveness of antibiotic and antifungal therapy, reduce adverse events, and help limit the emergence of antimicrobial resistance in children and adults. The TAILORED-Treatment project is designed to maximize impact on patients and physicians while integrating and synergising with current EU funded research strategies.
Methods: At the heart of the TAILORED-Treatment project is a prospective clinical study in which we will recruit 1200 patients (>2000 patient samples) presenting with respiratory tract infections and/or sepsis. Patient cohorts will include equal representation of genders, children and adults.
State-of-the-art molecular and biochemical technologies (transcriptomics, proteomics, genomics, microbiota analysis) will be developed and applied to characterize the host and pathogen at the genomic, transcriptomic, proteomic and clinical level. The data collected will be added to a large-scale unique multi-dimensional dataset which is stored in a publically available database, and is accessible to the EU scientific and clinical community.
The consortium partners will also develop and apply new computational tools to interrogate the data, in order to provide new insights into personalized host-pathogen interactions, including the discovery of novel biomarkers for patient diagnosis and disease monitoring. In this respect, we will construct a predictive personalized treatment algorithm that will lead to informed and personalized antibacterial, antifungal and antimicrobial treatment regimens (indication, dosage, and duration) that are tailored to the needs (type of infection, presence of novel biomarkers etc) of children and adults presenting with respiratory infections and sepsis.
Finally, the algorithm and large-scale unique multi-dimensional dataset will be built into an easily navigable web-based, free-to-use, decision support system ready for use by physicians to explore, test and assist in patient-tailored antimicrobial treatment decisions.
Results: The result will be a large-scale unique multi-dimensional dataset stored in a publically available database, which is accessible to the EU scientific and clinical community, as well as an easily navigable web-based, free-to-use, decision support system ready for use by physicians to explore, test and assist in patient-tailored antimicrobial treatment decisions.
Conclusion: Ethical approvals have been granted and clinical samples are being collected and processed. Preliminary results are expected to be available in 2016.

P044

Nanotherapeutics to treat antibiotic resistant gram-negative Pneumonia infections

J. Hays, I. Bakker-Woudenberg, H. van der Weide, W. Goessens, M. ten Kate
Erasmus MC, MMIZ, Rotterdam, PneumoNP Consortium

Introduction: PneumoNP (www.pneumonp.eu) is an FP7 funded European project involving 11 European partners. The main objective of the project is the development of a theragnostic (diagnostic and therapeutic) system for the treatment of multi-antibiotic resistant Gram-negative bacterial infections in pulmonary disease. According to the majority of international stakeholders from the healthcare sector, Gram negative multi-antibiotic resistant bacteria are one of the major global health threats facing the world today. Not least due to the lack of effective antibiotic therapy currently available. In this project, ESBL and carbapenemase resistant *Klebsiella pneumoniae* isolates will be used as proof of concept for multi-antibiotic resistant Gram negative bacteria.
The main objective of the PneumoNP project will be to generate a new inhalable nanotherapeutic system. An aerosol device will be specifically developed for this purpose that will enable topical administration of novel antimicrobial peptide antibiotics directly into the affected area i.e. the lung. The drug formulated in the aerosol will consist of a nanotherapeutic system combining a novel antimicrobial peptide antibiotic coupled to a soft nanocarrier. The nanocarrer will improve the transportation capacity of the active biomolecule to the alveolar surfaces.
Another objective of the PneumoNP project is to develop a new diagnostic system for a rapid identification of bacteria causing pulmonary infections. This early diagnostic

system will greatly reduce the time required for pathogen detection compared to conventional culture.
The 11 PneumoNP partners include 2 SMEs with novel antimicrobial peptides entering the clinical stage, 2 research organizations manufacturing different types of nanocarriers (with access to GMP facilities), a GLP compliant research institute for *in vitro* assays, a renowned medical centre with a validated *in vivo* respiratory infection models, an international molecular imaging centre for *in vivo* biodistribution, 2 SMEs dedicated to the commercialization of aerosol systems, a diagnostic kit developer, and finally 2 SMEs for dissemination and IP management.
Methods: Two nanocarrier systems (liposomes and single chain polymeric nanoparticles) and 2 different novel antimicrobial peptides will be investigated, developed and combined to assess their clinical ability to cure Gram negative pulmonary infections using a validated and novel rat model of pneumonia. The same novel rat pneumonia model will be used to develop a diagnostic kit for Gram negative pulmonary infections
Results: Preliminary results are expected to be available in 2016.
Conclusion: This novel nanotherapeutic, in combination with the diagnostic kit to be developed, will radically improve the speed of detection and cure rate for multi-antibiotic resistant Gram negative bacteial infections of the lung, including difficult to treat carbapenemase resistant infections.

P045

Zebrafish as a model for pneumococcal meningitis

K.K. Jim[1], M.C. Brouwer[2], A. van der Ende[2], D. van de Beek[2], C.M.J.E. Vandenbroucke-Grauls[1], W. Bitter[1], A.M. van der Sar[1]
[1]*VUmc, Department of Medical Microbiology and Infection Control, Amsterdam,* [2]*AMC, Department of Neurology, Amsterdam*

Introduction: Bacterial meningitis is a severe disease in both children and adults. *Streptococcus pneumoniae* is the most important cause of meningitis and carries a high rate of morbidity and mortality. Recently, the zebrafish embryo has emerged as a powerful vertebrate model to study host-microbe interactions. With this study we aimed to develop a representative pneumococcal meningitis model in zebrafish embryos.
Methods: Zebrafish embryos were inoculated in the bloodstream or hindbrain ventricle with increasing doses of *S. pneumoniae* serotype 2 colony forming units (CFU; n = 120, range 10^2-10^3 CFU). Outcome parameters were survival rate and bacterial growth. To determine bacterial growth zebrafish embryos were randomly collected and sacrificed at regular time points (2h, 7 h, 24 h, 48 h). To determine the localization of the bacterial infiltrates we inoculated zebrafish embryos with fluorescent *S. pneumoniae* and used fluorescence microscopy and confocal microscopy for imaging.
Results: Inoculation of *S. pneumoniae* in both bloodstream and hindbrain ventricle showed a dose-dependant mortality, where lower doses of CFU delayed onset of disease and time of death. Infection via the bloodstream was associated with a more rapid disease progression compared to hindbrain ventricle injection. Bloodstream injection resulted in a fulminant systemic disease that can progress to meningitis, while hindbrain ventricle injection causes an isolated infection of the central nervous system. Bacterial infiltrates were observed in the blood vessels and organs throughout the whole zebrafish embryo, including the central nervous system in bloodstream injected zebrafish. In hindbrain ventricle injected zebrafish bacterial infiltrates were mainly observed in the central nervous system.
Conclusions: 1. We developed and validated a zebrafish embryo model of pneumococcal meningitis. 2. Both bloodstream injection and hindbrain ventricle injection cause a fulminant, dose-dependant infection in zebrafish embryos. 3. This model can be used to study both host and bacterial factors involved in the pathogenesis of pneumococcal meningitis.

P046

Structure and function of RNase AS, a polyadenylate-specific exoribonuclease affecting mycobacterial virulence *in vivo*

R. van de Weerd
VUmc, Department of Medical Microbiology and Infection Control, Amsterdam

The cell-envelope of *Mycobacterium tuberculosis* plays a key role in bacterial virulence and antibiotic resistance. Little is known about the molecular mechanisms of regulation of cell-envelope formation. Here, we identify and characterize biochemically, structurally and functionally an enzyme of *M. tuberculosis*, here denoted as RNase AS, which modulates mycobacterial cell-envelope properties and strongly impacts bacterial virulence *in vivo*. The structure of RNase AS reveals resemblance to RNase T from *Escherichia coli*, an RNase of the DEDD family involved in RNA maturation. We show that RNase AS acts as a 3'-5'-exoribonuclease that specifically hydrolyzes adenylate-containing RNA sequences. Also, crystal structures of complexes with AMP and UMP reveal the structural basis for the observed enzyme specificity. Notably, RNase AS shows a novel mechanism of substrate recruitment, based on the recognition of the hydrogen bond donor NH2 group of adenine. Our work opens a field for the design of novel drugs able to reduce bacterial virulence *in vivo*.

P047

Immunogenic secreted protein (Isp) from Group A *Streptococcus* neutralizes the neutrophil-derived antimicrobial protein azurocidin

V.P. van Hensbergen[1], S. Bekkering[2], N. Borregaard[2], J. Soppe[1], E. Berends[1], S.H.M. Rooijakkers[1], N.M. van Sorge[1]
[1]*UMC Utrecht, Department of Medical Microbiology, Utrecht,* [2]*Copenhagen University, Department of Hematology, Copenhagen, Denmark*

Introduction: Group A *Streptococcus* (GAS), otherwise known as *Streptococcus pyogenes*, causes a wide range of diseases. It is estimated that at least 500,000 people die from severe infections annually making GAS a major human pathogen.[1] Little is known about immune evasion techniques used by GAS, however, GAS secretes many uncharacterized proteins that are predicted to be virulence factors.[2] We focused on immunogenic secreted protein (Isp), a conserved secreted protein among GAS serotypes. GAS also contains an Isp homologue located distantly in the chromosome, named isp2. Both proteins are found in GAS culture supernatants and induce antibodies upon infections in humans.[2,3] Furthermore, Isp is located near well-known virulence factors such as M1 and C5a peptidase in the genome.[2] Our research aims to unravel the molecular mechanisms by which Isp and Isp2 interact with the human host.
Methods: Isp (from GAS M3 serotype) and Isp2 (from GAS M1T1 serotype) were expressed and purified as recombinant proteins with an N-terminal his-tag in *E. coli*. Recombinant proteins were screened in several functional immunological assays like complement and coagulation assays. Using an ELISA-based assay, we evaluated possible binding of Isp to purified neutrophil granules and antimicrobial proteins. In addition, we generated Isp and Isp2 GAS deletion mutants to screen for phenotypes in assays that represent different steps in pathogenesis, such as whole blood survival, neutrophil killing and resistance to individual antimicrobial host proteins.
Results: We obtained small amounts of full-length recombinant Isp and Isp2 from *E. coli* whole cell lysates. Binding assays revealed that recombinant Isp specifically bound to neutrophil azurophilic granules, while a panel of 100 other recombinant bacterial proteins showed no interaction. Further analyses revealed that Isp bound to the bactericidal protein Azurocidin, a non-catalytic homologue of neutrophil Elastase. Isp had no effect on complement activation or coagulation in the assays tested. Functionally, addition of recombinant Isp protected *E. coli* from Azurocidin killing.
Conclusion: RecombinantIsp interacts with and neutralizes the neutrophil-derived antimicrobial protein-Azurocidin. We are currently performing similar assays using Isp2 as well as killing experiments comparing wild-type with Isp mutant GAS sensitivity for Azurocidin.

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P048

Rapid screening for carbapenemase genes by use of a real-time multiplex assay

E.M. Terveer, M.E.M. Kraakman, A.T. Bernards, E.C.J. Claas
LUMC, Department of Medical Microbiology, Leiden

Introduction: The prevalence of carbapenemase producing Enterobacterieaceae is rapidly increasing worldwide. Therefore, rapid detection of carbapenemase genes is essential for adequate antibiotic therapy and infection control measurements. This study describes the validation of a real-time multiplex PCR assay including five carbapenemase genes: bla_{KPC}, bla_{OXA-48}, bla_{NDM}, bla_{VIM} and bla_{IMP}.
Methods: The bla_{KPC} and bla_{VIM} primers and probes were used as previously described (1) bla_{NDM}, bla_{OXA-48} and bla_{IMP} primers and probes were designed based on the sequence variations of the carbapenemase genes described at the Lahey site. The DNA extraction method, PCR conditions, and multiplex combinations of targets were optimized. Subsequently, a collection of 71 Enterobacterieaceae and *Pseudomonas* isolates (including 67 isolates each producing one carbapenemase, two isolates containing two carbapenemase genes and two negative isolates) was tested. Additionally the assay was prospectively validated from August 2014 - December 2014 at the Leiden University Medical Centre.
Results: Optimal results were obtained using a triplex of bla_{KPC}, bla_{VIM} and bla_{NDM}, and two monoplex PCRs with bla_{OXA-48} and with bla_{IMP}. DNA was isolated by suspending a single colony in 150 µl lysisbuffer followed by incubation for 30 min at 65°C and 15 min at 95°C. After centrifugation, 10 µl of extract was subjected to real-time PCR amplification. Sensitivity and specificity were 100% with the 69 carbapenemase positive isolates. In seven of 23 patients a carbapenemase positive gram negative rod was found (10 of 34 isolates). In addition, 59 of 68 isolates from a single outbreak were found to have a bla_{VIM} carbapenemase gene.
Conclusion: The above described real-time multiplex carbapenemase PCR in combination with a rapid DNA

isolation method is a very fast (<3h after diagnosing carbapenem resistance), sensitive and specific method for detection of the most prevalent carbapenemase genes. A limitation of this study is that the less prevalent carbapenemase genes like Bla_{PER}, Bla_{GES}, Bla_{SPM}, Bla_{AIM}, Bla_{GIM}, Bla_{BIC}, Bla_{SIM}, Bla_{DIM} are not detected, and new variants of known carbapenemase families may not be detected.

P049

Synergistic pro-inflammatory effects of co-infections with *Haemophilus influenzae* and Respiratory Syncytial Virus on respiratory epithelial cells

C. Bellinghausen, F. Gulraiz, E.F.M. Wouters, G.G. Rohde, F.R. Stassen
MUMC, Department of Medical Microbiology/Respiratory Medicine, Maastricht

Infection with one microorganism can influence secondary infections with other, unrelated pathogens by for example, breaching mucosal barriers or altering the host's immune response. While this phenomenon has been studied in detail for respiratory viral infections facilitating bacterial superinfections, the reverse order is less well understood.
In order to investigate how the presence of bacteria affects a secondary viral infection, bronchial epithelial cells (BEAS-2B) were exposed to heat-inactivated (hi) suspensions of nontypeable *Haemophilus influenzae* (NTHI), *Pseudomonas aeruginosa* or *Streptococcus pneumoniae* and subsequently infected with Respiratory Syncytial Virus (RSV). Response to infection was measured by monitoring cytokine release and viral replication.
Exposure to bacteria and viral infection independently caused the release of the inflammatory cytokines IL-6 and IL-8 by BEAS-2B cells. When cells were first exposed to hi-NTHI and then infected with RSV, release of both cytokines was synergistically enhanced. Seventy-two hours after viral infection, measured cytokine levels exceeded a purely additive effect of the individual pathogens by 120% (IL-6) and 150% (IL-8), respectively. This exaggerated response was not linked to viral replication and was to such extent only seen for hi-NTHI but none of the other pathogens.
Our data suggest that bacteria can contribute to the severity of secondary viral infections in a pathogen-specific manner. Considering the substantial proportion of patients with chronic lung diseases whose lungs are colonized with bacteria, it seems likely that such colonization can affect their susceptibility and response to secondary viral infections.

P050

Extracellular vesicle release during macrophage infection with non-typeable *Haemophilus influenzae*

C. Volgers
Maastricht University, Department of Medical Microbiology, Maastricht

Introduction: Non-typeable *Haemophilus influenzae* (NTHi) colonizes the upper airways of most healthy adults and invades the lower airways when innate immunity is compromised. Control over infection is mainly conferred by alveolar macrophages and this involves a sound intercellular communication. Recently it has become clear that also extracellular vesicles (EVs) are involved in these processes, and they provide the cells with sophisticated means for communication. Such EVs, e.g. derived from macrophages infected with intracellular pathogens, have been shown to induce pro-inflammatory responses (Bhatnagar et al, 2007). Here we investigated whether macrophages also release immunostimulatory EVs in response to NTHi.
Methods: EVs released by macrophages during NTHi-infection or stimulation with heat-inactivated (hi) NTHi were assessed by flow cytometry to determine release kinetics. Moreover, EVs were isolated by ultracentrifugation (UC) for characterization by electron microscopy (EM), western blotting (WB). Additionally, also the functional activity was assessed.
Results: Flow cytometric analysis revealed a dose- and time-dependent release of $CD63^{+}$/$CD81^{+}$-EVs which peaked at 6 hours after NTHi (6-fold) or hi-NTHi (10-fold) challenge. EM and WB confirmed the presence of vesicles in the medium. Finally, EVs from both conditions induced a TNF-a response, which was comparable to an LPS-induced response.
Conclusion: Both infection with NTHi and stimulation with hi-NTHi resulted in an enhanced EV-release. The vesicles released possessed a strong pro-inflammatory character. However, we cannot exclude a contribution from NTHi-derived outer membrane vesicles.

P051

New AntiBacterials with Inhibitory activity on Aminoacyl-tRNA Synthetases (NABARSI)

C.G. Monteferrante, W.H.F. Goessens, J.P. Hays
Erasmus MC, Department of Medical Microbiology and Infection, Rotterdam

Introduction: Widespread resistance to antimicrobials is now a global threat. However, the number of new antimicrobials that have been brought to market has declined considerably in recent decades. Further, many of the antibiotics that are now in clinical development belong to existing families of compounds and their activity is likely

to be subjected to mechanisms of resistance already in existence in clinically relevant bacterial strains. Thus, there is an urgent need for chemically-novel antibacterial agents, preferably those which act upon novel bacterial targets.

The NABARSI consortium (www.nabarsi.eu) will undertake a cutting-edge drug discovery project of 36-month duration to identify such an agent. The main goal of NABARSI is to find proof-of-concept for new chemical entities (NCEs) as antibacterial drugs and to exploit the results in conjunction with industry. We will develop inhibitors that are active against multiple unexploited aminoacyl-tRNA synthetase enzymes; this 'multi-target' approach will restrict the emergence of resistance.

The NABARSI project is an FP7 funded EU project involving the cooperation of 5 European partners with expertise in aminoacyl-tRNA synthetase enzymes, *In silico* drug design, chemical synthesis, antimicrobial molecular drug resistance, clinical antimicrobial resistance and animal models of infection.

Methods: Proof of concept will be obtained by demonstrating the antibacterial efficacy of the new chemical entities in animal models of infection caused by multi-drug resistant pathogenic bacteria belonging to ESKAPE group of bacteria (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species) and *Escherichia coli*. Co-development of the molecules will be sought through collaboration and/or licensing with medium and large pharmaceutical and biotechnology companies, to the benefit of both parties.

Specifically, NABARSI will identify inhibitors of aminoacyl-tRNA synthetase enzymes. Validation of this protein family as a target for antibacterials has been provided by the already marketed drug mupirocin and by other compounds in clinical development (Hernandez, V., Crépin, T., Palencia, A. *et al.*, Antimicrob Agents Chemother 2013).

Novel computational and screening technologies, synthetic methodology and advanced mechanistic studies will be combined with state-of-the-art drug discovery approaches in order to provide selective NCEs with high antibacterial efficacy and low resistance potential.

Results: Three primary 'on-target' screening assays have been established, as well as growth susceptibility assays against MRSA (Methicillin resistant *Staphylococcus. aureus*), Escherichia coli and *Acinetobacter baumannii*.

The consortium is currently completing the primary screening of 2,145 NCEs, resulting in the identification of 49 interesting hits, comprising 15 inhibitors of bacterial growth and 36 enzymatic inhibitors.

One of the major difficulties encountered so far has been the fact that NCEs that have activity against the relevant targets do not have an effect on bacterial growth, and conversely, NCEs that have an effect on bacterial growth tend to display non-specific (off-target activity).

Conclusion: New molecules are continuing to be synthesized and tested, and new strategy designs are being explored.

Partners have currently started a new round of selection/synthesis of NCEs, which is based on a new powerful design approach.

P052

Eradication treatment of MRSA carriage following revised SWAB guideline rules

J.H. van Zeijl[1], R.R. van Heuvelen[2], J.W. Kapelle[2], N. Veeger[2]

[1]*Izore Centre for Infectious Diseases Friesland, Medical Microbiology, Leeuwarden,* [2]*Medical Centre Leeuwarden, Internal Medicine, Leeuwarden*

Introduction: Despite the high prevalence of MRSA in surrounding countries, the prevalence of MRSA in Dutch healthcare institutions is still among the lowest of Europe. In 2011 1,8% of all *S. aureus*-isolates were MRSA. This low prevalence is for the greatest part the result of the national search and destroy policy; actively tracing of potential MRSA-carriers, the isolation of suspected or confirmed MRSA carriers and eradication of MRSA carriage. For the approach of eradication treatment a guideline (SWAB) 'treatment of MRSA carriage' has been developed in the Netherlands. The type of treatment depends on the type of carriage: either uncomplicated or complicated. The effectiveness of treatment according to the guideline has been studied and was 60% after one treatment and 80% after multiple treatments. It was also shown that throat carriage should be treated as complicated carriage. In February 2012 the SWAB-guideline was adjusted, also for this detail. We examined the effectiveness of the new guideline rules retrospectively on a Frisian cohort of MRSA positive carriers. This was possible because at Izore throat carriage was already treated as complicated carriage.

Methods: In this retrospective descriptive cohort study we collected data from culture proven MRSA positive patients from the laboratory data system of Izore (Glims). De study period was from January 1 2005 until December 31 2011. We collected the basic characteristics of the included patients and analyzed the data of patients starting eradication treatment and compared this with the outcome. The primary endpoint was proven eradication by three consecutive sets of cultures from nose, throat and perineum, and from extra sites on indication, taken more than 48 hours after the last treatment day, with intervals of at least 7 days. Secondary endpoints were proven risk factors for failure of the treatment. Differences between patient groups were calculated by Fisher's exact test and Chi-square test. Multivariate logistic regression analysis was used to define risk factors which influenced the treatment results.

Results: We found success percentages of 76,8% after one treatment and 88,7% after multiple treatments with a mean number of 1,29 treatments. Young age (P = 0,003), throat carriage (P = 0,006) and skin conditions (P = 0,003) turned out to be the most important factors with a negative influence on the outcome of eradication treatment. Working in the healthcare sector (P = 0,003) was associated with a positive influence on the outcome of eradication treatment.
Conclusion: The conclusion of our study is that the adjustment of the SWAB MRSA guideline had led to an increase in effectiveness of eradication treatment of MRSA carriage. Young age, throat carriage, skin conditions and working in the healthcare sector are influencing the outcome of eradication treatment.

