nederlandse vereniging voor Medische Microbiologie

NEDERLANDS TIJDSCHRIFT VOOR MEDISCHE MICROBIOLOGIE

Supplement bij vierentwintigste jaargang, maart 2016

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

Papendal, 22 & 23 maart 2016
Programma-overzicht
Abstracts
Auteursindex

24e jaargang . maart 2016 . 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).

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Meeting secretariat

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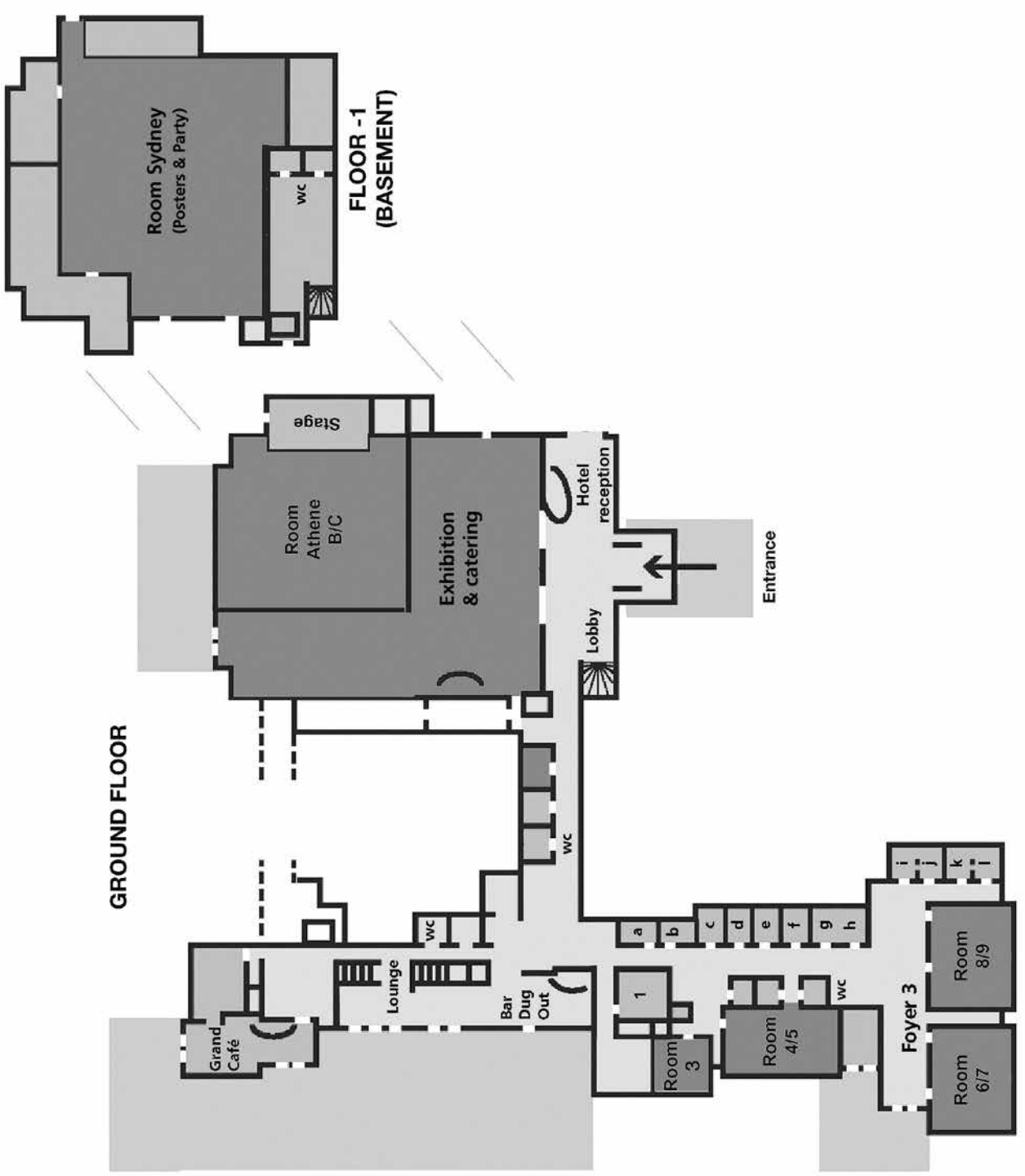
TUESDAY MARCH 22, 2016							
	EXHIBITION	ROOM ATHENE B/C	ROOM SYDNEY	ROOM 3	ROOM 4/5	ROOM 6/7	ROOM 8/9
09:00 - 09:30	Registration						
09:30 - 11:00		Plenary session					
11:00 - 11:30	Coffee/tea						
11:30 - 13:00		Molecular tools in (regional) outbreak prevention and control	The human gut microbiota in the first 1000 days of life	The role of micro-organisms in biogeo-chemical cycling and climate change	EcoHealth; Circulation of human pathogens across ecosystems	Evolutionary engineering of microbes	Clinical & antimicrobial microbiology
13:00 - 14:00	Lunch			KNVM Business Meeting			
14:00 - 15:30		Infection control in the 21st century: the added value of whole genome sequencing	Changing epidemiology and new treatment molities for *Clostridium difficile* infections (CDI)	Marine Microbiology	WAMM & NVP – One Health and emerging infections	Yeast: pathogen, industrial workhorse and model system	Environmental & general microbiology
15:30 - 16:00	Coffee/tea						
16:00 - 17:30		Clinical microbiology	Enteric infections: from pathogenesis to vaccines	Anaerobic microbes for health and the environment	Host associated fungal ecology	Experimental evolution and ecology of microbial ecosystems	Sectie onderwijs – How to cultivate future microbiologists?
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				

Clinical microbiology
General microbiology
Both: Clinical & general microbiology

SCHEMATIC PROGRAMME

WEDNESDAY MARCH 23, 2016							
	EXHIBITION	ROOM ATHENE B/C	ROOM SYDNEY	ROOM 4/5	ROOM 6/7	ROOM 8/9	
08:30 - 09:00	Registration						
09:00 - 10:30		Emerging infections affecting the Netherlands	Primary HPV cervical cancer screening	Pathogenesis 1	Bacterial morphogenesis regulation	Bachelor and Master (BAMA) Symposium	
10:30 - 11:00	Coffee/tea						
11:00 - 12:30		Clinical cases in medical microbiology: an interactive session	Novel approaches to combat viral infections	Pathogenesis 2	Bacterial competition and cooperation	Bachelor and Master (BAMA) Symposium	
12:45 - 13:45	Lunch			BBC-MMO Business Meeting			
14:00 - 15:30		Plenary session & Award Ceremony					
15:30 - 16:00	Coffee/tea						
16:00 - 18.00		NVMM Business Meeting					

Clinical microbiology
General microbiology
Both: Clinical & general microbiology


FLOOR -1
(BASEMENT)
Room Sydney
(Posters & Party)
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GROUND FLOOR
Stage
Room Athene B/C
Exhibition & catering
Hotel reception
Entrance
Lobby
wc
wc
Grand Café
Lounge
Bar
Dug Out
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Room 3
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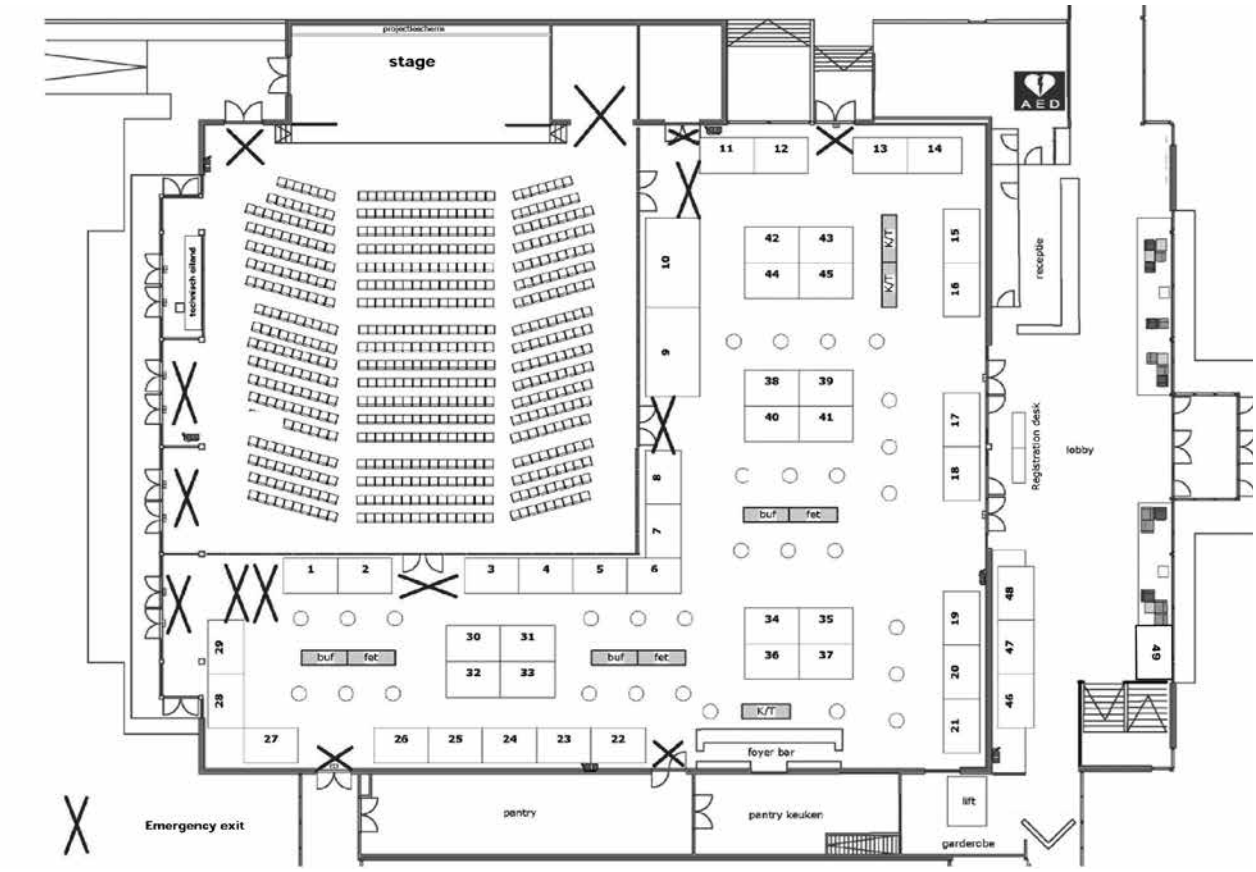
Room 6/7

EXHIBITION ROOM



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TUESDAY 22 MARCH 2016

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

09:30 - 11:00 PLENARY SESSION

Athene B/C *Chair: Menno de Jong*

09:30 - 10:15 **The power of networking: studying microbiomes for health**
O001 Gabrielle Berg (Austria)

10:15 - 11:00 **Evolution of stochastic phenotype switching**
O002 Paul Rainey (France/New Zealand)

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

11:30 - 13:00 PARALLEL SESSIONS

Athene B/C **Molecular tools in (regional) outbreak prevention and control**
Chairs: John Rossen & Paul Savelkoul

11:30 - 12:00 **Network analysis and molecular tools for regional outbreak control**
O003 Alex W. Friedrich

12:00 - 12:30 **Molecular tools for surveillance of antimicrobial resistance**
O004 Rene Hendriksen (Denmark)

12:30 - 12:45 **Clonal spread of vancomycin-resistant *Enterococcus faecium* between multiple Dutch hospitals inferred from whole genome sequence data**
O005 Malbert Rogers

12:45 - 13:00 **Molecular tools in (regional) outbreak prevention and control. Origin of fatal sepsis after a platelet transfusion traced by molecular typing**
O006 Lieke van Alphen

Room 3 **The human gut microbiota in the first 1000 days of life**
Chairs: Clara Belzer & Jan Knol

11:30 - 12:00 **Tba**
O007 Paul Ross (Ireland)

12:00 - 12:30 **Early stress and the microbiome**
O008 Carolina de Weerth

12:30 - 12:45 **Allergy management by protein hydrolysates and prebiotics - closer to breastfed**
O009 Harm Wopereis

12:45 - 13:00 **Gestational age of preterm infants is associated with intestinal microbiota composition and activity**
O010 Romy Zwittink

Room 3 **The role of microorganisms in biogeochemical cycling and climate change**
Chairs: Adrian Ho & Claudia Lüke

11:30 - 12:00 **Bacterial metabolism of isoprene: a much neglected atmospheric trace gas**
O011 Colin Murrell (United Kingdom)

12:00 - 12:15 **Rising CO_2 concentrations will intensify phytoplankton blooms in eutrophic and hypertrophic lakes**
O012 Jolanda Verspagen

12:15 - 12:30 **Happily ever after? How repeated subcultivation influences a methanotrophic marriage**
O013 Frederiek-Maarten Kerckhof

12:30 - 12:45 **Climate change effects on greenhouse gas balances of shallow lakes**
O014 Ralf Aben

12:45 - 13:00 **Discovery of Nitrospira as complete ammonium oxidizer (comammox)**
O015 Sebastian Lücker

Room 4/5 **EcoHealth; Circulation of human pathogens across ecosystems**
Chairs: Joop van Doorn & Leo van Overbeek

11:30 - 12:00 **Do organic fertilizer influence the abundance and diversity of resistance genes and mobile genetic elements in soil?**
O016 Kornelia Smalla (Germany)

12:00 - 12:30 **Back to the Roots: microbiology & chemistry at the plant-soil interface**
O017 Jos Raaijmakers

12:30 - 12:45 **Ecology of *E. coli* in plant seed production systems**
O018 Leo van Overbeek

12:45 - 13:00 **Cycling of EHEC bacteria across animal and plant ecosystems**
O019 Peter Willemsen

Room 6/7 **Evolutionary engineering of microbes**
Chairs: Herwig Bachmann & Bas Teusink

11:30 - 12:00 **Directed evolution workflows with microfluidic emulsions and their derivatives**
O020 Martin Fischlechner (United Kingdom)

12:00 - 12:30 **Constraints and trade-offs shape the evolution of bacterial growth and metabolism**
O021 Frank Bruggeman

12:30 - 12:45 **Laboratory evolution of constitutive acetic-acid tolerance in *Saccharomyces cerevisiae***
O022 Arthur Gorter de Vries

12:45 - 13:00 **Adaptation of a riboswitch platform to a new ligand**
O023 Sjoerd Creutzburg

Room 8/9 **Clinical & antimicrobial microbiology**
Chairs: Rob Rentenaar & Lieke Reubsaet

11:30 - 11:45 **A major reduction in the use of antimicrobials for livestock in the Netherlands since 2009: the critical success factors**
O024 Jaap Wagenaar

11:45 - 12:00 **Changing characteristics of LA-MRSA isolated from humans in the Netherlands. Emergence of a subclade transmitted without livestock exposure**
O025 Thijs Bosch

12:00 - 12:15 **Risk factors, duration of carriage and onward transmission of ESBL-producing Enterobacteriaceae acquired during travel: results of large prospective cohort study of travellers and their households**
O026 Jarne van Hattem

12:15 - 12:30 **Cycling with lantibiotics**
O027 Andrius Buivydas

12:30 - 12:45 **Dynamics of Extended Spectrum Beta-Lactamase/AmpC producing *E. coli* in broiler parent stock**
O028 Anita Dame-Korevaar

12:45 - 13:00 **Bactericidal activity of novel antimicrobial peptide M33 against *Klebsiella pneumoniae*: insight into the mode of action**
O029 Hessel van der Weide

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

Room 3

13:00 - 14:00 **KNVM Business Meeting**

14:00 - 15:30 PARALLEL SESSIONS

Athene B/C **Infection control in the 21st century: the added value of whole genome sequencing**
Chairs: Jan Kluytmans & John Rossen

14:00 - 14:30 **Investigating Staphylococcus aureus outbreaks using whole genome sequencing**
O030 Nicola Gordon (United Kingdom)

14:30 - 15:00 **Use of whole genome sequencing in the evaluation of isolation strategies for ESBL: results of the SoM study**
O031 Marjolein Kluytmans

15:00 - 15:15 **Presumed outbreak of colistin resistant *Enterobacter cloacae* during prolonged use of selective decontamination of the digestive tract**
O032 Mirjam Dautzenberg

15:15 - 15:30 **Next-generation sequence (NGS) analysis reveals methicillin-resistance transfer to a methicillin-susceptible *Staphylococcus aureus* (MSSA) strain that subsequently caused a methicillin-resistant *S. aureus* (MRSA) outbreak**
O033 Veronica Weterings

Sydney **Changing epidemiology and new treatment modalities for *Clostridium difficile* infections (CDI)**
Chair: Ed Kuijper

14:00 - 14:30 **The role of the intestinal microbiota for development of *Clostridium difficile* infections**
O034 Vincent Young (USA)

14:30 - 14:45 **Development of the Netherlands Donor Feces Bank (NDFB) for Fecal Microbiota Transplantation (FMT)**
O035 Josbert Keller

14:45 - 15:00 **Changing epidemiology of CDI in The Netherlands**
O036 Sofie van Dorp

15:00 - 15:15 **The role of asymptomatic carriership for spread of CDI in hospitals**
O037 Monique Crobach

15:15 - 15:30 **Long term effect of feces microbiota transplantations for recurrent CDI**
O038 Bram Goorhuis

Room 3 **Marine Microbiology**
Chair: Laura Villanueva

14:00 - 14:30 **Microbes and their genes in the global ocean: the deep ocean survey of the Malaspina 2010 cruise, with comparison to the Tara Oceans global surface ocean survey**
O039 Josep M. Gasol (Spain)

14:30 - 14:45 **Ecological genomics of coastal microbial mats**
O040 Henk Bolhuis

14:45 - 15:00 **Transmission of microbiota in marine invertebrates**
O041 Detmer Sipkema

15:15 - 15:30 **Characterization of the highly branched glycogen from the thermoacidophilic red microalga *Galdieria sulphuraria* and comparison with other glycogens**
O042 Marc van der Maarel

15:15 - 15:30 **Archaea as sources of tetraether membrane lipids in the water column and sediments across an oxygen minimum zone**
O043 Marc Besseling

Room 4/5 **WAMM & NVP - One Health and emerging infections**
Chairs: Titia Kortbeek & Joke van der Giessen

14:00 - 14:15 **One Health, to empower interdisciplinary approach**
O044 Wim van der Poel

14:15 - 14:45 **A case of tularemia: veterinary background and human diagnostics & therapy**
O045 Herjan Bavelaar & Miriam Koene

14:45 - 15:00 **A case of echinococcosis: veterinary background and human diagnostics & therapy**
O046 Joke van der Giessen & Titia Kortbeek

15:00 - 15:30 **A case of psittacosis: veterinary background and human diagnostics & therapy**
O047 Edou Heddema & Marloes Heijne

Room 6/7 **Yeast: pathogen, industrial workhorse and model system**
Chairs: Pascale Daran-Lapujade & Mickel Jansen

14:00 - 14:30 **Fundamental questions in metabolic regulation revisited in yeast**
O048 Bas Teusink

14:30 - 15:00 ***Cryptococcus* and *Malassezia*: two models to understand disease potential**
O049 Teun Boekhout

15:00 - 15:15 **Insights into organelle fission specificity from the peroxisomal membrane protein Pex11p**
O050 Chris Williams

15:15 - 15:30 **Engineering redox metabolism improves ethanol yield in acetate-reducing *Saccharomyces cerevisiae***
O051 Ioannis Papapetridis

Room 8/9 **Environmental & general microbiology**
Chairs: Laura van Niftrik & Boran Kartal

14:00 - 14:15 **Regime shifts between oxic and anoxic states in a microbial model ecosystem**
O052 Timothy Bush

14:15 - 14:30 **Bacterial interactions inside tomato xylem vessels: Understanding biofilm formation, cooperation and competition**
O053 Reindert Nijland

14:30 - 14:45 **Ultrastructure and (meta)virome of a bacteriophage infecting the anaerobic methane oxidizing bacterium *Methylomirabilis oxyfera***
O054 Lavinia Gambelli

14:45 - 15:00 **The effect of weak organic acid stress on the intracellular pH dynamics of *Bacillus subtilis* spores during germination and outgrowth**
O055 Stanley Brul

15:00 - 15:15 **Biomethaniation of syngas using a thermophilic co-culture**
O056 Martijn Diender

15:15 - 15:30 **Quantitative proteomic analysis of *B. subtilis* spores made in liquid and on solid growth media**
O057 Wishwas Abhyankar

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

16:00 - 17:30 PARALLEL SESSIONS

Athene B/C **Clinical microbiology**
Chair: Dries Budding

16:00 - 16:15 **Safety, immunogenicity, and protective efficacy of intradermal immunization with aseptic, purified, cryopreserved *Plasmodium falciparum* sporozoites in volunteers under chloroquine prophylaxis: A randomized controlled trial**
O058 Guido Bastiaens

16:15 - 16:30 **Epidemiology of multiple viremia in previously immunocompetent patients with septic shock**
O059 David Ong

16:30 - 16:45 **Non-typeable *Haemophilus influenzae* is an emerging invasive pathogen**
O060 Jeroen Langereis

16:45 - 17:00 **Prevention of *Staphylococcus aureus* biomaterial-associated infections using a polymer-lipid coating containing LL-37-derived antimicrobial peptides**
O061 Martijn Riool

17:00 - 17:15 **Application of eubacterial molecular detection to clinical routine**
O062 Dries Budding

17:15 - 17:30 **Conjugal transfer of VIM-2 carbapenemase-encoding plasmid in *Pseudomonas aeruginosa***
O063 Bart Kraak

Sydney **Enteric infections: from pathogenesis to vaccines**
Chairs: Han van den Bosch & Michiel Stork

16:00 - 16:30 **Mucosal immunity and vaccines against Enteric infections**
O064 Cecil Czerkinsky (France)

16:30 - 17:00 **Conjugation of a shigella flexneri 2a derived synthetic oligosaccharide to tetanus toxoid**
O065 Robert van der Put

17:00 - 17:30 **Vaccination: a way to protect swine from intestinal infections**
O066 Ruud Segers

Room 3 **Anaerobic microbes for health and the environment**
Chairs: Laura Villanueva & Cornelia Welte

16:00 - 16:30 **A fresh look at dissimilatory sulfate reduction**
O067 Inês Cardoso Pereira (Portugal)

16:30 - 16:45 **Microbiota for health: The case of *Akkermansia muciniphila***
O068 Kees van der Ark

16:45 - 17:00 **Anaerobic oxidation of methane in the paddy field dominated by *Methanoperedens nitroreducens***
O069 Annika Vaksmaa

17:00 - 17:15 **Extraction and characterization of extracellular polymers from anammox granular sludge**
O070 Marissa Boleij

17:15 - 17:30 **Novel sulfate-reducing bacteria create microniches in acidic environments**
O071 Irene Sanchez

Room 4/5 **Host associated fungal ecology**
Chair: Guus Roeselers

16:00 - 16:30 **Yeast evolutionary ecology revisited: the wasp connection**
O072 Ducio Cavalieri (Italy)

16:30 - 17:00 **Irritable Bowel Syndrome and the fungus among us**
O073 Rene van den Wijngaard

17:00 - 17:15 **On the oral microbiome and Candida interaction**
O074 Egija Zaura

17:15 - 17:30 **Differential kinetics of *Aspergillus nidulans* and *Aspergillus fumigatus* phagocytosis**
O075 Mark Gresnigt

Room 6/7 **Experimental evolution and ecology of microbial ecosystems**
Chairs: Irene de Bruijn & Marjon de Vos

16:00 - 16:30 **Evolution of species interactions in diverse bacterial communities**
O076 Thomas Bell (United Kingdom)

16:30 - 17:00 **Evolving symbiotic partnerships**
O077 Toby Kiers

17:00 - 17:15 **Meta-analysis of natural disease suppressive soils**
O078 Ruth Gómez Expósito

17:15 - 17:30 **Comparative genomics of Intestinimonas butyriciproducens, a lysine utilizing and butyrate producing bacteria in the human gut**
O079 Sudarshan Shetty

Room 8/9 **Sectie onderwijs - How to cultivate future microbiologists?**
Chairs: Loek van Alphen & Marie-Monique Immink

16:00 - 16:15 **The future of the Microbiological technician**
O080 Ellen Hilhorst

16:15 - 16:45 **General discussion onderwijsvernieuwing: Kiest u maar (Dutch spoken)**
O081 Ellen Hilhorst & Martine Reij

16:45 - 17:00 **Team-based learning in a medical microbiology course**
O082 Bas Zaat

17:00 - 17:15 **How to stimulate students to design their own microbiological experiment?**
O083 Ida Jongenburger

17:15 - 17:30 **Gamification in laboratory education**
O084 Eus van Hove

Exhibition

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

Restaurant

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

Sydney

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**

Sydney

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

WEDNESDAY 23 MARCH 2016

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

09:00 - 10:30 PARALLEL SESSIONS

Athene B/C **Emerging infections affecting the Netherlands**
Chairs: Chantal Reusken & Barry Rockx

09:00 - 09:30 **The emergence of Zika virus; epidemiology, diagnostics and clinical aspects**
O086 Remi Charrel (France)

09:30 - 10:00 **Competence for West Nile virus of mosquitoes in the Netherlands**
O087 Gorben Pijlman

10:00 - 10:15 **Environmental surveillance suggests a role for surface water in supporting endemic tularemia in the Netherlands**
O088 Ingmar Janse

10:15 - 10:30 **High prevalence of undetected highly resistant microorganisms among residents of long term care facilities in Amsterdam, the Netherlands**
O089 Aletta Tholen

Sydney **Primary HPV cervical cancer screening**
Chairs: Mariet Feltkamp & Rob Schuurman

09:00 - 09:30 **Evidence on HPV testing: Primary hrHPV detection versus cytology in cervical cancer population screening**
O090 Marc Arbyn (Belgium)

09:30 - 10:00 **Introducing the New Dutch Cervical Cancer Screening program**
O091 Nynke van der Veen

10:00 - 10:15 **Cobas hrHPV PCR testing in practice**
O092 Adriaan van den Brule

10:15 - 10:30 **BK polyomavirus seroreactivity of kidney donors predicts viremia and nephropathy in recipients**
O093 Herman Wunderink

Room 4/5 **Pathogenesis 1**
Chairs: Marien de Jonge & Jeroen Langereis

09:00 - 09:15 **Cell wall composition of Group A Streptococcus influences the bactericidal efficacy of human Group IIA-Secreted Phospholipase A**
O094 Vincent van Hensbergen

09:15 - 09:30 **The role of C5 convertases in MAC-dependent killing of Gram-negative bacteria**
O095 Dani Heesterbeek

09:30 - 09:45 **The true face of Cas9: a subtle killer**
O096 Chinmoy Saha

09:45 - 10:00 ***Streptococcus pneumoniae* infection of zebrafish embryos: a new model to visualize and study pneumococcal meningitis**
O097 Kin Ki Jim

10:00 - 10:15 **Investigating possibilities for control of pathogenic *Streptococcus suis* in piglets via the natural piglet microbiome**
O098 Peter van Baarlen

10:15 - 10:30 **Persistence of *Enterococcus faecium* outside the human host**
O099 Vincent de Maat

Room 6/7 **Bacterial morphogenesis regulation**
Chairs: Tanneke den Blaauwen & Jan Willem Veening

09:00 - 09:30 **Two short stories about bacterial cell division**
O100 Dirk-Jan Scheffers

09:30 - 10:00 **Coordination of envelope constriction during Gram-negative division**
O101 Alexander Egan (United Kingdom)

10:00 - 10:15 **The minimal Divisome**
O102 Terrens Saaki

10:15 - 10:30 **Accurate cell division in *Streptococcus pneumoniae* by an integrated cell cycle**
O103 Renske van Raaphorst

Room 8/9 **Bachelor and Master (BAMA) Symposium**
Chair: Girbe Buist & Liesbeth Nuyens

09:00 - 09:15 **Functional characterization of actin homolog MreB in the anaerobic ammonium oxidizing bacterium *Kuenenia stuttgartiensis***
BAMA-O01 Stijn Peeters

09:15 - 09:30 **A novel nitrite reductase: a 60-heme-containing heterododecameric protein complex in the anaerobic ammonium oxidizing bacterium *Kuenenia stuttgartiensis***
BAMA-O02 Rob Schmitz

09:30 - 09:45 **Identification and characterization of fungal components involved in immune evasion**
BAMA-O03 E. Keizer

09:45 - 10:00 **Microbicidal effect of LL-37 and teicoplanin in combination against *Staphylococcus aureus* and *Staphylococcus epidermidis***
BAMA-O04 Bruce Koppen

10:00 - 10:25 **Poster pitches BAMA Symposium**

10:25 - 11:15 **Poster session & coffee/tea break BAMA Symposium**

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

11:00 - 12:30 PARALLEL SESSIONS

Athene B/C **Clinical cases in medical microbiology: an interactive session**
Chairs & moderators: Jaap van Hellemond, Ed Kuijper Annelies Riezebos, Rolf Vreede

11:00 - 11:30 **A microbiological twist**
O104 Sjoerd Nauta

11:30 - 12:00 **An uncommon presentation of a common disease**
O105 Wianda Christoffers

12:00 - 12:30 **A sudden surprise from Surinam**
O106 Maurits van Meer

Sydney **Novel approaches to combat viral infections**
Chairs: Marion Koopmans & Hélène Verheije

11:00 - 11:30 **MERS: from discovery to prevention**
O107 Bart Haagmans

11:30 - 11:45 **CRISPR/Cas9-mediated genome editing of herpesviruses limits productive and latent infections**
O108 Robert Jan Lebbink

11:45 - 12:00 **Host target-directed antiviral strategies to combat coronavirus infections**
O109 Adriaan de Wilde

12:00 - 12:15 **Cap-snatching of a segmented (-)RNA plant virus: Perspectives for new antiviral drug design against Influenza viruses**
O110 Richard Kormelink

12:15 - 12:30 **Uncovering novel features of cytomegalovirus immune evasion: HLA class I degradation by the protein US2**
O111 Anouk Schuren

Room 4/5 **Pathogenesis 2**
Chairs: Suzan Rooijakkers & Bart Bardoel

11:00 - 11:15 **Redirection of ESX-1 substrates to ESX-5 system in T7SS of pathogenic mycobacteria**
O112 Trang H Phan

11:15 - 11:30 **Differential Interaction of the Staphylococcal Toxins Panton–Valentine Leukocidin and γ-Hemolysin CB with Human C5a Receptors**
O113 András Spaan

11:30 - 11:45 **Human Langerin interacts with a conserved glycan modification of *Staphylococcus aureus* Wall Teichoic Acid**
O114 Rob van Dalen

11:45 - 12:00 **Proteolytic-independent role of mycosins in formation of the ESX-1 and ESX-5 type VII secretion complexes**
O115 Vincent van Winden

12:00 - 12:15 **Genome-wide screening identifies PTS permease BepA to be involved in *Enterococcus faecium* endocarditis and biofilm formation**
O116 Fernanda Paganelli

12:15 - 12:30 **Major antibiotic stress operon iniBAC of mycobacteria is induced upon vitamin B12 and mutAB deficiency**
O117 Maikel Boot

Room 6/7 **Bacterial competition and cooperation**
Chairs: Nina van Sorge & Willem van Schaik

11:00 - 11:30 **Coevolution and conflict within the genome: interactions between bacteria and their mobile genetic elements**
O118 Ellie Harrison (United Kingdom)

11:30 - 12:00 **Hypermutation and the division of labour in Streptomyces colonies**
O119 Daniel Rozen

12:00 - 12:15 **Aggregation is a key factor leading to mycelial heterogeneity in Streptomyces**
O120 Boris Zacchetti

12:15 - 12:30 **Host glycans driven interspecies metabolic cross talk**
O121 LooWee Chia

Room 8/9 **Bachelor and Master (BAMA) Symposium**
Chairs: Girbe Buist & Liesbeth Nuyens

11:15 - 11:30 **Human monoclonal antibody against the staphylococcal complement inhibitor protein SCIN for the specific detection of *Staphylococcus aureus***
BAMA-O05 Hedzer Hoekstra

11:30 - 11:45 ***Mycoplasma genitalium*; prevalence of azithromycin resistance and development of genotyping in clinical samples**
BAMA-O06 Maarten Verhart

11:45 - 12:00 **The role of IL-1 family members on *Aspergillus fumigatus*-induced ROS production and LC3-associated phagocytosis**
BAMA-O07 Evelien Sprenkeler

12:00 - 12:15 **Interactions of *Mycobacterium marinum* with *Candida albicans***
BAMA-O08 Jasper van der Peet

12:15 - 12:30 **Posteraward ceremony BAMA Symposium**

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

Room 4/5

12:45 - 13:45 **BBC-MMO Business Meeting**

14:00 - 15:30 PLENARY SESSION & AWARD CEREMONY

Athene B/C *Chair: Wilbert Bitter*

14:00 - 14:30 **Sugars in immunity: good or bad?**
O122 Theo Geijtenbeek

14:30 - 15:00 **Can we make life in the lab?**
O123 Sijbren Otto

15:00 - 15:30 **Award ceremony**

Kiem – Category Microbial Ecology:

Stable and sporadic symbiotic communities of coral and algal holobionts
Eric Hester

Kiem – Category Medical Microbiology:

Structural basis for inhibition of TLR2 by staphylococcal superantigen-like protein 3 (SSL3)
Kirsten Koymans

Kiem – Category – General Microbiology:

Intrinsic challenges in ancient microbiome reconstruction using 16S rRNA gene amplification
Kirsten Ziesemer

Westerdijk Award – Category Medical Microbiology:

Hiding in plain Sight
András Spaan

Westerdijk Award – Category Microbial Ecology:

Cell Biology of anammox Plantomycetes and methanotrophic
Muriël van Teeseling

Westerdijk Award – Category General Microbiology:

Proteolytic regulation of Cell-Surface Signalling in Pseudomonas bacteria
Karlijn Bastiaansen

Van Leeuwenhoek Award:

Complete nitrification by a single microorganism
Sebastian Lücker

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

Athene B/C

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

O001

The power of networking: studying microbiomes for health

G. Berg
Graz University of Technology, Institute of Environmental Biotechnology, Graz, Austria

The plant microbiome is a key determinant of plant health and productivity. The microbiomes associated with plants form tight networks, which revealed strong species and niche specialization. Analyzing microbiome networks from healthy and diseased plants led to the identification of health indicators and pathogen-supporting microorganisms as well. This approach thus serves to open new opportunities for future targeted biocontrol studies and could fuel progress in sustainable agriculture, such as the development of microbial inoculants as biofertilizers, biocontrol, or stress protection products. The plant microbiome has not only an impact on plant health, it also influence the human microbiome, e.g. by raw-eaten fruits, vegetables and herbs. Moreover, it can have a positive impact on the microbiome of our built environment. Taken together, plant microbial networks are powerful networks with an impact on ecohealth.

O004

Molecular tools for surveillance of antimicrobial resistance

R.S. Hendriksen
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Antimicrobial resistance is a global emerging threat to people causing approximately 2.5 mill. extra hospital days within the European Union each year as a direct result of infections with antimicrobial resistant (AMR) bacteria and a burden of at least 25.000 annual deaths. For decades, countries around the world have established own surveillance systems for AMR following the concept of DANMAP – the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme. These surveillance systems have all been based on phenotypic methodologies. Proficiency test (PT) schemes for AMR have been developed and implemented to ensure reliable and accurate data. The general perception of PT results has been satisfactory if a deviation level below 10% were obtained.

In the last five years, the advancement of bench-top Next Generation Sequencing (NGS) and development of bioinformatics detections tools has revolutionized conventional microbiology and diagnostics consequently reducing time and costs. This advancement allows to set up AMR surveillance systems monitoring the occurrence and frequency of AMR close to real-time based on AMR genes. Bioinformatics tools for detection of AMR genes have been developed which to some degree enable none-bioinformaticians to operate the tools providing plain language reports. Currently, a handful of tools have been developed which include: ARG-ANNOT (Antibiotic Resistance Gene-ANNOTation), ARDB (Antibiotic Resistance Genes Database), Resistance Gene Identifier (CARD), Dantas Lab's Resfams, and the ResFinder. All of the tools have their strengths and limitations in usability and detection of AMR genes which have been highlighted in several benchmarking papers.

The ResFinder tool is a "plug and play" tool which enables search among 1800 antimicrobial resistance genes and variants. The tool provides an overview of the AMR genes detected, the homology percentage, and sequence length to the known references. The ResFinder tool was most likely the first one to be developed and belongs to the family of CGE tools by the Center of Genomic Epidemiology. In the month of August 2012, the tool had 60 external submissions which peaked in the month of October 2015 with more than 6018 submitted jobs. In 2015, the tool was used by a total of 69 countries including 18% from the US, 13% from the Netherlands, 10% from the UK and 7% from Australia.

In Denmark and the US, the ResFinder and CARD tools have been used to facilitate AMR surveillance based on resistance genes. In Denmark, the phenotypic susceptibility profile of 200 DANMAP isolates of Salmonella, E. coli and enterococci were compared with the ResFinder data which overall were 99.8% in concordance. In the US, 76 multi-drug resistant E.coli and 285 Salmonella from the NARMS project were genotypically tested and revealed a sensitivity of 99.6% and 98.6%, respectively with the expected phenotypical profile. This indicates the strength and sensitivity of an NGS approach in combination with bioinformatics tools. Recently, a commandline version of the ResFinder tool has also been used in a metagenomic project as proof of concept to survey larger populations for the presence of AMR bacterial pathogens. Here, wastewater from 18 incoming airplanes was investigated providing an insight to the presence of AMR pathogens. This approach could likely be expanded to potentially monitor parts of the global population for the presence of AMR pathogens and responsible genes. Overall, the advancement into NGS and bioinformatic tools will provide some future opportunities for AMR surveillance. However, the expansion also

brings some challenges which needs to be addressed such as detection of novel or unknown resistance mechanisms, curation of databases, truncated contigs, missing standards and QC thresholds, silent genes and the data in context to plasmids, integrons, IS, and the chromosome.

O005

Clonal spread of vancomycin-resistant *Enterococcus faecium* between multiple Dutch hospitals inferred from whole genome sequence data

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Introduction: Since 2011 vancomycin-resistant *Enterococcus faecium* (VRE) has rapidly emerged in hospitals in the Netherlands. VRE is of particular relevance, as they are often multidrug-resistant, which drastically limits treatment options. It is therefore of great importance to understand the spread of VRE's in, but also between hospitals. Previous studies showed traces of evidence of transmission of VRE clones between Dutch hospitals through multilocus sequence typing (MLST), but this method lacks the resolution to reliably resolve transmission events.
Method: Here we applied whole genome sequencing of 682 VRE (299 *vanA*, 265 *vanB*, 116 undetermined) from hospitalized patients from 42 different hospitals in the Netherlands. Isolates were sequenced with either an Illumina MiSeq or NextSeq and assembled. Core genome multilocus sequence typing (cgMLST) was performed using the previously described *E. faecium* cgMLST scheme that index variation in 1423 core genome genes (de Been et al, JCM 2015). Genetic relationships between cgMLST profiles were inferred by constructing a Minimum Spanning Tree (MST).
Results: A total of 20 VRE (11 *vanA*, 7 *vanB*, 2 undetermined) had to be excluded from the analysis due to the fact that more than 10% of cgMLST alleles were missing. cgMLST analysis of 662 VRE resulted in 167 unique profiles. Of these, 95 cgMLST profiles (57%) were singleton profiles, while 72 profiles (43%) could be grouped in 34 cgMLST clusters of clonally related isolates using the previously defined cluster threshold (de Been et al.) of containing at least 2 isolates differing in no more than 20 alleles. A total of 23 clusters (68%) contained strains from up to six hospitals. Moreover, of the 167 unique cgMLST profiles, 17 profiles (10%) represented isolates from multiple hospitals. In some cases isolates from hospitals with significantly distant geographical positions clustered together, with one specific cluster containing clonally related VRE from six hospitals ranging from the southeast (Venlo) to the northwest (Hoorn) of the Netherlands.
Discussion: Our results indicate clonal spread of VRE between Dutch hospitals. Whether transmission of VRE clones between different hospitals is a consequence of direct transfer of patients or staff between hospitals or that a sizable reservoir of VRE exists outside hospitals that can act as source of VRE is currently under investigation. Furthermore, the impact of horizontal gene transfer of vancomycin-resistance genes on the transmission of vancomycin-resistance among Dutch hospitals, in addition to clonal spread, is also currently being investigated.

O006

Molecular tools in (regional) outbreak prevention and control. Origin of fatal sepsis after a platelet transfusion traced by molecular typing

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Introduction: Transfusion-Transmitted Bacterial Infections (TTBI) occur rarely in the Netherlands. Only 20 reports of TTBI were made in the period from 2002-2013, most commonly after a platelet (PLT) transfusion. *Staphylococcus aureus* was involved once. In all TTBI cases, the involved blood products screened negative in bacterial screening.
Here, we describe a case of a man, 60 years, diagnosed with Multiple Myeloma, with an autologous stemcell transplantation in 2006 and since 2014 under palliative care. In November 2014 the patient presented at the First Aid department with epistaxis due to a low PLT count and was hospitalized for a PLT and RBC transfusion. Patient did not display any signs of fever or infection at the time of transfusion. After PLT transfusion (5 donorbuffycoat plateletconcentrate) an acute transfusion reaction occurred. Bacterial culture from patient blood and transfused PLT concentrate turned positive with *S. aureus* within one day. One day after transfusion the patient died.
Methods: To determine whether the same *S. aureus* isolate was found in the PLT concentrate as in the donor, molecular typing was performed using Spa and Multiple-locus variable number of tandem repeat analysis (MLVA) typing. In addition, staff attending the patient during transfusion and the 5 donors involved in the PLT concentrate were traced back and screened for nose carriage of *S. aureus* with subsequent typing in positive staff or donors.
Results: PLT concentrate and patient showed identical *S. aureus* strains: Spa type t3164/MLVA type MT5077. Nose

swabs of the staff did not show *S. aureus* carriage, while among the donors *S. aureus* carriage was seen in 4 of the 5 donors. Typing demonstrated that one of the 4 donors was carrier of *S. aureus* Spa type t3164, MLVA type MT5077.
Conclusion: This investigation is one of the few reported cases where *S. aureus* carriage in a donor might be related to a fatal acute sepsis by an immunocompromised patient after a platelet transfusion. However, additional investigations into the prevalence of this *S. aureus* strain in the community would further strengthen this remarkable result.

O008
Early stress and the microbiome
C de Weerth
Radboud University, Behavioural Science Institute, Nijmegen

Recent studies highlight the important role for health of bacteria in our gut, the *intestinal microbiota*. Moreover, animal studies show that this microbiota also modulates brain, behavior and cognitive development. These effects occur early in life and are vulnerable to stress. Comparable effects of the intestinal microbiota in humans have not yet been studied, but may be profound: whether we are easy or difficult babies, anxious or outgoing persons, or even whether we become depressed. Because the microbiota can be modified (e.g. by probiotics) such studies open monumental possibilities of using bacteria to positively guide human development.
In the present talk, I will present data from our ongoing prospective longitudinal study on infant and child development, the Bibo study. This healthy cohort (n = 193) was followed from pregnancy and 9 intestinal microbiota samples were collected from birth till the age of 4 months. In a first study we used these samples to follow the development of excessive crying, or colic, in the infants. We found that the microbiota of infants with colic was characterized by a specific signature of lower microbiota stability and complexity, and different microbiota composition than control infants. These differences were observed in the first weeks of life, before the colic has become established. Next, in the first human study relating maternal prenatal stress to infant microbiota and health, we showed strong relationships between maternal prenatal psychological stress and cortisol concentrations, and infant microbial signatures. Infants born from high-stress mothers had significantly higher relative abundances of potentially pathogenic Proteobacteria, and lower relative abundances of several groups of Lactobacilli, Actinobacteria, and Clostridia. Further, the aberrant colonization pattern appeared to predispose the infants to gastrointestinal illness and allergy.
The mechanisms underlying the links between early life stress, behavior and intestinal microbiota in humans are as yet to be discovered. In this talk, I will discuss potential mechanisms, together with several novel findings, and our ongoing studies in this field.

O009
Allergy management by protein hydrolysates and prebiotics – closer to breastfed
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Introduction: The protective effects of breastfeeding against infections and potentially also allergy, is in part attributed to the presence of non-digestible oligosaccharides in human milk which impacts the developing gut microbiota of infants. In this study the effects of early life nutrition on gut microbiota composition in infants at risk of atopy was investigated.
Methods: This study is part of a parent study registered with trial number ISRCTN65195597 investigating the effects of a partially hydrolysed formula containing specific oligosaccharides (pHF-OS) on the prevention of eczema in infants at risk for atopy. Infants were randomly assigned, if parents decided to (partially) stop breastfeeding (BF), to receive pHF-OS containing a specific mixture (0.8 g/100 ml) of oligosaccharides including short chain galacto-oligosaccharides and long chain fructo-oligosaccharides (9:1), or standard cow's milk formula (control) for the first 6 months of life. Gut microbial composition was investigated in a set of vaginally born infants, including breastfed infants (n = 30) and infants receiving pHF-OS (n = 51) or control formula (n = 57). Faecal bacterial compositions were analysed by 16S ribosomal RNA gene sequencing of DNA extracted from stool samples in the first 6 months of life. In addition major microbial metabolites (lactate and short-chain fatty acids) and stool pH were determined. Statistical analyses involved multivariate explorative data analysis using Canoco 5 software and differential abundance testing using the R-package MetagenomeSeq. All statistical comparisons between feeding groups were corrected for ethnicity and having siblings.
Results: Intake of pHF-OS was associated with a significant increase of Bifidobacterium and significantly decreased abundances of Clostridium and an unassigned genus of Lachnospiraceae when compared to infants receiving control formula. These changes were associated with marked differences in gut eco-physiology, characterised by a lower stool pH and increased proportions of lactate and decreased proportions of propionate, butyrate, isobutyrate and isovalerate. Overall the gut microbiota composition and

activity in infants receiving pHF-OS was more similar to breastfed infants than to infants receiving control formula. The bacterial biodiversity was not different between pHF and control formula and was significantly lower in BF infants compared to either of the formula fed groups.
Conclusion: Intervention with pHF-OS modulates the developmental gut microbiota towards a pattern closer to BF infants. Future investigations will be directed towards association of these gut microbiota changes with allergic outcomes in these subjects.

O010

Gestational age of preterm infants is associated with intestinal microbiota composition and activity

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Introduction: Development of the gastrointestinal tract and immune system in early life can be modulated by the gut microbiota. Compositional development of the gut microbiota, in its turn, is known to be affected by host- and environmental factors. As such, development of the gut microbiota is greatly impacted in preterm infants, who have an immature gut and are in many cases exposed to environmental factors like hospitalisation, antibiotic treatment and formula feeding. The extent to which preterm infants are associated with organ immaturity and special care depends greatly on gestational age (GA) and could therefore lead to divergent microbiota development in infants of varying GA. We aim to study the establishment and function of the intestinal microbiota of preterm infants born at varying GA.
Material and Methods: Faecal samples from five extremely preterm (EP, 25-27 weeks GA) and five very preterm (VP, 30 week GA) infants were collected during the first six postnatal weeks. Faecal microbiota was analysed by 16S rRNA gene sequencing and functionally characterised by analysing the metaproteome through LC-MS/MS.
Results: During the first six postnatal weeks, rapid bacterial colonisation occurred as indicated by the increase in bacterial derived proteins from 1.5% in meconium, towards 45.2% at postnatal week six. However, in EP infants born at 25-26 weeks gestation, bacterial proteins accounted for only 15% of total proteins at week six, suggesting delayed overall bacterial colonisation in these infants. During the first two postnatal weeks, microbiota composition showed high inter- and intra-individual variation, but dominance of facultative anaerobic bacteria in all preterm infants. From the third postnatal week, a GA-dependent microbial signature could be identified. In contrast to VP infants, where *Bifidobacterium* dominated the intestinal microbiota, EP infants were predominantly colonised by facultative anaerobic bacteria. *Streptococcus* and *Enterobacter* were dominant in infants born at 25-26 and 27 weeks respectively. In addition, a GA-dependent functional profile could be identified. High abundance of proteins involved in membrane transport and translation indicated generation and maintenance of biomass in EP infants. The VP infants *Bifidobacterium*-dominated microbiota directed its activity to carbohydrate- and energy metabolism, indicating an established microbiota with metabolic activity towards human milk fermentation.
Conclusion: These results indicate that GA of preterm infants is strongly associated with microbiota composition and function. As the gut microbiota plays a major role in development of the neonate, GA could set the state for early and later life health complications via the interference with microbiota development.

O011

Bacterial metabolism of isoprene: a much neglected atmospheric trace gas

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Isoprene (methyl isobutene), is a climate-active volatile organic compound that is released into the atmosphere in similar quantities to that of methane, making it one of the most abundant trace volatiles. Large amounts of isoprene are produced by trees but also substantial amounts are released by microorganisms. The consequences on climate are complex. Isoprene can indirectly act as a global warming gas but in the marine environment it is also thought to promote aerosol formation, thus promoting cooling through increased cloud formation. We have been studying bacteria that grow on isoprene. These aerobic bacteria appear to be widespread in the terrestrial and marine environment. *Rhodococcus* AD45, our model organism, oxidizes isoprene using a soluble diiron centre monooxygenase which is similar to soluble methane monooxygenase.
The physiology, biochemistry and molecular biology of *Rhodococcus* AD45 will be described, together with genome analysis, transcriptome analysis and regulatory mechanisms of isoprene degradation by bacteria. The distribution, diversity and activity of isoprene degraders in both the terrestrial and marine environment has been studied using functional gene probing and DNA-Stable Isotope Probing experiment. Results indicate that isoprene-degrading bacteria are widespread in soils, leaf surfaces and estuarine sediments.

O012

Rising CO2 concentrations will intensify phytoplankton blooms in eutrophic and hypertrophic lakes

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Dense phytoplankton blooms often deplete dissolved CO_2 concentrations and raise pH. Yet, quantitative prediction of the feedbacks between phytoplankton growth, CO_2 drawdown and inorganic carbon chemistry of aquatic ecosystems has received little attention. We developed a mathematical model to predict dynamic changes in dissolved inorganic carbon (DIC), pH and alkalinity during phytoplankton bloom development. We tested the model in chemostat experiments with the toxic freshwater cyanobacterium *Microcystis aeruginosa* at different CO_2 levels. The experiments showed that dense blooms sequestered large amounts of atmospheric CO_2, not only by biomass production but also by inducing a high pH and alkalinity that enhanced DIC storage capacity. We used the model to explore how phytoplankton blooms of eutrophic waters will respond to rising CO_2 levels. The model predicts that rising CO_2 levels will enhance phytoplankton blooms in low- and moderately alkaline waters. However, above some threshold, rising CO_2 will alleviate phytoplankton blooms from carbon limitation, resulting in less intense CO_2 depletion and a lesser increase in pH. These findings warn that rising CO_2 levels will intensify phytoplankton blooms in eutrophic and hypertrophic waters.

O013

Happily ever after? How repeated subcultivation influences a methanotrophic marriage

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Methane (CH_4) is an important greenhouse gas, with a global warming potential 34 times larger than carbon dioxide over a time horizon of 100 years.[1] Methane oxidizing bacteria (MOB) could be employed for both the mitigation of CH4 emissions and the recovery of the carbon and energy derived from CH_4. Recently it has been shown that methane oxidation is stimulated by non-methanotrophic partners in a methanotrophic interactome.[2,3]. Beneficial partnerships with the MOB are limited to specific strains.[4] The MOB offer the partners methane-derived carbon, while the partners can probably alleviate stress induced by self-inhibitory compounds from methane oxidation metabolism[5] or offer the MOB metabolites such as vitamins.[6] However, it is not yet elucidated what the exact mode of interaction between the MOB and their partners is.To gain a better understanding of the methanotrophic partnerships we used a synthetic ecology approach to constitute 'marriages' (multiple cycles of co-cultivation between MOB and their partners). We investigated how the community structure of an alpha- (type II) or a gammaproteobacterial (type I) aerobic MOB with 8 randomly partners evolved over time by means of 16S rRNA gene-DGGE (complex 'marriage'). We also investigated the interactions of the MOBs with 2 partners, 1 fixed and one variable (simple 'marriage') using qPCR-assays (pmoA/16S rRNA gene based) specific to each partner combined with Illumina MiSeq 16S rRNA gene amplicon sequencing. These 2 partners were selected from a preliminary screening of 38 random proteobacteria based on the compatibility with the MOB (highly, moderately and lowly compatible). In each cycle of the 'marriage', the MOB and the fixed partner (moderately compatible) were challenged with a variable partner. In total 6 variable partners (2 of each compatibility class) were evaluated. We showed that for the complex 'marriage', a selection of partners occurred, highlighting specificity of MOB and partner interactions. While no clear improvement of the methane oxidation rates (MOR) could be observed, the lag time until methane oxidation started was reduced upon co-cultivation with partners. In the simple 'marriage' a differential impact of the variable partner on the MOR was observed as the number of co-cultivation cycles increased, depending upon the type of the MOB. We observed that the initial partnership with the fixed partner was very easily outcompeted by a variable partner, regardless of the initial compatibility. This shows that adaptation through repeated co-cultivation generally did not offer an advantage to the fixed partner, although when the variable partner was moderately compatible a co-existence with the fixed partner was possible. Given the importance of these biological interactions for methane oxidation in the environment as well as in biotechnological applications, our insights could be employed for microbial resource management (MRM) to steer the composition and performance the methanotrophic microbiome *in situ*.

References

1. Myhre G., Shindell D, Bréon F-M, et al, 2013. Anthropogenic and natural radiative forcing. In: Climate change 2013. The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker TF, Qin D, Plattner G.-K., et al (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 659-740.
2. Ho A, de Roy K, Thas O, et al. The more, the merrier: heterotroph richness stimulates methanotrophic activity. ISME J. 2014;8:1945-8.

3. Oshkin IY, Beck DA, Lamb AE, et al. Methane-fed microbial microcosms show differential community dynamics and pinpoint taxa involved in communal response. ISME J. 2015;9:1119-29.
4. Stock M, Hoefman S, Kerckhof FM, et al. Exploration and prediction of interactions between methanotrophs and heterotrophs. Res Microbiol. 2013;164:1045-54.
5. Hanson RS, Hanson TE. Methanotrophic bacteria. Microbiol Rev. 1996;60:439-71.
6. Iguchi H, Yurimoto H, Sakai Y. Stimulation of methanotrophic growth in cocultures by cobalamin excreted by rhizobia. Appl Environ Microb. 2011;77:8509-15.

O014

Climate change effects on greenhouse gas balances of shallow lakes

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Shallow lakes play an important role in the global carbon (C) cycle by processing large amounts of carbon. It is therefore critical to understand how climate change affects C cycling in shallow lakes. The predicted global temperature increase (+2 to 4 °C) might have a substantial impact on C cycling and greenhouse gas (GHG) emissions. For instance, methanogenesis rates are expected to increase at higher temperatures. However, since methane oxidation rates are also temperature dependent and thus expected to increase, the net effect on diffusive methane (CH_4) emissions remains unclear. Besides diffusive emission, CH_4 can also be emitted via ebullition (bubble release). This emission pathway differs from diffusive emission by largely avoiding CH_4 oxidation by methanotrophic bacteria at oxic water and/or sediment interfaces, a process which is known to consume 30-99% of the CH_4 produced in lakes. This makes a direct relation between ebullitive CH_4 emission and methanogenesis likely to exist. We therefore hypothesize that a global temperature increase will imply a substantial further increase of the ebullitive emission, but not necessarily of the diffusive emission of CH_4, a 34 times more potent GHG than carbon dioxide (CO_2). To test our hypothesis we conducted a controlled indoor mesocosm experiment to unravel the effects of temperature on the C cycling and GHG fluxes at the sediment-water and water-atmosphere interface. The temperature controlled mesocosms (so called limnotrons) were set-up as to mimic phytoplankton dominated lakes and were kept at two temperature regimes simulating an average (temperature) year, based on Dutch conditions, and a warm (+4 °C) year. The experiment was monitored for a full year and included measurements of ebullitive and diffusive fluxes of CH_4 and CO_2. At the conference we will present the results of this experiment and discuss the effects of temperature on emissions of CH_4 and CO_2.

O015

Discovery of Nitrospira as complete ammonia oxidizer (comammox)

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Nitrification, the step-wise oxidation of ammonia to nitrate, is a process catalyzed by two physiologically distinct clades of microorganisms. First, ammonia-oxidizing bacteria and archaea convert ammonia to nitrite, which subsequently is oxidized to nitrate by nitrite-oxidizing bacteria. This division of labor was already described by Winogradsky in 1890 and is a generally accepted characteristic of the biogeochemical nitrogen cycle. Even though the existence of a single organism capable of catalyzing complete nitrification was not reported to date, this reaction is energetically feasible and was postulated to occur under conditions selecting for microorganisms with lower growth rates, but higher growth yields than canonical ammonia and nitrite-oxidizing bacteria. Thus, these organisms could have a competitive advantage in biofilms and other microbial aggregates growing at low substrate concentrations. In this study, we enriched for microorganisms responsible for nitrogen transformations in an ammonium-oxidizing biofilm, which was sampled from the anaerobic compartment of a biofilter connected to a recirculating aquaculture system. This enrichment culture contained two *Nitrospira* species that had all genes required for ammonia and nitrite oxidation in their genomes. Batch incubation experiments indicated that these *Nitrospira* indeed formed nitrate from the aerobic oxidation of ammonia, and FISH-MAR confirmed their ability to use the energy derived from ammonia and nitrite oxidation for carbon fixation. Their ammonia monooxygenase (AMO) enzymes were phylogenetically distinct from canonical AMOs, thus rendering recent horizontal gene transfer from known ammonia-oxidizing microorganisms unlikely. Instead, their AMO subunit A (AmoA) displayed highest similarities to the 'unusual' particulate methane monooxygenase from *Crenothrix polyspora*, thus shedding new light onto the function of this largely uncharacterized sequence group. Our results show by the recognition of a novel AmoA type that a whole group of ammonia-oxidizing microorganisms was previously overlooked and thus will lead to a better understanding on the environmental abundance and distribution of this functional group. Furthermore, the discovery of the long-sought-after comammox process will change our perception of the nitrogen cycle.

O016

Do organic fertilizers influence the abundance and diversity of resistance genes and mobile genetic elements in agricultural soil?

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Organic fertilizers, such as livestock manure, biogas digestates or sewage sludge were analyzed by cultivation-independent methods and shown to contain high abundances of bacteria carrying resistance genes (RGs) and mobile genetic elements (MGEs). Most importantly, transferable RGs could be captured into *E. coli* or *Pseudomonas putida* strains by exogenous plasmid isolation from most of the manure, digestate and sewage samples analyzed. Molecular characterization of the captured plasmids revealed that a large proportion belonged to the broad host range IncP-1 plasmids known to be efficiently transferred in soils and in the rhizospheres. Thus with organic fertilizers not only nutrients important for plant nutrition but also bacteria carrying transferable RGs and antibiotic residues are introduced into soil.

The effect of organic fertilizers on soil bacterial communities was investigated in microcosm and field experiments. Amplicon sequencing of rRNA gene fragments amplified from total community DNA revealed that the addition of organic fertilizers caused changes of the soil bacterial community composition. Furthermore, initially a strong increase of RGs and MGEs relative abundances in total community DNA from soil was observed that vanished with time. Both soil types and also the presence of antibiotics seemed to influence the extent of bacterial community shifts. The addition of manure clearly increased the frequency of capturing plasmids conferring multiple antibiotic resistances into recipients such as *E. coli*.

In a recently performed field study, the effects of different fertilizers (mineral, manure, digestate) on RG and MGE relative abundances and bacterial community composition were investigated in soils and in the rhizosphere of maize. In comparison to the mineral fertilized plots, a transient increase of the relative abundance of integrons and RGs was observed in the soil samples with organic fertilizer treatments. At harvest, no treatment effects were detected in bulk soil, while an increased relative abundance of some RGs and *intI1* was still detectable in the rhizosphere of maize grown in the soil plots treated with organic fertilizers in comparison to the plots treated with mineral fertilizers. This indicates that organic fertilizers have a transient effect on the composition of soil bacterial communities, and their potential contribution to RG spread in agro-ecosystems might depend on the co-introduced antibiotic residues and metal compounds (reviewed by Jechalke et al., 2014).

Reference

Jechalke S, Heuer H, Siemens J, et al. Fate and effects of veterinary antibiotics in soil. Trends Microbiol. 2014;22:536-45.

O017

Back to the Roots: microbiology & chemistry at the plant-soil interface

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Plants are colonized by an astounding number of microorganisms that can reach cell densities much greater than the number of plant cells. Specific members of the plant microbiome can have profound effects on plant growth and development, nutrition and tolerance to diseases and abiotic stress. For the vast majority of plant-associated microbes, however, there is limited knowledge on the mechanisms involved in modulation of plant growth and health. Novel omics technologies have provided more in-depth understanding of the diversity and functioning of the plant microbiome and significant advances are being made to uncover the multitrophic interactions on plant surfaces. To better understand this intriguing complexity, both systems ecology and reductionists' approaches are needed to identify biotic and abiotic factors involved in microbiome assembly and activity. Here, new results are presented on how soil and rhizosphere bacteria impact on plant root architecture, plant chemistry and tolerance to soil-borne pathogens. Also the impact of plant domestication on microbiome composition and functions will be presented.

O018

Ecology of *E. coli* in plant seed production systems

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The ehec strain responsible for the disease outbreak in Hamburg, 2011, was presumed to originate from Fenugreek seeds. Seeds thus can play a role in transmission of human pathogens to growing plants and to edible products derived from plants. We therefore explored the possibility of transmission of *Escherichia coli* from artificially contaminated seeds to mature plants and to

seeds derived from these plants. Pea (*Pisum sativum*) was chosen as model plant and pea seeds were inoculated with *E. coli* cells up to levels of between 10^7 - 10^8 per seed. Two experiments were conducted, one in the greenhouse and the other in the open field. In the greenhouse trial transmission from seed to plants and to the next generation of seeds of three different *E. coli* strains (one clinical isolate and two isolates originating from plants) were evaluated. Cells of all three strains were able to colonize shoots and roots up to 62 days of growth in the greenhouse, although *E. coli* was not consistently present in all analysed plants (n = 5 per treatment) after 62 d. Average numbers in roots were between log 0.21 and 2.20 CFU/ g and in shoots between log 0.64 and 0.88 CFU/g. However, a higher preference was shown for the root zone than for shoots and reproductive organs for all three strains. Artificial seed contamination never resulted in contamination of the next generation of seeds, even after a second inoculation event by injection of inoculant cells into flower receptacles for all three strains. In the field trial we evaluated one strain, the clinical *E. coli* O104:H4 strain, on transmission from seed to roots, rhizosphere, shoots reproductive organs and seeds of growing pea plants. Roots and rhizosphere soil were shown to be the preferred sites for colonization by *E. coli* O104:H4 and CFUs were shown to be present in the rhizosphere after 84 d of plant growth in the field up to an average level of log 0.17 (roots) and 0.24 (rhizosphere) CFU/ g roots or dry soil, respectively. No *E. coli* CFUs were found to be present in surface-sterilized stem samples or in the next generation seeds. We therefore concluded that all tested *E. coli* strains were able to colonize pea plants from seeds, irrespective of the origin of the isolate. The root zone was shown to be the preferred site for colonization by *E. coli*. Introduced *E. coli* cells were not demonstrated to contaminate next generation of seeds, indicating that the transfer of *E. coli* via seeds to next generations of plants will be an unlikely event.

O019

Cycling of EHEC bacteria across animal and plant ecosystems

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The hypothesis that human pathogens belonging to the class of *Enterobacteriaceae* are able to cycle across different habitats in plant and animal production systems and may acquire new traits like virulence and resistance factors via horizontal gene transfer formed the basis of this research. We tested this hypothesis at a dairy farm, holding calves in a stable and a cattle of heifers on grassland and followed the fate of EHEC bacteria in different habitats by taking samples from manure from the stable, from roots and shoots of grass with and without recent heifer droppings and from surface water from an adjacent ditch.

Samples were microbiologically analysed by cultivation dependent and cultivation independent techniques. We were able to isolate different *E. coli* serotypes from manure, grass rhizosphere and grass shoot samples by specific enrichment for *E. coli*. At least two different *E. coli* serotypes were found in grass shoots and rhizosphere soil, indicating that *E. coli* may have been transferred from manure to grass. Via molecular approaches, we detected virulence (eae, stx and hly genes) and plasmids of different incompatibility groups typical for *E. coli* in grass shoot, rhizosphere soil and manure DNA extracts and in extracts from isolates from the same habitats and ditch water. DNA sequences representative for prophages, as well as presence of coli-phages in grass shoot, rhizosphere soil and manure extracts and in ditch water were found.