P053

Understanding the mechanism of protein transport across the mycobacterial cell envelope by the type VII secretion system membrane complex

C.M. Bunduc, R. Ummels, W. Bitter, E.N.G. Houben
VU, Department of Molecular Microbiology, Amsterdam

Introduction: Pathogenic mycobacteria, such as *Mycobacterium tuberculosis*, use type VII secretion (T7S) systems to secrete important virulence factors across their distinctive cell envelope. In particular, this cell envelope includes a highly specific and impermeable outer membrane. These bacteria have up to five of such systems, which are known as ESX-1 to ESX-5. T7S does not resemble any of the known specialized secretion pathways in other bacteria, reflecting the unique properties of the mycobacterial cell envelope. Although these secretion systems are crucial for mycobacterial virulence, their composition and mechanism is still largely unknown. The aim of this study is to understand the mechanism of protein transport across the mycobacterial cell envelope by elucidating and characterizing the T7S membrane channel.
Methods: Here, we have isolated and analysed the ESX-5 membrane complex by subcellular fractionations, Blue Native PAGE and pulldown experiments.
Results: The ESX-5 membrane complex is composed of four conserved membrane components and has a size of 1.5 MDa. Isolation and characterization of the equivalent ESX-1 membrane complex showed that the composition and size is conserved. To more easily analyse the functioning of this membrane complex in secretion, we reconstituted the ESX-5 system in the avirulent and fast-growing mycobacterial species *Mycobacterium smegmatis* that lacks ESX-5. This reconstituted system showed to be relatively overexpressed and was efficiently secreting substrates. In addition, the ESX-5 membrane complex was properly assembled. We are currently mutating individual ESX-5 complex components and investigating their role in the secretion process.
Conclusions: Here we showed that ESX-1 and ESX-5 are conserved in terms of composition and size of the membrane complex. In addition, the functional reconstituted and overproduced ESX-5 system in *M. smegmatis* opens up the possibility to efficiently and more thoroughly analyse the functioning of this secretion system.

P054

Excellent virological and immunological response to Dolutegravir despite the presence of extensive resistance mutations in integrase, protease and reverse transcriptase

A.J. Stam[1,2], L.M. Hofstra[1,3], M. Nijhuis[1], T. Mudrikova[2], A. Fun[1], P.J. Schipper[1], M. Schneijder[2], A.M.J. Wensing[1]
[1]*UMC Utrecht, Department of Medical Microbiology, Utrecht* [2] *UMC Utrecht, Department of Internal Medicine and Infectious Diseases, Utrecht* [3] *Laboratory of Retrovirology, CRP-Santé, Luxembourg*

Introduction: Dolutegravir is a second-generation integrase inhibitor with a proposed high genetic barrier to resistance. However, in clinical trials decreased virological response was seen in a subset of patients with prior exposure to raltegravir and multiple integrase resistance mutations.
Methods: We describe two cases of HIV subtype B-infected patients starting dolutegravir after previous failure on a raltegravir-containing regimen with extensive resistance. Genotypic analysis was performed using population and 454 ultradeep sequencing of integrase at time of raltegravir exposure.
Results: Both patients were diagnosed in early 1990s and received mono- and dual therapy, followed by several cART-regimens. Due to presence of extensive resistance the genotypic susceptibility score of these regimens never reached a score >2 and never resulted in sustained virological suppression despite good adherence. Early 2012, the clinical condition of patient A worsened during persistent failure of a mega-cART regimen despite excellent drug levels. 6 major PI (M46L-I50V-I54V-V82A-I84V-L90M), 6 minor PI, 7 NRTI (M41L-D67N-T69i-L74V-M184V-L210W-T215Y), 6 NNRTI (A98G-K103N-V108I-E138G-Y181C-G190A) and 2 INI mutations (Q148R-E138K) plus DM-virus were detected. Ultradeep sequencing of integrase showed the selection of Q148R, E138K+Q148K, and N155H variants and phenotypic raltegravir resistance was demonstrated. After addition of dolutegravir and enfuvirtide to the failing regimen (zidovudine+lamivudine+tenofovir+etravirine+darunavir/ritonavir+maraviroc) viral load (VL) decreased from 244,000 to <20 cps/mL within 5 months, CD4-count increased (33 to 272 mm^3) and the clinical condition improved substantially. In patient B similar worsening

of the clinical condition was observed late 2012 during persistent failure on mega-cART. 5 major PI (M46I-I54V-V82C-I84V-L90M), 6 minor PI, 9 NRTI (M41L-D67d-T69G-K70R-L74I-M184V-L210W-T215Y-K219E), 7 NNRTI (A98G-L100I-K103N-V106I-V108I-E138K-G190A) and INI mutation N155H plus DM-virus were detected. Ultradeep sequencing showed selection of N155H, followed by Q95K and V151I variants and phenotypic raltegravir resistance was demonstrated. Dolutegravir was added to his failing regimen (zidovudine+lamivudine+etravirine+atazanavir/ritonavir+maraviroc) at a VL of 39,000 cps/mL. Sustained virological suppression was reached within 5 months with considerable increase of CD4-count (41 to 175 mm^3) and slight improvement of clinical condition.

Conclusion: We present the first patients with extensive integrase resistance who were treated with dolutegravir in clinical practice and who achieved excellent virological and immunological success. These cases demonstrate the high genetic barrier of dolutegravir.

P055

Comparison of three different methods for handling and processing of (positive) blood cultures

S.Q. van Veen, C.J. van der Mark
Onze Lieve Vrouwe Gasthuis, Department of Medical Microbiology, Amsterdam

Introduction: blood cultures are an important diagnostic tool to detect bacteraemia in patients. Interval between drawing blood and report of a positive culture should be as short as possible to provide optimal patient care. At our laboratory – affiliated to four hospitals – three different methods are currently used. Method A (2 hospitals): all bottles are sent 3 times/day to central laboratory (CL) during office hours for incubation. If positive, immediately processed and reported between opening hours (8.30-19.00). Method B (1 hospital): all bottles are sent to CL located at that hospital between 8.30 - 17.00 for incubation. If positive, immediately processed and reported between 8.30-19.00. Method C (1 hospital): all bottles are immediately incubated 24/7 at the chemical laboratory. If positive, sent to CL 3 times/day during office hours for processing and report. We would like to replace our blood culture machines. Which method results in fastest report of a positive culture?

Methods: We analysed the time between drawing blood and report of positive blood culture for all three methods for all positive blood cultures in 2013. A total of 35439 blood culture bottles of which 3968 (11.2%) were reported positive were analysed.

Results: see *Table 1*. Differences were the result of delay in transport and not incubation time in the blood culture machine and/or processing and reporting at CL (data not shown). It was noticed that in all hospitals most blood cultures were drawn between 14.00 and 22.00 hours. Therefore, for method A and B, the majority of bottles were not incubated the same day but the next morning. For method C time between drawing blood and start of incubation was not related to the moment of the day due to 24/7 loading of the blood culture machine. This might be in important factor to explain the differences.

Table 1. Time between drawing blood and reporting positive blood culture.

Method	Average (hours)	25th percentile (hours)	50th percentile (hours)	75th percentile (hours)
A	43	30	40	46
B	40	24	36	46
C	36	19	29	41

Conclusion: 24/7 loading and incubation of blood cultures at the location the are drawn, results in fastest report of positive blood cultures, despite some delay of transport to a central laboratory for processing and report.

For our new blood culture equipment, based on these results, we will switch to method C for all hospitals. So, all hospitals will have 24/7 loading and incubation in cooperation with the clinical chemistry department.

P056

Protein inventory of the oral pathogen *Porphyromonas gingivalis*

T. Stobernack[1], S. Junker[2], C. Glasner[1], A. Otto[2], G. Gabarrini[1,3], M.J. de Smit[3], A.J. van Winkelhoff[1,3], D. Becher[2], J.M. van Dijl[1]
[1]*University Medical Center Groningen, Department of Medical Microbiology, Groningen,* [2]*Ernst Moritz Arndt University Greifswald, Institute for Microbiology, Greifswald,* [3]*University Medical Center Groningen, Center for Dentistry and Oral Hygiene, Department of Periodontology, Groningen*

Background: Autoimmune diseases are among the leading causes of death worldwide. Although much is known about the pathology of immunological dysfunctions, the triggers for disease onset are not well understood. One example is rheumatoid arthritis (RA). Interestingly, patients suffering from RA have a higher risk of developing periodontitis and vice versa. Several studies showed a significant correlation between these two diseases without unravelling pathological mechanisms. Since there is a strong microbiological component in periodontitis, there is a clear need to investigate the possible involvement of oral pathogens in the pathology of RA. Therefore, the aim of this study was to analyse the proteome of *Porphyromonas gingivalis*, one of the most prominent periodontal pathogens. A special

emphasis was given to protein citrullination, because the presence of anti-citrullinated protein antibodies (ACPAs) is characteristic for RA patients.

Methods: Bacterial isolates were derived from periodontitis and RA patients, and also included two reference strains and two citrullination deficient mutant strains. First the growth of *P. gingivalis* isolates in liquid culture was optimized and subsequently their cellular and extracellular proteomes were analysed by SDS-PAGE, Western blotting and mass spectrometry.

Results: Culturing of *P. gingivalis* in liquid BHI medium was shown to be optimal for proteome analyses. The growth experiments revealed variations in growth rates and maximal OD_{600} values reached by the different clinical isolates. Furthermore, SDS-PAGE banding patterns of the proteome revealed differences between isolates, especially in the secreted fractions. Mass spectrometry analyses of the secreted fractions identified a set of approximately 100 proteins amongst others major virulence factors, including proteases, fimbriae and the citrullinating enzyme *P. gingivalis* peptidylarginine deiminase (PPAD). Citrullinated *P. gingivalis* proteins were identified in the secreted fractions of all clinical isolates.

Conclusion: A robust workflow for analysing the proteome of *P. gingivalis* isolates has been established. Altogether, the present data provide first insights into the protein composition of different fractions of clinical *P. gingivalis* isolates, leading to the conclusions that (i) the known major *P. gingivalis* virulence factors are secreted during *in vitro* growth and (ii) several of these secreted proteins are citrullinated. Further proteome analyses will be performed to define the role and exact function of protein citrullination in *P. gingivalis* as a potential link between periodontitis and RA.

P057

Evaluation of a new collection and enrichment swab device for direct testing in accordance with Dutch guideline on the laboratory detection of methicillin-resistant *Staphylococcus aureus*

K. Khan[1], N. Gizzie[2]

[1]*Heatherwood and Wexham Hospital, Microbiology and Immunology, Slough, United Kingdom* [2]*University of the West of England, Faculty of Health and Life Sciences, Bristol, United Kingdom*

Introduction: One of the routine procedures in the diagnosis of infections involves the collection and transportation of a clinical swab specimen from the patient to the laboratory. In particular, for swab specimens which are to be tested for methicillin resistant *Staphylococcus aureus* (MRSA), the first stage of processing is often to transfer the swab to an enrichment broth containing sodium chloride (NaCl). Most strains of *Staphylococcus aureus* (*S. aureus*) will grow in the presence of NaCl, while other organisms commonly picked up on swabs will be inhibited. In the Dutch guideline on the laboratory detection of methicillin-resistant *Staphylococcus aureus* an NaCl concentration of 6.5% (w/v) in an otherwise non-inhibitory broth such as tryptic soy broth (TSB) is recommended. In MWE Sigma-TSB with 6.5% NaCl, those two stages are combined into one device, so that for specimens intended only to be tested for MRSA, the swab specimen is collected directly into a transport tube containing TSB with 6.5% NaCl. Following incubation, the broth is inoculated directly on to a suitable chromogenic agar, and any MRSA or methicillin sensitive colonies readily observed.

The present study was designed to evaluate the ability of this device to recover and enrich *S. aureus*, while inhibiting *Escherichia coli* (*E. coli*). In many laboratories confirmation of presumptive positive MRSA is often performed using PCR, so the suitability of the swab device for such testing was also evaluated by real-time PCR using the BD Max™ System automated molecular platform.

Methods:

1. **Enrichment:** Suspensions of MSSA, MRSA and E.coli and were prepared at a concentration 10^6 cfu/ml. Serial dilutions of each suspension were made as follows - 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}. Swabs from each device were inoculated with a 50 µl aliquot and transferred back into a tube of medium. Two different types of swab were used (Sigma polyurethane foam bud and PurFlock®flocked polyester fibre bud.) The transport tubes were incubated for 24 hours at 37°C.
2. **Real-time PCR with BD Max™ System:** 10 swabs (5 of each type) were inoculated with MRSA (10^{-2} dilution) and incubated for 24 hours at 37°C before testing using BD Max MRSA XT kits.

Results: All swabs tested for enrichment showed a significant increase in numbers for MRSA and MSSA. They were easily distinguished on MRSA chromogenic agar. There was no increase in numbers for *E. coli* after 24 hours incubation.

On BD Max™ all swabs gave a positive identification for MRSA. An uninoculated control of the TSB medium was negative.

Conclusion: The results suggest that this device is suitable for use for MRSA screening in accordance with the Dutch guideline.

P058

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]*VUmc, Department of Medical Microbiology and Infection Control, Amsterdam,* [2]*VU, Department of Molecular Cell Biology, Amsterdam*

Tuberculosis (TB), caused by the eponymous pathogen *M. tuberculosis*, is responsible for over 1 million deaths annually. Due to the increasing prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains, novel anti-TB drugs are required. A promising new group of novel drug targets for *Mycobacterium tuberculosis* are the type VII secretion (T7S) systems. Mycobacteria can have up to five of these systems, ESX-1 to ESX-5, of which at least three are crucial for the virulence and/or viability of *M. tuberculosis*. The ESX systems have a set of conserved proteins, amongst which is the membrane-associated subtilisin-like serine protease mycosin (MycP), which is an essential part of the T7S system. Due to the proteolytic activity, conservation and essentiality of the mycosins they are promising targets for the development of novel drugs against TB. In this study we set out to functionally dissect the role of the mycosin proteases in T7S, by analyzing the phenotype of MycP1 and MycP5 mutants.

Deletion strains of the mycosins of the ESX-1 and ESX-5 secretion systems were created in the close relative of *M. tuberculosis*, *Mycobacterium marinum*. Subsequently, various MycP1 and MycP5 variants, amongst others proteolytic inactive versions, were introduced in the deletion strains. Functional complementation by these variants was verified by the effect on the secretion by the ESX-1 and ESX-5 systems.

While the deletion strains of MycP1 or MycP5 were defective in respectively ESX-1 or ESX-5 dependent secretion, the protease inactive variants were able to mediate secretion by ESX-1 or ESX-5. In contrast to this observation we did identify the protease domain to be responsible for the specificity of MycP for its respective ESX-system. We are currently testing the various MycP5 mutants in the zebrafish infection model to investigate the effect on virulence.

Our results show that the proteolytic activity of MycP1 and MycP5 is not essential for ESX-1 or ESX-5 dependent secretion. This indicates a dual function for mycosins, with a proteolytic role in substrate processing and a second, so-far unknown, role in the regulation of the secretion process. We have identified the proteolytic domain to be essential for this system-specific unknown function of the mycosin proteases and we are currently pinpointing which residues in this domain are involved in this second function in T7S.

P059

Presence of infection in patients with presumed sepsis at the time of intensive care unit admission

P.M.C. Klein Klouwenberg, O.L. Cremer, L.A. van Vught, D.S.Y. Ong, J.F. Frencken, M.J. Schultz, M.J. Bonten, T. van der Poll

UMC Utrecht, Department of Medical Microbiology, Utrecht

Introduction: A clinical suspicion of infection is mandatory for diagnosing sepsis in patients with a systemic inflammatory response syndrome. Yet, the accuracy of categorizing critically ill patients presenting to the Intensive Care Unit (ICU) as being infected or not is unknown. We therefore assessed the likelihood of infection in patients who were treated for sepsis upon admission to the ICU, and quantified the association between plausibility of infection and mortality.

Methods: We studied a cohort of critically ill patients admitted with clinically suspected sepsis to two tertiary ICUs in the Netherlands between January 2011 and December 2013. The likelihood of infection was categorized as none, possible, probable or definite by post-hoc assessment. We used multivariable competing risks survival analyses to determine the association of the plausibility of infection with mortality.

Results: Among 2579 patients treated for sepsis, 13% had a post-hoc infection likelihood of 'none', and an additional 30% of only 'possible'. These percentages were largely similar for different primary suspected sites of infection. In crude analyses, the likelihood of infection was associated with increased length of stay and complications. In multivariable analysis, patients with an unlikely infection had a higher mortality rate compared to patients with a definite infection (subdistribution hazard ratio 1.23; 95% confidence interval 1.03-1.49).

Conclusions: This study is the first prospective analysis to show that the clinical diagnosis of sepsis upon ICU admission corresponds poorly with the presence of infection on post-hoc assessment. A higher likelihood of infection does not adversely influence outcome in this population.

P060

Comparison between commercial versus in-house developed multiplex qPCR assays to detect *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium* and *T. vaginalis* – a retrospective study

F. Hagen, M. Kersten, F. Hagen, J.J.H.C. Tilburg, J.F. Meis

Canisius-Wilhelmina Hospital, Department of Medical Microbiology and Infectious Diseases, Nijmegen

Introduction: The Diagenode multiplex qPCR assays (Diagenode, Seraing, Belgium) have been developed to detect *Chlamydia trachomatis* plus *Neisseria gonorrhoeae* (S-DiaCTNG) and *Mycoplasma genitalium* plus *Trichomonas vaginalis* (S-DiaMGTV) in clinical samples. The setup of these commercial qPCRs is similar to those developed in-house and used in our laboratory. A comparison between the two tests was made based on samples that were historically scored positive for one or more of the target pathogens, this in order to determine if a switch

from in-house to commercially developed qPCR assays is worthwhile.

Material & methods: Samples that were scored positive in the past for one or more of the four targets were selected. These included 54 eswabs, 38 urines and 1 exudate positive for *C. trachomatis* (n = 58) or *N. gonorrhoeae* (n = 27) or *C. trachomatis* plus *N. gonorrhoeae* (n = 8), and 62 eswabs and 33 urines positive for *M. genitalum* (n = 75) or *T. vaginalis* (n = 18) or *M. genitalum* plus *T. vaginalis* (n = 2). Each sample (200µl) was supplemented with 20µl internal control prior to DNA extraction with the MagNA Pure 96 (Roche Diagnostics, Almere, The Netherlands). The commercial multiplex qPCR assays (S-DiaCTNG and S-DiaMGTV; Diagenode) were used to test samples that were historically scored positive for one of the targets with the in-house qPCR assays. For the in-house and commercial qPCR assays a reaction volume of 20µl was used, including 6µl sample DNA. Real-time PCRs were performed on LightCycler 480-II's (Roche Diagnostics). The multiplex in-house qPCR assays were regarded as the reference method.

Results: Ninety-three samples were included in this comparison. In both assays 65 and 34 samples were positive for *C. trachomatis* and *N. gonorrhoeae*, respectively and 26 and 58 samples remained negative. A few discrepancies were observed, with the reference method 2 samples were *C. trachomatis* positive but negative with the S-DiaCTNG assay, similarly for 1 sample that was found *N. gonorrhoeae* positive. Ninety-five samples were included with the multiplex assays to detect *M. genitalium* and *T. vaginalis*, with both assays 73 and 20 were found positive, respectively. Nineteen and 74 samples remained negative with both assays for *M. genitalium* and *T. vaginalis*, respectively. Some discrepancies were observed, 2 samples scored positive with the reference method for *M. genitalium* but negative with the S-DiaMGTV kit. With the S-DiaMGTV assay one sample was found positive for *M. genitalum*, while the reference method remained negative. This was also observed for one sample scored positive for *T. vaginalis* with the commercial assay, but remained negative with the reference method. All discrepancies were observed with samples that had high Ct-values (close to 40) for one of the methods.

Conclusion: Commercial and in-house methods were nearly in full agreement with each other. With both approaches only few false negative results were observed, albeit only in samples with high Ct-values. Since the samples were collected and stored for up to one year quality of the samples might be an explanation. We recommend to further validate the commercial assays in a prospective study.

P061

Developing a latent zebrafish embryo infection model

S. Commandeur[1], G.V. Mukamolova[2], M. Boot[1], A.M. van der Sar[1], W. Bitter[1]

[1]*VUmc, Department of Medical Microbiology and Infection Control, Amsterdam,* [2]*University of Leicester, Department of Infection, Immunity, and Inflammation, Leicester, United Kingdom*

Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis, is able to reside in the host without inducing any clinical symptoms of the disease. A so-called latent infection can be present for decades. It has been suggested that inside the host Mtb is able to adapt to the harsh environmental conditions it encounters, resulting in a dormant phase. This phase reflects, among others, a reduced metabolism (and growth) and induced resistance against antibiotics.

Our aim is to develop a model that can be used to identify antibiotics or compounds that efficiently kill such dormant mycobacteria. We use *Mycobacterium marinum* (Mm) and the zebrafish embryo host as model system because (i) Mm infection of zebrafish embryos induces similar pathology as observed for Mtb infection in humans and (ii) it is useful for high throughput screens in search for novel antibiotics. Because latent infections cannot be reached in the current zebrafish embryo model due to time constraints, we therefore have to establish the bacterial dormant phase *in vitro* and subsequently introduce a latent infection in the host.

To create dormant Mm we analyzed whether Mm is able to adapt to *in vitro* stress conditions. Responses to hypoxic and starvation stress were studied, conditions to which mycobacteria are thought to be exposed to in the host environment. Viability, growth and susceptibility to antibiotics were determined. Starved Mm ceased to grow (OD/CFU) but remained viable over time (live/dead stain flow cytometry). As expected, transfer of starved Mm to nutrient rich media resulted in re-growth of the bacteria. Similar results were detected when starved Mm were introduced in zebrafish embryos.

Mtb expresses resuscitation promoting factors (Rpfs) which are described to aid in resuscitation of dormant mycobacteria. Mm expresses homologous Rpfs and we hypothesize that Rpf knock-outs of Mm will prevent or delay the out-growth *in vitro* but also *in vivo* in the zebrafish embryos. Viability, growth and susceptibility to antibiotics were also determined for 4 Mm Rpf mutant strains as well as their growth capabilities *in vivo*. The use of Rpfs knock-outs will finalize the creation of a zebrafish embryo latency model and make the model ready for screening purposes.

Overall, we conclude that Mm is able to adapt to stress conditions and therefore can be used as a tool for identi-

fication of novel antibiotics and compounds efficiently targeting dormant Mtb which will ultimately lead to a reduction in TB cases.

P062

Identification and discrimination of ten most prevalent Candida species by multiplex real-time PCR

W.B. Kraak, L. Roorda, J.M. Duin, A. van der Zee
Maasstad Hospital, Department of Medical Microbiology, Rotterdam

Introduction: In current *Candida* diagnostics, often cultivation methods and selective media are used to identify different *Candida* species. Recent introduction by MALDI-TOF analysis of *Candida* species improved correct identification and shortened time for a final diagnosis. In addition a test that discriminates correctly between closely related species like *C. albicans* and *C. dubliniensis* and the *C. -psilosis* complex is not available. We developed a novel real-time PCR assay that discriminates between ten most commonly found *Candida* species (*C. albicans, C. dubliniensis, C. glabrata, C. lusitaniae, C. metapsilosis, C. orthopsilosis, C. parapsilosis, C. tropicalis, I. orientalis* and *P. guilliermondii*).

Methods: Based on genomic information different primers and probes were designed. Primer and probe design was performed in Primer Express v2.0 (Life Technologies, Bleiswijk, The Netherlands) and checked by BLAST analysis for specificity. The *C. meta-* and *orthopsilosis* species primers and probes were designed based on threonyl-tRNA synthetase (*ThrRS*) genes described by Prandini *et al.* 2013, were *C. parapsilosis* primers and probes are based on the *BCR1* gene. Manganese SuperOxide Dismutase (*MnSOD*) was used for creating different primers and probes, each specific for species *C. glabrata, C. lusitaniae, C. tropicalis* and *I. orientalis*. The following genes were used for primer and probe design; *C. albicans*: Secondary alcohol dehydrogenase (SADH), *C. dubliniensis*:Zeta-Carotene desaturase (*Zds1*) and *P. guilliermondii*: Topoisomerase II (*TopII*). Isolation of genomic DNA of multiple *Candida* strains was performed by MagNAPure96 (Roche Diagnostics, Almere, The Netherlands).