In this exploratory study, we demonstrated the presence of different *E. coli* serotypes harbouring virulence genes, such as for shiga toxin, in manure, grass, soil and ditch water samples, indicating the potential risk of these isolates for humans. The presence of (pro) phages and plasmids in these isolates as well as in community DNA extracts derived from manure, grass and soil show that genetic exchanges of *E. coli* strains with each other and / or with other members of the indigenous communities in analysed habitats probably occur. We demonstrated a part of the transmission cycle by showing that potentially pathogenic *E. coli* strains from cow manure could pass the soil barrier to reach grass.

O020

Directed evolution workflows with microfluidic emulsions and their derivatives

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Miniaturising biological assays in water-in-oil emulsions is a versatile means for working with populations of cells in high numbers. Compartmentalisation for example provides an isolating environment for encapsulated cells preventing direct competition for resources, which enables directed evolution of cellular phenotypes not accessible with other methods.[1] While selection regimes based on growth rate can simply be conducted by iterative cycles of emulsification, incubation, de-emulsification and dilution of sample, many other applications require active selection of improved variants based on the readout of a biological assay. For intracellular fluorescent phenotypes, Flow

Cytometry is a convenient means to screen large cell populations. With phenotypes based on the excretion of compounds, microfluidic emulsions provide a versatile technique to screen samples with comparable throughput, while maintaining genotype and phenotype linked.

Droplet microfluidics is a versatile toolkit that allows to process aqueous samples in extreme miniaturization and throughput. The monodispersity of microfluidic emulsions makes it possible to conduct quantitative measurements, which means that phenotypes can be assessed with high accuracy. Monodisperse droplets are produced with microfluidic chips in kHz throughput and a set of microfluidic building blocks has been created that allows droplet generation, incubation, droplet-droplet fusion, on-chip sorting and more. While droplet microfluidics is very powerful, it needs a considerable amount of specialist know-how because most of the technology is not commercially available.

An alternative to conducting workflows entirely on chip, is to use only microfluidic droplet generators and to convert emulsions into monodisperse double emulsions[2] or emulsion-templated beads.[3] The resulting samples are dispersions in an aqueous phase, which allows them to be processed in ways similar to cells, for example sorted with FACS. Templated beads in addition allow to conduct multiple biochemical reactions in a step-by-step fashion by iterative emulsification/de-emulsification cycles, while maintaining genotype-phenotype links intact.

The talk will introduce droplet microfluidics, show relatively simple workflows that are accessible to non-specialist labs, and discuss how the technology can be applied to directed evolution campaigns.

References

1. Bachmann H; Fischlechner M; Rabbers I, et al. Availability of public goods shapes the evolution of competing metabolic strategies. Proc Natl Acad Sci U S A. 2013;110:14302-7.
2. Zinchenko A, Devenish SR, Kintses B, et al. One in a million: flow cytometric sorting of single cell-lysate assays in monodisperse picolitre double emulsion droplets for directed evolution. Anal Chem. 2014;86:2526-33.
3. Fischlechner M, Schaerli Y, Mohamed MF, et al. Evolution of enzyme catalysts caged in biomimetic gel-shell beads. Nat Chem. 2014;6:791-6.

O022

Laboratory evolution of constitutive acetic-acid tolerance in *Saccharomyces cerevisiae*

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During hydrolysis of lignocellulosic material for the production of second-generation bioethanol, acetic acid is released with a potential negative impact on yeast growth and alcoholic fermentation. An important factor in acetic-acid tolerance is the fraction of the cells in the inoculum that are able to grow when *Saccharomyces cerevisiae* is freshly transferred to medium containing acetic acid. Yeast strains that are constitutively tolerant to acetic acid would therefore benefit the process economy. The aim of this study is to investigate how laboratory evolution and mutagenesis can be used to specifically obtain *Saccharomyces cerevisiae* strains with constitutive acetic-acid tolerance. After evolution, a combination of whole genome sequencing, and crossing and segregation studies was used to investigate the mutations underlying the constitutive acetic-acid tolerant phenotype. The impact of these mutations on acetic-acid tolerance was confirmed by reverse engineering the evolved phenotype into the wild type background. Based on this knowledge the acetic-acid tolerance of industrial yeast strains can either be improved through applying these novel evolution strategies or via direct introduction of the genetic changes that were identified in this study.

O023

Adaptation of a riboswitch to a new ligand

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Riboswitches found in nature most often control the expression on translation level, inhibiting translation unless induced or the exact opposite. Most riboswitches are part of the mRNA it controls, acting as a cis regulatory element. Regardless of their specific mechanism, riboswitches have in common that it changes its conformation upon either induction or repression by an external effector. The nature of riboswitch effectors ranges from metal ions like $Mg^{2}+$ to small molecules like amino acids; in addition to chemical stimuli, also physical shifts may trigger a riboswitch response (e.g. temperature). In case of a molecule, the effector binds the aptamer domain of the riboswitch and causes a conformational change that is transduced to a platform domain. The structure of this second domain then changes in such a way that it alters (either positively or negatively) the translation of the corresponding coding sequence.

For synthetic purposes it would be useful if the aptamer specificity of riboswitches could be engineered. However, when substituting the aptamer sequence, the ligand binding to the RNA alone generally does not induce the required allosteric change in the platform. The way the aptamer is grafted onto the platform dictates its behaviour. A generic way to successfully graft the aptamer onto the platform requires a communication module. This communication module can cause the aptamer to either allow translation upon binding or cause it to block translation, depending on the desired action.

An interesting application of riboswitch systems would be the high-throughput screening of large libraries of cells for variants that generate a product of interest. For that purpose, an in vivo system is required for selecting riboswitches with adjusted specificity. We developed a system that gives substantial growth advantage to bacteria with a functional riboswitch, allowing for the efficient selection of specific riboswitches. This selection system can also be applied to find aptamer variants with an altered ligand range.

O024

A major reduction in the use of antimicrobials for livestock in the Netherlands since 2009: the critical success factors

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Introduction: Use of antimicrobials in animals poses a potential risk for public health as it contributes to the selection and spread of antimicrobial resistance (AMR). In the Netherlands the total therapeutic antimicrobial use (AMU) (in mass sold) in farm animals doubled between 1990 and 2007. A series of measures and initiatives were triggered by the findings of widespread presence of multi-resistant bacteria (MRSA, ESBLs) and discussions in the general public regarding agricultural practices.
Methods: In 2008 joint initiatives by the Dutch Government, livestock sectors and the Royal Dutch Veterinary Association (KNMvD) resulted in a covenant describing measures for prudent use of antimicrobials. One of the decisions was to establish the Netherlands Veterinary Medicines Authority (SDa). This authority was founded with the tasks to i) collect the antimicrobial usage data of Dutch livestock, initially pig, poultry, veal calves and dairy farms (approximately 42,000 units) and define benchmark targets for AMU in these sectors (in defined daily dosages per animal year); ii) report annual trends; iii) identify frequent- or mis-users/prescribers; iv) assess the effect of improvement programs developed by the livestock sectors. Reduction targets were set by the government at 20%, 50% and 70% reduction in 2010, 2013 and 2015, respectively, with reference to 2009. Next to the founding of the SDa, several other actions were performed at different levels (e.g. advices of the Dutch Health Council and the Antibiotics Policy Working Group of the KNMvD, and the development of treatment guidelines of the KNMvD). Parallel to these actions, continuous monitoring of resistance in commensal *E. coli* had already been set up in livestock from 1998 onwards, enabling measurement of trends in resistance.
Results: The total reduction of AMU (in mass sold) between 2009 and 2014 was 58.1%. Compared to 2007, the year with the highest veterinary usage there was a reduction of 65%. The use of antimicrobials defined as ''critically important for human health'' (fluoroquinolones and 3rd and 4th generation cephalosporins) in livestock reduced to almost zero. These achievements have been made by Dutch farmers and their veterinarians through improved infection and health control measures, which resulted in overall less, and more individual treatments instead of group treatments. As a result of the enforced 1-to-1 relationship of farmers and veterinarians, it was possible to develop the Veterinary Benchmark Indicator allowing to compare prescription levels between veterinarians. The SDa has played a crucial role by making the reporting of AMU transparent for all farms, by benchmarking farms (action, signaling and target level) and by benchmarking veterinarians. The government played a crucial role by setting targets. Parallel to reduction of AMU there was a reduction of AMR in livestock observed.
Conclusion: The critical success factors were: clear targets defined by the government (created a sense of urgency), measures initiated by private animal production sectors and veterinary association (need for collaboration), having fully transparent usage data and the founding of an independent Netherlands Veterinary Medicines Authority (accepted by all parties involved). The reduction of AMU appears to be effective in reducing AMR in livestock.

O025

Changing characteristics of LA-MRSA isolated from humans in the Netherlands. Emergence of a subclade transmitted without livestock exposure

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Introduction: Since its first detection in 2003, livestock-associated MRSA (LA-MRSA) has become the predominant MRSA clade isolated from humans in the Netherlands since 2007. In this study, we molecularly characterized all submitted human LA-MRSA isolates for the national MRSA surveillance from 2003 to 2014 to assess the characteristics and possible temporal changes of the most predominant MRSA clade in the Netherlands.

Methods: Over 9,000 LA-MRSA isolates submitted from 2003 to 2014 for the Dutch MRSA surveillance were characterized by multiple-locus variable number of tandem repeat analysis (MLVA) and staphylococcal protein A (*spa*)-typing. The MLVA also includes the detection of the *mecA* and *mecC* genes and of the *lukF* gene, indicative of Panton Valentine leucocidin (PVL) production. Furthermore, next generation sequencing (NGS) was performed on 118 isolates and the presence of bacteriophage ϕ3 was determined for 1,538 isolates. Nearly 6,000 questionnaires provided epidemiological data.

Results: Over 80% of the Dutch LA-MRSA isolates belonged to one of three predominant MLVA/*spa*-types; MT398/t011, MT572/t108 and MT569/t034. After an initial rapid increase with a peak in 2009 (n = 1,368), the total number of submitted LA-MRSA isolates has been slowly decreasing to 968 in 2014. The decrease could be largely attributed to the drop in submitted MT398/t011 and MT572/t108 isolates and a geographical comparison showed that the decrease occurred in provinces where LA-MRSA is predominant. In contrast, the number of MT569/t034 isolates increased since 2008, surpassing MT572/t108 as the second most frequently found LA-MRSA in 2014. The increase in the number of submitted MT569/t034 isolates was not restricted to a particular province, but occurred in the entire Netherlands. NGS showed that MT398/t011 isolates clustered closely together as did MT572/t108 isolates. MT569/t034 isolates were genetically more diverse than MT398/t011 and MT572/t108 and did not partition in a single group. PVL positive LA-MRSA were only found in 23 isolates (0.3%) with 14 different MLVA/*spa*-types. The overall prevalence of ϕ3 among LA-MRSA was 2.2%, with the highest prevalence among MT569/t034 isolates (6.6%). Concurrent with the decrease in LA-MRSA in the Netherlands, fewer people reported having contact with livestock and this was most prominent for people from whom MT569/t034 LA-MRSA was isolated. The proportion of LA-MRSA isolated from infection-related materials increased from 6.3% in 2009, to 13% in 2014 and most of these isolates originated from patients older than 50 years of age. Remarkably, 83% of these patients reported not having contact with livestock.

Conclusion: There is an ongoing change in the genotypic and epidemiological characteristics of Dutch LA-MRSA isolated from humans with the emergence of a LA-MRSA subclade independent of livestock exposure. This suggests LA-MRSA starts to resemble non-LA-MRSA in terms of transmissibility and pathogenicity.

O026

Risk factors, duration of carriage and onward transmission of ESBL-producing *Enterobacteriaceae* acquired during travel: results of large prospective cohort study of travellers and their households

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Background: The COMBAT-study aims to examine the impact of international travel on the acquisition, persistence and onward transmission of extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-E).

Material/Methods: We performed a large-scale multicenter longitudinal cohort study among Dutch travellers (n = 2001) and their non-travelling household members (n = 215): the COMBAT-study. Faecal samples and questionnaires were collected before and immediately after travel, and at 1, 3, 6 and 12 months after return. Acquisition was defined as the absence of ESBL-E in pre-travel and their presence in post-travel samples as determined by phenotypical tests. ESBL genes were genotypically characterized using microarray, targeted PCR and DNA sequencing. Isolates with MICs for imipenem >1 mg/L or for meropenem >0,25 mg/L (confirmed with E-test) and isolates with MIC for colistin >2 mg/L (confirmed with E-test) were genotypically characterized for presence of carbapenemase-encoding genes and the *mcr-1* gene respectively, by PCR and sequence analysis..

Multivariable logistic regression analyses were conducted to identify risk factors for ESBL-E acquisition during travel. Transmission of ESBL-E within households was estimated with a maximum likelihood method using a Markov model including culture data of all samples immediately after travel until 12 months post-travel.

Results: The acquisition rate of ESBL-E during travel was 34% (CI_{95} 32-36%) among travellers (n/N = 633/1847). From these travellers, a total of 859 ESBL-positive strains (759 *E. coli*, 67 *K. pneumoniae* and 33 other species) were isolated, mainly carrying *CTX-M-15* (51%) and *CTX-M-14* (15%) genes. Three ESBL-producing E. coli (ESBL-Ec) isolates co-produced a carbapenemase, NDM-1/2, NDM 7

or OXA-244. Moreover, in six of nine ESBL-Ec isolates with a colistin MIC of 4-8 mg/L, the *mcr-1* gene was detected.
Acquisition rates of ESBL-E varied widely according to travel destination with highest rates among travellers to Southern Asia (75%, CI_{95} 64-89%) and Central/Eastern Asia (49%, CI_{95} 36-66%).
Risk factors for acquisition of ESBL-E were: antibiotic use during travel (OR 2.7, CI_{95} 1.8-4.1), traveller's diarrhoea (OR 2.3, CI_{95} 1.4-3.8), daily consumption of meals at food stalls (OR 1.8, CI_{95} 1.1-3.0) and pre-existing chronic bowel disease (OR 2.1, CI_{95} 1.1-3.9). Decolonization rate of acquired ESBL-E was 0.010 (CI_{95} 0.0092-0.011) per day, corresponding to a mean duration of colonization of 100 days. Transmission of ESBL-E from an ESBL-positive person to a household member occurred at a rate of 0.0013 (CI_{95} 0.0005-0.0024) per colonized person per day. The probability to transmit ESBL-E to a household member was 12%.

Conclusions:

1. ESBL-E are frequently acquired during travel, mostly involving *E. coli* producing CTX-M-15 ESBLs.
2. Travel destination was the strongest risk factor for acquisition of ESBL-E, with travel to Southern and Central/Eastern Asia having the highest acquisition rates.
3. Previously unidentified risk factors related to eating behaviour and chronic bowel disease were identified.
4. Despite prolonged carriage of ESBL-E after return from travel, the probability of onward transmission to household members was limited.
5. Our data indicate worrisome community-spread and acquisition of ESBL-E with co-resistance against carbapenems or colistin.

O027

Cycling with lantibiotics

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Nowadays, microbial antibiotic resistance is the Achilles' heel in the fight with infections caused by multiple antibiotic resistant bacteria. Some pathogenic bacteria harbor the resistance to almost every approved antibiotic.[1] There is a tremendous necessity to find new effective compounds and/or approaches to treat infections. Natural antimicrobials – bacteriocins – are clear points of interest in the current antimicrobials research field. Noteworthy, some of bacteriocins are already applied in clinics in all major disease fields.[2,3] Lantibiotics and microcins form different groups of posttranslationally modified bacterial peptides mainly produced by Gram-positive and Gram-negative bacteria, respectively. Most of these peptides exhibit a potent antimicrobial activity, even greater than some antibiotics.
Maturation of a peptide begins with a propeptide which is guided throughout specific modification events due to leader peptide (an amino acid sequence recognized by particular modification biomodule). The leader peptide is proteolitically removed in the last steps of peptide modification making the modified peptide active. Posttranslational modifications endorse lantibiotics and microcins with high target-specific activities and stability against proteolysis.
We used a synthetic biology approach to design plug-and-play biomodules for production of novel peptides containing both, lantibiotic- and microcin-specific posttranslational modifications. Peptide modification systems were cloned under different controlled promoters to assure gradual and temporal activity of designed biomodules. Codon optimized synthetic genes of designed model peptides to be modified were employed together with modification machineries.
This is a unique case where posttranslational modifications from two different peptide classes are fused together into a single peptide chain *in vivo*. Here we also show the possibilities of employing chimeric leader peptides for substrate recognition and processing by particular posttranslational modification machineries.

References

1. Cherkasov A, Hilpert K, Jenssen H, et al. Use of artificial intelligence in the design of small peptide antibiotics effective against a broad spectrum of highly antibiotic-resistant superbugs. ACS Chem Biol. 2009;4:65-74.
2. Montalban-Lopez M, Zhou L, Buivydas A, et al. Increasing the success rate of lantibiotic drug discovery by Synthetic Biology. Expert Opin Drug Discov. 2012;7:695-709.
3. Levengood MR, van der Donk WA. Use of lantibiotic synthetases for the preparation of bioactive constrained peptides. Bioorg Med Chem Lett. 2008;18:3025-8.

O028

Dynamics of Extended Spectrum Beta-Lactamase/AmpC producing *E. coli* in broiler parent stock

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Introduction: Extended Spectrum, and AmpC Beta-Lactamases (ESBL/AmpC) are enzymes produced by bacteria rendering it resistant to beta-lactam antimicrobials. ESBL/AmpC producing bacteria are found throughout the broiler production pyramid. In the Netherlands the prevalence of ESBL/AmpC-*E. coli* in broilers at slaughter was around 66% in 2014. The aim of this study is to determine the prevalence, concentration

and persistence of ESBL/AmpC-*E. coli* in broiler parent stock during the rearing and laying period.

Methods: About 3200 one-day old birds (broiler parent stock), hatched at a commercial hatchery in Germany, were moved to a poultry farm in the Netherlands, divided into four equally sized, separately housed groups. During the study the birds didn't receive antibiotics or coccidiostats. ESBL/AmpC-*E. coli* prevalence was determined at day 7 (week 1) of age by sampling 57 randomly selected birds in each group (n = 228), using individual cloacal swabs. In week 12 in each group 57 randomly selected birds were tagged and sampled at week 12, 16, 17, 18 and 19. From week 16 onwards, environmental samples were taken in each group using bootsocks. After the rearing period two groups of 30 hens and 3 males were made and moved to the layerhouse, both groups were housed separately. Cloacal swabs and environmental samples were taken at week 21 and 24. After culturing a selection of samples containing ESBL/AmpC-*E. coli* was typed using multi locus sequence typing (MLST) and for the samples collected during the rearing period the ratio between total *E. coli* and ESBL/AmpC-*E. coli* was determined.

Results: ESBL/AmpC-*E. coli* prevalence was 91% (86-94%) at day 7 (week 1) of age and decreased to 46% (39-52%) at week 12. The prevalence further decreased to 1% (0.1-3%) (week 19) and during the laying period (week 21 and 24) prevalence was 0% (0-6%). During the sampling period almost all (22 out of 24) environmental samples were positive, even at low ESBL/AmpC-*E. coli* prevalence. The concentration of ESBL/AmpC-*E. coli* varied between <10^3 to 2X10^4 CFU/gram faeces in week 16 and <10^3 CFU/gram in week 18 and 19. Total *E. coli* counts were between 10^4 and >10^8 CFU/gram. Almost all ESBL/AmpC-*E. coli* isolates carried the AmpC gene bla_{CMY-2}. The *E. coli* sequence types showed a large variation. This suggests that the occurrence and spread of resistance is determined by plasmid transfer within the *E. coli* population.

Conclusions:

1. We are the first to show a considerable decrease of ESBL/AmpC-E. *coli* prevalence in broiler parent stock during rearing and laying period.
2. The concentrations of ESBL/AmpC-*E. coli* found in the cloacal samples of individual birds were low. However, positive bootsocks were found, even in the absence of positive birds. This suggests that birds are excreting small amounts of ESBL/AmpC-*E. coli*, or that intermittent shedders are present. Another explanation would be that birds ceased shedding ESBL/AmpC-*E. coli*, but environmental contamination persisted, or contamination from sources outside the pen occurred.
3. In the absence of antibiotics the prevalence of ESBL/AmpC-*E. coli* in broiler parent stock may sharply reduce, suggesting a selective disadvantage of AmpC gene bla_{CMY-2}.

O029

Bactericidal activity of novel antimicrobial peptide M33 against *Klebsiella pneumoniae*: insight into the mode of action

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Introduction: The spread of multidrug-resistant bacteria has limited the effectiveness of antimicrobial therapy. Research into new antibiotics has focused on various novel compounds, among which antimicrobial peptides (AMPs). These molecules commonly exert their bactericidal activity through a mechanism similar to that of the drug of last resort colistin (CST). Unfortunately, the pharmaceutical development of AMPs has been limited due to their toxicity and stability issues. Here we evaluated the antimicrobial activity of the novel AMP antibiotic 'M33'.

Methods: Minimum Inhibitory Concentration (MIC) assays using M33 and CST were performed for 50 characterized clinical isolates of *K. pneumoniae* with different antibiotic resistance profiles. Time-kill kinetics (TKK) assays were performed using M33 and CST in a two-fold concentration range (0.125 to 64 mg/L) for a multidrug-resistant, but CST-sensitive, clinical isolate of *K. pneumoniae*. After 24 hours of drug exposure changes in drug susceptibility were assessed through MIC assays. The effect of M33 on the cellular morphology of *K. pneumoniae* was investigated using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). To assess whether M33 acts non-specifically, red blood cells were incubated with M33 and supernatant absorbance measured.

Results: The susceptibility (MIC) of the 50 *K. pneumoniae* clinical isolates, including isolates carrying plasmids for ESBL, KPC, OXA-48 and NDM resistance, towards M33 remained unchanged. A *K. pneumoniae* strain with MIC values for CST and M33 of 0.5 and 16 mg/L, respectively, was further investigated in the TKK assay. During the first 6 hours of exposure, CST and M33 showed rapid, concentration-dependent, bactericidal activity. Regrowth after 24 hours occurred at all concentrations of CST (except 64 mg/L), and the initially CST-susceptible bacteria had become CST-resistant. For M33, regrowth after 24 hours was only observed at concentrations ≤ 4 mg/L, and changes in susceptibility to M33 were not observed. Regarding cellular morphology *K. pneumoniae* cells showed blistering and porosity after exposure to M33, the bacteria also becoming turgid, with protrusions from their surface and aberrations in their periplasmic spaces. Red blood cells incubated with M33 did not show significant hemolysis.

Conclusion: Susceptibility towards M33 remained the same across different resistance profiles of *K. pneumoniae*,

including isolates with multidrug resistance that are currently major health concerns. *K. pneumoniae* developed CST resistance after 24 hours exposure, whereas the susceptibility to M33 after 24 hours exposure remained unchanged. Exposure of *K. pneumoniae* to M33 resulted in obvious disturbances in the bacterial cell membrane. The absence of hemolytic activity agrees with previous studies that M33 does not act upon the eukaryotic cell membrane and does not cause the type of toxicity associated with other AMPs. Summarizing, M33 appears to be a promising new AMP antibiotic for the treatment of *K. pneumoniae* infections.

O030

Investigating *Staphylococcus aureus* outbreaks using whole genome sequencing

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Several studies have used whole genome sequencing (WGS) to investigate outbreaks of *S. aureus* and to investigate transmission. It now seems inarguable that, in comparison with standard typing techniques (*spa*, PFGE, phage-typing), WGS has enhanced resolution and therefore can determine, with a very high degree of certainty, whether isolates are closely related to each other or otherwise. Consequently, there is much enthusiasm for the adoption of WGS into routine outbreak investigation.
However, studies to date have focused on intensive care and neonatal units. There is no standardised approach and no consensus for the interpretation of WGS data or how it relates to traditional techniques, particularly in the wider hospital setting or in the community.
Using historical outbreaks previously characterised by standard typing methods, we have developed an approach to outbreak analysis using WGS. We have examined the effect of within-host diversity to determine whether current sampling strategies are adequate, and by comparing WGS data with epidemiological data have identified outbreaks where WGS provided additional information to enhance the outbreak investigation. In some outbreaks, WGS may be able to predict transmission routes which may be of particular value where epidemiological data is incomplete.

O031

Use of whole genome sequencing in the evaluation of isolation strategies for ESBL-producing Enterobacteriaceae: results of the SoM study

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In the Netherlands, resistance rates are traditionally lower than in most other countries. This is achieved by a combination of restrictive antibiotic use and active infection control in health care. In addition to standard precautions, hospitals take transmission-based precautions in order to prevent the spread of antimicrobial resistance. In 2005, the Dutch Workingparty on Infection Prevention (WIP) developed a guideline for the prevention of nosocomial transmission of highly-resistant microorganisms. According to this guideline it is recommended to apply contact precautions for patients that are colonised or infected with ESBL-producing Enterobacteriaceae (ESBL-E), where the use of a single room is preferred over a multiple room.
A multicentre, cluster-randomised, cross-over, non-inferiority study was performed between 2011 and 2014 in 14 Dutch hospitals to evaluate whether contact isolation of patients colonised with extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-E) in a multiple room is not inferior to contact isolation in a single room with respect to the transmission of ESBL-E to other patients (SoM study). Whole genome multilocus sequence typing (wgMLST) was used to identify nosocomial transmission of ESBL-E.

O032

Presumed outbreak of colistin resistant *Enterobacter cloacae* during prolonged use of selective decontamination of the digestive tract

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Background: A high prevalence of colistin resistance among *E. cloacae* isolates in two intensive care units (ICU) (of 16 and 6 beds) using selective digestive decontamination (SDD) since 1990 instigated a retrospective and prospective investigation. SDD is topical application of antibiotics (colistin and tobramycin) and systemic use of cefotaxim during the first days of ICU-admission. It was hypothesized that the use of SDD in this ICU since 1990 had contributed to this high incidence, either due to repeated events of endogenous selection or due to incidental introduction of colistin resistant strains followed by clonal transmission. A retrospective and prospective investigation was performed in combination with whole genome sequencing (WGS) to investigate the epidemiology of antibiotic resistant *E. cloacae*.
Material/Methods: Multi-resistant Enterobacter species (MREb) was defined as a strain with ESBL production and/

or tobramycin non-susceptibility (MIC>=8 mg/L) and/or colistin non-susceptibility (MIC>=4 mg/L) as determined by Vitek 2 and corfirmed by Etest. Conversion rates of colistin susceptible to resistant *E. cloacae* were calculated per 1000 patientdays at risk. Whole genome sequencing (WGS) was performed using Illumina NextSeq.

Results: Colistin-resistant *E. cloacae* was first detected in November 2009 and carriage was demonstrated in 141 patients until October 2014. Mean incidence of MREb acquisition was 4.61 and 1.86 per 1000 days at risk in ICUs 1 and 2, respectively, and the mean monthly prevalence of MREb in both ICs was 19.6 per 282 patientdays (7.0%) and 2.5 per 80 patientdays (3.1%), respectively, without a discernible trend in time. Among the MREb isolates proportions of non-susceptibility were 68.8% for colistin, 89.3% for tobramycin and 71.2% had ESBL-production. Of the MREb isolates, 82.3% was susceptible to either ciprofloxacin or trimethoprim/sulfamethoxazole, 99.1% to ciprofloxacin, trimethoprim/sulfamethoxazole or meropenem, and 99.5% to ciprofloxacin, trimethoprim/sulfamethoxazole, meropenem or colistin. A change in colistin susceptibility (from susceptible to resistant) of *E. cloacae* detected in screening cultures was observed in three patients with rectal colonization and in two other patients with respiratory colonization, corresponding to conversion rates of 0.20 and 0.13 conversions per 1000 patient days. All five patients received SDD. WGS of 84 isolates revealed two major clones with number of SNPs ranging between 11 and 126 for clone 1, and 6 and 96 for clone 2, among isolates derived between 2009 and 2014. Within the clusters there was a high level of concordance in colistin and tobramycin susceptibility, but not for ESBL-production.

Conclusions: This study demonstrates a stable low-level endemicity of MREb in two Dutch ICUs with prolonged use of SDD, which was characterized by the persistent presence of two clusters, suggesting incidental clonal transmission.

O033

Next-generation sequence (NGS) analysis reveals methicillin-resistance transfer to a methicillin-susceptible *Staphylococcus aureus* (MSSA) strain that subsequently caused a methicillin-resistant S. aureus (MRSA) outbreak

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Background: Horizontal, interspecies transfer of the staphyloccal cassette chromosome mec (SCCmec) is supposed to be an important factor in the emergence of new clones of MRSA but has been rarely observed in real life.

In September 2012 a patient of the oncology ward developed a methicillin-resistant *Staphylococcus aureus* (MRSA) bacteraemia. Active contact tracing revealed three (asymptomatic)MRSA carriers: two patients and one HCW. No known risk factors for MRSA acquisition were present in the patients or HCW. All MRSA isolates were resistant to fusidic acid. This is a relatively rare susceptibility pattern. In the past five years only 1% of all clinical isolates of Staphylococcus aureus (including MSSA and MRSA) in our laboratory, were resistant to fusidic acid. Spa-and MLVA-typing showed all isolates were t586 and MT4065-MC0008, a type of MRSA, which had not been found before in the Netherlands.

One and half year prior to this outbreak, a nose swab from the involved HCW had been cultured. A fusidic acid resistant MSSA was detected, however this isolate was not available for further testing. Prior to decolonisation of the HCW, the partner of the HCW was tested for MRSA carriage. No MRSA was found, only a fusidic acid resistant MSSA was detected in the nose. AFLP genotyping showed that the MRSA isolate of the HCW was identical to the MSSA strain of the partner of the HCW. Whole genome mapping showed a similarity of 94.9% between the MSSA and MRSA isolates, just below the cut-off value for highly-related strains.

To obtain definite proof that the MSSA isolate acquired a SCCmec-cassette, NGS of the MSSA and MRSA isolates was performed.

Methods: A complete reference genome (isolate from first patient) was obtained by a hybrid approach combining PacBio (Pacific Biosciences,Menlo Park,CA) and Illumina data (Illumina,San Diego,CA,USA). Additional NGS was performed with an Illumina HiSeq on two MRSA isolates of the HCW (one from 2012; one from 2014, isolated after two MRSA-treatment cycles), the MRSA isolate of patient 2, and the MSSA isolate of the partner. NGS data was used for single nucleotide polymorphism (SNP) analysis, determination of SCCmec types and detection of possible plasmids and bacteriophages.

Results: SNP analysis revealed that the isolates of patient 1,2 and HCW-2012 were nearly identical, with a maximum of 17 SNPs between the isolates. Compared to the reference sequence, the HCW-2014 isolate had 52 SNPs, while the MSSA isolate from the partner had 44 SNPs. Furthermore, analysis revealed two deletions of 87 bp and 818 bp, in the MSSA genome.

SCCmec was absent in the MSSA isolate, while the MRSA isolates carried a type V(5C2&5C). However, there was an 8.5 kb deletion in the region where the second ccrC-gene is located in isolates belonging to patient 1,2 and HCW-2012, but not in the isolate of HCW-2014.

Further analysis showed the presence of two plasmids (4.3kb and 3.1kb) in the isolates of patients 1,2 and HCW-2012, whereas the isolate of HCW-2014 contained the plasmid of 3.1kb only. No plasmids were detected in the MSSA isolate. A bacteriophage was present in the isolates of HCW-2014 and the partner of the HCW(MSSA) but was absent in the other isolates.

Conclusion: This study revealed a relatively large difference between the MSSA and the MRSA outbreak isolates (patient 1,2 and HCW-2012). Unexpectedly, relatively large differences in both SNPs and mobile genetic elements were found between the two MRSA isolates of the HCW. This shows that in relatively short timeframe more variation may occur within the *S.aureus* genome than previously assumed. However, the results, in combination with a clear epidemiologic link, support the hypothesis that the MSSA isolate acquired a SCCmec-cassette

O034

The role of the intestinal microbiota in the development of *Clostridium difficile* infection

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It is clear that the complex community of bacteria that inhabits the human gut plays a key role in maintaining health. Disturbances of the gut microbiota play an important role in the pathogenesis of *Clostridium difficile* infection (CDI). While it is clear that disruption of the gut microbiota by antibiotics underlies the majority of cases of CDI, pathogen characteristics and the epithelial response is also central to the development of colitis. I will review how studies of the dynamics and function of the gut microbiota influences the development of CDI via interactions with the pathogen and the host epithelium.

A key node in the interactions between a host and its gut microbial community is metabolism. The gut bacterial community is integral to the metabolic potential of the host. Disruption of the microbiota can also shift host metabolic profiles towards one that supports the growth of bacterial pathogens such as *C. difficile*. We have shown that communities that are drastically different in terms of membership can provide equivalent resistance to colonization by *C. difficile*. Rather than similarities in the community structure, the commonality between these resistant communities was their metabolic profile. A number of investigators have recognized the central role that bile acid metabolism has in influencing the physiology of *C. difficile* in the intestinal tract. Disruption of the microbiome can shift the bile acid pool to create an environment that supports both the germination of *C. difficile* spores as well as vegetative outgrowth and toxin production.

With the realization that an altered microbiota can result in disease, the desire to manipulate the microbiome therapeutically has increased. One of the ways in which this has been attempted has been through the transplantation of the microbiota from a healthy individual (obtained in the form of fecal material) into a patient who is suffering from a disease that is felt to result from a 'dysbiotic' microbiome. Fecal microbiome transplantation (FMT) has been shown to have remarkable efficacy for patients with recurrent *Clostridium difficile* infection. In patients with recurrent CDI, the results from multiple case series and a handful of randomized trials, FMT can result in cure of over 90%, a much higher response than is observed using traditional antibiotic therapy. In patients with recurrent CDI who are treated with FMT, analysis of the fecal microbiome reveals that the transplanted microbiota is rapidly established in the recipient and remains detectable for a significant period of time.

The study of the interaction between *C. difficile*, the indigenous microbiota and the host is providing more insight into the complex interactions between host and microbes that determines human health and disease. A more complete understanding of this complex system is likely to lead to novel means to prevent and treat a wide variety of diseases beyond gastrointestinal infections.

O035

Development of the Netherlands Donor Feces Bank (NDFB) for Fecal Microbiota Transplantation (FMT)

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Development of the Netherlands Donor Feces Bank (NDFB) for Fecal Microbiota Transplantation (FMT)

J.J. Keller, E.M. Terveer, and E.J. Kuijper; on behalf of the *NDFB working group*

Fecal Microbiota Trasplantation is the only effective treatment for a subset of patients with recurrent *Clostridium difficile* infections (CDI), with a cure rate > 90%. The mechanism of action of FMT seems restoration of the healthy microbiota, which is importantly disturbed in patients with recurrent CDI. The effect of FMT against other dysbiosis-associated disorders is currently extensively investigated. In the near future, FMT may also be applied for other indications such as Inflammatory Bowel disease. To date, there are no internationally standardized guidelines for FMT. Careful donor screening and selection appears the principal step for FMT, and (un)availability of screened donor feces seems an obstacle to offer FMT to patients who may benefit from this new treatment approach. Therefore, the Netherlands Donor Feces Bank (NDFB) was initiated to enable safe and cost-effective FMT

for patients with recurrent CDI, and to support further research. A protocol was set up based on the experience obtained during the FECAL trial, combined with expert opinions of gastroenterologists, infectiologists.
Potential donors are extensively screened by questionnaire and laboratory analysis for (potential) fecal or blood transmitted diseases and factors that can affect a healthy microbiota. Donor feces is collected and processed within 2 hours after defecation. The feces is homogenized with saline, sieved and concentrated by centrifugation. The cryoprotectant glycerol is added and a ready-to-use suspension is stored at -80°C in the Leiden University Medical Center Biobank. In addition, a sample of the donor feces is stored as part of the quality and safety protocol. After a second negative screening round of the donor, the donor feces suspension can be send to out for treatment of patients after consultation and approval by the FMT working group. Administration of the fecal suspension via nasoduodenal route is recommended. The NDFB will actively collect follow up data of patients receiving FMT.
The NDFB is supported by ZonMW.

O036

Changing epidemiology of CDI in The Netherlands; six years of sentinel surveillance

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Background: In one decade, *Clostridium difficile* infection (CDI) emerged as an important cause of diarrhoea in acute care hospitals in the Netherlands. In 2005, an excessive number of CDI outbreaks occurred, related to the spread of the fluoroquinolone resistant ribotype 027 strain from Canada and the U.S. towards Europe. In 2006, the incidence of ribotype 027 declined. Ribotype 078 is one of the three most isolated ribotypes from hospitalised patients since, and is predominant in pig(let)s as well. A rise of ribotype 078 was also noted in Scotland, Ireland and England, but its sources are still unknown. It has been suggested that the high incidence of ribotype 078 CDI in the Netherlands is related to pig(let) industry.
Methods: Patient data were collected in 26 of the circa 90 hospitals in the Netherlands between May 2009 to May 2015. All hospitalised patients above two years old, with diarrhoea and a positive toxin test for *C. difficile* were included. Stool samples or isolates were investigated by PCR ribotyping, and results were linked to patient data. Ribotypes 078 and 126 were clustered due to limited pattern discrimination. Pig(let) density was defined as the number of pig(let)s per hectare (using StatLine database of Statistics Netherlands). The relation between the number of ribotype 078 CDIs per 10,000 patient-days and the provincial pig(let) density was assessed by weighted linear regression.
Results: A total of 4,691 CDIs were identified by 26 hospitals. Ribotype 078 was isolated in 493 of the 3,756 patients (13%) of whom a PCR ribotyping result was obtained. The mean age of 078-infected patients was 69 years (95% CI: 67-70), similar to those infected by other types (67 years; 95% CI: 67-68). A community onset was reported for 37% (95% CI: 33-42) of the 078-infected patients, compared to 33% (95% CI: 31-35) of other patients. Ribotype 078-infected patients had more often severe CDI (RR 1.29; 95% CI: 1.10-1.50), and a higher mortality (RR 1.33; 95% CI: 1.05-1.68) compared to others. The median CDI incidence rate was 2.70 per 10,000 patients-days (IQR: 2.06-3.95), and 0.28 (IQR: 0.19-0.39) for ribotype 078 CDI only. No significant relation between ribotype 078 incidence rate and provincial pig(let) density was found.
Conclusions: Ribotype 078-infected patients had a similar age and proportion of community-onset CDI compared to others, but a worse clinical outcome. We found no higher incidence of ribotype 078 in hospitals located in provinces with a high pig(let) density. However, our indicator of pig(let) exposure may have been too imprecise, and results do not exclude occurrence of ribotype 078 pig-human transmission at a smaller scale or beyond provincial borders (e.g. through meat consumption), or restricted to the community setting.
Acknowledgements: We thank all infection control personnel, laboratory technicians, and medical microbiologists who contributed to this study.

O037

The role of asymptomatic carriership for spread of CDI in hospitals

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Clostridium difficile spores are ubiquitous in the environment, but *Clostridium difficile* infection (CDI) was long regarded as transmitted primarily from other symptomatic CDI patients. Cross-infection of patients via direct contact with a CDI patient, via contaminated hands or via contaminated environment could all occur

and were for a long time regarded the main routes of transmission. However, recent studies (notably based on highly discriminatory techniques such as whole genome sequencing), have reported that only 25-55% of new CDI cases could be linked to previously identified CDI patients. A large proportion of CDI cases are therefore not due to transmission from other CDI cases. These findings highlight that the traditional assumptions about the sources and transmission of *C. difficile* may not be correct and that there is a need to re-examine the many possible sources and modes of transmission. Diverse sources of acquisition other than symptomatic CDI patients have been suggested. One significant alternative mode of transmission could be *C. difficile* colonised patients. These are patients without any CDI symptoms but a positive culture of *C. difficile*. The prevalence of *C. difficile* colonisation among populations varies widely, with especially high rates reported among infants and patients in long-term healthcare facilities. The scale of asymptomatic colonisation at admission to a hospital was reported to range from 4 to 21 percent. Although asymptomatic, these individuals may have skin contamination and may shed spores into the hospital environment and thus disseminate the bacterium to susceptible individuals who could develop CDI. Moreover, patients colonized at admission by toxigenic *C. difficile* strains may progress to CDI themselves during admission due to disturbances in their microbiome (for example as a result of antibiotic use during admission). For these reasons, risk factors to be colonised at admission are of interest. Recent hospitalization, chronic dialysis, use of immunosuppressants, use of gastric acid suppressant medication and having lose or frequent stools are among the identified risk factors for colonisation at admission. Also, patients recovering from a CDI episode should be regarded as potential *C. difficile* carriers. Although colonisation at admission and risk factors for colonisation have gained attention recently, there is still need to further elucidate risk factors for colonisation at time of admission and to identify possible sources of acquisition. At the moment, a multicentre case control study is performed in the Netherlands to identify risk factors for colonisation at admission. This study is also taking into account the possible zoonotic acquisition of *C. difficile* as whole genome sequencing provided evidence of direct transmission of a certain ribotype *C. difficile* (078) between farm animals, the environment and humans. Also, the risk of colonized patients to develop CDI will be investigated in this study. Furthermore, using data from the national reference laboratory for *C. difficile*, the relatedness between isolates from patients colonised at admission and clinical isolates from patients diagnosed with CDI will be determined to learn about the role of colonised patients in transmission of the disease.

This and other studies will hopefully contribute to a more complete understanding of how *C. difficile* is acquired and transmitted and in particular how asymptomatic colonisation contributes to the epidemiology of CDI. Possibly, specific measures to prevent progression to CDI would be useful for colonised patients when admitted. Also, it has to be determined whether specific control measures for colonised patients may be justified to prevent spread.

O038

Long term effect of feces microbiota transplantations for recurrent CDI

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Fecal microbiota transplantation (FMT) is an effective treatment for recurrent *Clostridium difficile* infection (CDI), but data on procedure related complications and long-term outcome are scarce. In this presentation and based on the AMC data so far, these two issues will be addressed.

O039

Microbes and their genes in the global ocean: the deep ocean survey of the Malaspina 2010 cruise, with comparison to the Tara Oceans global surface ocean survey

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We know about just a fraction of the ocean. Particularly the ocean's dark depths, which constitute the largest habitat in the biosphere, contain a large share of the Earth's microbes, and are the sites of microbially-driven important processes in Earth biogeochemistry, regulating e.g. the time carbon spends sequestered in the ocean. The Malaspina 2010 cruise was designed to extensively look at the deep waters of the global Ocean, and sampled the North and South Atlantic and Pacific as well as the Indian Ocean in a 7-month trip. We explored the abundances of prokaryotes and those of small protists, their activity, and the factors determining activity. In addition we used 18S and 16SrRNA and DNA tagging as well as metagenomics to explore which microorganisms and genes inhabit globally the deep ocean and how do they depend on the geographical, physical and oceanographic factors, including water-mass biogeographical patterns. Almost simultaneously, the Tara Oceans schooner sampled the

surface and some mesopelagic waters of the ocean. An unprecedented microbial sequencing effort resulted in a large dataset of microbial genes, the Ocean Microbiome Reference Gene catalogue. We will also compare the degrees of species and gene novelty in the deep ocean uncovered by the Malaspina cruise with those inventoried by the Tara Consortium, and, in particular we will look at organism and gene distribution in the free-living and attached fractions, and the degree of phylogenetic conservation of that lifestyle in the prokaryotes.

O040

Ecological genomics of coastal microbial mats: The case of *Lyngbya aestuarii*

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Coastal microbial mats develop on sandy beaches worldwide and can be seen as extreme environments in several ways. These intertidal areas are irregularly flooded and experience strong salinity fluctuations ranging from almost freshwater, marine to desiccation induced hypersaline conditions. They also experience high irradiation and large fluctuations in temperature (up to 40°C difference in 24 hours). Microbial mats are further characterized by steep and fluctuating physicochemical micro-gradients formed under influence of environmental factors and by the metabolic activities the microorganisms. These extreme conditions may explain the huge (micro)-diversity in these microbial ecosystems. Oxygenic photo-autotrophic Cyanobacteria colonize the coastal intertidal sandy sediments, fix carbon dioxide and exude excess organic carbon as extracellular polymeric substances (EPS). This EPS forms the matrix in which the microbial mat organisms are embedded, stabilizes the sand and offers protection against the above mentioned stressors.
The filamentous cyanobacterium *Lyngbya aestuarii* is a cosmopolitan organism found in coastal microbial mats. In the dark, under anoxic conditions, *L. aestuarii* ferments its storage carbohydrate and excretes low molecular weight carboxylic acids such as lactate and acetate and ethanol. *L. aestuarii* also fixes atmospheric nitrogen and it has therefore minimal growth requirements while it enriches the mat with essential nutrients to support other micro-organisms. A combination of Sanger sequencing and 454 pyro-sequencing resulted in a nearly closed genome of less than 50 contigs for *L. aestuarii* strain CCY9616. In addition we performed a metagenome and metatranscriptome analysis of natural microbial mat samples in which *L. aestuarii* dominates. I will discuss the major findings and their significance in light of adaptation of L. aestuarii to its extreme fluctuating habitat.

O041

Transmission of microbiota in marine invertebrates

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Perhaps the most well-known example of bacteria living in symbiosis with a marine invertebrate are the luminescent Vibrio fischeri in the bobtail squid. This is a classic example of two organisms doing a complicated dance. However, many symbiosis escape from the classic paradigm in that a multitude of partners is involved. Marine sponges represent the oldest symbiotic associations between bacteria and animals and host a wide variety of species-specific communities of microorganisms. These bacteria are often vertically transmitted from mother to embryo. Horizontal transmission has often been proposed to co-occur in sponges, but the mechanism is poorly understood and little experimental evidence is available to support this hypothesis. Issues that obscure a clear picture of the transmission mechanism are:

- presence of bacteria in sponge larvae is usually mentioned in ultrastructure studies, while the absence is rarely reported;
- sponges may combine vertical and horizontal transmission, in such way that some bacteria are vertically transmitted, while others are horizontally transmitted;
- the same bacterial species that are vertically transmitted may also be acquired horizontally from the water column;
- some bacteria that are detected in sponges may be present solely as part of the sponge diet.

To assess the impact of the mode of transmission on the microbial assemblages of sponges, we analysed the microbiota in sympatric sponges that have previously been reported to acquire bacteria via either vertical (*C. candelabrum* and *C. crambe*) or solely horizontal transmission (*P. ficiformis*). The comparative study was performed by pyrosequencing of barcoded PCR-amplified 16S rRNA gene fragments. We found that *P. ficiformis* and *C. candelabrum* each harbor their own species-specific bacteria, but they are similar to other high-microbial-abundance sponges. In addition, nearly 50% of the reads obtained from *P. ficiformis* were most closely related to bacteria that were previously reported to be vertically transmitted in other sponges and comprised vertical-horizontal transmission phylogenetic clusters (VHT clusters). Therefore, our results provide the first evidence for the hypothesis that similar sponge-associated bacteria can be acquired via both vertical and horizontal transmission.

O042

Characterization of the highly branched glycogen from the thermoacidophilic red microalga *Galdieria sulphuraria* and comparison with other glycogens

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Red algae (Rhodophyta) are a group of ancestral photosynthetic eukaryotes that synthesize a storage glucan molecule known as floridean starch.[1] Despite being called starch, it shares more common features with glycogen, since both are synthesized from UDP-glucose and are stored in the cytosol of the cell. *Galdieria sulphuraria* is the only eukaryotic phototroph capable of thriving in extreme environments with relatively high temperatures (up to 56°C) and pH values of 0-4.[2] Species belonging to the genus Galdieria are particularly interesting due to their metabolic flexibility, being able to grow both autotrophically and heterotrophically on a wide variety of substrates.[3] The structure of the storage molecule synthesized by *G. sulphuraria* growing under heterotrophic conditions was characterized and compared to several glycogens from bacteria and oyster. Glycogen was extracted from *G. sulphuraria* cells growing on glycerol by harvesting the cells through centrifugation, lysing the cells by bead beating, removing cell debris by centrifugation and mixing the supernatant with an equal volume of ice cold absolute ethanol. The glycogen precipitated and was harvested by centrifugation at 4°C. Similarly, glycogen was harvested from glucose grown cells of *Escherichia coli* DSMZ 10235 and *Arthrobacter globiformis* DSMZ 20124. Glycogens from *Pseudomonas* V-19, *Sphaerotilus natans* and *Arthrobacter viscous* have been extracted by Dr. L. Zevenhuizen (Laboratory of Microbiology, Wageningen University) in the 1960s and 1970s and were a kind gift of dr. M. Breedveld (Groningen). The amount of alpha-1,4 and alpha-1,6 *O*-glycosidic linkages was determined by H-NMR (500 MHz). The side chain distribution was analysed by treating the glycogens with isoamylase/pullulanase and subsequently running the debranched samples on a Dionex HPLC system. Finally, the molecular weight distribution was measured by size exclusion chromatography combined with a multi-angle laser light scattering and refractrometric index detectors.

The *G. sulphuraria* glycogen clearly differs from the other glycogens in its unusually high percentage of branching linkages, i.e. the amount of alpha,1-6 per 100 alpha,1-4 *O*-glycosidic linkages, of around 18% and an abundance of short chains (between 5 and 10 anhydroglucopyranose residues). The microbial glycogens showed a degree of branching ranging from 8% for *E. coli* to 13.5% for *A. viscous*. The weight average molecular weight of the *G. sulphuraria* glycogen was about 2.5 x 10^5 kDa, being at least one order of magnitude lower than all the microbial glycogens. In conclusion, the *G. sulphuraria* glycogen is small, compact, highly branched molecule that is very different from other microbial glycogens so far. The particular structure of this glucan could play a role in the ability of *G.sulphuraria* to adapt to nutrient-limited conditions and cope with different stresses that are characteristic from the environments that this alga colonizes.[4,5]

References

1. Viola R, Nyvall P, Pedersén M. The unique features of starch metabolism in red algae. Proc Biol Sci. 2001;268:1417-22.
2. Yoon HS, Müller KM, Sheath RG, et al. Defining the major lineages of red algae. J Phycol. 2006:42;482-92.
3. Gross W, Schnarrenberger C. Heterotrophic growth of 2 strains of the acidothermophilic red alga *Galdieria Sulphuraria*. Plant Cell Physiol. 1995b;36:633-8.
4. Schönknecht G, Chen WH, Ternes CM, et al. Gene transfer from bacteria and archaea facilitated evolution of an extremophilic eukaryote. Science 2013;339:1207-9.
5. Gross W, Küver J, Tischendorf G, et al. Cryptoendolithic growth of the red alga *Galdieria sulphuraria* in volcanic areas. Eur J Phycol. 1998;33:25-31.

O043

Archaea as sources of tetraether membrane lipids in the water column and sediments across an oxygen minimum zone

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Archaea are known to occur ubiquitously on Earth. They are characterized by unique membrane lipids with two main structures: archaeol and glycerol dibiphytanyl glycerol tetraether (GDGT). GDGTs can be preserved for longer time scale than nucleic acid and thus they can be used as biomarkers of the presence of archaeal groups in past and present ecosystems. In the marine environment, Thaumarchaeota (marine group I, MGI) ammonia oxidizers are abundant and involved in the carbon and nitrogen cycles. Other archaeal groups, such as the Marine Group II and III Euryarchaeota (MG-II, III) have been detected in oceanic surface and deep waters, respectively. Recent studies have also described uncultured archaeal groups such as Miscellaneous Crenarchaeotic Group (MCG) and Marine Benthic Group-D (MBG-D) in the marine sub-sea floor. However, the environmental factors that determine their distribution and contribution to the total archaea population, and thus to the marine GDGT-pool are still unknown. Here we determined the diversity of archaea in suspended particulate matter (SPM) and sediments (top and 10 cm deep) across the Arabian Sea oxygen minimum zone (OMZ) by means of 16S rRNA amplicon sequencing and sequencing of metabolic genes and gene-coding enzymes involved in the GDGT synthesis. Abundance (DNA) and potential activity (RNA) of archaeal groups were determined

by quantitative PCR of 16S rRNA and metabolic genes. This was compared with the abundance and diversity of intact polar lipid GDGTs considered as biomarkers for living Archaea as they are thought to quickly degrade after cell death.

Sequencing analyses revealed that most of the archaeal reads detected above and below the Arabian Sea OMZ SPM were affiliated to Thaumarchaeota MGI while MGII comprised 13-20% of the archaeal reads, indicating coexistence of these two archaeal groups. Within the OMZ, with oxygen concentrations below detection limit, the % of reads of MGI and MGII decreased, while the MGIII comprised up to 30% of the archaeal reads, which suggests a strong effect of oxygen availability on the archaeal diversity. In the sediments across the OMZ, the % reads of MGI increased in sediments with higher oxygen penetration depth. In contrast, in anoxic sediments MCG and MBG-B and D were dominant, which is compatible with an anaerobic metabolism based on organic matter degradation. The abundance and activity of the MGI archaea was highest in the upper interface of the OMZ, characterized by microaerophilic conditions, and also in surface sediments with higher oxygen availability. These results were in agreement with higher abundance of GDGTs with a hexose-phosphohexose (HPH) headgroup in the water column and in sediments where oxygen is available, which has been previously suggested to indicate in situ production due to the labile nature of the phosphate-ester bond of the HPH-GDGT. This combined lipid/genomic analysis has proven to be very informative to evaluate the diversity and abundance of archaeal groups and their potential role in marine settings and also it will also shed light on the lipid membrane composition of uncultured archaeal groups to evaluate their role both in present and past marine ecosystems.

O044

One Health, to empower interdisciplinary approach

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Global change includes our socio-economic environment but also our biologic and our physical environment. Changes in the human way of live with increasing mobility and trade, Changes in agriculture, animal husbandry, land use or wildlife result in increasingly more changes in the interactions between humans and animals, plants and other organisms of the ecosystem (Tylianakis et al 2008). As part of the One-Health concept these changing interactions are subject of research but in many cases interactions between people and other organism populations such as animals and plants are studied separately.

Wageningen University has many different science groups and over the last few years a number of research projects have been developed in which internal research units work together on important health issues. In collaborative efforts between different internal and external research disciplines it is aimed to study the different interactions within ecosystems in a coherent way taking into account the many different relationships within these ecosystems. This approach should anticipate the development of interventions for the overall health improvement which might lead to opposite effects on the different elements within ecosystems. It is envisaged that a multidisciplinary approach in life sciences research will support a more effective protection of the health of people, animals and plants within the 'One Health' concept. The ongoing 'One Health' projects, integrate disciplines to enhance the overall scientific progress to be made for the quality of life. In workshops for life sciences researchers within Wageningen University have produced vision documents and developed a primary 'One Health' strategy.

Februari 4th, 2015 in The Hague the Netherlands Centre for One Health (NCOH) was launched. The NCOH was founded by the Utrecht University and the Wageningen University and will be a virtual centre focussing on collaborations in four important One Health research themes.

Another important Wageningen University initiative is the 'Global One Health' approach. This wider multidisciplinary approach will also consider the health of animals, plants and sustainable ecosystems for human prosperity and not only for human health. There is a need to weigh all possible effects of interventions on the health of humans, animals, plants and the environment, while taking ecosystem sustainability into account. 'Global One Health' will use multiple disciplines to seek transnational solutions for improving the health of humans, animals and plants worldwide

References

1. Koplan JP, Bond TC, Merson MH, et al. Towards a common definition of global health. Lancet. 2009;373:1993-5.
2. Tylianakis JM, Didham RK, Bascompte J, et al. Global change and species interactions in terrestrial ecosystems. Ecol Lett. 2008;11:1351-63.

O045

A case of tularemia: veterinary background and human diagnostics & therapy

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In most European countries tularemia is an infrequent disease and for decades the causative agent, *Francisella tularensis*, was thought to be absent in the Netherlands. However autochthonous infections have been reported in

five patients since 2011. Additionally the disease has been confirmed in a number of brown hares (*Lepus europaeus*) throughout the country. The latter findings have been the result of a non-targeted monitoring starting in 2011 as a collaboration between the Dutch Wildlife Health Centre (DWHC), Central Veterinary Institute of Wageningen UR (CVI) and the National Institute for Public Health and the Environment (RIVM). An overview of the findings will be presented.

Francisella tularensis can be divided in a number of subspecies and biovars. The two main subspecies are *F. tularensis* subspecies *tularensis* (also known as type A), which is restricted to North America, and *F. tularensis* subsp *holarctica* (type B) which is prevalent throughout the Northern hemisphere and the only subspecies causing tularemia in Europe. Tularemia has a complex ecology, many aspects of which are still poorly understood. Although numerous animal species can be infected, hares as well as small rodents are considered to be the key reservoirs of *F. tularensis* in Europe. The disease can be transmitted by arthropods such as mosquitoes, ticks, and bloodsucking flies; the relative importance of these vectors in disease transmission may differ per region.

Recent cases indicate the re-emergence of *F. tularensis* in the Netherlands. From the start these findings have been discussed in the monthly "Signaleringsoverleg Zoönosen" meetings, in which experts in the veterinary and medical domain (RIVM , GGD, GD, CVI , FD and NVWA) exchange signals from their various monitoring systems. When an outbreak of tularemia among hares was reported in a restricted area in Friesland (Akkrum and vicinity) in the first months of 2015, the risk for inhabitants and tourists as well as for domestic animals was discussed there. It led to a collaborative study by members of the already existing Working Group on Tularemia in which medical, veterinary, wildlife, and environmental professionals partake. This one health collaboration has shown its great value in risk assessment, investigating sources of infection, and for informing medical and veterinary professionals and hunters.

Awareness of the current status of tularemia and its clinical variants is vital in diagnosing this potentially lethal infectious disease. Most patients present themselves with atypical symptoms like fever, myalgia, headache and focal complaints depending on the site of infection. If no clinical suspicion arises through careful medical history many cases will remain undetected, particularly in the case of self-limiting infection. A typical case of ulceroglandular tularemia in a Dutch hunter is presented. With the help of this case the more atypical and less frequently diagnosed clinical variants like pneumonic and typhoidal tularemia are discussed.

Microbiological diagnosis of this elusive pathogen can be challenging. Available diagnostic methods including culture, serology and molecular detection and their application will be discussed. Infection of laboratory workers with this highly infectious pathogen poses a threat, again establishing the importance of awareness among microbiologists and clinicians.

Historically the most widely accepted antibiotics in managing infection are aminoglycosides and tetracylines. Theoretical and clinical evidence supporting a role for quinolones is increasing. Recommendations on the management of tularemia and its naturally occurring resistance patterns will be reviewed in conclusion of the presentation.

O046

A case of echinococcosis: veterinary background and human diagnostics & therapy

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In The Netherlands, Echinococcus multilocularis (Em), a small tapeworm and the causative agent of alveolar echinococcosis (AE), was detected for the first time from a population of foxes in 1997 in the northern region of Groningen adjacent to Germany and the southern region of Limburg adjacent to Belgium. These areas are considered the geographical westernmost border area of the parasite distribution in Europe. Alveolar echinococcosis is a sporadic but severe disease which needs lifelong treatment. Incubation time is between five and fifteen years and symptoms might remain atypical untill severe pathology develops especially in the liver. Lesions can be misinterpreted for metastatic lesions, as Em lesions usually present on CT with irregular borders and various densities. Increase in red fox populations in many parts of Europe due to the successful rabies control campaigns might have extended the parasite distribution. The number of human cases is increasing not only in the endemic central area in Europe but are also found now in Western and Northeastern Europe. Notification is not mandatory in many countries in Europe including the Netherlands and diagnosis is base don the combination of ultrasound and serology, although sensitivity and specificity are not optimal. Detection of Em DNA in fecal samples of foxes or pathological lesions confirms the diagnosis. Studies in foxes in The Netherlands and Belgium were used to analyse the spatial distribution of the parasite in both countries and various studies in foxes were carried out to determine the spread of the parasite and the risk for public health. In 2013, a study was performed to assess the prevalence of Em in red foxes in the area east of Maastricht. Results showed a steep increase in prevalence compared with the 2006 from 11% (95% confidence interval 6.7-18.4%) to 59% (95% confidence interval

43-74%) in the same area. Using a predictive risk model, the first human authochtonous case was predicted and found in 2008 in Limburg. After that two other human cases have been identified in 2011 in the central part of the Netherlands. Underreporting and misdiagnosis might complicate the identification of human cases. It remains to be determined how the increased prevalence in Limburg influences the infection pressure, and how this translates to the estimated number of human cases of AE, because parasitic biomass and not prevalence is the most relevant parameter for humans risk. Since Em is emerging in the wildlife population and AE is increasing in incidence in many countries in Europe, increased awareness at the medical site and collaboration between the medical and veterinary domain is a prerequisite.

O047

A case of *psittacosis:* veterinary background and human diagnostics & therapy

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In the Netherlands the first report on human psittacosis dates from 1930.[1] The import of parrots from Argentina was thought to be the source of infection. This led to quarantine and hygienic rules for the importation of psittacine birds. Currently psittacosis is a notifiable disease in man and pet birds but not in poultry. The disease is caused by the gram negative intracellular bacterium *Chlamydia psittaci*. On a yearly base thirty to seventy animal cases and forty to eighty human cases are reported to veterinary or public health authorities, but this might be an underestimation of the actual number of cases.

Psittacosis has been described in more than 460 bird species, but is mainly associated with psittacine birds and pigeons.[2] Recent publications from Belgium and France show the bacterium is also highly prevalent in poultry. Seroprevalences of over 90% in broiler and turkey farms have been reported.[3] Other studies indicate the recently described *Chlamydia gallinacea* is more commonly found in chickens.[4,5] The role of this new species as an avian pathogen or a possible zoonotic agent has to be elucidated. So far, data about the presence of *C. psittaci* or *C. gallinacea* in Dutch poultry are lacking. This is one of the aims of the research project Plat4m-2Bt-Psittacosis.

In this project the prevalence and presence of different genotypes of *C. psittaci* in poultry and pet birds will be investigated. *C. psittaci* is currently divided into nine different genotypes based on the analysis of the variability of the ompA gene. Each genotype is associated with a preferred animal host and can therefore be used to aid source finding.

The genotyping system is already used in human diagnostics[6], but further harmonization between medical and veterinary labs is needed. At present not all medical microbiological labs (MML's) perform molecular testing to detect *C. psittaci*. Furthermore, community-acquired pneumonia (CAP) is not routinely confirmed by laboratory diagnosis. The current first choice treatment of CAP are beta-lactam antibiotics, for which *C. psittaci* is resistant. The platfm-2Bt-psittacosis therefore encourages MML's to implement a *C. psittaci* PCR to reduce the human diagnostic deficit.

The collected human and animal data will be used to determine the disease burden in humans, map psittacosis incidence and genotypes in animals and humans, and identify the main animal reservoirs for zoonotic transmission. Within the project a platform is built to improve this data exchange.

The final goal is to implement this platform into the daily work routine of the public and animal health authorities. This one health based project is considered a proof of principle to improve the control and prevention of notifiable zoonosis by connecting medical and veterinary data.

References

1. Peeters H. Een en ander over de epidemiologie van de psittacosis. Nederlands Tijdschrift voor Geneeskunde. 1930;74:931-4.
2. Kaleta EF, Taday EM. Avian host range of Chlamydophila spp. based on isolation, antigen detection and serology. Avian Pathol. 2003;32:435-61.
3. Yin L, Kalmar ID, Lagae S, et al. Emerging Chlamydia psittaci infections in the chicken industry and pathology of Chlamydia psittaci genotype B and D strains in specific pathogen free chickens. Vet Microbiol. 2013;162:740-9.
4. Hulin V, Oger S, Vorimore F, et al. Host preference and zoonotic potential of Chlamydia psittaci and C. gallinacea in poultry. Pathog Dis. 2015;73:1-11.
5. Guo W, Li J, Kaltenboeck B, et al. Chlamydia gallinacea, not C. psittaci, is the endemic chlamydial species in chicken (Gallus gallus). Sci Rep. 2016;6:19638.
6. Heddema ER, van Hannen EJ, Bongaerts M, et al. Typing of Chlamydia psittaci to monitor epidemiology of psittacosis and aid disease control in the Netherlands, 2008 to 2013. Euro Surveill. 2015;20:21026.

O048

Fundamental questions in metabolic regulation revisited in yeast

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Saccharomyces cerevisiae is an interesting model organism for both biotechnology and medicine, for applied and fundamental questions. One such question is the reason why this yeast exhibits the so-called Crabtree effect: the puzzling phenomenon that at high growth rates the energetically-inefficient fermentative pathway is preferred – even in the presence of oxygen. Recent work, especially in *E coli* [see e.g. Basan, Nature 2015] suggests

that limitations related to protein partitioning causes trade-offs between glucose usage (yield) and protein costs. In my talk I will explain these ideas about the cellular protein economy, present corresponding computational models and discuss to what extent these concepts may explain metabolic adaptations in *Saccharomyces cerevisiae*.

O049

Cryptococcus and *Malassezia*: two models to understand disease potential

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The yeast domain holds approximately 2000 species and only a small part of these can cause infection in humans and animals. Recent biodiversity studies, however, unfolded the presence of hitherto unknown pathogenic yeast species and pathogen species complexes. Here we present data on some basidiomycetous yeast pathogens belonging to the genera *Malassezia* and *Cryptococcus*. Extensive phylogenetic analyses of the *Cryptococcus neoformans* species complex revealed 7 monophyletic lineages that represent species which differ in their disease potential, but also various hybrid lineages. One of the species is involved in an ongoing and expanding outbreak in Canada and USA that is emerging in Mediterranean Europe. Using coalescence methods the origin of the outbreak lineages could be traced back to the Amazon forest. *Malassezia* species are commensals on human and animal skin, but they can also cause disease and some species may cause sepsis. All phylogenetic neighbors of *Malassezia* spp. are plant pathogens, thus during evolution a host shift from plants to the animal may have occurred. We will present data on a genomics-based phylogeny of *Malassezia* in order to understand patterns of speciation, including hybridization, and involvement in skin disorders.

O050

Insights into organelle fission specificity from the peroxisomal membrane protein Pex11p

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Mitochondria and peroxisomes are cell organelles that occur in all eukaryotes and play a central role in many metabolic processes. Regulating organelle numbers is vital for cell viability, since irregularities are known causes of disease.[1,2] Cells increase organelle numbers via a process called fission and a remarkable aspect of organellar fission is that many of the proteins involved are shared by both mitochondria and peroxisomes.[3] While a shared fission machinery is extremely interesting, studying specific fission events can be challenging, particularly when in relation to human disease. Therefore, mitochondria and peroxisomes require factors that infer specificity onto the shared components of the fission machinery. Identifying and characterizing such factors will increase our understanding of how problems in organelle fission lead to disease.

We propose that the peroxisomal membrane protein Pex11p is such a factor. Previous results from our group show that Pex11p initiates peroxisomal fission by remodeling the peroxisomal membrane.[4] Here, we demonstrate that yeast Pex11p is necessary for the function of the GTPase Dnm1p *in vivo*.[5] In addition, our data indicate that Pex11p physically interacts with Dnm1p and that inhibiting this interaction compromises peroxisomal fission. Finally, we demonstrate that Pex11p functions as a GTPase activating protein (GAP) for Dnm1p *in vitro*. Crucially, similar observations were made for mammalian Pex11β and the corresponding GTPase Drp1, indicating that GTPase activation by Pex11p is conserved. Taken together, our data demonstrate that Pex11p is a master regulator of peroxisome fission, while also establishing that fission can be fine-tuned by specific factors on the organelle membrane.

References

1. Scorrano L. Keeping mitochondria in shape: a matter of life and death. Eur J Clin Invest. 2013;43:886893.
2. Thoms S, Gartner J. First PEX11beta patient extends spectrum of peroxisomal biogenesis disorder phenotypes. J Med Genet. 2012;49:314-6.
3. Schrader M. Shared components of mitochondrial and peroxisomal division. Biochim Biophys Acta. 2006;1763:531-41.
4. Opalinski L, Kiel JA, Williams C, et al. Membrane curvature during peroxisome fission requires Pex11. EMBO J. 2010;30:5-16.
5. Williams C, Opalinski L, Landgraf C, et al. The membrane remodeling protein Pex11p activates the GTPase Dnm1p during peroxisomal fission. Proc Natl Acad Sci U S A. 2015;112:6377-82.

O051

Engineering redox metabolism improves ethanol yield in acetate-reducing *Saccharomyces cerevisiae*

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Acetic acid, an inhibitor of sugar fermentation by yeast, is invariably present in lignocellulosic hydrolysates that are used or considered as feedstocks for yeast-based bioethanol production. *Saccharomyces cerevisiae* strains have been constructed in which anaerobic reduction of acetic acid to ethanol replaces glycerol formation as a mechanism for

reoxidizing NADH formed in biosynthesis. In this study, strategies to increase the amount of acetate that can be reduced to ethanol were investigated stoichiometrically and subsequently tested in vivo. The results of this work can be used to further improve the fermentation of lignocellulosic hydrolysates at industrial scale.

O052

Regime shifts between oxic and anoxic states in a microbial model ecosystem

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Despite their environmental and industrial importance, the response of microbial communities to environmental change remains poorly understood. A small number of experimental studies (for example, in the human microbiome) suggest that microbial communities may develop into very different ecosystem states over time in response to environmental change, because they can contain alternate stable states. Ecological regime shifts between community states can be identified by certain behaviors. Firstly, ecosystems containing alternate stable states exhibit extreme sensitivity to initial conditions. Secondly, environmental changes can cause these ecosystems to become "stuck" in a community state, such that simply reversing the environmental change is not sufficient to return the ecosystem to its original community state, a phenomenon known as 'hysteresis'.
Here, we present a simple mathematical model demonstrating the existence of alternate stable states in a microbial ecosystem. Our model contains cyanobacteria, sulfate reducing bacteria and phototrophic sulfur bacteria, and would be applicable to many different environments, such as freshwater lakes, marine environments, microbial mats or coral reefs. In response to changes in the availability of oxygen or total sulfur, this model abruptly transitions between an oxic stable state in which cyanobacteria dominate, and an anoxic stable state in which sulfate reducing bacteria co-exist with phototrophic sulfur bacteria. We then demonstrate the validity of our model by comparing our results to 16S amplicon sequence data from the metaliminion of a stratified lake; co-occurrence network analysis on this experimental data shows that the community divides into the same two states that our model predicts. Although this does not prove the experimental existence of *alternate* stable states as found for our mathematical model, it does suggest that alternate stable states may be widespread in microbial ecosystems.

O053

Bacterial interactions inside tomato xylem vessels: Understanding biofilm formation, cooperation and competition

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Background: A remarkable variety of bacteria can be found inside plants. These bacteria 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. Many plant bacteria form aggregates and encase themselves in a sticky extracellular matrix, a process called biofilm formation. Biofilm-growing bacteria have an increased tolerance to environmental stresses and antimicrobials.
Objectives: To develop biocontrol strategies aimed at pathogenic microorganisms in tomato xylem vessels, we study how xylem microbiome bacteria are competing by targeting each other's biofilm. As bacterial biofilms are held together by hydrated polysaccharides, proteins and extracellular DNA, the breakdown of these matrix compounds is an important competition mechanism. Also inhibition of positive regulators of biofilm formation and secretion of specific antimicrobials can play a role.
Methods: Bacteria were isolated from tomato xylem. Colonies were picked after incubation at 25°C on TSB or LB plates for 2-10 days. Isolated strains were identified by 16s rRNA gene sequencing. Selected strains were inoculated on a lawn of fungal, bacterial or oomycete plant pathogens and the release of antimicrobial compounds was scored.
To study the interaction between these bacteria in controlled conditions mimicking the plant xylem, an innovative culturing system using biofilm flow cells is being developed. The flow cells contain hollow membrane fibres, in which the flow rates and nutrient availability are set based on the daily fluctuation naturally occurring in xylem.
Results and Conclusions: We isolated and identified over 200 bacterial species from tomato xylem. A third of them have antifungal, antibacterial and/or anti-oomycete properties during *in vitro* co-culture. Most of the species isolated from above ground parts tomato xylem are gram-positive. The majority of strains with strong antimicrobial properties are bacilli. We have sequenced the genomes of two *Bacillus amyloliquefaciens* strains which promote plant growth and produce antimicrobial compound using the Oxford Nanopore Minion sequencing device. Several of the strains isolated from tomato xylem will be further studied in the diurnally fluctuating membrane flow cell system. This flow cell system is currently being fine-tuned and tested. Our research will contribute unique insights in the adaptations bacteria have evolved to survive and compete inside plant xylem vessels.

O054

Ultrastructure and (meta)virome of a bacteriophage infecting the anaerobic methane oxidizing bacterium *Methylomirabilis oxyfera*

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Introduction: Microorganisms play a major role in driving biogeochemical cycles. In the carbon cycle, methanotrophs metabolize methane, a well-known greenhouse gas, as their main carbon and energy source and are ecologically relevant as methane sink. Methane oxidation can be performed aerobically or anaerobically. The newly-discovered anaerobic methane-oxidising bacterium '*Candidatus* Methylomirabilis oxyfera' oxidises methane to carbon dioxide coupled to nitrite reduction to dinitrogen gas via an intra-aerobic pathway. *M. oxyfera* is unique also with respect to its cell biology. It is a rod- and polygonal-shaped bacterium with ridges running all along the cell length and ending in a cap-like structure at the cell poles. Compared to microorganisms, the relevance of viruses in elemental cycles remains largely unexplored. However, by shaping microbial communities through mortality, horizontal gene transfer and metabolic reprogramming, viruses are postulated to be main drivers in most ecosystems.

Little attention has been given to the (metavirome) study of virus-host interactions in continuous bioreactor systems, mainly due to the high amount of viral biomass needed to construct metaviromes.

In the present study we describe for the first time a bacteriophage infection occurring in *M. oxyfera*.

Methods: We used (advanced) electron microscopy techniques including electron tomography, molecular tools and metagenomics to study the bacteriophage and host interaction at different stages of infection.

Results and Conclusion: The *M. oxyfera*-infecting bacteriophage is ~70 nm in diameter and has a proteinaceous capsid with an icosahedral morphology. Underneath the capsid, a lipid bilayer encloses the genome, appearing as an electron dense core in 2D sections. The bacteriophage does not have a tail. Electron tomography on infected *M. oxyfera* cells showed that, in addition to completely assembled bacteriophages, entities were observed that contained only the central core, i.e. the capsid was not assembled yet. Moreover, during the bacteriophage replication, the host cytoplasmic membrane appeared extremely patchy, an indication that the bacteriophages may use host bacterial lipids to build their own internal membrane. Finally, the bacteriophage population was isolated from the bioreactor and used to obtain a metaviriome. Eventually, we speculate on the significance of the viral infection for the bacterial community in the bioreactor system.

O055

The effect of weak organic acid stress on the intracellular pH dynamics of *Bacillus subtilis* spores during germination and outgrowth

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The internal pH (pH_i) critically determines bacterial cell physiology. Hence, a variety of food preservation strategies aim at perturbing pH_i homeostasis.[1,2] Unfortunately, accurate pH_i quantification is difficult and existing methods are suboptimal: since they average across populations of cells and do not take into account inter-individual heterogeneity. The latter is key as differences in growth and division kinetics make that there is physiological diversity in genetically homogenous populations that leads to a variable response to external stressors.[3] This holds in particular for bacterial spores, ultimate survival capsules of live and important determinants of microbial food stability (spoilage and safety).[4] Here we have developed a robust method to be able to quantify pH_i at the level of single cells emanating from germinating spores of the Gram+ model organism *Bacillus subtilis*. *B. subtilis* strains may spoil foods, are genetically well accessible and their spore structure stands model for food borne aerobic spore forming pathogens such as *Bacillus cereus*, *Bacillus weihenstephanensis* and even *Bacillus anthracis*.[5] Using an improved version of the genetically encoded ratiometric pHluorin (IpHluorin), we quantified pH_i in *B. subtilis* cells, in the absence or presence of weak acid stresses. In the presence of low concentrations of sorbic acid, a decrease in pH_i and increase in the generation time of growing cells were observed. Similar, effects were observed when cells were stressed with acetic acid, albeit at a higher concentration (25 mM). However a large spread was observed which calls for more detailed analyses using molecular (gene-expression)markers for physiological states of the bacteria. Finally, sorbic acid but not acetic acid was in population based analyses and pilot experiments at single spore level, able to extensively postpone a rise in the intra-spore pH in conjunction with a postponed germination and outgrowth process. Currently we are exploring, together with the group of prof. Setlow[6], the use of super resolution microscopy for a refined assessment at single spore level of the effects of weak organic acids

on the initial phases of spore germination at its inner membrane(IM). These cover at least the binding of the germinant to Germination Receptor proteins as well as the inferred interaction of the latter with the SpoVA channel. This channel mediates the uptake of water in exchange for the calcium chelate of dipicolinic acid and hence marks 'the point of no return' in spore germination. Further (novel) IM proteins that may have to be considered are being identified from the recently by our laboratory published data of the Bacillus spore IM proteome.[7] The presentation will illustrate and discuss the microcopy approaches being used for this purpose.

References

1. Brul S, Coote P. Preservative agents in foods. Mode of action and microbial resistance mechanisms. Int J Food Microbiol. 1999;50:1-17.
2. Stratford M, Nebe-von-Caron G, Steels H, et al. Weak-acid preservatives: pH and proton movements in the yeast Saccharomyces cerevisiae. Int J Food Microbiol. 2013;161:164-71.
3. Ackermann M. A functional perspective on phenotypic heterogeneity in microorganisms. Nat Rev Microbiol. 2015;13:497-508.
4. Eijlander RT, Abee T, Kuipers OP. Bacterial spores in food: how phenotypic variability complicates prediction of spore properties and bacterial behavior. Curr Opin Biotechnol. 2011;22:180-6.
5. Abhyankar W, Hossain AH, Djajasaputra A, et al. In pursuit of protein targets: proteomic characterization of bacterial spore outer layers. J Proteome Res. 2013;12:4507-21.
6. Troiano AJ Jr, Zhang J, Cowan AE, et al. Analysis of the dynamics of a Bacillus subtilis spore germination protein complex during spore germination and outgrowth. J Bacteriol. 2015;197:252-61.
7. Zheng et al. 2016. J. Proteome Res. (in press, DOI: 10.1021/acs.jproteome.5b00976).

O056

Biomethaniation of syngas using a thermophilic co-culture

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Synthesis gas, or syngas, is a gas mixture consisting primarily of CO, H_2 and CO_2. Syngas can be formed from any carbohydrate source by gasification, this includes conversion of materials currently inaccessible for biotechnological processes (e.g. plastics, lignin). Syngas fermentation by microbial catalysts allows for conversion of a broad spectrum of wastes into useful bio-chemicals and biofuels, making it an interesting technology for future bio-based economy. Additionally, via the syngas route about 30% more products can be obtained from biomass than via conventional fermentation processes, as lignin is anaerobically poorly degradable and even prevents degradation of easily degradable biopolymers such as hemicellulose. The microbial conversion of syngas to methane allows for production of a biofuel that is easily storable and can be used in the current gas infrastructure. Only a few methanogenic archaea are known to utilize CO, and are in general quickly inhibited by its presence. This makes the conversion of syngas to methane an inefficient and slow process. In order to increase the efficiency and rate of biomethaniation of syngas, we established a synthetic thermophilic co-culture of a methanogenic archaea and a hydrogenogenic bacterium. This co-culture converts syngas to methane at a high rate. Additionally, in contrast to traditional biogas production, gas produced via this method has a high CH_4:CO_2 ratio, approaching the composition of natural gas. This research shows biomethaniation of syngas can be performed efficiently and at a high rate, potentially posing an alternative way for biogas production.

O057

Quantitative proteomic analysis of B. subtilis spores made in liquid and on solid growth media

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Introduction: Spores of *Bacillus subtilis* are dormant cellular entities formed by the process called sporulation. Sporulation is trigged by unfavourable environments such as starvation. Spores are of high interest to the food and health sectors because of their extreme resistance to harsh conditions and especially their heat resistance. The multiple layered structure of the spores contributes in part to this high resistance. Earlier research has shown that spores prepared on solid agar plates have a higher heat resistance than those prepared in a liquid medium. It has also been shown that the more mature a spore is the higher is the heat resistance, which is suggested to be due to cross-linking of coat proteins. The current study for the first time enlightens the effect of growth conditions on spore proteins.

Methods: Spores were prepared on solid (Sporulation & Growth agar supplemented with ^{14}N) and in liquid (3-(N-morpholino) propane sulfonic acid i.e. MOPS buffered medium supplemented with ^{15}N) medium. The spore surface layer proteins were quantified using metabolic labelling technique in a combined LC-FT-ICR-MS/MS approach. The ratio ^{14}N:^{15}N was determined for all the identified proteins and peptides.

Results: The coat protein composition of spores prepared on solid medium was different from that of spores prepared in liquid medium. Most of the identified inner coat and crust proteins were significantly up regulated for spores prepared on solid medium, while most of the outer coat proteins were significantly down regulated. A

marginal but significant difference in the heat resistance of the ^{14}N and ^{15}N spores was observed where the spores prepared on solid medium (^{14}N spores) possessed a higher thermal resistance than the spores prepared in liquid medium(^{15}N spores). The core DPA content was similar in both spore population.

Conclusions: Thus this research confirms the notion that the protein composition of spore coat and the inherent wet heat resistance of spores seem to be affected by the sporulation medium conditions. The large variations in the peptide ratios for certain proteins are suggested to be due to inter protein cross-linking.

O058

Safety, immunogenicity, and protective efficacy of intradermal immunization with aseptic, purified, cryopreserved *Plasmodium falciparum* sporozoites in volunteers under chloroquine prophylaxis: A randomized controlled trial

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Immunization of volunteers under chloroquine prophylaxis by bites of Plasmodium falciparum sporozoite (PfSPZ)-infected mosquitoes induces > 90% protection against controlled human malaria infection (CHMI). We studied intradermal immunization with cryopreserved, infectious PfSPZ in volunteers taking chloroquine (PfSPZ chemoprophylaxis vaccine [CVac]). Vaccine groups 1 and 3 received 3× monthly immunizations with 7.5×10^4 PfSPZ. Control groups 2 and 4 received normal saline. Groups 1 and 2 underwent CHMI (#1) by mosquito bite 60 days after the third immunization. Groups 3 and 4 were boosted 168 days after the third immunization and underwent CHMI (#2) 137 days later. Vaccinees (11/20, 55%) and controls (6/10, 60%) had the same percentage of mild to moderate solicited adverse events. After CHMI #1, 8/10 vaccinees (group 1) and 5/5 controls (group 2) became parasitemic by microscopy; the two negatives were positive by quantitative real-time polymerase chain reaction (qPCR). After CHMI #2, all vaccinees in group 3 and controls in group 4 were parasitemic by qPCR. Vaccinees showed weak antibody and no detectable cellular immune responses. Intradermal immunization with up to 3×10^5 PfSPZ-CVac was safe, but induced only minimal immune responses and no sterile protection against Pf CHMI.

O059

Epidemiology of multiple viremia in previously immunocompetent patients with septic shock

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Introduction: Systemic reactivation of cytomegalovirus (CMV) has been frequently described in intensive care unit (ICU) patients, even in those without known prior immune deficiency. However, the incidence of viremia episodes by other herpes viruses has not been explored extensively. We studied the epidemiology of CMV, Epstein-Barr virus (EBV), human herpesvirus 6 (HHV-6), herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), and varicella zoster virus (VZV) in the blood of previously immunocompetent patients with septic shock, and assessed their associations with clinical outcome.

Methods: From January 2011 to June 2014 we prospectively included consecutive adults who were admitted with septic shock to the mixed ICUs of two tertiary care hospitals in the Netherlands for more than four days. We excluded patients who had received antiviral treatment in the week preceding ICU admission and those with known immunodeficiency (i.e., a history of solid organ or stem cell transplantation, infection with the human immunodeficiency virus, hematological malignancy, use of immunosuppressive medication, chemotherapy or radiotherapy in the year before ICU admission, and any known humoral or cellular immune deficiency). Viral loads were determined in plasma weekly by polymerase chain reaction.

Results: Among 329 included patients, 223 (68%) subjects developed viremia at some time during their ICU stay, of whom 112 (50%) had multiple viremia events simultaneously. CMV, EBV, HHV-6, HSV-1, HSV-2, and VZV were detected in the blood of 60 (18%), 157 (48%), 80 (18%), 87 (24%), 13 (4%) and 2 (0.6%) patients, respectively. Patients with multiple viremia events during ICU admission had higher ICU mortality (36%) compared to those with single type viremia (15%) or either no viremia (23%) ($p < 0.01$). Length of stay in the ICU was 16 days (IQR 11-26), 11 days (IQR 7-17) and 8 day (IQR 5-12) in the multiple, single and no viremia groups ($p < 0.01$), respectively.

Conclusions: Multiple herpes viruses may frequently be detected in the blood of previously immunocompetent patients with septic shock. Among these events, patients with multiple viremia had the highest risk of death.

O060

Non-typeable *Haemophilus influenzae* is an emerging invasive pathogen

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Introduction: The incidence of severe *Haemophilus influenzae* infections such as sepsis and meningitis has declined substantially since the introduction of the *H. influenzae* serotype b (Hib) vaccine. However, the Hib vaccine fails to protect against non-typeable *H. influenzae* (NTHi) strains that have become increasingly frequent causes of invasive disease.

Methods: We summarized nation-wide surveillance of invasive *H. influenzae* disease recorded by The Netherlands Reference Laboratory for Bacterial Meningitis and we systematically searched Pubmed using the following queries: "Invasive nontypeable *Haemophilus influenzae*"and "Invasive non-typeable *Haemophilus influenzae*". All papers published from 2000 to 2014 were reviewed and we analyzed all surveillance studies that: 1) Were written in English; 2) Recorded invasive *H. influenzae* cases in the post-Hib vaccine era; 3) Spanned at least four years; 4) Discriminated between serotype b, non-serotype b, and NTHi strains. We describe mechanisms that may explain the increasing prevalence of invasive NTHi infections over the last two decades.

On the experimental side, we determined resistance of invasive NTHi isolates to complement-mediated killing and determined the presence of bactericidal antibodies in serum from patients with NTHi bacteremia.

Results: In 1992, 93% (294 cases) of *H. influenzae* isolates causing sepsis or meningitis in The Netherlands were attributed to Hib alone. The introduction of Hib vaccination in 1993 drastically decreased the incidence of infections to 19% (30 cases) of invasive *H. influenzae* in 2014. However, with the near-elimination of invasive disease caused by Hib, the number of recorded invasive NTHi cases increased almost 6-fold in the last two decades, from 20 in 1992 to 117 in 2014. The vast majority of these NTHi isolates (87%) were collected from blood. NTHi invasion was detected mainly among individuals >50 years of age (75%).

Emergence of NTHi as cause of invasive disease is by no means restricted to The Netherlands alone and was consistently found to be the most prevalent *H. influenzae* causing invasive disease in all studies analyzed. Seven out of eighteen studies showed a clear increase in either absolute number of invasive NTHi cases or increased incidence rates of invasive NTHi during the study periods, whereas four studies did not. Whereas Hib predominantly causes bacterial meningitis in children under the age of 5, the clinical presentation of invasive NTHi cases is different. Most invasive NTHi disease is found the elderly, in whom it develops as a pneumonia or bacteremia without apparent focus of infection.

We propose that multiple (or combination of) mechanisms contribute to the apparent increase in the number of invasive NTHi cases: 1) Strain replacement; 2) Advances in bacterial detection and characterization; 3) Increased virulence for selection of NTHi strains; 4) Diminished immune response to NTHi. Recent experimental data from our group support that both host immune status and bacterial complement resistance are important determinants of invasive disease, such as bacteremia, caused by NTHi.

Conclusion: Based on the available literature, we conclude that invasive NTHi disease is emerging worldwide and should be considered as a potential invasive pathogen, especially in patients with a dysfunctional immune system.

O061

Prevention of *Staphylococcus aureus* biomaterial-associated infections using a polymer-lipid coating containing LL-37-derived antimicrobial peptides

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Despite the immense progress in the field of biomaterial sciences, infection occurs in 0.5 - 10% of patients that receive an indwelling biomedical device ("biomaterial") – depending on the type and location of the biomaterial. *Staphylococcus aureus* and coagulase-negative staphylococci are the most common causative agents of biomaterial-associated infection (BAI). Bacteria that colonize the biomaterials may form biofilms and also colonize the surrounding tissue due to local immune deficiency induced by biomaterial in combination with bacterial compounds (Riool et al. 2014).

The scarcity of current antibiotic-based strategies to prevent BAI and their risk of resistance development prompted us to develop a novel antimicrobial implant-coating to prevent *S. aureus*-induced BAI. In the EU project BALI we have therefore developed novel synthetic antimicrobial antibiofilm peptides (SAAPs) based on the primary structures of the human antimicrobial protein LL-37.

We selected the LL-37-derived antimicrobial peptide OP-145, previously named P60.4Ac (Nell et al. 2006), which has proven efficacy as treatment for patients with

therapy-resistant chronic otitis media (Peek et al. 2009), to use as a scaffold for the design of SAAPs with improved antimicrobial and anti-biofilm activities in physiological conditions *in vitro*. We incorporated two of these novel SAAPs, P145 and P276, into a polymer-lipid encapsulation matrix (PLEX)-coating (Metsemakers et al. 2015) to obtain high peptide levels for prolonged periods at the implant-tissue interphase, like we recently showed to be an effective approach for OP-145 (de Breij et al. 2015).

In this study, we first confirmed that P145 and P276 have potent *in vitro* bactericidal and anti-biofilm activities, essential traits in view of the application for biomedical devices, against *S. aureus* JAR060131, an isolate collected from BAI. When tested in a mouse model for BAI, P145, but not P276, injected along *S. aureus*-inoculated titanium alloy implants significantly reduced the number of CFUs on the implants. Next, P145 and P276 were released from the PLEX coating in a controlled zero-order kinetic rate after an initial burst release. Lastly, we assessed the antimicrobial activity of the SAAP-PLEX-coated implants in the same mouse model as a prophylactic against implant-related infection caused by either a clinical *S. aureus* isolate or a drug-resistant MRSA, as a first step towards the clinical development of these SAAPs. When incorporated in a coating, P145 as well as P276 showed to be effective against both *S. aureus* isolates. Thus, incorporation in the PLEX coating enhanced the effectivity of the SAAPs.

Together, we conclude that the SAAP-PLEX coatings hold promise for further (pre)clinical development as an alternative for coatings releasing conventional antibiotics associated with resistance development.

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O062

Application of eubacterial molecular detection to clinical routine

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Introduction: Culture has been the mainstay of clinical bacteriology since it was founded more than a century ago. The past decade has seen an increasing application of molecular techniques -most notably qPCR- for the detection of bacterial pathogens in clinical samples. The greatest advantages of qPCR over culture are its speed and the ability to detect species when culture cannot, e.g. when patients have received antibiotics or when species are refractory to culture. However, current molecular techniques are typically directed at specific species. Thus, anything that is not explicitly searched for will be missed. This greatly limits the flexibility and universal application of these techniques. A common solution to this is performing a universal PCR followed by sequencing. However, this significantly increases cost, complexity and turnaround time. Additionally, this approach typically has a low sensitivity and can be applied only when a single bacterial species is present in a sample. Here we investigated the application of a rapid universal bacterial molecular identification method, IS-pro, to clinical practice in 220 patient samples received in a clinical microbiology laboratory.

Methods: We used IS-pro is a fully automated and internally controlled eubacterial technique based on the detection and categorization of Interspace regions between 16S and 23s ribosomal DNA, that are specific for each microbial species. IS-pro was performed on 220 clinical samples received in normal clinical routine. Results were compared to culture and clinical records.

Results: Of the 220 samples that were analysed, 30% of samples that were negative by culture were positive by IS-pro. Species found included many common pathogens, such as *Staphylococcus aureus*, *Streptococcus pyogenes* and *Escherichia coli*, but also many species less established as pathogens, such as *Faecalibacterium prausnitzii* and *Ailstipes putredinis*. The finding of cultivable species with IS-pro while culture was negative could often – but not always – be explained by administration of antibiotics to the patient before sampling. The species that were less well established as pathogens were often species that were refractory to standard culture techniques applied in clinical routine. These species commonly derived from endogenous microbiota.

Conclusion: Here we demonstrate that the application of a broad range molecular detection technique, IS-pro, can be of great benefit to clinical diagnostics.

IS-pro can very often detect pathogens when culture cannot (30% of culture negative samples).

Not only uncultivable, but also common pathogens that are missed by culture are detected by IS-pro.

Additionally detected species are highly clinically relevant: IS-pro results often guided clinical decisions.

O063

Conjugal transfer of VIM-2 carbapenemase-encoding plasmid in *Pseudomonas aeruginosa*

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Introduction: In 2012 van der Bij et al. reported the presence of Verona integron-encoded metallo-β-lactamase

2 (VIM-2)-producing *P. aeruginosa* in several Dutch hospitals. Genome sequencing of a representative strain, S04 90, yielded a 7.01-Mbp chromosome and a single plasmid of 160 Kbp. Strain S04 90 has O-antigen serotype O11 and Multi-locus Sequence Type ST446. The plasmid was further analysed.

Methods: The annotation of the plasmid was performed using PGAAP, RAST and BLASTP. Conjugation experiments were performed making use of acceptor strain PAO1 which was made fosfomycin resistant, needed for counter selection. The presence of the VIM gene was confirmed using PCR. Susceptibility determinations of the donor and transconjugant strains was performed by using VITEK2 analysis. Amplified fragment length polymorphism (AFLP) was used for genotyping of the donor, acceptor and transconjugants.

Results: The genetic marker bla_{VIM-2} was found on the plasmid, embedded in a class I integron sequence. Other integron-related antibiotic resistance genes were *aacA29b* (aminoglycoside resistance), and *sul1* (sulphonamide resistance). BlastP revealed the presence of two toxin-antitoxin systems, designated DinJ-YafQ and ChpBS. Both systems were found in a putative prophage region on the plasmid.

The presence of a type IV secretion system and type IV pili is often related to transmissible plasmids. To test the transferability, conjugation experiments were performed using a fosfomycin-resistant derivative of strain PAO1 as the recipient. After conjugation, the colonies resistant to meropenem and fosfomycin were further analysed. Two mutants proved to be VIM positive and showed a similar AFLP profile as PAO1.

Susceptibility testing of the mutants showed MICs for piperacillin/tazobactam, ceftazidime, tobramycin of respectively 128 µg/ml, 64 µg/ml and 64 µg/ml. The MICs for meropenem and imipenem were respectively, >16 µg/ml. Partial resistance was acquired for cefepime.

Conclusion: The conjugation experiments showed a transferrable plasmid carrying the VIM-2 gene which resulted in carbapenem resistance in PAO1. The presence of two toxin-antitoxin systems on the plasmid increases stability of the plasmid.

O064

Mucosal immunity and vaccines against Enteric infections

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As of today, all vaccines but one (oral polio vaccine) recommended by the Expanded Program on Immunization are administered by injections. Such vaccines induce immunity in blood and in peripheral tissues but are relatively inefficient for eliciting immune responses in the gastrointestinal tract, especially in young infants. On the other hand, mucosal administration of a number of experimental as well as few licensed enteric vaccines has been shown to be more efficient for inducing intestinal immunity in animals and in humans.

Modern biotechnology has yielded an abundance of vaccine candidates against enteric infections but few vaccines have been registered for human use. Efforts are being deployed to design safe mucosal adjuvants, to identify alternative delivery routes, to evaluate prime-boost strategies including mucosal-systemic vaccination, and to develop vectors and delivery systems for tissue- and cell-specific targeting of vaccines.

If these candidates are to reach those in need in developing countries, several lessons from field research done in these settings must be considered. These include the need to develop vaccines that avoid storage in a cold chain and can be administered without needles or trained heath care workers. These vaccines must be safe and affordable for the world's poorest, including undernourished children, should confer long-term protection and herd immunity, and must be able to contain epidemics following complex emergencies.

O065

Conjugation of a *Shigella flexneri* 2a derived synthetic oligosaccharide to tetanus toxoid

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Bacterial infections causing diarrhoeal diseases account for over three million deaths annually. One of the major diarrhoeal diseases is bacillary dysentery, or shigellosis caused by *Shigella* ssp. Of which *S. flexneri* 2a is most prevalent. Several efforts have been made to develop a potential vaccine. Examples of these are the use of attenuated strains, native *S. flexneri* 2a lipopolysaccharide complexed to meningococcal outer membrane proteins and other O-antigen specific Polysaccharide-Protein Conjugate Vaccines. The consensus of these approaches is that they intend to induce protection by raising antibodies specific for the O-antigen (O-Ag). The O-Ag is part of the lipopolysaccharide (LPS), which is one of the major bacterial surface antigens.

We aim to develop a vaccine against *S. flexneri* 2a based on a synthetic derivative of the LPS O-antigen conjugated to tetanus toxoid. Previous work showed that a pentadeca oligosaccharide specific to the O-antigen of *S. flexneri* 2a conjugated to TTd gave a good immune response in mice.[1] In order to test this experimental vaccine in a phase I clinical trial the process steps were optimized and altered to comply with current GMP standards.

In order to couple the pentadecasaccharide moiety to the tetanus toxoid the primary amines are first modified using N-gamma-(maleimidobutyryloxy)succinimide ester (GMBS). Using design of experiments (DoE), the modification reaction was thoroughly studied. The goal to have a maximum amount of modified primary amino groups (> 22) without inducing additional aggregation (< 7.5%) was achieved. The pentadecasaccharide is then coupled to the modified TTd through a conjugation reaction. The conjugations reaction was optimized and we have full control on how many pentadecasaccharides are coupled to the TTd. In fact for the first time, full occupation of all the modified groups by the pentadecasaccharide was obtained.

Immunizations of mice were performed using four individual conjugates with different loadings of pentadecasaccharide (5, 9, 17 and 26 per TTd), with and without absorption to alum. The vaccine with the lowest ratio elicited almost no immune response; the conjugates with higher ratios did elicit an immune response that was much even higher with Alum. However, the glycoconjugate with 17 pentadecasaccharide per TTd had a higher antibody titer than the fully loaded glycoconjugate with 26 pentadecasaccharide per TTd.

Reference

1. Phalipon A, Costachel C, Grandjean C, et al. Characterization of functional oligosaccharide mimics of the *Shigella flexneri* serotype 2a O-antigen: implications for the development of a chemically defined glycoconjugate vaccine. J Immunol. 2006;176:1686-94.

O067

A fresh look at dissimilatory sulfate reduction

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The dissimilation of sulfur compounds is likely to have been one of the first energy metabolisms on the early Earth. However, many questions remain about how sulfur-metabolizing organisms obtain energy for growth from reducing, oxidizing or disproportionating sulfur compounds. In the case of sulfate reduction, the terminal reductases involved (APS reductase (AprAB), and dissimilatory sulfite reductase (DsrAB)) have long been recognized, but how these two reactions are coupled to energy conservation is still not clear. DsrAB, in particular, is a key enzyme in dissimilatory sulfur metabolism, being present not only sulfate/thiosulfate/sulfite reducing organisms, but also in sulfur-oxidizers (where it is thought to operate in reverse) and sulfur disproportionators. The mechanism of sulfite reduction by DsrAB has long been the subject of controversy, until our recent work showing that a protein trisulfide is involved and that the pathway of sulfate reduction is not a three-, but a four-step pathway.[1]

Our lab has studied several of the key proteins involved in sulfate reduction, including DsrAB and two respiratory membrane complexes specific to sulfur-metabolizing organisms, QmoABC and DsrMKJOP, which are likely involved in the electron transfer pathways with AprAB and DsrAB.[2] These complexes point to menaquinone involvement and chemiosmotic energy conservation during sulfate reduction. In addition, a genomic analysis of energy metabolism genes in sulfate reducers indicates that the recently recognized process of electron bifurcation may also be involved, and that there are conspicuous links between sulfate reducers and methanogenic organisms.[3,4] In this talk I will present recent results on the role of these proteins in sulfate reduction, with a special focus on the function of the small protein DsrC as a physiological partner of DsrAB and the DsrMKJOP complex, and on the involvement of electron confurcation/bifurcation processes during sulfate reduction.

References

1. Santos A, Venceslau SS, Grein F, et al. A protein trisulfide couples dissimilatory sulfate reduction to energy conservation. Science. 2015;350:1541.
2. Rabus R, Venceslau SS, Whlbrand L, et al. A Post-Genomic View of the Ecophysiology, Catabolism and Biotechnological Relevance of Sulphate-Reducing Prokaryotes. Microb Physiol. 2015;66:55-321.
3. Pereira IAC, Ramos AR, Grein F, et al. A comparative genomic analysis of energy metabolism in sulfate reducing bacteria and archaea. Front Microbiol. 2011;2:69.
4. Ramos AR, Grein F, Oliveira GP, et al. The FlxABCD-HdrABC proteins correspond to a novel NADH dehydrogenase/heterodisulfide reductase widespread in anaerobic bacteria and involved in ethanol metabolism in *Desulfovibrio vulgaris* Environm Microbiol. 2015;17:2288-305.

O068

Microbiota for health: The case of *Akkermansia muciniphila*

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Akkermansia muciniphila is an anaerobic gut microbe that degrades the host mucus layer. Studying the interaction between *A. muciniphila* and the host has led to novel insights in the use of host compounds, such as mucosal glycans, mothers milk and oxygen. Although *A. muciniphila* was characterized as a strictly anaerobic bacterium, we have shown that it can use oxygen for respiration via cytochrome bd. We used two parallel fermentor systems to grow *A. muciniphila* anaerobically, after which a small amount of oxygen was added to one, while the other remained anaerobic. By transcriptomic comparison of these aerated versus non aerated cultures, we identified a suite of genes involved in oxygen metabolism. Initially, genes involved in detoxification were upregulated, including those coding for superoxide dismutase and hydroperoxidase. After prolonged exposure to oxygen, the cytochrome bd genes were expressed more

in the aerated fermentor compared to the strictly anaerobic condition. To test the functionality of this cytochrome complex, we expressed the genes for both subunits in the cytochrome-deficient *Escherichia coli* ECOM4. By analysing the metabolites produced by *E. coli* ECOM4 and the strain complemented by the cytochrome bd genes, we were able to show that the *A.muciniphila* cytochrome bd changed the aerobic metabolism of strain ECOM4, indicating the functionality of the respiration complex. This complex, together with the detoxification mechanisms, enable *A. muciniphila* to tolerate and even utilize mucosal oxygen concentrations.

Further analysis of the metabolic capacities of *A. muciniphila* testified for its adaptation to the gastrointestinal mucin, a complex glycoprotein. Mucosal glycans contain many different sugar residues, while the peptide backbone largely consists of only three amino acids: proline, serine and threonine. By testing all compounds individually we were able to design a minimal medium that will allow us to study this bacterium in detail. Understanding the metabolic requirements of *A. muciniphila* paved the way to create an animal component-free medium that is essential for human trials. Using this approach we have now started a human intervention trial with *A.muciniphila* in collaboration with Prof Patrice Cani in Belgium (see https://clinicaltrials.gov/ct2/show/NCT02637115).

O069

Anaerobic oxidation of methane in the paddy field dominated by *Methanoperedens nitroreducens*

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Methane is the second most important greenhouse gas in the atmosphere and is 25 times more potent in the radiative forcing than CO_2. Paddy fields contribute about 20% to the global methane emission, making them a significant source of methane. Paddy fields provide several distinct habitats for microorganisms: the surface soil, the bulk soil, rhizosphere soil and roots. In order to mitigate methane emissions from paddy fields, it is important to understand the sources and sinks. Most paddy fields are heavily fertilized with nitrate which via nitrite can be used as electron acceptor by bacterial (NC10 phylum) and archaeal anaerobic methanotrophs. Here we collected paddy field soil and rhizosphere samples at the Italian Rice Research Institute, Vercelli Italy. We showed that slurry incubations of Italian paddy field soil with nitrate and ^{13}C-labelled methane have the potential for nitrate-dependent anaerobic oxidation of methane. Community analysis based on 16S rRNA amplicon sequencing and qPCR of the water-logged soil and the rhizosphere showed that AOM-associated archaea (AAA), including *Methanoperedens nitroreducens,* comprised 9% (bulk soil) and 1% (rhizosphere) of all archaeal reads. The NC10 phylum bacteria made up less than 1% of all bacterial sequences.

The phylogenetic analysis was complemented by qPCR with newly developed primers and showed that AAA ranged from 0.28 to 3.9 x 10^6 16S rRNA copies g^{-1}_{dw} in bulk soil and 0.27 to 2.8 x 10^6 in the rhizosphere. The abundance of NC10 phylum bacteria was an order of magnitude lower. The bulk soil was used to start enrichment cultures in bioreactors supplied with nitrate and methane. After 1 year of continuous operation nitrate-AOM could be measured and both NC10 bacteria and AOM archaea could be visualized by FISH. Currently the metagenome of the reactor is being analysed. Together our data suggests that AOM archaea may play an important role in methane cycling in paddy fields.

O070

Extracellular polymeric substances of anammox granular sludge contain glycoproteins and have a gel-forming property

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Anammox (anaerobic ammonium oxidation) bacteria belong to the phylum of *Planctomycetes*. They are able to convert ammonium and nitrite to nitrogen gas. Anammox bacteria have a strong tendency to grow in aggregates. To have a better understanding of the formation of aggregates like flocs and granules, it is important to elucidate the composition of extracellular polymeric substances (EPS). However, due to the limitation in the present methodologies of extraction and characterization, the EPS of anammox granules are still a black box. The aim of this study was to explore the EPS of anammox granules and to get more insights into the structure of the EPS matrix.

An alkaline extraction was used to extract EPS from the granules. To get a high extraction yield while avoiding protein hydrolysis, different concentrations of NaOH were tested. The extracted EPS were characterized by elemental composition analysis and by quantification of the proteins and polysaccharides with the BCA and phenol-sulphuric acid assay, respectively. The proteins were further analysed using SDS-PAGE. Besides Coomassie blue staining for the detection of proteins, periodic acid-Schiff (PAS) and Alcian blue staining were applied to detect respectively neutral and acidic glycoproteins. The monomer composition of polysaccharides was determined using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The mechanical properties of the extracted EPS were investigated by rheological characterization.

The results showed that extraction with 0.1 M NaOH for 5 hours gives a relatively high yield of 205 21 mg/g VSS of which the main part is proteins. Using SDS-PAGE a glycoprotein of 80 kDa was found to be a dominant protein. Different monomers of sugars were detected by HPAEC-PAD. The rheology measurements showed that the EPS form a gel at pH 8 and 25°C. The strength of the gel increased with multiple frequency sweeps.
This research provides first insights into the investigation of glycoproteins in EPS of anammox granules, and the gel-forming property of the EPS. The gel-forming property of EPS is important since it contributes to a gel matrix in granular sludge for anammox bacteria to be imbedded in. Furthermore, the finding of a dominant glycoprotein in the EPS is a very interesting starting point to study the EPS composition. Future research will focus on identifying if the glycoprotein of 80 kDa is a surface layer protein of anammox bacteria, and its function in anammox granule formation.

O071
Novel sulfate-reducing bacteria create microniches in acidic environments

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Exposure of mineral ores (or waste material from mining industry) to water and air leads to the formation of acidic and metallic waters, referred to as acid rock/mine drainage. The formation of this acid drainage is a chemical process greatly enhanced by the action of various species of iron- and/or sulfur-oxidizing prokaryotes and nowadays it constitutes a world-wide severe threat to the environment. Fortunately, sulfate-reducing bacteria (SRB) can essentially reverse this process producing sulfide, which precipitates the metals, and producing alkalinity, which raises the pH. Despite the high interest in acidophilic SRB for their potential in application, thus far, only three acidophilic species of SRB have been formally described: *Desulfosporosinus acidiphilus, D. acididurans* and *Thermodesulfobium narugense*. By using as inoculum sediments from an acidic environment (Tinto River) we isolated several strains of acidophilic SRB. One of our isolates, strain INE, represents a new genus of acidophilic SRB, phylogenetically related with the *Desulfosporosinus* and *Desulfitobacterium* genera, proposed as a *Desulfobacillus* sp. Genome-guided characterization of our isolate, shows a very versatile metabolism; it is able to use multiple electron donors and electron acceptors for respiratory growth, and to perform fermentation and disproportionation. Strain INE shows a unique metabolism compared to other acidophilic SRB by being able to oxidize acetic acid through the reverse acetyl-CoA cycle. In strain INE^T monoglycerolethers membrane lipids are present, which seem to be related with cell resistance by conferring a highly impermeable membrane. The monoglycerolethers lipids are especially present during growth at pH 3.8 in comparison with pH 5, accounting for up to 13% of the total cellular lipids. As a consequence, strain INE, is the most acidophilic strain described so far, able to grow while reducing sulfate at a pH as low as 3.8 (optimum at pH 5).
Its high tolerance to low pH and its acetate detoxifying metabolism together with the alkalinity generation coupled to growth of SRB suggest its important role in promoting microbial niches with milder conditions in acidic environments. Related rRNA gene sequences are often found in acid mine drainage (AMD) environments, indicating their ecological importance.

O073
Irritable Bowel Syndrome and the fungus among us

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There is vast attention for the gut microbiome in relation to a wide range of diseases. Possibly because they are a minor component of the gut ecosystem (0.1% of the microorganisms), fungi were largely excluded from these investigations so far. This also counts for irritable bowel syndrome (IBS), were gut fungi were ignored but bacterial microbiome dysbiosis was repeatedly described and became an important target for treatment. IBS is a highly prevalent gastrointestinal disorder characterized by abdominal pain and altered bowel habits. The diagnosis of IBS is made based on symptoms. Treatment options are limited mostly due to the lack of pathophysiological understanding. Nevertheless, increased sensitivity to distension of the distal gastrointestinal tract (so called visceral hypersensitivity) is present in 35-60% of the IBS patients. It can be triggered by stress and is hypothesized to be a key mechanism in the generation of abdominal pain symptoms. Recent evidence that resident fungi can play a role in inflammatory bowel disease (IBD) led us to assess the possible role of fungi in inducing abdominal pain in IBS.We used a rat model for IBS that mimics early adverse life events that have been shown to be associated with increased risks of IBS later in life. In this model, Long Evans rat pups are separated from the dam for 3 hours daily from postnatal day 2 to 14 (maternal

separation: MS) and left undisturbed throughout further development. Acute water avoidance stress at adult age induces visceral hypersensitivity in MS rats whereas non-handled rats remain normally-sensitive. Earlier, our preclinical results were successfully translated into clinical trials, demonstrating that this animal model can be predictive for human IBS. When, in our most recent investigations we treated MS rats with different fungicides prior to water avoidance, there was no visceral hypersensitivity. In addition, rats that already developed post water avoidance visceral hypersensitivity could be normalized by fungicide treatment. Subsequent repopulation experiments indicated that visceral hypersensitivity was associated with a unique mycobiome composition and this was confirmed by mycobiome analysis. Last, we obtained evidence that recognition of fungi/fungal antigens by the host immune system was key to the observed visceral hypersensitivity. To investigate the possible role of fungi in human IBS we then determined the fungal composition in feces of IBS patients and controls. This analysis showed that IBS patients have a mycobiome that is distinct from that of healthy volunteers. Together, our data strongly suggest a role for fungi in the development of abdominal pain in IBS. Based on our results we now investigate novel therapeutic avenues to treat abdominal pain in this disorder.

O074

On the oral microbiome and Candida interaction

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A recent study on variability and stability of oral fungal communities in time (Monteiro-da-Silva et al., 2014) described 100% incidence of moulds and 92.5% incidence of yeasts in a group of healthy participants with good oral health. However, only the bacterial component (generally referred to as 'microbiome') has been studied extensively, with the fungal component being largely neglected or addressed mainly in relation to diseases such as HIV and candidiasis. In this presentation a current state of knowledge on diversity of fungal communities in a healthy oral ecosystem, as well as the ecological relation between oral *Candida* and oral microbiome will be addressed.

O075

Differential kinetics of *Aspergillus nidulans* and *Aspergillus fumigatus* phagocytosis

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Introduction: *Aspergillus* species differ in their pathogenicity: invasive and allergic aspergillosis is most commonly caused by *A. fumigatus,* while *A. nidulans* is only rarely found. Only in patients lacking the NADPH-oxidase activity, like chronic granulomatous disease (CGD), *A. nidulans* is a frequent cause of aspergillosis, and is associated with a higher mortality. We investigated whether these species result in differential immune recognition and phagocytosis, which might explain their diversity in pathogenicity.
Methods: Using live-cell imaging we tracked phagocytosis events of *A. fumigatus* and *A. nidulans* by the murine macrophage cell line J774A.1. Differences in the dynamics of recognition, engulfment and migration of macrophages to perform phagocytosis were assessed. In addition, we investigated the capacity to induce an oxidative burst and cytokine responses in peripheral blood mononuclear cells (PBMCs) of healthy volunteers.
Results: A delayed recognition and phagocytosis of *A. nidulans* spores compared to *A. fumigatus* spores was observed, which could be attributed to a slower macrophage migration towards *A. nidulans* spores. Moreover, we observed that phagosome acidification is significantly slower upon phagocytosis of *A. nidulans*. In line with this, a less pronounced oxidative burst was observed following exposure of PBMCs to *A. nidulans*. Yet in contrast, *A. nidulans* was able to induce significantly higher levels of innate and adaptive cytokines than *A. fumigatus*.
Conclusion: Collectively, *A. nidulans*, which primarily causes aspergillosis in CGD patients, has a higher immunostimulatory and lower ROS-inducing capacity, but is slower phagocytosed than *A. fumigatus* conidia. On top of defective phagocytosis machinery in CGD patients, there is a less effective clearance of *A. nidulans* by macrophages in general. This helps to explain the unique susceptibility of CGD patients to *A. nidulans* infections, and the higher mortality associated with *A. nidulans* infections in CGD patients.

O076

Diversity and functioning of bacterial communities in the lab and in the wild

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Bacterial communities are incredibly complex, with thousands of interacting species in every pinch of soil. A major challenge in microbial ecology is to understand how these thousands of interacting parts influence key ecosystem properties (e.g. rates of nutrient cycling). We have conducted a series of experiments to understand how ecological and

evolutionary processes interact to mould both broad (e.g. respiration) and specific (e.g. activity of specific metabolites) ecosystem properties. The presentation will summarise these results, outline current methods for linking structure and function in laboratory microcosms, and will also develop new methods for understanding ecosystems in nature.

O077

Evolving symbiotic partnerships

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The formation of symbiotic partnerships among unrelated species has been instrumental in shaping biological complexity and diversity across the tree of life. From the evolution of mitochondria that define eukaryotic complexity, to algal symbionts that power the world's oceans, symbiotic partnerships have driven the evolution of organismality. While genomic tools have significantly advanced our ability to identify these symbiotic partnerships, we still do not understand the selection pressures driving the evolution of symbiosis. For hosts (humans and other organisms alike), associating with symbiotic microbes can have both costs and benefits. In most cases, symbiosis provides clear benefits and new functions to both partners, leading to positive selection. However, the degree to which symbionts benefit their hosts can vary dramatically, with enormous variation both across and within species. Because associations generally involve multiple microbial genotypes varying in mutualistic benefit, a potential tragedy of the commons can arise. How do hosts maintain cooperation with the most beneficial microbes over the course of evolution? Specific host mechanisms may be employed that reduce the fitness benefits to microbes from defection. A major aim of our group is to study the trade of carbon and nutrients between the hosts and microbes. We are exploring the use of quantum dot technology to track trading strategies across space and time. Ultimately, we are interested in understanding how cooperation is maintained among hosts and their symbiotic microbial communities.

O078

Meta-analysis of natural disease suppressive soils

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Disease suppressive soils are soils in which plant pathogens cause little or no plant infections due to microbial activities in the soil or rhizosphere. This worldwide phenomenon has been described for many plant species and a range of plant pathogens, including fungi, oomycetes, bacteria and nematodes. For most disease suppressive soils, however, the responsible microbial communities and the underlying mechanisms of disease suppression still remain unknown. In this study, we induced disease suppressiveness in soils against the soil-borne fungus *Rhizoctonia solani* by growing the host plant (sugar beet) successively in presence of the pathogen. The induced suppressiveness could be transferred to a conducive soil, suggesting that it has a (micro)biological basis. To study the dynamics and *in situ* activities of the microbial communities during the transition from a disease conducive to a disease suppressive state, total DNA and RNA were isolated from the rhizosphere of sugar beet and subjected to metataxonomic and metatranscriptomic analyses. 16S amplicon analyses revealed only minor changes in the rhizobacterial community composition during transition from a disease conducive to a suppressive state, with most OTU changes observed in the Bacteroidetes phylum. Results of the ongoing metatranscriptomic analyses will be presented to highlight which functions in the rhizobacterial community are induced during rebiosis, i.e. the transition from a diseased to a healthy soil.

O079

Comparative genomics of *Intestinimonas butyriciproducens*, a lysine utilizing and butyrate producing bacteria in the human gut

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Introduction: *Intestinimonas* is a newly described bacterial genus with representative strains present in the intestinal tract of human and other animals. Although members of this genus carry out important metabolic functions including the production of butyrate from both sugars and amino acids, there is to date no data on their diversity, ecology and physiology.
Methods: *Intestinimonas butyriciproducens* strain AF211 was obtained from a stool sample of a healthy individual and strain SRB521^T was isolated from the caecum of a mouse. Bacteria were routinely grown in anaerobic Reinforced Clostridium Medium (RCM, Difco) in 120-ml serum bottles sealed with butyl-rubber stoppers at 37ffIC under

a gas phase of 1.7 atm of N_2/CO_2 (80:20, v/v). Growth of both strains were compared on sugars and amino acids and tested for production of short chain fatty acids. The genomes of two strains of *Intestinimonas butyriciproducens* AF211 and SRB521^T was sequenced using PacBio RS II instrument using P4/C2 chemistry. Genome sequences were also collected using a HiSeq2000 and a hybrid assembly was done to obtain complete genomes for both the strains. Genome annotation was done using RAST server and the core genome was calculated using Spine and the pan-genome was identified using AGEnt. CRISPR and prophage genes were identified using CRISPRfinder and PHAST (PHAge Search Tool) while, genomic islands were identified using online IslandViewer and a standalone program i.e. SeqWord Gene Island Sniffer. The carbohydrate-active enzymes (CAZymes) were annotated using the amino acid sequences with default search parameters in CAZYmes Analysis Toolkit (CAT).

Results: The complete 3.3-Mb genomic sequences of both strains were highly similar with 98.8% average nucleotide identity, testifying for their assignment to one single species. The pan-genome of *I. butyriciproducens* was calculated to contain 4,762 coding DNA sequences (CDS), of which 2,612 CDS (2,603,042bp) were considered as core. Approximately 22.8% of the genome sequences were unique to one of the isolates signifying the strain level differences in the genomic content of AF211 and SRB521T. Further detailed analysis revealed significant genomic rearrangements including large inversions and integration of foreign regions of DNA, variations in phage-derived sequences, and the presence of new CRISPR sequences and metabolic genes present only in the human strain. While both strains were capable of producing butyrate from sugars and lysine, the human strain AF211 was more efficient in these conversions than the mouse isolate. Notably, this was observed with arabinose and galactose, two sugars found abundantly in the human but not mouse diet.

Conclusion: The two strains of *I. butyriciproducens* contained 2 butyrate synthesis pathways, where both lysine and simple sugars can serve as energy source. Impact of exposure to different host environment was evident mostly at genomic level (differences in GC skew, prophage sequence, etc.) while functionally AF211 was more efficient in producing butyrate from sugars and lysine. In conclusion, the present genomic and physiological study of *I. butyriproducens* provides evidence for different host-specific features of strains originating from the mouse and human gut.

O080

The future of the microbiological technician

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How do we prepare our students for a future as a microbiological technician? In order to answer that question, it is important to know how the specific bachelor programmes are organized in the Netherlands.

The Applied Science Domain (DAS = Domein Applied Science) is the national collaboration between higher professional educational establishments (Universities of Applied Sciences = HBO) that award a Bachelor of Science degree, formerly (before September 2015) Bachelor of Applied Science. This domain consists of 15 Universities of Applied Sciences offering 9 different bachelor programmes and two master programmes (and additionally one master is being developed). More than 11000 students are attending one of these study programmes at different locations and 27 senior lecturers are conducting applied research in this field.

Biological and Medical Laboratory Research (about 4680 students) is the programme which can be attended at 11 different locations. About 1400 new students have started to participate in this programme in September 2015. Part of this programme (one of the major specialisations) is responsible for the training of microbiological technicians. On regular basis the domain consults the professional field about the required qualifications, competencies and skills of our students for the different specialisations. Not only the qualitative matching is important, but also the quantitative matching.

The number of newly registered students for Biological and Medical Laboratory Research has increased by a factor 1,6 in a period of four years (1048 in 2010 and 1717 in 2014), while the number of internships positions decreased. As a result five Universities of Applied Sciences introduced in september 2015 a Numerus Fixus for this programme, resulting in a decrease of the number of students: 1400 new students entered the programme in September 2015.

In this presentation an overview is given of the outcomes of the discussion with the professional field about the position and requirements of the microbiological technicians. For instance: there appears to be a decrease in the number of positions for microbiological technicians in medical laboratories, due to automatization and robotization. A decrease in research positions is also observed in the academic hospitals, due to less funding and replacement of our bachelor hbo-students by academic students. Other qualifications are required, for instance analytical chemistry (in general chemistry) and molecular biology in the academic hospitals. The importance of knowledge of bio-informatics and big data (ICT) is increasing.

The University of Applied Sciences in our domain have to take measures to anticipate for the decrease in job positions. These measures have to be taken in close collaboration with the professional field.
So how do we prepare our students Microbiology for the future? What kind of competences, knowledge and skills is required?

O081

General discussion onderwijsvernieuwing: Kiest u maar!

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Hoe gaan we de toekomstige generaties microbiologen in Nederland opleiden? Nu veel microbiologisch werk geautomatiseerd is en deelonderzoeken uitbesteed worden aan gespecialiseerde laboratoria, zullen de taken van microbiologen over paar jaar anders zijn dan voorheen. Waarschijnlijk zullen zij veel minder klassieke technieken toepassen en meer tijd doorbrengen achter de computer om moleculaire data te analyseren. En hoe bereiden we onze collega's microbiologen van de toekomst voor op de snelle technische ontwikkelingen op het gebied van big data?
Onderwijsinstellingen volgen de arbeidsmarkt, maar zij moeten nog verder vooruitkijken dan de markt. En dat willen we vandaag doen samen met u, de specialisten in het werkveld. Wat verwacht u van uw toekomstige collega arts-microbioloog? Of, welke eisen stelt u over een paar jaar aan een sollicitant die uw onderzoeksteam wil komen versterken?
Aan de hand van een aantal stellingen willen we met u bediscussiëren welke competenties en vaardigheden prioriteit hebben en welke niet meer in de opleiding van de aanstaande microbioloog hoeven voor te komen. Kiest u maar!

O082

Team-based learning in a medical microbiology course

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Team-based learning is a novel approach to teaching, designed to enhance active participation and motivation of students in their learning process, make them more accountable for their performance (and presence), make them learn how to effectively work together as a team, learn from each other in the process, and have fun in doing so!
The basic steps in team-based learning are (i) individually study materials (book chapters, articles) selected by the teacher to provide the student with basic knowledge on a particular subject. This knowledge is tested in a socalled iRAT, the individual Readiness Assessment Test, consisting of 10-20 multiple choice questions. The students make the test but do not yet get the answers. (ii) They then immediately do the same test as a team, the tRAT, and are subsequently provided with the answers. In case they have objections to particular questions or answers they can file written, argumented appeals which are discussed with the entire group. This can lead to question retraction or change in scoring. (iii) The third step is a much more elaborate exercise, the 'Application'. The student groups all receive the same assignment and need to find answers to a number of questions. By studying and discussing the provided documents they need to reach one answer to each question which the entire Group agrees upon. In a plenary session, the groups then need to defend to the other groups why they have chosen a particular answer. The teachers will provide different viewpoints to deepen the discussion and to help reach a final well-argumented conclusion.
We have designed and performed a short team-based learning module in the 2015 course 'Advanced Medical Microbiology' of the Master Biomedical Sciences of the University of Amsterdam. The subject of this module was 'Vaccination against Group B meningoccal disease'. The outline of the module and the experience in running the program will be presented. The experience of both the students and the teachers, all novices to team-based learning, was absolutely positive.

O083

How to stimulate students to design their own microbiological experiment?

I. Jongenburger, W.C. Hazeleger
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In the course Advanced Food Microbiology at Wageningen University, Master students design their own experiments from scratch based on their prior knowledge gained in previous courses. This group of students has diverse backgrounds, levels, and nationalities (25 different nationalities in group of 93 students). The number of students has increased from 57 (2010) to 93 (2015) and is expected to increase even further in the future. During the practical course of two weeks, the students (groups of 3) design two experiments. One experiment is the preservation experiment, in which the students apply a preservation technique on a chosen fresh product. The development of spoilage microorganisms in the preserved product is investigated during a specific storage time and temperature. After the preservation they observe the structural characteristics of the food product. The students have one

afternoon to work on the design. In the current set up, we experienced the following issues:

- Students have difficulties in designing the experiments.
- Students need approval by staff for the experiments they need to perform, which is time consuming.
- Students have to hand in a list of media and confirmation test for their experiments.

To solve these issues we developed an e-learning case using the online LabBuddy™-tool (http://www.labbuddy.net). During this case, students designed their preservation experiment in the experiment designer (ExperD). In the laboratory, they were supported by the web laboratory manual (webLM). Both ExperD and webLM are part of LabBuddy™.The aim of the tool is to design an experimental set-up for the determination of certain groups of spoilage microorganisms. The design tool has the following features:

- to assist student to choose a combination: product, preservation technique, storage conditions, relevant spoilage microorganisms to investigate.
- to assist student to order the correct number of media
- output is a shopping list of media, which can be used to order the media (Excel file).

The outline of preservation case in LabBuddy™ is as follows. To brush up prior knowledge, the linear case starts with an introduction containing the topics: microbial spoilage, shelf life, growth rate and its influencing factors, spoilage associations, characteristics of specific spoilage organisms, including 5 questions. Then, the experiment is discussed: explanation of the preservation experiment, demonstration of ExperD in a clip. Subsequently, the students use ExperD to design their experiment by choosing a fresh product, preservation technique(s), storage temperature(s) and microbiological tests. The microbiological tests are linked to an online manual with protocols. The output is a shopping list with correct number of media. Although not obligatory, the students can discuss their design or shopping list with the supervisors.

In January 2016, the new set-up of the practical course was introduced. The design tool reduced an overload of information, and provided just in time (JIT) feedback, and a link between design and protocols. The students asked less questions and after filling in the shopping list correctly, they did not need approval by the staff to order media. Furthermore, the media for all groups could easily be ordered and distributed by using a clear and coherent digital shopping list. The experiences of supervisors and students have been evaluated, as well as a survey among 80 students. These results will be presented. Our first impression was very positive, and this innovation is promising for other courses.

O084

Gamification in laboratory education

E.M. van Hove
ROC Midden Nederland, Laboratoriumonderwijs, Amsterdam

Reimagine education with gamification! Gamification, the use of online game elements, is not yet adopted in education. The tool 'Achievements' recently became available in the virtual learning environment of Blackboard. With this new tool a digital badge system can be designed within Blackboard, in which students are challenged to learn more. Students can 'win' digital badges by completing assignments and tests. In classes microbiology every week my students make a formative test in Blackboard. They do the test as many times as they want. Blackboard generates everytime different questions and provides immediate feedback. If the test is completed, students earn a digital badge on one of the three levels: *novice, intermediate* or *expert*. Making progress tests is one of the most effective learning strategies. Using digital badges is an extra incentive by which the progress tests are made even better. In this presentation the use of digital badges in classes microbiology is demonstrated.

O087

West Nile and Usutu viruses: high chance of transmission by north-western European mosquitoes

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Background: West Nile virus (WNV) is a highly pathogenic flavivirus (family *Flaviviridae*). The introduction of lineage 1 WNV into North America caused the largest outbreak of neuroinvasive disease to date. In southern Europe, a highly pathogenic lineage 2 strain has recently established itself, causing annual outbreaks. Additionally, the related flavivirus Usutu virus (USUV), has also recently emerged in Europe. Both WNV and USUV are transmitted between avian amplifying hosts by *Culex* species mosquitoes and infect humans and horses incidentally. USUV and WNV co-circulate in parts of southern Europe, but the distribution of USUV extends into central and north-western Europe. To estimate if WNV can also expand its distribution it is key to know if mosquitoes from WNV-free areas can support further spread.

Methods: We determined the vector competence of north-western European and American *Culex pipiens* mosquitoes for both WNV lineages and compared it to that of USUV. We orally infected mosquitoes with lineage 1 or 2 WNV or USUV and determined the infection and transmission rates. We explored reasons for vector competence differences by comparing intrathoracic injections versus

oral infections and we investigated the influence of temperature.

Results: North-western European mosquitoes were highly competent for both WNV and USUV. Notably, transmission rates for lineage 2 WNV were significantly elevated in European mosquitoes, compared to those in American, due to better virus dissemination and a shorter extrinsic incubation time. The infection rates of both WNV and USUV were enhanced at higher temperatures. Interestingly, at higher temperatures USUV infected significantly more mosquitoes compared to WNV.