Results: Analysis of 103 different *Candida* species, previously tested by cultivation on selective media, were correctly identified by our real-time PCR assay. The multiplex PCRs, including the internal control Phocine Herpes Virus, showed no cross-reactivity. The lower limit of detection by real-time multiplex PCR's was found between 1 pg and 100 fg, corresponding respectively to 87-65 and 8.7-6.5 genomic copies. Specificity of the assay was further investigated by analysis of viral and bacterial panels. No aspecific reactions were observed.

Conclusions: 1 The multiplex PCR assay is a reliable and rapid method to identify and discriminate the most prevalent *Candida* species. 2 Performance of our assay in various clinical materials will be further investigated.

P063

Comparison of automated blood culture systems for rapid detection of bacteria and yeasts: Bact/ALERT® versus Bactec™

T.J.H. Siebers, A.W. Friedrich, G.A. Kampinga
University Medical Center Groningen, Department of Medical Microbiology, Groningen

Introduction: The purpose of this study was to compare the time to detection (TTD) of several types of micro-organisms by two commonly used blood culture systems: Bactec™ FX-40 (Becton Dickenson, BD) and BacT/ALERT® (BioMerieux, BM). As patients at risk for candidemia are hospitalized in the UMCG, special attention was paid to the detection of Candida species.

Methods: A total of 12 aerobic and 5 anaerobic bacterial species (one strain each), and 4 Candida species (n = 9 to 13 strains) were studied. At first BacT/ALERT FA Plus (aerobic; BM-aer), Bact/ALERT SN (anaerobic; BM-an), BacT/ALERT PF Plus (BM-ped) bottles were compared with Bactec Plus Aerobic/F (BD-aer), Bactec Lytic/10 Anaerobic/F (BD-an) and Bactec Peds Plus/F (BD-ped). BM-an bottles are used in our routine as they show superiority over BM-aer and BacT/ALERT FN Plus (anaerobic) vials for yeast detection (previous study, data not shown). All bottles were inoculated with 3 ml (paediatric bottles) or 5 ml defibrinated horse blood combined with 0.5 ml bacterial suspension containing a median of 235 (range 10-2500) colony-forming units (based on subculture of the suspensions). All bottles were tested in triplo and compared for positivity as well as TTD. To check for purity all positive bottles were subcultered on an appropriated medium.

When it was noticed that the BD bottles failed to detect all Candida species as early as the BM-an bottle, additional tests were performed using the Bactec Mycosis IC/F bottle (BD-myc). In this study, 13 strains were tested: C. albicans (n = 5), C. glabrata,(n = 2 plus 4 additional strains), C. parapsilosis (n = 1), and C. tropicalis (n = 1).

Results: Common blood-cultered aerobic bacteria were detected earlier in BD-aer bottles compared to BM-aer (median difference in TTD 2.0; range 0.1-2.7 h). The obligate aerobic species Stenotrophomonas maltophilia, Neisseria meningitides, Pseudomonas aeruginosa, and Micrococcus luteus, were missed in the BD-an bottles. Remarkably, all these species grew in the BM-an bottles.

BM-an had a shorter TTD for Candida species (8/9 cases) as compared to BM-aer (median difference in TTD 3.2;

range -1.7 - 38.5 h) and BD-aer vials (median 3.7; range -2.3 - 34.5 h). Except for C.glabrata, all yeasts were missed in BD-an bottles. This species was typically detected faster (29.5 to 38.5 h) in anaerobic bottles as compared to aerobic vials. Using the BD-myc bottle a reduction in TTD was obtained (median 22.2; range 0.8 - 49.1h) compared to the BD-aer bottles and for *C. glabrata* the reduction was 2.7 - 9.9 h compared to BM-an.
BD-an vials were superior to BM-an bottles for detection of anaerobic species (difference in TTD 1.9 - 59.1 h). Peptoniphilus harei was missed in the BM-an.
Conclusions: The Bactec aerobic bottle demonstrated the shortest TTD for common aerobic bacteria. We observed differences between the two systems regarding yeasts and anaerobes: the BM-an bottle was superior to the aerobic (both systems) and BD-an vials for yeasts, but appeared less optimal for detection of anaerobic bacteria. With the Bactec system however, a reduced TTD for yeast can be achieved by adding a mycosis bottle and this may benefit high-risk patients.

P064

Introducing a multipurpose sample collection and transport system compatible with lab automation

S.J. Mulder, T.N.H. Nguyen, W. Flisijn, R. Muiser, R. van Rhee-Luderer, E. Mooi Kokenberg, T.A. Schuurs
Izore, Centrum infectieziekten Friesland, Molecular Microbiology, Leeuwarden

Introduction: In our laboratory, the automated inoculation system, InoqulA, (Becton Dickinson, BD) was purchased while in the molecular department the Roche FLOW system, is employed. Optimal use of these automated systems requires an uniform multipurpose sample collection and transport buffer/kit. Additional benefit is that sampling once is less invasive for the patient. Liquid Amies, is described as one of the best transport medium for culture. Among the commercially available liquid Amies media are media/kits from Copan (ESwab), BD (BD ESwab), Puritan (Liquid Amies) and Medical Wire(S transwab).The aim of this study was to test the suitability of these different sample collection kits for culture of bacteria/yeasts and molecular diagnostics from the same sample by analysing stability and different storage temperatures.
Methods: For STD testing, dilutions were made of positive samples for *Chlamydia trachomatis* and *Trichomonas vaginalis* and added to the different liquid Amies media. For *Neisseria gonorrhoeae* different concentrations of culture suspensions were made. Nucleid acid extraction (MagnaPure 96, Roche) and PCR testing were performed after 0, 24, 48 hours and after one week. The *Neisseria gonorrhoeae* samples were also cultured.For culture testing dilutions of *Streptococcus pneumoniae*, Haemophilus influenza, *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacteroides fragilis* and *Candida albicans* were made. Using the flocked swab an amount of this dilution was added to the Liquid Amies and stored at room temperature and at 4 degrees. Culture and plate counting were performed after 0, 24 and 48 hours.
Results: For *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis* no significant differences in Cp values were observed after 0, 24, 48 hours of storage at room temperature, when the different Amies media were compared. Results of Neisseria culture show dramatic decrease of viability of the gonococci: After 48 hours at room temperature, only culture-growth was detected when gonococci had been stored/incubated in Amies medium from Copan or BD.As the ESwab form Copan and BD turned out to be identical, further culture-suitability was only tested for the BD ESwab. Results of culture and platecount show degredation in time of *Heamophilus influenza* and *Bacteroides fragilis* when the sample is stored at roomtemperature as well as at 4 degrees. When stored at roomtemperature *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa* are still replicating resulting in overgrowth of the culture-plate. Cultures of the replicate samples which were stored at 4 degrees showed no replication..
Conclusion: Culture and molecular testing for diagnostics of infectious diseases can reliably be performed out of one tube.For molecular STD testing all tested commercially available Amies media are stable for at least one week at room temperature without sample degradation. Culture of *Neisseria gonorrhoeae* must take place within 48 hours. For general culture the samples are stable for 48 hours when stored at 4 degrees. Therefore it is important to place the sample at 4 degrees at the samplinglocation if transport to the lab takes more than 24 hours.

P065

Diagnosing intrauterine parvovirus B19 infection in neonatal dried blood spots

A. Russcher, L.G. Rusman, D. Oepkes, A.C.M. Kroes, A.C.T. Vossen
LUMC, Department of Medical Microbiology, Leiden

Introduction: Approximately 40% of infections with parvovirus B19 (B19V) in pregnancy result in fetal infection. Fetal infection can cause severe fetal anemia, which can be managed by intrauterine erythrocyte transfusion (IUT). Until recently, long term outcome after intrauterine B19V infection was thought to be unaffected. However, recent data suggest an increased incidence of neurodevelopmental disorders in children treated by IUT for intrauterine B19V infection. Diagnosing intrauterine

B19V infection after birth would provide possibilities to establish prevalence and disease burden of (asymptomatic) fetal infection. The aim of this study was to provide a 'proof of principle' of the detection of B19V DNA in neonatal dried blood spots (DBS) following intrauterine fetal infection.

Methods: Patient selection: Patient records were reviewed to identify all patients who underwent IUT in the period 2009-2014 for a confirmed intrauterine B19V infection (PCR positive amniotic fluid and/or fetal plasma). Neonatal DBS, collected in the first week after birth as part of the neonatal screening program for metabolic and endocrine disease, were obtained from the National Institute for Public Health and the Environment (RIVM, Bilthoven, the Netherlands) after parental informed consent.

DNA extraction and PCR: One entire DBS (equivalent to 50 μL whole blood) was punched. DNA extraction was performed using spin columns (QIAmp DNA Mini Kit, Qiagen). An in-house developed real-time B19V PCR, targeting the NS region of the genome, was performed in triplicate.

Results: Twenty-one IUT's were performed in the period 2009-2014. Twelve DBS from twelve children with confirmed intrauterine B19V infection were available. IUT's were performed at a mean gestational age of 21.7 weeks. All fetuses were hydropic. All twelve DBS tested positive. Seven DBS tested positive in all triplicates, and three DBS tested positive in two triplicates. Viral load in DBS correlated with viral load in amniotic fluid. Also, viral load in DBS corresponded inversely with time from IUT to birth.

Conclusions: B19V DNA can be detected in neonatal DBS of children treated by IUT for B19V-related fetal anemia.

B19V DNA load in neonatal DBS correlates with fetal load and timing of infection.

P066

Is intraventricular gentamicin useful in the treatment of drain associated bacterial meningitis due to Gram negative rods?

M.B. Haeseker, Z. Safipour, V. Hira, F.H. van Tiel, T.R.A. Havenith

MUMC, Mmb, Maastricht

Introduction: External ventricular and lumbar drains are commonly used to decrease intracranial pressure. The risk of meningitis from external ventricular catheter is approximately 8%. The most common pathogens isolated are *Staphylococci*. Gram negative bacteria are less frequently isolated. Intraventricular administration of vancomycin is often used in combination with vancomycin intravenously. The use of intraventricular aminoglycosides, adjusted to cerebrospinal fluid (CSF) concentration is advocated in guidelines as treatment of last resort, but in contrast to intraventricular vancomycin, intraventricular aminoglycosides are hardly used. Furthermore, little is known about peak and trough levels in CSF. Here, we describe our experience with intraventricular gentamicin in a patient with severe Gram negative drain associated meningitis. Trough levels of gentamicin in CSF were measured.

Methods: Telephone inquiry of experience with intraventricular gentamicin was made in Dutch University Medical Centres (UMC).

Gentamicin levels were measured in surplus CSF samples with a fluorescence polarization immunoassay Roche Cobas Integra® 800. Blank CSF was spiked with gentamicin and different concentrations were used as quality controls.

Results: Six UMCs responded to the telephone inquiry. Five UMCs had no experience with intraventricular gentamicin. Only one UMC had used intraventricular gentamicin once before.

Case description: A 67 year old man was admitted to our hospital with a subarachnoid hemorrhage. On the day 0, an external ventricular drain (EVD) was placed, which was revised on day 2. A hygroma was released on day 4. The patient developed fever and signs of meningitis on day 13. CSF cultures were taken, ceftazidime and vancomycin iv were started empirically on day 13. The CSF culture became positive with *Enterobacter cloacae*, ceftazidime and vancomycin was stopped and meropenem iv was started on day 15. The patient did not improve and intraventricular gentamicin (8 mg/24h) was started on day 17. The four measured gentamicin trough levels were between 2-4 mg/L. Peak levels were not measured, but are believed to be >20 mg/L. CSF cultures remained positive with *Enterobacter cloacae* until day 19 and remained negative from that day onwards. Intraventricular gentamicin was stopped on day 31 and meropenem iv was stopped on day 42. The patient slowly recovered, without developing side effects, such as seizures or chemical ventriculitis.

Discussion: Intraventricular gentamicin is seldom used in Dutch UMCs. Even in severe and difficult to treat Gram negative bacterial drain associated meningitis, intraventricular gentamicin is not used in Dutch UMCs. Randomized prospective clinical trials evaluating different antibiotic regimes for Gram negative bacterial drain associated meningitis do not exist, because of the relative low incidence. Based on retrospective studies and expert opinion, several guidelines advocate the use of intraventricular aminoglycosides in severe Gram negative bacterial drain associated meningitis. Our case showed a complete microbial recovery and slow neurological recovery of a severe Gram negative bacterial drain associated meningitis. We believe that in such cases, intraventricular gentamicin in combination with iv antibiotics might improve clinical outcome. However, further research on

pharmacokinetics and adverse drug effects of intraventricular aminoglycosides is urgently needed to define clear indications of intraventricular administration of aminoglycosides.

P067

Chlamydia antibody titre (CAT) screening; can commercial ELISA's be considered highly specific in the detection of specific IgG against *Chlamydia trachomatis*?

J. Roelofs-van den Ing, K. Fleischer, J.C. van Pelt-Koops, F.F. Stelma

Radboudumc, Department of Medical Microbiology / Virology, Nijmegen

Background: Previous infection with *Chlamydia trachomatis* can cause infertility in women. Earlier an association was demonstrated between Chlamydia IgG and ovarian tubal pathology. Chlamydial antibody titers (CAT) was shown to have a high negative predictive value (85-90%) and therefore appears to be an acceptable tool for screening purposes in infertile women. However, cross-reactivity with other Chlamydia species may result in low positive predictive values as was shown earlier (30-65 %). *Chlamydia pneumonia* sero-prevalence may reach up to 60% in females between 20 and 40 years, possibly influencing CAT-screening in infertile women. This study aimed to investigate *C. trachomatis* specificity in four commercial ELISA's (Mikrogen *recom*Well Chlamydia trachomatis IgG, Euroimmuun Anti-Chlamydia trachomatis ELISA (IgG), Medac Chlamydia IgG recombinant ELISA and Serion ELISA classic *Chlamydia trachomatis*).

Methods: The assumed negative samples were obtained from infants between 1 and 2 years (n = 15) and from healthy pregnant women (n = 14). The assumed positive samples were obtained from patients with confirmed tubal pathology (n = 2) and from patients with a positive PCR for *C. trachomatis* (n = 18). All samples were submitted to Chlamydia IgG testing by the above mentioned commercial tests.

Results: The specificities obtained for the Mikrogen-, Euroimmuun-, Medac- and Serion ELISA's were 90%, 100%, 90% and 100%, respectively. One infant showed reactivity in the Mikrogen ELISA. The sensitivities obtained were 80%, 70%, 75% and 45%, respectively. Healthy pregnant women tested IgG positive in 2, 0, 3 and 0 samples, respectively. Patients with tubal pathology tested IgG positive in 1, 0, 2 and 0 samples, respectively. Patients with a positive PCR for *C. trachomatis* tested positive in 15, 14, 13 and 9 samples, respectively.

Conclusion. High specificities were obtained for all four commercial assays; Medac- and Euroimmuun ELISAs showing the best properties. The high specificity found, indicates that serological cross-reactivity with other chlamydia species is not an important issue in CAT screening.

P068

VRE screening of environmental samples of a hospital outbreak; increased sensitivity with PCR compared to screening by culture

R. van Rhee-Luderer, J. Duinkerken-Kinderman, O.J. Lindeboom, S.J. Mulder, K. Waar, J.H. van Zeijl, T.A. Schuurs

Izore, Centrum infectieziekten Friesland, Medical Microbiology, Leeuwarden

Introduction: In spring 2014 *vanB* positive Vancomycin resistant *Enterococcus faecium* (VRE) was detected in specimens from several patients on an intensive care unit. Typing analysis revealed that all strains were MLVA type 012. An outbreak investigation was started and swabs from patients and the environment were analysed by culture. Enrichment broth was analysed retrospectively by PCR for comparison.

Methods: For routine analysis by culture, specimens were inoculated onto Brilliance VRE plates (Oxoid) and in a modified esculine bile broth (Media Products) containing 10 mg/l amphotericin B, 16 mg/l ampicillin and 2mg/l oxacillin. Plates and broths were incubated at 35-37°C and examined after 24 and 48 hours. Brown or black broths were subcultured onto Brilliance VRE plates. Broths were stored at 4 degrees till PCR analysis. Based on the method described by Fang et al. (2012) a multiplex real-time PCR assay was developed for detection of *vanA*, *vanB* and an internal control. Extraction of DNA from 500 µl of broth was performed with a MagNaPure 96 (Roche) instrument and PCR was performed on a LightCycler 480 (Roche). Broths generating an amplification curve with a Cp < 35 were regarded as PCR positive and were subcultured onto Brilliance VRE plates for confirmation. Indigo-purple colonies on Brilliance VRE plates, which were inoculated with either brown or black broths or PCR positive broths, were further analysed and *vanB* gene presence was confirmed by PCR analysis. Specimens from which a *vanB* positive VRE strain was obtained were regarded as true positives.

Results: During outbreak analysis, broths from 25 patient samples and 58 environmental swabs were saved after analysis by culture. In five patient samples VRE was detected by culture and PCR. With PCR one extra *vanB* positive broth was identified, but VRE presence could not be confirmed by subculturing of the broth onto Brilliance VRE plates.

Eight of the 58 environmental specimens were shown to contain VRE by culture analysis and the broths of these

eight samples were also positive in the PCR analysis. With PCR 10 extra positive specimens were identified of which 6 could be confirmed by subculturing the broth onto Brilliance VRE plates. These broths were still clear after 48 hours of incubation during the analysis process based on culture and were not subcultured for that reason during culture screening. These results show that the sensitivity of VRE screening of environmental samples could be improved from 57% with culture analysis to 100% with PCR analysis and the negative predictive value from 88% to 100%. Specificity of PCR analysis of environmental samples is 91% compared to 100% with culture analysis.

Conclusion: For environmental samples, PCR screening of enrichment broths has an increased sensitivity compared to culture analysis. The enrichment broths of this comparison were incubated for 24 or 48 hours before they were stored for retrospective PCR analysis. Additional experiments indicate that overnight incubation of enrichment broth is sufficient to obtain a negative predictive value of 100% with PCR analysis, resulting in a significant decreased turn around time of negative specimens.

P069

One-step cloning and overexpression system for flexible, secretable and removable his-tagged fusions of heterologous proteins in *Lactococcus lactis*

G. Buist[1], J. Neef[1], F. Milder[2], D.G.A.M. Koedijk[1], M. Klaassens[1], E. Heezius[2], J.A.G. van Strijp[2], J.M. van Dijl[1]

[1]University Medical Center Groningen, Department of Medical Microbiology and Infection Prevention, Groningen, [2]UMC Utrecht, Department of Medical Microbiology, Utrecht

For the identification of potential targets for immunotherapy and/or vaccination strategies against many pathogenic bacteria, isolation and functional analysis of surface exposed or secreted proteins is needed. With the purpose to generate a simple and flexible Histidine tag fusion approach, a set of expression vectors for extracellular production by *Lactococcus lactis*, has been adapted from an available *Escherichia coli* vector set. Vectors for the expression of N- or C-terminally His_6-tagged fusion proteins or an N-terminal His_6-tag followed by a TEV-cleavage site to remove the His_6-tag after protein purification, were constructed. The well-known T7-promoter was used in the *E. coli* vector set for optimal inducible intracellular protein production, resulting in efficient production and functional analysis of the Staphylococcal Complement Inhibitor from *Staphylococcus aureus*. Upon nisin induced expression various His_6-tagged proteins originated from *S. aureus* were secreted using a natural signal peptide for Sec-dependent export from *L. lactis*. Staphylococcal antigens, which are naturally secreted, membrane- or cell wall-bound (covalently or non-covalently), or function as a pro-peptide have been successfully produced and/or isolated using the *L. lactis* vector set. The functional activity of the secreted Staphylococcal peptidoglycan hydrolases SA0620 and SA2100 and the naturally cell wall bound ClfB have been shown upon secretion from *L. lactis*. The removal of the tag from the His-tagged FtsL protein of *S. aureus* upon secretion from *L. lactis* showed the functionality of the TEV-cleavage-site. FtsL and the propeptide of the staphylococcal protein Atl were produced in high amounts by *L. lactis* indicating that proteins with a different codon uage than L. actis can be succefully expressed in this host. Finally, the naturally phosphorylated protein IsdB of *S. aureus* was shown to be also phosphorylated after secretion from *L. lactis* as a His-tagged fusion.

The use these expression vector sets will be beneficial for a flexible approach for the successful overexpression and isolation of tagged, active heterologous proteins with the possibility to use a Gram-negative and/or a Gram-positive bacterium as the production host.

P070

Invasive fungal disease due to azole-resistant *Aspergillus fumigatus* in haematological patients – a retrospective study

D.W.L. Scoop, P.A. von dem Borne, E.J. Kuijper, M.T. van der Beek

LUMC, Department of Medical Microbiology, Leiden

Introduction: Invasive fungal disease due to *Aspergillus fumigatus* is a large problem in immunocompromised patients. Increasing azole-resistance is reported, with higher resistance rates in The Netherlands than in other countries. In the Leiden area, azole resistance rates are higher than elsewhere in the country (14% vs. 6.8%). A recent study performed at the ICU of the Leiden University Medical Center revealed azole-resistance rates as high as 26%, with 100% overall mortality at 90-days. For adequate empirical treatment of invasive fungal disease, risk assessment of the possibility of azole-resistance is therefore urgently needed. Because haematological patients are an important risk group for invasive fungal disease, we wanted to determine the prevalence of and risk factors for azole-resistance in order to guide empirical treatment in these patients.

Methods: Clinical and microbiological data were collected retrospectively on all haematological patients at risk for invasive aspergillosis (IA) between January 2010 and July 2014 with culture-proven IA. Susceptibility of *Aspergillus fumigatus* to triazoles was tested by 4-well azole-agar dilution provided by the Microbiology department at Nijmegen University. Suspected triazole-resistance was confirmed by phenotypical and genotypical testing at the

Mycology Reference Laboratory in Nijmegen. Decreased susceptibility was defined according to EUCAST clinical breakpoints for azoles.

Results: Between January 2010 and July 2014, 56 cases of IA occurred among 570 haematological patients at risk (9.8%). In 10 of these 56 cases culture showed *Aspergillus fumigatus* strains with decreased susceptibility to triazoles (17,9%). In 9 out of these 10 cases, the isolate showed resistance to itraconazole, voriconazole, as well as posaconazole. In 1 case the strain had reduced susceptibility to voriconazole only (MIC 2 mg/L).

Six of 10 patients with azole-resistant *A.fumigatus* had previous exposure to triazoles, either as prophylaxis or as treatment. Serum levels of prophylactic itraconazole were adequate in 2 of 5 patients that were monitored.

Empirical antifungal treatment was adequate in only 2 cases. Treatment adjustment based on susceptibility testing occurred in 5 of the 8 patients with inadequate empirical treatment, at a median of 10 days after treatment initiation. Overall mortality at 90 days in the haematological patients with IA due to azole-resistant *A.fumigatus* was 80% (8/10).

Conclusion: The high incidence of 17.9% azole-resistant *A.fumigatus* as a cause of IA with a positive culture found in haematological patients, is comparable to earlier studies performed in the same area of The Netherlands. The mortality among patients with IA caused by azole-resistant *A.fumigatus*, illustrates the importance of monitoring the prevalence of azole-resistance in clinical strains, so empirical treatment can be adjusted to the local situation. Determining risk factors for infections with azole-resistant *A.fumigatus* in haematological patients, will be an important tool for this objective.

P071

Occurrence of antibiotics and antibiotic resistant bacteria in wastewater streams

S.K. van den Hengel, L. Hernandez Leal, B. de Vries, H. Sombekke
Wetsus, N.a., Leeuwarden

Objective: Prevention of dissemination of antibiotic resistance (AR), especially in the clinical setting, receives great attention whereas only little attention is given to the spread of AR through the sewage system into the environment. Sewage streams of concern include household wastewater as well hospitals wastewater. Especially,toilet wastewater from hospital wards where antibiotics are intensively used (i.e. internal medicine and the pulmonary ward) may contain high concentrations of AR and antibiotics. To what extend each source contributes to the spread is currently unknown.

In order to obtain an increased understanding of the current situation different wastewater streams are characterized in terms of antibiotic resistant bacteria and antibiotic residues. This may form a fundament for wastewater collection and treatment strategies to prevent the spread of AR in the environment.

Methods: For this purpose, samples were collected from hospital wastewater streams (combined sewer and a stream of toilet wastewater from internal and pulmonary medicine), vacuum collected toilet wastewater from households, and both influent and effluent from the municipal wastewater treatment plant in Sneek, The Netherlands.

Within 4 hours after collection samples were processed for microbiological quantification of extended spectrum β-lactamase (ESBL) producing *E.coli* and *Klebsiela* and of vancomycin-resistant *Enterococcen* (VRE). In addition, specific genes responsible for AR within these isolates were determined. Wastewater streams were analyzed for the presence of antibiotics including ciprofloxacin, amoxicillin and clavulanic acid, gentamicin and meropenem. Also, chemical oxygen demand (COD), total phosphate, total nitrogen, anions and heavy metals were measured.

Results: Preliminary data show that in mixed hospital wastewater up to 2% of the total colony count is ESBL producing *E.coli* or *Klebsiela*. Out of the 22 ESBL positive *E.coli* or *Klebsiela* isolates analyzed, 17 contained the *bla*SHV gene and five contained a CTX-M group 1 gene. The genes responsible for carbapenem resistance were not found in any of the isolates. Results of antibiotic residue studies showed that amoxicillin was present ranging from 1-169 μg/l and ciprofloxacin from 3-53 μg/L. In addition, centamicin and more importantly meropenem levels remained below detection limits.

Conclusion: These preliminary data show that antibiotic resistant pathogens are present in hospital wastewater as well as residues of important antibiotics. How this compares to households remains to be evaluated.

To conclude, it is not clear yet to what extend separation of streams would be effective on the spread of antibiotics and AR in the environment. Therefor a more thorough characterization is needed to shed light on the impact of source separation of wastewater streams.

P073

Metagenomic analysis of iron-dependent anaerobic methane oxidation in the Bothnian Sea sediment

O. Rasigraf, M. Egger, C. Lüke, T. Jilbert, C.P. Slomp, B. Kartal, K.F. Ettwig, M.S.M. Jetten
Radboud University, Department of Ecological Microbiology, Nijmegen

Anaerobic oxidation of methane (AOM) is the major biological methane sink in marine sediments. So far, most AOM research has focused on sulfate-dependent AOM, a

process which has been recognized as a major methane removal mechanism in the sulfate transition zone where sulfate and methane are co-occurring. However, the potential of solid-phase electron acceptors, such as iron oxides, for methane oxidation has not been fully explored. Here we provide geochemical evidence for iron-dependent AOM in the Bothnian Sea sediment below a shallow sulfate-transition zone. This sediment layer is characterized by non-sulfidic conditions, methane availability and high amounts of reactive iron oxides. Batch incubations with and without ^{13}C-labeled methane and nanoparticulate iron hydroxide were performed with sediment slurries from different depths. The incubations from the zone where iron oxides and methane co-occur showed significant production of $^{13}CO_2$, indicating the potential for iron-mediated AOM. DNA from both the original sediment and the active incubations was sequenced by using Ion Torrent technology. Phylogenetic comparison of 16S rRNA genes derived from metagenomes obtained from the original sediment and from active sediment slurries showed a significant increase in reads related to *Archaea*, Clostridia and iron-reducing Proteobacteria after the 3 month incubation period. Together, our results indicate that iron-dependent AOM may play an important role in the biogeochemical cycling of iron in sulfate-poor iron-rich marine sediments.

P074

Prevalence of antibiotic resistance and virulence factors in commensal Enterococcus isolates from the gut microbiota

T.D.J. Bello Gonzalez[1], P. Pham[2], J. Top[3], M.W.J. van Passel[4], R.J.L. Willems[3], W. van Schaik[3], H. Smidt[1]

[1]Wageningen University, Department of Microbiology, Wageningen, [2]University of Tsukuba, Tsukuba, Japan, [3]UMC Utrecht, Utrecht, [4]National Institute for Public Health and Environment, Bilthoven

Introduction: Enterococci are present in the oral cavity, gastrointestinal tract and vagina in humans, as a normal commensal. In recent years, enterococci have become one of the most frequent causes of acquired nosocomial infections worldwide. Nowadays, the use or misuse of antibiotics for the treatment of infectious diseases caused by enterococci and the presence of genes conferring resistance and virulence, leads to the increase of antibiotic resistance and the ineffectiveness of medical therapies.

Methods: In this study, we investigated the presence of glycopeptide and macrolide antibiotic resistance genes and four virulence factors: enterococcal surface protein (*esp*), aggregation substance (*asa1*), hyalorunidase (*hyl*) and cytolysin (*cylB*) in commensal *Enterococcus* isolated from faecal samples of 11 hospitalized patients that received antibiotic prophylactic therapy including: Tobramycin (80mg), Polymyxin (100mg), Cefotaxime (4x 1000mg) and Amphotericin B (500mg). In addition, *E. faecium* and *E. faecalis* isolates were typed by Multi Locus Sequence Typing (MLST).

Results: A total of 47 isolates from faecal samples were identified by 16S rRNA gene sequencing and subsequently subclassified by $(GTG)^5$ PCR; 27 isolates were classified as *E. faecium*, 17 as *E. faecalis*, and single isolates clustered with *E. canintestini, E. dispar* and *E. avium*, respectively. Resistance to macrolides was detected by double disk test in 46 isolates. All isolates showed constitutive expression of resistance (cMLSb), and in 33 isolates the ermB gene was detected by PCR. The resistance to glycopeptides, specifically vancomycin was investigated by Diffusion Test (10μg) and the Minimal Inhibitory Concentration (MIC) by E-test; however, none of these isolates were resistant to vancomycin (MIC < 1 μg/ml). The *asa1* gene was detected in 13 isolates, *esp* in 28 isolates, and *hyl* in 1 isolate. In addition, all 47 isolates showed alpha hemolysin activity after 18h incubation at 37°C but the corresponding *cylB* was not detected. Based on MLST, 4 types were identified in *E. faecalis* and 7 types in *E. faecium* (including type 17 and 117 belonging to the clonal complex of hospital-associated strains). In 4 *E. faecalis* isolates, a new type 589 was identified.

Conclusion: In our study we showed that a significant number of virulence factors and antibiotic resistance genes are present in these isolates, which ones could contribute to the ability of a given enterococcal species to cause infection, however, these genetic factors are not necessarily found in clinical isolates but in commensals, which highlights the importance that enterococcal infections are multifactorial and even the host contributes to the spread of infection; besides, the emergence of virulence factors could lead to increased colonization potential of *Enterococcus*.

P075

Surveillance system for zoonotic pathogens and antimicrobial resistance in companion animals in the Netherlands: trends and signals

M.A.M. van Dijk, J.A. Wagenaar, A.H.W. Schoormans, E.M. Broens

Utrecht University, Department of Infectious diseases and Immunology, Utrecht

Background: The risk of transmission of zoonotic pathogens from pets to humans is potentially high considering their close contact. A surveillance system for zoonotic pathogens and antimicrobial resistance (AMR) in Dutch companion animals was put in place in 2011. The aim of the surveillance is to observe trends in prevalence of zoonotic pathogens and AMR and inform stakeholders.

Methods: The surveillance system consists of 3 pillars:
Passive surveillance, based on data of routine tests performed at the Veterinary Microbiological Diagnostic Centre (VMDC). (Retrospective) analysis was performed from the year 2010 onwards.
A helpdesk for (veterinary) healthcare professionals concerning zoonoses. Consults are categorised and analysed to be able to detect an increase of cases with comparable clinical features and (suspected) etiology.
Participation in the monthly meeting of the Signaling Forum Zoonoses, a network of representatives of the main Dutch institutes involved in veterinary and human infectious disease control.
Results: 1.Passive surveillance
Annual analysis was reported to the ministries of Economic Affairs and Public Health, Welfare and Sport. Quarterly analysis secured early detection of trends and was reported to the Dutch Food and Consumer Product Safety Authority (NVWA). Prevalences of the majority of detected agents were stable over the study period. A significant rise ($p < 0.05$) was observed for *Giardia intestinalis* in dogs with a positive fraction of 3.8% in 2010 to 9.3% in 2013. In cats a significant rise ($p < 0.05$) was seen for methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) with a positive fraction of 10.0% in 2010 to 28.6% in 2013. A significant decreasing prevalence ($p < 0.05$) was observed for extended spectrum beta-lactamase producing *Enterobacteriaceae* (ESBLs) from clinical cases in dogs, with a positive fraction of 4.9% in 2011 to 2.6% in 2013. These findings were discussed with policy makers/risk managers. For both *Giardia* and ESBLs existing research projects were extended to investigate the reasons for prevalence changes. The increased MRSP prevalence in cats could be explained by more targeted submission.
In the third quarter of 2014 a rise in the number of leptospirosis positive samples from dogs was noticed. With 7 positive samples in the 3rd quarter of 2014, this number clearly exceeded the average number of 1.5 positive sample per quarter in the years 2010-2014. This could be explained by both an increase of submissions for canine leptospirosis and a higher positive fraction. The rise paralleled the increase of autochtonous leptospirosis cases in humans.
2.Helpdesk
The helpdesk was consulted over 600 times per year. In 2013 the most important subjects were MRSA, MRSP, the new policy regarding veterinary antimicrobial therapy, a new leptospirosis vaccine and *Dientamoeba fragilis*. Actions were taken to improve the information provided to practitioners; such as preparing a paragraph in a healthcare professionals' guideline (Landelijke Coördinatie Infectieziektebestrijding [LCI]-Richtlijn), regarding companion animals in a household with MRSA positive humans.

Conclusions: The surveillance system for zoonoses and AMR in companion animals is a useful tool enabling the identification of trends in prevalence of selected pathogens and thus providing information for (veterinary) health professionals and risk managers.

P076

Evaluation of Thermo Scientific™ Sensititre™ susceptibility MIC plates for the MIC determination of gram negative isolates for amoxicillin, amoxicillin/clavulanic acid, cefotaxime, meropenem and piperacillin-tazobactam

L. Scopes, M. Oleksiuk, J. Screen, J. Bursens
Thermo Fisher Scientific, Trials, Basingstoke, Thermo Fisher Scientific, Erembodegem, Belgium

Introduction: This study compared the performance of Thermo Scientific™ Sensititre™ susceptibility MIC plates (Thermo Fisher Scientific), using both manual (Thermo Scientific™ Sensititre Vizion™ system) and auto (Thermo Scientific™ Sensititre ARIS™ System) reading methods to broth microdilution MIC panels manufactured according to ISO 20776-1 to determine the MIC of Enterobacteriaceae, Acinetobacter and Pseudomonas spp.
Methods: Sensititre susceptibility MIC plates and broth microdilution MIC panels were manufactured to contain amoxicillin, amoxicillin/clavulanic acid (constant 2), cefotaxime, meropenem and piperacillin-tazobactam. *Enterobacteriaceae*, Acinetobacter and Pseudomonas spp were cultured onto Columbia Blood Agar and incubated at 36 ±1°C for 18-24 hr. A 0.5 McFarland suspension of each isolate was prepared using Sensititre demineralised water and diluted to give a final inoculum of 5x10E4 - 5x10E5 cfu/ml for the Sensititre susceptibility MIC plates (according to the technical insert) or 2x10E5 - 8x105 cfu/ml for broth microdilution MIC panels (according to ISO 20776-1). Sensititre susceptibility MIC plates and broth microdilution MIC panels were inoculated using the Thermo Scientific™ Sensititre AIM™ and incubated at 36 ±1°C for 18-19 hr. Sensititre susceptibility MIC plates were read both manually (using the Sensititre Vizion system) and automatically (using the Sensititre ARIS System) and broth microdilution MIC panels were read manually. The essential agreement (EA) and categorical agreement (CA) of the Sensititre susceptibility MIC plates was calculated using the broth microdilution MIC panels as the reference method according to ISO 20776-2 criteria.
Results: *Table 1* shows the EA and CA of all antibiotics tested was greater than 90% with the EA of amoxicillin, cefotaxime and piperacillin-tazobactam (constant 4) being and the CA for amoxicillin, amoxicillin/clavulanic acid (constant 2) and cefotaxime equal to or greater than 95%.
Table 1: Comparison of Sensititre susceptibility MIC plates against broth microdilution MIC panels (EA manual read, EA automatic read, CA manual read, CA automatic read)

Amoxicillin: 99.1%, 99.1%, 99.3%, 99.6%; amoxicillin/clavulanic acid (constant 2), 93.7%, 93.7%, 96.0%, 95.6%; cefotaxime: 97.0%, 97.3%, 96.3%, 96.3%; meropenem: 94.0%, 92.5%, 92.8%, 93.1%; piperacillin-tazobactam (constant 4): 96.7%, 96.1%, 93.4%, 94.3%.

Conclusion: Sensititre susceptibility MIC plates are a suitable alternative to broth microdilution MIC panels manufactured according to ISO 20776-1 for the determination of MICs of Enterobacteriaceae, Acinetobacter and Pseudomonas spp. for amoxicillin, amoxicillin/clavulanic acid (constant 2), cefotaxime, meropenem and piperacillin-tazobactam (constant 4).

P077

Dendritic cell induced T-cell responses to biomaterials in presence of *Staphylococcus aureus* infection

P. Balraadjsing
AMC, Department of Medical Microbiology, Amsterdam

Staphylococcus aureus is a major pathogen in medical device (biomaterial)-associated infection. The combination of a biomaterial and bacteria can provoke inflammatory reactions of non-expected nature, resulting in increased susceptibility to infection. A major cell type orchestrating these immune responses are dendritic cells (DCs), which activate and instruct T-cells. We therefore aimed to obtain insight into the potency of DCs to activate and polarize T-cells in response to biomaterials, staphylococci, and to their combination. We used poly(trimethylene-carbonate) (PTMC), a high interest experimental material.

Human monocyte-derived DCs were cultured with or without PTMC in the presence or absence of *S. aureus*. Activation state of DC was determined by measuring maturation markers; HLA-DR, CD83, CD86 with flow cytometry and by measuring secreted cytokines; TNF-a IL-10, IL-6, IL-23, IL-12p70, IL-1β with ELISA. Activated DCs were subsequently co-cultured with CFSE-labeled autologous naive $CD4^+$ T-cells, and T-cell proliferation was assessed by flow cytometry. For T-cell polarization, DCs were co-cultured with autologous naive CD4+ T-cells with or without PTMC in presence or absence of *S. aureus*. The resulting intracellular IFN-γ and IL-4 levels of the T cells were analyzed by flow cytometry, as markers for Th1 or Th2 polarization, respectively.

DC stimulation with PTMC alone did not induce DC activation or T-cell proliferation and polarization whereas the combination of PTMC and *S. aureus* induced DC activation as well as the capacity of the DCs to induce naive T-cell proliferation and polarization. DCs cultured with the combination of PTMC and *S. aureus* resulted in not significantly increased DC activation and no difference in the numbers of proliferating T-cells and the percentage of Th1 or Th2 polarized T-cells than DCs cultured with *S. aureus* alone.

PTMC does not significantly change the capacity of *S. aureus*- activated DCs to activate or polarize Th-cells.

P078

Archaea are dominant among anaerobic methanotrophs in a paddy field

A. Vaksmaa, C. Lüke, T. van Alen, M.S.M. Jetten, K.F. Ettwig
Radboud University, Microbiology, Nijmegen

Paddy fields contribute approximately 20% to the global methane emission, making them a relevant source of methane. They provide several distinct habitats for microorganisms: the surface soil, the bulk soil, rhizosphere soil and roots. They are dynamic ecosystems where (nitrogen) fertilization, rice cultivar, flooding and draining seasons affect the presence and activity of microbial communities associated with methane production and consumption.

Most research efforts in paddy fields so far have targeted methanogenesis and aerobic methane oxidation, but less is known about anaerobic oxidation of methane (AOM). Nitrite-dependent AOM by NC10 bacteria has been demonstrated for some paddy fields, whereas the role and importance of nitrate-dependent methanotrophic archaea is not yet explored.

Here, we collected paddy field bulk soil and rhizosphere samples from the fields of the Italian Rice Research Institute, Vercelli (Italy) during the flooded (anaerobic) season. Soil slurry incubations of different depths were carried out using nitrate and ^{13}C-labelled methane. Q-PCR was performed to quantify known anaerobic bacterial and archaeal methanotrophs as percentage of total bacteria and archaea.

Our results showed that anaerobic methane oxidizing microorganisms were present in the paddy field, and that the archaea clearly dominated over (NC10 phylum) bacteria at all depths. Stable isotope assays showed that nitrate additions stimulated AOM indicating a significant activity of methanotrophic archaea. Future studies should be aimed to quantify the contribution of bacterial and archael AOM to microbial methane oxidation in paddy fields.

P079

Assessing the public health risk of Shiga toxin producing *Escherichia coli* using a molecular-based pathotyping approach

A.M.D. Kooistra-Smid[1,2], R.F. Boer de[1], M. Ferdous[2], I.H.M. Friesema[3], A. Ott[1,2], P.D. Croughs[4], J.W.A. Rossen[2], A.W. Friedrich[2]
[1]*Certe, Medical Microbiology, Groningen,* [2]*University Medical Center Groningen, Department of Medical Microbiology,*

Groningen, [3]National Institute for Public Health and the Environment, Bilthoven, [4]STAR-mdc, Department of Medical Microbiology, Rotterdam

Introduction: Clinical manifestations of Shiga toxin producing *Escherichia coli* (STEC) range from mild diarrhea to severe disease. Particularly O-serogroups O157, O26, O111, O103, O104, O145 and O121, pose a serious public health concern because of their ability to cause outbreaks. Microbiological risk assessment and categorization is needed to discriminate virulent serotypes from less virulent ones.
A multicenter, prospective study, STEC-ID-net, was performed during 12 months (2013-2014) in The Netherlands. A rapid diagnostic screening algorithm that enables categorization into pathotypes, was used to discriminate STEC infections associated with a high to moderate risk for severe disease from infections with less virulent STEC.
Methods: A total of 23153 stool samples from patients with presumed infectious gastroenteritis were screened using qPCR targeting the stx1/stx2/escV genes. Subsequently, PCR positive stool specimens were enriched (>16hrs) in BRILA broth, followed by culture on SMAC and CHROMagar STEC medium. On BRILA broth confirmatory multiplex qPCR were performed targeting stx1/stx2/escV/bfpA/aggR/aat/O26/O103/O104/O111/O121/O145/O157. STEC suspected colonies were tested with mPCRs.
STEC isolates were characterized using sero/genotyping, stx subtyping and genetically virulence and resistance profiling using DNA-micro-array and Next Generation Sequencing.
Molecular risk categorization was performed using a pathotyping scheme for categorization into presumptive PathoType (PT) groups ranging from PT I (high risk for diarrhea and severe disease) to PT IV (low risk).
Results: The detection frequency of stx1/stx2/escV genes and stx1/stx2 genes was 11.9 % (n = 2766) and 1.8 % (n = 425), respectively.
A total of 336 samples were categorized in PT I (n = 56 (17.2%)), PT II (n = 60, (18.4%)), PT III (n = 170, (52.1%)), and PT IV (n = 50, (15.3%)).
In PT I the detection frequencies of *stx1a, stx2a,* stx2c and *stx2e* subtypes were 10.7% (n = 6), 12.5% (n = 7), 12.5% (n = 7) and 3.6% (n = 2), respectively.
In PT II the detection frequencies of *stx1a, stx2a, stx2b* and *stx2f* subtypes were 11.7% (n = 7), 1.7% (n = 1), 1.7% (n = 1) and 50% (n = 30), respectively.
In PT III the detection frequencies of *stx1a, stx1c, stx2a, stx2b, stx2c,stx2d, stx2e, stx2f* and *stx2g* subtypes were 9.4% (n = 16), 2.9% (n = 5), 0.6% (n = 1), 1.8% (n = 3), 1.2% (n = 2), 0.6% (n = 1), 4.1% (n = 7), 1.8% (n = 3) and 0.6% (n = 1), respectively.
In PT IV the detection frequencies of *stx1a, stx1d* and stx2f subtypes were 32.0% (n = 16), 10.0% (n = 5) and 2.0% (n = 1), respectively.
A combination of different *stx1* and *stx2* subtypes were detected in 38 (11.7%) of the samples.
Among PT group I-III the O-serogroups O26, O91, O103, O104, O111, O121, O145 and O157 were detected in 11 (3.4%), 29 (8.6%), 13 (4.0%), 3 (0.9%), 3 (0.9%), 2 (0.6%), 9 (2.8%) and 21 (6.4%) of the samples, respectively.
Conclusions: 1. Fast microbiological risk assessment of STEC enables discrimination of virulent STEC (PT I and II) from less virulent ones (PT III). 2. Subtypes *stx2a* and *stx2c* seem to be more associated with PT group I than to the remaining PT groups. 3. Most detected O-serogroups were O91, O157, O103, O26 and O145.