Conclusions: Our study provides experimental evidence to indicate markedly different risk levels between the American and European continents for lineage 2 WNV transmission. Our results explain the current localized WNV activity in southern Europe, yet imply further epidemic spread during periods with favourable climatic conditions. Finally, as both viruses utilize the same vector and reservoir species, the higher infection rate of USUV suggests that this virus may precede WNV transmission in Europe. This presses the need for intensified surveillance of virus activity in current WNV-free regions and warrants increased awareness in clinics throughout Europe.

O088

Environmental surveillance suggests a role for surface water in supporting endemic tularemia in the Netherlands

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Tularemia is an emerging zoonosis in Europe with most cases reported from Scandinavia and Turkey. The disease is caused by the intracellular pathogen *Francisella tularensis*, which has a wide host range but also seems to be tied to one or several yet unknown environmental reservoir(s). The organism has been detected in various types of surface waters and sediments where it is potentially hosted by free-living protozoa. In the Netherlands, several cases of endemic tularemia among hares and humans have been detected since 2011, after a period of more than 50 years without such endemic infections. In order to investigate a potential role of the aquatic environment as a reservoir of *F.tularensis*, we initiated an environmental surveillance pilot.

Surface waters and sediments samples from ditches, canals and lakes, were collected from a region in Friesland where a cluster of tularemia-infected hares had been recognized in 2015. *F. tularensis* DNA was shown to be present in water samples and sediments from a limited geographical area where tularemia-positive hares had been found, while *F. tularensis* DNA was not detected at greater distances from this cluster. In addition to the Friesland region, *F. tularensis* DNA was detected in water samples that had been collected at two different sites in the middle and Southwest of the Netherlands.

Even though not all sampled locations were positive for F. tularensis, we can conclude that F. tularensis does reside in aquatic environments in the Netherlands, and was in some cases shown to be present over time. It is however currently unknown which environmental conditions are favorable for growth of *F. tularensis* to detectable levels.

More detailed sampling schemes and analyses are required to substantiate temporal and spatially restricted occurrence of *F. tularensis* and to elucidate potential environmental conditions favoring its growth. As such, environmental surveillance will likely contribute to elucidate whether detectable levels of *F. tularensis* in surface waters indicates an elevated health risk, both for animal and public health.

O089

High prevalence of undetected highly resistant microorganisms among residents of long term care facilities in Amsterdam, the Netherlands

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Introduction: Dutch national guidelines for infection control measures for carriers of Highly Resistant Microorganisms (HRMO) in Long Term Care Facilities (LTCFs) were published late 2014. A recent study in Amsterdam (2010) showed a prevalence of extended-spectrum beta-lactamase producing Enterobacteriaceae (ESBL-E) of 10.6% (9.7-11.5 95% CI) in patients with gastrointestinal symptoms. Our aim was to study the prevalence and risk-factors of carriage of HRMO among residents of LTCFs in Amsterdam.

Methods: We performed an observational cross-sectional study in which we determined the prevalence of HRMO-carriage in residents of LTCFs in Amsterdam. Swabs from nose and faeces were cultured for phenotypical analysis of meticillin-resistant *Staphylococcus aureus* (MRSA), faecal swabs were analysed for multidrug-resistant Gram-negative organisms (MRGN) and vancomycin-resistant enterococci (VRE) according to the Dutch guidelines.

Results: From November 2014 to august 2015, 385 residents from 12 LTCFs were enrolled of whom 355 could be analysed for MRSA colonization, 346 for rectal carriage

of MRGN and 347 for rectal carriage of VRE. Prevalences for MRSA, MRGN and VRE were 0.8% (range 0-7%), 18.2% (0-47%), and 0% respectively. 47 Out of 346 (13.6%, 10-17.2 95% CI) samples yielded ESBL-E. In total, 68 MRGN strains were cultured from 63 residents; 50 (74%) were ESBL-E, of which 13 strains were as well resistant to fluoroquinolones and aminoglycosides and one strain harboured also carbapenemase. Of the remaining strains 17 were Enterobacteriaceae resistant to the combination of aminoglycosides and fluoroquinolones and one strain was identified as *Pseudomonas aeruginosa* additionally resistant to piperacillin. Infection control measures were only applied in 9 out of 59 (15%) carriers of HRMO at the time of sampling. Additional analyses on resident and institutional related risk factors will be presented.

Conclusions: Our data shows that the prevalence of MRSA and VRE in Amsterdam LTCFs is low. The carriage rate of MRGN in LTCFs is at least as high as in the general population. If we consider the infection control measures taken in patients, the majority of MRGN-carriers (85%) are currently undetected. Therefore continuation of infection control measures without surveillance or additional screening for HRMO seems inappropriate.

O090

Evidence on HPV testing: Primary hrHPV detection versus cytology in cervical cancer population screening

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Over recent years, a series of Cochrane reviews and meta-analyses were conducted in order to establish evidence on questions related to secondary prevention of cervical cancer, which were used to develop the second edition of the European Guidelines for Quality Assurance in Cervical Cancer Screening, published in 2008.[1] These guidelines were pivotal to promote organized screening in the member states of the European Union. In these guidelines, evidence was recognized regarding superior accuracy (higher sensitivity and similar specificity to diagnose CIN2/3+) of hrHPV testing compared to cytology in two clinical indications: 1) triage of women with ASC-US and 2) follow-up after treatment for cervical precancer.[2] It was recognized in 2007, when evidence of primary HPV screening was assessed that testing with high-risk HPV assays was more sensitive but less specific than cytology to detect precancer.[2] However, it was judged that cross-sectional data was insufficient to recommend primary HPV-based screening, since it could not be proven that HPV testing mainly would generate overdiagnosis.1 Recently, longitudinal data from four randomized European trials were pooled which revealed a lower cumulative incidence of CIN3+ and of invasive cervical cancer in women who were at baseline hrHPV negative versus cytology negative in the first screening round (proof of higher efficacy).[3,4] Moreover, when hrHPV-positive women were triaged with cytology, the detection rates of CIN3+ over the first and second screening round were comparable in the experimental and control arms, indicating a balanced amount of over-diagnosis.[3] Based on these observations, HPV-based cervical cancer screening is recommended in EU member states.[5] Equivalency criteria were established which new HPV assays have to demonstrate: good reproducibility and non-inferior accuracy for CIN2+ compared to the standard comparator tests which were validated in the randomized trials (HC2 assay and GP5+/6+ PCR-EIA assay).[6] A list of assays fulfilling these criteria was published recently.[7]

HPV testing has the additional advantage that it can be done on self-samples which shows similar accuracy compared to clinician-collected samples, under the condition that a validated PCR assay is used.[8]

References

1. Arbyn M, Anttila A, Jordan J, et al. European Guidelines for Quality Assurance in Cervical Cancer Screening. Second Edition - Summary Document. Ann Oncol. 2010;21:448-58.
2. Arbyn M, Sasieni P, Meijer CJ, Clavel C, Koliopoulos G, Dillner J. Chapter 9: Clinical applications of HPV testing: a summary of meta-analyses. Vaccine. 2006;24:S78-S89.
3. Arbyn M, Ronco G, Anttila A, et al. Evidence regarding HPV testing in secondary prevention of cervical cancer. Vaccine. 2012;30 Suppl 5:F88-F99.
4. Ronco G, Dillner J, Elfstrom KM, et al. Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials. Lancet. 2014;383:524-32.
5. Von Karsa L, Arbyn M, De Vuyst H, et al. European guidelines for quality assurance in cervical cancer screening. Summary of the supplements on HPV screening and vaccination. Papillomavir Res. 2015;22-31.
6. Meijer CJLM, Castle PE, Hesselink AT, et al. Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. Int J Cancer. 2009;124:516-20.
7. Arbyn M, Snijders PJ, Meijer CJ, et al. Which high-risk HPV assays fulfil criteria for use in primary cervical cancer screening? Clin Microbiol Infect. 2015;21:817-26.
8. Arbyn M, Verdoodt F, Snijders PJF, et al. Accuracy of human papillomavirus testing on self-collected versus clinician-collected samples: a meta-analysis. Lancet Oncol.2014;15:172-83.

O091

Introducing the New Dutch Cervical Cancer Screening program

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Changes in the cervical population based screening

In The Netherlands cervical cancer and preliminary stages of cervical cancer are detected early by means of the population screening. At the request of the Minister of Health, Welfare and Sport (VWS), the Health Council of the Netherlands i.e. Gezondheids Raad (GR) has advised on various (medical-technological) developments with

regard to the population screening on cervical cancer. The GR has released the report 'Screening for Cervical Cancer' on 24 May 2011

On the advice of the GR, the following changes in the renewed population screening are made:

Switching from cytological screening to hrHPV-screening. HrHPV-screening is more sensitive than cytological screening whereby (precancerous) stages of cervical cancer are better to detect. Applied is a clinically validated test focused on the detection of DNA of high-risk hrHPV-types. After a first positive test result on hrHPV, a second analysis is performed on the sampled material. The second analysis consists of a cytological assessment to see if abnormal cells are present.

The chances of (precancerous) cervical cancer after a negative hrHPV-test are lower than after a negative cytological assessment, as is done now in the current population survey. In the renewed population screening women of 45 and 55 years old are only invited if they were tested positive for hrHPV on the previous screening. It is expected to be less than 5 percent of the relevant women in Netherlands. Hence with most women of 40 and 50 years old the screening interval is extended from five to ten years.

In order to lower the thresholds for participation in population screening, the GR advises to deploy a self (smear collection) tool for women who do not respond to an invitation. With a self (smear collection) tool the woman herself takes off vaginal material. This material will be tested in the laboratory on the presence of hrHPV. Women, who are tested positive for hrHPV, will then receive an invitation for a Pap smear at the general practitioner's (GP) provision for the second analysis, the cytological assessment.

Organisation: It is estimated that during the term of the agreement 560,000 samples per year are collected. The vast majority of the body material will be collected at GP's posts (approximately 97%). Remaining samples of body material are collected by the woman herself.

560,000 samples will be dispersed to 5 screening laboratories. The number of tests will equally be dispersed to these 5 laboratories.

Quality Assessment Programme for Primary hrHPV Screening: The Quality assessment exists of an accreditation quality system and an acceptation protocol for critical test components (before use). There will be internal run controls (first line), external run controls (second line) and interlaboratory comparison (thirth line)

O092

Primary HPV cervical cancer screening in the Netherlands: determination of HPV prevalence using three different systems

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Primary high risk (hr)HPV screening will be introduced in The Netherlands in the beginning of 2017. Our aim was to determine the hrHPV prevalence and workflow in a representative population based screening population.

A total of 11,802 residual PreServCyt cervical samples from the Dutch population based cytology screening program (women aged 30-60 years) were rendered anonymous, randomized and tested for hrHPV using 3 completely automated HPV detection systems: Qiagen Hybrid Capture 2 (HC2, signal amplification), Roche Cobas4800 (DNA target amplification) and Hologic Aptima (RNA target amplification). All HPV assays fulfill the international guidelines and guidelines of the Dutch Society for Pathology; all systems consist of the complete workflow from sample processing to HPV results.

The selected samples were representative for the population based screening program with respect to age distribution and cytology classification. Employing the 3 systems, the mean hrHPV prevalence was 7.9±0.3% and therefore, independently of the assay used, higher than the previously reported 4-5% using the GP5+/6+PCR-EIA (POBASCAM) and HC2 (VUSA-Screen) HPV tests. As expected, a clear age dependency was found, with an hrHPV prevalence ranging from 18.6±1.1% in women 29-33 years of age to 3.9±0.3% in women 59-63 years of age. Also for severity of cytology a correlation with hrHPV prevalence was observed, ranging from 5.4±0.3% in normal cytology to 92.2±3.1% in severe dysplasia. Medio 2016 the tender for the HPV assay resulted in the automated Cobas 4800 solution (Cobas p480 and x480) as test of choice for the primary HPV screening. The HPV prevalence found with Cobas4800 appeared to be 8% and preliminary performance data will be presented as well using this system.

In conclusion, in contrast to the report of the Dutch Health Council, a higher hrHPV prevalence of approximately 8% was found in this population based screening cohort using 3 different HPV assays, including the Cobas4800, which has consequences for the cost-effectiveness of the Dutch screening program.

O093

BK polyomavirus seroreactivity of kidney donors predicts viremia and nephropathy in recipients

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Introduction: Kidney transplant (KTx) donors are not implicated in predicting BK polyomavirus (BKV) infection

in the immunocompromised recipient. It has been suggested, however, that BKV-infection originates from the kidney allograft. Since BKV-seroreactivity correlates with BKV-replication and, therefore, may mirror the infectious potential, we investigated whether baseline BKV-seroreactivity of KTx-donors predicts viremia and BKV-associated nephropathy (BKVAN) in recipients.

Methods: In a retrospective cohort of 407 living kidney allograft donor-recipient pairs, transplanted between 2003-2013, pre-KTx donor and recipient sera were tested for BKV IgG-levels. Baseline IgG-levels were correlated with the occurrence of BKV-viremia and BKVAN during the first year after transplantation.

Results: Baseline BKV-seroprevalence of both donors and recipients was high, ≥ 95%. A strong, statistically significant association was observed between donor BKV-IgG level and occurrence of viremia ($p < 0.001$) and BKVAN. This resulted in a sevenfold increased hazard ratio for BKV-viremia, which increased even further in case of a low BKV-seroreactive recipient. Baseline recipient BKV-seroreactivity as such was not associated with viremia or BKVAN. Multivariate analysis showed donor BKV-seroreactivity to be the strongest baseline factor associated with BKV-viremia and BKVAN.

Conclusion: Donor level of BKV-IgG is a strong predictor of BKV-infection in KTx-recipients. The proportional relation between donor BKV-seroreactivity and recipient infection suggests that donor BKV-seroreactivity reflects the infectious load of the kidney allograft. This finding promotes the use of BKV-serological testing pre-KTx, in order to assess the risk of BKVAN and to personalize BKV-plasma load-monitoring. Furthermore, it emphasizes the relevance of strategies aimed to increase BKV-immunity in kidney allograft-recipients.

O094

Cell wall composition of Group A *Streptococcus* influences the bactericidal efficacy of human Group IIA-Secreted Phospholipase A

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Introduction: Human Group IIA-Secreted Phospholipase A_2 ($sPLA_2$-IIA) is a potent cationic bactericidal enzyme that hydrolyses phospolipids of the bacterial membrane. $sPLA_2$-IIA kills both Gram-positive and Gram-negative bacteria, however, activity against Gram-negative bacteria requires additional host defense proteins. The strong positive charge of $sPLA_2$-IIA suggests that negatively charged moieties on the bacterial surface are targets for initial interaction, after which $sPLA_2$-IIA penetrates through the cell wall to reach the cell membrane to exert its activity. $sPLA_2$-IIA is present in plasma and tissue fluids at concentrations below 1 nM in resting conditions. Upon inflammation or infection, $sPLA_2$-IIA levels are upregulated to mM concentrations, which is required for their bactericidal effect against most Gram-positive bacteria. Group A *Streptococcus* (*Streptococcus pyogenes*; GAS) is remarkably resistant to $sPLA_2$-IIA activity compared to other Gram-positive bacteria. This human-specific commensal can cause severe infections resulting in the death of 500,000 people globally each year. Previous research has demonstrated that loss of all LPXTG-linked surface proteins, through mutation of the enzyme sortase A (srtA), renders GAS 30-fold more susceptible to $sPLA_2$-IIA killing. Currently, the molecular mechanism behind this resistance mechanism is unknown.

Methods: The bactericidal effect of $sPLA_2$-IIA was assessed using classical plating assays using recombinant active or enzyme-dead $sPLA_2$-IIA and a clinically relevant GAS M1T1 serotype. To identify GAS $sPLA_2$-IIA resistance determinants, we screened a mutant transposon library as well as defined GAS mutant strains. Mutants included a srtA insertion mutant, seven individual LPXTG protein mutants, and a *gacI* mutant, which expresses a modified cell wall-anchored Group A Carbohydrate (GAC). Binding of $sPLA_2$-IIA to bacterial strains was performed using using the enzyme-dead $sPLA_2$-IIA and a specific anti-$sPLA_2$ antibody.

Results: In accordance with previous data, we confirmed that $sPLA_2$-IIA is bactericidal towards GAS M1T1 serotypes. Importantly, $sPLA_2$-IIA enzymatic activity was required for its bactericidal effect since the enzyme-dead $sPLA_2$-IIA or addition of a specific inhibitor did not affect GAS survival. Deletion of srtA increased susceptibility of GAS for $sPLA_2$-IIA in the GAS M1T1 background by approximately 20-fold. Addition of $sPLA_2$-IIA to serum in concentrations reached in inflamed conditions inhibited GAS growth. None of the individual LPXTG protein mutants showed altered $sPLA_2$-IIA susceptibility compared to wild-type (WT) GAS. Surprisingly, GAS fj*gacI* was completely resistant to $sPLA_2$-IIA up to concentrations of 10 μg/ml. $sPLA_2$-IIA binding to WT and fj*gacI* bacteria was similar, but undetectable in the GAS fj*srtA* mutant.

Conclusion: SrtA and GacI have opposing effects on GAS $sPLA_2$-IIA susceptibility in the clinically relevant GAS M1T1 serotype. Both proteins have a major impact on the composition of the GAS cell wall. We are currently investigating how these cell wall changes impact $sPLA_2$-IIA penetration to the cell membrane.

O095

The role of C5 convertases in MAC-dependent killing of Gram-negative bacteria

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The complement system is important for immune defenses against invading bacteria. Gram-negative bacteria are effectively killed by complement through the Membrane Attack Complex (MAC), a pore-forming complex that disintegrates bacterial membranes. The generation of the MAC on bacteria is triggered by surface-bound C5 convertases that cleave C5 into C5b, which interacts with components C6, C7, C8 and multiple copies of C9 to form the MAC (C5b-9). Despite decades of research, surprisingly little is known about the molecular events that drive bacterial killing by the MAC.

Here we developed highly purified systems to obtain molecular insights into MAC dependent killing of bacteria. Using genetically modified bacteria expressing a streptavidin-binding peptide fused to the Outer Membrane Protein A, we were able to specifically coat bacterial cells with purified (biotinylated) C5 convertases. Dynamics of inner membrane (IM) versus outer membrane (OM) disruption by (pre)pores were studied using fluorescent DNA dyes in combination with flow cytometry, dynamic fluorescence analyses and conventional plating assays.

Although it was previously indicated that C5b6 assembly takes place in solution, our data show that C5b needs to be actively formed by a C5 convertase on the bacterial surface to form a bactericidal MAC. Whereas purified C5b-9 can effectively lyse erythrocytes, Gram-negative bacteria are only killed through an actively generated C5b-9 complex. This implies that the hydrophobic domains of C7 and C8 alone are not enough for the MAC to properly anchor into the bacterial membrane. Our data also hints that complement components C5b-C8 specifically disrupt the OM of *Escherichia coli* cells whereas C9 is essential to disrupt the IM. Using bacteria specifically coated with purified C5 convertases, we rule out the requirement of other serum components in MAC-dependent killing given that we can successfully lyse *E. coli* cells with only complement components.

In conclusion, we show that C5 convertases are essential for proper docking of the MAC into Gram-negative membranes.

O096

The true face of Cas9: a subtle killer

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Introduction: Human bacterial pathogens are able to induce DNA damage upon infection of host cells, but coincidently also harbor Cas9, a nuclease that with the help of small RNA guides is able to induce double stranded DNA breaks. For the zoonotic human bacterial pathogen *Campylobacter jejuni* an important pathological feature is the ability to induce DNA damage and apoptosis in eukaryotic cells. Earlier we established that the pathogenic features of *C. jejuni* were crucially dependent on Cas9, however, the precise mechanism and whether this was related to the ability to induce DNA damage remained unknown.

Methods: To study whether Cas9 of *C. jejuni* is responsible for the induction of DNA damage and apoptosis of eukaryotic cells, two *C. jejuni* wild type strains, their isogenic and Cas9 complemented mutants were allowed to infect eukaryotic cells after which the infection process was microscopically followed for 48 hours. Microarray analyses was used to identify which cellular pathways were up or down regulated in the presence or absence of Cas9 upon infection. To study whether the DNA damage related pathological feature was induced by *C. jejuni* Cas9, two *C. jejuni* wild type strains, their isogenic and Cas9 complemented mutants were used to infect eukaryotic cells after which these cells were stained for γ-H2AX and 53BP1, markers that are used to identify double stranded DNA breaks. To confirm the induction of double stranded DNA breaks upon *C. jejuni* infection a technique called BLESS was used. Cas9 was fused to GFP to study after plasmid transfection whether Cas9 of different bacterial species were able to localize into the eukaryotic nucleus using live imaging and fluorescent microscopy. Plasmid transfection, γ-H2AX staining and the BLESS technique were used to analyze whether the induction of double stranded DNA breaks in the eukaryotic genome were truly dependent on Cas9 alone.

Results: Here we show with infection assays, microarray analyses, immunohistochemistry and the BLESS technique that upon *C. jejuni* infection of human epithelial cells, Cas9 is able to cause severe DNA damage, resulting in eukaryotic cell death within 24 - 48 hours. Plasmid based transfections revealed that not only *C. jejuni* GFP-Cas9, but also GFP-Cas9 of *Francisella novicida* were both able to localize into nucleus and nucleolus of eukaryotic cells, although this characteristics was absent when Cas9 of

Streptococcus pyogenes and *Neisseria meningitidis* were fused to GFP. The addition of a nuclear localisation signal to Cas9 of *S. pyogenes* reversed this observation and established together with (GFP)-Cas9 of *C. jejuni* that the ability to localize into the eukaryotic nucleus was accompanied with the induction of double stranded DNA breaks in a host guide RNA dependent or independent manner as visualized with γ-H2AX staining and the BLESS technique.

Conclusion: The reported novel insights are crucial for a more complete appreciation of cross-kingdom functionality of bacterial Cas9 and its role as a virulence factor. Our findings also contribute to the discussion how to safely use Cas9 in the genome editing technology.

O097

Streptococcus pneumoniae infection of zebrafish embryos: a new model to visualize and study pneumococcal meningitis

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Introduction: *Streptococcus pneumoniae* is the most important cause of meningitis in children and adults, with high morbidity and mortality. The aim of our study was to develop a pneumococcal meningitis model in the zebrafish embryo, to study host-microbe interactions. Zebrafish embryos provide a unique opportunity to image and study central nervous system infectious diseases, because these embryos are translucent.

Methods: Zebrafish embryos (n = 60 per group) were infected in the bloodstream or hindbrain ventricle with green fluorescent *S. pneumoniae* D39 (range 10^2-10^3 CFU). To visualize blood vessels by red fluorescence, a *kdrl:mCherry* transgenic zebrafish line was used. Phagocytic cells were visualized by anti-L-plastin staining. Embryos were imaged using fluorescence and confocal microscopy. A double-labeled zebrafish line with green fluorescent neutrophilic granulocytes and red fluorescent macrophages was used and time-lapse imaging was performed to study the innate immune response after pneumococcal meningitis. A time-resolved dual transcriptomics approach was used on *S. pneumoniae* and human alveolar epithelial cells during early infection to screen for putative novel pneumococcal virulence genes and mutants were subsequently tested in zebrafish embryos.

Results: Inoculation of *S. pneumoniae* in bloodstream and hindbrain ventricle of zebrafish embryos induced a dose-dependent mortality. Whereas bloodstream injection resulted in fulminant systemic disease with meningitis, hindbrain ventricle injection mainly caused brain infection. A pneumolysin deficient strain was attenuated and difference in phagocyte recruitment upon injection was observed. After pneumococcal meningitis, the initial innate immune response consists mainly of neutrophilic granulocytes without involvement of macrophages. Analysis of dual transcriptomics data revealed adherence-specific transcriptional changes during early pneumococcal infection of both *S. pneumoniae* and alveolar epithelial cells. Knockout mutants of these adherence-specific operons were attenuated in the zebrafish model compared to the wild-type strain.

Conclusions: 1. Both bloodstream and hindbrain ventricle injection with wild-type pneumococci cause a fulminant, dose-dependent infection with meningitis in zebrafish embryos. 2. *In vivo* screening of pneumococcal mutants in zebrafish embryos is a useful tool to identify new pneumococcal virulence genes. 3. The zebrafish embryo infection model appears as a powerful model to visualize and study host-pathogen interactions in pneumococcal meningitis.

O098

Investigating possibilities for control of pathogenic *Streptococcus suis* in piglets via the natural piglet microbiome

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Introduction: *Streptococcus suis* is an important swine pathogen and an emerging zoonotic agent of human infectious diseases, causing meningitis, infection of joints, endocarditis and septicaemia. Colonisation of the tonsils of young pigs during weaning poses a high risk of infection whereas carriage of *S. suis in* abundances of up to 5 percent of the total microbiome in the throat and intestine of adult pigs is usually asymptomatic. We hypothesised that the complications caused by *S. suis* are the result of a weaning-induced shift from a commensal to a pathobiont lifestyle, enabling S. suis blooms that are inadequately counterbalanced by a sufficiently robust antagonistic microbiome.

Methods: To investigate this hypothesis, about 10.000 bacterial isolates were obtained using a robotic colony-picker platform, from the oral cavity and small intestine of healthy pre-weaning and weaned piglets with lower and higher *S. suis* load, as determined by 16S rRNA targeted pyrosequencing determination of the microbiota composition in 40 tonsil and 41 intestinal samples.

Results: Using *S. suis* agar overlay assays, we recovered 10 bacterial isolates inhibiting seven *S. suis* serotypes,

identified three toxins responsible for the inhibitory phenotype, and obtained genome sequences of three isolates. We identified high abundances of specific bacterial taxa in piglets with no or very low abundance of *S. suis* 16S sequences suggesting these taxa might suppress blooms of pathobiont *S. suis*.
Conclusion: Specific microbial taxa, naturally present in piglets, can antagonise multiple serotypes of *S. suis*.
Outlook | We propose that modulation of the microbiome may offer a rational way to avoid complications with *S. suis* in weaned piglets, and outline ongoing research in our lab to validate this approach.

O099

Persistence of *Enterococcus faecium* outside the human host

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Background: *Enterococcus faecium* is a Gram-positive commensal of the gut of human and animals. In the last few decades, it has acquired resistance to several antibiotics and has become an important cause of nosocomial infections. Although infections caused by *E. faecium* are not particularly severe, they can be difficult to treat due to its antibiotic resistance. Notably, *E. faecium* can survive outside the human host for extended periods of time, which increases its ability to spread throughout the hospital and increase the chance of outbreaks.
Objectives: Our goal is to uncover the mechanisms used by *E. faecium* to survive outside the human host.
Methods: Using high-throughput transposon sequencing (Tn-seq), we determined which genes are important for the survival of *E. faecium* E745 (a vancomycin-resistant clinical isolate) under nutrient-limiting conditions at ambient temperatures. A transposon library of *E. faecium* E745 was incubated in nutrient-free phosphate buffered saline (PBS) at 20°C for up to 7 days after which genomic DNA was isolated and transposon insertion sites were determined by sequencing on an Illumina HiSeq system. A computational pipeline was developed to determine which genes contribute to survival of *E. faecium*.
Results: We show that *E. faecium* E745 is able to persist for five days under nutrient-limiting conditions at ambient temperatures with 80% survival rate. Using Tn-seq we revealed several genes which appear to be essential for survival under these conditions. The functions cover a broad range of functions including, metabolism, membrane stability, and transcription regulators.
Conclusion: We found that there are several different mechanisms that contribute to survival outside the human host.

O100

Two short stories about bacterial cell division

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Cell division in bacteria is an essential process that is carried out at mid-cell by a group of cell division proteins referred to as the divisome. In *Escherichia coli,* over two dozen cell division proteins have been identified of which ten are essential. These division proteins localize sequentially and interdependently to the division site, after which constriction eventually produces two daughter cells. In the first part of my talk, I will discuss our recent identification of a large (1 MDa) multi-protein complex by native page that contains seven essential cell division proteins (FtsZ, ZipA, FtsK, FtsQ, FtsB, FtsL, and FtsN). As this complex is only present in rapidly dividing cells, and absent in mutants in which division is blocked, we postulate that the complex is (a subassembly of) the long hypothesized divisome. In the second part of the talk I will address peptidoglycan composition at the division site. The cell wall grows inward during constriction (gram-negative) or septum formation (gram-positive), which implies a change in architecture of the peptidoglycan. This architectural change should be the result of local differences in PG chemistry that confer structure to the molecule. I will discuss recent work that shows that a specific subtype of PG, in which the stem-peptides that are used for crosslinking are not processed, is present at bacterial cell division sites. Whereas these peptides are normally shortened after incorporation into PG, this activity is reduced at division sites indicating either a lower local degree of PG cross-linking or a difference in PG composition which could be a topological marker for other proteins.

O101

Coordination of envelope constriction during Gram-negative cell division

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During cell division Gram-negative bacteria must carefully coordinate constriction of their multi-layered cell envelope composed of the inner membrane (IM), peptidoglycan (PG) and outer membrane (OM). The molecular mechanisms of cell division and how the constriction process is coordinated have remained largely unknown. We have shown that envelope machines facilitating septal PG synthesis (PBP1B-LpoB complex) and OM constriction (Tol-Pal system) are physically and functionally coordinated via the Tol-associated protein CpoB. CpoB (formerly YbgF) is the product of the last gene in the *tol-pal* gene operon and was previously shown to interact with TolA[1], however its cellular function was unclear.

CpoB localizes to the site of cell division concurrent with PBP1B-LpoB and Tol proteins at the onset of constriction, and interacts directly with PBP1B in addition to TolA. CpoB selectively modulates the peptide cross-linking (transpeptidase) activity of PBP1B in the presence of the outer-membrane anchored regulator, LpoB. LpoB is essential for PBP1B function in the cell[2,3] and has been shown to stimulate both of its PG synthesis activities.[2,4] CpoB partially prevents stimulation of PBP1B transpeptidase activity without impacting stimulation of glycosyltransferase activity. TolA, which also interacts directly with PBP1B, reverses the CpoB effect on transpeptidase activity and further enhances PBP1B glycosyltranferase activity, synergistically with LpoB. Furthermore, these regulatory interactions are responsive to the function of the Tol system in the cell, suggesting that the Tol system can modulate peptide cross-linking in coordination with its own activity during constrictive peptidoglycan synthesis. Coordination of the PBP1B and Tol machines by CpoB contributes to effective PBP1B function *in vivo* and maintenance of cell envelope integrity during division.[5]

References

1. Krachler AM, Sharma A, Cauldwell A, et al. TolA modulates the oligomeric status of YbgF in the bacterial periplasm. J Mol Biol. 2010;403:270-85.
2. Typas A, Banzhaf M, van den Berg van Saparoea B, et al. Regulation of peptidoglycan synthesis by outer-membrane proteins. Cell. 2010;143:1097-109.
3. Paradis-Bleau C, Markovski M, Uehara T, et al. Lipoprotein cofactors located in the outer membrane activate bacterial cell wall polymerases. Cell. 2010;143:1110-20.
4. Egan AJ, Jean NL, Koumoutsi A, et al. Outer-membrane lipoprotein LpoB spans the periplasm to stimulate the peptidoglycan synthase PBP1B. Proc. Natl. Acad. Sci. U. S. A. 2014;111:8197-202.
5. Gray AN, Egan AJ, Van 't Veer IL, et al. Coordination of peptidoglycan synthesis and outer membrane constriction during Escherichia coli cell division. eLife. 2015;4:e07118.

O102

Constructing the *Bacillus* minimal divisome: what regulatory genes are really required for cell division?

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The essential tubulin homologue FtsZ is the key coordinator of cell division in almost all bacteria. FtsZ polymerizes to form the so-called Z-ring at midcell, which serves as scaffold to recruit the divisome machinery; an array of proteins necessary for an efficient and successful cell division. The Z-ring formation and positioning are tightly regulated and maintained throughout the cell division process by accessory cell division proteins to guarantee its efficiency and success. Although these accessory proteins are required for efficient midcell positioning of the Z-ring, they are not essential for cell division. We consecutively deleted eight regulatory cell division genes using a marker-free deletion approach to obtain a *Bacillus* minimal divisome strain. The genome of the *Bacillus* minimal divisome strain has been confirmed by whole genome sequencing. Here, we conclude a *Bacillus* subtilis minimal divisome strain lacking the noc and min systems, the metabolic sensor UgtP, ClpX, and ZapA with SepF as the sole known anchor of the Z-ring to the membrane.

O103

Accurate cell division in *Streptococcus pneumoniae* by an integrated cell cycle

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The cell cycle of the important human pathogen *Streptococcus pneumoniae* is poorly understood. The chromosome cycle of *S. pneumoniae* depends on other mechanisms than known for rod-shaped bacteria, as does the division cycle. We are therefore studying how chromosome segregation is mediated in *S. pneumoniae* and how this process is connected to and coordinated with DNA replication and cell division. We constructed new tools for visualizing chromosomal locations based on a fluorescence repressor-operator system and a ParB-*parS* plasmid segregation system. Furthermore, fluorescent fusions to different replisome proteins were constructed. We show that the *S. pneumoniae* chromosome has a longitudinal organization with the origin and terminus located near the old and new cell pole, respectively. The origins split early after initiation of DNA replication and moves to the quarter positions of the cells. Strikingly, the replicated origin arrives at the quarter positions earlier than cell division protein FtsZ. High temporal resolution imaging shows that the replisome is highly dynamic, but mainly localizes at midcell. Using these tools, we show that the highly conserved SMC complex is pivotal for the timing of origin segregation. Perturbed origin localization leads to aberrant cell division and inaccurate FtsZ localization,

suggesting an intimate connection of the chromosome cycle to the cell division machinery.

O110

Cap-snatching of a segmented (-)RNA plant virus: Perspectives for new antiviral drug design against Influenza viruses

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The world of negative strand (-)RNA viruses contains many well known and important human pathogens like Influenza, Ebola, Lassa Fever, Measles, Rabies and Mumps. In contrast to these, negative strand (-)RNA viruses from plants are less well known. Nevertheless, a few of those are of high economic importance and rank within the top ten of most devastating plant viruses worldwide. One of these is the plant-infecting bunyavirus Tomato spotted wilt and studies on viral genome transcription initiation will be described that advance the insight into this process, named Cap-snatching, and provide possibilities for new antiviral drug design against Influenza viruses.
'Cap-snatching' represents a specific transcription initiation mechanism found among the segmented, single-stranded (ss) (-)RNA viruses. During this process, the viral transcriptase cleaves m^7G-capped RNA leader sequences from host mRNAs to prime transcription of the viral genome segments.
In vivo and *in vitro* transcription studies on tomato spotted wilt virus (TSWV) in the presence of alfalfa mosaic virus (AlMV) or AlMV RNA3 (AMV3), have demonstrated a requirement for base complementarity of AlMV3-derived capped RNA leaders to the ultimate or penultimate 3' residue of the TSWV RNA template. Furthermore, pair-wise competition of several (mutant) cap donors have shown that TSWV prefers capped leaders with multiple base complementarity to the vRNA template, even when offered at relative low amounts compared to others with less base complementarity. The requirements for alignment of capped leader sequences along the viral genome during Influenza transcription initiation (cap-snatching) have long been an enigma. In light of the highly conserved nature of cap-snatching, a preference for multiple base-pairing cap donors during transcription initiation was also analyzed for Influenza A virus. Results from *in vitro* and *in vivo* transcription initiation assays show that, like TSWV, Influenza A displays a preference for multiple base-pairing cap donors (Geerts-Dimitriadou et al., 2011). Furthermore the occurrence of prime-and-realign for Influenza virus was observed, as well as internal priming at the 3'-penultimate residue. Recently, similar findings were obtained for the Schmallenberg orthobunyavirus which strengthens the idea that the mechanistic mode of cap-snatching with segmented (-) ssRNA viruses is very similar for all these viruses and offers interesting possibilities for new antiviral drug design. Support for this is provided by experiments in which the application of favoured cap donor molecules to the *in vitro* system showed stronger reduction of globin leader RNA initiated influenza genome transcription.

O111

Uncovering novel features of cytomegalovirus immune evasion: HLA class I degradation by the protein US2

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Herpesviruses have evolved various strategies to evade the immune system of their hosts. As a member of the *Herpesviridae*, human cytomegalovirus (HCMV) evades immune recognition by specifically downregulating antigen-presenting HLA class I molecules (HLA-I).
During its synthesis, HLA-I is translocated into the endoplasmic reticulum (ER), where it is loaded with an antigenic peptide. Once loaded with a peptide, the HLA-I complex travels to the plasma membrane, where it can activate CD8+ T cells.
The HCMV US2 protein prevents this by degrading ER-resident HLA-I molecules, hijacking the quality control mechanism for misfolded proteins. In this process, called ER-associated degradation (ERAD), misfolded proteins are recognized in the ER and transported back into the cytosol where they are degraded by the ubiquitin-proteasome system. The mechanisms of ERAD are complex and only partially understood. With US2-mediated HLA-I degradation as a model for ERAD, we identified novel players in this protein degradation route. This study provides new insights into the role of the ubiquitin system in ERAD.
Using CRISPR/Cas9 libraries we show that multiple E2 and E3 enzymes catalyze ubiquitination during US2-mediated degradation of HLA-I. Recent preliminary data suggest that multiple E2 enzymes may cooperate. These findings indicate the complexity of cellular quality control mechanisms, which are elegantly exploited by HCMV to elude the immune system.

O112

Redirection of ESX-1 substrates to ESX-5 system in T7SS of pathogenic mycobacteria

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Type VII secretion (T7S) is employed by mycobacteria, including the important pathogen Mycobacterium tubercu-

losis, to export protein effectors across their highly unusual cell envelope. There are five paralogous T7S systems in M. tuberculosis, named 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. To further investigate the substrate specific binding of the EspG chaperone we obtained the crystal structure of ESX-5 chaperone EspG5 with the ESX-5 substrate pair PE25/PPE41. The structure revealed that the chaperone exclusively interacts with the elongated end of PPE41, a region containing several hydrophobic residues that are conserved in PPE substrates of different secretion systems. Introducing point mutations in these conserved residues of multiple ESX-5 PPE substrates and an ESX-1 dependent PPE protein abolished the interaction with the cognate EspG chaperone. Disrupting the interaction with EspG also blocked their secretion by Mycobacterium marinum, indicating that chaperone binding is an important prerequisite for protein secretion. Subsequently, when we exchanged the complete EspG-binding domain between an ESX-1 (PPE68) and ESX-5 (PPE18) PPE protein we were able to alter chaperone-binding specificity. Further secretion analysis in different M. marinum mutant strains of ESX-1 and ESX-5 secretion systems revealed that EspG5-binding-domain-containing PPE68 is secreted independently on its original system of ESX-1, yet entirely dependent on ESX-5 system. This result suggested us that EspG5 chaperone is the primary factor on determining ESX-5 system specificity in M. marinum. In summary, we have elucidated the molecular determinants for EspG binding to PPE substrates and the key role of chaperone binding in secretion. By understanding how EspG chaperone works, for the first time we have been successful in rerouting the substrates from ESX-1 to ESX-5 system.

O113

Differential interaction of the Staphylococcal toxins panton-valentine leukocidin and γ-Hemolysin CB with human C5a receptors

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Introduction: *Staphylococcus aureus* is well adapted to the human host. Evasion of the host phagocyte response is critical for successful infection. The staphylococcal bicomponent pore-forming toxins Panton-Valentine leukocidin LukSF-PV (PVL) and γ-hemolysin CB (HlgCB) target human phagocytes through interaction with the complement receptors C5aR1 and C5aR2. Currently, the apparent redundancy of both toxins cannot be adequately addressed in experimental models of infection because mice are resistant to PVL and HlgCB. The molecular basis for species specificity of the two toxins in animal models is not completely understood.
Methods and Results: We show that PVL and HlgCB feature distinct activity toward neutrophils of different mammalian species, where activity of PVL is found to be restricted to fewer species than that of HlgCB. Overexpression of various mammalian C5a receptors in HEK cells confirms that cytotoxicity toward neutrophils is driven by species-specific interactions of the toxins with C5aR1. By taking advantage of the species-specific engagement of the toxins with their receptors, we demonstrate that PVL and HlgCB differentially interact with human C5aR1 and C5aR2. In addition, binding studies illustrate that different parts of the receptor are involved in the initial binding of the toxin and the subsequent formation of lytic pores. These findings allow a better understanding of the molecular mechanism of pore formation. Finally, we show that the toxicity of PVL, but not of HlgCB, is neutralized by various C5aR1 antagonists.
Conclusion: This study offers directions for the development of improved preclinical models for infection, as well as for the design of drugs antagonizing leukocidin toxicity.

O114

Human Langerin interacts with a conserved glycan modification of *Staphylococcus aureus* Wall Teichoic Acid

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Introduction: *Staphylococcus aureus* (*S. aureus*) is an important cause of hospital- and community-acquired infections in humans, ranging from mild infections to severe disease such as toxic shock syndrome and sepsis. The cell wall of *S. aureus* is abundantly decorated with glycans that play an important role in the virulence and immunogenicity of the pathogen. These structures include polysaccharide capsule, peptidoglycan, lipoteichoic acid, wall teichoic acid (WTA) and glycosylated proteins. C-type lectin receptors (CLRs) are a family of host pattern-recognition receptors specialized in detection of carbohydrate patterns. In an initial screening with recombinant CLR constructs, we identified human Langerin (hLangerin, CD207), but not murine Langerin, to interact with

S. aureus. Langerin is predominantly expressed by Langerhans cells, which are a subtype of dendritic cells that reside in the epidermis, where they play an important role in barrier immune surveillance. We aim to identify the bacterial ligand of hLangerin on *S. aureus*, and unravel the functional consequences of this interaction for disease.

Methods: The interaction between Langerin and *S. aureus* was examined by incubation of FITC-labelled soluble Langerin constructs with live bacteria, in the presence or absence of EDTA, mannan or blocking hLangerin antibody, and analyzed by flow cytometry. Alternatively, hLangerin was ectopically expressed on different cell lines to assess whether overexpression conferred *S. aureus* binding. The bacterial ligand of hLangerin was determined by screening a panel of *S. aureus* knockout strains with characterized deficiencies in cell wall associated glycosylated structures.

Results: The interaction between hLangerin and *S. aureus* is Ca^{2}+-dependent and blockable by either hLangerin ligand mannan or a blocking hLangerin antibody. Interaction was observed in all tested strains of human *S. aureus* isolates but not in other Gram-positive species, suggesting a conserved *S. aureus*-specific ligand. Ectopic expression of hLangerin on different human cell lines conferred the ability to bind *S. aureus*.

By screening a panel of characterized *S. aureus* glycosylation-deficient mutants, we identified β-linked *N*-acetylglucosamine (β-GlcNAc) on WTA as the target for hLangerin. Importantly, in approximately 35% of *S. aureus* isolates WTA is additionally modified with α-linked GlcNAc.[1] The reason for the presence of GlcNAc in two configurations is currently unknown. We observe that interaction of *S. aureus* with hLangerin is significantly increased in the absence of α-GlcNAc, suggesting that WTA α-GlcNAc shields recognition of β-GlcNAc by hLangerin.

Conclusion: hLangerin binds specifically GlcNAcylated WTA of *S. aureus* in an anomeric-specific fashion. Future work aims to clarify the functional consequences of this specific glycan-based interaction between *S. aureus* and Langerhans cells.

Reference

1. Winstel V, Xia G, Peschel A. Pathways and roles of wall teichoic acid glycosylation in Staphylococcus aureus. International journal of medical microbiology. IJMM. 2014;304:215-21. Epub 2013/12/25.

O115

Proteolytic-independent role of mycosins in formation of the ESX-1 and ESX-5 type VII secretion complexes

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Introduction: The pathogen *Mycobacterium tuberculosis*, the eponymous agent of tuberculosis (TB), is responsible for over 1 million deaths annually. As a result of the recently increased prevalence of (multi-)drug resistant strains, current research majorly focuses on the development of novel anti-TB drugs. A promising new group of drug targets of *M. tuberculosis* are the type VII secretion (T7S) systems, which are involved in the secretion of proteins and the uptake of nutrients across the unusually hydrophobic mycobacterial cell envelope. 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 the pathogen. The ESX systems consists of a set of conserved components. One of these components is the membrane-associated subtilisin-like serine protease mycosin (MycP), which is an essential part of the T7S system. Due to their proteolytic activity, conservation and essentiality, mycosins are promising targets for the development of novel drugs against TB. In this study we set out to elucidate the role of the mycosin proteases in T7S, by analyzing the phenotype of mycosin knock-outs of the ESX-1 and ESX-5 system and their complementation with various mutant constructs.

Methods and Results: Deletion strains of the mycosins of the ESX-1 and ESX-5 secretion systems, <i*e. mycP1* and *mycP5* respectively, were created in *Mycobacterium marinum*, a close relative of *M. tuberculosis*. 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 secretion of ESX-1 and ESX-5 substrates and the proper assembly of the 1.5 MDa ESX-1 and ESX-5 membrane complexes, through which substrates are probably transported across the cell envelope. While the deletion strains of *mycP1* or *mycP5* were defective in respectively ESX-1 or ESX-5 dependent secretion, the proteolytic inactive variants were still able to mediate secretion by ESX-1 or ESX-5. Furthermore we found that the ESX-1 and ESX-5 membrane complexes were not detectable in the *mycP1* or *mycP5* deletion strains after detergent extraction and native PAGE analysis.

Conclusion: Our results show that the proteolytic activity of $MycP_1$ and $MycP_5$ is not essential for ESX-1 and ESX-5 dependent secretion, respectively. This indicates a dual function for mycosins, with a proteolytic role in substrate processing, which is not essential for the secretion process, and a second, so-far unknown, essential role for T7S functioning. The absence of the ESX membrane complexes in the *mycP* deletion strains indicates that this second role is involved in the formation or stability of the ESX membrane complexes.

O116

Genome-wide screening identifies PTS permease BepA to be involved in *Enterococcus faecium* endocarditis and biofilm formation

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Enterococcus faecium is a common cause of nosocomial infections of which infective endocarditis is associated with substantial mortality. In this study, we applied a Microarray-based Transposon Mapping (M-TraM) approach in a rat endocarditis model, and identified a gene, originally annotated as *fruA* and renamed to *bepA* (Biofilm and Endocarditis-associated Permease A), putatively encoding a carbohydrate Phosphotransferase System (PTS) permease, as important in infective endocarditis. This gene is highly enriched in *E. faecium* clinical isolates and absent in commensal isolates that are not associated with infection. Confirmation of the phenotype was established in a competition experiment of wild-type and a markerless *bepA* mutant in a rat endocarditis model. In addition, deletion of *bepA* impaired biofilm formation *in vitro* in the presence of 100% human serum and metabolism of β-methyl-D-glucoside. β-glucoside metabolism has been linked to the metabolism of glycosaminoglycans that are exposed on injured heart valves where bacteria attach and form vegetations. Therefore, we propose that the PTS permease BepA is directly implicated in *E. faecium* pathogenesis.

O117

Major antibiotic stress operon iniBAC of *mycobacteria* is induced upon vitamin B12 and mutAB deficiency

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Introduction: *Mycobacterium tuberculosis* is the causative agent of tuberculosis (TB), an infectious disease that kills over 1.5 million people annually. The disease can be treated with a six-month regimen of antibiotics. Although the targets of most of the first-line antibiotics have been identified, less research has focused on the intra-bacterial stress response that follows upon treatment with antibiotics. Studying the roles of these stress genes will lead to the identification of crucial stress-coping mechanisms that may provide additional drug targets to increase treatment efficacy. A three-gene operon with unknown function that is strongly up-regulated upon treatment with isoniazid and ethambutol is the *iniBAC* operon. In this study we aim to elucidate the genetic network that the *iniBAC* operon operates in.

Methods: RNA sequencing was used to confirm that 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 and assessed fluorescence induction by flow cytometry. Transposon mutagenesis was performed on *Mycobacterium marinum* cells containing this indicator plasmid. Colonies that showed increased expression were isolated and the transposon insertion site was determined by ligation-mediated PCR.

Results: Firstly, we confirmed that the *iniBAC* operon is induced *in vitro* by EMB and INH in *M. marinum*. When the indicator bacteria were phagocytosed by macrophages they showed a similar induction phenotype when treated with EMB and INH. Transposon mutagenesis screen revealed that the majority of the mutations that showed constitutive induction of the *iniBAC* operon were affected in vitamin B12 biosynthesis or the vitamin B12-dependent methylmalonyl -CoA-mutase mutAB. MutAB is a key enzyme that plays a role in the degradation of proprionate to synthesize important precursors for the biosynthesis of outer membrane lipids. Two of these lipids are phthiocerol dimycocerosate (PDIM) and phenolic glycolipid (PGL), both crucial for mycobacterial virulence. Lipid analysis showed that an *M. marinum mutA::tn* mutant has decreased PDIM and PGL levels, suggesting a link between *iniBAC* induction and the production of these outer membrane lipids. Moreover, we found that mutations in the PDIM biosynthesis operon in *M. bovis* BCG cause upregulation of the indicator plasmid. Based on these data, we propose that *iniBAC* is induced in response to mutations that cause defects in the biosynthesis of important outer membrane lipids. The resulting stress caused by these mutations, or caused by ethambutol or isoniazid treatment, may be relieved by *iniBAC* to increase the chance of bacterial survival.

Conclusions: 1. The *iniBAC* operon is induced by EMB and INH in *M. marinum*, both *in vitro* and in cell infection experiments. 2. The *iniBAC* operon is induced in a vitamin B12 and MutAB-dependent manner in *M. marinum* 3. Mutations in the vitamin B12 biosynthesis cluster or MutAB cause a decrease in PDIM in *M. marinum*. 4. In *M. bovis* BCG the operon is induced by mutations in the PDIM biosynthesis cluster.

O119

Hypermutation and the division of labour in *Streptomyces colonies*

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The Streptomycetes are a diverse group of filamentous bacteria that live at high densities in the soil. Streptomyces produce spores that germinate to produce branching vegetative hyphae that extend into the soil where they secrete a large repertoire of proteases, cellulases and chitinases that liberate insoluble organic materials arising from the decay of fungi, plants and animals. When these resources are exhausted, the substrate mycelium undergoes a developmental shift from vegetative growth to sporulation. This shift is accompanied by the production of millions of environmentally-resistant spores as well as the production of an enormous diversity of secreted secondary metabolites, including some two-thirds of the antibiotics used in clinical practice. As with multicellular eukaryotes, the complex differentiation during Streptomyces development can be understood as a division of labour that maximizes colony fitness by allowing specialized cells to segregate different tasks. Here we argue that a similar division of labour occurs between colony subsections during the production of secondary metabolites. We present results showing that spatial differentiation across developing colonies permits the segregated expression of secreted products like antibiotics. Remarkably, this division of labour arises through a unique and high-frequency form of genomic instability. This mechanism results in colony sectors that hyper-express expensive antibiotics but which comes at the cost of vastly reduced individual fitness. Via this metabolic division of labour, Streptomyces colonies reduce the metabolic burden of secreted products while maximizing the colony-wide yield and diversity of these costly metabolites.

O120

Aggregation is a key factor leading to mycelial heterogeneity in *Streptomyces*

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Streptomycetes are filamentous microorganisms that provide us with a wide range of useful metabolites, including the vast majority of antibiotics that are used in the clinic. Growth of these microorganisms in bioreactors is characterized by the formation of complex mycelial networks of hyphae, which are heterogenic in size. Given the strong correlation between specific productivity and morphology, this heterogeneity is a major drawback in industry. Recent work indicates that mycelial morphology is controlled by a number of genes that encode for proteins required for the synthesis of extracellular glycans. Using a quantifiable system based on fluorescent markers, we here provide evidence that these glycans mediate aggregation of germlings and mycelia only at the early stages of growth. The absence of these glycan synthases yields particles that are more uniform in size. Given that aggregation is widespread within streptomycetes and can also occur between different *Streptomyces* strains, our work paves the way to improve *Streptomyces* as a cell factory for the production of known metabolites, but possibly also to discover new ones.

O121

Host glycans driven interspecies metabolic cross talk

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Introduction: Host microbe interactions have co-evolved into a symbiotic state in which specialized members of intestinal microbiota can utilize host-derived compounds as substrates for growth. *Akkermansia muciniphila* that resides in large abundance at intestinal mucosal layer has adapted to the consumption of host mucin glycans, owing to refined capability to secrete mucin-degrading enzymes. It is hypothesized that host-glycan degraders can have direct effect on other community members through cross-feeding and syntrophic interactions. Here we elucidate trophic interactions between *A. muciniphila* and beneficial butyrate-producing microbes that are found in the human intestinal tract.
Methods: Co-cultures of *A. muciniphila* with non-mucolytic butyrate producing gut isolates (*Anaerostipes caccae*, *Eubacterium hallii*, and *Faecalibacterium prausnitzii*) were performed in minimal media supplemented with mucin. Growth measurements (OD), HPLC analysis of metabolites, FISH staining and q-PCR were conducted.
Results: *A. muciniphila* degradation and fermentation of mucin glycans resulted in butyrate production of non-mucolytic butyrate-producing gut isolates. By using qPCR and FISH, we observed over 100-fold increase of butyrate-producing species within the co-cultures after 8 days, coupled with a significant increase in butyrate production. *A. muciniphila* degrades mucin glycans resulting in metabolites (including galactose, fucose, mannose, N-acetylglucosamine and acetate) that supported growth of syntrophic partners. In addition, a co-culture of *A. muciniphila* with *E. hallii* specifically, showed metabolites shift of succinate to propionate in comparison

to *A. muciniphila* mono-culture, indicating mutualistic syntrophic interactions.

Conclusion: We concluded that syntrophic interaction between microbes driven by host glycan can lead to butyrate production that is beneficial for host health.

O123

Can we make life in the lab?

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How did life start? Can we make life? These are among the most fundamental questions in contemporary science. In this talk I will show how, starting from a mixture of relatively simple interconverting organic molecules, a set of self-replicating molecules can emerge spontaneously. Importantly, the process of self-replication was found to be exponential, which is an important characteristic in the context of Darwinian evolution. Mutation of the replicators was enabled by providing the system with different building blocks. When mixed, these systems gave rise to the emergence of two different replicator sets (that bear resemblance to quasi-species), of which one is the ancestor of the other. Molecular-level insight into this diversification process showed that outliers in the mutant distribution of the first quasi-species induced the formation of the second quasi-species. The next step is now to achieve replication far from equilibrium, by allowing concurrent replication and destruction processes to take place. The first results of replication in such replication/destruction regime will be presented.

References

1. Carnall JMA, et al. Mechanosensitive self-replication driven by self-organization. Science. 2010;327:1502-06.
2. Malakoutikhah M, et al. Uncovering the selection criteria for the emergence of multi-building-block replicators from dynamic combinatorial libraries. *J. Am. Chem. Soc.* 2013;135:18406-17.
3. Colomb-Delsuc M, Mattia E, Sadownik JW, et al. Exponential self-replication enabled through a fibre elongation/breakage mechanism. Nat Commun. 2015;6:7427.
4. Leonetti G, Otto S. Solvent composition dictates emergence in dynamic molecular networks containing competing replicators. J Am Chem Soc. 2015;137:2067–72.
5. Sadownik JW, Mattia E, Nowak P, et al. Diversification of self-replicating molecules. Nature Chem. doi:10.1038/nchem.2419

P001

Improve quality by using a selective agar for Group B streptococcus in pregnant woman: a prospective study about sensitivity, specificity, cost effectivity and time to detection

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Introduction: Group B streptococcus (GBS) are commonly found in the genital flora of women. During delivery the bacteria can pass to the infant and develop a GBS infection in the newborn. In the Netherlands, if there are risk factors present during pregnancy, women are screened by culturing a vaginal or cervical swab. In this study we compared two different agar plates (our routine Columbia CAP agar and a new selective agar from Oxoid, the Brilliance ™GBS agar) and incubated the samples at both O2 and CO2, resulting in four methods. We determined the difference in sensitivity, specificity, cost effectivity and time to detection between these methods.

Methods: First, we compared 18 predefined micro-organism (10 GBS, 1 *Streptococcus dysgalactiae* and 2 *Streptococcus pyogenes* and 5 enterococcus species) that were all cultured with the four different methods. On different time moments (6h, 8h, 12h, 16h, 18h, 20h, 24h, 48h) the plates were analyzed. Second, we used 20 previously collected clinical samples (11 GBS positive and 9 GBS negative) to compare the methods. Third, we performed a prospective study in which 98 clinical samples were included. In the three different steps the definition of positivity was: suspected growth of GBS and determination of GBS with the MALDI-TOF. The golden standard for a GBS positive sample was defined as GBS growth (and MALDI-TOF determination) in one or more of the four methods.

Results: In step 1 no difference was seen between the four **Methods:** all showed a sensitivity of 100%. The best time to analyze the plates was t = 20h. In step 2, the selective agar-O2 method showed a higher sensitivity (100%) compared to the selective agar-CO2 (91%), the CAP-O2 (64%) and the CAP-CO2 method (64%). The difference in sensitivity between the selective agar O2 and CO2 method was due to one colony that was missed in the CO2 stove (but this sample was probably affected by chemicals). In step 3, 14 of the 98 clinical samples were GBS positive. The selective agar (both with O2 and CO2) showed a higher sensitivity (93%) compared to the routine CAP agar (79%).

Conclusions:

Our data showed that the selective agar (Oxoid) was more sensitive than our routine CAP agar. Using the selective agar may decrease the number of GBS positive women who will be missed and consequently the number of infants who will not be protected with antibiotics during delivery. However, the analyses of the 98 clinical samples showed that the selective agar was not cost-effective, also not, if the extra costs for the determination of all GBS suspected bacteria were incorporated.

However, the price difference is not much if we realize that our mission is to offer high standard healthcare and the extra costs by missing a positive woman with a GBS infected infant are taken into account.

P002

Congenital *Plasmodium vivax* malaria in a non-endemic country, an unique case-report in the Netherlands

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Introduction: Upon presentation at the age of 20 days, an irritable newborn male was seen. The child had a temperature of 38.9 degrees Celcius with normal heart rate and respiratory frequency. Auscultation of heart and lungs showed no abnormalities. No enlarged liver or spleen was noticed. Due to the fact that at birth his mother was diagnosed with *P.vivax* malaria, a malaria screening was included besides a sepsis workup. Blood results revealed elevated infection parameters, mild anemia and thrombocytopenia. Malaria testing turned out to be positive for *P. vivax* (see attached picture).
Methods: Malaria screening comprised the microscopic examination of thick and thin blood smears, the Quantitative Blood Concentration test (QBC-malaria test) and the BinaxNow ICT malaria test. Retrospectively *P. falciparum, P. vivax, P. ovale* and *P. malariae* PCR was performed on blood samples of the child at birth and mother after labour. Unfortunately umbilical cord blood was not available anymore by that time. In addition, malaria serology and PCR's were performed on the 12th week pregnancy serum.
Results: The parents of the neonate immigrated from Eritrea ten months before his birth. In the year prior to her pregnancy the mother was treated for malaria (Plasmodium species unknown) with unknown medication in a refugee camp in Ethiopia. At our hospital in the Netherlands she gave birth to a healthy boy, with a birth weight in the 50th percentile. During labour however the mother developed fever; which appeared to be based on a *P. vivax* infection (PCR confirmed). Due to maternal infection the newborn was tested for malaria on the first day of life; malaria screening revealed no indications for infection (PCR confirmed). At follow up of the neonate one week post partum no symptoms of malaria infection were noticed; a malaria screening was not performed. After that, the child was discharged from outpatient monitoring.
Conclusion: Since the Netherlands is a non-endemic country for malaria neonatal infection of the child presented here can be excluded. The presented case describes a newborn with a proven congenital infection with *P. vivax* in the Netherlands. Although the mother of the child appeared to be positive in malaria serology(PCR's negative), she developed an overt *P vivax* infection most probably caused by activation of the liver hypnozoites due to pregnancy and/or diminished immunity resulting from moving from an malaria endemic area to a malaria free country. Since malaria screening (including PCR) of the child at birth was negative, transmission of *P. vivax* erythrocytic stages probably occurred *durante partus*. Consecutive malaria testing in the newborn child might have prevented clinical symptomatology due to the infection.

P003

Bacterial DNA load and other infection markers in blood to diagnose and predict clinical course of community-acquired pneumococcal pneumonia

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Streptococcus pneumoniae is the major cause of community-acquired pneumonia (CAP) worldwide. Routine diagnostics to detect *S. pneumoniae* are culture from sputum and/or blood, Gram stain and pneumococcal antigen test (PnAT; BinaxNow) from urine. Molecular assays using blood could be a valuable addition to culture methods in determining the microbiological cause of CAP, especially in patients who received antibiotic therapy. It remains difficult to identify CAP patients who are at risk of developing major complications such as septic shock. *S. pneumoniae* DNA load combined with a (set of) biomarker(s) may assist in identifying CAP patients who need extra care.
In this pilot study, we analysed *S. pneumoniae* DNA load, C-reactive protein (CRP), procalcitonin (PCT), neutrophil-lymphocyte count ratio (NLCR) and soluble urokinase plasminogen activator receptor (suPAR) in patients of whom the result of a PnAT was available.
Until now 100 patients were included in the study, 45 with a positive and 55 with a negative PnAT. Of the PnAT positive patients, 24 had a negative blood culture and 13 had a positive blood culture. Of these, respectively 2 and 8 had a positive PCR. Of 8 PnAT positive patients no blood cultures were obtained, but were positive in PCR in 4 cases. In the group with a negative antigen test, 40 patients had a negative blood culture and 39 of these 40 were also negative in the PCR (98%). Of 30 of 45 PnAT positive patients follow up samples were obtained. Seven of these 30 were PCR positive and in 6 patients bacterial DNA load decreased after treatment. CRP and PCT values were significantly higher in patients with a positive PnAT ($p < 0.002$). Bacterial DNA load, NLCR and suPAR values were not significantly different between the groups.
In **Conclusion:** 1) in the PnAT positive and PnAT negative group blood culture and PCR results were similar in 82% and 96%, respectively. 2) PnAT positivity is significantly related with increased CRP and PCT levels. 3) It remains difficult to predict the clinical course of CAP based on

infection markers. More samples are currently being included and investigated.

P004

Direct entry of blood culture vials at the emergency department leads to earlier results

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Introduction: Early appropriate antimicrobial treatment of patients with sepsis has a large impact on clinical outcome. To enable prompt and efficient processing of blood cultures, the inoculated vials should be placed into an automated continuously monitoring blood culture system immediately after sampling. To accommodate this for a category of seriously ill patients outside laboratory operating hours, we placed an extra BACTEC FX instrument at the emergency department (ED) of our hospital after validating the re-entering of ongoing vials from this instrument into the BACTEC FX at the laboratory.
We subsequently assessed the benefits of shortening the transport time between sampling and monitored incubation of blood culture vials.
Methods: From July up to and including October 2015, we registered turnaround times of blood cultures collected from patients consulting the ED (ED2015). The time between sampling and entering into the BACTEC FX, the time between entering and a positive signal (time to detection, TTD) and the times between sampling and, respectively, reporting of Gram stain results, identification by Matrix-assisted Laser Desorption/ionization Time of Flight (MALDI-TOF) and first antimicrobial susceptibility results were compared with the same time intervals found for ED patients during the same months in 2014 (ED2014), when the external BACTEC was not placed at the ED yet.
According to normal practice, Gram stain results are available at 30 minutes after a BACTEC positive signal, but only during laboratory operating hours, i.e. daily between 8:00 a.m. and 5:00 p.m. If Gram stain results are available before noon, a blood vial subculture is started from which after four hours of incubation a MALDI-TOF identification can be obtained the same day.
Results: In total 601 blood cultures were analysed. The median transport times for ED2014 (n=309) and ED2015 (n = 292) were respectively 11.2 and 0.1 hours, the TTDs 10.9 and 13.6 hours. The median time intervals between sampling and positivity, and respectively Gram stain results, MALDI-TOF results and susceptibility results were between 6.5 and 8.5 hours shorter for ED2015 ($p < 0.05$).
As a result the percentage of cultures with known Gram stain results on the working day following sampling was increased significantly for ED2015 (74% vs. 50% for ED2014). The percentage of same day MALDI-TOF results increased from 30% to 52%. This difference was largest for cultures collected between 5 p.m. and 8 a.m. (ED2014 0%, ED2015 34%).
Conclusion: Immediate entering of blood culture vials into a BACTEC FX instrument results in significantly earlier available Gram stain and identification results. For a large proportion of patients this means that decision making regarding appropriate antimicrobial therapy is accelerated by 24 hours.