P080

Pedriatic gut resistome development explored in a cohort study

C.J.H. von Wintersdorff[1], J. Penders[1], P.H.M. Savelkoul[1], R.R.R. Nijssen[1], E. Hamelmann[2], S. Lau[2], P.F.G. Wolffs[1]
[1]MUMC, Department of Medical Microbiology, Maastricht, [2]Ruhr-Universität Bochum, Bochum, Germany

Introduction: The antibiotic resistance of pathogenic bacteria has been studied extensively. However, recently the collection of antibiotic resistance genes (ARGs) in the gut microbiota (resistome) has been acknowledged as an important reservoir of ARGs. The establishment of the human gut resistome is ideally studied in the pediatric fecal microbiota. We therefore aimed to investigate the presence of various ARGs in the microbiota of newborns in the weeks following birth.
Methods: Within a prospective cohort among neonates born between May 2002 and September 2007, fecal samples collected at the ages of 5, 13 and 31 weeks postpartum were analyzed from a random selection of 120 infants. Inclusion criteria were healthy newborns at term, birth weight of >=2500g, 1 or 2 parents with atopic disease (criteria not related to the current study), and informed consent. Fecal samples were subjected to qPCR for the detection of ARGs *cfxA, tetM, tetQ, aac-aph, ermB* and *qnrS*. Prevalence was compared by using a McNemar's test for paired samples. Risk factors were analyzed by using logistic regression.
Results: Overall, the prevalence of ARGs gradually increased over the three time points. The prevalence of *cfxA* showed a non-significant increasing trend from 16.7% at week 5 to 25.0% at week 31. The *ermB* gene increased from 25.0% to 55.0% ($p < 0.01$). For *tetM* and *tetQ*, the prevalence increased from 76.6% and 43.3% respectively at week 5, to 92.5% ($p = 0.01$) and 55.8% ($p < 0.05$) at week 31. Contrarily, a decrease was observed for the *aac(6')-aph(2')* gene, of which the prevalence dropped from 25.8% to 15.8% ($p < 0.05$). Furthermore, *qnrS* was detected in only 1 sample at both week 5 and 31. Infants delivered by cesarean section showed a significantly lower prevalence of

tetQ at all three time points (14.3%, 25.0%, 39.3% respectively) when compared to vaginally born infants (52.2%, $p = 0.001$, OR = 0.15, 95%CI = 0.049-0.475; 60.9%, $p = 0.002$; 60.9%, $p < 0.05$ respectively), which seemed to be normalizing over time. Infants breastfed for longer than 26 weeks showed a lower prevalence of *tetQ* (49.0%) when compared to infants breastfed for 0-26 weeks (83.3%; $p < 0.05$, OR = 0.19, 95%CI = 0.061-0.603). Preliminary data suggest that the observed fluctuations in resistance genes are at least partly due to shifts in the gut microbial composition. For example, the prevalence of *tetQ* showed a positive correlation ($p < 0.001$) with the concentrations of *Bacteroides fragilis* in microbiota samples.

Conclusion: Our study shows that acquisition of ARGs in the gut microbiota occurs already within the first weeks after birth and that the prevalence of these genes in the microbiota of newborns is highly variable over the course of a few weeks. These findings warrant for a more extensive investigation including more resistance genes and relating their prevalence to changes in the microbial composition in order to further characterize the establishment of the resistome of the human gut microbiota.

P081

Oral amoxicillin for suppressive treatment of a complicated *Enterococcus faecalis* infection

V. Hira, T.R.A. Havenith, J. van Os, M.B. Haeseker, F.H. van Tiel, V.P.M. van Empel, S.H. Lowe

MUMC, Department of Medical Microbiology, Maastricht

Introduction: A 66 year old female with a history of aortic and mitral valve replacement and a pacemaker was admitted to our hospital after two weeks of malaise and fever. Blood cultures drawn on admission yielded an *Enterococcus faecalis*, and transesophageal echo (TEE) showed vegetations on her mitral valve. Surgical replacement of the mitral valve was not an option. Antibiotic treatment with amoxicillin and gentamicin was started, but gentamicin had to be discontinued due to nephrotoxicity. A subsequently performed TEE showed an increase in vegetation size on the mitral valve and therapy was switched to daptomycin iv. After nine weeks of daptomycin therapy, she was discharged in good health, without any clinical or laboratory signs of infection. However, two weeks later she was readmitted with fever and blood cultures yielded a daptomycin resistant *E. faecalis*. She was treated with amoxicillin and ceftriaxone iv for 12 weeks. This therapy was complicated by an *Enterococcus faecium* central venous line infection. Due to ambiguous echocardiographic results and the recurrence of the *E. faecalis* infection, we decided that lifelong antimicrobial suppressive therapy was justified. Here, we describe our experience with several antibiotic regimens.

Methods: Linezolid therapy was accompanied with frequent controls of haematological and inflammation blood values. Teicoplanin dosing was based on serum trough levels (target 20 - 40 mg/L). To determine the optimal amoxicillin dose, we performed an individual dosing simulation with MW Pharm 3.60, adjusting for kidney function, body weight and MIC of the *E. faecalis*. Subsequently, blood serum levels of amoxicillin were determined for verification.

Results: Since linezolid has an almost 100% oral resorption and is dosed independently of kidney function, suppressive treatment was started with linezolid 600mg po q12h. However, after two weeks the patient suffered from severe pancytopenia, which recovered after discontinuation of linezolid. As teicoplanin has a long half-life and is presumably less nephrotoxic than vancomycin, therapy was continued with teicoplanin iv. Based on serum trough levels of teicoplanin, she received 300mg (6mg/kg) iv q48h at home. However, within 6 months therapy was complicated twice with *Enterobacter aerogenes* catheter sepsis and an infected thrombus. As intravenous therapy repeatedly showed complications, amoxicillin orally was considered. The MIC of the *E. faecalis* for amoxicillin was 0.75mg/L. Computer simulation showed that dosing amoxicillin at 1000mg po q8h would result in continuous serum amoxicillin levels savely above the MIC. This was verified with a measurement, which showed an amoxicillin trough level of approximately 15mg/L. The patient was discharged and after seven months of amoxicillin orally, she was in good health, without signs of recurrence of the *E. faecalis* infection and without any side effects of amoxicillin.

Conclusions: 1. Amoxicillin is adequate for suppressive oral treatment of complicated infections, when dosed on MIC and pharmacokinetic monitoring. 2. Linezolid pharmacokinetics in patients might be more variable than in healthy individuals and therefore TDM might be of use to avoid the risk of treatment failure or of dose-dependent toxicity. 3. Focusing mostly on optimal effectiveness of therapie can be harmful for the patient.

P082

Shifting in the force of infection of dengue in Curacao

T.D.K. Thai, M. Limper, I. Gerstenbluth, A.J. Duits, E.C. van Gorp

Erasmus MC, Department of Medical Microbiology and Infectious Diseases, Rotterdam

Background: Knowledge of the force of infection is essential for planning public health interventions such as vector control or possible future use of a vaccine. This study aims to estimate the force of infection (λ) and transmission potential (R_0) in Curacao for 1973 and 2008.

Methods: For estimating both, the λ and R_0, a simple compartment model was employed. Two epidemiological data sets were analyzed, i.e. age-specific sero-prevalence and surveillance data in which patients with serologically confirmed dengue were stratified by age and infection parity.

Results: A decrease in λ from 10.7% (95% CI: 10.0-11.3) per year to 7.0% (95% CI: 6.8-7.1) per year was observed, resulting in an increase in the mean age at primary infection from 9.4 to 14.3 years of age. Based on the obtained λ, the estimated average R_0 were 8.8 (95% CI: 8.3-9.3) and 6.5 (95% CI: 6.4-6.6) in 1973 and 2008, respectively, indicating a minimal changes in mean critical vaccination fraction.

Conclusions: Changes in the average age of infection of dengue will have implications for clinical practice and disease control in Curacao. Recent decline in λ, not only resulted in a reduction of the overall reduction of the herd immunity, but also poses a paradoxical problem of an increase in the incidence of clinical disease by exposing a higher proportion of adults to infection.

P083

Anorectal *Chlamydia trachomatis* load is higher in women reporting anal sex than women who do not

J.A.M.C. Dirks[1], G.A.F.S. van Liere[2], N.H.T.M. Dukers-Muijrers[2], P.F.G. Wolffs[1], C.J.P.A. Hoebe[2]
[1]*MUMC, Department of Medical Microbiology, Maastricht,* [2]*Public Health Service, Sexual Health and Infectious Diseases, South Limburg*

Introduction: Guidelines recommend anorectal *Chlamydia trachomatis* (CT) testing in women because of frequent reports of anal sex (11-26%) and substantial prevalence of anorectal chlamydia in women (6-15%).
A recent study demonstrates that of all women with urogenital chlamydia, 71.2% also have anorectal chlamydia. Furthermore, two-thirds of these anorectal infections were diagnosed in women without anal sex or symptoms, and would thus be missed on indication-only-testing. The question arises whether these infections are clinically relevant, or a mere superficial presence. Therefore, we investigated whether the anal bacterial CT load was different in women with and without anamnestic anal sex.

Methods: A convenience sample of 116 women with a concurrent ano-genital CT infection were included from the STI-clinic in South Limburg. CT load (log_{10} number of CT bacteria/ml) was determined via qPCR from self-collected anorectal and cervicovaginal swabs. Unquantifiable samples were set to cycle-treshold-value >42 (n = 23/116). Student's T-test, univariable- and multivariable linear regression analyses were performed to identify determinants associated with load.

Results: The majority 56% (65/116) of women did not report anal sex. Anal symptoms were present in 15.8% (n = 8) of women with- and 3.3% (n = 2) of women without anal sex. The mean anorectal CT load was significantly higher in women who reported anal sex compared to women who did not (median 3.8±1.7 vs 2.73±1.60, p = 0.001). However, both groups had a similar range in CT load (max. 7.3). Urogenital CT load was not associated with anorectal CT load in women who reported anal sex (median 5.1) or women who did not report anal sex (median 4.8). Anorectal CT load was not associated with having anorectal symptoms.

Conclusion: Anal sex is associated with a higher anorectal CT load in women. The CT load range was similar in women with- and without anal sex, and comparable to that found in prior studies conducted in men-who-have-sex-with-men.
Nonetheless, anorectal CT infections in women who did not report anal sex still remain unexplained. Possible explanations for this are underreporting of anal sex or autoinoculation from vaginal infections. However, our results do not indicate a correlation between the genital and anorectal CT load. Regardless of its origin, once infection is established the similar range in load seems to indicate similar clinical relevance.

P084

Comparison of whole genome sequencing with DiversiLab and AFLP for typing of MDR *Pseudomonas aeruginosa*

R. te Witt[1], M.E.M. Kraakman[2], A. Boogaard[1], S. Bernards[2], M.C. Vos[1], K.E. Veldkamp[2], E.C.J. Claas[2]
[1]*Erasmus MC, MMID, Rotterdam,* [2]*LUMC, MM, Leiden*

We evaluated the value of next-generation sequencing for typing of *Pseudomonas aeruginosa*. A representative selection of 20 multi-drug resistant *P. aeruginosa* (MDR-PA) carrying the VIM metallo-β-lactamase gene, originating from 2 Dutch university hospitals (Erasmus MC (n = 10) and LUMC (n = 10)), was analyzed using the DiversiLab™ System, Amplified Fragment Length Polymorphism (AFLP) and whole-genome sequencing (WGS). From both the Erasmus MC and the LUMC 7 MDR-PA strains were chosen from a total of 4 different outbreak clusters, completed with 3 unique strains. Outbreak was defined according to a combination of epidemiological data and previous Diversilab and AFLP results.The library for WGS SNP typing was prepared with the Illumina Nextera kit. Sequences were obtained with a Nextseq500 that provides paired end read lengths of 2x149bp. Sequencing on the Nextseq500 rather than the MiSeq resulted in an extensive coverage ranging from 187 - 986 on a 7.5 Mb genome. Subsequently, the sequences were mapped to a published genome as a reference.The

overall concordance between (presumed) outbreak isolates as analysed by WGS SNP typing showed to be similar to Diversilab and AFLP results. Three different clusters were detected of which isolates from one cluster were present in both hospitals. Earlier this cluster was known to belong to a widespread clone belonging to ST 111.[1] Next to this, each hospital harboured a hospital-specific cluster and several unique isolates. WGS makes it possible to provide more detailed information on the correlation by including analysis of the number of identical SNPs between the strains. In this way, more information on the epidemiology of the outbreak can be obtained. WGS proves to be the typing method of the future, with a high discriminatory power and high reproducibility. However, the challenge will not be to produce the sequence data, but to analyze these data and distill the relevant information from large data sets within an acceptable and relevant timeframe. For use in clinical diagnostics, a standardized and simple pipeline should be available. Costs may be high for now, but since these continue to drop, WGS will lead to accurate identification and characterization of bacterial isolates for clinical use in the near future.

Reference

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P085

Is carnival a risk factor for chlamydia infections?

J.A.M.C. Dirks[1], T.H. Geelen[1], P.F.G. Wolffs[1], C.J.P.A. Hoebe[2], V. Hira[1]

[1]*MUMC, Department of Medical Microbiology, Maastricht,* [2]*Public Health Service, South Limburg*

Introduction: It is a popular belief that unsafe sex is practised widely at carnival celebrations in South-Limburg, increasing the risk for sexually transmitted infections (STIs). To see if carnival is indeed a risk factor for unsafe sex and thus STIs, we investigated the number of *C. trachomatis* (CT) tests, assuming this correlated with risk behaviour, and positive CT PCR during the year.

Methods: We retrospectively analysed all records from CT-tests performed by the laboratory of the academic hospital in Maastricht from January 2011 to December 2013.

34173 samples were tested from January 2011 to December 2013. After exclusion of all patients younger than 12 years, all biopsy samples and all samples that were tested for academic research reasons, 33278 samples were included in the analysis (♀ 51.5%). This resulted in 2437 CT-positive samples (7.3%; ♂ 55% vs. ♀ 45%).

As carnival is in February or March and patients are recommended to get tested 3 weeks after their exposure, we focused on the number CT tests and incidence of CT positivity in March and April. Correlations were assessed by Student's T-test and Pearson's Chi-square analyses where appropriate.

Results: Combined over the three years, every month an average of 2773 (8.3%) CT tests were performed. In March and April 3066 (9.2%) and 2921 (8.8%) CT tests were performed, respectively. March, October and November were the busiest months for CT-testing, with over 3000 tests.

In March and April, the incidence of CT positivity was 7.7% and 6.7%, respectively, which was not significantly higher than in other months. The overall incidence of CT positives was highest in September (8.2%) and November (8.0%). April and October had significantly fewer CT positives than average (6.4% and 6.0%, respectively, $p < 0.05$).

The average age for CT testing and positivity was 33.0 and 27.3 years, respectively. In September the highest percentage (68.4%) of CT-positives is seen in patients under 25 years (average 61.3%, $p = 0.027$).

Conclusions: 1. As the number of CT tests is higher after carnival, but the incidence of CT positivity is not, carnival is probably a risk factor for unsafe sex, but not for CT infections.

2. The increase in CT positivity among young people in September is probably due to unsafe sex in the summer holidays and new students unsafely enjoying their introduction week. Therefore, efforts should be increased to notify these groups of the risk of unprotected sexual intercourse.

P086

How to handle oxygen: The case of the mucosal gut anaerobe *Akkermansia muciniphila*

K.C.H. van der Ark, J.P. Ouwerkerk, C. Belzer, W.M. de Vos

Wageningen UR, Laboratory of Microbiology, Wageningen

The Gram-negative anaerobic gut bacterium *Akkermansia muciniphila* is a dedicated mucus degrader in the colon of many mammals. In line with its metabolic capacity, *A. muciniphila* has found to be located in the mucus layer. To be able to survive in this environment, it has to be able to cope with oxygen that diffuses from the host epithelial cells into the mucus layer. The genome of *A. muciniphila* has found to contain several genes for electron transport chain and other respiratory components that might enable the organism to cope with the oxygen levels. Hence, we addressed the possibility that *A. muciniphila* is able to survive and grow at micro-aerophilic conditions and is able to reduce oxygen.

Oxygen sensitivity was tested in fermentor systems. The oxygen was introduced during growth when an OD_{600}

of 0.1 was reached. Samples were taken for metabolite characterization by HPLC, growth rate measurements by OD_{600}, and determining the transcriptional response by RNA sequencing using an Illumina HiSeq platform. The protocols were also executed for a culture without oxygen. These integrated data sets resulted in a complete overview of the transcriptional and metabolic response of *A. muciniphila* to oxygen.
Our results indicate that the growth of *A. muciniphila* is influenced by the introduction of oxygen to the medium, as growth rate and yield were higher in the oxygenated fermentor. The metabolic profiles of both oxic and an-oxic conditions showed minor differences. Equal amounts of 1,2-propanediol were found in both conditions, but the ratio acetate:propionate shifted towards more acetate. The RNA sequencing data indicated an induction of gene expression of the cytochrome bd gene cluster under micro-aerophilic conditions. Similarly, several genes that are involved in the detoxification of oxygen were upregulated in the presence of oxygen, including those encoding Superoxide Dismutase (SOD), catalase and cytochrome c peroxidase. Redox measurements also showed that the medium remained anaerobic in both fermentors, indicative of oxygen reduction by *A. muciniphila* during growth. The oxygen-exposed culture reduced oxygen to an amount of 2.6 mU/mg protein. This is in the same range as can be reduced by characterised cytochrome bd systems from other aerobic and facultative aerobic microorganisms.
We hypothesise that *A. muciniphila* uses the cytochrome bd complex to regenerate NAD^+ to gain more energy by producing acetate instead of propionate and support for this derived from slightly increased growth rate and yield following oxygen sparging. Moreover, toxic reactive oxygen species that result from metabolic oxygen reduction can be detoxified by SOD and catalase. The combined metabolic, growth and expression data suggest that *A. muciniphila* is capable of reducing oxygen in amounts equal to the presence in its ecological niche. Whether a proton motive force is generated by the NAD^+ oxidation by the cytochrome bd complex remains to be determined and we are presently studying the functionality of the cytochrome bd genes by heterologous expression in *Escherichia coli* ECOM4, a cytochrome deficient strain.

P087

Astrovirus type 8 outbreak in hospitalized immunocompromised pediatric patients and medical personnel at a pediatric hematology and hematopoietic stem cell transplant unit

H.P.J. van der Doef, E. Bathoorn, M.P.M. van der Linden, T.F. Wolfs, A.L.C. Minderhoud, A.M.J.W.E Wensing, C.A. Lindemans
UMC Utrecht, Department of Medical Microbiology, Utrecht

Introduction: Recently we had a large astrovirus outbreak on our pediatric hemato-oncology ward involving both patients and personnel members. The aim of this study was to describe the clinical features of patients and the genetic features of the virus during this outbreak.
Methods: A retrospective cohort study of the astrovirus outbreak was performed in patients and personnel members with astrovirus infection. Human astrovirus sequencing and phylogenetic analysis was performed.
Results: During the outbreak astrovirus infection was proven in 6 immunocompromised patients and 3 personnel members. Three patients had a complicated course; two patients had prolonged and severe diarrhea (62 and 82 days) and one patient developed gastro-intestinal bleeding. Phylogenetic analysis shows clustering with human astrovirus genotyope 8 sequence and sequence analysis yielded a 99.8-100% nucleotide similarity between each of the nine astrovirus strains.
Conclusion: We report the first human astrovirus outbreak involving both immunocompromised pediatric patients and personnel members. These patients have a high frequency of severe gastrointestinal symptoms (prolonged and severe diarrhea, intestinal bleeding). Phylogenetic and sequence analysis showed a genotype 8 and strongly suggest the presence of one single outbreak strain.

P088

Staphylococcal modulators of the kallikrein-kinin system

A. Kuipers, T. Sentner, R.T. Urbanus, S.H.M. Rooijakkers
UMC Utrecht, Department of Medical Microbiology, Utrecht

The kallikrein-kinin system of coagulation plays a role in the host defense against bacterial infections. Similar to the complement system, this system comprises a cascade of plasma proteins and results in numerous responses that cause bacterial clearance. Activation of the kallikrein-kinin system on bacteria leads to formation of antimicrobial peptides and, through activation of the common pathway of coagulation, results in the entrapment and killing of bacteria in fibrin clots. The kallikrein-kinin system consists of three different proteins: the serine protease zymogens factor XII (FXII) and plasma prekallikrein (PK), and a non-enzymatic cofactor called high molecular weight kininogen (HK). The cascade is initiated by the auto-activation of FXII into FXIIa on negatively charged surfaces. Active FXIIa converts PK into active kallikrein, which circulates in complex with HK. Kallikrein then cleaves HK to release the proinflammatory peptide bradykinin (BK) and activates FXII to amplify the contact system. In the last decade it has become clear that pathogenic bacteria, like *Staphylococcus aureus*, have evolved mechanisms to avoid immune clearance by secreting small evasion molecules. The role of the

kallikrein-kinin system in innate immunity may also have resulted in the development of bacterial evasion strategies against this system. We therefore set out to identify new bacterial modulators of the kallikrein-kinin system.
By using a functional assay with a specific fluorogenic substrate for plasma kallikrein we found an inhibitory effect of *S. aureus* supernatant on kallikrein activity. Furthermore, we screened a collection of recombinantly produced bacterial proteins that were selected based on the criteria for being small, secreted molecules. From a pool of 105 different proteins, we identified several *S. aureus* proteins as potential inhibitors of the kallikrein-kinin system, of which *S. aureus* lipase was the most potent. *S. aureus* secretes two lipases, *Geh* and *Lip*, which are 45% homologous. Both proteins are conserved and will be secreted as a precursor protein of 74 kD and cleaved into a mature protein of approximately 40 kD. These lipases are known to cleave lipids and are shown to be *S. aureus* virulence factors in a mouse model. Both the precursor protein as well as the mature protein inhibited the activity of kallikrein in our assay. Also a mutant of the precursor protein of *Lip*, which has no lipase activity anymore, showed an inhibitory effect on kallikrein activity.
In conclusion, *S. aureus* secretes two lipases which can inhibit the kallikrein-kinin system in a manner that seems unrelated to their ability to cleave lipids. We are currently working on further examining the exact mechanism and target of this inhibition.
This work may provide a better understanding of the complex pathogenesis of *S. aureus*, especially on its ability to evade host immune processes, and can be useful in the development of antibacterial strategies.