P005

Infectious complications after esophagectomy: treatment, outcome and costs

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Introduction: Esophagectomy is faced with high post-operative morbidity, mainly caused by infectious complications, increased hospital stay and costs, or even death. Reports addressing the optimal antimicrobial prophylaxis and treatment of these infectious complications are scarce. In the context of a quality improvement program and considering the increasing antimicrobial resistance of bacterial pathogens, we reviewed the current protocols for antimicrobial prophylaxis and empiric therapy in relation to infectious complications and their associated pathogens of esophagectomy and assessed the impact of these infections on outcome and cost.
Methods: A retrospective study of patients who underwent elective esophagectomy for esophageal cancer between 2009 and 2013. Infectious complications up to 30 days after surgery were scored. Antimicrobial prophylaxis consisted of cefazolin and metronidazole. Empiric treatment was administered according to hospital antibiotic policy and was continued based on culture reports.
Effectiveness of treatment was assessed independently by two clinical microbiologists.
Results: In total 123 patients were included in the study, of whom 51 (42%) had one or more infections after esophagectomy, consisting of surgical site infections, pneumonia, urinary tract infection, catheter related infection and sepsis. From 82 positive cultures 145 pathogens were isolated, mostly *Enterococcus faecium*, *Staphylococcus* species and *Candida albicans,* which were not covered by standard antimicrobial prophylaxis.
Patients with infectious complications had a significantly longer hospital stay and higher costs than non-infected patients. In 78% of patients with an empirically treated infection, the antibiotic spectrum did not completely cover all isolated pathogens.
Patients were categorized into four groups: those without infection (costs: € 12,742); infection and no positive

cultures (€ 15,777); infection and complete antimicrobial coverage of the pathogens (€ 16,007); and infection and incomplete coverage (€ 35,954). Especially this last group demonstrated a disproportionally high mortality, surgical site infection rate, ICU stay, ward stay and cost.

Antimicrobial prophylaxis consisting of piperacillin/tazobactam and fluconazole would have resulted in almost complete coverage. For routine empiric treatment full coverage of pathogens would have been provided by meropenem, vancomycin and anidulafungin combined.

Conclusion: Prophylactic and therapeutic antimicrobial protocols for prevention or empiric treatment of infectious complications after esophagectomy routinely used in our hospital often had insufficient coverage of relevant pathogens.

This relative undertreatment was associated with a prolonged length of hospital stay and significantly higher overall cost. Adjusting protocols to broad-spectrum prophylaxis consisting of topical mupirocin and intravenous piperacillin/tazobactam and fluconazole, and, respectively, broad-spectrum empiric treatment consisting of meropenem, vancomycin and anidulafungin may significantly reduce morbidity, hospital stay and costs. These findings underline the value of careful periodic review of antimicrobial protocols, and may also be applicable to other institutions and other types of abdominal or thoracic surgery.

P006

Point prevalence survey of *Clostridium difficile* and multidrug-resistant bacteria in a Dutch elderly care facility

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Introduction: Little is known about the prevalence of multidrug-resistant (MDR) bacteria and *Clostridium difficile* (*C. difficile*) in Dutch nursing homes. The aim of this pilot study was to determine the prevalence of intestinal colonisation with *C. difficile,* vancomycin-resistant enterococci (VRE) and MDR Gram-negative bacteria in an elderly care facility in preparation of a larger subsequent survey.

Methods: A nursing home was selected in Delft, the Netherlands with 7 wards and 316 beds. MDR Gram-negative bacteria were defined as *Enterobacteriaceae* producing extended-spectrum beta-lactamase (ESBL), *Enterobacteriaceae* resistant to both aminoglycosides and fluoroquinolones and Gram-negative bacteria producing carbapenemase. Stools of residents of 3 rehabilitation clinics within the Dutch nursing home were collected and analysed for aforementioned bacteria using a selective enrichment broth containing vancomycin and cefotaxime (bioMérieux, Marcy-l'Étoile, France), chromogenic media (ChromID ESBL agar, bioMérieux), MacConkey agar with tobramycin (bioMérieux) and selective *C. difficile* culture media (CLO agar, bioMérieux). Potential risk factors for colonisation with these micro-organism were also assessed by a standardised questionnaire which was completed by the geriatric specialist of the participating departments. Furthermore, information on recent infections and antibiotic use was retrieved from the electronic client records.

Results: The participation rate of the 60 inhabitants was 47% (n = 28). The prevalence of *C. difficile,* VRE and MDR Gram-negative bacteria was 18%, 0% and 11% respectively. The majority (n = 4) of the *C. difficile* strains were non-toxinogenic and belonging to PCR ribotype 039 (n = 3) and 067 (n = 1), while the only toxinogenic strain belonged to PCR ribotype 002. All 3 isolated MDR Gram-negative bacteria were ESBL producing *E. coli,* of which 1 was already known due to the diagnosis of culture-proven urinary tract infection. Eight residents (29%) had an indwelling urinary catheter and 14% had recently used antibiotics. No associations were found between the investigated risk factors and colonisation with MDR bacteria and *C. difficile* .

Conclusion: The prevalence of MDR Gram-negative bacteria and VRE in Dutch nursing home residents of this pilot study was 11% and 0% respectively. *C. difficile* carriage was found in a high number of inhabitants (18%).

P007

High concordance of two Lyme serology assays among proven untreated Lyme neuroborreliosis patients, but lower concordance among treated Lyme neuroborreliosis patients and healthy individuals

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Introduction: Diagnosis of Lyme disease is challenging because of the often nonspecific symptoms, persisting antibodies and background seroprevalence. Moreover, many different diagnostic assays are available, but studies comparing serological assays on well-defined patient populations are lacking. Therefore, we compared the performance of the current gold standard two-tier testing (enzyme-linked immunosorbent assay (ELISA) followed by immunoblot) with two ELISA systems on a well-defined study population in The Netherlands, where seroprevalence is 4-8%.

Methods: Our study population consisted of 151 healthy volunteers (HV), 38 patients treated for Lyme neuroborreliosis (LNB) and 21 patients with active LNB. LNB criteria as defined by the European Federation of Neurological Societies were used. Definite LNB included the presence of neurological symptoms, cerebrospinal fluid pleiocytosis and intrathecally produced *Borrelia*-specific antibodies. Possible LNB included two of the aforementioned criteria. We subjected sera of all study subjects to analysis with the C6 ELISA (C6 Lyme ELISA™ Kit, Immunetics, Massachusetts, US) and the Serion/Virion IgM and IgG ELISA (ELISA *classic Borrelia burgdorferi* IgM (without VlsE) and IgG (with VlsE), Serion/Virion, Würzburg Germany). Equivocal and positive results were confirmed by using the RecomLine IgM and IgG immunoblot (Mikrogen GmbH, Neuried, Germany).
Results: 37/38 (97.4%) treated and 19/21 (90.5%) active LNB patients had definite LNB, the remaining three patients were defined as having possible LNB. 130/151 (86.1%) HV had concordant results when comparing both ELISA systems; 116 HV were negative, one was equivocal and 13 were positive. 7/21 discordant results were either positive or negative, and 14/21 were either equivocal in one system and negative or positive in the other. 4/21 discordant ELISA results led to a different serological outcome. In those cases, C6 ELISA was negative, and since immunoblot testing would not be necessary, would be recorded as seronegative. In contrast, Serion/Virion ELISA results were either equivocal (n = 2) or positive (n = 2), leading to three seropositive and one equivocal immunoblot result. 20/38 (52.6%) treated LNB patients had concordant ELISA results; ten treated LNB patients were positive and ten were negative. 11/18 discordant ELISA results were either positive or negative, and 7/18 were either equivocal in one ELISA and negative or positive in the other. 4/18 discordant results, in which the C6 ELISA was negative and the Serion/Virion ELISA was either equivocal or positive, subsequent immunoblot analysis identified three seropositive and one equivocal result. Among the active LNB patients, 20/21 (95,2%) had concordant (positive) ELISA results. For the single patient with discordant results, the C6 ELISA was positive, while the Serion/Virion ELISA was equivocal. The immunoblot for this case was positive. All active LNB patients were confirmed as being seropositive (n = 20) or equivocal (n = 1) by immunoblot.
Conclusion: First of all, the two ELISA systems performed equally well in proven, untreated LNB patients. Secondly, the performance of these assays in patients treated for LNB, or healthy volunteers, were much more diverse complicating the interpretation of antibody tests. This variation is probably due to the high background seroprevalence and variable persistence of antibodies even years after the infection had been treated.

P008

Functional and structural characterization of rhamnose biosynthesis enzymes in Group A Streptococcus

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Introduction: The bacterial human pathogen Group A *Streptococcus* (GAS) ranks in the top 10 of infection-related causes of mortality worldwide. The Group A Carbohydrate (GAC) is the molecular signature and a virulence determinant of GAS. The production of dTDP-L-rhamnose is critical for GAC biosynthesis but also more broadly for the viability or virulence of other medically-important bacteria including *Mycobacterium spp.* and *Enterococcus faecalis*. dTDP-L-rhamnose is synthesized from α-glucose-1-phosphate through a four-step enzymatic process catalyzed by the enzymes RmlA-D. In GAS, homologues of all four dTDP-L-rhamnose biosynthesis enzymes can be identified through bioinformatics. We recently demonstrated through biochemical and structural analysis that GacA is the dTDP-4-dehydrorhamnose reductase of GAS, which catalyzes the final step of dTDP-L-rhamnose biosynthesis.[1] The aim of this study was to characterize two additional enzymes in the dTDP-L-rhamnose biosynthesis pathway of GAS, RmlB and RmlC.
Methods: We expressed recombinant GAS RmlB and RmlC proteins for biochemical assays and for protein structure determination using X-ray crystallography. The function of RmlB and RmlC in intact bacteria was studied through heterologous expression of GAS *rmlB* and *rmlC* in *S. mutans rmlB* and *rmlC* knockout strains, respectively. Our set of *S. mutans* wild-type, mutant and complemented bacteria were analyzed for growth, morphology by scanning electron microscopy, and cell wall carbohydrate composition by chromatography.
Results: Structural and biochemical comparison of GAS RmlB and RmlC with characterized homologues of *Salmonella enterica* and *Streptococcus suis* revealed a high sequence identity and conservation of all catalytic residues. Both RmlB and RmlC were functional as metal independent enzymes and confirmed their role as monomeric dTDP-D-glucose 4,6-dehydratase and dTDP-4-dehydrorhamnose 3,5 epimerase, respectively. Since *rmlA-C* are essential genes for GAS[2], we confirmed the enzymatic function of RmlC through heterologous expression in a *S. mutans rmlC* knockout strain. Overexpression of GAS RmlC restored growth, cell division and the presence of rhamnose in the cell wall of the *S. mutans* RmlC mutant. In addition, we confirmed the critical role of one of the RmlC catalytic residues (Y140F) using the S. mutans heterologous expression system.

Conclusion: *rmlB* and *rmlC* are essential rhamnose biosynthesis genes in GAS. Our data provide a framework for future drug screens to identify novel inhibitors that interfere with dTDP-rhamnose biosynthesis.

References

1. Van der Beek SL, Le Breton Y, Ferenbach AT, et al. GacA is essential for Group A Streptococcus and defines a new class of monomeric dTDP-4-dehydrorhamnose reductases (RmlD). Mol Microbiol. 2015;98:946-62.
2. Le Breton Y, Belew AT, Valdes KM, et al. Essential Genes in the Core Genome of the Human Pathogen Streptococcus pyogenes. Sci Rep. 2015;5:9838.

P009

SSL3 as a virulence factor *in vivo*

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Introduction: In recent years *Staphylococcus aureus* has become a major health threat to both humans and domestic animals. Increased antibiotic resistance and a high amount of virulence factors secreted by *S. aureus*, amongst which are immune evasion molecules, contribute to this. Staphylococcal superantigen-like protein 3 (SSL3) was identified as a potent inhibitor of the pattern recognition receptor toll-like receptor 2 (TLR2). TLR2 recognizes bacterial lipopeptides and is important in the defense against *S. aureus*. SSL3 is the first molecule described to block TLR2 through direct interaction with the receptor. We recently solved the crystal structure of the extracellular LRR-domain of murine TLR2 with SSL3 134-326 and this revealed that SSL3 acts in a dual mechanism by both inhibiting ligand binding to and also subsequent dimerization of TLR2 with TLR1 and TLR6. Now that we have solved the mechanism of action there is still one important matter that remains unknown; the expression and *in vivo* relevance of SSL3. *In vitro* studies have proven difficult and expression *in vitro* cannot be measured well using promoter reporter constructs. Therefore we set out to study the *in vivo* importance of SSL3 by using mouse models.

Methods: The presence of neutralizing antibodies in human serum was determined by incubating HEK293T-TLR2 cells with an anti-TLR2 antibody and different concentrations of SSL3, in absence or presence of 10% heat inactivated serum. To measure expression of SSL3 in the mouse, total RNA was extracted from *S. aureus* isolated from infected kidney tissue of two different mice strains (A/J1 and C57BL/6). RNA was transformed to cDNA and sequenced using Illumina next generation sequencing. A super-producing SSL3 strain was created by electroporation of *S. aureus* with a plasmid containing the SSL3 gene under the constitutive active LukM promoter (pLukM-SSL3). C58BL/6 mice were intravenously injected with $4x10^7$ WT, SSL3 knock-out, or pLukM-SSL3 SH1000 bacteria. At 24 h post infection organs were harvested and CFU's were determined in liver, kidneys, and heart.

Results: Serum of human healthy volunteers contains high levels of neutralizing antibodies against SSL3, indicating that it is expressed *in vivo* in the human host. SSL3 however does not appear to be expressed in the mouse, limiting further *in vivo* studies. Therefore, we developed an over-expressing SSL3 strain where SSL3 expression is regulated by a constitutive active promoter. pLukM-SSL3 produces approximately 30-100 µg/ml functionally active SSL3. The super-producing strain is significantly more virulent and causes higher CFU's in liver, kidneys, and heart.

Conclusion: This data shows that SSL3 acts *in vivo* and is an important virulence factor when expressed. More research is needed to determine in which phase of infection SSL3 plays a role and where and when it is expressed in the human host.

P010

Mixed culture studies on the anaerobic fermentation of lignocellulosic sugars: Testing the influence of batch feeding as opposed to continuous feeding using mixed substrates

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Mixed culture studies on the anaerobic fermentation of lignocellulosic sugars: Testing the influence of batch feeding as opposed to continuous feeding using mixed substrates.

Introduction: Annually, on a global scale, a total of 1,500,000,000 tonnes of lignocellulosic biomass waste are generated from agriculture and other industries (Kim and Dale 2004). Glucose and xylose constitute a large saccharide fraction of lignocellulosic biomass. When cascading lignocellulosic biomass to products, carbohydrate rich waste streams can emerge to offer a great opportunity for mixed-culture fermentation (MCF). In order to understand this bio conversion better, it is of value to identify the micro-organisms that drive this process. In this study, a continuous stirred tank reactor (CSTR) and sequential batch reactor (SBR) are used as model systems, using xylose and glucose as limiting substrates. Two hypothesises are studied, assuming no symbiosis, parasitism or mutualism to be significantly present:

Limited feeding a mixture of two or more substrates to a MCF CSTR system will theoretically lead to the dominance of a single species (Kuenen and Johnson 2009). Limited here means, that the C-source and electron donor acceptor couple, which can be glucose, is limited in the feed.

In the MCF SBR, one xylose and one glucose specialist will occur This division of uptake by two specialists can be explained by the diauxic uptake of for example xylose and glucose, which shows the preference of single organisms for one or the other substrate

Methods: MCF CSTR and MCF SBR using a limited feed of glucose and xylose. The medium composition of (Temudo, Kleerebezem, and van Loosdrecht 2007) is used. 4M NaOH is used as base reagent. 215 mL/min N_2 gas is sparged to maintain anaerobic conditions. The reactor is stirred at 300 rpm. Enrichment can result in 1 or more organisms, which are likely to belong to the genus *Clostridia* (Temudo et al. 2008). HPLC and GC are used to quantify the main catabolic products and limiting substrates. TSS/VSS method is used to quantify biomass concentration. H_2/CO_2 online gas analyzer is used to quantify main gaseous products. 16S based identification of micro-organisms will be used, most likely 16S amplicon sequencing and/or 16S based DGGE.

Results: Literature research performed, and two valuable microbial research opportunities are identified, of which one is formulated as hypothesis here.

4 g/L glucose enrichment is performed. H_2 production is in the same range as previous experiments (Temudo et al. 2008). Stable base dosage after enrichment, indicating the production of volatile fatty acid's.

Conclusion: First steps towards running and analysing a mixed substrate MCF system are taken.

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P011

Intestinal bacteria of cabbage root fly larvae break down plant's main defense isothiocyanate compound

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Introduction: Plants of the Brassicaceae family produce toxic isothiocyanates (ITC) to provide protection against herbivorous insects and mammals, as well as pathogenic bacteria. The larvae of the cabbage root fly (*Delia radicum*) are one of few specialist insects that are able to feed on Brassicaceae. We hypothesized that *D. radicum* copes with high ITC concentrations through the action of microbes residing in the gut of the insect larvae.

Methods: The gut microbial community of *D. radicum* larvae was profiled by 16S rRNA gene sequence analysis obtained by metagenomic sequencing. ITC resistant strains were isolated from *D. radicum* intestines by successive plating on 2-phenylethyl isothiocyanate (2PE-ITC) containing medium. Naturally occurring plasmids were isolated from these strains and subsequently sequenced, assembled and annotated. A gene encoding a putative hydrolase (called *saxA*) found on one of those plasmids was expressed in *E. coli* cells. *saxA*-vector containing *E. coli* cells were then tested for increased resistance against 2PE-ITC. Purified SaxA protein activity on 2PE-ITC was determined by GC-MS.

Results: Four strains capable of metabolizing 2PE-ITC were isolated from the gut. Plasmid Drgb3 contained an operon with three genes with close homology to the *saxCAB* region associated with aliphatic ITC resistance. Heterologous expression of the *saxA* gene in *E. coli* transformants increased their resistance to 2PE-ITC compared to empty vector controls. Purified SaxA protein was shown to catalyze the hydrolysis of several isothiocyanate species.

Conclusions: Four 2PE-ITC resistant bacterial strains in the *D. radicum* gut carry a natural plasmid containing genes that facilitate ITC breakdown. The SaxA protein that was produced from one of these genes is the first representative of a novel family of ITC hydrolase enzymes and is hypothesized to catalyze the first step in a previously undescribed ITC breakdown pathway. Our findings suggest that the gut bacteria that are the source of these ITC hydrolase enzymes may play an important role in detoxification of the insect diet.

P012

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

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Introduction: Community acquired pneumonia (CAP) patients are at high risk for readmission due to pneumonia after discharge from the hospital. Since *S. pneumoniae* is the most common cause of CAP, pneumococcal vaccination post-CAP may prevent readmission. It is therefore of interest to determine if post- pneumococcal CAP vaccination with a pneumococcal conjugate vaccine (PCV) will show efficacy.

Methods: Informed consent was obtained from 60 CAP patients: 34 pneumococcal CAP patients and 26 CAP patients with another known pathogen (cCAP) as control. Serum samples were obtained before and 3-4 weeks after vaccination with PCV13. Anti-polysaccharide (PPS) IgG antibodies were measured using Luminex technology. Vaccine responses were categorized according to qualitative and quantitative criteria.

Results: Baseline characteristics, including mean age and comorbidities, were similar. Prior to vaccination, anti-PPS

concentrations were occasionally higher in pneuCAP compared with cCAP patients, e.g. serotypes 19A and 19F (t-test p-values 0.042 and 0.014, respectively). Following vaccination, 76% had a good vaccine response in the pneuCAP group based on qualitative criteria, compared with 81% in the cCAP group. Quantitatively, distribution of pooled fold-increases for all serotypes showed an overall less strong response by the pneuCAP group (chi-square p-value <0.01). PneuCAP patients responded less well against several serotypes compared with cCAP patients, especially serotypes 1 and 9V (t-test p-values <0.01 and 0.036, respectively).

Conclusion: Our results show that in both CAP groups, PCV13 elicits an adequate overall antibody response. The response against several serotypes is reduced in former pneumococcal CAP patients. This may indicate a predisposition for reduced anti-polysaccharide responses in these patients.

P013

Hepatitis E virus seroprevalence differs in Dutch and first generation migrant populations in Amsterdam, the Netherlands

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Introduction: In the last decade hepatitis E virus (HEV) is increasingly recognized as a cause of acute viral hepatitis in developed countries. HEV is transmitted via the fecal-oral route. In countries like the Netherlands, HEV infection is suspected to be a zoonosis but HEV may also be introduced by migrants. We aimed to study the seroprevalence of HEV among different migrants, mainly Moroccans and Turks, and compared this to that of the native Dutch population in Amsterdam, the Netherlands.

Methods: Data were obtained from a cross-sectional survey performed in 2004 among adults, the Amsterdam Health Monitor (AHM). Plasma samples were tested for IgG-and IgM antibodies to HEV using the Wantai kit. Basic demographic data (gender, age, country of birth, and age at immigration) were used in the analyses. Previous hepatitis A virus (HAV) serology data were available.

Results: The weighted anti-HEV IgG seroprevalence was 25%, based on 1294 adult AHM participants. HEV seroprevalence increased significantly with age. First-generation Moroccan migrants (44%) had a higher weighted HEV seroprevalence than the Dutch (30%) whereas first generation Turks (19%) and first generation migrants from other countries (17%) this seroprevalence was lower. None of the second generation Moroccan and Turks were HEV seropositive. The weighted HEV seroprevalence in second generation migrants from other countries was 14%. Only 0.5% (n=7) had IgM antibodies to HEV, showing few acute infections. There was no relation between HEV and HAV seropositivity, despite fecal-oral transmission for both viruses.

Conclusions: HEV seroprevalence in migrant populations differed from each other and from the Dutch population. First generation Moroccans had a higher HEV IgG seroprevalence than Dutch or Turkish adults. Second generation migrants had a lower seroprevalence than Dutch adults.

P015

Comparison of three commercial PCR assays for the detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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Introduction: Molecular diagnostic methods can reduce the turnaround time for detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA), leading to earlier identification of carriers. The use of a molecular method in the MRSA control strategy may reduce the time MRSA negative patients spent in isolation.

In this study three commercially available PCR assays for the detection of MRSA were compared. These included the BD GeneOhm™ MRSA ACP Assay (BD Diagnostics, Breda, Netherlands), Sentosa® SA direct MRSA/SA PCR Test (VELA Diagnostics, Hamburg, Germany) and RIDA® GENE MRSA real-time PCR Kit (R-Biopharm AG, Darmstadt, Germany).

Materials and **Methods:** A total of 32 bacterial cultures were tested. These consisted of 22 MRSA positive strains and 10 mixed cultures of Methicillin Sensitive *S. aureus* strains and Methicillin Resistant *S. epidermidis* strains (with a MecA gene present). All MRSA positive strains were typed by means of Multiple-Locus Variable number tandem repeat Analysis (MLVA) by RIVM (Dutch National Institute for Health and Environment). In the study, different MLVA types were included.

Furthermore a total of 50 nasal swabs were tested (E-swab, Copan, Brescia, Italy). These included 14 samples which were MRSA positive in culture and 36 culture negative samples.

Results: Out of the 22 MRSA positive strains 2 were negative in the BD assay, these were both MecC positive strains. The Sentosa assay also missed 2 out of 22 MRSA positive strains and 1 additional strain was redeemed invalid. These 3 strains were all MecA positive strains. The Rida Gene assay detected all of the positive strains.

All mixed cultures tested negative in the BD assay, whilst the Sentosa assay showed 1 out of 10 mixed cultures

positive compared to 3 out of 10 in the Rida Gene assay. Out of the 14 MRSA-positive nasal swabs, the BD assay was negative in 2 (both contained MecC-positive strains), the Sentosa assay was negative in 3 (all MecA-positive strains) whilst the Rida Gene assay detected all 14 positive samples. Furthermore, one out of the 36 MRSA-negative samples was found positive by the BD assay compared to none in the Sentosa assay and 11 in the Rida Gene assay. Therefore the overall sensitivity (sn) and specificity (sp) of the assays were as follows: BD: sn 94%, sp 98%, Sentosa: sn 86%, sp 100% and the Rida Gene: sn 100%, sp 70%

Conclusion: Overall the BD assay had the highest combined sensitivity and specificity which could even be increased by including the MecC gene in their detection panel. Furthermore the Rida Gene assay showed a high rate of false positive results when using mixed cultures or nasal swabs, making it not applicable as a method for MRSA-screening directly on patient material.

P016

Important gender differences in invasive pneumococcal disease and the impact of pneumococcal conjugate vaccination, the Netherlands

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Introduction: Implementation of the 7-valent pneumococcal conjugate vaccine (PCV7) for Dutch infants (2006) led to a major shift (''replacement disease'') from vaccine to non-vaccine serotypes causing invasive pneumococcal disease (IPD) in both vaccinated children (by direct effects) and in other age groups (by indirect effects). This changed serotype-specific IPD incidences in all age groups. The objective of this study was (1) to explore potential differences in IPD susceptibility between genders in the pre -and post-PCV7 period and (2) to explore differences between genders in direct and indirect effects after PCV7 introduction.

Methods: Pneumococcal isolates from hospitalized IPD patients, obtained from nine sentinel microbiology laboratories covering 25% of the Dutch population, were collected on behalf of the national IPD surveillance team (in total 4853 cases). Structural gender differences (i.e. present in both pre -and post-PCV7) in IPD incidence (stratified by clinical syndrome/age) were assessed by calculating the relative risks (RR) of incidences comparing males and females within the pre-PCV7 (June 2004-May 2006) and post-PCV7 period (June 2008-May 2012). Also, the change in IPD incidence comparing post-PCV7 to pre-PCV7 was separately assessed for males and females by calculating RR. Differences in these changes between genders were assessed by calculating the ratio of RR.

Results: The largest structural difference between genders in IPD incidence was observed in those at highest risk i.e. children <5 years old and elderly ≥65 years old. Childhood males had a higher incidence of meningitis and elderly males a higher incidence of invasive pneumonia. Overall both within the pre- and post-PCV7 period, and for all age groups, IPD incidence was higher in males. The exception was IPD incidence in women aged 20-39 years in the post PCV7 period (that increased from 3.2 to 4.6/100.000 persons/per year) and became slightly higher than the male incidence in the same age group (4.2/100.000 persons/per year). This was caused by an increased invasive pneumonia incidence in 20-39 year old females comparing post-PCV7 to pre-PCV7, whereas in men of the same age IPD incidence (including invasive pneumonia) decreased (ratio of RR 1.72 95%CI:1.04-2.84, indicating a difference in indirect effects). Women 20-39 years of age significantly more often attracted non-PCV7 serotype IPD mainly due to non-vaccine serotypes 1, 3, 5 and 7F, which was not observed in males.

Conclusion: IPD incidence is structurally higher in males compared to females. PCV7 introduction in children caused higher IPD incidence in women aged 20-39 years old likely due to more exposure of women with close contact to PCV7 vaccinated children. Whether this is temporary needs to be established. Overall, we have shown that shifts in serotypes in groups with high exposure can cause (temporary) increased IPD incidence rates. Ongoing surveillance of IPD incidence by gender is crucial to evaluate the long-term effects.

P017

Google Trends as surveillance tool for seasonal rotavirus in the Netherlands and Belgium

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Introduction: Google Trends is a free online monitoring system that shows how often a specific search-term has been queried over a specific period and geographic region. Our aim was to assess the correlation between Google Internet queries for rotavirus and rotavirus laboratory surveillance in two European countries: the Netherlands, without universal rotavirus vaccination (URV) and Belgium, who has offered URV since 2006.

Methods: As traditional surveillance data source, we used weekly reports on the number of rotavirus detections from

the Dutch and Belgian national virologic surveillance systems. Monthly search query volumes containing the word 'rotavirus' or 'rota virus' for the respective countries were collected from Google Trends. We restricted our analyses to the years after January 2007 for Belgium and January 2008 for the Netherlands, because from these time-points onwards the overall Google search volume for the respective countries was sufficiently high to produce meaningful query fractions for rotavirus. We assessed the correlation between Google Trends and the laboratory surveillance data. Next, time series regression analysis was performed to optimize the explanatory value of search query volumes in estimating the time series of traditional surveillance. Ordinary regression models, including search query volumes as a regressor were fitted that accounted for seasonal trends using sine and cosine terms, and allowed for lagged effects of search query volumes for up to three months. We calculated confidence intervals that accounted for residual correlation.

Results: For both countries, high correlation was observed between the time series of Google Trends and those of traditional surveillance (squared correlation (R^2): 0.89 and 0.69 for the Netherlands and Belgium, respectively). The time-series models further optimized estimation of rotavirus detections based on Google Trends data. The R^2 for these models was 0.92 for the Netherlands and 0.86 for Belgium. The fitted values accurately followed the actual peaks and troughs in rotavirus detections. For the Netherlands, a 5% proportionate change in relative search volume was associated with a 4% change in rotavirus detections in the same month (RR = 0.80; 95% CI = (0.69-0.92)). Similarly, the model for Belgium predicted a 3.6% proportionate change in rotavirus detections in the same month for a 5% change in search volume (RR = 0.72; 95% CI = (0.37-1.07)).

Conclusion: Google-based rotavirus search volume, as summarized in Google Trends, correlates well with rotavirus activity in the population as measured by traditional rotavirus surveillance in settings both with and without universal rotavirus vaccination. Time series regression models based on search query volume data accurately reflect actual changes in rotavirus activity. Google Trendscould be a useful, inexpensive and easily accessible tool for real-time rotavirus surveillance and early detection of rotavirus epidemics, thereby facilitating public health planning.

P018

Development of tailored antimicrobial treatment regimens and novel host- pathogen insights for respiratory tract infections and sepsis. (TAILORED-Treatment)

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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.

P019

Towards understanding hydrazine synthesis in anaerobic ammonium oxidizing bacteria

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Anaerobic ammonium oxidizing (anammox) bacteria are versatile chemolithoautotrophs that are ubiquitously present in manmade and natural ecosystems, and contribute substantially to the global release of fixed nitrogen to the atmosphere. The biological novelty of the anammox process is the anaerobic activation of relatively inert ammonium (NH_4+). Instead of oxygen, the oxidizing power of nitric oxide (NO) facilitates this purpose, resulting in the production of the highly reactive intermediate hydrazine (N_2H_4). Hydrazine synthesis by anammox bacteria is the second known biological process that can forge an N-N bond -the other one being nitrous oxide production by denitrifiers- and the only one to do so from two different nitrogen species. The aim of this study is to investigate the molecular mechanism of hydrazine synthesis by anammox bacteria. The novel protein complex that catalyzes the formation of N_2H_4 from nitric oxide and ammonium is comprising the three most abundant gene products in the *Kuenenia stuttgartiensis* proteome (kuste2859-61) and was purified to homogeneity as a dimer of heterotrimers $(\alpha\beta\gamma)_2$. Analyses of the crystal structure of the native complex of hydrazine synthase (HZS) at 2.7 Å resolution together with enzymatic assays and spectroscopic studies suggested that hydrazine synthesis occurs *via* a two-step mechanism facilitated by two *c*-type heme-containing active sites. First, NO undergoes a three-electron reduction to hydroxylamine (NH_2OH) at the active site of the γ-subunit. Hydroxylamine is then transferred to the active site of the α-subunit through a tunneling system that is presumably regulated by the β-subunit. At this site, ammonia (NH_3) and hydroxylamine are condensed into N_2H_4.These results provide the first insight into the molecular mechanism of hydrazine synthesis by anammox bacteria. Based on the grounds of the structural model described herein, further research will be focused on elucidating the exact catalytic mechanism of hydrazine synthase.

P021

Genome wide Single Nucleotide Polymorphism analysis of *Campylobacter fetus* showed the classification of *C. fetus* in different clades

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Introduction: The pathogen *Campylobacter fetus* (*C. fetus*) can cause disease in both animals and humans. The species *C. fetus* is divided into three subspecies; *C. fetus* subsp. *venerealis* (Cfv), *C. fetus* subsp. *fetus* (Cff) and *C. fetus* subsp. *testudinum*. Cfv includes a biochemical variant designated *C. fetus venerealis* biovar intermedius (Cfvi). The control of Bovine Genital Campylobacteriosis (BGC) relies on the identification of Cfv, as this subspecies has been associated with BGC. However, *C. fetus* subspecies identification is hampered by insufficient discrimination by the available assays.

Methods: In this study, we performed whole genome alignment analysis of 41 *C. fetus* strains from cattle, sheep and human to investigate the presence of subspecies-specific SNPs in the *C. fetus* core genomes, and if specific SNPs or genes could be associated with biochemical and clinical characteristics of the *C. fetus* strains.

Results: Phylogenetic reconstruction of the SNPs in the core genomes shows the separation of *C. fetus* strains in different clades and suggests that the Cfv clade and a successful Cff clade evolved from a single Cff ancestor. The presence of multiple *C. fetus* clades suggests closer evaluation if the current subspecies separation is still valid for the applied BGC control programs. In the 41 *C. fetus* genomes, 5 Cfv specific SNPs were found and 282 SNPs specific for the Cfv/Cfvi clades compared to the Cff clades. No SNPs could be associated with the biochemical characteristics and source or clinic of the strains.

We also show that the H_2S production biochemical test, which defines separation of Cfv biovar intermedius from Cfv strains, is tightly linked to the presence of two cystine transporter genes. Using phylogenetic reconstruction, the deletion of these cystine transporter genes has occurred multiple times as independent events, suggesting that the genetic region encoding the cystine transporters is sensitive to deletion events.

Conclusions: We conclude that a genome-wide SNP analysis can reliably identify *C. fetus* strains to the

subspecies level and that Cfv appears to be a niche specific clade of Cff. Furthermore, we suggest that the use of the biochemical H_2S production test to identify Cfvi strains is not preferable.

P022

Automation of microbial whole genome assembly

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In recent years the use of whole genome sequencing in microbiological laboratories has become feasible by the wide application of next-generation sequencers. The accuracy and depth of information provided by whole genome sequencing is a clear advantage for molecular diagnostics and molecular epidemiological studies. However the development of a matching and dedicated bioinformatics infrastructure lacks behind and hinders the routine use of whole genome sequencing in a real-time surveillance system for microbial infections. For many clonal species reference-based mapping is a viable option, but species with open pangenomes and novel species benefit from de novo assembly of short reads to contigs. De novo assembly can be challenging because it requires computational infrastructure that goes beyond a standard PC, and assembly parameter selection can vary for different sequencing technologies and organisms. Nonetheless assembled microbial genomes are the basis for many further analyses such as genomic or genetic characterization, generation of reference genomes, comparative genomics, antimicrobial resistance determination, outbreak determination and many more.Here we describe the development of a basic bioinformatic infrastructure for bacterial whole genome sequencing using high-performance computing. It consists of a workflow that requires minimal and controlled user input. It automatically backs up raw sequencing reads to a data repository, and performs quality check, trimming and assembly with automated parameter selection for a wide range of bacterial species. To confirm the identity of the sample, a taxonomic determination and multi-locus sequence typing of the resulting assembly is automatically performed. All results are reported back in a user-friendly format. The intended turnaround time for the assembly pipeline for a single sequencing run containing up to 96 samples is two hours.The automated assembly pipeline will improve the speed, reproducibility and robustness of microbial whole genome sequence-based analysis and constitutes a first step in the automated interpretation of microbial whole genome sequencing data.

P023

Metagenome analysis of a methane emitting coal seam

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About seven percent of total annual methane emissions originates from coal mines, making these systems significant contributors to the global greenhouse effect.
This study focuses on reconstructing the complex microbial food web of an anaerobic, nutrient amended Australian coal seam. The system showed continuous methane production over a period of 25 months. DNA was extracted from coal samples and sequenced using Ion Torrent technology.
Species composition analyses on 16S rRNA and functional genes retrieved from the metagenome indicated relatively low species complexity with the main groups being fungi, metal reducers, organic compounds degraders and methanogenic archaea. The genome of a metal reducing *Geobacter* species and the methanogens core genome could be reconstructed from the metagenomics dataset for further analysis.
In more detail, functional gene analyses indicated pathways involved in metal reduction, degradation of complex aromatic compounds to methanogenic substrates and methanogenesis. In-depth analysis of methyl-coenzyme M reductase A (McrA) protein sequences indicated presence of both a *Methanosaeta* species and an uncultured methanogenic archaeon. The abundance of methanogens was surprisingly low, but they appear to be highly active, and are therefore one of the focus points of this study.
This metagenomics study provided insight in the species diversity of an amended methanogenic coalbed and the microbial mechanisms that are responsible for coal degradation and methane production.

P024

Shewanella spp. from aquatic environment and food-producing animals hold chromosomal blaOXA-48-like genes: a real threat for public health?

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Introduction: Carbapenems are considered last resort antibiotics in health care and resistance by production of carbapenemases is a serious threat to human health.

Food-producing animals, environmental water from the Netherlands and imported ornamental fish were screened to assess the presence of carbapenemase-producing bacteria and their potential public health risks.

Methods: Faecal samples of broilers, slaughter pigs, veal calves, dairy cows, imported ornamental fish, surface fresh water and fish transport water were analyzed with commercial RT-PCR to detect KPC, NDM, VIM, IMP and OXA-48 carbapenemase families. In OXA-48 positive samples allele variants were confirmed by PCR and sequencing. Gene localization was established by Southern blot hybridization of chromosomal DNA and plasmid transformation and conjugation.

Results: No KPC, NDM, VIM or IMP enzymes were detected. bla_{OXA-48}, $bla_{OXA-48b}$, $bla_{OXA-181}$, $bla_{OXA-199}$, $bla_{OXA-252}$ and $bla_{OXA-416}$ were found in water and/or faecal samples. Selected bacterial isolates were identified as *Shewanella* spp. Chromosomal location for all bla_{OXA-48}-like variants was confirmed, independent of the source. Reduced susceptibility to imipenem, meropenem and/or temocillin was observed for three *Shewanella* isolates.

Conclusions: The presence of bla_{OXA-48}-like genes in ubiquitous aquatic bacteria is not routinely screened during antibiotic surveillance which represents a chance for these carbapenemases to go undetected. Yet, given their chromosomal location in *Shewanella* spp., the bla_{OXA-48}-like genes found are not transferable and they do not pose a threat to public health. They represent the environmental reservoir from which these genes have evolved in the past decades.

P025

Paediatric causes of encephalitis and meningitis, the first PACEM study results

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Introduction: Meningitis and encephalitis are potential life-threatening diseases. Since effective vaccines significantly reduced the incidence of bacterial meningitis, other non-bacterial pathogens (such as viruses) have increasingly emerged as important causes of central nervous system (CNS) infections. Routine testing of these pathogens is not performed, therefore insights in incidence numbers and clinical relevance are missing. The aim of this PACEM-study is to systematically register clinical and laboratory findings of children with meningitis and/or encephalitis in order to increase insight in the causes and pathogenesis of these diseases.

Methods: This prospective observational study aimed to include children with a clinical suspicion of a CNS infection. Cerebrospinal fluid (CSF) was tested for the presence of bacteria and neurotropic viruses. Demographic, clinical and laboratory characteristics were collected from the patient's files.

Results: Between January 2012 and January 2014, 70 children (median age 55 days) were included. In 37% of the children a pathogen was detected in CSF, with 20% positive for enterovirus (EV), 8% for human parechovirus (HPeV), 3% for human herpesvirus 6, and 2% for herpes simplex. One child had a positive bacterial culture, and one an CNS-infection with Borrelia. Most children with an EV and HPeV meningitis/encephalitis were younger than 3 months. Leukocyte counts in CSF were normal in all children with a viral meningitis/encephalitis.

Conclusion: Viruses are frequent causes of meningitis/encephalitis, although in 2/3 of the children a disease-causing pathogen could not be identified. Negative CSF-samples should therefore be further analysed to identify possible new pathogens to develop novel intervention strategies that improve survival and reduce brain injury.

P026

Nitrate/nitrite driven anaerobic methane oxidation: a promising technology for wastewater treatment

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Introduction: Methane and ammonium are major end products of anaerobic digestion. Currently, ammonium is removed from these effluents through conventional nitrification/denitrification or more efficient partial-nitritation anammox processes. Methane is collected from the flue gas and used for electricity production. Still, remaining dissolved methane in effluents from anaerobic digestion is slowly released to the atmosphere, contributing to global warming. Here, we aimed to develop an integrated oxygen-limited system that removes ammonium and methane simultaneously by combining nitrate/nitrite-dependent anaerobic methane and ammonium oxidation processes. These system offers many advantages such as lower costs, reduced sludge production, less energy consumption, reduced greenhouse gas emissions and a much smaller resource footprint.

Microorganisms that couple the anaerobic oxidation of methane to nitrite and/or nitrate reduction were recently discovered. The first reaction is performed by *Methylomirabilis oxyfera* with N_2 as end product, whereas the latter is performed by *Methanoperedens nitroreducens*.

Methods: Nitrogen and methane conversions were investigated in a stainless steel and glass bioreactor (Applicon Biotechnology BV, Applisens, Schiedam, the Netherlands),

inoculated with a pre enriched effluent from a bioreactor performing nitrite dependent anaerobic methane oxidation (Ettwig *et al*. 2008) composed by *M. oxyfera*-like bacteria (70%) and *M. nitroreducens*-like archaea (30%). The reactor was kept anoxic by continuous supply of methane (95% CH_4, 5%CO_2), and Ar:CO2 (95:5) in the medium. The system was fed with mineral medium containing nitrate as sole electron acceptor.

Results: After 1.5 years of enrichment the co-culture has favoured AAA (≈60%) and N-damo bacteria as side population (≈30%) and ≈10%. The consumption rates of nitrate increased from 0.3 µmole per day to 14 µmole/day. Complete removal of the supplied nitrate was observed, 90% of which was converted to N_2. Further analyses revealed that *M. nitroreducens*-like archaea could reduce nitrate all the way to ammonium and under the present conditions we could show that the remaining 10% of nitrate was indeed converted to ammonium. Surprisingly, despite the presence of ammonium and transient production of nitrite, anaerobic ammonium-oxidizing (anammox) bacteria were not detected. Nevertheless, these findings indicate that it is possible to create an environment where anammox and nitrate/nitrite reducing methanotrophs could coexist.

Conclusions: Nitrate as sole electron acceptor favors the enrichment of anaerobic methanotrophic archaea related to Methanoperedens nitroreducens.

Archaea perform nitrate dependent methane oxidation, producing nitrite and ammonium as end product, thus providing M. oxyfera like bacteria with the necessary nitrite for nitrite dependent anaerobic methane oxidation, converting nitrite into dinitrogen gas.

The production of ammonium in addition to nitrite creates an ideal niche for anammox bacteria but this were not detected in our enrichment culture.

P027

Experimental evolution of heavy metal tolerance in *Saccharomyces cerevisiae* exposed to different rates of environmental change

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Directional environmental change is a ubiquitous phenomenon, with climate change as a notorious example. To investigate the effect of different rates of change on evolution, we grew *Saccharomyces cerevisiae* for 500 generations in the presence of (i) constant high, or (ii) gradually increasing, concentrations of cadmium, nickel or zinc. We anticipated that, depending on the nature of underlying genotype-by-environment interactions (GxE), these regimes would result in different evolutionary dynamics and endpoints. More specifically, we considered the consequences of magnitude versus reranking GxE. Both types of GxE predict that gradual change, as opposed to abrupt change, delays fitness increase, but only under reranking GxE may evolution under both regimes lead to different endpoints. Our data show that gradual change delays fitness increase compared to abrupt change, but fitness of evolutionary endpoints is the same under both regimes. For cadmium this is due to smaller fitness differences at low metal concentrations (magnitude GxE), while for nickel different isolates are selected at different concentrations (reranking GxE). Whole-genome sequencing has uncovered SNPs and structural changes that provide additional support for our hypotheses. Our findings imply that the rate of environmental change and the nature of the stressor are crucial determinants of evolutionary dynamics and outcomes. We are currently following up on this work by reconstructing the fitness landscapes underlying adaptation in our selective environments. This will provide additional insight into evolution in changing environments in general, and the observed dynamics in particular.

P028

Development of a Zika virus specific serological assay on a protein micro-array platform

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Introduction: In 2013 and 2014 outbreaks of zika virus (ZIKV) were reported on several islands in the Pacific region, including French Polynesia with an estimated 32,000 patients presenting to health facilities between October 2013 and April 2014. In addition, spread to New Caledonia, the Easter Island (Chile) and in 2015 to Latin America ZIKV has shown its potential of spreading outside its usual geographic range in Africa and Southeast Asia. Autochthonous cases have been reported in several countries in Latin America and the Caribbean ever since. The likelihood of travel-related cases of ZIKV infection in the Netherland increases as demonstrated by the recent import of ZIKV cases from Suriname. Clinical manifestations of ZIKV infection are very similar to those of DENV and CHIKV infections, but usually milder so ZIKV infection represents a diagnostic challenge since no specific commercial serological tests are available and cross-reactivity with DENV has been described.

To overcome this cross reactivity we developed a serological assay based on recombinant NS1 protein of ZIKV instead of the whole virus to increase specificity. We used a protein

microarray platform because it enables us simultaneous serological testing for multiple viruses within a clinical syndrome.
Methods: NS1 protein of ZIKV and DENV as well as E proteins of CHIKV were used as antigens in a protein microarray. Sera from ZIKV PCR confirmed patients and from travelers with serologically confirmed arbovirus infections were used for validation of the assay.
Results: ZIKV specific antibodies were detected in sera from confirmed patients. For sera from patients known to be infected with other flaviviruses, some NS1 reactivity was observed in the IgG responses but low reactivity in de IgM responses.
Conclusion: Serological differentiation between flaviviruses is a serious challenge in the serological diagnostics of a ZIKV infection. These preliminary results show that the use of recombinant NS1 protein of ZIKV is a promising tool to increase the specificity significantly. The protein micro array platform allows us to expand the number of viruses that can be tested simultaneously with the use of a small amount of sample increasing the differential diagnosis.

P029
Metagenomics of methane oxidizing bacteria in paddy field soil

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Methane is an important greenhouse gas, playing an essential role in atmospheric chemistry and climate change. Its emission from natural wetlands and flooded rice-fields is significantly mitigated by methanotrophs, acting as a bio-filter. Methanotrophs are methane oxidizing bacteria that have been studied for over a century, but their diversity is still far from being fully understood. For a long time, methanotrophy was found exclusively within the alpha- and gamma-proteobacteria. However, discoveries of the last few years changed this view dramatically, describing aerobic or intra-aerobic methanotrophs belonging to the verrucomicrobia or the NC10 phylum. This project focuses on enrichment and isolation of novel methanotrophs from the paddy field in Vercelli under oxygen limitation using laboratory scaled microcosms. This system allows for a less biased enrichment method compared to conventional enrichment strategies used previously. Furthermore, the focus will be on growth and physiology of selected enriched methanotrophs in bioreactors. Soil slurries were prepared and put in microcosms to be enriched for a total of three months. At each month, DNA was extracted and used for the metagenomic analysis. Using differential coverage binning, we found that already after two months there is a shift in the community within soil. A difference in oxygen diffusion was observed along with the enirchment process as well. Extracted 16S sequences were compared to look at the key players involved in methane oxidation in paddy field. Longer enrichment period was found to be required for the binning of bacterial genome and isolation of pure methanotrophic culture.

P030
Detection of human papillomavirus in (un)vaccinated young women using the highly sensitive SPF10- Line probe assay: First effects of vaccination and evaluation of the assay

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Introduction: Vaccination against HPV using the bivalent HPV16 and -18 vaccine (Cervarix) was introduced in the Netherlands in 2009 for young girls aged 12-16 years. To monitor the prevalence of specific HPV types in the population of adolescents and young adults in the Netherlands, several HPV prevalence surveys were initiated in 2009. Here we show the results of the HPV prevalence in vaccinated and unvaccinated young women detected by using the highly sensitive SPF_{10}- Line probe assay and evaluate the use of this assay.
Materials & Methods: In 2009 and 2010 girls aged 14-16 years who were eligible for HPV vaccination during the catch-up campaign were invited to participate in a longitudinal study: HPV Amongst Vaccinated and Unvaccinated Adolescents (HAVANA). In total 1802 girls participated of which 55% were vaccinated. The girls were asked to fill in a questionnaire and to take a vaginal self-sample yearly. HPV DNA status in the self-samples was determined annually. The samples were stored at -20 °C. Total DNA was isolated from 200µl of the self-sample using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche, Mannheim, Germany). DNA was eluted in 100µl elution buffer. Broad-spectrum HPV DNA amplification with the highly sensitive SPF_{10}-PCR was performed on 10µl of DNA extract. Amplified HPV DNA was detected with a DNA enzyme-linked immunoassay (Labo Biomedical Products). HPV-DEIA-positive amplicons were subsequently analyzed in a reverse line blot assay (HPV-$LiPA_{25}$, Labo Biomedical Products). The reverse line blot assay is able to detect 25 HPV genotypes.
Results: The HPV-DNA prevalence in this group of girls increased each year ranging from 4.4% in the first year to 32% in 2015. A significant difference in HPV-DNA prevalence for HPV types 16 and 18 among vaccinated and unvaccinated participants was found after three years of follow up. The HPV DNA prevalence was significantly

higher under unvaccinated individuals. HPV type 51 was the HPV type with the highest prevalence in the HAVANA-study among both vaccinated and unvaccinated participants. The reproducibility of typing results was evaluated by retesting 5% of all positive and negative samples with the SPF_{10} LiPA system. All HPV16 and -18 positive samples were retested with a HPV16 or HPV18 type specific qPCR, concordant results were found in 88% of these samples. All HPV16 and -18 negative samples of persons tested positive for HPV16 and/or -18 were also tested with HPV16 and -18 type specific qPCR. Of these samples 6.6% were HPV16 positive with the HPV16 qPCR while tested negative in the SPF_{10} PCR. For HPV18 0.7% were positive in the HPV18 qPCR while tested negative in the SPF_{10} PCR.

Conclusions: HPV vaccination is very effective in protection against HPV 16/18 infections. HPV genotyping methods used in the HPV surveillance study are reliable. In coming rounds HPV16 and -18 type specific qPCR will be performed on all HPV-DEIA positive samples.

P031

Kinetics after a chikungunya virus infection and the use for a reliable diagnostic algorithm during an outbreak

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Introduction: Chikungunya virus (CHIKV) is an alphavirus which is transmitted by mosquitos and can cause high fever, severe muscle and joint pain and rash. CHIKV was originally found in Africa and Asia where it caused sporadic outbreaks. However, since the introduction of CHIKV in the Americas in December 2013 over 1 million suspected cases of autochthonous transmission have been reported.

Several methods can be used for diagnosis of CHIKV. The virus may be detected in the blood during the acute phase of infection. Serological tests may confirm the presence of CHIKV-specific IgM and IgG. IgM antibody levels are highest 3 to 5 weeks after the onset of illness and persist for about 2 months. During an outbreak, the decision of which diagnostic assay to be used would depend on the kinetics of the response after an infection with the virus. This includes the duration of the viremic phase and the appearance of antibody responses in an infected person after onset of symptoms. During the outbreak of CHIKV in the Americas, the Pan American Health Organization (PAHO) published an algorithm for detection of CHIKV testing serum samples by RT-PCR and for detection of IgM in samples taken between day 1-8 after onset of symptoms based on historical data. In order to determine whether the kinetics of CHIKV during the most recent outbreak corresponded with this algorithm, we determined the kinetics of viremia and antibody responses.

Methods: Serum samples submitted to us for CHIKV testing from the Dutch Caribbean with known first day of illness in the period 2014 to 2015 were used. Serum samples from day 1-8 day after onset of clinical symptoms were tested by quantitative (q)RT-PCR for detection of CHIKV RNA by indirect fluorescent antibody assay (IFA) for detection of CHIKV specific IgM and IgG, according to the PAHO algorithm.

Results: Viremia following a CHIKV infection could be detected up to 4 days after onset of illness. Following clearance of the virus in serum, IgM antibodies appeared as early as day 5 followed by the IgG response detected by day 7.

Conclusion: The diagnostic algorithm used during the outbreak of CHIKV in the Americas can detect all CHIKV cases however this algorithm results in unnecessary testing by serology prior to day 5 and by qRT-PCR after day 5. Therefore, the current algorithm can be refined by testing serum samples collected 1-5 days after onset of symptoms by qRT-PCR and testing samples collected after this period by serological techniques for detection of CHIKV specific IgM and IgG antibodies.

P032

Multiplex real-time PCR for the rapid detection and differentiation of Salmonella species, *Salmonella* Typhimurium and *Salmonella* Enteritidis in samples from the food chain

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Introduction: An early and fast detection of *Salmonella* species and identification of the pathogenic serovars Typhimurium and Enteritidis in the food chain facilitates effective intervention and prevents further distribution of contaminated food products. Therefore a multiplex real-time PCR was developed for the rapid and simultaneous detection of *Salmonella* spp., *S*. Typhimurium and *S*. Enteritidis in samples from the food chain and compared to cultured-based methods.

Methods: Three primer and probe sets were designed to target the *InvA* gene, the STM4200 gene and the SEN1392 gene to detect *Salmonella* spp., *S*. Typhimurium and *S*. Enteritidis, respectively. The multiplex real-time PCR was validated and the selectivity was analysed by using 225 *Salmonella* isolates and 35 non-*Salmonella* isolates from various sources. Furthermore the limit of detection (LOD) was examined for ten different matrices by artificial contamination of samples of 25 gram at four different inoculation levels. The enrichments of these artificial contaminated samples were also analysed by the

Salmonella detection method according to ISO:6579:2002 and the Modified Semisolid Rappaport-Vassiliadis (MSRV) method according to ISO:6579:2002 Annex D.

Results: The inclusivity of the multiplex real-time PCR was 100% for all 225 *Salmonella* isolates, including 72 *S*. Typhimurium and 53 *S*. Enteritidis isolates. The exclusivity was 100%, 93,9% and 100% for *Salmonella* spp., *S*. Typhimurium and *S*. Enteritidis, respectively. The LOD of the multiplex real-time PCR for the matrices poultry, fish products, minced meat and swabs from carcasses was 0,6 CFU/ml and for milk products, eggs, spices and animal feed 1,0 CFU/ml. For these eight matrices the LOD of multiplex real-time PCR and the traditional detection methods ISO:6579:2002 and MSRV medium, were the same. However, for the matrixsocks with faeces the LOD of the real-time multiplex PCR was higher compared to the traditional methods, i.e. 1,6 CFU/ml versus 0,6 CFU/ml. And for the matrix down the LOD of the traditional methods was 0,9 CFU/ml, where the real-time multiplex PCR failed to detect *Salmonella*.

Conclusion: The developed real-time multiplex PCR has a desirable selectivity for *Salmonella* spp., *S*. Typhimurium and *S*. Enteritidis. Moreover, it has been shown that the method is horizontal applicable for *Salmonella* detection in the food chain, since for more than five different matrices the performance of the multiplex real-time PCR was comparable to the performance of the ISO6579:2002 and the MSRV method. The advantages of the real-time multiplex PCR over the traditional methods is that it is faster, use of less materials and that it simultaneous differentiates the pathogenic serovars Typhimurium and Enteritis next to the detection of *Salmonella* spp.

P033

Shiga toxin-producing *Escherichia coli* infections in the Netherlands; recommendations to re-establish a reliable and feasible surveillance system

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Introduction: Clinical manifestations of Shiga toxin-producing *Escherichia coli* (STEC) range from mild diarrhea to extraintestinal severe complications, such as hemolytic uremic syndrome (HUS). Surveillance is needed for early detection of clusters, source finding, and prevention of illness in contacts. In the Netherlands, the surveillance was broadened from O157 to all STEC serotypes in 2007, as molecular methods for the detection of all STEC became more widely available in medical microbiology laboratories. Consequently, a sharp increase in STEC notifications took place, which caused an increased workload for public health services, and which often were missing epidemiological and serotyping information. This decreased the reliability of the national surveillance system.

Methods: A multicenter prospective study consisting of a laboratory and a public health component, STEC-ID-net, was performed in two regions in the Netherlands, to investigate the virulence of STEC and the public health relevance of *stx1*/*stx2* genes. Stool samples from patients with presumed infectious gastroenteritis arriving at one of the two participating laboratories between April 1, 2013 and March 31, 2014 were screened using qPCR targeting the *stx1*/*stx2*/*escV* genes. Samples tested positive were further analysed for additional virulence factors and typed. Patients with *stx*-positive stool were interviewed by phone, and in case they could not be reached, a questionnaire was sent by mail.

Results: *Stx*-genes were detected in 425 of 23153 stool samples (1.8%). The median age of the STEC-positive patients was 40 years (0-92 years), and 59% were female. A questionnaire was obtained from 246 patients (58%). A sample was sent in within 3 weeks after onset in 45% of the patients, further referred to as "acute illness". Diarrhea, blood in stool, vomiting, less frequent urination, paleness and tiredness were significantly more often reported by patients with acute illness. Additional to the presence of *stx* genes, the *escV* gene was associated with acute illness. The *stx1* gene -without *escV* and *stx2*- was only rarely detected in HUS-associated STEC serotypes.

Conclusion: Based on the results of the present study and data from the national surveillance, we propose to limit the notification of STEC infections to patients with acute illness (< 3 weeks) with at least diarrhea, blood in stool and/or vomiting, with a STEC harboring the *stx2* gene, and/or both the *stx1* gene and *escV* gene. This change in notification will narrow down the reports to the most public health relevant infections and will lead to an expected reduction in notifications of 60-70%.

P034

Digging the archives: new O-types associated with entero-invasive *Escherichia coli* (EIEC) are discovered

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Introduction: Strains received for identification or serotyping of *Shigella species* were archived at the National

Institute of Public Health and the Environment (RIVM) since 1970. Entero-invasive *Escherichia coli* (EIEC) and *Shigella species* share an infection mechanism and are genetically closely related. They can be separated from other noninvasive *E. coli* with different phenotypical tests and a PCR targeting the *ipaH*-gene. Since standard testing of the *ipaH*- gene by PCR is routine since 2009, more EIEC strains were identified. To investigate if and which EIEC strains were sent to our laboratory before 2009, a search in the archives was conducted.

Method: A total of 736 strains from 1970 until 2008 were included in the search, they were identified formerly as: *E. coli*, *E. coli* inactive, non-*Shigella species*, provisional *Shigella* or Alkalencens dispar. Because invasiveness correlates with a negative result for Lysine Decarboxylase (LDC), the strains with negative or unknown LDC results were selected for *ipaH*-gene PCR. This PCR, targeting a conserved core of *ipaH7,8*, was performed using a validated PCR program. The LDC of the *ipaH* positive strains was repeated and the strains were subjected to different phenotypical tests. The strains were also subjected to classical *Shigella* serotyping with all available *Shigella* antisera from Denka Seiken, completed with *S. flexneri* MASF IV-1, MASF IV-2, MASF 1c and MASF B from Reagensia. Next to this, a classical *E. coli* O-serotyping was performed with antisera for O1 until O187. Based on the results of all these phenotypic and serological tests together, the strains were identified.

Results: 513 strains had a negative or unknown result for LDC. 115 of these strains were *ipaH*-gene positive and they were, along with 25 possible EIEC strains from 2009-2015, subjected to extensive phenotypical testing and serotyping. In total, 37 EIEC, LDC negative strains were identified. 33 of them had a known EIEC associated *E. coli* O-type. However, 4 of these strains had an O-type not earlier described as EIEC associated. These strains are typed as O23, O62, O70 an O176. Next to this, 3 EIEC, LDC positive strains were found; they had the not earlier described O-types O1, O44 and O102. An additional 7 strains had Shigella-like characteristics; they were negative in *Shigella* serotyping; and had an O-type not earlier described as EIEC associated. In these cases, the distinction between provisional *Shigella* and newly described EIEC associated O-types cannot be made with the tests used in this study. The O-types of these strains are O16, O42, O87, O127, O132, O150 and O180.

Conclusion: In this study, four *E. coli* O-types that are associated with EIEC are described for the first time. Also three EIEC strains which are LDC positive with not earlier described O-types were discovered. The clinical relevance of these strains is not clear as LDC positive EIEC strains are not associated with invasiveness. Additional testing is necessary for the strains that cannot be identified as provisional *Shigella* or EIEC with an unknown associated O-type.

P035

Membrane protein complexes in the anaerobic methanotroph *Candidatus* Methylomirabilis oxyfera

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The bacterium *Candidatus* Methylomirabilis oxyfera couples the oxidation of methane to the reduction of nitrite, without any microbial partner, under anaerobic conditions. Instead of reverse methanogenesis utilizing MCR, as methanotrophic archaea do, this bacterium is proposed to employ a unique intra-aerobic pathway for methane activation. Nitrite is first reduced to NO via NirS. Rather than further reducing NO to N2O as in denitrification, two molecules of NO are enzymatically disproportionated into N2 and O2. The generated oxygen is then partly used to oxidize methane via the classical aerobic methane oxidation pathway. As this postulated pathway includes a novel biological reaction, the dismutation of NO, we can only speculate about its catalyst. Two putative candidates have been identified and are presumed to reside in the membrane together with most of the key proteins involved in catabolism. In addition there are a number of apparent redundancies within the genome of M. oxyfera related to membrane bound respiratory complexes, including three quinol-dependent NO-reductases, one canonical haem copper O2-reductase, two additional divergent haem copper oxidases, three Rieske-cytochrome *b* complexes and two NADH dehydrogenases. To investigate if the membrane proteins proposed to be involved in the M. oxyfera metabolism are functionally expressed and determine if the apparent redundancy is not only present at a genomic level, but also at a protein level, the whole membrane complexome was profiled. Protein complexes were isolated from the membrane using the detergent Lauryl-beta-D-maltoside in an optimal detergent to protein ratio, and separated by Blue Native PAGE. The gel was sliced into 20 slices after which an in-gel tryptic digest is performed. The isolated peptides were analyzed using liquid chromatography coupled to tandem mass spectrometry. This technique allowed hierarchical clustering of protein migration patterns and identification of complex subunit composition. Our initial analysis showed that the NADH dehydrogenase, the Rieske-cytochome *b* and the succinate dehydrogenase complexes are all functionally expressed in the *M. oxyfera* membranes, which was confirmed by in-gel activity assays. Further analysis will result in the identification of all functionally expressed protein complexes residing in the membranes of *M. oxyfera*.

P036

Evaluation of molecular and culture diagnostics for *Shigella* species and Entero-invasive *Escherichia coli*, used by medical microbiological laboratories in the Netherlands

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Introduction: Most Medical Microbiological Laboratories (MMLs) in the Netherlands use molecular diagnostics for the detection of *Shigella* species in feces samples. However, *Shigella* and entero-invasive *Escherichia coli* are genetically closely related. Culturing, serotyping and extended phenotypical testing is required for distinction. Culturing of *Shigella* and EIEC is difficult and extended phenotypical tests are not performed routinely by MMLs as they are labor-intensive and not cost-effective. In January 2016, the multicenter study IBESS (Invasive Bacteria *E.coli-Shigella* Study) has started. Aims of the study are to characterize *Shigella* and EIEC strains and to gain more insights into its diagnosis. To get insight in the current diagnostic tests used by the participants of the IBESS study and their performance, a survey and a standardized sample set was sent out to fifteen participating MMLs throughout the Netherlands.
Methods: Five pooled, standardized 10-fold dilutions of a DNA extract from *Shigella flexneri* 2a, CIP 82.48 (typestrain of *S. flexneri*) was made. Next to this, feces negative for routinely tested bacterial, parasitical and viral targets were pooled and spiked with five different dilutions of a *S. flexneri* 2a strain. The DNA-extracts and spiked feces samples were sent to the participating MMLs, together with a survey with detailed questions about the used molecular and culture protocols. Participants were asked to perform molecular diagnostics on these samples.
Results: Almost all MMLs use a real-time PCR method targeting the *ipaH* gene for diagnosing *Shigella species* or EIEC; however different extraction and amplification platforms are used. More than half of the MMLs use an enrichment broth for culturing, and most of them inoculate the feces also directly on selective agar plates. The selective agar plates that are used in different combinations are Hektoen enteric agar (HEA), Salmonella-Shigella (SS) agar, Xylose Lysine Desoxycholate (XLD) agar, chrom SS agar and Desoxycholate Citrate agar (DCA). Almost half of the MMLs have a protocol for culturing EIEC from feces, of which most differed from each other. For identification of suspected colonies, Bruker mass spectrometry, Vitek mass spectrometry, VITEK 2/XL, molecular tests and classical phenotypical tests are used. Most MMLs use more than one identification technique, in very diverse combinations. A vast majority of the MMLs also use *Shigella* serotyping, most used antisera are polyvalents for *S. flexneri* and *S. sonnei*. Susceptibility testing is mostly done with a VITEK 2 or VITEK XL. In February 2016, the Ct-values of the sent DNA-extracts and spiked feces samples of the participating laboratories will be collected and analyzed.
Conclusions: The methods for molecular detection of *Shigella* and EIEC in feces samples used in the Netherlands are very similar but performed on different platforms. In contrast, used culture techniques are very diverse, and the combinations of used techniques are almost unique for each MML.

P037

Feasibility of a quantitative polymerase chain reaction assay for diagnosing *Streptococcus pneumoniae*-associated CAP

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Introduction: *S. pneumoniae* is the most important pathogen causing community-acquired pneumoniae (CAP). The current diagnostic microbial standard detects *S. pneumoniae* in less than 30% of CAP cases due to the insensitivity of the methodology. A polymerase chain reaction (PCR) targeting autolysin (LytA) might be suitable to increase this rate of detection. Colonization with *S. pneumoniae* in the upper airways may influence the interpretation of a PCR, and therefore, a quantitative test is needed. The aim of this study is to validate a quantitative PCR and to evaluate the feasibility of this PCR in a clinical pilot-setting.
Methods: Intra- and inter-run variability, analytical specificity and sensitivity of quantitative PCRs of Fast-Track Diagnostics and an in-house PCR using the LytA gene as target were tested by serial dilution series of *S. pneumoniae* ATCC 49619 and a panel of well-characterized streptococci species. After in-vitro validation a pilot-study was performed using samples from 28 patients with pneumococcal pneumonia and 28 patients with a pneumonia caused by either unknown or other pathogens.
Results: Intra- and inter-run variability are relatively low (SD's ranging from a difference of 0.08 to 0.96 cycle thresholds). The lower limit of detection was 1-10 DNA copies/reaction. In vitro sensitivity and specificity turned out to be 100%. In the pilot-study, in which oropharyngeal swabs were used, 18/28 patients with another pathogen

causing the pneumonia had a negative PCR in contrast to 5/28 patients with a pneumococcal pneumonia. A cut-off value of 6.000 copies/ml would lead to a diagnostic sensitivity of 57.1% and a diagnostic specificity of 85.7%.
Conclusion: Quantitative PCRs targeting LytA have good in-vitro test characteristics that meets the requirements for discriminating colonization and infection of *S. pneumoniae*-associated CAP. Further research is needed to set a proper cut-off value that differentiates between colonization and infection. Using such cut-off will make the qPCR targeting LytA to be a rapid and reliable tool for diagnosing pneumococcal CAP.

P038

Ecology and evolution of microbial communities in Mabisi, a traditional Zambian fermented food product

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My PhD project aims to characterize the microbial ecology of the traditional fermented Zambian dairy product Mabisi and use its microbiological communities as a model system for experimental evolution to address long-standing questions in evolutionary biology. Mabisi is prepared in batch-to-batch propagation of raw milk in calabashes. As Mabisi producers do not share their products, the bacteria of the various Mabisi bacterial communities have co-evolved in isolation. Besides, the six to nine micro-organisms present in Mabisi form a stable community, which most likely had years of co-evolution. Most evolution experiments are based on one or only a few (co-operating) micro-organisms. Therefore, using the complex microbial community of Mabisi as an experimental model system is expected to give new insights in fundamental principles of co-evolution, which are essential to improve the nutritional and sensory quality of fermented foods in general, and Mabisi in particular.
As a first step, I will investigate the link between ecosystem complexity and the different ecosystem functions, for example the influence of fermentation method on the microbial stability of the end product. The keystone species and functional groups will be identified and the niches they occupy will be determined. Then, I will use these microbial communities to gain understanding of the population dynamics of the various communities, reproducibility of evolution, stability of co-evolved communities against invaders and pathways of evolution in the framework of adaptive landscapes. This knowledge will support the formulation of a Mabisi starter culture and further fundamental research into the co-evolution of complex microbial communities.

P039

Diagnosing clinically relevant STEC/EHECs; rapid molecular differentiation between mild and severe pathogenic types

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Introduction: Shiga-toxin producing Escherichia coli's (STECs) are a serious public health concern because of their ability to cause outbreaks, hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS). In most cases however, STEC only causes mild symptoms (diarrhea) or may even be asymptomatic. Introducing PCR for detecting STEC, in patients with gastrointestinal complaints, has led to more than 10-fold increased number of positive samples, when compared to culture.
Here, we present a 2-year experience of a strategy developed to generate rapid and clinically relevant STEC results.
Methods: The following algorithm was validated and employed in our laboratory: Stool samples from patients with gastrointestinal complaints were screened by PCR for *stx1*/*stx2* genes. PCR-positive feces was cultured o.n. in BRILA enrichment medium. Enriched medium was further analysed using STEC-differentiation-PCR and culture on CSTEC medium. Mauve-colored colonies were serological agglutinated by O157. When O157 negative, STEC-differentiation-PCR was used for confirmation of isolated strains. Isolates were send to the National Institute for Public Health (RIVM) for further typing. For molecular differentiation of the STEC-types a pentaplex PCR discerning *stx1*, *stx2*, *stx2f*, *escV* and intern control, was developed. Presence of (one or more of) these genes enables differentiation (1,2) into STECs associated with mild (normally diarrhea without mucus or blood: *stx1 or stx2f*, or *escV* together with *stx2f*), moderate (normally mild disease but infrequently HC or HUS: *stx2*, or *escV* in combination with *stx1*) or severe clinical symptoms (frequently HC or HUS but sometimes mild disease: *escV* in combination with *stx2*).
Results: From October 2013 till October 2015, 235 STEC-PCR-positive (*stx1* or *stx2*) stool samples were detected in our laboratory. After enrichment and STEC-differentiation-PCR, 63 (27%) were grouped into STECs associated with mild, 87 (37%) associated with moderate and 39 (17%) associated with severe clinical symptoms. In the latter group, percentage of bloody diarrhea was highest (33%). Moreover, the majority (84%; 16/19) of the isolates cultured from this group belonged to serotype O157 or to the 'big 6' (serotypes associated with outbreaks, HC or HUS). In contrast, these serotypes were absent in the STEC group associated with mild clinical symptoms (as assessed by STEC differentiation PCR).

By using BRILA enrichment and CSTEC plates, STEC culture strongly improved from 10% (no enrichment and only SOMCT plates) to 29% of the PCR positives. The CSTEC plate preferentially recognizes STECs associated with severe disease: 49% of the PCR positives from this group were cultured. STEC-differentiation-PCR results, on isolates, corroborated with the initial findings obtained on enriched feces.

Summary:

- Molecular detection on (enriched) feces of the *stx1*, *stx2*, *stx2f* and *escV* genes enables reliable and rapid differentiation between STECs associated with mild, moderate or severe disease (outbreaks, HC, HUS).
- Rapid differentiation facilitates clinicians to make a proper estimate on expected disease severity, crucial in offering proper treatment and prevention of further spread.
- STEC culture strongly improved using BRILA enrichment and CSTEC plates on STEC PCR-positive-stool samples, thereby facilitating further isolate-typing purposes.

References

1. Karmali MA, Mascarenhas M, Shen S, et al. Association of genomic O island 122 of Escherichia coli EDL 933 with verocytotoxin-producing Escherichia coli seropathotypes that are linked to epidemic and/or serious disease. J Clin Microbiol. 2003;41:4930-40.
2. Andreoletti et al. Scientific Opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment. EFSA Journal. 2013;11:3138.

P040

Hydrazine dehydrogenase from the anammox bacterium *Kuenenia stuttgartiensis*

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Anammox bacteria derive their energy for growth from the anaerobic oxidation of ammonium with nitrite as electron acceptor and nitric oxide (NO) and hydrazine (N_2H_4) as intermediates. N_2, the end product of this metabolism, is produced from hydrazine oxidation. We purified and characterized the N_2-producing hydrazine dehydrogenase (KsHDH) from the anammox bacterium *Kuenenia stuttgartiensis*. KsHDH is the gene product of kustc0694 and its close relatives are found in the genomes of all other anammox species. KsHDH is isolated as a covalently cross-linked homotrimeric octaheme protein (201 kDa), highly similar to hydroxylamine (NH_2OH) oxidoreductase (HAO) from aerobic ammonium oxidizers and the hydroxylamine-oxidizing enzyme kustc1061 (KsHOX) from *K. stuttgartiensis*. Whereas HAOs and the KsHOX are also capable of hydrazine oxidation, KsHDH was highly specific in this activity and inhibited by NH_2OH and NO. To understand this specificity, we performed detailed amino acid sequence analyses and investigated the catalytic and spectroscopic (electronic absorbance, EPR) properties of KsHDH in comparison with the structurally and functionally well-defined HAO and KsHOX. We conclude that HDH substrate specificity is most likely derived from small structural changes around the catalytic heme 4 ("P460") and of an electron-wiring circuit comprising seven His/His-ligated *c*-type hemes in each subunit.

P041

Identification of novel antibiotics that affect protein trafficking in Gram-negative bacteria

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Introduction: The emergence of drug-resistant bacteria is a major threat to human health that necessitates the development of antibiotics acting on novel bacterial targets. Promising targets are bacterial virulence factors, which are required to establish and maintain an infection within the host and to spread to a new host. Most virulence factors are secreted across the bacterial cell-envelope to exert their function in the host, for instance as adhesins or toxins. Hence, interference with bacterial secretion systems would be a promising strategy to interfere with bacterial infections. In Gram-negative bacteria most secreted virulence factors are secreted via the autotransporter (AT) pathway, also known as type V secretion. AT secretion is semi-autonomous and only requires the assistance of two membrane-based protein complexes: Sec and Bam. Importantly, ATs play a crucial role in the virulence of severe human pathogens, like meningitis, whooping cough and peritonitis. Although several small molecules have already been found interfering with bacterial secretion systems, the AT pathway has not yet been explored as a target for intervention.

Methods: To screen for AT inhibitors we developed a high-throughput assay with a simple fluorescence intensity readout. The assay reports on the stress induced by the accumulation of the model AT hemoglobin protease (Hbp), expressed from a plasmid in *Escherichia coli (E. coli)*, in the periplasm when secretion is inhibited at the level of the outer membrane. Accumulation of Hbp in the periplasm leads to the activation of the so-called cell-envelope stress response, leading to increased expression of periplasmic chaperones and proteases to degrade the accumulated Hbp molecules. To monitor this stress in the same bacteria a second plasmid is introduced, containing the gene encoding the fluorescent protein mNeonGreen fused to a stress regulated promoter, in this case the *rpoE* promoter. The

rpoE gene encodes the SigmaE factor, the key signalling protein in a cell-envelope stress response pathway. Here, we present data demonstrating successful application of the assay to identify cells affected in Hbp biogenesis.

Results: We first validated the fluorescence-based stress assay by expressing the Hbp secretion incompetent mutant Hbp-110C/348C, known to accumulate in the periplasmic space and to trigger the cell-envelope stress response. After extensive optimization we found a robust increase in fluorescent intensity upon expression of Hbp-110C/348C compared to the fluorescent intensity upon expression of wildtype Hbp, showing that *E. coli* cells that experience cell-envelope stress can be identified. After validation we screened a small fragment-based compound library and found one compound that leads to cell-envelope stress upon Hbp expression. With a secondary assay we confirmed that this compound indeed inhibits AT secretion in *E. coli*.

Conclusion: (1) We developed and validated an assay that can be used to screen for compounds that interfere with secretion of virulence factors via the Type V secretion pathway. (2) Furthermore, in a preliminary screen we already identified a potential AT inhibitor.

P042

A multi-centre prospective evaluation of the Check-Direct ESBL Screen™ as a rapid molecular screening method for extended spectrum beta-lactamase producing Enterobacteriaceae rectal carriage

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Background: In the Netherlands preemptive isolation of patients with a high risk of carriage of extended spectrum beta-lactamase producing Enterobacteriaceae (ESBL-E) is recommended when patients get hospitalized. Isolation measurements are costly, inconvenient for patients, and put a high work load on health care professionals. Current guidelines concerning detection of ESBL-E carriage are culture dependent and therefore time-consuming and laborious. We prospectively compared a rapid multiplex real-time ESBL-E nucleic acid amplification test (NAAT) used directly on rectal swabs, to a culture based protocol and studied the discrepancies between both methods.

Material & Methods: In two teaching hospitals in the Netherlands a rectal swab (eSwab®) was collected from three patient populations: i) 210 patients hospitalized ≥ 14 days; ii) 201 patients who started selective digestive decontamination (SDD); and iii) 143 patients that were checked for carriage of multi-drug resistant organisms (MDRO). Our culture based protocol consisted of consecutively inoculation of selective culture media (Brilliance ESBL agar®), cephalosporins susceptibility screening (Vitek®) and ESBL confirmation with a combination disk test (Beldico). It was compared to the Check-Direct ESBL Screen for BD MAX™ (Check-Points), that detects $bla_{\text{CTX-M1}}$, $bla_{\text{CTX-M2}}$, $bla_{\text{CTX-M9}}$ and bla_{SHV} genes. Both tests were processed simultaneously. Culture and NAAT were repeated for all discrepancies. All culture positive samples were tested with the Check-MDR CT103XL (Check-Points) to confirm or identify the responsible resistance gene. To assess the performance of the NAAT test, we considered our culture based protocol as the golden standard.

Results: Of the 571 rectal swabs tested, 69 (12.1%) were ESBL-E culture positive. Fifty-nine of these 69 samples also tested ESBL-E positive with the Check-Direct ESBL Screen™. In addition, 17 samples were NAAT positive but culture negative. Eight out of 10 culture positive/NAAT negative discrepancies, were confirmed as true ESBL-E positive samples with the Check-MDR CT103XL (n =3 bla_{TEM}, n = 2 $bla_{\text{CTX-M1}}$, n = 2 $bla_{\text{CTX-M9}}$, n = 1 bla_{VEB1}). The other 2 samples were considered false positive and showed an *ampC* gene (CMY-II). The three patient populations had large variance in ESBL-E prevalence (10.0%, 4.0% and 26.6% respectively), resulting in different performances of the NAAT test i) sensitivity 90.5%, specificity 96.8%, positive predictive value (PPV) 76%, negative predictive value (NPV) 98.9%; ii) sensitivity 87.5%, specificity 97.4%, PPV 58.3%, NPV 99.5%; iii) sensitivity 84.2%, specificity 94.3%, PPV 84.2%, NPV 94.3%.

Conclusion: In this prospective study, the performance of the Check-Direct ESBL Screen for BD MAX™ was assessed in comparison to a culture based protocol. 1. In total, 571 rectal swabs were analyzed, of which 27 (4,7%) showed a discrepant result between NAAT and culture. 2. It was observed that the performance of the Check-Direct ESBL Screen™ was influenced by the ESBL prevalence. 3. The sensitivity was relatively low, resulting in suboptimal negative predictive values when the ESBL-E prevalence was higher, making the assay less suitable as a carriage exclusion test. 4. We believe that a thorough knowledge of the local ESBL-E genes and prevalence is essential before implementing this test as a screening tool.

P043

Current practice of infection control in primary care in the Netherlands: results of a questionnaire survey

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Background: Good infection prevention (IP) is an important aspect of quality of medical care and is effective in reducing of transmission of infectious agents in health care settings. Little is known about implementation of infection control (IC) practice in primary healthcare. The aim of this study was to evaluate IC procedures and structure among Dutch general practitioners (GPs).
Materials & Methods: Based on the current professional guideline (WIP), we developed a self-administered on-line questionnaire, which was sent to GPs in the Nijmegen region. The questionnaire consisted of 28 questions, with regard to basic characteristics of the GP office, current performance of IC measures and questions about motivating factors for better practice. The questionnaire was composed of closed questions and disagreement/agreement questions using a six point Likert scale, respectively. Questionnaires were included in analysis when ≥ 50% of questions were answered.
Results: 100 from 118 returned questionnaires (85%) were included to our analysis. 54% of responders were men. The age of the GPs ranged from 30 to 65 years (mean 51.2 years). The preferred method of hand hygiene was soap and water (56%) versus 44% for alcohol-based hand rub (ABHR). Gender, age or duration of work experience were not significantly associated with the choice of preferred hand hygiene agent. The frequency of cleaning of the working areas at GP offices was performed in 42% of the cases in accordance with national infection control guidelines (WIP). 84% of GP offices cleaned and disinfected non-disposable instruments according to WIP. The availability of personal protective *equipment* (PPE), ABHR, and skin disinfectant was 62%, 95% and 89% during patient contact in the GP office versus 25%, 57% and 50% at patient home, respectively. Of the GP's 10.5% (10/95) wear white coats during patient contact and only 34.4% of responders (32/93) wear sterile gloves during a minor surgical procedure. Of the GPs 83.0% (76/92) were content with their present use of hand hygiene and PPE use/availability. Barriers, which were listed as comments from GP's to improve IC were: lack of evidence and doubts on the efficacy of IC measures as stated in the guidelines and the fear of side effects such as hand eczema when using ABHR. Some GPs suggested that the IC measures are not needed for non-hospitalized patients. Still 89% of GPs believed that clear and GP population-based guidelines would be helpful to improve their IC performance.
Conclusions: The WHO recommended switch from hand wash to ABHR was not implemented among majority of GPs. PPE is available in 62% of the GP offices, but was not available in 1/3 of cases during home visits of patients. Presently not all national IC guidelines are followed to the same extent, and the current situation leaves room for improvement with regard to the implementation of IC measures in GP offices. Future interventions to improve uptake of national IC guidelines should take into account the already mentioned barriers and motivating factors.

P044

The value of fast genotyping to detect and control outbreaks of Highly Resistant Microorganisms (HRMO) in the hospital

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Introduction: Nosocomial outbreaks of (multi-resistant) micro-organisms are a threat to patient's health and come with a substantial financial burden. To detect spread of bacteria in the hospital it is necessary to have a tool available that can swiftly identify whether true cross-transmission has occurred or not. Amplification fragment length polymorphism (AFLP) is a reliable and cheap fingerprint genotyping method that is routinely performed in our laboratory twice a week. AFLP typing results can be available within 24 hours after obtaining the isolate. A large database of all AFLP types found in the past is available for comparison. We describe the usefulness of fast typing in our laboratory on the basis of three recent examples.
Methods: AFLP was used to identify possible outbreaks in three suspected cases of nosocomial cross-transmission.
Example 1: A patient on haemodialysis was found to be carrier of vancomycin-resistant enterococci (VRE). During a first round of contact tracing among 19 patients, VRE was detected in two other patients; this led to a second round of contact tracing among 39 more patients. In this round a fourth VRE positive patient was identified.
Example 2: A patient that had been admitted at the emergency department and the Intensive care unit (ICU) was found to be an anal carrier of MRSA. The patient was placed in strict isolation. First ring contact tracing (n = 22) resulted in one MRSA positive health care worker at the ICU and a second ring of contact tracing was started (n = 42).
Example 3: MRSA was detected in a second healthcare worker from the emergency department that was involved in another contact screening (n = 28) around a different MRSA positive patient. A third positive patient with MRSA was detected in the ring around the second health care worker (n = 6).
Results: AFLP typing of four VRE strains from different patients showed four clearly unique ALFP types. This

indicated that no nosocomial cross-transmission of VRE had occurred. Guided by this result, no further action was taken, besides isolation precautions around the patients.
Five MRSA strains that were detected after contact tracing around two different unexpected MRSA patients resulted in four different AFLP types. The first patient and the health care worker at the ICU harboured strains with identical AFLP types. However, the second health care worker and the other two patients harboured different MRSA AFLP types. This indicates nosocomial cross-transmission had occurred in the ICU but not in other departments. Besides, the contact tracing around the second positive healthcare worker needed to be extended, since this was not related to contact with a known MRSA positive patient.
Conclusion: AFLP provided rapid and clear results that allowed swift decision making on infection prevention measures like closing wards, and initiating further contact-screening rounds. While whole genome sequence typing holds promise for the future, for now AFLP is a cheap, reliable and fast method to rely on for infection prevention measures in case of a (possible) nosocomial outbreak.

P045

Intestinal microbiome landscape of more than 1000 Western adults

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Introduction: The human intestinal microbiome is a complex and dynamic ecosystem, consisting of trillions of microbes, which is characterized by high temporal stability in healthy adults. Perturbations in the microbial balance have been linked to intestinal disorders, autoimmune diseases, and other common health complications. However, the high individuality and limited understanding of the mechanisms underlying microbiome function remain major challenges for designing modulation strategies.
Methods: We analysed the microbial composition of more than 1000 Western adults gathered over the past decade using the Human Intestinal Tract phylogenetic microarray (HITChip). We assessed the phylogenetic core with blanket analysis (Salonen et al CMI 2012) applying various criteria for the microbial group abundance or prevalence. We also studied the influence of technological (notably DNA extraction and processing) and subject parameters (age and health status) on resulting core microbiota. We further extended our previous observation of "tipping elements"(Lahti et al Nature Comm 2014), bistable taxonomic groups that are frequently observed either in very low or very high abundance in any given individual, and exhibit reduced temporal stability at intermediate abundances. We analysed the two modes of low and high abundance in these bacteria on differentiating the Western population into sub-groups that can be clearly identified as discrete combinations of specific bacterial sub-populations across a thousand Western adults based on kernel density estimates, a non-parametric method used to characterize the community structure.
Results: The core microbiota analysis revealed that the DNA extraction method and health status had a small but significant influence on the ranked abundance of genus like groups. In addition, these factors also influenced the core microbiota determination. We observed that 81 out of the 130 genus-like groups were shared in 90% of the subjects. Bacteria related to *Faecalibacterium prausnitzii* were the most abundant and constituted also the most prevalent bacterial group in this cohort followed by bacteria related to *Ruminococcus obeum* and *Subdoligranulum variable*, which complete the top three in healthy lean individuals. Previously, the tipping elements analysis revealed that bacteria related to *Prevotella melaninogenica* and *Dialister* exhibit robust bistable distribution. Visualizations of two-dimensional density estimations of abundance frequency of these two bacterial taxa bacteria, we observed that the Western population studied here seem to exist as discrete subpopulations i.e. 1) low *Dialister*, low *P. melaninogenica*; 2) high *Dialister*, low *P. melaninogenica*; 3) low *Dialister*, high *P. melaninogenica*. A high *Dialister*, high *P. melaninogenica* population was not clearly separated.
Conclusion: The wealth of data generated from large-scale studies results in hidden patterns and structures in large data sets. We used unbiased statistical approaches to look beyond the obvious observations and observed that in an over 1000-subject cohort of Western individuals a distinct common phylogenetic core consisting of 81 genus-like taxa. We also demonstrated that the relative abundance of two bistable bacteria (related to *Prevotella melaninogenica* and *Dialister*) shape the ecological landscape in the cohort of Western adults into three distinct clusters, reminiscent of the earlier reported enterotypes (Arumugam et al Nature 2011).

P046

Prior exposure to respiratory bacteria alters the airway epithelial response to subsequent viral infection

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Introduction: A number of chronic lung diseases, such as COPD and cystic fibrosis, are associated with bacterial colonization of the lower respiratory tract. Acute viral infections on the other hand are considered a trigger for the onset of acute exacerbations of these diseases. Relatively little is known about the effect of bacterial colonization on the susceptibility and outcome of secondary viral infections. In this study, we investigated if previous exposure to bacteria influences the airway epithelial response to subsequent viral infection.

Methods: Respiratory epithelial cells (BEAS-2B cells and primary bronchial epithelial cells) were first exposed to heat-inactivated preparations of non-typeable *Haemophilus influenzae, Pseudomonas aeruginosa* or *Streptococcus pneumoniae* reference strains. Subsequently, cultures were infected with either respiratory syncytial virus (RSV), human adenovirus type 2 or influenza B virus. Epithelial response was assessed by monitoring the release of the pro-inflammatory cytokines IL-6 and IL-8, and by measuring expression of key pattern recognition receptors and viral replication.

Results: Stimulation of BEAS2B cells with multiple microbial stimuli had distinct effects on airway epithelial cells, depending on the combination of pathogens. Stimulation of BEAS-2B cells with non-typeable *H. influenzae,* and to lesser extent *P. aeruginosa* but not with *S.pneumoniae,* led to an exaggerated inflammatory response to subsequent infection with RSV. Likewise, *H. influenzae* and RSV synergistically increased IL-6 production in primary bronchial epithelial cells from four out of seven donors. We did not observe a significant effect of bacterial priming on viral replication, nor an interaction of the pathogens on expression of TLR2, TLR3, TLR4 or RIG-I in BEAS-2B cells.

The impact of microbial interactions was not only determined by the bacterial stimulus, but also distinct for different respiratory viruses. Secondary infection with human adenovirus reduced bacterially-induced cytokine secretion by more than 50%. On the other hand, cultures superinfected with influenza B after exposure to heat-killed bacteria produced levels of IL-6 and IL-8 similar to those of cells stimulated with heat-inactivated bacteria only.

Conclusion: Pre-exposure of epithelial cells to bacteria induces a differentiated response to subsequent viral infection depending on the combination of pathogens. These findings highlight the complexity of microbiome interactions in the airways, which might contribute to the susceptibility of exacerbations as well as the natural course of airway diseases.

P047

Bet-hedging strategies in Bacillus subtilis

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Introduction: Many (micro) organisms live in environments that can change over time. To cope with these changes organisms can apply strategies such as phenotypic heterogeneity. Phenotypic heterogeneity describes the simultaneous occurrence of different phenotypes in a uniform environment and originates from noise in gene expression and regulatory networks. In an ever changing environment the occurrence of different phenotypes can be beneficial and can be seen as a bet-hedging strategy. Evolutionary theory predicts that the frequency, magnitude and predictability of the changes in an environment determine the degree of phenotypic heterogeneity within a population.

This research aims to elucidate the dynamics of regulation of gene expression and how this enables a bet-hedging survival strategy.

We have chosen to use sporulation in *Bacillus subtilis* as our model system. In response to nutrient limitation induced stress conditions only a fraction of the population will produce endospores as a mean of survival. This phenomenon can also occur stochastically, while other cells keep growing and dividing. This means that the overall fitness of the population in the current environment might not be optimal, but the survival chances increase in case of sudden and rapid environmental changes.

Methods: In this experiment we culture UV-mutagenized *B. subtilis* in different fluctuating environments. Availability of carbon-source (glucose) is the fluctuating, growth limiting factor. Influx of glucose is either with fixed intervals (predictable), or with varying intervals (unpredictable). When influx of glucose is unpredictable, the environmental glucose concentration holds no information about the length of the future starvation period in which case stochastic switching is hypothesized to be fitness increasing. Submitting mutants to these fluctuations should result in selection for strains with beneficial mutations. Mutant strains fitter than the parental strain are sequenced and further characterized.

Results: Strains that evolved under both predictable and unpredictable conditions outcompeted the parental strain in the conditions they evolved in. Strains evolved in unpredictable environments have on average a slower growth rate than the parental strain, however they sporulate sooner, and the percentage of cells that do sporulate during exponential growth is higher than in the parental strain.

Strains that evolved in a predictable environment have a growth rate that is slightly higher than the parental strain,

and have a lower percentage of cells that sporulate during exponential growth.
Conclusion: The strains that evolved in this experiment show different characteristics depending on whether they evolved in predictable or unpredictable environments, and have shown to be significantly fitter than the parental strain. These results show that evolving *B. subtilis* under differently fluctuating conditions lead to a different degree of bet-hedging. Further analysis of the mutations that cause this effect is currently being studied.

P048

Genome annotation of the novel nitrifier *Nitrospira lenta* BS10

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All described members of the genus *Nitrospira* are chemolithotrophic nitrite-oxidizing bacteria (NOB), catalyzing oxidation of ammonium or nitrite to nitrate. The *Nitrospira* genus is diverse and present in many different ecosystems. Sublineage I and II *Nitrospira* are the dominant NOB in most wastewater treatment plants (wwtp's) playing a key role in the efficient removal of nitrite in conventional wastewater treatment systems. However, despite their ubiquitous abundance and importance their physiology is understudied. *Nitrospira lenta* BS10 was isolated from a municipal wwtp and represents the first genome-sequenced sublineage II *Nitrospira* from activated sludge. Compared to the other two cultured sublineage II *Nitrospira*, *N. lenta* grows at lower temperatures (10-32 °C) and is adapted to lower nitrite concentrations. To understand the microorganism's core metabolism and obtain data for comparative (meta-)genomics studies, we sequenced and analyzed the high-quality draft genome of *N. lenta* BS10. The key enzyme for nitrite oxidation by *Nitrospira* bacteria is a periplasmic nitrite oxidoreductase, which consists of three different subunits. The *N. lenta* BS10 genome contains two copies of the NXR alpha (NxrA) and beta (NxrB) subunits. Three different candidate genes encoding putative gamma subunits (NxrC) were also identified. Although denitrification is not demonstrated in *N. lenta*, *nirK* and *nirA* genes were present in the genome. Interestingly, the *N. lenta* BS10 encodes an operon for a functional urease (ureABC) as well as a complete gene set for a high affinity urea ABC transporter (*urtABCDE*). This implies that *N. lenta* is able to hydrolyze urea to ammonium and CO_2. The *N. lenta* urease is closely related to the *N. moscoviensis* enzyme, but phylogenetically differs significantly from other known ureases. The oxidative and reductive TCA cycles are present for energy conservation and CO_2 fixation, respectively. Complete glycolysis/glyconeogenesis and pentose phosphate pathways were also identified. The genome features defense mechanisms against diverse toxins, including acriflavine. Furthermore, the *N. lenta* BS10 genome contains a superoxide dismutase, which plays a key role in oxidative stress defense. The genome analysis of *N. lenta* BS10 is clearly a step towards a description of the core metabolism enabling *Nitrospira* to grow by nitrite oxidation and might furthermore lead to a better understanding of adaptations required to proliferate in activated sludge.

P049

Enterococcus faecium genome dynamics during long-term patient gut colonization

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In recent decades, *Enterococcus faecium*, normally a gut commensal, has emerged as an important multi-drug resistant hospital-associated pathogen. 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 96 multi drug-resistant *E. faecium* patient isolates over timespans ranging from days to years using whole genome sequencing.
We found host-specific colonization pattern, where some patients were colonized for 2 years with one *E. faecium*-clonal population while other patients with multiple populations. Arecombination filtered core genome-based phylogenetic tree together with 70 previously published strains showed high diversity of *E. faecium*genomes and of the 96 isolates 95 belong to a hospital-associated sub-population of *E. faecium*. Using highly similar subsets of patient isolates, we describe micro-evolution in closely related strains. In one clonal population, we found the gain of a prophage and loss of a plasmid, as well as rapid rearrangements of IS elements. Using a root-to-tip analysis, we estimate an evolutionary rate for these isolates of 12 SNPs/year/genome.
This study highlights the different mechanisms that contribute to the genomic flexibility of clinical *E. faecium* strains, a crucial factor in the ability of *E. faecium* to rapidly acquire antibiotic resistance determinants and adapt to new ecological niches.

P050

Novel putative virulence factors expressed in invasive *Enterococcus faecium*

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Since the introduction of antimicrobial agents, enterococci, *Enterococcus faecium* in particular, have undergone a transition from harmless gut commensals to leading causes of multiple drug resistant hospital infections. Recent European surveys have documented a pronounced increase in bacteremia caused by invasive *E. faecium* represented by hospital-adapted clonal lineages, high-risk clones (HiRiCs). The transition of commensals to pathogenic HiRiCs is also associated with acquisition of genes that contribute to destabilization of the microbe-host relationship as well as mobile genetic elements. The initial recognition of a pathogen by immune cells may be counteracted by bacteria through immune evasion factors such as bacterial TIR (Toll/interleukin-1 receptor)-domain containing proteins that interfere with early host cell signaling. Further factors that disrupt the interaction between host and microbe, thus promoting virulence, might be encoded by mobile genetic elements such as megaplasmids.

Our objectives were I) to identify enterococcal TIR-domain containing proteins (TirEs) as well as secretory proteins of unknown function (SfE) encoded by megaplasmids and to compare their prevalence in nosocomial versus community-associated strains using a large-scale enterococcal sample collection; II) to confirm expression on RNA level in different growth phases as well as during exposure to human blood; III) to study the megaplasmids putative contribution to bacterial survival within human blood.

TirE encoding genes were identified through searching databases for proteins with conserved TIR domains. Putative soluble protein factors were identified from *E. faecium* megaplasmids by using BLAST combined with SignalP analyses on open reading frames encoding putative proteins with no known homology. A large and diverse strain collection was used for prevalence screening. It consists of about 300 enterococcal isolates, differing in source, country of origin, year of isolation, species and sequence type of bacteria. To gain templates for PCR screening, total DNA was isolated. Screening was performed utilizing PCRs with specifically designed TirE and SfE primers or by WGS. To confirm the expression of TirEs and SfEs transcriptional analysis was performed using bacterial mRNA as template for qRT-PCR. Two different genes encoding putative TirEs, found in different *E. faecium* strains, and thirteen putative soluble SfEs were selected for further analyses. Blood survival assays were conducted by comparing the survival of *E. faecium* BM4105-RF with and without a SfE-encoding megaplasmid.

The same distribution pattern was found for TirEs and SfEs; a significant higher prevalence in clinical samples, especially in blood culture isolates. All TirEs and SfEs were found in sequence types belonging to HiRiCs, whereas non-HiRiCs were mostly negative for TirEs and OrfEs. The transcription of TirEs and OrfEs was confirmed on RNA level by qRT-PCR in standard growth conditions as well as in blood. *E. faecium* BM4105-RF with the SfE-encoding megaplasmid survived significantly better than BM4105-RF without this plasmid in human blood.

Our findings of high prevalence of TirEs and SfEs in clinical isolates, particularly invasive HiRiCs isolates, in combination with expression of these factors in blood and better blood survival of *E. faecium* with SfE-encoding megaplasmid, suggests an important contribution of these factors for the invasive properties of *E. faecium*.

P051

The *Bacillus subtilis* spore inner membrane proteome

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Introduction: The endospore is the dormant form of *Bacillus subtilis* and many other Firmicutes. By sporulation these spore formers can survive very harsh physical and chemical conditions. Yet they need to go through germination to return to their growing form. The spore inner membrane (IM) has been shown to play an essential role in triggering the initiation of germination. In this study, we isolated and analyzed the proteome of the *B. subtilis* spore IM, in parallel with the membrane of vegetative cells.

Methods: The spores were chemically decoated and enzymatically deprived of the cortex. Subsequently the core was mechanically disrupted and the IM fraction was collected by differential centrifugation. Tryptic peptides, acquired from in-gel digestion, were analyzed using Ion trap LC-MS/MS, which led to identification of the spore IM proteins. Bioinformatics tools were used to predict the membrane proteins among the identifications.

Results: From the *B. subtilis* spore IM fraction 929 proteins were identified in at least two of the three replicates, out of the which 334 proteins were assigned as candidate membrane

proteins. The fraction we obtained contained 6 out of 9 germinant receptor proteins corroborating its enrichment for the spore IM. Compared to the proteins identified from the vegetative cell membrane fraction, there was a large portion of proteins, general or membrane predicted, overlapped in both cell types as well as unique to either.

Conclusions: We have adapted the spore IM isolation protocol to proteomics studies.

A number of newly identified proteins in the IM containing fraction provide starting points to gain new insights into the IM compositions and functions, in particular into the spore germination machinery.

P052

Microbial-plant interactions under differential nitrogen loads

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Due to intensive agricultural fertilization, Dutch wetlands have become impacted by increased nitrogen loads. The effect of eutrophication on plant-microbe interactions remains unclear. In order to characterize the diverse microbial assemblage within the rhizosphere, in particular those microbes involved in the nitrogen cycling, we investigated how an increased nitrogen load influences the microbial community associated with *Juncus acutiflorus* compared to bulk soil. A three month experiment was performed in which nutrient, elemental and greenhouse gas emission data was collected. In addition, samples were taken for metagenomics analysis. The results from this study will be combined to understand the influence of increased nitrogen loads on microbial community structure and function associated with *Juncus acutiflorus*.

P053

Microbiology of extremely acidic terrestrial volcanic ecosystems

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The word 'volcano' originates from the little island of Vulcano in the Mediterranean Sea off Sicily. Vulcano was believed to be the chimney of the forge of Vulcan – the blacksmith of the Roman gods. Terrestrial mud volcanos are extreme environments with pH values below even 1, and temperatures up to 70 °C. Despite the hostile conditions, mud volcanos harbour very unique microbial communities involved in the cycling of elements. As such the microbial collective of volcanic ecosystems has a major impact on geochemical cycles and represent 'hotspots' of greenhouse gas emissions. However, especially the unique microbial communities shaped by high temperatures and extreme acidic conditions with their exceptional properties have hardly been explored and not been exploited to date. The aim of our research is to obtain a fundamental understanding of the microbial ecology of extremely acid terrestrial mud volcanos with special emphasis on the elemental cycles of sulfur, methane and nitrogen. After identification and isolation, the microbial key players will be investigated to unravel the molecular mechanisms by which they adapt to extreme (thermo)acidophilic conditions.

Microbial communities in extreme environments are characterized by low biodiversity and as a consequence serve as good models to study linkages between genomic potential and environmental parameters. Metagenome studies have shown that most of the microorganisms in extreme environments are only distantly related to cultivated bacteria. The identity, diversity, ecophysiological properties and interactions of the microorganisms present in these volcanic ecosystems are in urgent need of investigation.

Initial experiments started with isolating DNA from the Solfatara mudpot (Naples, Italy). Because of the composition (high in positive ions) of the soil, different cleaning steps and methods were tried to extract the DNA from the sample. DNA was sequenced using IonTorrent technology and the reads were assembled into contigs using CLC-Bio. Binning of the contigs based on GC-content and coverage resulted in seven different bins. Two showed high identity to *Thermoplasma acidophilum* and *Acidithiobacillus caldus*, respectively. Blast searches with the contigs from the other bins showed much lower identity to *Parvarchaeum*, *Acidiplasma*, *Acidithiobacillus*, *Thermoplasma* species. From these data it became clear that the meta-omics approach has an enormous potential for understanding the regulation of microbial activities in response to environmental conditions. Furthermore it is the first step to explore and exploit the genetic potential of these extreme ecosystems.

The metagenome information will guide state-of the-art enrichment techniques using chemostat and sequencing batch cultivation with inocula from different geothermal sites. This approach was already successful in the isolation of both mesophilic and thermophilic acid-loving methanotrophs and CS_2-hydrolyzing bacteria and archaea.

P054

Decreasing the turnaround and hands-on time of laboratory analysis for the *Escherichia coli* surveillance by implementing the LEAN philosophy

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Introduction: LEAN was introduced in the 1950s and is a philosophy that focusses on creating the maximum added value for the customer with a minimum of waste in the production process. The LEAN philosophy was invented by Toyota and has since been applied in many different sectors worldwide, including healthcare facilities. In 2013, LEAN was introduced in the National Institute for Public Health and the Environment. Here, we describe the successful implementation of the LEAN philosophy in our *Escherichia coli* surveillance leading to a decrease in turnaround time (TAT) of our laboratory analysis and in the hands-on time by laboratory staff.

Methods: This project consisted of 5 phases, namely define, measure, analyse, improve and control. In the first phase, the scope, voice of the customer and the problem(s) in the main process were defined. In the measure phase, the current process was mapped by means of a value stream map (VSM) and the current process performance and bottlenecks were determined based on the VSM. Subsequently, the movement of personnel was monitored to achieve the optimal laboratory setting. The most important causes contributing to the problems were identified during the analyse phase and solutions were discussed in a brainstorm session with the project team. In the improve phase, the solutions were prioritized by an ease/impact matrix and tested in a pilot. Finally, the pilot was evaluated and the new process performance was determined in the control phase.

Results: The TAT of the laboratory analysis and the hands-on time for the *E. coli* surveillance were identified as main problems during the define phase. Throughout the VSM and analyze phase, it became clear that the execution of the PCR once a week (TAT = possible 7 days), the outsourcing of the PCR fragment-analysis (TAT = 2 days) and the preparation of the lysates and PCR (hands-on time) were the main bottlenecks for an optimal process performance. Other bottlenecks were the lack of digitalization within the *E. coli* surveillance and the sub-optimal movement of personnel in the laboratory. After brainstorm and analysis of the ease/impact matrix, it was decided that to decrease the TAT, the PCR should be done twice a week and fragment-analysis should be performed in-house. To decrease the hands-on time, the preparation of the lysates was optimized by adapting a new protocol. Furthermore, ready-made mixes that only required the adding of lysate replaced the previous preparation of the PCR mix based on single components. Finally, the entire *E. coli* surveillance was digitalized and the laboratory setting was optimized, to strive for a 'paperless' laboratory. After a pilot of 6 weeks, the average TAT was decreased by seven days, while the hands-on time decreased from an average of 90 minutes to 45 minutes.

Conclusion: In this project, the added value for the customer was a decrease in TAT of seven days, while the hands-on time for analysis could be halved. Furthermore, increased awareness of laboratory staff for process efficiency and reduction of 'waste' and subsequent adaptation of attitude and behavior was noted.

P055

Sequencing of HPV 16 allows discrimination between same-type reinfection events and persisting infections

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Objectives: Vaccine efficacy has been shown against persisting HPV16 infections. However, incident HPV16 infections can still occur in vaccinated persons. Repeat incident infections with the same HPV type could incorrectly be considered persistent infections. Here we aim to investigate if discriminating between persisting HPV16 infections and same type re-infection events is possible by HPV16 whole genome sequencing.

Methods: Vaginal self-samples were collected from women (16-29y) participating in the *Chlamydia trachomatis* Screening Implementation program in the Netherlands. Collection occurred annually in up to four rounds. HPV-DNA was detected using the SPF_{10}-PCR DEIA $LiPA_{25}$ system. HPV16 persistent infections were selected and subjected to long-template PCR generating two 4.6kb fragments covering the full HPV16 genome. Sanger sequencing of PCR fragments was performed.

Results: We have sequenced the genome of 21 HPV16 infections persisting in at least two rounds. Identical genomes were found across all rounds for 20 persisting infections. One of 21 persisting HPV16 infections was identified as a same type re-infection event, since the sample collected in the first round differed from the second sample by 143 nucleotides. Moreover, the first and second round samples clustered with different HPV16 sublineages (A1 and D3 respectively).

Conclusions: Within persisting HPV16 infections sequences are highly conserved. Same type reinfection events occur and have led to false classification of a persistent infection. Discrimination between incident and persistent infections is possible via whole genome sequencing.

P056

(Im)possibilities of identification of highly pathogenic bacteria using fatty acid analysis

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Introduction: *Bacillus anthracis, Burkholderia mallei, B. pseudomallei, Francisella tularensis, Yersinia pestis,* and *Brucella* species are highly pathogenic and classified as potential agents for bioterrorism. Infections with those bacteria occur occasionally in The Netherlands, mainly through import, as they are still endemic in different parts of the world. To react appropriately to infection and prevent unnecessary actions and panic, fast and specific identification of these bacteria is necessary. The specificity of identification tests is difficult for two reasons. Many of the highly pathogenic bacteria have closely related, but less pathogenic, counterparts, like *B. cereus, B. thailandensis* or *Y. pseudotuberculosis*. In addition, pathogenicity is based on (sub)species identification, like *F. tularensis* and *Brucella*. In this study, we have validated the possibilities to use the Microbial Identification System (MIS; MIDI, Newark, U.S.A.) as a specific, fast and cheap method for the identification of highly pathogenic bacteria. This method is less vulnerable to naturally or deliberately induced differences in the target DNA than e.g. PCR, because the whole cellular fatty acid content is assessed.

Methods: With the MIS, the fatty acid composition was determined of culture collections- and wild-type strains. The identity of the reference strains was confirmed by sequence determination of the 16S-rRNA-gene. The identity of the wild-type strains was verified with conventional biochemical techniques, specific real-time PCRs and 16S rDNA-sequencing. Genetically and phenotypically related strains, including possible false positive results based on literature and our experience, were included to determine the specificity of the method. With the fatty acid composition, the identity of the bacteria was determined using the following Sherlock-libraries (MIDI, Newark, U.S.A.): BA-BTR3.10, CLIN6.10, and, if appropriate, the TSBA6.10. The CLIN6.10 and the TSBA6.10 libraries do not contain all bacteria determined in this study. In addition, the profiles were manually searched for specific biomarkers. MANOVA-analysis in BioNumerics (Applied Maths, Sint-Martens-Latem, Belgium) was used to focus on the differences between closely related bacteria.

Results: *B.anthracis* could be differentiated from *B. cereus* using the BA-BTR3.10 library and with MANOVA. Identification of *Brucella, B. mallei* and *B. pseudomallei* demonstrated a lower accuracy using the BA-BTR3.10 library with respectively 90%, 0% and 83% being correctly identified as the target organism. However, analysing the data with MANOVA improved this to 100% which is higher than found by MALDI-TOF MS (unpublished data). Applying MANOVA made distinguishing *F. tularensis* subspecies and differentiating *F. tularensis* from other *Francisella* species except *F. hispaniensis* possible. However, it was not possible to distinguish *Y. pestis* from *Y. pseudotuberculosis* neither with one of the libraries nor with MANOVA. The *Brucella* species could only be determined correctly in 64% (23/36) of the strains. For none of the bacteria biomarkers could be found to accurately distinguish them from their less pathogenic counterparts.

Conclusion: Analysing the fatty acid content of highly pathogenic bacteria using the MIS is a specific method to differentiate *B. anthracis* from the rest of the *B. cereus*-complex using the BA-BTR3.10 library. *Brucella* species, *B. mallei* , *B. pseudomallei*, and *F. tularensis* subspecies can be identified using MIS with subsequent MANOVA-analysis.

P057

A Dutch multi-center study reveals a high prevalence of azithromycin resistant *Mycoplasma genitalium*

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Introduction: *Mycoplasma genitalium* infection is a sexually transmitted disease (STD) and an important cause of non-gonococcal urethritis (NGU), cervicitis and related upper genital tract infections. Although treatment with an extended cure of the macrolide antibiotic azithromycin (500 mg on day 1, followed by 250 mg daily on day 2-5) is recommended, often a single-dose therapy with 1 g azithromycin is used. Studies show that single-dose therapy increases the risk of development of resistance, as is evident from macrolide resistance in *M. genitalium* being high in countries where 1 g azithromycin is used (up to 40% in Denmark and the UK) while still being low in countries where *Chlamydia trachomatis* infections are primarily treated with doxycycline (10-15% in France and Sweden). Unfortunately, resistance data are often based on isolates obtained from STD clinics and systematically collected data on the primary resistance of *M. genitalium* resistance in the open population are still scarce.

In this study we analyzed the current prevalence of macrolide resistance-associated mutations in primary *M. genitalium* isolates from Dutch patients.

Methods: During 2014-2015, urogenital and urine samples that were submitted to four different laboratories in the Netherlands (CWZ Nijmegen, Maasstad Rotterdam, PAMM Veldhoven en Rijnstate Velp) for the detection of *M. genitalium* with nucleic acid amplification techniques, were collected. *M. genitalium* positive samples were used for DNA sequencing of region V of the 23S rRNA gene to analyze the presence of macrolide resistance-associated

mutations. Only the first *M. genitalium* positive sample from a patient was included and only the first approximately 75 sequentially collected positive isolates from each laboratory were included. Samples from patients from STD clinics were excluded as were those from individuals who had a history of *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae* infection in the previous three months.

Results: In total, 353 *M. genitalium* positive samples were included. For 257/353 (72,8%) samples, the relevant portion of the 23S rRNA gene could successfully be sequenced, leaving 257 isolates for inclusion on this study. Overall, 58/257 (22,6%) samples harboured a macrolide resistance-associated mutation in the 23S rRNA gene.The most frequently observed macrolide resistance-associated mutation was A2059G (n = 27/58; 46,6%), followed by A2058G (n = 20/58; 34,5%), A2058T (n = 9/58; 15,5%) and A2058C (n = 2/58; 3,4%).The prevalence of macrolide resistance in the isolates of the four individual laboratories were 14,3 % (CWZ), 32,8% (Maasstad), 24,5% (PAMM) and 21,7% (Rijnstate).

Conclusion: The high prevalence for macrolide resistance in *M. genitalium* necessitates adequate information regarding resistance in relevant clinical isolates prior to treatment. Therefore, rapid detection of resistance mechanisms might help to better predict the adequacy of empirically started azithromycin therapy.

P058

The occurrence of the colistin resistance gene mcr-1 in *Salmonella enterica* and *E. coli* isolates from Dutch livestock

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Introduction: Colistin is one of the last treatment options against human infections caused by resistant bacteria. Hence, the recent finding of a transferable colistin resistance gene called mcr-1 in E. coli isolates in pigs and chickens in China is considered a major concern to human health. In response, we retrospectively screened our bacterial strain collection with PCR for the presence of mcr-1. This study is initiated to gather more information on the occurrence and spread of this novel gene in livestock and meat products in the Netherlands.

Methods: Colistin resistant Salmonella and E. coli isolates (MIC: ≥ 4 mg/L) were selected from a large database with MIC-results determined in the Dutch national surveillance program on antibiotic resistance in animals. Salmonella isolates were collected from 2010 – 2015 in various sources (mainly human, animals and food). E. coli isolates from fecal samples collected from 2010 – 2015 obtained in broilers, laying hens, turkeys, slaughter pigs, veal calves and dairy cattle. E. coli isolates from meat were obtained in chicken meat (n = 1211), turkey meat (n = 114), pork (n = 607), veal (n = 54), beef (n = 725), lamb (n = 56) in the period 2012 - 2014. These samples included both imported products and fresh meat from Dutch retail. Isolates were screened by PCR for the presence of mcr-1 according to the protocol of the European Reference Laboratory on Antimicrobial Resistance (EURL-AR).

Results: A selection of 120 colistin resistant Salmonella (out of 12.100 strains, 1249 from poultry sources) and 67 colistin resistant E. coli (out of 2767 strains) were tested with PCR. As a result thirteen Salmonella (= 0.1% of the total) were found positive for mcr-1 consisting of eleven Paratyphi B variant Java collected from broilers or broiler meat, one Schwarzengrund and one Anatum isolate both obtained from turkey meat. The earliest detected Salmonella isolate with mcr-1 was a Paratyphi B variant Java isolate collected in 2010. The mcr-1 gene was not detected in human Salmonella isolates. The screening of E. coli from fecal samples from livestock is pending. The screening of E. coli from meat resulted in 47 mcr-1 positive isolates (= 1.7% of the total) almost all obtained from chicken meat or turkey meat either detected in imported frozen meat or in fresh meat from Dutch retail. One mcr-1-positive E. coli isolate was detected in veal sampled in 2013 from Dutch retail. The earliest detected mcr-1-positive E. coli was collected in 2012.

Conclusions:

1. The colistine resistance gene mcr-1 was in low frequency (3.5%) detected in E. coli isolated from imported and domestically produced fresh poultry meat, and in veal collected at Dutch retail.
2. The gene was incidentally (1%) identified in Salmonella from poultry sources.
3. The mcr-1 gene was not detected in isolates from slaughter pigs and humans.
4. The earliest mcr-1-positive bacterial isolate was collected in 2010.
5. Prospective screening with selective isolation procedures is needed to reveal the prevalence of this gene in bacterial populations from different sources.

P059

Molecular screening for ESBL and Carbapenemase during a prevalence study in two Dutch Hospitals

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Background: Globally, the prevalence of extended spectrum β-lactamase (ESBL) and carbapenemase producing *Enterobacteriaceae* (CPE) has been rising. Traditionally, selective culture methods are used to screen for colonization of patients. However, faster molecular testing methods are now available for this purpose. This study describes the comparison of selective culture and molecular testing for ESBL colonization using the BD MAX system. Furthermore, screening for CPE using qPCR is described.

Material/Methods: During a period of one month, in two Dutch hospitals, two rectal swabs (Eswab, Copan) were taken from 479 patients as part of a crossectional prevalence survey. The swabs were pooled and ESBL and CPE qPCR was performed using the Check-Direct ESBL screen and Check-Direct CPE screen qPCR kits on the BD MAX system. These kits can detect the most common ESBL and CPE resistance genes (CTX-M1, CTX-M2, CTX-M9, SHV, KPC, VIM, OXA-48 like and NDM, respectively). Also, all samples were cultured for ESBL. For CPE, only CPE qPCR positives samples were cultured. ESBL and CPE cultures were performed using a selective broth followed by a subculture on a selective agar. Bacteria suspected for ESBL or CPE according to elevated MIC for Cefotaxim, Ceftazidime, Meropenem or Imipinem, were confirmed for ESBL or CPE by qPCR. Sensitivity, specificity, positive, and negative predictive value and Cohen's Kappa coefficient were calculated for qPCR. Selective culture is used as a the gold standard for the analyses.

Results: Out of 479 samples, 3% was inhibited in either the ESBL (n = 6) or CPE (n = 9) qPCR. These samples were excluded from the analyses. Of the remaining 473 ESBL samples, 36 were found positive by culture, and 42 by qPCR .The additional ESBL positives found by qPCR had Cq values between 23 and 43. All discrepant samples were retested in the Check-Direct ESBL screen assay. Results after discrepant analysis showed a sensitivity, specificity, positive and negative predictive value of 97.2%, 98.4%, 83.3% and 99.8%, respectively. The Kappa coefficient was calculated and resulted in a inter-rater agreement of 0.9.
A total of eight CPE positives were found in qPCR with Cq values between 39 and 49. None of these CPE's could be cultured.

Conclusions: Our study showed a very good strength of agreement between qPCR and culture for ESBL screening (Kappa coefficient of 0.9). Furthermore, molecular screening is less laborious then culture, and it has a high NPV of 99.8%. This makes the Check-Direct ESBL screen kit a promising pre-screening method for ESBL directly from clinical material. The Check-direct CPE screen kit needs more investigation.

P060

Enrichment model based investigation of microbial cooperation among carbon, sulfur and nitrogen cycles

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Microbial processes such as, denitrification, dissimilatory nitrate reduction to ammonium (DNRA), anaerobic ammonium oxidation (anammox) and nitrite- as well as nitrate-dependent anaerobic methane oxidation (AOM), all require nitrate or nitrite as electron acceptors. These nitrogen-transforming microorganisms compete with each other and are co- dependent for the supply of their substrates. Coupling of partial denitrification to anammox is one example of such microbial interaction. The cooperation among sulfide-driven autotrophic denitrifiers and anammox was successfully established in a laboratory scale bioreactor setting. The co-culture of anammox and *Sulfurimonas denitrificans* DSM 1251 was supplied with only ammonium and nitrate, under marine incubation conditions with continuous inflow of non-toxic sulfide concentrations. Assessment of community composition through several molecular methods (PCR, fluorescence *in situ* hybridization (FISH) and metagenome sequencing) revealed proliferation of anammox and denitrifiers. Furthermore, stable isotope-labeling experiments showed that denitrification converted nitrate and sulfide in the expected stoichiometry, thereby supplying anammox with sufficient nitrite to stay active despite an influx of sulfide. To further expand our understanding of microbial interactions, the aforementioned reactor was slowly adapted to freshwater growth conditions by reducing salt concentrations in the medium. We hypothesize that subsequent methane introduction along with addition of anaerobic methanotrophic biomass will lead to competition for the substrates nitrate and nitrite. Nitrite would be available either through autotrophic denitrification or nitrate-dependent methane oxidation. Similarly, anammox and nitrite-dependent anaerobic methanotrophs would compete for nitrite. The feasibility of these microbial interactions among nitrogen, sulfur and carbon cycle organisms will be investigated through application of an array of molecular as well as biochemical tools.

P061

***Methylacidiphilum fumariolicum* SolV: methanotroph and 'knallgas' bacterium**

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Methanotrophs form an important sink for the greenhouse gas methane and they play a key role in keeping the

atmospheric methane concentration in. The most studied group comprises the aerobic methanotrophs that phylogenetically belong to the Gammaproteobacteria (type I) and the Alphaproteobacteria (type II). However, the methanotrophic world was extended once three research groups independently described novel thermoacidophilic methanotrophs isolated from geothermal areas in Italy, New Zealand, and Russia which represented a distinct phylogenetic lineage within the Verrucomicrobia. They belong to a single genus for which the name *Methylacidiphilum* was proposed. The discovery of a verrucomicrobial methanotroph was exciting, since for the first time the widely distributed Verrucomicrobia phylum, from which most members remain uncultivated, was coupled to a geochemical cycle.

It has long been assumed that methanotrophs are very strict in their diet, consuming only methane or methanol and occasionally other C1 compounds, despite the fact that most of the time a range of simple potential multicarbon substrates is available in their environments. In addition, in volcanic ecosystems another source of reducing equivalents is available as a potential energy source for methanotrophs, i.e. molecular hydrogen.

Methylacidiphilum fumariolicum SolV was shown to use the Calvin-Benson-Bassham (CBB) cycle for carbon fixation, and is capable of fixing nitrogen gas. The full genome of strain SolV revealed the presence of two hydrogenases. The presence of the CBB-cycle, together with the uptake hydrogenase suggested us to investigate the possibility to grow strain SolV as a real 'knallgas' bacterium without addition of methane. In this study, we showed that under oxygen limitation another oxygen sensitive hydrogenase is expressed in addition to the constitutively expressed oxygen insensitive one. Based on dry weight and organic carbon measurements a value of 3.3 g DW.Mol H_2-^{1} was obtained. We also reported apparent hydrogen Ks values of 0.6 μM and 1.1 μM at dO_2 value of 0.8 % and 0.2 %, respectively. Using phylogenetic analysis, we showed that strain SolV, *Methylacidiphilum infernorum* V4 and *Methylacidiphilum kamchatkense* Kam 1 contain a [NiFe]-hydrogenase (Group 1d) encoded by the genes *hup*S (small subunit) and *hup*L (large subunit). In addition, we identified a second [NiFe]-hydrogenase (Group 1h/5) encoded by the genes *hya*A (small subunit) and *hya*B (large subunit), only present in our thermophilic strain SolV and its close relative strain Kam 1. We also showed that the sensitive hydrogenases were up-regulated under oxygen limited conditions compared to the cells grown at the highest growth rate (μmax). These findings show that *Methylacidipohilum fumariolicum* SolV is a methanotroph and 'knallgas' bacterium possessing a mixotrophic growth strategy.

P062

Genotyping of hepatitis B and C virus and detection of the hepatitis C virus Q80K polymorphism

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Introduction: Hepatitis refers to an inflammatory condition of the liver, which is usually caused by viruses. Viral factors, such as viral load, genotype, and certain viral mutations, are known to affect disease progression.

In this study we describe the optimization and validation of genotyping assays for hepatitis B virus (HBV) and hepatitis C virus (HCV), and an assay to detect the HCV Q80K polymorphism, associated with a reduced response to NS3/4A protease inhibitors, such as Simeprevir.

Method: After lysing of the pasma for 30 minutes at 37°C, DNA and/or RNA were isolated using EasyMag extraction system (BioMérieux). RNA was translated into cDNA with random hexamers and Superscript II. The primers for amplification and subsequent sequencing were derived from published assays, targeting the S-gene[1] (HBV), NS5B-region[2] (HCV) and the NS3/4A-region[3] (Q80K).

PCR was performed using a 96-well cycler (Verity, Applied Biosystems). For sequence analysis the ABI 3130 GA system was used (Applied Biosystems).

The minimal viral load for genotype assignment was determined using serial dilutions of viruses belonging to different genotypes. A blinded test panel of 28 samples (14 clinical samples, 8 QCMD-samples and 6 NIBSC samples) was analysed for technical and clinical validation of the assays.

Results: PCRs were optimized by varying annealing temperatures. The genotypes were assigned to the obtained sequences using NCBI blast, NCBI genotyping tool and the geno2pheno from the Max-Planck-Institut für Informatik. HBV genotypes A2, B3, B4, C1, C2, D1, D2 and D3 were readily assigned and corresponded to the externally assigned genotypes, as did HCV genotypes 1a, 1b, 2b, 3a, 4a, 5a, 6a en 6e.

One sample did not yield a PCR product, this sample was externally assessed as HBV genotype C. An additional oligo-nucleotide was designed with which the HBV genotype C was detected. One sample was assigned HCV 4h by NCBI blast, while the other two programs assigned HCV 4k. External evaluation of this sample yielded genotype 4h. This "apparent" discrepancy might be due to the absence of 4h in Smith et al.[4], on which the Geno2pheno database is based. Of another sample, NCBI yielded 2j, NCBI genotyping tool and Geno2Pheno yielded 2, while this sample was externally assessed as 2a/2c. Genotypes were obtained for all samples with viral load ≥ 1000 I.U./ml. No double infections were evident.

HCV samples of genotype 1a (n = 1) and 1b (n = 2) were tested for the presence of the Q80K polymorphism. The two 1a samples tested negative for the Q80K polymorphism, while the 1b sample tested Q80K positive.

Conclusion: The HBV and HCV genotyping assays and the assay for the detection of the HCV Q80K polymorphism are suitable for genotyping of plasma samples with viral loads ≥ 1.000 I.U./ml. The assays were successfully implemented in the Jeroen Bosch Hospital.

References

1. Pas SD, Tran N, de Man RA, et al. Comparison of reverse hybridization, microarray, and sequence analysis for genotyping hepatitis B virus. J Clin Microbiol. 2008;46:1268-73.
2. Murphy DG, Willems B, Deschênes M, et al. Use of sequence analysis of the NS5B region for routine genotyping of hepatitis C virus with reference to C/E1 and 5' untranslated region sequences. J Clin Microbiol. 2007;45:1102-12.
3. Beloukas, A, King S, Childs K, et al. Detection of the NS3 Q80K polymorphism by Sanger and deep sequencing in hepatitis C virus genotype 1a strains in the UK. Clin Microbiol Infect. 2015;21:1033-9.
4. Smith DB, Bukh J, Kuiken C, et al, Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. Hepatology. 2014;59:318-27.

P063

Prevalence of integrase Sa3 containing prophages in community acquired and livestock associated methicillin resistant *Staphylococcus aureus*

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Introduction: Prophages are integrated viral genomes that can contribute important biological properties to their bacterial hosts. Prophages appearing in *S. aureus* can be classified according to variety among integrase genes. Previous studies have shown that prophages of the Sa3 integrase group (Sa3int) carry human innate immunomodulatory genes that play crucial roles in human niche adaptation.

Most livestock associated MRSA strains (LA-MRSA) belong to the ST398 lineage. While LA-MRSA is known to be limited in its transmissibility between humans, the presence of aforementioned virulence genes could increase the chance of human transmission.

Recently, the emergence of an animal-independent ST398 clone has been documented. Whole genome sequencing revealed the presence of Sa3int in this clone (Uhlemann A. et al. MBio. 2012 Vol. 3(2): e00027-12). For regular diagnostics it might be useful to determine the presence of Sa3int prophages in LA-MRSA strains because of the supposedly higher risk of transmission.

In this study we developed a real-time PCR for the detection of Sa3int prophages in *S. aureus* strains. Using this assay we determined the prevalence of this prophage in ST398 MRSA strains and non-ST398 strains found in the area of 's Hertogenbosch.

Methods: The primers and probe for detection of prophage Sa3int were designed based on previously published sequences (Ziebandt A. et al. Proteomics 2010, 10, 1634-1644).

For DNA isolation one colony from an overnight culture was suspended in TE-buffer. This suspension was heated to 100°C for 15 minutes. After centrifugation, 10µl of the supernatant was used for amplification using the ABI 7500 FAST (Applied Biosystems). Linearity and sensitivity of the assay were determined using dilution series of extracted DNA.

In total, a collection of 139 MRSA strains (91 ST398 and 48 non-ST398 strains) were tested for the presence of prophage Sa3int.

Results: Positive or negative results obtained from real-time PCR were consistent with conventional PCR test (Ziebandt A. et al. Proteomics 2010, 10, 1634-1644) results. The reproducibility and linearity of the assay were according to expectation with a variance coefficient below 5%. The detection limit was approximately 32 bacterial cells per reaction.

In 5 out of 91 ST398-positieve strains Sa3int was detected (5,49%). Based on the information available for these 5 patients there was no indication of transmission of these strains. The prevalence of Sa3int in the non-ST398 strains was significantly higher, since 41 out of 48 strains tested positive for Sa3int (85,42%).

Conclusions: The real-time PCR assay can be used to determine the presence of Sa3int in *S. aureus* strains.

The group of non-ST398 MRSA strains show a considerably higher prevalence of the Sa3int-gene compared to LA-MRSA strains. The 5 LA-MRSA strains that tested positive for Sa3int, however, were most likely not involved in hospital transmission events. To further evaluate the additional value of the test we now routinely analyse all new clinical MRSA isolates for Sa3int carriage.