P089

Inhibition of lipoteichoic acid synthesis of multi-drug resistant *Enterococcus faecium* isolates as potential novel treatment for enterococcal infections

A.P.A. Hendrickx, M.J.M. Bonten, R.J.L. Willems
UMC Utrecht, Department of Medical Microbiology, Utrecht

Background: *Enterococcus faecium* is recognized as a multi-drug resistant pathogen with significant human morbidity and mortality. New antimicrobials are needed, since antibiotic treatment options are decreasing. *E. faecium* incorporates lipoteichoic acid (LTA, 1,3-polyglycerol-phosphate linked to glycolipid) in its cell wall. Richter *et al.* demonstrated that the small molecule inhibitor 1771 (2-oxo-2-(5-phenyl-1,3,4-oxadiazol-2-ylamino)ethyl 2-naphtho[2,1-b]furan-1-ylacetate) specifically blocks the activity of the *Staphylococcus aureus* LtaS synthase, the enzyme that polymerizes 1,3-polyglycerol-phosphate into LTA polymers. Little is known of the effects of LTA synthesis inhibition by 1771 on *E. faecium* growth. Therefore, the objective was to characterize the effects of LTA inhibition using inhibitor 1771 on 19 *E. faecium* isolates in combination with either vancomycin, daptomycin, ampicillin or linezolid antibiotics.
Methods: Effect of inhibitor 1771 on growth inhibition of 19 (8 commensal, 7 hospital- associated, 1 linezolid (E7127)-, 1 daptomycin (E7130)-, 1 ampicillin (E1162)- and 1 vancomycin (E745)-resistant) *E. faecium* isolates was studied using the Bioscreen automated system by growing enterococci in the presence and absence of varying concentrations of 1771, and in combination with 4 different antibiotics. Phenotypic effects of LTA inhibition on cell wall integrity were analyzed by confocal immuno fluoresence (IF) microscopy with anti-LTA antibodies or BODIPY-vancomycin lipidII stain, and scanning electron microscopy (SEM).
Results: Nineteen *E. faecium* strains were incubated with 20 μM inhibitor 1771 or mock treated and grown for 15hrs. Growth of all 19 tested enterococcal strains was reduced on average by 43%, without significant differences in growth inhibition between 8 commensal and 7 hospital-associated strains. Incubation of *E. faecium* E745 with increasing concentrations (0, 25, 50, 100 uM) of 1771 showed a 1771-dependent inhibitory effect on growth and IF using anti-LTA antibodies confirmed a 1771-dependent reduction of LTA polymers at the surface of the cell. SEM showed detrimental effects of inhibitor 1771 on the *E. faecium* cell wall; the cells adopted undulating shapes and structures, often leading to lysis. A combination of 20 μM 1771 inhibitor and either 5 ug/ml linezolid, 10 ug/ml daptomycin, 15 ug/ml ampicillin or 5 ug/ml vancomycin lead to additive growth inhibitory effects on corresponding resistant *E. faecium* strains E7127, E7130, E1162 and E745, respectively. In particular, a combination of daptomycin and inhibitor 1771 lead to a 60% growth reduction of the daptomycin-resistant strain E7130. IF and BODIPY-vancomycin staining of these daptomycin/1771 treated cells revealed punctated distribution of lipidII around the surface of the cell, while untreated cells revealed lipidII localization at the septum.
Conclusions: The LTA synthesis inhibitor 1771 has (1) an on average 43% growth inhibitory effect on the 19 tested *E. faecium* isolates, (2) a 1771-concentration-dependent growth reduction effect of E745 and (3) reduces LTA polymers at the surface of the cell. (4) 1771 leads to severe alterations in cell wall morphology and (5) 1771 has in combination with in particular daptomycin a strong (60%) growth inhibitory effect and (6) leads to an aberrant lipidII distribution around the cell. In conclusion, a combination of daptomycin and LTA synthesis inhibition may represent a potential novel therapy to treat enterococcal infections.

P090

Regional differences in Human Papillomavirus prevalence and type distribution among sexually active young women in three regions in the Netherlands

F.M.H.P.A Koene[1], P.W. Wolffs[1], M.M. Mollers[2], N.D. Dukers-Muijrers[3], A.K. King[2], P.W. Woestenberg[2], B.B. van Benthem[2], C.H. Hoebe[3]

[1]*MUMC, Department of Medical Microbiology, Maastricht,* [2]*National Institute for Public Health and the Environment (RIVM), Bilthoven,* [3]*South Limburg Public Health Service, Department of Sexual Health, Geleen*

Introduction: In the Netherlands a pre-vaccination study among sexually active women aged 16-29 years participating in the CSI program (*Chlamydia trachomatis* Screening Implementation program) was executed showing a higher HPV prevalence compared to the 14-16 year olds. This study, performed in three different regions in the Netherlands allowed us to compare prevalence and type distribution in sexually active women from three different regions to investigate local differences in type distribution and its related risk factors.

Methods and materials: From the CSI program database 3299 self-sampled cervical swabs from women from the city of Amsterdam (n = 1964), the city of Rotterdam (n = 897) and South-Limburg (n = 438) were tested for HPV. Ninety-three women were excluded because of missing demographic data. All participating women fulfilled a questionnaire about sexual behaviour, number of sex partners and previous sexual transmitted diseases (STDs). DNA was isolated by MagnaPure LC®, Roche and a processing control was added. To ensure appropriate self-sampling, the presence of human DNA was evaluated via betaglobin PCR. The samples were processed immediately and kept at - 20°C. Broad-spectrum HPV DNA amplification and HPV genotyping were performed using the SPF_{10} DEIA-$LiPA_{25}$ system(version 1, Labo Bio-medical Products, Rijswijk).

Results: In total 62% of 3299 women was HPV positive, of which 38% was infected with high risk types only, 33% with low risk types and 29% with mixed high and low risk types.

Per region the HPV prevalence was 67% in Amsterdam, 59% in Rotterdam and 48% in South-Limburg. Comparing Amsterdam to South-Limburg and Rotterdam, women in Amsterdam have a 2.3 (CI 95% 1.9-2.8), respectively 1.5 (CI 95% 1.2-1.7) fold higher chance of being HPV positive than women in South-Limburg, respectively Rotterdam ($p < 0{,}001$).

Comparing type distribution the frequency of type occurrence is different between the three regions. The most notable differences are found between Amsterdam and South Limburg, where five high risk types(18, 52, 53, 59 and 68) and three low risk types(43,54 and 74) are significantly more prevalent in Amsterdam than in South Limburg. Furthermore women in Amsterdam have a two times higher chance on high risk HPV type 33 and 56 than women in Rotterdam ($p = 0.011$, resp. $p = 0.032$). Comparing Rotterdam to South Limburg women from Rotterdam have a significant higher risk to contract high risk HPV types compared to women from South Limburg.

Conclusion: This study shows that there are significant HPV prevalence and type distribution differences between the three regions with women from Amsterdam having the highest chance on a high risk infection compared to the other two regions. The differences between the populations might be due to ethnicity, but we have to analyze the risk factors per region. These risk factors might enable us to directly target high risk subpopulations within the HPV vaccine program and therefor improve vaccination coverage.

P091

Endophytic bacteria of tomato: a source of antifungal and antibacterial compounds

R. Nijland, A. Ruaud, C. Papazlatani, S. Nieuwesteeg, H. Gibriel

Wageningen University, Laboratory of Phytopathology, Wageningen

Introduction: Plants support a diverse array of bacteria and fungi inside their roots, vascular system, stem, and leaves. These microbes are known as endophytes, and play an important role in maintaining plant health. Endophytes are in a continuous struggle for survival. Not only do they encounter the plant immune system, they also compete with other endophytes and pathogenic microorganisms in the same niche.

To be able study the mechanism of this *in planta* inter-microbe competition, we have isolated a large collection of endophytic bacteria from tomato plants. We have focused on two underexplored above ground niches inside the tomato plant, the xylem vessels and the leaf apoplast. Xylem vessels are the primary habitat of vascular wilt causing pathogens such as the bacterium *Clavibacter michiganensis* and the fungus *Verticillium dahliae,* whereas the leaf apoplast is the niche for the fungus *Cladosporium fulvum*, causing leaf mould, and the oomycete *Phytophthora infestans*, causing late blight. To understand the biological warfare inside the plant, and to identify biocontrol species, we are screening for bacteria that can protect tomato from these pathogens. Our isolated endophytes originate from the same niche occupied by the pathogens, making it likely we will identify direct interactions.

Methods: Endophytic bacteria were isolated from tomato stem- and leaf xylem vessels, and leaf apoplastic space using niche specific isolation methods after surface steri-

lization. Samples were plated on Tryptic Soy Agar, and colonies were picked after incubation for 2-10 days at 25°C. Pure cultures were provisionally identified using 16 S rRNA-gene sequencing. Isolated strains were inoculated on agar plates with a lawn of the fungi *Verticillium dahliae* or *Cladosporium fulvum*, the oomycete Phytophthora infestans or the bacteria *Micrococcus luteus* or *Bacillus pseudomycoides* and incubated at 25°C. A halo surrounding the inoculated bacteria was interpreted as the release of antimicrobial compounds. Germinating garden cress seeds were inoculated with selected strains on rockwool plugs. Seedling development was followed, and after 20 days stems were surface sterilized and incubated on plates to re-isolate endophytic bacteria.

Results: We have isolated and identified over 100 tomato associated bacteria, of which more than half have antifungal, antibacterial and/or anti-oomycete properties during *in vitro* co-culture. In the above ground parts of the tomato 3/4 of all species isolated were gram-positive. The majority of strains with strong antimicrobial properties were Bacilli. Fifteen strains with strong *Verticillium* inhibiting properties were inoculated with garden cress. Almost all promoted growth compared to the mock control, and could be re-isolated from the plant stems, indicating a true endophytic lifestyle.

Conclusion: Our collection of tomato endophytic bacteria contains a large amount of species with antagonistic properties against a wide range of tomato pathogens. From the species tested further the majority promote plant growth, and are able to re-colonize plants. Currently we are elucidating the antagonistic mechanism employed and testing their potential for inhibiting pathogens *in planta*. Although this work is very much in progress, it is already obvious that the underexplored above ground tomato endophytic bacteria are a promising source of antimicrobial compounds.

P092

HIV and HCV viral- load random access testing: optimizing the workflow and reducing the turn around time

R. van Houdt, M.K. Bomers, J. Algra, R. Roosendaal, A.M. Pettersson, W. Ang

VUmc, Department of Medical Microbiology and Infection Control, Amsterdam

Background: Viral load assays, like for HIV and HCV, are preferably performed on high throughput automated real-time PCR platforms. However, the throughput of not all laboratories is high enough to be cost-effective when run on such a platform. Therefore, such hospital laboratories either have in-house assays or send their samples to other laboratories. Random access testing can be used for an ever increasing number of molecular diagnostic assays, and as of 2015 also for determining the HIV and HCV load. This study was performed to assess what effect random access testing would have on the turn around time of HIV and HCV viral load assays, and consequently the possible advantages for both patient and physician.

Methods: In our hospital, the determination of HIV and HCV loads is being. Both the HIV and HCV loads are determined on the Roche CAP-CTM platform. The results and turn around time of this method, including transportation, are being compared to the random access system of Cepheid, the GeneXpert. In total, 100 HIV and 100 HCV assays will be compared.

Results: Thus far, 42 HIV and 4 HCV tests have been tested using both the current outsourcing procedure and the random access procedure. The rest of the tests will be performed in the first two months of 2015. Test results were comparable between the two systems. However, one has to bear in mind that this is not a validation study. The mean turn around time, including transportation and processing of the results, of the current procedure in our hospital was 8.5 days (IQR: 4-12 days). The mean turn around time of the random access procedure was 0.5 day (IQR: 0-1 day).

Conclusion: Random access testing for determining the HIV and HCV load will dramatically decrease the turn around time in a setting where no high throughput platform is available and where this kind of testing is normally being outsourced. Although this will not improve the standard of care for the majority of the patients who respond well on their anti-retroviral therapy, there is now the possibility for rapid diagnostics for a smaller, but more critical group of patients. Furthermore, it will decrease the amount of time spent on closing a patients dossier. However, the real advantage of random access testing is that the workflow can easily be adjusted to the number of samples that are being processed in a laboratory, making the laboratory much more flexible.

P093

Results of methicillin-resistant *Staphylococcus aureus* eradication treatment

E.C. Bowles, W. Maathuis – de Haan

Gelre Hospitals, Department of Medical Microbiology and Infection Prevention, Apeldoorn

Introduction: In the Netherlands an active 'methicillin-resistant *Staphylococcus aureus* (MRSA) search and destroy' policy is in place, consisting of case finding, contact tracing and eradication treatment of MRSA carriers.

In 2013 Gelre Hospital started a 'infection prevention outpatient clinic' that had decontamination of MRSA carriers as its main goal. At this clinic MRSA carriers receive information about MRSA and instruction about their treatment.

Methods: MRSA carriers were treated according to the national guideline on treatment of MRSA carriers, combined with hygienic measures at home and some additional instructions. The direct contacts of the carriers were screened for MRSA and, if positive, treated at the same time as the index case.
Results: Sixtyfour persons underwent a decontamination treatment. Fourty-one of them (64%) were MRSA-free after 1 year of follow up. In twelve persons (19%) the treatment failed. Eleven persons (17%) were MRSA negative after 2 months but failed to be cultured after 12 months.
Conclusion: MRSA decontamination treatment was succesful after one year of follow up in 64% of cases.

P094

Real-time PCR for *Sarcoptes scabiei*, the causative agent of scabies

A.M.C. Bergmans[1], N. Brabers-Bax[2], A. Vrolijk[2], W. Theune[1], M. van der Ent[1], S. van Leest[1], R.G.F. Wintermans[1], P.H.J. van Keulen[2]
[1]Bravis Hospital, Laboratory of Medical Microbiology Roosendaal-Bergen op Zoom, Roosendaal, [2]Amphia Hospital, Laboratory of Medical Microbiology, Breda

Scabies is a neglected parasitic disease that can be a major public health problem in places where people live in close contact with one another such as nursing homes, prisons and schools. Scabies is easily spread. It just requires direct, extended skin-to-skin contact with someone who is infected. The World Health Organization estimates that there are more than 300 million cases of scabies worldwide each year.
Scabies is caused by *Sarcoptes scabiei*. After a person is exposed to scabies, it can take four to six weeks before the symptoms start appearing. This is because the symptoms are mostly caused by an allergic reaction to the mites, their saliva, eggs, or waste products. Symptoms of scabies include intense nighttime itching and a rash. Continuous scratching of the infected area can create sores that become infected.
Disease control requires treatment of the affected individual and all people they have been in contact with, but is often hampered by inappropriate or delayed diagnosis.
The main clinical signs of scabies are rash and burrows. The mites dig burrows in the skin where they lay their eggs. A person with a scabies infestation usually will have between 10 and 15 mites. Norwegian or crusted scabies is a more severe and extremely contagious type of scabies. People with crusted scabies develop thick crusts of skin that contain thousands of mites and eggs. Crusted scabies does not always create itchy skin or rash. It is usually found in people with weakened immune systems, such as the elderly. Clinical symptoms may vary between patients and often the burrows are not recognisable anymore by intensive scratching. Therefore laboratory diagnosis is desirable. Currently microscopic examination of skin scrapings to confirm the presence of mites (feces) and/or eggs is the only diagnostic tool for scabies. Therefore we developed a real-time PCR based on the ITS2 region for detection of *Sarcoptes scabiei* DNA in skin scrapings of patients suspected of having scabies.
We analyzed 119 skin scrapings and swabs from humans and dogs suspected from having scabies, and from close contacts of these suspects, by PCR. Additionally, a skin biopsy of a goat with confirmed scabies was analyzed by PCR. Fifty-one of these 120 samples were also examined by microscopy. Forty of these 51 samples (78.4%) gave concordant results with microscopy and PCR (17 positive, 23 negative). Seven samples were positive by PCR and negative by microscopy (all 7 clinically highly suspected), and 4 samples were negative by PCR and microscopy-positive (2 samples from 1 nurse having had contact with a scabies-positive patient). Six of the 69 samples on which no microscopy was performed, were positive by PCR (all clinically suspected of having scabies).
The real-time PCR for diagnosis of scabies showed promising results. However, more, well-defined clinical samples need to be analyzed in order to determine the performance of this new molecular diagnostic tool further.

P095

Dynamics of ESBL/AmpC carriage in healthy cats

J. Hordijk, V.O. Baede, A.J. Timmerman, E.M. Broens, B. Duim, J.A. Wagenaar
Utrecht University, Department of Infectious Diseases and Immunology, Utrecht

Introduction: Companion animals have been suggested as a potential source of Extended Spectrum Beta Lactamase-producing *Enterobacteriaceae* (ESBLpEc) for humans due to their close contact. Healthy dogs were shown to be frequent, non-persistent carriers (Baede *et al*; ASM General Meeting '14). Data on carriage in healthy cats is very limited. The aims of this study were to: (i) Determine persistence of shedding of ESBLs, (ii) identify dominant plasmid-mediated ESBL genes and (iii) quantify ESBLpEc in faecal samples from healthy cats.
Methods: Fecal samples from 23 cats from 21 households were collected monthly for 6 months. From 13 of these cats samples were also collected on a weekly basis for 6 weeks to identify potential short-term shifts in shedding. Changes in presence of ESBLpEc between consecutive faecal samples were defined as shift in shedding. Samples were cultured quantitatively on MacConkey agar (MC), MC supplemented with 1 mg/L cefotaxime (MCC) and cultured

in LB-broth supplemented with 1 mg/L cefotaxime with subsequent inoculation onto MCC. The average ratio of ESBLpEc compared to the total number of *E. coli* was calculated as the mean of ratios. The presence of ESBL-genes was screened by PCR and sequence analysis.

Results: In total 191 faecal samples were collected of which 9 (5%) tested positive for ESBLpEC. One of the 23 cats (K006A) was positive for *E. coli* carrying TEM-52-StPaul on one sampling moment. Cat K006B from the same household was negative. Cat K009 was positive for ESBLpEc on eight out of ten sampling moments. In the first 6 consecutive weekly samplings this cat carried CMY-2 in all samples. In week 2 and 5, SHV-12 and CTX-M-1 were found respectively in addition to CMY-2. In the subsequent two monthly samplings, K009 was positive for SHV-12 and CTX-M-55 respectively. In the final two sampling moments, this cat was negative. All remaining cats (n = 21) were negative for ESBLpEc throughout the whole study. K009 was the only cat that was fed with raw meat. Therefore, a feed sample was taken 2 times (sampling moment 8 and 9). Furthermore, two dogs lived in the same household as K009. These dogs were sampled once (sampling moment 6). One feed sample was positive for CTX-M-55 at the same sampling moment the cat was positive for CTX-M-55. Both dogs were negative for ESBLpEc. The mean cell count of ESBL-producing *E. coli* was ~10^5 CFU/gram faeces (range 10^2 to 10^6). The mean ratio of ESBLpEc was 0.1% (range 0.00002% to 0.4%).

Conclusion: The prevalence of ESBL-producing *E. coli* in healthy cats was shown to be low. One cat was positive for CMY-2 for six consecutive weeks, suggesting potential stable colonization. Raw feed should be included in further study as potential source for ESBL-producing *E. coli*.

P096

The role of surface layer proteins in anammox granular sludge

M. Boleij, Y.M. Lin, M.C.M. van Loosdrecht
TU Delft, Department of Biotechnology, Delft

Anammox (Anaerobe ammonium oxidation) bacteria are autotrophic bacteria that are able to convert nitrite and ammonium to nitrogen gas. The anammox process is already widely applied for nitrogen removal from wastewater. It has advantages over the conventional nitrification-denitrification process since the addition of an electron donor is not required and energy costs for aeration are saved. Because anammox bacteria have a low growth rate, granular sludge reactors are applied in order to have sufficient biomass retention. It is generally accepted that extracellular polymeric substances (EPS) play an important role in the granulation process but the mechanism of the granulation process is not well understood. In previous research EPS was extracted from anammox granular sludge and a surface (S) layer like structure was observed. S-layers are crystalline arrays which form the outermost layer of the cell envelope of many bacteria. They are formed by monomers of surface layer proteins (SLPs) which have the property to self-assemble. Because of their self-assembling property, SLPs are potential products for application in bio- and nanotechnology. Since the S-layer is the outermost layer of the cell it is in direct contact with the environmental and therefore it is interesting to investigate the role of SLPs in the granulation process. The aim of this research is to have a better understanding in the granulation process of anammox.

In order to investigate the role of S-layer based EPS in granule formation EPS will be extracted from anammox granular sludge. The polysaccharide and protein fractions of the isolated EPS will be characterized. Also a methodology for the isolation of SLPs will be set up. The self-assembling behaviour of the isolated SLPs will be analysed under different conditions. Furthermore the interactions of SLPs with extracellular polysaccharides will be studied.

In conclusion, previously an S-layer like structure was found in EPS that was isolated from anammox granular sludge. Further research will focus on the properties of the S-layer based EPS in order to clarify the role in the granulation process. Knowledge on these properties will also provide opportunities for the production of biopolymers from wastewater.

P097

The ecological role of IncP-1β plasmids in the mycosphere

J.K. Brons, M. Zhang, J.D. van Elsas
University of Groningen, Groningen

Horizontal gene transfer (HGT) is a major evolutionary force, which enables bacteria to adapt to fast-changing environments. Conjugative plasmids are important contributors to HGT in soil. Plasmids from IncP-1β group are broad-host-range and can confer varying phenotypes to their hosts. We isolated plasmid pHB44 from *Variovorax paradoxus* HB44 in the mycosphere of *Laccaria proxima*. This typical IncP-1β plasmid was analyzed as to sequence and ecological function and evolution. Plasmid pHB44 revealed a canonical backbone consisting of three main regions involved in replication, central control/partitioning and transfer, interspersed with three insertional hot spots. Phylogenetic inference on the basis of concatenated backbone genes revealed plasmid pHB44 belongs to the IncP-1β1 group, with as its closest relative plasmid pB10. A comparative analysis of the sequences present in each of the three insertional hot spots across plasmids pHB44, pB10 and pADP1 showed the insertions to be

different across the plasmids. Hot spots I and III were devoid of major inserts in pHB44, whereas hot spot II was dominated by one key gene cassette denoted mmf. Our analyses of this 10-gene insert revealed a two-component regulatory system, several transmembrane proteins, a secreted protein, a ferrichrome receptor and a UPP phosphatase. The predicted phenotypes, i.e. enhaced iron uptake and bacitracin resistance, were experimentally proven. Thus IncP-1β1 plasmid pHB44 isolated from mycosphere provides two novel beneficial traits to bacterial hosts under particular soil conditions.

BAMA-O1

Delia radicum resistance to the mustard oil bomb: Bacterial gut symbionts help to degrade isothiocyanate

T.J.M. van den Bosch, R.M. de Graaf, H.J.M. Op den Camp, M.S.M. Jetten, C. Welte
Radboud University, Department of Microbiology, Nijmegen

Introduction: The glucosinolate-myrosinase system (GMS, also known as mustard oil bomb) is a defense mechanism found in plants of the *Brassica*ceae family. This defense is based on the universally toxic isothiocyanates (ITCs) that are produced when a plant is attacked by a herbivore, providing protection not only from insects and mammals, but also from pathogenic bacteria.
The maggots of the root fly (*Delia radicum*) are one of few specialists that are able to feed on *Brassica*ceae. However, the reason for the *D. radicum* resistance against the GMS is not explored, as they do not employ any of the currently known modes of defense against the GMS observed in other resistant insect species. It was hypothesized that *D. radicum*'s resistance to the GMS depends on the maggots' intestinal microbiome, causing harmful ITCs to be broken down by intestinal microbial symbionts.
Methods: Four bacterial strains capable of breaking down ITC were isolated from *D. radicum* intestines. These strains were tested for naturally occurring antibiotic resistant properties, coupled with the isolation and next-generation sequencing of their plasmids using Ion Torrent technology. Plasmids were assembled *In silico* and genes were annotated by comparison with a BLAST or HMMER algorithm. One gene (*saxA*) was of particular interest as it showed a close relation to a gene known to be involved in ITC resistance. This gene was cloned into a *strep*-tag fusion vector, which was subsequently transformed into competent *Escherichia coli* cells. These transformants were then tested for increased resistance against 2-phenylethyl isothiocyanate (2PE-ITC).
Results: All four strains were resistant to ampicillin, cefazolin, trimethoprim and nitrofurantoin. The underlying reasons for these resistances were in part explained by antibiotic resistance genes found on the plasmids. Plasmid sequencing resulted in the assembly of over 8 different plasmids, of which 5 were present in all four of the strains. 2 of these were small (2-5kb) plasmids, the other three were over 100kb, with the largest being approximately 185kb. This plasmid also contained a gene that showed close homology to an 'Aliphatic isothiocyanate resistance hydrolase, *saxA*' (e-value of $2.9e^{-86}$ according to the HMMER algorithm).
Successful production of the SaxA-*strep*-tag fusion protein by the *E. coli* transformants was confirmed with a chromogenic reaction. Interestingly, these transformants were shown to be more resistant to 2PE-ITC than cells containing only an empty vector, indicating that SaxA may be responsible for the increased tolerance of *D. radicum* larvae to dietary ITCs.
Conclusion: 1. These findings provide evidence for the existence of a newly discovered mode of defense by breaking down ITCs in the intestinal tract of *D. radicum* maggots with the help of enzymes that are produced by bacterial symbionts.
2. Four isolated bacterial strains carry a natural megaplasmid containing a saxA gene that encodes a metallo-β-lactamase-like protein.
3. This protein is likely responsible for the enzymatic breakdown of isothiocyanates, as cloning of the saxA gene into a vector and consecutive expression in *E. coli* resulted in an increased resistance to 2PE-ITC.