P064

Human papillomavirus (HPV) prevalence among high-risk youngsters in the Netherlands

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Introduction: Human papillomavirus (HPV) infections are the most common sexually transmitted infections (STIs). Over hundred different HPV types exist, which can be divided into two groups: low-risk and high-risk. Persistent infection with high-risk HPV-types can lead to lesions in the anogenital region and after many years to anogenital cancers in males and females. In 2009 vaccination against

the high-risk HPV-types (HPV-16 and -18) causing most of the cervical cancers was introduced in the Netherlands in young girls. To monitor the effects of vaccination on HPV dynamics in a highly sexual active population we started a biannual study in STI clinics in the Netherlands in 2009, the PApillomavirus Surveillance among STI clinic YOungsters in the Netherlands (PASSYON) study. Here we present the results of the HPV prevalence in STI clinic visitors and evaluate the methodology used.

Methods: Anogenital swabs were collected in 2009, 2011, 2013 and 2015 from attendees (m/f age 16 -24y) of 12 STI clinics across the Netherlands. Type specific HPV-DNA in swabs was determined in 4-7 different medical microbiology laboratories including the RIVM using uniform PCR-based protocol SPF10/LIPA version1 (DDL) with high analytical sensitivity. The method is able to detect 25 different HPV types. A standardized protocol for spiking (PhHV-1), extraction (MagnaPure 32/96, Roche), amplification (SPF10), detection (DEIA) and genotyping (LiPA) was used in all laboratories. Trained technicians performed the assay uniformly. To test for accuracy among the different laboratories 5% of both the HPV-positive and -negative DNA extracts were retested at the RIVM.

Results: The total number of anogenital swabs collected was n = 1684 in 2009, n = 2237 in 2011, n = 2300 in 2013 and n = 2449 in 2015. The percentage of HPV positive and typable samples was comparable in each test year (2009: 65%, 2011: 61%, 2013: 61%, 2015: 64% (results from 2015 are preliminary). However, a few changes in predominance of some HPV types over the years was seen. The prevalence of HPV-16 shows a declining trend from 2013 onwards. For HPV-18 no decline was seen. Statistical analyses for 2015 were not yet available. With retesting inter-laboratory reproducibility of HPV-detection method in samples across different laboratories was assessed. For the rounds 2009, 2011 and 2013 a concordance of > 87% was found.

Conclusion: The overall percentage of HPV-DNA positivity in young STI-clinic attendees was around 60% in the past six years. A statistically significant decline in prevalence of the vaccine HPV type HPV-16 was seen since 2013. The observed trend could be explained by (in)direct effects of the introduction of vaccination against HPV-16 and -18. Further analyses of the 2015 data are required in order to determine if the decline in prevalence of HPV-16 will be continued and if a decline for HPV-18 prevalence will be seen. In this multicenter surveillance study, inter-laboratory HPV detection using standardized HPV detection methods was shown to lead to reproducible results for the rounds 2009, 2011 and 2013.

P065

Effective diagnosis of lower respiratory tract infections using a combination of 16S rDNA based next-generation sequencing and species specific real-time qPCRs

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Introduction: Next-Generation Sequencing (NGS) is a quickly growing field, and is commonly used to obtain insight into the full spectrum of bacteria present in microbial communities by sequencing the gene encoding the 16S ribosomal RNA (rRNA). Combining NGS with a panel of real-time quantitative PCRs (qPCRs) enables, besides characterization of complex microbial communities at genus level, also identification and quantification of a number of specific bacterial species by targeting unique DNA sequences. Our aim was to evaluate the use of a combination of NGS and real-time qPCRs for the diagnosis of lower respiratory infections (LRTIs), by comparison of these novel methods to the current standard practice, which is based on culture of bacteria from sputum samples and species identification using MALDI-TOF technology.

Methods: Clinical sputum samples and the corresponding primary inoculated agar plates of 62 patients with respiratory symptoms were collected. Whole sputum samples were subjected to NGS, by amplifying the V3-V4 regions of the 16S rRNA gene, and subjected to a panel of real-time quantitative PCRs (qPCRs) targeting Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa. Results were considered concordant when the same micro-organism was detected by the routine laboratory and NGS/real-time qPCR. Samples with discrepant results were further investigated performing NGS/real-time qPCR on the total bacteria loads collected from the primary inoculated agar plates.

Results: With NGS, a broad spectrum of genera was detected, including Haemophilus, Moraxella, Streptococcus, Staphylococcus and Pseudomonas. With the combination of NGS and real-time qPCR, Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa were identified in 15 (24%), 8 (13%), 13 (21%), 1 (2%) and 2 (3%) sputum samples, while this was the case in 10 (16%), 5 (8%), 3 (5%), 3 (5%) and 2 (3%) sputum samples by the routine laboratory, respectively. The micro-organisms detected by the routine laboratory were all present at a relatively high concentration as determined by real-time qPCR. Discrepancy analysis of 17 discordant

cases confirmed the presence of a specific micro-organism in 12 sputum samples, which was detected with NGS/real-time qPCR. For two sputum samples, the presence of Streptococcus pneumonia detected by the routine laboratory could not be confirmed. The other three sputum samples were negative with NGS and contained a relatively low concentration of Staphylococcus aureus or Moraxella catarrhalis, while the micro-organism was present in a relatively high concentration in the total bacteria loads collected from the corresponding agar plates, indicating selection by the growth-media.

Conclusion: The combination of NGS and real-time qPCR is a sensitive and specific approach that can be effective for diagnosis of LRTIs.

P066

Spread of multiple-antibiotic resistant *Clostridium difficile* type 078 between animals and humans at global scale

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Background: *Clostridium difficile* recently emerged globally as a significant cause of infectious antibiotic-associated diarrhea and a leading cause of healthcare infections. *Clostridium difficile* PCR ribotype 078 is commonly found in both humans and farm animals raising the possibility of zoonotic transmission. We performed a comprehensive whole genome phylogenetic and comparative analysis of a global collection of *C. difficile* 078 strains from diverse sources.

Material & Methods: We sequenced the genomes of 263 *C. difficile* type 078 isolates that were globally collected (North-America, Europe, Australia and Asia) between 1992 and 2012 from diverse sources (human, animal, environment and food). The sequence reads were bioinformatically mapped against strain M120, after which we extracted the variation (SNPs). Bayesian analysis (BEAST and BAPS) was done to evaluate the population structure and to estimate a time scaled phylogeny. The core and accessory genomes of animal and human type 078strains were compared using Roary, a tool that rapidly builds large-scale pan genomes.

Results: Our results show that *C. difficile* 078 has a clonal population structure and spread globally through repeated long-range transmissions between North-America, Europe and Australia beginning in the 1940s at the start of the antibiotic era. Interestingly, human and animal strains do not form distinct clades in the global phylogeny, as is expected for host-adapted strains, but instead they are intermixed indicating frequent spread between host populations. Interspecies transmission was confirmed by several cases of clonal spread (<*ie.* zero to two SNP differences). In line with the core genome, the accessory genome of human and animal strains are indistinguishable, including a wide variety of antibiotic resistance genes. Identical tetracycline resistance genes in human and animals type 078 genomes are present on the mobile element Tn916 transposon that possesses the potential for horizontal transfer.

Conclusions: Antibiotic use is associated with the global spread of multiple antibiotic resistant *C. difficile* 078 between humans and farm animals, and potentially the widespread dissemination of antibiotic resistance genes. The whole genomes (core and accessory) from animal and human samples are highly similar supporting the idea of frequent transmission between both populations.

P067

Adaptation of the proteome of awakening spores of *Bacillus subtilis*

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Introduction: In response to nutrient limitation Gram positive organisms like *Bacillus subtilis* form dormant spores. These cellular entities are survival capsules, resistant to chemical and environmental assaults. They pose challenges to the food and medical sectors. Upon contact with germinants spores return to vegetative life through a process called 'germination and outgrowth'. The vegetative cells may cause food spoilage and food borne diseases. The aim of the study is to obtain detailed information on the adaptation of the proteome of germinating *Bacillus subtilis* spores.

Method: Using a quantitative proteomics approach a study of the time resolved break down of spore proteins during early stages of germination was initiated. Time-resolved monitoring of ^{14}N incorporation in newly synthesised proteins during germination of ^{15}N metabolically labelled spores in ^{14}N medium is done using Fourier transform ion cyclotron resonance mass spectrometry.

Result: Amino acids were recycled in the germination activation phase. The recycling was mainly from small acid soluble proteins (SASP). Amino acid synthesis started before 60 min as shown by the onset of the ^{14}N incorporation in the newly synthesised proteins. The early set of proteins synthesized were mainly important to start the transcription of later proteins. New synthesis of proteins seemed to be consistent with the timing of the transition from phase-bright to phase-dark spores indicating that water with the ^{14}N source can enter the spore. After 180 minutes it was shown that all amino acids were ^{14}N synthesised.

Conclusion: Timing of the incorporation of ^{14}N for different proteins reflects the difference in start and end of the protein synthesis. No incorporation of the ^{14}N lysine added to the germination medium indicates no transport of lysine over the inner membrane can occur during the amino acid recycle phase.

P068

Identification of a novel beta-lactamase, Axy_1, involved in resistance towards carbapenem in a clinical isolate of *Achromobacter xylosoxidans*

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Introduction: In 2014, an immunocompromised patient was admitted to the Leiden University Medical Center while suffering from an opportunistic pulmonary *Achromobacter xylosoxidans* infection. The initial isolated and identified strain was susceptible to carbapenems. Treatment with meropenem was initiated but proved unsuccessful and the patient died due to septicaemia with positive bloodcultures containing meropenem resistant *A. xylosoxidans*. Using an in-depth proteomic approach followed by functional assays, we aimed to identify the underlying cause and mechanism of this resistance.
Methods: Various proteomic methods were used to get an in-depth view of the differentially expressed proteins between the meropenem sensitive and resistant clinical isolates. These included gel electrophoresis followed by tryptic digestion and in-solution tryptic digests of whole cells, both followed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Following data base searches for protein identification, semi-quantitative protein analysis was performed using spectral counting. In addition, PCR, heterologous expression in *Escherichia coli* and classical antibiotic resistance assays (E-tests) were performed.
Results: Proteomic analysis revealed high expression of a Class A beta-lactamase, which we called Axy_1, in the meropenem resistant strain which was absent in the susceptible strain. Based on the predicted enzyme class there was no indication that this enzyme would be involved in carbapenem resistance, but the clear difference prompted further investigation. First of all, PCR analysis revealed that *axy_1* was present in both strains and the sequences were identical. Second, heterologous expression of Axy_1 in *Escherichia coli* resulted in higher resistance to meropenem and imipinem. The minimum inhibitory concentration (MIC) for meropenem increased 5-32 fold and the MIC for imipenem resistance increased 16-20 fold. The difference in MIC between the resistant and susceptible clinical *A. xylosoxidans* isolates is even stronger, indicating the involvement of other proteins in the acquired resistance. The proteomic spectral count analysis hints at a down regulation of outer membrane porin of 40 kDa, which could be a potential co-factor.
Conclusions: We have observed the development of resistance towards carbapenems during hospital stay of a patient suffering from an *A. xylosoxidans* infection.
Using proteomic analyses, we have identified Axy_1, a PenP class A beta-lactamase, in the carbapenem resistant *A. xylosoxidans* isolate which was absent in the sensitive isolate. Expression of Axy_1 in *Escherichia coli* resulted in enhanced resistance to imipenem and meropenem.

P069

Performance of an isothermal SIBA®-based *Clostridium difficile* assay in comparison to Real-Time PCR and antigen-based assays

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Objective: To evaluate the Orion Genread® *C. difficile* assay in comparison to the BDMax Cdiff assay and the Techlab C.diff Quik Chek Complete antigen assay.
Methods: Fecal samples were send to the Regional Laboratory for Medical Microbiology and Public Health Kennemerland in the period November 2015 and tested for the presence of *C. difficile* toxin (A/B) for routine diagnostic purposes. The samples were obtained from patients to diagnose antibiotic associated diarrhea, recurrent diarrhea complaints or a suspicion of pseudomembranous colitis.
All samples were tested by the Techlab® C.diff Quik Chek Complete antigen assay (AG), the BDMax™ Cdiff Real-Time PCR assay (BD) and the Orion Genread® *C. difficile* assay based on isothermal Strand Invasion Based Amplification (GR) according the manufacturer's instructions. Discrepant results are retested using all three methods and were send to an external reference lab for confirmation.
Results: Ninetyeight consecutive fecal samples were tested for *C. difficile* toxins (A/B) by the Techlab® C.diff Quik Chek Complete antigen assay and the tcdB-gene of *C. difficile* by the BDMax™ Cdiff assay and the Orion Genread® *C. difficile* assay. Six samples were indeterminant in the AG assay and are confirmed by the BD assay and GR assay. One of them were observed to be positive in both assays. Positivity of the sample was confirmed by PCR and cultivation by an external reference lab (Université Catholique de Louvain, Brussels, Belgium).
Six samples were found to be positive using the BD assay as reference method. The GR assay showed the same 6

samples to be positive and the AG assay 5. As a result of the positivity rates the GR assay was found to be 100% specific according to the BD assay. Using the AG as a reference assay, the GR had a sensitivity of 100% also. Comparing the AG assay to either the GR and the BD assay, the sensitivity was 83,3%.

Conclusion: Based on the present results the Orion Genread® C. difficile assay has a 100% specificity and sensitivity according to the BDMax Cdiff assay. The Techlab C.diff Quik Chek Complete antigen assay showed in 6 percent a non-conclusive result from which one confirmed toxin positive sample which was missed. If an antigen assay like the The Techlab C.diff Quik Chek Complete is used in routine practice, a confirmation assay as the Orion Genread® *C. difficile* assay or the BDMax Cdiff assay is recommended to elucidate non-conclusive results.

P070

Are time to culture positivity and the molecular bacterial load assay telling us different stories in a mouse tuberculosis model?

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Introduction: Mycobacterial population within Tuberculosis (TB) patients is considered to be heterogeneous; several subpopulations of bacteria have been identified in different microenvironments. Environmental stress and antibiotic pressure can drive *Mycobacterium tuberculosis* (Mtb) into a non-replicating state. As a consequence, extensive and long-term treatment is required to eradicate all subpopulations of Mtb. For the analysis of new drugs and novel combinations it is important to predict at an early stage of development the long-term efficacy and relapse of infection. Current methods to monitor treatment response such as sputum smear or sputum serial colony counting have their drawbacks. Furthermore, it is unlikely that all different subpopulations of Mtb are detected using these methods. Therefore more research and new techniques are necessary to provide more information about the different subpopulations.

Methods: In this study, we compared with matched lung samples of our murine TB model, Colony Forming Unit (CFU) counting on solid media, Time To Positivity (TTP) using the Bactec MGIT 960 and the Molecular Bacterial Load (MBL) assay which determine the presence of mycobacterial 16S ribosomal RNA. Mice were treated for 24 weeks and treatment arms were designed as follows: Isoniazid (H) - Rifampicin (R) - Pyrazinamide (Z), HRZ-Streptomycin (S), HRZ – Ethambutol (E) or ZES.

Results: Our study revealed inverse correlations between TTP with CFU and MBL and positive associations between CFU and MBL. Description of the net elimination of bacteria was performed for CFU *vs.* time, MBL *vs.* time and 1/TTP *vs.* time during treatment with mathematical models (fitted by nonlinear regression). CFU *vs.* time and 1/TTP *vs.* time showed bi-phasic declines with the exception of HRZE. A similar rank order, based on the initial elimination rate (alpha slope), was found comparing CFU *vs.* time and TTP *vs.* time, respectively HRZE, HRZ, HRZS and ZES. In contrast, MBL *vs.* time showed a mono-exponential decline with a relatively flat gradient of elimination and a different rank order respectively, ZES, HRZ, HRZE and HRZS.

Conclusions: We conclude that the correlations between methods reflecting the ability of each to discern a general load of Mtb.

Based on the description of net elimination, we conclude that the MBL assay tells "a different story" in a mouse TB model to that of CFU counts and TTP, by detecting a subpopulation of Mtb which is not detected by CFU or TTP assays and indicating a slower general elimination of the total bacterial load.

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P071

Molecular diagnosis of bacterial vaginosis using an in-house and a commercial multiplex PCR

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Introduction: Bacterial vaginosis (BV) is a common condition where the vaginal environment is depleted of lactobacilli and overgrown with anaerobic bacteria. Neither clinical nor research settings currently make wide use of molecular tools for BV diagnostics. Here we evaluated an in-house and a commercially developed multiplex PCR for the diagnosis of BV. We, therefore, conducted a case-control study in women reporting urogenital complaints at the sexually transmitted infection (STI) clinic in Amsterdam, the Netherlands.

Methods: BV diagnosis was based on the Amsel criteria: vaginal discharge, "clue"cells and/or a positive whiff test. We included 83 women with BV and 92 women without BV. The cases and controls were included so as to be similar in age, ethnicity and date of clinic

visit. An in-house PCR was developed targeting four BV associated bacteria (*Gardnerella vaginalis, Atopobium vaginae,* Megaspheara and BVAB-2) and *Lactobacillus crispatus*. The PCR outcomes were interpreted as follows: 1) at least two BV-associated bacteria with Ct≤30 and *L. crispatus* with Ct≥25 qualified as BV positive; 2) all BV-associated bacteria Ct≥30 and *L. crispatus* Ct≤25 were BV negative. Other outcomes were termed 'intermediate'. The commercial multiplex PCR targeted, quantified and compared the bacterial load of BV associated bacteria *G. vaginalis* and *A. vaginae* to the bacterial load of Lactobacilli, in relation to overall bacterial load in each sample. We compared both molecular tools with the STI clinic BV diagnosis. Additionally, the commercial multiplex PCR was tested in 128 cervico-vaginal samples for which clinical BV diagnoses and microbiome data – using the 16srRNA gene – were available, enabling us to describe the microbial composition of a BV negative, intermediate and positive commercial test outcome.

Results: The study population had a median age of 24 years (interquartile range: 21-27) and consisted predominantly of Caucasians. Cases and controls had similar STI prevalence: *Trichomonas vaginalis*: 0%; *Chlamydia trachomatis:* 8.6%; *Neisseria gonorrhea:* 1.1%. We found high sensitivity (>96%) and NPV (>96%) for both molecular tools, relative to the STI clinic diagnosis. The in-house PCR had a specificity of 93.1% and a PPV of 89,9%, whereas the commercial kit had a specificity and PPV of respectively 77,2% and 79,4%. The cervico-vaginal microbiome composition differed significantly between samples with different test outcomes (p≤0.001); BV negative samples were mostly dominated by *L. crispatus*, samples with intermediate outcome were mostly dominated by *Lactobacillus iners,* and BV positive samples were mostly dominated by BV-associated bacteria and sometimes by *L. iners* (n = 15; 22,7%). None of the commercial test BV positive samples that were dominated by *L. iners,* had a clinical diagnosis of BV.

Conclusion: The in-house PCR has both high sensitivity and specificity. When comparing the commercial mutliplex test outcomes to clinical BV diagnosis, sensitivity was high, but specificity and PPV were relatively low. This either indicated that the commercial test over-diagnoses BV, as was seen in samples that were dominated by *L .iners*, or that the molecular tool detects pre-clinical asymptomatic BVs in which patients do not yet fulfill the Amsel criteria.

P072

Improve BCG vaccination by heterologous secretion: using LipY as a carrier

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Introduction: *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG) is well known for its use in vaccine treatment against tuberculosis and for its use in intra-vesical therapy to prevent recurrence of bladder tumors. Although BCG has been used for years in both therapy strategies, there is still an urgent need for improvement. Both vaccination and bladder cancer treatment could benefit from heterologous secretion by BCG. Secretion of specific antigens, human cytokines or tumor targeting proteins are promising strategies to improve therapy.
To enable heterologous secretion by mycobacteria we looked for an endogenous protein that could serve as a carrier protein to facilitate heterologous secretion. As a proof-of-concept we examined LipY as a carrier for the secretion of egg white protein Ovalbumin (OVA) and investigated which domains of LipY are essential for secretion and which mutations can enhance secretion of the fusion protein.

Methods: *Mycobacterium marinum* was used as a model to study the secretion of the LipY-OVA-HA fusion protein. Deletions in the linker domain were made to study the effect on secretion of the fusion protein and random mutations were introduced by error-prone PCR. To analyze secretion of the fusion protein two different assays were performed. First, secretion was assessed by double filter assay, in which secreted proteins were captured on a nitrocellose membrane and were stained by immunohistochemistry. Second, bacterial cultures were fractionated and analyzed by SDS PAGE and Western blot.

Results: *M. marinum* showed low levels of ESX-5 dependent secretion of LipY-OVA-HA. Since secretion of proteins can be hampered by protein structure, we hypothesized that small changes in the structure introduced by error-prone PCR might enhance secretion. Surprisingly, we found that extension of the HA-tag also enhanced secretion, as different random mutations caused the same frameshift in the HA-tag, leading to an extension and subsequent enhanced secretion. Furthermore we found that complete deletion of the linker domain enhanced secretion of the fusion protein.

Conclusion:

1. We showed that in *M. marinum* the secretion signal of LipY can be used as a carrier for heterologous secretion of OVA.
2. Secretion can be enhanced by deletion of the linker domain of LipY or by extension of the HA-tag.

P073

Methane production and oxidation in wetland ecosystems

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Background: Methane is an important greenhouse gas in the atmosphere and 25 times more potent in the radiative forcing than CO_2, thus contributing up to 20% to

global climate change. Wetland ecosystems are one of the most significant methane sources. Furthermore pristine wetlands systems are under pressure due to high nitrogen loading. In such wetlands there is an apparent unbalance in both methane and nitrogen cycling. Recently new groups of bacteria and archaea were discovered that are able to convert methane and nitrogen compounds under oxygen limitation or even oxygen absence with nitrite or nitrate as electron acceptors[1-3] but their contribution to methane and nitrogen cycling in wetland systems has not been well explored. In this study we aim to investigate the microbial diversity and ecophysiology of methanogenic archaea and both aerobic and anaerobic methane-oxidizing or ammonium-oxidizing micro-organisms.

Methods: Several wetland systems were selected for sampling (nutrient loaded estuaries, nutrient loaded river sediment and fertilized paddy fields). The samples were used to obtain nutrient profiles of methane, ammonium, and nitrate. Total DNA was extracted from selected sub samples, and used for amplicon sequencing of 16S rRNA and diagnostic functional genes (mcrA for methanogens and AOM archaea, amoA for nitrification and comammox, pmoA for aerobic methanotrophs, hzsA for anammox). Furthermore total DNA was sequenced using ion torrent technology to obtain a metagenome of the ecosystems. Soil slurries amended with 15N-nitrate, 15N-ammonium or 13C-methane were used to estimate activities of the various processes. Most promising soil samples were used to start enrichment cultures. The bioreactors were monitored by qPCR, FISH, and metagenome sequencing.

Results: Anoxic soil slurries of nutrient loaded paddy field and estuaries showed relative rapid conversion of ^{13}C-labelled methane into $^{13}CO_2$ indicating a good potential for anaerobic oxidation of methane. Community analysis based on 16S rRNA amplicon sequencing and analysis of functional gene diversity showed that AOM archaea were present in significant numbers. Quantification by qPCR with newly developed primers confirmed to AOM archaea numbers. Soil samples with the highest number of AOM archaea were used to start either batch or bioreactors supplied with methane and appropriate electron acceptors to enrich these particular AOM archaea. After several months of continuous operation AOM could be measured and AOM archaea could be visualized by FISH. Currently the metagenome of the reactor is being analysed. Together thesedata suggests that AOM archaea may play an important role in methane cycling in various nutrient loaded ecosystems.

References

1. Raghoebarsing AA, Pol A, van de Pas-Schoonen KT, et al. A microbial consortium couples anaerobic methane oxidation to denitrification. Nature. 2006;440:918-21.
2. Haroon MF, Hu S, Shi Y, et al. Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage. Nature. 2013;501:578.
3. Van Kessel MA, Speth DR, Albertsen M, et al. Complete nitrification by a single microorganism. Nature. 2015;528:555-9.

P074

Phage display screening for innate immune evasion molecules of *Staphylococcus aureus*

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Introduction: *Staphylococcus aureus* is a Gram-positive common human pathogen that cause of variety infection diseases ranging from mild skin infections to more serious life-threating wound and bloodstream infections. The prerequisite for *S. aureus* to successfully colonize and induce infection is to secrete evasion molecules. Therefore, unravel the nature of these evasion molecules contributes to a better understanding of the pathological processes underlying infectious and inflammatory diseases.

Methods: A high-throughput selection strategy involving secretome phage display method was introduced in this study. Only secreted and anchored proteins of a bacterial genome are displayed on the surface of filamentous phage by fusion of phage coat proteins to secreted proteins. Based on this method, a phage display library of *S. aureus Newman* strain was constructed. Evasion molecules were then selected by applying the human neutrophils as a bait, and sequencing the enriched phages. Next generation Illumina sequencing was used to identify phage selected proteins. The protein was produced in *E. Coli* with a His-tag for isolation and detection.

Results: During a screening of a human *S. aureus* phage library on human neutrophils, the secreted hypothetical protein NWMN_1076 was identified. NWMN_1076 is a hypothetical protein with unknown function. Binding of purified NWMN_1076 to human leukocytes was tested and confirmed binding to PMNs and Monocytes. Competition experiment with several monoclonal antibodies to surface antigens on leukocytes did not reveal a potential binding target. However, experiments conducted at 37°C showed a strong activation phenotype for PMNs and Monocytes. Furthermore, NWMN_1076 can enhance phagocytosis by human neutrophils, which confirms the potential immune stimulating activity.

Conclusion: The secreted hypothetical protein NWMN_1076 binds to human PMNs and Monocytes resulting in cell activation, and enhancement of phagocytosis by PMNs. The mechanism of action and role of NWMN_1076 in *S. aureus* pathogenesis needs to be determined.

P075

Low prevalence of urethral lymphogranuloma venereum infections among homosexual men visiting the STI clinic in Amsterdam, the Netherlands

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Introduction: Several guidelines (BASHH, WHO/USTI and CDC) recommend routine screening for anorectal lymphogranuloma venereum (LGV) in men who have sex with men (MSM). None recommend screening of urethral LGV infections. However, urethral LGV infections might play an important role in the ongoing transmission of LGV infections among MSM. Therefore our aim was to study the prevalence of urethral LGV among MSM visiting the sexual transmitted infection (STI) clinic in Amsterdam, the Netherlands.

Methods: We prospectively collected all urethral Chlamydia trachomatis (Ct)-positive samples from MSM visiting the Amsterdam STI clinic between March 2014 and April 2015. Samples that tested positive by transcription mediated amplification (TMA) screening assay (Aptima Combo test, Hologic, USA) were typed using an in-house pmpH qPCR to differentiate between LGV and non-LGV type infections.

Results: During the inclusion period, 13197 MSM were tested for urethral Ct infections of whom 431 (3.4%) tested chlamydia positive. Of these, 339 (78.7%) were negative for an LGV type infection, 84 (19.5%) were inconclusive and 8 (1.9%) samples were positive for an LGV-type infection. Overall, we found a urethral LGV-type prevalence of 0.06% (8/13197) among MSM visiting the Amsterdam STI clinic. During the same period we found an anorectal LGV-type prevalence of 0.75% (120/16008) among MSM visiting the Amsterdam STI clinic, which is significantly higher compared to the urethral LGV-type prevalence ($P < 0.001$).

Conclusion: The observed prevalence of urethral LGV infections (0.06%) is significantly lower compared to the prevalence of anorectal LGV infections (0.75%) found among MSM visitors of the STI clinic. Despite the low prevalence, these infections would be missed in the current diagnostic algorithm and could subsequently contribute to the ongoing transmission of LGV infections. Routine screening of urethral LGV infections might not be feasible for all MSM, however, awareness, screening of partners, and prompt treatment of LGV infections is crucial for the individual patient and prevents ongoing transmission and possible severe outcomes.

P076

Mucosal and fecal microbiota in patients with compensated and decompensated liver cirrhosis

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Introduction: Increasing data show perturbations in the fecal microbiota of liver cirrhosis patients. Whether these findings can be useful in daily clinical practice needs further study. Furthermore, data on the mucosal microbiota composition are limited. The 16S-23S interspacer (IS) region-based profiling method (IS-pro) has potential as a clinical tool to assess and monitor the intestinal microbiota, as it enables relatively fast analysis of individual samples. The aim of this study was to investigate differences in the fecal and mucosa-associated microbiota in a heterogeneous group of (de)compensated liver cirrhosis patients and healthy controls, taking into account disease-related factors.

Methods: Fecal samples, sigmoid and duodenum biopsies of healthy controls (n = 26, n = 22 and n = 18, resp.) compensated (n = 25, n = 12 and n = 13, resp.) and fecal samples of decompensated cirrhotic patients (n = 16) were available for analysis. Microbiota composition was assessed by IS-pro.

Results: In fecal samples, a higher median richness (number of observed peak) were found for *Firmicutes* in compensated (5.0(0.9-12.0)) and decompensated cirrhotics ((6.5(1.0-22.0)) versus controls (3.0(0.0-10.0), all $P<0.05$). Lower median richness for *Bacteroidetes* in fecal samples was found in compensated (20.0(4.0-39.0)) and decompensated cirrhotics (18.5(0.0-26.0) versus controls (23.5(13-32), all $P<0.05$). In duodenal biopsies, only an increased median richness for *Firmicutes* was found in compensated cirrhotic (10.0(3.0-13.0)) versus control subjects (5.0(0.0-14.0), $P=0.01$). No significant microbial differences were found in sigmoid biopsies. Constrained ordination showed distinct clusters of fecal samples based on disease severity by Child Pugh score, but not by demographics, etiology or medication use. An increase of *Streptococcus bovis/gallolyticus* and *Streptococcus mitis* in feces and an increase of *Enterococcus* spp., *Staphylococcus* spp., *Lactobacillus Johnsonii* and *Lactobacillus plantarum* in the duodenum was associated with compensated liver cirrhosis. A significant

positive correlation was found between the permeability of the small intestine, indicated by the lactulose/rhamnose ratio, and *Lactobacillus johnsonii* (peak 264: P = 0.03, r = 0.43 and peak 265: P = 0.02, r = 0.51)

Conclusion: Differences in the fecal and duodenal, but not sigmoid microbiota composition were found between liver cirrhosis patients and healthy controls, pointing to increased Firmicutes/Bacteroides ratios. The results indicate involvement of the duodenal microbiota in cirrhosis and possible associations with the small intestinal permeability. Alterations in the fecal microbiota were more pronounced with progression of liver cirrhosis, suggesting a stronger dysbiosis of the fecal microbiota with liver cirrhosis progression. This potential of profiling the fecal microbiota as a disease progression marker should be further validated in prospective follow-up studies.

P077

Bacterial lysis through interference of peptidoglycan synthesis increases biofilm formation by non-typeable *Haemophilus influenzae*

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Background: Nontypeable *Haemophilus influenzae* (NTHi) is an opportunistic pathogen that causes otitis media in children and community-acquired pneumonia or exacerbations of chronic obstructive pulmonary disease in adults. A large variety of studies suggest that biofilm formation by NTHi may be an important step in the pathogenesis of this bacterium, however, the underling mechanisms involved in this are poorly elucidated.

In this study, we used a transposon mutant library to identify bacterial genes that were involved in biofilm formation.

Materials & Methods: Biofilm formation was determined by static growth on 96-well plates and crystal violet staining. A mutant library was generated in NTHi strain 13/4, which was collected from the nasopharynx of a healthy child. Growth and biofilm formation was determined for 4172 transposon mutants. Transposon mutants with normal growth (A_{620} less than 35% lower compared to wild-type) but an altered biofilm formation (A_{560} at least 1.5-fold difference compared to wild-type) were selected for a second and third round.

The involvement of the identified genes in biofilm formation were validated in NTHi strain R2866 bin various in vitro experiments.

Results: Transposon insertion site was successfully identified for 30 transposon mutants that showed altyered biofilm formation. Interestingly, R2866Δ*ampG*, R2866Δ*amiB* and R2866Δ*mrdA* code for genes involved in peptidoglycan synthesis and remodeling, and disrupting these genes with a spectinomycin cassette increased biofilm formation significantly.

The presence of DNA in R2866Δ*ampG* biofilms was increased and DNAse abrogated biofilm formation to wild-type level. We showed that interference of peptidoglycan synthesis or remodeling increased bacterial lysis during static growth, supplying genomic DNA, which was required for increased biofilm formation. Interestingly, similar results were obtained with sub-inhibitory concentrations of beta-lactam antibiotics, known to interfere with peptidoglycan synthesis, but not with other classes of antibiotcs.

Conclusion: We provide evidence that interference with peptidoglycan synthesis and remodeling, either by making gene deletion mutants or by using sub-inhibitory concentrations of beta-lactam antibiotics, increases sensitivity to bacterial lysis, releasing bacterial DNA that contributes to biofilm formation *in vitro*.

P078

Clinical and microbiological characteristics of *Clostridium difficile* infection among hospitalised children in the Netherlands; a six-year surveillance

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Background: In contrast to *Clostridium difficile* infection (CDI) in adults, few studies have been performed in children. We describe the reporting rates, clinical and microbiological characteristics of CDI among hospitalised children in the Netherlands.

Methods: In the period May 2009 to May 2015, 26 of the circa 90 hospitals in the Netherlands registered clinical characteristics of all paediatric CDIs (aged 2-18 years) as part of a national sentinel surveillance. PCR ribotyping of all available strains was performed, and paediatric results were compared to those of adults by proportion 95% confidence interval (CI). Multiple-Locus Variable number tandem repeat Analysis (MLVA) was used to study CDI transmission on specific cases. Time-trend in paediatric CDI reporting was evaluated by Poisson regression.

Results: Paediatric CDIs were reported in 17 of the 26 participating hospitals (n = 135, 3% of all CDI); reporting rates were constant in time. The median age of children with CDI was 10 years (IQR: 4.7-14.5 years). Fifty-five percent of the children had community-onset, and 31% had severe CDI. Lower complication and mortality 30-day rates were observed in paediatric CDI compared to adults

(n = 4,556). Two children died within 30 days, due to other causes than CDI. Thirty-six different PCR ribotypes were identified. Ribotype 265 was most prevalent in children (15%; 95% CI: 9-24%), but rarely found in adults (1%; 95% CI: 0.8-2%). Most paediatric ribotype 265 cases were genetically related according to MLVA. Ribotype 265 has, as far as we know, have not been reported by any other country in human, animal or environmental studies. PCR ribotype 014/020 was equally common, yet some ribotypes (e.g. 001, 027, and 078/126) were less abundant in children than in adults.

Conclusion: The number of paediatric CDI in hospitals was stable during the last six years, and paediatric CDI-related mortality did not occur. Further studies need to confirm and elucidate the differences in ribotype distribution (in particular for ribotype 265) found compared to adults.

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P079

Colistin and tigecyclin susceptibility among carbapenem resistant microorganisms

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Introduction: The emergence and spread of antimicrobial resistance is a worldwide public health threat. With the increasing occurrence of carbapenem resistant microorganisms (CRMO), the use of colistin and tigecyclin as a last resort for treating infections caused by them is on the rise as well. Not surprisingly, resistance to these true last resort antibiotics is also reported more frequently, mainly by mutations in the genome. However, the recent discovery of a plasmid-mediated colistin resistance gene, *mcr-1* reported from China, showed that resistance to colistin can be acquired and exchanged between different microorganisms. Furthermore, other studies have also found this gene in isolates from Denmark and the Netherlands, indicating a widespread dissemination of this novel resistance gene. Triggered by these findings, we examined susceptibility to colistin and tigecycline among a selection of CRMO and we have also tested these and other isolates submitted as part of the national CRMO surveillance for the presence of the *mcr-1* gene.

Methods: Susceptibility to colistin and tigecycline was assessed by Etest according to EUCAST guidelines on a selection of 213 gram-negative isolates, comprising not only *Klebsiella, Enterobacter* and *Escherichia* isolates, but also *Acinetobacter* and *Pseudomonas* species. These isolates were all either found positive for carbapenemase activity with the CIM (carbapenem inactivation method) or had an MIC of > 8 for meropenem and were subjected to next-generation sequencing (NGS) via Illumina HiSeq. The presence of *mcr-1* was determined either by examination of NGS data or by PCR using primers as described in the original report combined with primers for 16S as an internal control. In addition to this selection, the following isolates from the CRMO surveillance were also screened for *mcr-1*: all *E. coli* isolates received since 2012, all *Klebsiella* isolates received in 2015, and all other isolates from November 2015 on.

Results: In total, 15 isolates were found to be resistant to colistin (11 *Klebsiella* and 4 *Enterobacter*). None of these were found to be resistant to tigecyclin as well, although two were intermediately susceptible. The *mcr-1* gene was not found in the NGS data of any of these isolates. Furthermore, the *mcr-1* gene was also not detected in any of the isolates ($n = 363$) by PCR.

Conclusion: The recent discovery of a mobile genetic element conferring resistance to colistin poses a serious threat to public health and it has now even been found in a human *Escherichia coli* isolate carrying the carbapenemase encoding gene $bla_{\text{KPC-2}}$. However, this isolate and the CRMO found to be phenotypically resistant to colistin in our study were not resistant to tigecycline, leaving this treatment option open. Nonetheless, the potential of colistin resistance to cause truly untreatable infections justifies close monitoring.

P080

Can one vanA/B qPCR on enrichment broth replace 5 rectal swab cultures if the qPCR is negative?

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Introduction: In the last year more than ten vancomycin resistant enterococci (VRE) outbreaks have been reported in The Netherlands. Many patients (contacts) have been labeled 'suspected VRE carrier' and are offered VRE screening. It is therefore essential to optimize VRE screening methods to minimize costs, safe time and keep it as patient friendly as possible. Recently the 'NVMM Guideline HRMO VRE' has been published which states that 3-5 rectal swabs should be taken, on separate days, when screening for VRE. After incubation in an enrichment broth, samples can be pre-screened using *van* qPCR or directly be offered for culture using a selective (chromogenic) agar. We hypothesized that the negative predictive value of 1 *va* qPCR on the first enrichment broth, is high enough to replace the subsequent 3-5 cultures advised by the guideline, assuming no ongoing VRE exposure.

Methods: During a large VRE outbreak in our region, sample sets of 5 rectal specimens from separate days

were routinely cultured using an enrichment both with 16mg/L amoxicillin. Subsequently, a chromogenic agar was used which was incubated for 48 hours. Suspected colonies were inoculated on a blood agar plate. Colonies (from blood agar) were identified with MALDI-TOF and a vancomycin e-test was performed if *Enterococcus spp*. were detected. If the vancomycin minimal inhibitory concentration was >4 mg/L and/or the borders were 'fuzzy' the strain was offered for *van* qPCR and an amoxicillin e-test was performed. If enterocci were amoxicillin resistant and the *va* qPCR was positive, the sample was determined VRE positive. A set of 5 specimens was considered VRE positive if VRE was detected in at least one culture (gold standard). All enrichment broths of the first rectal swab were offered for *vanA/B* qPCR .

Results: Between December 2014 and December 2015, 151 sets of 5 rectal specimens from 142 patients, were included. *van* qPCR was performed on the first enrichment broth of each set and 43% had a positive *va* qPCR (*VanA* n = 2, *VanB* n = 63). Based on culture data, 12 (7.9%) sets were VRE positive. The negative predictive value of *van* qPCR on the first enrichment broth, for predicting a VRE negative set of 5 specimens, is 98.8% (CI 94.6-99.9%). One enrichment broth was *va* qPCR negative, but the corresponding set of 5 culture positive. The enrichtment broths of the other four samples (out of 5) had Cq values ranging from 21-25, suggesting sampling error of the first swab (which was culture negative). All other VRE positive sets had Cq values between 17-34 on the first enrichtment broth. The culture negative sets had Cq values between 21-44. Six culture negative sets (n = 6 patients) had Cq values below 30 on the first enrichment broth. Five of these patients were known VRE positive patients, but all had at least 11 negative VRE cultures in the past year.

Conclusion: One *van* qPCR on enrichment broth can replace 5 rectal swab cultures if the *va* qPCR is negative. One qPCR instead of 5 cultures has a shorter turnaround time and is more patient friendly.

P081

Altering human specific Chemotaxis Inhibitory Protein of *Staphylococcus aureus* host tropism to study its contribution to pathogenesis in animal models

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The Gram-positive bacterium *Staphylococcus aureus* is a successful opportunistic pathogen that causes a wide spectrum of acute and chronic diseases in humans and several animal species. *S. aureus* pathogenesis depends on an arsenal of secreted virulence factors that play a role in host invasion, cellular adherence, colonization, nutrient scavenging and immune evasion. These secreted proteins also enable the bacterium to survive in multiple hosts. However, clinical isolated *S. aureus* strains differ genetically in the genes associated with virulence factors compared to animal isolated strains. Till now, 15 *S. aureus* virulence factors have been described that interact with high affinity with human proteins but are not compatible with the homologous proteins in other mammalian species.

The human specific Chemotaxis Inhibitory Protein of *Staphylococcus aureus* (CHIPS) plays a crucial role in host immune evasion. *In vitro* data suggests that CHIPS specifically inhibits human neutrophil migration to the site of infection by binding to the N-terminal region of the C5a receptor (C5aR) present on these neutrophils. This CHIPS-C5aR interaction obstructs the binding of the potent chemoattractant C5a *in vitro*. However, the human specificity of CHIPS makes it difficult to study its role in pathogenesis in *in vivo* infection models. Nevertheless, mice and other animal models are crucial for the *in vivo* study of *S. aureus* infections and how virulence factors contribute to pathogenesis.

We hypothesize that by adapting certain amino acids present in CHIPS would raise its affinity towards zebrafish C5aR. Structural and binding analysis revealed that the amino acid regions D42-K61 and K95-N111 on CHIPS interact with human C5aR. Selectively randomizing these regions and incorporating them in the phagemid pDJ01 resulted in a complex CHIPS phage library consisting of approximately 5x10^8 CHIPS variants. Selection of the zebrafish adapted CHIPS was performed using the previously mentioned phage library and U937-cells expressing zebrafish C5aR, human C5aR or blank U937-cells. After selection, a specific amplification of CHIPS was observed containing 5 mutations; N47I, Y48S, K51R, A57G and F59I. Subsequent His-tagged protein expression and binding analysis using FITC labelled anti-His antibody on zebrafish C5aR showed a specific increase in binding towards zebrafish C5aR. However, even though an improved binding was observed, CHIPS containing N47I, Y48S, K51R, A57G and F59I did not show any improvement in inhibiting zebrafish C5a *in vitro*. Nevertheless, preliminary data suggests that Phage Display is a powerful tool to predict amino acid mutations needed to alter host tropism of CHIPS and possibly other human specific virulence factors. Additional experiments are needed to improve binding towards zebrafish C5aR resulting in a biologically functional zebrafish adapted CHIPS. Better understanding and knowledge of the mechanisms of action of CHIPS and other human specific virulence factors would not only identify new targets that could be exploited for anti-virulence strategies to limit *S. aureus* infections, but also improve current *in vivo* infection models.

P082

Elucidating the composition of the outer membrane of the anammox Planctomycete *Kuenenia stuttgartiensis*

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The organization of the cell envelope of *Planctomycetes* in general and anammox *Planctomycetes* in particular has been a matter of debate. Anammox bacteria have three compartments of which the innermost is a prokaryotic organelle in which the anaerobic ammonium oxidation (anammox) reaction takes place (Neumann et al, 2014). The outermost compartment has for a long time been known as the *Planctomycetes*-specific paryphoplasm (Lindsay et al, 2001), but the recent detection of peptidoglycan in this compartment suggested that this compartment should be interpreted as a periplasm (van Teeseling et al, 2015; Jeske et al, 2015). This viewpoint suggests that the outermost membrane of *Planctomycetes* is in fact an outer membrane.

Here we investigated the composition of the outer membrane in order to elucidate if OMPs and LPS are present in the outer membrane of the anammox bacterium *K. stuttgartiensis*. Therefore, purification, immunogold localization and functional characterization of a putative OMP were performed. In addition, the presence of LPS was investigated.

This research gives the first comprehensive description of two main outer membrane components of an anammox *Planctomycete* and substantiates the emerging image of (anammox) *Planctomycetes* as Gram-negative bacteria.

References

1. Jeske O, Schüler M, Schumann P, et al. Planctomycetes do possess a peptidoglycan cell wall. Nat Commun. 2015;6:7116.
2. Lindsay MR, Webb RI, Strous M, et al. Cell compartmentalisation in Planctomycetes: novel types of structural organisation for the bacterial cell. Arch Microbiol. 2001;175:413-29.
3. Neumann S, Wessels HJCT, Rijpstra WIC, et al. Isolation and characterization of a prokaryotic cell organelle from the anammox bacterium *Kuenenia stuttgartiensis*. Mol Microbiol. 2014;94:794-802.
4. Van Teeseling MCF, Mesman RJ, Kuru E, et al. Anammox Planctomycetes have a peptidoglycan cell wall. Nat Commun. 2015;6:6878.

P083

Molecular comparison of *Listeria monocytogenes* sequences from regional Listeriosis cases to an international database by core genome multilocus sequence typing

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Background: Invasive listeriosis is a sporadic life-threatening disease caused by *Listeria monocytogenes*. Listeriosis may occur in clusters as a result of a common contaminated food source. There are 13 different *Listeria*-serogroups. Almost all human *Listeria* infections are caused by isolates belonging to serogroups IIa, IIb, IIc and IVb. It is not fully clear which specific virulence factors explain the associated pathogenicity of isolates to these serogroups. In our hospital, two cases of invasive listeriosis occurred on consecutive days. This is exceptional, since only two other cases had occurred in the preceding two years. Hospitals in the region were alerted, and cases in the following two months were assessed as well. Isolates were sequenced by next generation sequencing (NGS) in the UMCG Groningen, and sequence files were sent to the Austrian Agency for Health and Food Safety for comparison to each other, and to an international database by core genome multilocus sequence typing (cgMLST). Next, we assessed diversity in virulence-associated genes.

Methods: In total, six *Listeria monocytogenes* isolates from unique cases from 2014-2015 were assessed by next generation sequencing (NGS). DNA extraction, NGS, *de novo* assembly, and MLST were performed as previously described (Zhou, *CMI*, 2015). A core genome scheme of *Listeria monocytogenes* including 1701 allele targets was used for cgMLST comparison using Seqsphere-software. Isolates were considered clonally related if there were less than 10 allelic mismatches (Ruppitsch, *JCM* 2015). MLST types, cluster types and serogroups were derived from NGS data. To assess diversity in virulence-associated genes, we used pairwise comparison of genes of all isolates for functional roles using RAST (Aziz, *BMC Genomics*, 2008).

Results: The isolates belonged to the following MLST types (cluster types/serogroups): ST1 (CT2758/IVb), ST3 (CT2756/IIb), ST4 (CT2755/IVb), ST91 (CT88/IIa), ST91 (CT2754/IIa) and ST123 (CT2795/IIc). All isolates showed more than 25 allelic mismatches compared to each other, and compared to sequences in an international database containing 999 isolates. Isolate pairs had on average 2202

(range 2175-2244) genes for functional roles in common, and 27 (range 1-51) different genes. Virulence-associated genes encoding for Streptolysin S biosynthesis (SagBCD operon) were found in 3/6 isolates, rhamnose-containing glycans (rfb operon) in 4/6 isolates, iron-acquisition cluster (EfeUOB operon) in 4/6 isolates, internalins (inlG, inlH) in 3/6 isolates, and sortase in 3/6 isolates.

Conclusions: 1) The *Listeria monocytogenes* strains isolated from patients in hospitals in our region in a short time period were not clonally related, nor to any other strain in the database. 2) CgMLST is useful for public health purposes, because it allows for rapid response and real-time surveillance to identify clusters of listeriosis cases. 3) NGS is a powerful tool to assess epidemiology of virulence-associated genes. We found diversity in virulence-associated genes between isolates. A future case-control study based on this method may identify specific virulence factors for listeriosis.

P084

Exploring the sponge holobiont *Dysidea avara* with molecular tools and by microbial cultivation

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Introduction: Marine sponges (*Porifera*) belong to the most ancient metazoan animal phylum, dating back approximately 600 million years into pre-Cambrian times. These active filter feeders are currently known for their ability to produce a high diversity of secondary metabolites as a means of chemical defence. A high number of these compounds are of interest to the pharmaceutical industry due to their anti-viral, anti-cancer or antimicrobial activities.

It is now generally accepted that microorganisms can make up to 40% of the sponges biomass and live permanently associated with the sponge. Cultivation of sponges and their associated microbes has been in focus for some time as these organisms have the potential to constitute 'biological fermenters' for the production of interesting compounds. However, an immortal sponge cell line has yet to be established, and the cultivation of the microbial symbionts has, in many cases, not been achieved."

In this study we investigate the bacterial community associated to the Mediterranean sponge *Dysidea avara* by culture independent methods and aim at increasing the cultivable fraction of key symbionts.

Methods: Sponge cell suspensions and the corresponding bacterial fraction were sequenced using 16S rRNA gene community profiling on an Illumina MiSeq platform. Sequencing results were analyzed using modified versions of the QIIME pipeline. OTUs were picked based on 100% sequence identity.

Sponge cell suspension was plated on undiluted and ten times diluted marine agar and Mueller Hinton agar with and without the addition of 6 antibiotics. Inoculated plates were incubated with and without natural light for three weeks. Colonies were picked from all conditions and identified by 16S rRNA gene sequencing using Sanger. Comparison between cultivation dependent and cultivation independent data was conducted using nBLAST considering only hits with ≥99% sequence identity.

Results: Amplicon sequencing resulted in 277 OTUs and revealed that the *D. avara* associated bacterial community was dominated by the phyla *Proteobacteria* (63%), *Bacteroidetes* (19%) and *Cyanobacteria* (6%). More than 70% of all sequences were comprised of only 30 highly abundant OTUs. The cultivation experiment yielded 37 isolates and included two novel *Flavobacteria* strains with less than 97% sequence identity to the closest cultured isolate. Furthermore, *Pseudovibrio* and *Ruegeria* species were isolated. Comparison to amplicon data revealed that 5 out of the top 24 OTUs were retrieved in culture which reflects 15% of the *D. avara* associated bacterial community based on relative abundance data.

Conclusion: Although *D. avara* is considered a low microbial abundance sponge, we conclude that this sponge associates to a distinct bacterial community which differs from the surrounding seawater. Furthermore, the overlap between amplicon sequencing data and OTUs retrieved in cultivation suggests that we can now study a significant part of the symbiotic community *in vitro*. This opens new perspectives for co-cultivation approaches in future attempts towards sponge cell line development.

P085

Prevalence and detection of plasmidal AmpC-producing *Escherichia coli* and *Klebsiella pneumoniae* from clinical isolates in Lahore, Pakistan

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Objective: AmpC β-lactamases cause resistance to third generation cephalosporins. Detection of plasmidal AmpC β-lactamase production is important for adequate treatment and effective infection control. The aim of this study was to estimate the prevalence of plasmidal AmpC producing *E. coli* and *K. pneumoniae* in Lahore, Pakistan.

Methods: From July 2014 to June 2015 all *E. coli* and *K. pneumoniae* from blood, urine, sputum, CSF and pus resistant to cefotaxime and/or ceftazidime were collected at the Al-Razi healthcare center, Lahore, Pakistan. Isolates

were screened for AmpC production using the cefoxitin disc zone (<18 mm). AmpC production was phenotypically confirmed using an inhibitor based method with 3-aminophenylboronic acid and a cefoxitin disc. All phenotypically confirmed isolates were genotypically tested using real-time multiplex and singleplex PCRs for the plasmidal AmpC genes CIT, MOX, FOX, DHA, ACC and EBC.

Results: In total, 2013 isolates (1230 *E. coli* and 783 *K. pneumonia*) were collected. Of these, 1500 were resistant to cefotaxime and/or ceftazidime and 900 were suspected for AmpC production using cefoxitin disc. In 222 isolates AmpC production was phenotypically confirmed. In 130 isolates AmpC genes could be identified (108 in *K. pneumonia*e (14%) and 22 in *E. coli* (2%)).

Eighty-one isolates were positive for CIT (37%), 8 for DHA (4%), 16 for EBC (7%). Twenty-five isolates had multiple AmpC genes: 10 CIT and DHA (5%), 15 CIT and EBC (7%). The overall prevalence of AmpC was (130/2013) 7%.

Conclusion: This study shows a prevalence of plasmidal AmpC producing *E. coli* and *K. pneumoniae* in Lahore, Pakistan of 7%, 14% in *K. pneumoniae* and 2% in *E. coli*. The most frequent plasmidal AmpC gene detected was CIT, followed by EBC and DHA.

P086

Comparison of MicroPlex beads with MagPlex beads in a Luminex multiplex assay

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Introduction: Multiplex bead based flow cytometry Luminex technology is a very attractive way for simultaneous, rapid and cost-effective analysis of multiple analytes in a single sample. In our previous studies, we have gained ample expertise with Luminex assay using the polystyrene MicroPlex beads coated with *Staphylococcus aureus* and *Streptococcus pneumoniae* antigens for the detection of serum antibodies. In the present study, we compared the performance of the Luminex assay based on the MicroPlex beads with that based on the newly developed MagPlex beads (Microplex beads imbedded with superparamagnetic particles). In addition, we adjusted the MagPlex beads coupling procedure and Luminex assay for *S. pneumoniae* antigens for our purposes and as a proof of concept, we measured IgG values from human pooled serum against a series of *S. pneumoniae*-derived antigens.

Material/Methods: A series of *S. pneumoniae* antigens were coupled to either Microplex or MagPlex beads at different concentrations (ranging from 2 µg-5 µg/10^6 beads). The final bead concentration and bead count per assay varied from 500-3000 beads/color and from 50-100 beads/color, respectively. We assessed the IgG (derived from human pooled serum) binding to these *S. pneumoniae* antigen coated beads and we analyzed the samples on a Luminex BioPlex 200 System.

Results: Luminex assays using the MagPlex beads compared to Luminex assays using MicroPlex beads showed: i) in the majority of the cases (13 of the 17 tested *S. pneumoniae* antigens) significantly higher IgG values, ii) lower detection limits, iii) lower coefficient of variation (CV: 12% vs 7% for MicroPlex vs. MagPlex) so lower inter-assay variation and higher reproducibility. Finally, MagPlex bead coupling procedure is very cost effective, as we used ¼ of the antigen and ½ of the beads in comparison to MicroPlex assay.

Conclusions: This optimized MagPlex Luminex technology combines ease of use with an improved assay performance. Luminex technology in combination with MagPlex beads allows a detection of antibodies with a low titer that are missed with the MicroPlex Luminex technology.

P087

Lysine and fructoselysine fermentation by Intestinimonas AF211

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Introduction: Human intestinal bacteria produce butyrate that impacts colonic health since it has enterocyte fuelling as well as signalling properties. However, the way intestinal butyrate is produced, from what substrates, and by what bacteria is not fully understood.

Methods: We used stool samples that are easy to collect, to isolate novel butyrogenic bacteria using defined strict anaerobic mineral salt media as a selective factor. Subsequently, the butyrogenic pathways of this isolate were investigated employing genome, proteome and metabolome analysis in combination with ^{13}C NMR of ^{13}C-labeled isotopomer and activity assays.

Results: We isolated a butyrate-producing bacterium, strain AF211, belonging to the new genus *Intestinimonas* from the human intestine that can convert lysine stoichiometrically into butyrate and acetate when grown in a synthetic medium. Moreover, *Intestinimonas* AF211 was found to convert the Amadori product fructoselysine, which is abundantly formed in heated foods via the Maillard reaction. The lysine and fructoselysine pathway was elucidated. The genomic analysis indicated the presence

of many copies of CoA transferase genes; however, the proteomic analysis showed that only three CoA transferases were overproduced during growth on lysine. The genetic coding capacity for the lysine conversion is present in most of the colonic samples of healthy dutch adults while that of the fructoselysine conversion is less abundant and only found in a small fraction of these. This is confirmed by enrichments on lysine from fecal samples, most of which showed butyrate production.

Conclusion: Our results indicate that lysine and fructoselysine can serve as a source of butyrate in the human colon.

Po88

Ecology of nitrogen-fixing methanotrophs associated with *Sphagnum* peat mosses

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Peatlands are wetland ecosystems that store 33% of the world's terrestrial carbon, while covering only 3% of the Earth's surface (Gorham 1991). On the other hand, natural wetlands are an increasing source of greenhouse gasses (Kirschke et al. 2013) and therefore contribute to global warming. The role of the microbiome of *Sphagnum* peat mosses is becoming more evident and the interplay between microorganisms and peat mosses may be very important in the production and conversion of greenhouse gasses like CO_2, CH_4 and N_2O. It is very likely that the epi- and endophytic microorganisms support the important role of bryophytes in this ecosystem.

The exchange of important and/or limiting nutrients, between plants and microorganisms is of high importance for these ecosystems. Methane oxidized to CO_2 by *Sphagnum* associated methanotrophs can provide up to 15% of the carbon assimilated by *Sphagnum* during photosynthesis (Raghoebarsing et al. 2005, Kip et al. 2010). Furthermore several studies have shown that the nitrogen acquisition by mosses over time is too high to be solely originating from atmospheric deposition (Vile et al. 2014, Larmola et al. 2014), and nitrogen-fixing microorganisms (diazotrophs) could be the potential missing link in this discrepancy (Ho et al. 2015; Kox et al. *Subm.*; van den Elzen et al. *Subm.*).

Our goal is to characterize the diazotrophic and methanotrophic community of Sphagnum mosses and to determine whether (diazotrophic) methanotrophs contribute significantly to the *Sphagnum* mosses' N- and C-content. Therefore we compared the microbial community of peat mosses originating from N-rich (the Netherlands) and N-poor (Finland) peatlands after DNA extraction and amplicon sequencing of 16S rRNA, and functional genes (pmoA & nifH). In addition we isolated nitrogen-fixing methanotrophs and heterotrophs from these mosses by extinction dilution series on nutrient poor media followed by plating on agarose and floating filters. Furthermore, the *in situ* activity and the activity of the isolated microorganisms was investigated by performing assays using labeled isotopes. Heavy labeled dinitrogen gas $^{30}N_2$ was used to monitor diazotrophs, and ^{13}C labeled carbon compounds to monitor methanotrophs and heterotrophs.

Isotope-tracer studies showed that methanotrophs and diazotrophs were active in all of the studied sites. The diazotrophic activity was neither affected by methane addition, nor by reduced oxygen concentration. These results indicate that methane dependent nitrogen fixation may only be important under certain environmental conditions in *Sphagnum*-dominated peatlands. Furthermore, community analysis revealed that the *Sphagnum*-associated community mainly consistsed of Proteobacteria in both sites. The N-poor site contained a larger fraction of Verrucomicrobia than the N-rich site, which indicates that these microorganisms thrive best under nutrient limited conditions. Gamma- and alphaproteobacteria made up the largest part of the methanotrophic community in both sites, whereas cyanobacteria and Burkholderia were the most abundant diazotrophs. Further studies on enriched and isolated (diazotrophic) methanotrophs will give us more insight into the ecophysiology of *Sphagnum*-associated (diazotrophic) methanotrophs and will ultimately help to understand their ecological significance.

Po89

Development of the Netherlands Donor Feces Bank for fecal microbiota transplantation

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Introduction: Recurrent *Clostridium difficile* infection (CDI) is difficult to treat and failure rates for antibiotic therapy are high. Fecal Microbiota Transplantation (FMT)

is however proven to be very effective with cure rates close to 90% and is considered as standard therapy in CDI treatment guidelines. The effect of FMT against other dysbiosis-related disorders is currently extensively investigated. Protocols concerning donor screening, fecal preparation and FMT administration differ between countries and institutions, and internationally standardized guidelines have not yet been established. In addition, donor feces is not easily available. Supported by a ZonMW grant, we developed a National Donor Feces Bank, in order to provide a standardized product for treatment of patients with recurrent CDI.

Methods: A literature search was performed to collect information about available standard operation procedures for production of fecal suspensions for transplantation. Using experiences obtained during the only conducted randomized controlled FMT trial; the FECAL trial, combined with expert opinions of gastroenterologists, infectiologists, biobank experts and microbiologists, a protocol was developed for the Netherlands Donor Feces Bank.

Results: Donors are recruited from the Leiden blood bank and extensively screened by questionnaire for factors that can affect a healthy microbiota. Laboratory analysis is performed for (potential) fecal or blood transmitted diseases. Additionally, donors give permission to the blood bank to share results from blood donor screening tests with the national feces bank. Donor feces is collected and processed within 2 hours after defecation. The feces is homogenized with saline, sieved and concentrated by centrifugation. The cryoprotectant glycerol is added to a final concentration of 10%, and stored at -80offlC for a maximum of 2 years. The Leiden University Medical Centre Bio bank facilitates storage and delivery of the products. A portion of the donor feces and fecal suspension is stored to ensure traceability in case of an adverse event. After a second negative screening of the donor 2 months later, the donor fecal suspension is released for patient treatment. After consultation and approval by the FMT team, the requested frozen suspension is delivered to physicians throughout the Netherlands on dry ice. FMT training sessions are offered at hospitals with FMT experience. Administration of the fecal suspension via nasoduodenal route is recommended, avoiding the risk of colonoscopy and anaesthesia. The Netherlands National Donor Feces Bank will actively collect follow up data of patients receiving FMT.

Conclusion: A standardized FMT protocol was developed ensuring a safe treatment of recurrent CDI. Long-term follow-up of patients and donors is included in the procedure. The FMT database can also be used for further research, including microbiota analysis.

P090

Experimental evolution of no-cost resistance at sub-MIC concentrations of streptomycin in Streptomyces coelicolor

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Introduction: At the high concentrations used in the clinical environment, antibiotics exert strong selection on bacterial populations for the evolution of resistance. However, it is likely that these lethal concentrations are not representative of the concentrations bacteria face in soil, where endogenous antibiotic-production occurs at much lower levels. This recognition has lead to questions of the role of antibiotics in soil environments as well as the dynamics of resistance evolution during sub-lethal antibiotic challenge. Here we examine the evolution of resistance to sub-MIC concentrations of the aminoglycoside antibiotic streptomycin in the filamentous soil bacterium *Streptomyces coelicolor.*

Methods and Results: First, we show that spontaneous resistance to streptomycin is highly costly to cells in the absence of drugs, causing an average fitness deficit of ~21%; however, these costs are entirely eliminated at concentrations as low as 1/10 the MIC of susceptible strains. Next, using an experimental evolution approach, we show that *de novo* resistance can readily evolve at these low, non-lethal doses and more importantly, that this resistance is cost-free.

Conclusion: Resistance acquired at high streptomycin concentrations is highly costly, while, in stark contrast, *S. coelicolor* strains that evolve resistance at sub-MIC doses bear no fitness costs of streptomycin resistance. Our results significantly broaden the conditions under which resistance can evolve in nature and suggest that the long-term persistence of these strains is facilitated by the absence of pleiotropic fitness costs. Finally, our data cast doubt on arguments that low-concentration antibiotics in nature are signals, instead supporting alternative models that resistance evolves in response to antibiotics used as weapons.

P091

Mimicking classical complement activation in purified model systems

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The complement system plays an important role in the innate immune response to microbial infections.

Complement proteins label bacteria for phagocytosis and form chemo-attractants to recruit immune cells to the site of infection. Furthermore, complement proteins can assemble into membrane attack complexes (MAC) to directly lyse Gram-negative bacteria.
The most important event of the complement cascade is formation of C3 convertases on the bacterial surface for cleavage of C3 to opsonin C3b. At high C3b density, the C3 convertases can turn into C5 convertases that cleave C5, the second major protein in the cascade. As the convertases are exclusively formed on (microbial) surfaces detailed analysis of these complement complexes is difficult.
In this study, we aim to elucidate the molecular details of complement activation via antibodies (classical pathway) and the formation of C3 and C5 convertases. We developed a bead-based system to study these events on a surface and in a purified and controllable environment. By labeling bacteria-sized streptavidin beads with biotinylated complement opsonins or antibody epitopes, we are able to induce step-by-step formation of functional (classical pathway) C3 and C5 convertases on the bead surface. Similarly, we intend to use streptavidin-coated bacteria to study these complement events on a more natural surface. A better understanding of complement activation in the classical pathway is of great importance for development of antibody therapies against pathogenic bacteria. Using the bead- and bacterium- based models we can now examine the complement cascade step by step and in molecular detail. Moreover, the model systems provide a way to study the effect and mechanisms of bacterial complement evasion molecules.