BAMA-O2

Synthesize lyso-phosphatidylserine from phosphatidylcholine and test it for activation of the Toll Like Receptor 2

R.S. van Schuijlenburg
Streeklaboratorium Haarlem, Research and development, Haarlem

The parasite Schistosoma occurs mainly in the tropics. Schistosoma is able to evade the immune system of humans. The way Schistosoma evades the immune system, can be very interesting for people with auto-immune diseases. For a solution to this problem it would be nice to copy some of the mechanism of Schistosoma, to make the immune system less active. But how does Schistosoma evade the immune system?
From previous research it is known that lyso-phosphatidylserine (which is a lysophospholipid in the tegument of the Schistosoma) is responsible for the activation of Toll Like Receptor 2. The activation of Toll Like Receptor 2 will ultimately activate the up regulation of expression of IL-10. This interleukin is responsible for the suppression of chronic infections, but also allergies and chronic inflammations. The increased expression of IL-10 is most likely involved in the immune evasion of Schistosoma. The main purpose of this research was to synthesize lysophosphatidylserine from phosphatidylcholine and test it for

activation of the Toll Like Receptor 2. Toll Like Receptors 1,2 and 6 are expressed in the Hek-Blue cell line, which is used for this research. For the synthesis of lyso-phophatidylserine we started with phophatidylcholine. The choline group of phosphatidylcholine was hydrolysed by using phospholipase D. This reaction occurred in the presence of high concentration of serine, which reacted with the hydrolysed bonds, thereby effectively replacing choline with serine. This new molecule, phosphatidylserine was further treated with phospholipase A2, which removes the fatty acid tail on the second position. Thereby we have synthesized lyso-phosphatidylserine from phophatidylcholine. We tested the synthesized lyso-phophatidylserine on the Hek-Blue cell line. To investigate which of the Toll Like Receptors were activated, several Toll Like Receptors were blocked by anti-Toll Like Receptor antibodies, and exposed to our products and several positive controls. We started with the synthesis of lyso-phophatidylserine from a phophatidylcholine with a fatty acyl tail of 18 C-atoms long and with 3 double bonds, 18:3 and a phosphatidylcholine 24:1. We did not find any activation on the Hek-Blue celline by stimulation with lyso-phophatidylserine 18:3. The synthesized lyso-phophatidylserine 24:1 together with the fraction 6 gave a higher activation on the Hek-Blue cell line than fraction 6 or lyso-phophatidylserine 24:1 alone. To know which Toll Like Receptor will be activated by fraction 6, the Toll Like Receptors 1,2 and 6 were blocked with anti-Toll Like Receptor antibodies. When Toll Like Receptor 2+6 were blocked there was no longer activation by fraction 6, which indicates that these two Toll Like Receptors are involved in the binding of fraction 6. Thus far, we have shown that lyso-phosphatidylserine is needed for the activation on the Hek-Blue cell line. Synthesized lyso-phophatidylserine, 18:3, was not able to activate Toll Like Receptor 2 in our assay. The lack of activation of pure synthesized lyso-phosphatidylserine could be caused by a missing co-factor, or a wrong combination of fatty acid tails/ double bonds.We can also conclude most of the activation of Toll Like Receptor 2 occurs via hetero dimerization with Toll Like Receptor 6.

BAMA-O3

Why gastro-esophageal reflux disease is related to otitis media: exploring the nasopharyngeal and middle ear microbiota in children with otitis media

A.M. de Zeeuw[1], J.P. Hays[2], S.J.C. Verhaegh[2]

[1]*Hogeschool Rotterdam, Department of Biology and Medical Laboratory Research, Rotterdam,* [2]*Erasmus MC, Rotterdam*

Introduction: Otitis media is one of the most frequent diseases of childhood, often being associated with severe pain and occasionally with serious complications such as meningitis, mastoiditis and hearing loss. The pathogenesis of otitis media is multifactorial, involving genetic, microbiological and environmental factors. As an example, a relatively unknown, though potentially important factor associated with otitis media is gastro-esophageal reflux disease (GERD). However, the majority of studies currently associating GERD with otitis media have been observational studies only – describing the association between otitis media and GERD, but not actually defining a cause-effect mechanism for the initiation and promotion of otitis media. In order to bridge this gap in our current understanding of the relationship between GERD and otitis media, we intend to identify both otitis media and GERD susceptible children and compare their nasopharyngeal and middle ear microbiota to that of a cohort of children suffering otitis media only ('traditional' otitis media). The results will provide valuable information on the etiology of GERD-associated otitis media, identify novel bacterial pathogens, and help provide novel information for clinicians with respect to future, alternative, treatment options.

Methods: Nasopharyngeal swabs and middle ear effusions have been collected from children (aged 0-8 years) who were listed for tympanostomy tube placement in the operating rooms of the department of Otolaryngology of the Erasmus MC-Sophia Children's Hospital, Rotterdam. Adenoid tissue was also obtained from children who were scheduled for adenoidectomy surgery jointly with tympanostomy surgery. Ethical approval was obtained before the study began and written informed consent was obtained from all parents or caregivers. Questionnaires were used to define the GERD-associated otitis media cohort of children from children suffering from 'traditional' otitis media. The nasopharyngeal swabs, middle ear effusions and adenoid tissue were cultured according to standard laboratory protocols as performed in the department of Medical Microbiology and Infectious Diseases (the 'gold standard' method). MALDI-TOF mass spectrometry was used for the identification of cultured bacterial species.

Results: Forty-two children were included in this pilot study. Twenty-four percent of these children (10/42) experienced reflux events according to questionnaires. Thirty-one percent (13/42) of middle ear effusions were found to be culture negative. Micro-organisms were however cultured from both middle ear effusions and nasopharynx and/or adenoid tissue in nineteen percent (8/42) of the children. Interestingly, the proportion of culture-positive (*Haemophilus parainfluenzae, Moraxella catarrhalis* and *Streptococcus pneumonia*) children was higher in the otitis media with GERD group compared to the otitis media non-GERD group. In contrast, the otitis media non-GERD group contained a higher proportion of children presenting with *Haemophilus influenza* infections.

Conclusion: Children suffering from otitis media and GERD appear to have a wider diversity of traditional

bacterial otitis media pathogens in their nasopharynx, adenoid tissue and middle ears compared to children suffering from 'traditional' otitis media. Future plans include the use of Next Generation Sequencing technology to determine the absence/presence, relative proportions, and diversity of the middle ear microbiota in children suffering from GERD-associated versus 'traditional' otitis media, and also to increase the population size of the study.

BAMA-05

The Silver Winning iGEM-2014 project: BananaGuard

B. van Sluijs, R. van Rosmalen

On behalf of the i-GEM team Wageningen University, Wageningen

The iGEM team Wageningen 2014 presents 'BananaGuard', a bacterial platform designed to control the Panama disease threatening banana cultivars all over the world by forming a protective biofilm around banana plant roots. A combination of strategies will prevent the fungus *Fusarium oxysporum f. sp. cubense* from infecting banana plants. First, its detection in the soil by sensing fusaric acid, a compound unique to pathogenic Fusarium species. After detection four fungal growth inhibitors are produced attacking *F.oxysporum*. Once *F.oxysporum* is removed from the soil, a kill-switch is activated causing the genetically modified host to self-destruct. Several aspects of this project were analysed computationally in order increase the chance of constructing a stable and functioning system.

BAMA-O6

The outer surface capsule modulates binding of *Campylobacter jejuni* to Siglec-7-expressing cells

S.F.A. Franch-Arroyo, R. Huizinga, B.C. Jacobs, H.P. Endtz, A.P. Heikema

Erasmus MC, Department of Microbiology and Infectious Diseases, Rotterdam

Introduction: *Campylobacter jejuni (C. jejuni)* is the major cause of bacterial gastroenteritis worldwide. *C. jejuni* infection can result in the acute and severe polyneuropathy Guillain-Barré syndrome (GBS). Sialylation of lipo-oligosaccharides (LOS), present in the outer membrane of *C. jejuni*, enhances activation of the innate immune system and is critical for the induction of GBS. Disialylated LOS can bind to the immune receptor Siglec-7 in a sialic acid specific manner. However, several cellular and bacterial factors seem to determine whether this binding takes place. In this study, we determine the optimal cell conditions for binding of *C. jejuni* to Siglec-7, whether the outer surface capsule of *C. jejuni* affects the binding and whether *C. jejuni* binds to Siglec-7 expressed on dendritic cells, important antigen presenting cells which link innate and adaptive immunity.

Methods: Two disialylated and two monosialylated *C. jejuni* strains were selected because of their high and low binding to Siglec-7 respectively. Capsule and sialic acid transferase knock-out mutants from these strains were generated or were available from previous studies. Live or heat-inactivated bacteria were FITC-labelled and incubated with Siglec-7-transfected chinese hamster ovary cells (CHO-Siglec-7). Neuraminidase treatment of the cells and incubation time were optimized. Then, binding of *C. jejuni* to Siglec-7 was quantified using flow cytometry. Established optimal binding conditions were used to assess binding of *C. jejuni* to human monocyte-derived dendritic cells.

Results: Neuraminidase treatment of the cells and 30 or 120 minutes as incubation time, for live or heat-inactivated bacteria respectively, optimized the binding of *C. jejuni* to CHO-Siglec-7. Absence of the capsule enhanced the binding of live *C. jejuni* with disialylated LOS to CHO-Siglec-7. In contrast, when *C. jejuni* was heat-inactivated the difference in binding between wild-type and non-capsulated *C. jejuni* was not observed. Monosialylated strains and sialic acid transferase mutants, either live or heat-inactivated, showed low binding to CHO-Siglec-7. Binding to wild-type CHO cells was low for all *C. jejuni* strains, demonstration that the binding was Siglec-7 specific. Disialylated *C. jejuni* also showed enhanced binding to dendritic cells that were neuraminidase-treated compared to non-treated cells. In contrast, monosialylated *C. jejuni* and the sialic acid transferase mutants showed low binding to dendritic cells, which was independent of neuraminidase treatment.

Conclusions:

1. The optimal conditions for binding of *C. jejuni* to Siglec-7 are neuraminidase treatment of the cells and 30 or 120 minutes as incubation time for live or heat-inactivated bacteria respectively.
2. Decapsulated live- and heat-inactivated *C. jejuni* with disialylated LOS bind to Siglec-7.
3. Absence of the capsule on live but not on heat-inactivated *C. jejuni* enhances the binding of *C. jejuni* to Siglec-7-expressing CHO cells.No difference in Siglec-7 binding observed for the heat-inactivated strains is may be explained by a loss of the outer surface capsule during heat-inactivation.
4. *C. jejuni* binds to Siglec-7-expressing dendritic cells in a sialic acid dependent manner.

These results define the capsule as a bacterial factor that may influence GBS onset and give insight in how *C. jejuni* is recognized by the immune system.

BAMA-O7

Response of sediment bacterial communities to polycyclic aromatic hydrocarbons, evidenced by the analysis of phylogenetic and functional biomarker genes

R.V.J. Kuin[1], M. Lozada[2], M. Marcos[2], H. Dionisi[2]
[1]University of Amsterdam, Department of Biological Sciences, Amsterdam, [2]CENPAT – CONICET, France

We analysed shifts in sediment bacterial community structure and hydrocarbon biodegradation potential as a result of hydrocarbon exposure. Experimental systems were built with intertidal sediments from three sites of the Patagonian coast with different hydrocarbon exposure history. The conditions included the addition of phenanthrene, pyrene or crude oil, or no pollutant addition, followed by incubation at 15°C for 20 days. Large-scale sequencing of bacterial 16S rRNA gene amplicons evidenced that an important fraction of the original bacterial diversity was maintained in the experiments, and the response of the bacterial community was dependent on the sediment of origin. The community from a non-impacted site did not show changes in response to individual polycyclic aromatic hydrocarbons (PAHs), only to crude oil. A novel dioxygenase α-subunit gene from an uncultured bacterium identified in Patagonia was relatively abundant in the polluted sediments, and further increased 0.5-1.5 orders of magnitude after exposure to PAHs or crude oil. These results point to the possible role of poorly described or yet-unidentified bacteria in natural attenuation, and highlight the importance of functional redundancy in shaping community response to PAHs.

BAMA-O8

Mycelium design

F.V.W. Appels, K. Lucasiewicz, P. Krijgsheld, H.A.B. Wösten
Utrecht University, Department of Microbiology, Utrecht

Aim: Our economy that is based on finite resources should convert to a sustainable system making use of recycling and eco-friendly products. The use of fungal mycelium can be part of this change. Fungi degrade plant-based materials such as low quality agricultural waste and turn these in an interconnected network of hyphae, called mycelium, that can serve as novel biomaterials.
Approach: In our research, *Schizophyllum commune* mycelium is grown in liquid media and subsequently subjected to chemical and physical treatments. We have assessed the effects of these treatments on the properties of the mycelium by tractile measurements. In addition, the effect of gene deletions, involved in the synthesis of the cell wall, are being studied.
Results: Both chemical and physical treatments impact the properties of the mycelium, as did deletion of particular genes. Mycelium material with plastic, rubber, leather and paper-like properties have been obtained. With these materials different fungal-based Design objects have been made.
Conclusion: The use of fungal mycelium to produce biomaterials is a very attractive concept for the transformation to a sustainable economy.

BAMA-P1

Validation of a Lues screenings test, using the Cobas 8000 system

L.A.J. Sijben
Academic Hospital Maastricht, Department of Medical Microbiology, Maastricht

The purpose for this research was to validate a Lues screenings test using the Cobas 8000 system
(Roche). The test is compared with the lues screening elisa (Biokit). The reason for validation of the test on the Cobas 8000 system is that this screenings assay is done on the same platform as for the other screening tests e.g. hepatitis B and HIV, which results in a lower turn around time per sample.
First of all in a validation plan the acceptance criteria have been formulated: the sensitivity and
specificity should be equal or before 90% and the interrun and intrarun variation should be less than 20%. For this validation 85 samples were selected that also have been used for another validation of a lues screening test.
Of those, 35 samples were tested negative with the current method, 35 samples were tested positive and 15 samples were tested positive or threshold, but appeared to be negative in the confirmation tests. This 15 samples are actually false positives.
The analytical sensitivity was 72% and specificity 97% (n = 85). However, in this results the false positive results of the current method were seen as positive. Taking into account that these samples are actually negative, this results in a clinical sensitivity of 100% and a specificity of 96%.
For the intrarun variation. 4 samples were measured again on the Cobas 8000 system twice on the same day as the first measurement. The maximum variation for each sample was 1,95%, 5,05%, 2,91% and 1,02%.
To determine the interrun variation 5 samples were measured 6 times during 2 weeks. The maximum percentage variation per sample was: 5,00%, 10,75%, 7,88%, 5,41% and 6,97 %.
Comparison of the results were with the predetermined acceptance criteria result in the following **Conclusions:** Both the sensitivity and the specificity are higher than 90% and meet the acceptance criteria. The intrarun variation and the interrun variation both give a maximum

percentage variation lower than 20%. So both the intrarun variation and the interrun variation also meet the acceptance criteria. Thus the new method is at least as good as the current method and will be implemented as a routine diagnostic test.

BAMA-P2

Developing a Master program Microbiology

M.H. in 't Zandt, M. Kox, M.S.M. Jetten, C.U. Welte
Radboud University, Department of Microbiology, Nijmegen

Background: Microbiology is an important science for many aspects of life on our planet. As Louis Pasteur already stated 'Life would not long remain possible in the absence of microbes'.[2] Microbiology addresses many of the grand societal challenges including establishing a sustainable bio-based economy and improving human health. Continuing the Dutch/Delft school of Microbiology to educate new generations of microbiologist is a vital prerequisite to solve these challenges.
Aim: In the Netherlands no dedicated master program in microbiology exists today. At Radboud University, we want to create and develop a new master program in microbiology focusing on molecular, medical and environmental microbiology to improve our health and environment and provide in-depth insight into present-day microbial research.
Results: The program will start with five mandatory courses covering an introduction to general microbiology, highlighting principles and fundamental aspects of molecular microbiology, host-microbe interactions, environmental microbiology and medical microbiology. After these courses the students are qualified to enter internships at the participating research groups. By completing the program, students have gained insight into principles of environmental & molecular microbiology, are independent microbial academics that can work within an international setting and are qualified for advanced training in microbial sciences as PhD students. The admission requirements are: BSc degree in a relevant field of study, sufficient relevant courses passed, submission of a motivation letter & letter of reference and fluency in English.
Conclusion: After training a new generation of microbiologists in the MSc program, we will be able to continue the strong and leading school of Dutch Microbiology that will undoubtedly unravel many more exciting secrets of our fascinating newly discovered anaerobic microorganism.[3]

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BAMA-P3

Human Langerin receptor specifically recognises *Staphylococcus aureus*

M. Rumpret[1], B. Lepenies[2], C. Rademacher[2], N.M. van Sorge[1]
[1]*UMC Utrecht, Department of Medical Microbiology, Utrecht,* [2]*Max Planck Institute of Colloids and Interfaces, Biomolecular Systems, Potsdam; Freie Universität Berlin, Institute of Chemistry and Biochemistry, Department of Biology, Chemistry and Pharmacy, Berlin, Germany*

Introduction: *Staphylococcus aureus* (*S. aureus*) is a Gram-positive bacterium and a major cause of human disease. *S. aureus* can invade a wide range of tissues causing a spectrum of infectious diseases ranging from minor skin infections to potentially life-threatening diseases such as pneumonia, meningitis, infective endocarditis, bacteraemia and sepsis. Importantly, emerging pathogenic *S. aureus* strains are becoming increasingly resistant to antibiotics, which largely contributes to the gravity of *S. aureus* infections to public health. A deeper understanding of the underlying mechanisms promoting *S. aureus* pathogenesis in humans is thus needed.
S. aureus expresses multiple glycosylated structures, including glycosylated wall-teichoic acid (WTA),[1] a polysaccharide microcapsule,[2] and a recently identified subset of glycosylated proteins,[3] known as serine-aspartate dipeptide repeat (SDR) proteins. Loss of glycosylation by genetic mutation changes bacterial virulence, suggesting that these glycans are important for bacterial-host interaction. We speculate that glycosylated structures of *S. aureus* interact with carbohydrate-specific pattern-recognition receptors like C-type lectin receptors (CLRs). Here, we aim to unravel the *S. aureus* CLRs fingerprint and the consequences of this interaction for pathogenesis.
Methods: A panel of 14 CLR-IgG Fc fusion constructs as well as Streptactin-tagged and FITC-labelled human and mouse Langerin constructs were screened for interaction with several wild-type (WT) *S. aureus* strains by flow cytometry and immunofluorescence microscopy. Interacting lectins were screened in concentration-dependent binding curves on *S. aureus* Newman and MW2. To identify the interacting *S. aureus* ligand, we performed lectin blotting experiments using whole cell lysates of *S. aureus* strains and screened specific *S. aureus* mutant strains for loss of binding, including mutants lacking Fc-binding spa/sbi or lacking all glycosylated proteins (panSDR knockout).
Results: Flow cytometry experiments identified strong binding of human, but not murine, Langerin-FITC constructs to all tested *S. aureus* WT strains. Also, none of the CLR-IgG Fc constructs interacted with a *S. aureus spa/sbi* mutant strain. Human Langerin binding was concentration- and calcium-dependent, as binding activity of human Langerin-FITC was completely lost in presence

of EDTA. Binding was independent of bacterial growth phase and was not lost using a *S. aureus* strain Newman ΔpanSDR mutant strain.

Conclusion: We have identified human Langerin as a CLR with significant calcium- and concentration dependent binding to all tested *S. aureus* strains. Experiments conducted with the *panSDR* mutant imply that the recently identified glycosylated proteins are not the target for human Langerin. Future experiments aim to identify the bacterial ligand for human Langerin and to verify the interaction on human Langerin transfected cell lines and primary human Langerhans cells to unravel the contribution of this interaction to *S. aureus* pathogenesis.

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BAMA-P4

Functional characterization and cellular localization of actin homolog MreB in the anaerobic ammonium oxidizing bacterium *Kuenenia stuttgartiensis*

S. Peeters, M.C.F. van Teeseling, L. van Niftrik

Radboud University, Department of Microbiology, Nijmegen

Anammox bacteria perform the anaerobic oxidation of ammonium with nitrite to nitrogen gas. They play an important role in the global nitrogen cycle and are applied in wastewater treatment for the removal of toxic nitrogen compounds. Anammox bacteria consist of three cell compartments of which the innermost, the anammoxosome, has an unusually curved membrane and is analogous in function to the eukaryotic mitochondria. This intriguing anammox cell plan is paired with a lack of most canonical cell division proteins such as FtsZ. Recent studies have revealed that anammox bacteria divide by binary fission and form an FtsZ-less cell division ring in the outermost cell compartment.

In *Bacteria*, both maintenance of cell shape and division are paired with strictly regulated lysis and assembly of the peptidoglycan cell wall. The actin homolog MreB plays an important role in this process as a scaffold that guides insertion of new peptidoglycan strands. Anammox MreB is very divergent, and its function in anammox bacteria is unknown.

Here we investigated the function and location of MreB in the anammox bacterium *Kuenenia stuttgartiensis* using MreB inhibitor studies in a continuous bioreactor system, immunogold localization and advanced (cryo) transmission electron microscopy techniques.

BAMA-P5

Genome-guided analysis of bacterial isothiocyanate breakdown in phytopathogenic root fly larvae

J.S. Simon

Radboud University, Department of Microbiology, Nijmegen

Brassicaceae contain the glucosinolate-myrosinase-system as a defense mechanism to prevent feeding by herbivores. Upon herbivore feeding, toxic isothiocyanates (ITC) are formed. Most insects are inhibited by ITC whereas *Delia radicum* larvae are not. Our hypothesis is that the larvae contain microorganisms in their gut, which secrete enzymes to detoxify ITC. Several microorganisms were isolated from the *D. radicum* gut. And after testing some of the isolates appeared to be resistant to ITC ('resistance strains') whereas other isolates could break down ITC ('breakdown strains'). SaxA is a protein first described from the plant pathogen *Pseudomonas syringae*. The *saxA* appeared to be encoded on a plasmid and was shared between the breakdown strains, and seemed essential for the degradation of ITC.

The *saxA* gene sequence encoded by the plasmid found in our breakdown strains was cloned into the vector pASK-3, expressed in *E. coli* BL21 and purified via Streptactin affinity chromatography. The yields for the purified enzyme were high with 6 mg SaxA purified from 100 mL culture. The identity of the protein was confirmed by Western blotting and MALDI-TOF analyses. The purified enzyme was used for enzyme assays with allyl-isothiocyanate (AITC) and 2-phenylethyl-isothiocyanate (PE-ITC) as substrates. After adding enzyme to the assay, samples were taken every 2.5 min. The substrate degradation was measured with gas chromatography coupled to mass spectrometry (GC-MS). The degradation of PE-ITC was catalyzed by SaxA with an activity of 1.6 U/mg SaxA while AITC was degraded at around 5.7 U/mg.

Next to the enzyme assays, the genomes of the resistance strains were sequenced. Based on the sequencing data and the BLASTx-search of the assembled sequences to the different Sax amino acid sequences of *P. syringae*, it became apparent that the resistance strains did not contain the *saxA* gene. However, they contained genes coding for multidrug efflux pumps, which were similar to the *saxD*, *saxF* and *saxG*-genes of *P. syringae*.

In summary, the breakdown strains contained the SaxA-protein, which may be responsible for the degradation and detoxification of PE-ITC and AITC, which are the main isothiocyanates found in Brassica root tissue. The sequencing results supported that the strains only resistant to ITCs did not contain the *saxA* gene but contained other sax genes encoding multidrug efflux pumps which might be responsible for the efflux of isothiocyanates out of the cell.

BAMA-P6

Molecular typing of clinical isolates by Amplification Fragment Length Polymorphism

D. Vodegel[1], C.M.C. van Herk[2], J.H.B. van de Bovenkamp[2]
[1]*Fontys Hogeschool, Department of Applied Science, Eindhoven,* [2]*Stichting PAMM, Veldhoven*

Introduction: Bacterial outbreak can occur in hospitals. These outbreaks can pose problems for patients in the hospital who have a weakened immune system. For infection prevention purposes it is important to act directly on an outbreak. To speed up the diagnostic process a Fragment Length Polymorphism (AFLP) assay is developed and validated. AFLP is a finger-printing technique which can be used for typing of clinical isolates without fully genomic knowledge of the micro-organism.

Methods: An AFLP assay with the following ATCC control strains was used to setup the assay: *C. koserii, C. freundii, E. faecalis, E. faecium, E. cloacae* and *E. aerogenes*, Clinical samples of earlier outbreaks (confirmed by a reference laboratory) were used to validate the assay. The following clinical strains were used: *C. freundii* (n = 21), *E. faecium* (n = 18), *E. cloacae* (n = 10), *C. braakii* (n = 3), *C. koserii* (n = 2), *C. amalonaticus* (n = 1). The genomic DNA is incubated (3 hr, 37°C) with EcoRI and MseI restriction enzymes and corresponding adapters, followed by a PCR to specifically amplify restriction fragments. The MseI primer has an additional cytosine and the EcoRI has an additional adenine and a 6-FAM fluorophore. The amplified fragments were separated by capillary electrophoresis (3130 Genetic Analyser, Applied Biosystems, Foster City, USA) and cluster analyses was performed with the software programme BioNumerics vs7.1 (Applied Maths, Sint-Martens-Latem, Belgium).

Results: The AFLP assay showed the following **Results:** i) for *Enterobacter sp.* one cluster with 5 similar strains; ii) for *Enterococcus sp.* among one main cluster consisting of 11 strains, 3 additional clusters were detected consisting of 2 similar strains; iii) for *Citrobacter sp.* a large cluster of fourteen strains was found. The cluster analysis results of of this AFLP assay were in concordance with the AFLP results of the reference laboratory with a cut-off value of 90% similarity.

Conclusion: An AFLP assay is developed and clinically validated for the bacterial species *Citrobacter, Enterobacter* and *Enterococcus*.

With the introduction of this AFLP assay the laboratory has a fast molecular typing method to investigate the possible outbreak which is invaluable for infection control management.

BAMA-P7

Point prevalence and epidemiology of extended-spectrum beta-lactamase producing Enterobacteriaceae and AmpC beta-lactamase producing *Escherichia coli* in a Dutch teaching hospital: 2010 to 2014

S. Oome
Avans University of Applied Sciences, Bachelor Biology and Medical Laboratory Research, Breda & Amphia Hospital, Laboratory for Microbiology and Infection Control, Breda

Background: Worldwide, the prevalence of extended spectrum beta-lactamase producing Enterobacteriaceae (ESBL-E) increases rapidly. In addition, more multi resistant, AmpC beta-lactamase producing *Escherichia coli* have been found over the last years. Since 2010, the laboratory for microbiology and infection control of the Amphia hospital runs an annual point prevalence to monitor the prevalence and outbreaks of ESBL-E. The prevalence of AmpC producing *Escherichia coli* is included in this study since 2013.

Methods: Rectal swabs were taken from all participating patients hospitalised (including day-care) on the day of survey. The swabs were incubated during 18-24 hours at 37°C in a selective tryptic soy broth (TSB-VC, Mediaproducts), containing cefotaxime (0,25mg/L) and vancomycin (8mg/L). After incubation, 10µl of the TSB-VC broth was subcultured on selective ESBL agar (MacConkey, ceftazidime 1mg/L or cefotaxime 1mg/L, vancomycin 64mg/L and cloxacillin 400mg/L, EbSA, Mediaproducts) and selective AmpC agar (MacConkey, cefotaxime 1mg/L, cefoxitin 8mg/L, Mediaproducts) and incubated for 18-24 hours at 37°C. All isolates that grew on the EbSA agar and AmpC agar were identified using Maldi-tof MS (bioMérieux) and susceptibility testing was performed using Vitek 2 (bioMérieux). For all isolates, isolated from the EbSA agar, with a MIC > 1mg/L for ceftazidime and/or cefotaxime the presence of ESBL was phenotypically confirmed using the double disk method for cefotaxime 30µg, cefotaxime 30µg + clavulanate 10µg, ceftazidime 30µg, ceftazidime 30µg + clavulanate 10µg (group I Enterobacteriaceae), cefepime 30µg, cefepime 30µg + clavulanate 10µg (group II Enterobacteriaceae) (Neo-Sensitabs, Rosco). All *Escherichia coli* isolates, coming from the AmpC agar, with a MIC ≥ 8mg/L for cefoxitin the presence of AmpC was phenotypically confirmed using the D68C AmpC & ESBL Detection Set (cefpodoxime 10µg, cepodoxime + clavulanate 1µg, cefpodoxime + cloxacillin 500µg, cefpodoxime + clavulanate + cloxacillin, Mastdiscs, Mastgroup). All positive isolates were genotypically confirmed with the Check-MDR CT103 microarray (Check-Points). Transmission of strains within a department was determined using molecular typing based on Amplification Fragment Length Polymorphism (AFLP, VU Amsterdam).

Results: Over a period of five years, a total of 2731 rectal swabs were obtained (559 of 668 patients in 2010 (83.7%), 560 of 639 in 2011 (87.6%), 510 of 598 in 2012 (85.3%), 516 of 601 in 2013 (85.9%) and 586 of 652 in 2014 (89.9%)). The prevalence of ESBL producing Enterobacteriaceae (excluding transmissions) was 4.5% in 2010, 4.1% in 2011, 2.5% in 2012, 5.0% in 2013 and 6.3% in 2014. In 2011 one outbreak was detected with two identical CTX-M-1-1 producing *Escherichia coli*. In 2012 two outbreaks were detected; four identical CTX-M-9 producing *Enterobacter cloacae* and four CTX-M-9 producing *Escherichia coli*. In 2014 one small outbreak was detected with two CTX-M-1-15 producing *Klebsiella pneumoniae*. In all years, *Escherichia coli* was the predominant species isolated with an average of 79.8%. The prevalence of AmpC producing *Escherichia coli* (including transmissions) was 3.7% in 2013 and 2.7% in 2014. In 2014 one possible outbreak was detected involving three patients with an AmpC producing (phenotypically confirmed) *Escherichia coli*.

Conclusion: The prevalence of ESBL-E was more or less stable over a five year period and no major shifts in the ESBL genes were detected. A relatively high rate of AmpC producing *Escherichia coli* was found in 2013 and 2014. The emergence of plasmidal AmpC poses a further threat to patient safety that deserves more attention.

BAMA-P8

The effects of microbiota composition on allergic sensitization in mice

L. van de Pol[1], L. Kleinjans[1], P.V. Jeurink[2,3], B.C.A.M. van Esch[2,3], J.H.M. van Bilsen[4], J. Garssen[2,3], J.J. Smit[5], R.H.H. Pieters[5], C. Belzer[1], J. Knol[1,3], L.M.J. Knippels[2,3]

[1]*Wageningen University, Wageningen,* [2]*Utrecht University, Faculty of Science, Utrecht Institute for Pharmaceutical Sciences, Utrecht,* [3]*Nutricia Research, Utrecht,* [4]*TNO, Zeist,* [5]*Utrecht University, Institute for Risk Assessment Sciences, Utrecht*

Introduction: To validate whether a mouse model of cow's milk allergy is suitable to assess the sensitizing capacity of native proteins and processed proteins with reduced allergenicity, a ring trial has been performed at four locations in the Netherlands (van Esch, Tox letters 2013). Within this study, it was shown that, although all circumstances were standardised as much as possible (e.g. same batch of mice, feeding pellets, cholera toxin, whey source, etc.), the sensitizing capacity at the different locations was different. As the gut microbiota plays an important role in shaping the host immunity, the aim of this study is to gain more insight in the location-dependent alterations within the microbiota.

Methods: C3H/HeOuJ mice were orally treated with a blunt needle on days 0, 7, 14, 21 and 28 with 0.5 ml PBS mixed with 10 µg cholera toxin. On day 33, blood was collected and used to determine the immunoglobulin levels in serum. Animal care and use were performed in accordance with the guidelines of the Dutch Committee of Animal experiments (DEC 2011.III.08.89). Faecal samples were collected at day 0 and 27 at the four different research centres. Microbiota composition was determined with the Mouse Intestinal Tract chip (MITchip) phylogenetic microarray. In short, the procedure is as follows: DNA was extracted by bead-beating and using the Maxwell MDx from Promega. The 16s rRNA genes were amplified with universal primers. The amplified 16s genes were transcribed into RNA after which it was labelled with Cy3 and Cy5 dyes. Samples were then hybridized with the MITchips and scanned.

Results and discussion: Results from the scans are expected to show a difference in the microbiota composition based on the observed immunological differences at the four locations; the samples from day 0 are expected to be more similar than the day 27 samples, while the day 27 samples are expected to cluster by research centre. The next step will be to link these alterations to the ability of allergic sensitization in mice.

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Verkorte productinformatie Dificlir® 200 mg

▼Dit geneesmiddel is onderworpen aan aanvullende monitoring. Daardoor kan snel nieuwe veiligheidsinformatie worden vastgesteld. Beroepsbeoefenaren in de gezondheidszorg worden verzocht alle vermoedelijke bijwerkingen te melden via de website van het Nederlands Bijwerkingen Centrum Lareb (www.lareb.nl). **Samenstelling:** elke filmomhulde tablet bevat 200 mg fidaxomicine. **Farmacotherapeutische groep:** Antidiarreemiddelen, intestinale anti-inflammatoire/anti-infectiemiddelen, antibiotica, ATC-code: A07AA12. **Therapeutische indicatie:** Behandeling van Clostridium difficile-infecties (CDI), ook wel C. difficile-geassocieerde diarree (CDAD) bij volwassenen. Er dient rekening te worden gehouden met officiële richtlijnen betreffende het juiste gebruik van antibacteriële middelen. **Dosering en wijze van toediening:** Dosering voor volwassenen en ouderen (≥ 65 jaar) is 200 mg (één tablet) tweemaal daags (om de 12 uur), oraal, gedurende 10 dagen. Dificlir kan met of zonder voedsel worden ingenomen. **Contra-indicaties:** Overgevoeligheid voor het werkzame bestanddeel of voor één van de hulpstoffen. **Waarschuwingen en voorzorgen bij gebruik:** Overgevoeligheidsreacties waaronder ernstige angio-oedeem zijn gerapporteerd. Indien er een ernstige allergische reactie met Dificlir optreedt dient het gebruik gestaakt te worden en passende maatregelen te worden genomen. Sommige patiënten met overgevoeligheidsreacties hebben een voorgeschiedinis van allergie voor macroliden gemeld. Voorzichtigheid is geboden bij patiënten met een bekende macrolidenallergie. Dificlir dient met voorzichtigheid gebruikt te worden bij patiënten met ernstig verminderde nierfunctie, matig tot ernstig verminderde leverfunctie, pseudomembraneuze colitis, inflammatoire darmziekte en fulminante of levensbedreigende CDI. Uit voorzorg heeft het de voorkeur het gebruik van Dificlir te vermijden tijdens de zwangerschap. Ondanks dat er geen effecten op met moedermelk gevoede pasgeborenen/zuigelingen worden verwacht, kan een risico voor de pasgeborenen/zuigelingen niet worden uitgesloten. Er moet worden besloten of borstvoeding moet worden gestaakt of dat behandeling met Dificlir moet worden gestaakt dan wel niet moet worden ingesteld (zie volledige SPC). **Interacties:** Gelijktijdige toediening van potente P-gp-remmers waaronder ciclosporine, ketoconazol, erytromycine, claritromycine, verapamil, dronedarone en amiodaron wordt niet aanbevolen. Dificlir is mogelijk een milde tot matige remmer van intestinaal P-gp. **Bijwerkingen:** Vaak: misselijkheid, braken, obstipatie. Soms: huiduitslag, pruritus, verminderde eetlust, duizeligheid, hoofdpijn, dysgeusie, opgezette buik, flatulentie, droge mond, verhoogd alanine-aminotransferase. Frequentie niet bekend: Overgevoeligheidsreacties (angio-oedeem, dyspneu). Dificlir is uitsluitend verkrijgbaar op recept. Volledige productinformatie op www.astellas.nl Astellas Pharma B.V. Sylviusweg 62, 2333 BE Leiden. Tel.: 071-5455854 SPC 26 juni 2014 14-DIF-010

Referentie: 1. DIFICLIR samenvatting van de Productkenmerken, juni 2014. **2.** www.swab.nl

Verkorte productinformatie Mycamine® 50 mg/100 mg (gebaseerd op SmPC van 18 december 2013) **Samenstelling:** Mycamine® 50 mg/100 mg poeder voor oplossing voor infusie (in natriumvorm). De toe te dienen hoeveelheid na reconstitutie is 10 mg/ml en 20 mg/ml, resp. (in natriumvorm). **Farmacotherapeutische groep:** Overige antimycotica voor systemisch gebruik, ATC-code: J02AX05. **Therapeutische indicaties:** *Volwassenen, adolescenten ≥ 16 jaar en ouderen:* Behandeling van invasieve candidiasis. Behandeling van oesofageale candidiasis bij patiënten voor wie intraveneuze therapie geschikt is. Profylaxe van *Candida*-infectie bij patiënten die allogene hematopoëtische stamceltransplantatie ondergaan of van wie wordt verwacht dat ze aan neutropenie (absolute neutrofielentelling < 500 cellen/µl) lijden gedurende 10 dagen of langer. *Kinderen (inclusief neonaten) en adolescenten < 16 jaar:* Behandeling van invasieve candidiasis; Profylaxe van *Candida*-infectie bij patiënten die allogene hematopoëtische stamceltransplantatie ondergaan of van wie wordt verwacht dat ze aan neutropenie lijden (absolute neutrofielentelling < 500 cellen/µl) gedurende 10 dagen of langer. Bij de beslissing Mycamine te gebruiken dient rekening gehouden te worden met het potentiële risico voor de ontwikkeling van levertumoren. Mycamine dient daarom uitsluitend te worden gebruikt als andere antifungale middelen niet in aanmerking komen. **Dosering en wijze van toediening:** Behandeling van invasieve candidiasis: 100 mg/dag bij lichaamsgewicht > 40 kg, 2 mg/kg/dag bij een lichaamsgewicht ≤ 40 kg. Als de patiënt in onvoldoende mate reageert, bv. indien de kweken positief blijven of de klinische toestand niet verbetert, dan mag de dosis worden verhoogd tot 200 mg/dag bij patiënten met een lichaamsgewicht > 40 kg of tot 4 mg/kg/dag bij patiënten met een lichaamsgewicht ≤ 40 kg. Behandeling van oesofageale candidiasis: 150 mg/dag lichaamsgewicht > 40 kg, 3 mg/kg/dag bij een lichaamsgewicht ≤ 40 kg. Profylaxe van *Candida*-infectie: 50 mg/dag bij lichaamsgewicht > 40 kg, 1 mg/kg/dag bij een lichaamsgewicht ≤ 40 kg. Er zijn onvoldoende gegevens beschikbaar over de farmacokinetiek van micafungine bij patiënten met ernstige leverfunctiestoornis. **Contra-indicaties:** Overgevoeligheid voor het werkzame bestanddeel, voor andere echinocandinen of voor één van de hulpstoffen. Zie de volledige SmPC. **Waarschuwingen en voorzorgen bij gebruik: Hepatische effecten:** De ontwikkeling van foci van veranderde hepatocyten (FAH) en hepatocellulaire tumoren werd bij ratten waargenomen na een behandelperiode van 3 maanden of langer. De veronderstelde drempelwaarde voor tumorontwikkeling bij ratten ligt ongeveer in het bereik van de klinische blootstelling. De relevantie van deze bevindingen voor het therapeutisch gebruik bij patiënten kan niet worden uitgesloten. De leverfunctie dient zorgvuldig te worden gecontroleerd tijdens behandeling met micafungine. Om het risico op adaptieve regeneratie en mogelijk daaropvolgende levertumorvorming te minimaliseren, wordt vroegtijdig staken aanbevolen indien significante en persisterende verhoging van ALT/AST optreedt. De micafungine behandeling dient uitgevoerd te worden na een zorgvuldige bepaling van risico's en voordelen met name bij patiënten met ernstige leverfunctiestoornissen of chronische leverziekten die preneoplastische aandoeningen vertegenwoordigen, zoals gevorderde leverfibrose, cirrose, virale hepatitis, neonatale leverziekte of congenitale enzymdefecten, of bij het tegelijkertijd ondergaan van een behandeling met hepatotoxische en/of genotoxische eigenschappen. Er kunnen anafylactische/anafylactoïde reacties optreden, met inbegrip van shock. Bij het optreden van dergelijke reacties moet infusie van micafungine worden stopgezet en de juiste behandeling worden ingesteld. Exfoliatieve huidreacties zijn gemeld. Als patiënten huiduitslag ontwikkelen dan dienen zij nauwkeurig geobserveerd te worden en dient de behandeling met micafungine gestopt te worden als de laesies verergeren. In zeldzame gevallen is er hemolyse met inbegrip van acute intravasculaire hemolyse of hemolytische anemie gerapporteerd. In dit geval dient nauwlettend te worden gevolgd of er geen verslechtering optreedt en er dient een risico/baten analyse gedaan te worden van voortzetting van de therapie. Micafungine kan nierproblemen, nierfalen en afwijkende nierfunctietests veroorzaken. Patiënten dienen nauwlettend te worden gecontroleerd op verslechtering van de nierfunctie. **Interacties:** Micafungine bezit een gering vermogen tot interactie met geneesmiddelen die via CYP3A-gemedieerde routes worden gemetaboliseerd. Gelijktijdige toediening van micafungine met amfotericine B-desoxycholaat is alleen toegestaan wanneer de voordelen duidelijk opwegen tegen de risico's, met een scherpe controle op mogelijke toxiciteit van amfotericine B-desoxycholaat. Patiënten die Mycamine in combinatie met sirolimus, nifedipine of itraconazol ontvangen, dienen te worden gecontroleerd op toxiciteit van sirolimus, nifedipine of itraconazol. Indien noodzakelijk moet de dosering van deze middelen worden verlaagd. **Bijwerkingen:** De volgende bijwerkingen deden zich vaak voor: leukopenie, neutropenie, anemie, hypokaliëmie, hypomagnesiëmie, hypocalciëmie, hoofdpijn, flebitis, misselijkheid, braken, diarree, buikpijn, verhoogd bloedalkaline-fosfatase, verhoogd aspartaataminotransferase, verhoogd alanineaminotransferase, verhoogd bilirubine in het bloed (inclusief hyperbilirubinemie), afwijkende leverfunctietest, uitslag, pyrexie, koude rillingen. Naast bovengenoemde bijwerkingen zijn bij kinderen tevens vaak thrombocytopenie, tachycardie, hypertensie, hypotensie, hyperbilirubinemie, hepatomegalie, acuut nierfalen en verhoogd bloedureum gemeld. Kinderen < 1 jaar toonden ongeveer 2 keer zo vaak een verhoogde ALT, AST en AP dan oudere kinderen. De volgende bijwerkingen kwamen soms voor: pancytopenie, trombocytopenie, eosinofilie, hypoalbuminemie, anafylactische/anafylactoïde reactie, overgevoeligheid, hyperhidrose, hyponatriëmie, hyperkaliëmie, hypofosfatemie, anorexia, slapeloosheid, angst, verwardheid, slaperigheid, tremor, duizeligheid, dysgeusie, tachycardie, palpitaties, bradycardie, hypotensie, hypertensie, blozen, dyspneu, dyspepsie, obstipatie, leverinsufficiëntie, verhoogd gammaglutamyl-transferase, geelzucht, cholestase, hepatomegalie, hepatitis, urticaria, pruritus, erytheem, bloedcreatinine verhoogd, bloedureum verhoogd, verergerde nierinsufficiëntie, trombose op injectieplaats, infuusplaats ontsteking, injectieplaats pijn, perifeer oedeem, verhoogde bloedlactaatdehydrogenase. De volgende bijwerkingen kwamen zelden voor: hemolytische anemie en hemolyse. Van de volgende bijwerkingen kan de frequentie met de beschikbare gegevens niet worden bepaald: gedissemineerde intravasculaire stolling, shock, hepatocellulaire schade inclusief gevallen met dodelijke afloop, toxische huideruptie, erythema multiforme, het syndroom van Stevens-Johnson, toxische epidermale necrolyse, nierfunctie-stoornissen, acuut nierfalen. **Afleverstatus:** UR. **Overige productinformatie:** Astellas Pharma B.V. Sylviusweg 62, 2333 BE Leiden. Tel.: 071-5455854 Fax: 071-5455850.

Referenties: 1. Aantal patiëntendagen berekend over aantal verkochte Kg (Bron: IMS Midas verkochte Kg - MAT 12 maanden sales 09/14)/gemiddelde dagdosering gedurende 14 aanbevolen behandelingen (bron: SmPC). Veronderstelde behandelduur is 14 dagen. **2.** SmPC Mycamine december 2013 15-MYC-002