P092

Nitrogen cycle in the Bothnian Sea sediment: a metagenomic perspective

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The biological nitrogen cycle is driven by a plethora of reactions transforming nitrogen compounds between various redox states, which can be performed by a variety of different microorganisms. Whereas some of these processes are fairly widespread (e.g. denitrification), some others are only restricted to narrow phylogenetic guilds (e.g. anammox). Here we investigated the metagenomic potential of nitrogen cycle of the *in-situ* microbial community in an oligotrophic, low salinity environment of the Bothnian Sea sediment Total DNA from three depths below the sediment surface was isolated and sequenced with the Ion Torrent technology. The characterization of the total community was performed based on 16S rRNA gene inventory using SILVA database as reference. The diversity of diagnostic functional genes coding for nitrate reductases (*napA*;*narG*), nitrite reductases (*nirK*;*nirS*;*nrfA*), nitric oxide reductase (*nor*), nitrous oxide reductase (*nosZ*), hydrazine synthase (*hzsA*), ammonia monooxygenase (*amoA*), hydroxylamine oxidoreductase (*hao*) and nitrogenase (*nifH*) were analyzed by blastx analysis against curated reference databases. In addition, PCR-based amplification was performed on the *hzsA* gene of anammox bacteria. Our results reveal high genomic potential for full denitrification to N_2, but minor importance of anaerobic ammonium oxidation (anammox) and dissimilatory nitrite reduction to ammonium. Genomic potential for aerobic ammonia oxidation was dominated by *Thaumarchaeota* while bacterial *amoA* genes were scarce at al sediment depths. In general, phylogenetic composition of core microbial communities correlated well with biogeochemical characteristics of particular depths. Moreover, despite their lower abundance in the Bothnian Sea sediment, we detected a higher diversity of anammox bacteria in metagenomes than with PCR-based technique. Our metagenome results reveal the quantitative importance of various N-cycle driving processes and highlight the advantage in detection of novel microbial key players which might be overlooked by using traditional PCR-based methods.

P093

Impact of temperature on microbial community dynamics in bioreactors producing polyhydroxyalkanoates

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Polyhydroxyalkanoate (PHA) production by mixed microbial communities is a promising and cost-effective approach, because it circumvents cost-intensive procedures like genetic engineering of microorganisms or operating bioreactors under sterile conditions. In order to further increase the general competitiveness, it is crucial to enhance the PHA production of those microbial communities. One approach is to optimize the selective conditions for superior PHA producers. In this study the effect of different temperatures (30 and 40°C) on microbial dynamics was assessed by means of Next Generation Illumina paired-end sequencing of the V3-V4 region of the 16S rRNA gene. Sequence batch bioreactors operated under 'feast and famine' conditions and acetate as a carbon source were used for selection of a microbial community with high PHA production. Parallel bioreactors were operated as replicates to investigate the reproducibility.
The inoculum provided 80% of the abundant biosphere recovered in all bioreactors, implying the absence of contamination during operation time. Microbial diversity between both temperature experiments differed

strongly, showing a lower diversity at 40°C than at 30°C. Predominant OTUs (operational taxonomic units) at 40°C consisted of *Chryseobacterium* spp. (9 - 73%) and *Acinetobacter baumannii* (8 - 79%), while at 30°C *Chryseobacterium pallidum* (1 - 23%), *Flavobacterium* sp. (4 - 22%) and different *Acinetobacter* species (1 - 73%) dominated. The contribution of these abundant OTUs differed strongly in some phases of the experiment. In both experiments the biological replicates showed a similar community structure after one day of incubation, although the structure was different between the two treatments. Thereafter, the community structures diverged, and biological replicates differed. At the end of the experiment the structures in the 30°C bioreactors were different, while in the 40°C bioreactors they were similar.
Concluding, the difference in microbial community structures at 30°C and 40°C showed that temperature is a strong selective force, able to shape the community of PHA producers. Variations within the abundant populations implied an unstable community. The different community structure at the end of the experiment indicated a diverging effect for the microbial communities incubated at 30°C, and a converging effect for the microbial communities incubated at 40°C.

P094

Impact of staphylococcal complement inhibitors on *S. aureus* infection in zebrafish

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The complement system can be activated by three different pathways which all converge at the generation of a C3 convertase. C3 convertases cleave complement component C3 into the opsonin C3b and the chemo-attractant C3a. Subsequently recruited and activated neutrophils show enhanced phagocytosis and killing efficiency. To circumvent recognition by the complement system and avoid neutrophil activation, *S. aureus* secretes several proteins. Usually, they are highly host-specific like SCIN (staphylococcal complement inhibitor) which generates a complex with C3b and Bb and thereby inhibits the formation of further C3 convertases and opsonisation by C3b. This host-specificity made it difficult to use animal models but during the last years, the zebrafish (*Danio rerio*) came into focus since it allows faster genetic modifications than conventional murine model systems. Therefore, the question arose whether it is possible to humanize the zebrafish to use it as an *in vivo* system for interaction of the complement system with *S. aureus*. The final aim would be the rebuilding of the human interaction $(C3bBb\text{-}SCIN)_2$ in zebrafish. Since this interaction is an intricate tripartite complex, we first aimed to confirm the general complement inhibitory activity of Efb (extracellular fibrinogen binding protein) and Ecb (extracellular complement binding protein) in a zebrafish infection model. The staphylococcal proteins Ecb and Efb seem to be less host-specific as previous mouse experiments showed. In contrast to SCIN, they block the C3b-containing convertases of the alternative pathway only.
To address this question 48 hpf *Danio rerio* larvae (*Danio rerio* Casper line, pMPO:gfp) were injected into the 4th hindbrain ventricle either with 850 cfu wild type *S. aureus* Newman, Newman f*ecb,efb* or co-injected with Newman and recombinant Efb/Ecb (0.5-0.25 ng/larvae). Neutrophil migration was assessed by fluorescence microscopy 3 h post injection and quantified with ImageJ. Experiments were performed with two independent replicates; 15-25 larvae were used per group. Statistical analysis (one-way-ANOVA with Tukey's post test) was carried out with GraphPad Prism 6. Injection of both Newman and Newman f*ecb,efb* lead to significantly increased neutrophil influx into the 4th hindbrain ventricle compared to the PBS-injected control group. However, there was no difference in neutrophil migration between Newman-injected and Newman f*ecb,efb*-injected larvae. In contrast, co-injection of Newman and Ecb/Efb significantly reduced the neutrophil influx into the hindbrain when compared with injection of Newman solely. Administration of the recombinant proteins only resulted in neutrophil migration similar to the PBS-injection.
Although the evolutionary distance between zebrafish and men is quite long, the complement components are structurally conserved. The complement-inhibitory property of Ecb and Efb was shown in the fish by reduced neutrophil migration upon co-injection of the proteins with Newman compared to injection of bacteria only. The reasons why injection of wild type and Efb/Ecb knock-out *S. aureus* induced similar levels of neutrophil influx may be various. It is conceivable that the time span between injection and complement activation in the fish might be too short for the bacteria to secrete sufficient amounts of those immune evasion molecules.

P095

Brachyspira pilosicoli is associated with diarrhea in dogs

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Background: *Brachyspira pilosicoli* is a gram-negative spirochete that can colonize the large intestines of both humans and various animal species. The role of *B. pilosicoli* as a causative agent of enteritis is well established in pigs and poultry and there are strong indications that *B. pilosicoli* is zoonotic and can cause intestinal spirochetosis in humans. Although the presence of *B. pilosicoli* has been described in dogs, little is known about the pathogenic potential of *B. pilosicoli* in dogs. Therefore, the aim of this study was to investigate the association between *B. pilosicoli* and the occurrence of diarrhea in dogs.
Method: Faecal samples from dogs without diarrhea (n = 446) were collected between 2011-2014 from a study in Dutch household dogs. Samples from Dutch household dogs with diarrhea (n = 446) consisted of faeces submitted to the Veterinary Microbiological Diagnostic Center of Utrecht University for routine microbiological/parasitological diagnostics between June 2015 and August 2015. DNA was extracted and amplification was performed with a newly designed SYBR Green based *B. pilosicoli* specific PCR on the NOX gene and the 16S rDNA gene. Dogs were regarded as positive if PCR results tested positive for both genes. SPSS (version 21) was used to test the association between *B. pilosicoli* and the occurrence of diarrhea in dogs.
Results: The NOX and 16S PCRs for *B. pilosicoli* was positive (Ct < 40) for only 0.2% (1/446) of the non-diarrheic dogs. In contrast, the dogs with diarrhea showed an overall positivity rate of 6.5% (29/446). The prevalence of *B. pilosicoli* in dogs with diarrhea was thus significantly higher ($p < 0.05$) than in healthy dogs.
Discussion and Conclusion: While the feces of 29/446 dogs with diarrhea were positive in both PCRs, only 1/446 dogs without diarrhea was positive. We did however observe a slight difference in the outcomes of the two independent PCR assays as the feces of three dogs from the diarrhea group were PCR positive, albeit for the 16S PCR only. As the Ct-values for all three discrepant samples was >35 we believe this to be a sensitivity issue. We considered these three dogs as negative. Including them in our statistical analysis only increased the observed association. To our knowledge, this is the first large study providing a statistically significant association between the presence of *B. pilosicoli* and the occurrence of diarrhea in dogs. Further investigations are required for definitive proof.

P096

High number of discrepancies in routine antimicrobial susceptibility testing for meropenem

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Introduction: Carbapenem resistance in Enterobacteriaceae and Pseudomonas is a serious concern for health care. Carbapenem resistance can be caused by different resistance mechanisms. In the Netherlands, microbiology laboratories are requested to send Enterobacteriaceae with elevated MICs for carbapenems to the national reference laboratory (RIVM), where carbapenemase genes are tested and the minimal inhibitory concentration (MIC) of meropenem is determined. In this study, results of meropenem routine susceptibility by Phoenix™ (Becton-Deckinson Diagnostic Systems, Sparks, USA) and by Etest® (Biomerieux, La Balme-les-Grottes, France) were compared with results of the RIVM to determine the acccuracy of routine susceptibility testing for meropenem.
Methods: National guidelines for screening for carbapenemase-producing Enterobacteriaceae were followed: In case Phoenix™ showed an MIC for meropenem >0.25 mg/L for Enterobacteriaceae, and/or, for *E.coli*, *Klebsiella* spp., *Enterobacter* spp. and *Citrobacter* spp. an MIC for imipenem > 1mg/L, E-test® was performed. If the elevated MIC was confirmed by Etest®, isolates were sent to the RIVM. Furthermore, Pseudomonas- isolates with meropenem MIC> 2 mg/L and/or imipenem MIC> 4 mg/L were sent to the RIVM after confimation of Phoenix™-MICs by Etest®. MIC results from RIVM were compared with results of Phoenix™ and Etest®. Essential agreement (EA), category agreement (CA), Major error(ME), Very major error (VME) and minor error (mE) were determined for Phoenix™ and Etest®.
Results: Over a period of two years, 94 isolates were sent to the RIVM. For 70 isolates (45 *Pseudomonas aeruginosa* and 25 Enterobacteriaceae), Phoenix™ was performed. Comparison with RIVM results showed EA for 20 strains (29%), CA for 43 strains (61%), ME for 1 strain (1%), VME for 1 strain (1%), and mE for 5 strains (7%). For 50 isolates, (41 *Pseudomonas aeruginosa* and 9 Enterobacteriaceae) Etest® meropenem was performed. Comparison with RIVM results showed EA for 28 strains (56%), CA for 12 strains (24%), ME for 2 strains (4%) and mE for 8 strains (16%).
Conclusion: Comparison of routine susceptibility results for meropenem with MIC results of the RIVM shows a high number of discrepancies for Phoenix™ as well as for Etest®. This is in agreement with literature where problems with carbapenem susceptibility in routine diagnostics are described. Based on the results there is a clear evidence showing the bad performance of Etest®. This raises a relevant question whether confirmation of meropenem- susceptibility results of automated systems by Etest® should be included in carbapenemase screening. This study shows the importance of sending strains with elevated MICs for carbapenems to the reference laboratory. In addition, a broad evaluation for discrepancies may be of added value for interpretation of carbapenem resistance.

P097

Performance of a molecular diagnostic, multicode based, sample-to-answer assay for the simultaneous detection of Influenza A, B and Respiratory Syncytial Viruses

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Introduction: Rapid diagnostics is required in cases with respiratory failure for clinical decision making regarding isolation and antiviral therapy. Techniques like immune-chromatographic test (ICT) and direct immunofluorescence assay (DFA) have lower sensitivities and specificities than molecular diagnostic assays, but have the advantage of quick turnaround times and ease-of-use. Here, we evaluated the performance of an automated, easy to use, sample-to-answer system, which preforms an Influenza Avirus (flu, respiratory syncytial virus (RSV) and internal control multiplex RT-PCR of 1-12 samples within 2 hours.
Methods: The analytical performance of the FluA/B/RSV assay on the Aries (Luminex), a system using multicode technology (a probe-free real-time RT-PCR method with melting curve confirmation), was evaluated using published laboratory developed automated real-time RT-PCR assays (LDA) for fluA, fluB,, RSV-A and RSV Genotype inclusivity of the Aries was tested using 16 avian (H1-H16) and 33 human fluA strains, 3 fluB strains and the two RSV (strains. Specificity was assessed using 40 high positive non-fluA/fluB/RSV-viruses and analytical sensitivity was compared to LDA assays by testing 0.5 log dilution series. The clinical performance was compared to both LDA + ICT (BinaxNOW influenza A/B and RSV test) + DFA using selected (pretreated), -80°C stored, respiratory tract samples from 2006 until 2015 (retrospective) and prospective testing of original respiratory tract samples from December 2015 onwards.
Results: All fluA, fluB and RSVAstrains tested for analytical performance evaluation were detected and no aspecific reactions were identified. Aries FluRSV assay was 0.5 log less sensitive for fluA, 1 log for RSV-A, 2 logs for RSV-B and 2.5 logs for fluB compared to LDA. In total, 227 samples were included in the clinical performance evaluation, of which 17.9% tested positive for fluA, 14.2% for fluB and 42.7% for RSV, (RSV-A , 24.5% and RSV-B 20.4%) in both LDA and Aries. Confirmed discrepant results were found in 7 samples (3 fluB and 4 RSV-A), which tested positive in LDA and negative in Aries (3.1%, LDA Ct values 27.9 -33.9), resulting in an overall clinical sensitivity and specificity of 100 and 100% for fluA, 91.2 and 100% for fluB and 96.0 and 100% for RSV, respectively. If compared to the DFA (n = 156) and ICT (n = 116), Aries detected 17 (10.6%; 3 fluA, 5 fluB, 9 RSV) and 32 (28.1%; 7 fluA, 3 fluB, 22 RSV) more samples respectively, all confirmed by LDA (Ct range 16.5-31.6). In terms of robustness, 1.8 % cassettes failed during operation.
Conclusion: 1. The Aries influenza A/B/RSV assay is a specific and rapid molecular assay. 2. Although analytically the Aries is less sensitive for fluB and RSV-A and RSV-B than the LDA assays, the performance in clinical samples is comparable to LDA and better than those of the established rapid assays.

P098

Evaluation of five commercially available chikungunya serology tests on samples of travelers returning from endemic regions with symptoms of a possible chikungunya virus infection

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Introduction: Chikungunya virus (CHIKV) infections among travelers returning from endemic CHIKV regions has increased in our hospital due to the recent outbreak in the Caribbean's and its further spread through North and South America. Symptoms include acute febrile illness, typically accompanied by a rash and arthralgia. Arthralgia can be severe and last for months to more than a year. The increase of returning travelers with a possible chikungunya infection has led to the demand of fast and reliable chikungunya serology in our hospital. Therefore, we set out to test different CHIKV serology test to select the optimal test for CHIKV diagnostics in our patient group.
Method: The following IgM and IgG CHIKV ELISAs were tested: Euroimmun, IBL, Novalisa, Abcam. In addition, a rapid diagnostic test of Standard Diagnostics was included which only screens for CHIKV IgM. We retrospectively selected samples from returning travelers presenting themselves in the AMC with symptoms and travel history suggestive for a CHIKV infection and a performed immunofluorescence test (in the virology laboratory in the Erasmus Medical Center). In addition, we included different control panels, such as patients with a confirmed dengue virus or Epstein-barr virus infection or autoimmune disease. In total 85 samples were tested. True positives were defined as positive in the majority of the tests (IF and ELISAs). In case of a grey zone result the sensitivity and specificity was calculated twice, as being positive or negative, resulting in a worst and best case sensitivity and specificity.
Results: The IBL and NovaLisa ELISAs had similar results, also the test kits looked alike. After discussing this with one of these companies these assays turned out to be identical. Thus in the analysis we combined the IBL and NovaLisa test results. Abcam IgM and the RDT had a relative poor sensitivity of 62-77% and 74% respectively. The specificity of the IgM Abcam and RDT was 96-98% and 87% respec-

tively. Euroimmun, IBL/NovaLisa IgM and IgG and Abcam IgG all had a good sensitivity and specificity of at least 88%. The RDT was the fastest test to perform. The Euroimmun ELISA IgM and IgG had one incubation step of 30 minutes less compared to the other ELISAs.

Conclusion: For CHIKV serology testing in our laboratory we selected the Euroimmun IgM and IgG ELISAs, because these tests have a good sensitivity and specificity in our patient population (sensitivity IgM 100 %, IgG 96% and specificity IgM 94%, IgG 98%). Also the 'time-to-result' was shorter compared to the IBL/NovaLisa ELISAs.

P099

Reversible metamorphosis in *Streptomyces*

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Streptomyces are filamentous bacteria that grow by extension of hyphae at the tip. New hyphae arise as branches from pre-existing ones by the establishment of new polarity centers and subsequent outgrowth. This leads to the establishment of a complex and multicellular network, called a mycelium. DivIVA, a tropomyosin-like protein, orchestrates this apical growth. To gain further insights in this atypical mode-of-growth, we have recently generated so-called Streptomyces L-forms, which can proliferate without the peptidoglycan layer that normally envelopes the hyphae. As a consequence, such cells are round and lack any obvious form of polarity. We reasoned that DivIVA might be obsolete in such L-form cells, and in order to confirm this, we deleted the *divIVA* gene, as well as a larger part of the conserved gene cluster of which divIVA is a member. In both L-form strains, no growth defects were observed. However, unlike the parental L-form strain, reversion to the mycelial form was blocked in the absence of *divIVA*, while an additional copy in the parental L-form strain increased the reversion frequency. Reintroduction of the *divIVA* gene at an ectopic locus in the deletion mutant restored the capability to initiate polar growth, although the frequency of reversion to mycelium was greatly reduced. We are currently analyzing if this reduction is due to changes in the expression level of the *divIVA* gene at the ectopic locus. Altogether, these results unambiguously show that DivIVA is crucial for initiation of mycelium formation.

P100

Retrieval of chronic Hepatitis C infection in a tertiary medical center

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Introduction: Recent advantages in the development of antiviral agents have revolutionized treatment for chronic hepatitis C infection. New treatment strategies have high success rates and are well tolerated, thus broadening the indication for treatment. However, the number of patients who might benefit from new treatment strategies is unknown, as patients with chronic hepatitis C infection might remain undetected, or, if detected, might not be under medical care. Public health benefits are therefore difficult to estimate. At the Leiden University Medical Center (LUMC) we explored one approach of structured case finding of previously diagnosed but untreated hepatitis C patients.

Methods: *Patient detection*: A query was performed using the electronic laboratory information management system to identify all unique patients who tested positive for hepatitis C (positive antibody test and/or PCR detection of RNA) in the period June 2007 - June 2015.

Patient evaluation: Current patient status was evaluated using the electronic patient record and personal communication with the last known treating physician at the LUMC. Patients were either defined as under medical care (i.e. in contact with a hepatitis specialist in 2015 or documented SVR), deceased or with unknown status. From the population with unknown status, a random sample was taken to contact the general practitioner (GP) to update patient status. If the patient was known to the GP and had not yet been treated successfully, we discussed the option of renewed referral to a hepatitis specialist.

Results: The query identified 628 unique patients. For 238 patients, diagnostics were performed as a specialist back-office service for external laboratories, implicating clinical responsibility in a different medical institution. 393 patients were tested at the request of physicians at the LUMC. Of these 393 patients, 257 were under medical care, 57 had died and 79 patients had an unknown status. After detailed personal review with the most recent treating physician, status of 50 patients remained unknown. As a pilot-study, the GP was contacted for 8 of these 50 patients, yielding 1 case in which the GP would discuss referral. The remaining 7 patients had either died (1), already been treated (1), were no longer known to the GP (3) or the GP suggested the LUMC contact the patient directly (2).

Conclusion: Extrapolation of results from our pilot-study in contacting GP's shows a hypothetical yield of 6 - 18 out of 393 patients (1.5 - 4.5%) that are eligible for renewed referral, but we aim to confirm this number in a follow-up project including all 50 unknown cases. How many of these will subsequently start and finish treatment successfully remains unknown. Though a relatively low yield, some may benefit from active retrieval. Regional agreements are now under way to guide referral and collaboration with GP's.

P101

Methicillin-resistant *Staphylococcus aureus* in primary care: the Dutch landscape

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Introduction: While the prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) in Dutch hospitals is consistently <1%, it varies considerably by country with rates of up to 20% in neighboring countries. Traditionally, MRSA has been considered a hospital-associated pathogen, but MRSA has now also emerged in the community setting (CA-MRSA) and in livestock (LA-MRSA). Infections are no longer confined to healthcare settings, but may also appear in healthy community-dwelling individuals without known risk factors for MRSA acquisition. Little is known about the occurrence of MRSA infections in Dutch primary healthcare. Therefore, we used national antimicrobial resistance surveillance data to describe the epidemiology of MRSA among patients visiting a general practitioner (GP) and to study regional trends.

Methods: The Infectious Disease Surveillance Information System for Antibiotic Resistance (ISIS-AR) database was used to select clinical *Staphylococcus aureus* isolates from patients visiting a GP between January 2008 and December 2014. Only the first isolate of *S. aureus* per patient per year was selected. Twenty-seven laboratories, which continuously provided data throughout the study period, were selected. We estimated the proportion of resistance and linear time trends over the years 2008 to 2014 and per province using logistic regression.

Results: In total 54,808 unique *S. aureus* isolates were included. The overall proportion methicillin resistance in *S. aureus* was 1.5% (n = 799), and ranged from 0.9% in Utrecht province to 2.1% in Noord-Brabant. The proportion of MRSA was higher in men than in women: 1.8% versus 1.2%, respectively ($p < 0.001$). Mean age of MRSA positive patients was 48.7 years versus 44.9 years in MSSA patients ($p < 0.001$). There were no significant differences in the proportion MRSA between adult age groups (18-40, 41-64 and >64 years) whereas the proportion MRSA among patients between the age of 0 to 7 years was significantly lower (1%, $p < 0.001$). The proportion MRSA among rural residents (1.7%) was higher than among city residents (1.3%) ($p < 0.001$). The national six-year trend showed a slight increase ($p = 0.080$). A significantly increasing six-year trend of MRSA was seen in province Groningen ($p = 0.003$), whereas a decreasing trend was seen in province Zeeland ($p = 0.021$). Over the years, the proportion of MRSA in wound or pus samples increased from 1.6% in 2009 to 2.0% in 2014 ($p = 0.017$). For 594 (74.3%) MRSA isolates information about genotyping was available, of which 118 isolates (19.9%) belonged to the MLVA-complex MC398 (defined as LA-MRSA).

The proportions of co-resistance in MRSA was 35% for ciprofloxacin, 23% for co-trimoxazole, 11% for clindamycin and 16% for fusidic acid. Susceptibility to all these antibiotics remained stable over the six-year period.

Conclusion: The proportion methicillin resistance in *S. aureus* from clinical samples from patients from Dutch GPs remains low (1.5%) and varies by province. The increasing rate of MRSA from wound or pus samples could be an indication of increasing clinical significance.

P102

Registration of proposed interventions and measured effects on antimicrobial prescribing behaviour following antimicrobial stewardship in a Dutch general hospital

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Introduction: Antimicrobial Stewardship was introduced in 2014 in a Dutch general hospital (14.000 admissions including 6000 day care stays). The goal was to optimize antimicrobial prescribing in order to improve individual patient care, to decrease the spread of antimicrobial resistance and to reduce adverse events, e.g. infections with Clostridium *difficile*. The effectiveness of the antibiotic team (A-team) and the new antimicrobial formulary was measured by registration of the interventions and the antibiotic use over 2014 and 2015.

Methods: Strategies for changing antimicrobial prescribing behaviour included education of prescribers regarding the new antimicrobial guidelines and stimulation of the appropriate use of antimicrobial drugs. 1. The antimicrobial committee created a new antimicrobial formulary with restricted prescribing of antimicrobial agents. 2. The A-team was installed, including an infectious diseases specialist, a hospital based pharmacist and a medical microbiologist. 3. The A-team visited all clinical wards twice a week. All hospitalized patients aged 18 years and above receiving antibiotics were discussed. The current results of the microbial diagnostic tests were available. The A-team started reviewing antimicrobial prescribing with feedback to prescribers in Q1 2014 and the registration of five different interventions in Q3 2014. At the end of 2014 the A-team aimed to reduce the use of amoxicillin/clavulanic acid because of the increasing resistance of microorganisms. Amoxicillin/clavulanic acid was removed from the antibiotic formulary as much as possible and

the change was discussed with the prescribers. The prescribing behaviour of amoxicillin/clavulanic acid was measured by registering the dispensing of the antibiotic from the hospital pharmacy to the department stocks of the hospital wards. The dispensing to individual patients from the hospital pharmacy was not registered.

Results: In 2014 and 2015, respectively 413 and 416 patients were discussed by the A-team and the interventions registered (table 1). The majority were internal medicine, surgery and orthopaedic patients. The decision to continue the antibiotic regime was not registered. The dispensing of intravenous amoxicillin/clavulanic acid decreased 49% in 2015 (from 1992 to 1014 DDD). The antibiotic use density (DDD/100 patient days) was altered 49% too. The restricted use of ciprofloxacin did not change significantly.

Conclusion: The A-team had direct control over antimicrobial use, resulting, if necessary, in switch or stop of the prescribed drug. The mean intervention per patient was reduced from 0.51 to 0.41. The decision making process of switching the antibiotic drug (mostly it was streamlining to an antibiotic with smaller spectrum) and of determining a quit date for the antibiotic regime was more and more delayed until the day the A-team visited the ward. The reduction from 37,0% to 13,5% of the frequency of immediate stopping of the prescribed antibiotic by the A-team and the reduced prescribing of intravenous amoxicillin/clavulanic acid demonstrate a positive change in antimicrobial prescribing behaviour.

P103

Pneumococcal colonization and invasive disease studied in the porcine model

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Introduction: *Streptococcus pneumoniae,* a Gram-positive bacterium carried in the human nasopharynx, is an important human pathogen causing mild diseases such as otitis media and sinusitis as well as severe diseases including pneumonia, meningitis and sepsis. Piglets are the natural host for *Streptococcus suis* infections. There is a strong resemblance between the anatomy, immunology and physiology of the pig and human species. Furthermore, there are striking similarities between *S. suis* pathogenesis in piglets and *S. pneumoniae* pathogenesis in humans. We here investigated the use of piglets as a model for pneumococcal colonization and invasive disease.

Methods: Twenty piglets were purchased from a pre-screened farm with a defined microbiological porcine pathogen free status. At the start of the experiment tonsil swabs of piglets were screened for the presence of *S. suis*. Animals negative for the most prevalent serotypes were selected. Piglets were inoculated with *S. pneumoniae* at the age of 5 weeks, either intravenously (10 pigs) or intranasally (10 pigs), using two different doses. Intravenously inoculated piglets were injected with $4.2x10^6$ colony forming units (CFU) or $2.9x10^8$ CFU of *S. pneumoniae* strain PBCN214 into the vena jugularis. To establish colonization, piglets were inoculated intranasally with $2.5x10^6$ CFU or $2.9x10^8$ CFU of *S. pneumoniae* BHN418. Aerosols of the inocula were produced by a commercial, gravity-fed, single trigger airbrush with a nozzle of 0.2 mm, creating an aerosol with about 10% droplets which are smaller than 26 μm and about 50% of droplets which are smaller than 50 μm in diameter.

Results: Intravenous inoculation of piglets with an invasive pneumococcal isolate led to bacteraemia during 5 days, showing clear bacterial replication in the first two days. Bacteraemia was frequently associated with fever and septic arthritis. Systemic pro-inflammatory immune responses confirm the presence of pneumococcal induced inflammation in piglets. Intranasal inoculation of piglets with a nasopharyngeal isolate led to colonization for at least six consecutive days.

Conclusion: The data demonstrate that central aspects of human pneumococcal infections can be modelled in piglets enabling the use of this model for studies on colonization and transmission but also on development of vaccines and host-directed therapies. Moreover this is the first example of an animal model inducing high levels of pneumococcal septic arthritis. There is growing evidence that *S. pneumoniae* infections are associated with an increased risk for cardiovascular events and stroke. Since the porcine and human cardiovascular system are very similar, the porcine pneumococcal model can be used to gain insight into the pathogenesis of this serious health problem.

P104

Dynamics of complement-mediated pore formation and killing of Gram-negative bacteria

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The complement system consists of around 30 proteins present in human plasma that rapidly kills Gram-negative (GN) bacteria through pore formation via the membrane attack complex (MAC). Although complement-dependent killing is an evolutionary conserved and

highly effective mechanism to lyse bacterial cells, the dynamics of MAC-dependent killing of bacteria is only poorly understood. The widely used serum bactericidal assay provides limited information on dynamics of MAC-dependent killing. In the current study, we developed several methods to measure the killing of GN bacteria in more detail. We assayed pore formation using an impermeable dye that becomes fluorescent upon binding to DNA. Bacterial viability was measured by transforming bacteria with the lux operon of *Photorhabdus luminescens*. We focussed on *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*, three clinically important GN bacteria.

MAC-dependent pore formation occurred around 20 min after addition of serum to *E. coli* and *K. pneumoniae*, whereas for *P. aeruginosa* strain PAO1 this was much slower. We determined the bacterial viability of these bacteria at various serum concentrations by analyzing light production of the lux operon. Bacterial luminescence corresponded with bacterial killing as determined by the traditional plating method. In contrast to the plating assay, that only provides an endpoint measurement, the lux assay provided information on bacterial viability over time. This showed that viability depends on serum concentration and decreases over time, whereas with the plating assay discrimination was impossible since all bacteria were killed. When comparing pore formation with bacterial survival, there was a delay of approximately 30 minutes between start of pore formation and decrease in bacterial viability.

With the described assays we obtain more detailed information on MAC-mediated lysis of GN bacteria. Moreover, these assays can be used to determine bactericidal activity of serum in a time-dependent and high-throughput fashion.

P105

Bacterial diversity and pathogen distribution in wild symptomatic and asymptomatic *Acanthaster planci* (Echinodermata: Asteroidea) populations

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The sea star wasting disease that is devastating wild populations of asteroids is prompting greatly renewed interest in potentially pathogenic agents among wild sea stars. For crown-of-thorns sea star, *Acanthaster planci*, the occurrence of naturally-occurring pathogens has possible application for understanding and managing periodic outbreaks, which are a major contributor to coral loss at many reef locations. Symptomatic wild sea stars, exhibiting matting of spines, curling arms, melanisation of digestive glands, loss of skin turgor, lethargy and excessive mucous production were collected from the Great Barrier Reef, Australia. Additionally, asymptomatic crown-of-thorns sea stars at different life stages (juveniles – adults) were collected in Kimbe Bay, Papua New Guinea. Using Illumina 16S rRNA amplicon sequencing a comprehensive identification of the microbial community structure and pathogen distribution in wild asymptomatic and symptomatic *A. planci* was conducted. In total, twenty-four bacterial phyla were associated with all *A. planci* analysed. Our results show clear differences between symptomatic and asymptomatic *A. planci* bacterial assemblages based on 97% OTUs. High abundances of gammaproteobacterial bacteria that are well known echinoderm pathogens, such as *Vibrio* spp., *Pseudomonas* spp., and *Alteromonas* spp., are present within all individuals. Moreover, the Vibrionaceae composition significantly correlates with the *A. planci* health status.

P106

Type-Ned MRSA, implementation of national surveillance of methicillin resistant *Staphylococcus aureus* (MRSA) using electronic data exchange

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Introduction: Since 1989, the National Institute of Public Health and the Environment (RIVM) collects MRSA isolates for the national MRSA surveillance. Medical microbiology laboratories (MMLs) send the first MRSA isolate of a patient or healthcare worker to the RIVM for molecular typing with multiple-locus variable number of tandem repeat analysis (MLVA). Isolates are sent by mail accompanied by a paper form with data on the isolate and person from whom MRSA was isolated. In addition, a separate questionnaire with epidemiological data on risk factors for MRSA colonization or infection is filled out by infection control practitioners either on paper or via an electronic online system. To streamline MRSA surveillance and prevent unnecessary delays the MRSA surveillance was converted to a system with electronic data transfer to disclose results for MMLs and the health care institutes they serve.

Methods: Type-Ned, an acronym for the Dutch term 'Typeernetwerk Nederland' was built on the molecular platform software of the RIVM. Type-Ned does not require locally installed software, but access to the internet in essential. Usage starts with log in by the MML on the central, limited access Type-Ned MRSA database, to

electronically submit data on the isolate and on the person from whom the MRSA was isolated. After submission the software creates a unique Database ID which is printed as a barcode label and stuck on the agar plate containing the MRSA isolate. As soon as the Database ID has been issued, the infection control practitioner receives an email with a request to fill out the questionnaire in the same database. The labelled agar plate is sent paperless to the RIVM in a special envelope and at arrival the bar code is scanned to identify and register the isolate. Thereafter, MLVA is performed and the typing result will be available for the MML within 48 hours after the RIVM received the isolate.
Results: By January 2016, 32 MMLs had access to the Type-Ned MRSA database and 27 were actively sending in isolates via the Type-Ned system. The Type-Ned MRSA database has been filled with typing data from more than 29,000 MRSA isolates collected from all MMLs in the Netherlands since 01-01-2008. Consequently, MMLs can access their own historical data, including epidemiological data from their own isolates. In addition, queries can be performed on own and national data and results can be downloaded or analyzed in a geotool to map distribution of MRSA types in time and place.
Conclusion: Type-Ned MRSA is fully operational and is currently used by more than a third of MMLs in the Netherlands sending in over 50% of the Dutch MRSA isolates. It provides means for MMLs to actively analyze data to assess spread of MRSA at national level. Due to the rapid turnaround time of MLVA, they can also utilize the system to assess nosocomial transmission in health care centers. The system is generic and can be used for any pathogen. A Type-Ned system for carbapenemase-producing Gram-negative bacteria, currently operational in pilot phase, illustrates its potentially broad applicability.

P107

vPCR as a novel approach to evaluate *Chlamydia trachomatis* viability

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Introduction: *Chlamydia trachomatis* (CT) infections are the most commonly reported bacterial sexual transmittable infections (STIs) in the world. According to the current guidelines for laboratory diagnosis of STIs, nucleic acid amplification tests (NAATs) are the preferred diagnostic method for CT infections. However, NAATs amplify the available target DNA without discriminating between DNA originating from viable or non-viable CT. In a recent prospective cohort study with azithromycin treated CT patients, Dukers-Muijrers et al. demonstrated that frequent intermittent patterns of CT positivity could be detected up to 8 weeks post treatment. Cases showing intermittent positive patterns could reflect treatment failure or re-infection. Until today the true clinical implications (e.g., transmission, sequelae, treatment and screening management) of such results are still unexplored. Assessing CT viability will provide more insights in the true meanings of these results. One promising strategy to assess microbial viability is the viability PCR (vPCR) method. Prior to DNA purification, samples are treated with the membrane impermeable DNA binding dye propidium monoazide (PMA). Upon exposure to light, PMA irreversibly binds DNA which in turn interferes with its amplification, allowing the selective detection of viable intact organisms. To our knowledge, vPCR, has never been applied in the field of bacterial STI diagnostics. Moreover, vPCR has only rarely been applied for the assessment of intracellular pathogen viability. Therefore we implemented and evaluated the vPCR method as CT viability assay.
Methods: Aliquots of CT culture were subjected to heat-treatment at 95 °C for 15 minutes resulting in a decrease of inclusion forming (IFU) units to zero. Heat inactivated CT culture was mixed with the untreated viable CT culture in defined ratios of viability representing 0%, 0.1%, 1%, 10%, 50%, and 100% viability, respectively. Ratios were spiked in different media, namely 2SP transport medium, urine, and MEM culture medium. Two different serotypes of CT were used to conduct experiments, namely serotype D and serotype LGV II. The DNA intercalating dye PMA was used as sample pretreatment prior to DNA purification following quantitative PCR (qPCR).
Results: Conform expectations, the DNA yields of all ratios were comparable without PMA treatment, as equal amounts of DNA were present. In contrast, when applying vPCR, increasing proportions of viable CT culture led to a substantial increase in DNA yield, i.e. decrease in Ct values in qPCR. As expected, PMA treatment almost completely inhibited amplification of DNA from heat-inactivated CT (i.e. a signal reduction of 99.9%). Additionally, PMA treatment of the 100% viable sample showed no significant reduction of amplifiable DNA, indicating that all DNA from viable CT was amplifiable.
Conclusion: vPCR showed to be a fast and sensitive method for assessing CT viability without the need of labor intensive, difficult to perform, and insensitive culture methods. Future work will focus on the assessment of the viability of CT in clinical samples by the vPCR method.

P108

Power of SIRS criteria to capture bacteremia at a Dutch emergency department

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Introduction: In patients with suspected sepsis, blood cultures are performed with the aim to tailor individual treatment upon pathogen identification. Blood cultures are generally collected at the emergency department (ED), where evaluation of patients with suspected sepsis takes place according to the criteria of the systemic inflammatory response syndrome (SIRS). Our objective was to determine the occurrence of identified SIRS cases that present at the ED, and to study whether blood culture positivity can be predicted by using SIRS criteria.
Methods: All adults admitted to the ED of the Canisius-Wilhelmina Hospital in Nijmegen between 2009 and 2014 in whom SIRS was identified (fulfilled ≥2 of the SIRS criteria) were retrospectively included in the study. Corresponding blood culture results were related to both qualitative and quantitative SIRS parameters.
Results: The annual number of identified SIRS cases tripled over the study period from 1,069 cases in 2009 to 3,470 cases in 2014 (Kendall tau p = 0.02). Variance in SIRS prevalence across month of identification was modest (monthly average 235 ± 18 cases). Blood cultures were collected in 47% of SIRS cases, of which 20% yielded a microorganism, corresponding with pathogen identification in 9% of SIRS cases. Among those with all 4 SIRS criteria assessed (33% of the 16,898 SIRS cases identified), the proportion of blood cultures that was positive increased with the number of SIRS criteria fulfilled (2: 16% (90/549); 3: 21%(278/1,334); 4: 26%(364/1,392); Chi square p < 0.00001). However, addition of qualitative and quantitative SIRS parameters improved the model for blood culture positivity. The optimal model exclusively contained absolute body temperature (odds ratio 1.24 per degree Celsius, p = 4E-9; Nagelkerke R^2 = 0.017; Hosmer and Lemeshow p = 0.87). In the total cohort, collection of blood cultures was 66% in patients with abnormal body temperature (<36°C or ≥38°C), compared to 29% with normal body temperature (≥36°C and <38°C). Importantly, blood cultures collected at normal body temperature still yielded 17% positivity.
Conclusion: While the identification of possible sepsis cases increased between 2009 and 2014, collection of blood cultures lingered at half of the cases, and was focused on patients with abnormal body temperature. Because blood culture positivity was just marginally explained by increasing body temperature, improvement in pathogen identification requires universal collection of blood cultures across all suspected sepsis cases, to enhance tailored treatment.

P109

Optimizing growth of *Nitrospira moscoviensis* by cultivation in a continuous stirred tank reactor

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Due to the central position of nitrite at the connection point of aerobic nitrification and anaerobic denitrification pathways, nitrite-oxidizing bacteria (NOB) play a significant role in the nitrogen cycle. Members of the genus *Nitrospira* appear to be the most widespread and diverse NOB, and have been found as predominant NOB in wastewater treatment systems. Nitrifiers are generally fastidious to culture under laboratory conditions and *Nitrospira* can quickly become inhibited by nitrate accumulation. Despite their environmental and biotechnological importance, *Nitrospira* are scarcely studied and we still lack detailed knowledge about their exact mechanism of energy conservation. Genome analyses of *Nitrospira* revealed an unusual periplasmic nitrite oxidoreductase (NXR) that is more closely related to the enzyme of anaerobic ammonium-oxidizing bacteria than to the cytoplasmic NXR of *Nitrobacter*. Besides multiple copies of the NXR alpha and beta subunits, *Nitrospira* genomes encode several candidates for the heme-containing gamma subunit. Furthermore, they lack a canonical haem-copper oxygen reductase, which however is indispensible for energy conservation.
The aim of this study was to optimize growth rates and biomass yields of *N. moscoviensis* by establishing a cultivation system in a continuous stirred tank reactor (CSTR). A 7 l bioreactor was operated at a working volume of 5 l and stirred at 150 rpm. pH was maintained at 7.7 by constant flushing with Ar/CO_2 (95%/5% v/v) and buffering with $KHCO_3$. Dissolved oxygen was kept at 30% by providing filtered air or N_2 gas. Temperature was upheld at 39°C with a loop type heat exchanger connected to a water bath. After inoculation with 0.5 l of an active stationary-phase batch culture, the reactor was manually supplied with 3 mM nitrite, which was replenished when consumed completely. After 41 days, the feeding regime was switched to continuous substrate supply, maintaining an approximate nitrite concentration of 0.3 mM. After nitrite had been depleted the first time, the bioreactor was constantly supplied with fresh mineral NOB medium at an exchange rate of 10% (day 11), which was subsequently increase to 15% (day 116) and finally 20% (day 118) Growth and activity were monitored by measuring optical density, protein, nitrite, and nitrate concentrations.
Using this culturing approach in a CSTR, *N. moscoviensis* could successfully be grown to high densities

(OD = 0.064), approximately 3 times higher than achieved with batch cultures. The continuous medium supply precluded trace element limitations, and the optimization of the exchange rate prevented end-product inhibition by nitrate. Planktonic growth of *N. moscoviensis* was maintained throughout the cultivation period, indicating a successful washout of quorum sensing molecules that could otherwise induce biofilm growth. Furthermore, the continuous flow ensured a constant supply of biomass.
In conclusion, this study provides a novel method for continuous biomass production of *N. moscoviensis* by culturing in a CSTR. This is an essential prerequisite for a detailed physiological, proteomic and biochemical characterization of these understudied and enigmatic organisms.

P110

The first year of a longitudinal population-based study of Extended-Spectrum Beta-Lactamases in the Netherlands

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Background: Extended-Spectrum ß-lactamases (ESBLs) are a public health concern, yet reliable estimates of ESBL prevalence and duration of carriage in the general population and associated risk factors are largely unknown. This is an interim report of an ongoing observational study in the Netherlands to provide these data.
Materials & Methods: Since November 2014, a random sample of ~2000 inhabitants of the Netherlands was drawn monthly from Dutch population registries and invited to complete online an epidemiological questionnaire and to provide a faecal sample. If applicable, subjects were also asked to submit one sample from their dog or cat and additional questions were asked in case of a participating dog or cat. All ESBL-carriers, and a random sample of non-ESBL-carriers were asked to provide follow-up faecal samples after 1 and 6 months. Faecal samples were plated either directly or after enrichment in 3 mL Luria Bertani broth with 1 mg/L cefotaxime onto MacConkey agar both supplemented with 1 mg/L cefotaxime. If there was growth, colonies were differentiated by using matrix-assisted laser desorption/ionisation time-of-flight analyser (MALDI-TOF). *Escherichia coli*, *Enterobacter cloacae* and *Klebsiella pneumoniae* isolates were screened for CTX-M-9 or CTX-M-1 group ESBL genes by PCR. Isolates negative for these ESBLs were screened by micro-array analysis for CTX-M-group 2, 8 and 25, TEM and SHV. Gene types were subsequently determined by sequencing.
Results: 25,218 subjects were invited till October 2015 (13 months of study), of which 4,644 (18.4%) completed the questionnaire, 2,073 (44.6%) provided a faecal sample and 415 provided a sample of a dog or cat (from 2,141 dog/cat owners). The median age of subjects providing a faecal sample was 57 years (Q1-Q3 33-65) and 44.3% was male. 79 participants were ESBL-carrier (3.8% 95%CI: 3.1-4.7%). 63 samples (out of the 79 ESBL-carriers) were sequenced till now and the most prevalent genes were $bla_{CTX\text{-}M\text{-}15}$ (n = 22; 34.9%), $bla_{CTX\text{-}M\text{-}14}$ (n = 10; 15.9%) and $bla_{CTX\text{-}M\text{-}27}$ (n = 8; 15.9%). A follow-up faecal sample after 1 month was available from 335 participants (373 invited) including 41 from the ESBL-carriers; 6 subjects (2.0% 95%CI: 0.9-4.4%) acquired ESBL-carriage, and 23 (56.1% 95%CI: 41.0-70.0%) lost carriage. Data from the 6-month follow-up is not yet available.
Conclusions: In this population-based study, the (preliminary) unadjusted ESBL prevalence in the open population was 3.8%. The main ESBL-type found is $bla_{CTX\text{-}M\text{-}15}$, and 56.1% of carriers had lost carriage, while 2.0% of non-carriers acquired carriage after 1 month.

P111

Emergence of and response to an outbreak of New Delhi metallo-beta-lactamase-producing Enterobacteriaceae in a Dutch teaching hospital

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Introduction: The Jeroen Bosch Hospital is a 730-bed teaching hospital in 's-Hertogenbosch. In a surgical drain fluid culture obtained 23 November 2015, we isolated a New Delhi metallo-beta-lactamase (NDM)-producing extended-spectrum beta-lactamase (ESBL)-positive *Klebsiella pneumoniae*. At identification, the patient was already discharged. Shortly after, screening cultures of long-term admitted surgical patients revealed two additional patients with NDM-producing *K. pneumoniae*. Here, we describe subsequent measures towards patient screening, outbreak management and infection prevention.
Methods: On 8 December, an outbreak management team was established. Both surgical wards were temporarily closed for admissions. The Health Care Inspectorate was informed and a press release issued. Rectal screening cultures were obtained for NDM-screening. Swabs were cultured overnight in ertapenem-broth after which NDM-PCR was performed. NDM-PCR-positive broths were subcultured onto chromID™ ESBL plates to isolate NDM-producing strains.

Results: Contact tracing and weekly screening rounds of all in-hospital patients were performed, identifying additional NDM-carriers. Weekly screening rounds revealed seven wards with uncontrolled NDM-transmission, i.e. two or more NDM-carriers (two surgical wards and the internal medicine, oncology, gastroenterology, cardiology and pulmonology wards). Based on the epidemiological curve of NDM-carriers detected, the outbreak period was defined as starting on 1 October 2015.

All patients admitted to one of these wards from 1 October onwards were defined at risk for carrying NDM and were flagged in the electronic patient system in order to allow adequate precautions upon (re-)admission until proven negative. All healthcare workers had to be aware of the outbreak and the importance of the interventions needed to control transmission, i.e. isolation and screening of risk-patients, improved hand hygiene, dresscode, disinfection of medical equipment and patient rooms. Once all interventions were implemented and strict adherence was ensured, the outbreak period could be defined: 1 October till 30 December. By then, almost 3,000 patients were flagged as risk-patient and 23 NDM carriers were identified. Not only NDM-producing *K. pneumoniae* was found, also other NDM-producing *Enterobacteriaceae* were isolated. All NDM-carriers tested positive in rectal swabs, two NDM-carriers also tested positive in clinical cultures: one had an asymptomatic bacteriuria with several NDM-producing *Enterobacteriaceae* and one had a positive blood culture with an NDM-producing *K. pneumoniae* but recovered from this bloodstream infection.

In 2016, weekly screening rounds will be continued on risk wards to confirm the outbreak was successfully controlled. Approximately 3,000 non-hospitalized patients received information and material for sampling that can be returned through mail. Although many NDM-carriers have a suspected medical travel history to foreign countries, as of yet no such patient explaining the introduction of the outbreak strain into our hospital has been identified.

Conclusions: An NDM-producing bacterium has been introduced to our hospital by an as of yet unidentified index patient.

Up till now uncontrolled in-hospital transmission seems to have ended after implementation of a comprehensive outbreak management strategy including identification and isolation of NDM-carriers and patients at risk for carriage.

P112

Detection of the plasmid-mediated colistin-resistance gene mcr-1 in faecal metagenomes of Dutch travellers

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Introduction: In November 2015, the first plasmid-mediated colistin resistance gene (*mcr-1*) was reported in China. Shortly after, *mcr-1* was reported in isolates from retail meats, animals and humans in various countries. Colistin is increasingly used as a last resort antibiotic for the treatment of infections with carbapenem resistant bacteria. Considering the successful dissemination of genes encoding extended-spectrum beta-lactamases (ESBLs) and carbapenemases by horizontal gene transfer, the finding of *mcr-1* may have major implications for public health. The studies performed thus far have focused on either detecting *mcr-1* in existing collections of cultured isolates, or by mining metagenomic sequencing data. As these existing collections were mostly restricted to specific micro-organisms, resistance profiles (e.g. ESBLs) or patient populations, and sensitivity of sequence-based metagenomics is relatively low, we hypothesize that the prevalence of *mcr-1* as reported in these studies might be an underestimation of the carriage rates in the community. In the current study, we applied a PCR-based targeted metagenomic approach to detect the presence of the *mcr-1* gene in the faecal metagenomes of healthy Dutch travellers between 2010 and 2012.

Methods: Pre- and post-travel stool samples of 122 healthy Dutch long-distance travellers were collected and submitted to metagenomic DNA extraction. Detection of *mcr-1* and CTX-M genes was performed by real-time PCR. All *mcr-1* positive samples were confirmed by sequencing of the amplicons.

Results: Screening of the faecal metagenomes of 122 participants, both before and after travel, yielded 7 samples positive for the *mcr-1* gene. One participant was found to be positive both before and after travel, whereas 5 participants were positive for *mcr-1* only after travel, reflecting a post-travel prevalence of 4.9% (95% CI 0.021-0.105) and an acquisition rate of 4.1% (95% CI 0.015-0.096). Travelers with suggested *mcr-1* acquisition visited destinations in South(east) Asia or southern Africa. In 4 out of the 6 *mcr-1*-positive post-travel metagenomes, the ESBL-encoding CTX-M genes were also detected. Moreover, ESBL-producing *E. coli* (harbouring CTX-M) were previously cultured from faecal samples of 5 out of 6 *mcr-1*-positive individuals. However, none of these ESBL-producing *E. coli* were positive for the *mcr-1* gene, indicating that the *mcr-1* genes detected in the microbiome of these individuals are located on other bacterial genomes.

Conclusion: 1) With a prevalence of 4.9%, our study reports the highest *mcr-1* prevalence in human microbiomes thus

far. This highlights the potential of PCR-based targeted metagenomics as an unbiased and sensitive method to screen for the carriage of *mcr-1*.
2) *mcr-1* has already been present in human microbiomes since 2010.
3) The high acquisition rate confirms a previous report from the Dutch COMBAT-study that international travel significantly contributes to the dissemination of *mcr-1* and suggests that it is widespread in various parts of the world.
4) Although prior travel could not be excluded, the participant positive for *mcr-1* before travel indicates that *mcr-1* may already be disseminated to the microbiomes of Dutch residents at a low prevalence, warranting for a more extensive investigation of its prevalence in the general population and possible sources.

P113

IS-pro for *Clostridium difficile*: towards simultaneous assessment of the intestinal microbiome and strain ribotyping

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Introduction: Assessment of the intestinal microbiome in patients diagnosed with *Clostridium difficile* infection (CDI) might be of predictive value for treatment failure and recurrence of CDI. Some *C. difficile* ribotypes are more virulent than others and strain ribotyping is important for hospital epidemiology. Ideally, microbiome analysis and ribotyping would be performed using a single test. We evaluated the intergenic spacer profiling (IS-pro) technique for assessment of the intestinal microbiome and ribotyping of *C. difficile* isolates.
Methods: The IS-pro technique uses phylum-specific fluorescent labelled PCR-primers for instant species identification. With IS-pro, we analysed the intestinal microbiota composition on the day of diagnosis in 21 patients with CDI. In addition, the *Firmicutes* primer was used to generate intergenic spacer (IS) peak profiles of 37 *C. difficile* isolates that were previously ribotyped in a reference center. Seven ribotypes, including 027 and 014, were included in this study.
Results: The composition of the intestinal microbiome of patients with recurrent CDI differed from that of patients without recurrent CDI: there was less diversity in the *Firmicutes* phylum in patients that had a recurrence of CDI. The ribotyping study showed clustering of 35/37 peak profiles of isolates as expected by reference ribotyping. Furthermore, the peak profiles matched with profiles that were recently published in a study on standardized capillary gel-based electrophoresis PCR-ribotyping.
Conclusion: IS-pro can be used for both intestinal microbiome analysis and *C. difficile* ribotyping. Our ultimate aim is to construct a risk model for (recurrent) CDI based on intestinal microbiome analysis combined with *C. difficile* ribotyping. This will be evaluated in a prospective clinical trial.

P114

The ongoing adaptation of *Bordetella pertussis* to vaccination: the emergence of strains that do not express pertactin

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Introduction: The gram-negative bacterium *Bordetella pertussis* is the causative agent of whooping cough or pertussis, a highly contagious infection of the upper respiratory tract. Pertussis is most severe and sometimes lethal for young unvaccinated infants. Despite high vaccination coverages since the 1950s, whooping cough is still one of the most prevalent vaccine-preventable diseases worldwide. In the Netherlands, the infection frequency has been estimated to be 9% per year. Since 1996, a dramatic increase in pertussis notifications has been observed in the Netherlands and we have proposed that a combination of waning vaccine-induced immunity and pathogen adaptation have contributed to this phenomenon. Antigenic variation has been found in genes coding for surface proteins; pertussis toxin (Ptx), pertactin (Prn) and fimbriae (Fim). One of the most recent findings is the emergence of *B. pertussis* strains that do not express pertactin, one of the components of the acellular vaccine that is used in the Netherlands since 2005. Whole genome sequencing was used in this study to characterize the pertactin-negative strains and to investigate the genetic relationship between Dutch *B. pertussis* strains from the period 2010-2015.
Methods: 286 Dutch *B. pertussis* isolates were used in this study, isolated from the period 2010-2015. Production of pertactin was determined using a multiplex bead-based immunoassay. Whole genome sequencing was performed of a limited number of 130 strains isolated from the period 2010-2015 to investigate the mutations that were responsible for the inactivation of pertactin. Moreover, phylogenetic analysis was carried out to assess the genetic relationship between the Dutch *B. pertussis* isolates.
Results: The number of pertactin-negative strains increased from 4% in 2010 to 20% in 2015. Out of 130 selected strains, 14 strains did not produce pertactin. Whole genome sequencing revealed different mutations to inactivate pertactin which suggests a selective advantage for *B. pertussis* strains not producing pertactin. A phyloge-

netic tree based on 130 *B. pertussis* strains showed a closely related Dutch *B. pertussis* population, where all strains harbored the alleles *ptxP3*, *ptxA1* and *prn2*. The pertactin-negative strains were found in different branches.

Conclusion: The dramatic resurgence of whooping cough in the Netherlands is associated with the ongoing adaptation of *B. pertussis* to vaccination and the bacterium is very well able to persist in a highly vaccinated population. The number of pertactin-negative strains is increasing and we can expect a continuing increase of pertactin-negative strains in the next years. *B. pertussis* is able to inactivate pertactin independently in indifferent lineages which indicates positive selection. The ongoing adaptation of *B. pertussis* to vaccination emphasizes the importance of strains surveillance and to keep monitoring *B. pertussis* populations, also in other countries were different immunization strategies are used. An important question that should be addressed in the future is the effect of pertactin-negative strains on the efficacy of currently used acellular vaccines.

P115

No need for confirmatory testing of amplification positive gononoccal test results in a low-prevalence population or in extra-genital samples

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Introduction: Conflicting statements regarding the need of confirmatory testing of positive gonococcal nucleic acid amplification results exist. UK guidelines advise to confirm positive test results with a second test when the test was originally performed in low risk groups or when extra-genital samples were tested. We assessed the need for such confirmatory testing in both high and low risk patients.

Methods: As a screening test to detect gonorrhea the Aptima Combo 2 (AC2) test (Hologic) was used. Consecutive *Neisseria gonorrhoeae* (NG) positive tonsillar and rectal swabs from patients visiting the STD outpatient department and urogenital samples from patients visiting general physicians were subjected to confirmatory testing, Two confirmatory tests were used: the NG Aptima single test (Hologic), in which a different target is detected,and a DNA PCR targeting the *opa* genes. If any of these tests was positive or equivocal, the positive AC2 screening test was considered confirmed.

Results: The incidence of positive NG test reults by AC2 in the studied populations during the study period were 4.7% and 5.8% for tonsillar and rectal specimens, respectively, from patients visiting the STD outpatient clinic and, 3.3% and 0.8% for urogenital samples from male and female patients visiting their general physician. Of the AC2 positive tonsillar and rectal specimens, 233/240 (97.1%) and 222/224 (99.1%), respectively, were confirmed. Regarding urogenital samples from male and female patients from general physicians, the number of confirmed positive NG Ac2 results were 154/155 (99.3%) and 69/70 (98.6%).

Conclusion: False-positive NG nucleic acid amplification results are rare when the AC2 test is used as a screening test, also with extra-genital samples or in a low-prevalence population (0.8%) of female patients visiting their general physicians. There is no need for confirmatory testing when a positive AC2 result is obtained.

P116

Variable susceptibility of *Enterococcus faecium* against the antimicrobial polypeptide RegIII?

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Background: *Enterococcus faecium* is an inhabitant of the mammalian microbiota in the gastrointestinal tract and a common cause of multidrug-resistant nosocomial infections. Hospital-associated *E. faecium* isolates are almost exclusively grouped in a distinct subpopulation of *E. faecium*, and substantially differ from commensal strains. The first step preceding infections is successful intestinal colonization of the host. Intestinal homeostasis is maintained by the mucosal immune system, through production of antimicrobial peptides (AMPs), including RegIIIγ, which is bactericidal to Gram-positive bacteria. Little is known about the interaction of AMPs with *E. faecium*. Therefore, the objective was to study the interaction of RegIIIγ with antibiotic-resistant *E. faecium* isolates.

Methods: RegIIIγ was purified from *Escherichia coli* BL21-CodonPlus (DE3)-RILP with expression vector pET3a-RegIIIγ from inclusion bodies and using a HiTrap SP XL column. The susceptibility of 6 commensal and 6 hospital-associated *E. faecium* strains and *E. coli* DH5α (negative control) to RegIIIγ was tested by incubating the cells with and without RegIIIγ for 2 hours at 37ffiC and subsequent enumeration of bacteria from TSB blood agar plates. For each strain, the killing assay was performed at least in two biological replicates. The effect of RegIIIγ on the bacterial integrity was analyzed by scanning electron microscopy (SEM) and immuno fluorescense (IF).

Results: RegIIIγ was bacteriocidal against most but not all *E. faecium* strains. Exposure of hospital-associated *E. faecium* isolates to RegIIIγ with a concentration of 20 μM reduced viability by 40 to 70%. Commensal isolates

exhibited a broad range of susceptibilities against RegIIIγ. Some commensal isolates were very susceptible to RegIIIγ, with a percentage of survival of only 10 to 20%, while other strains were more resistant with up to 100% survival. As expected, RegIIIγ did not affect the viability of *E. coli,* since Gram-negative bacteria are naturally resistant against this AMP. IF using specific anti-RegIIIγ IgG antibodies demonstrated binding of RegIIIγ to the surface of *E. faecium* strains and SEM revealed that RegIIIγ treated cells agglutinated, caused lysis, and displayed cell wall abnormalities. **Conclusion:** Strains of *E. faecium* display variable levels of RegIIIγ susceptibility, regardless their source of isolation from either hospitalized patients or non-hospitalized persons. The mechanism responsible for decreased susceptibility in particular *E. faecium* strains is not known and is currently under investigation.

P117

Central nervous system tuberculosis

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Introduction: Central nervous system tuberculosis (CNS-TB) occurrence depends on the prevalence of tuberculosis, which varies highly across regions of the world, totalling up to nine million worldwide. Despite decreasing rates of CNS-TB in the Netherlands, clinical attention for CNS-TB should not decrease. Therefore we discuss three cases of CNS-TB.

Case descriptions: *Case 1:* A 70 year old Dutch male diagnosed with intracerebral neurosarcoidosis in 2007, presented with progressive loss of consciousness and an increasing cerebral tumor. A lumbar puncture (LP) showed a positive tuberculosis complex PCR and a non-MDR Mycobacterium tuberculosis strain was cultured. Revision of brain biopsies from 2007 and 2014 showed negative and positive PCR results, respectively. Ziehl-Neelsen (ZN) stains had been negative. The revised diagnosis was tuberculoma and quadruple therapy with dexamethasone was started. Clinical improvement was evident within one month and the patient was discharged. The patient died three months later.

Case 2: A 32 year old Eritrean male refugee presented with progressive nausea, headache and vision loss since 9 months. Cerebral MRI showed an occipital tumour. A dura biopsy showed granulomatous inflammation, but a negative ZN stain. With a positive biopsy-PCR tuberculoma was diagnosed. Subsequent culture proved positive for non-MDR TB. The patient was without signs of pulmonary involvement and HIV negative. He commenced treatment with isoniazid, rifampicin, pyrazinamid, moxifloxacin, pyridoxin plus dexamethason. The tuberculoma regressed. Because of deteriorating liver biochemistry, quadruple therapy has been interrupted.

Case 3: A 52 year old Dutch male presented with severe, progressive back pain and 8kg weight loss since 2 months. Spondylodiscitis was diagnosed. The biopsy culture showed no growth and empirical antibiotic treatment was started. An MRI for progressive pain showed an abscess which was treated surgically. Bacterial cultures were negative. After three weeks the patient was discharged. One week later he was readmitted with neurologic symptoms and an increasing abscess. Aspiration of the abscess showed a positive ZN and TB-PCR. A LP also showed a positive TB-PCR. Disseminated Mycobacterium tuberculosis infection was diagnosed and treatment commenced with quadruple therapy plus moxifloxacin. Because of deteriorating neurology due to a pressure hydrocephalus, the patient was referred to our hospital. External ventricular drains were placed. This enabled experimental therapeutic drug monitoring of the antituberculous drugs. Nevertheless the patient recovered poorly.

Discussion: Diagnosing CNS-TB can be difficult as clinical features usually are non-specific. Previously, 37% was diagnosed on an initial positive ZN stain; the diagnostic yield increased to 87% with four serial specimens independent of treatment (4). CSF and biopsies should be submitted for PCR whenever possible, particularly in the setting of high clinical suspicion and negative ZN staining, as shown above.

Optimal treatment for CNS-TB is unknown. Although clinical evidence is lacking, Dutch and WHO guidelines advise treating CNS-TB similarly to pulmonary tuberculosis, with extended consolidation therapy for minimally 7-10 months. Moxifloxacin instead of, or next to, ethambutol, can be used because of superior brain penetration. However, appropriate therapy should be initiated based upon strong clinical suspicion without delay until diagnostic proof has been obtained.

P118

Cooperation of anaerobic methane and ammonium oxidizing microorganisms

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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, anaerobic ammonium oxidizing (anammox) bacteria and nitrite-dependent anaerobic oxidation of methane (n-damo) performing bacteria were discovered. In addition, nitrate-dependent methane oxidizing archaea were enriched together with n-damo bacteria, and are now known to perform anaerobic oxidation of methane (AOM) with nitrate as electron acceptor. Studies in our lab indicate that the AOM performing archaea, may also produce ammonium, making it ideal collaborators for anammox bacteria. Combining these three processes has great potential for waste watertreatment, as ammonium, nitrite, nitrate and methane are removed simultaneously without the need of external input of electron donors or acceptors. To assess the feasibility of coculturing anammox, n-damo bacteria and the AOM performing archaea, an existing anaerobically methane oxidizing enrichment culture (1.6 liter) was used to inoculate a 3 liter sequencing batch reactor (SBR). After 18 months, Kuenenia stuttgartiensis single cells (0.5 L) were added to the n-damo enrichment and ammonium was added to the medium to stimulate the growth these anammox bacteria. After 14 months of anammox enrichment in the culture, two reactor assays with labelled nitrite were performed to determine the contributions of anammox and n-damo bacteria to nitrite removal. Fluorescence in situ hybridization (FISH) was used to detect the n-damo bacteria, anammox bacteria and the archaea in the culture at different timepoints.After 14 months of anammox enrichment, the coculture consumed 2.2 mmol NO2- hour-1, and roughly 75% of the produced dinitrogen gas originated from anammox activity. Examination of the biomass with FISH indicated that n-damo bacteria and anammox bacteria both represented about 40% of the biomass, while the AOM performing archaea constituted about 10% of the biomass. Increased knowledge on the interactions within the coculture mentioned above, will help to implement these processes in wastewater treatment systems.

P119

An outbreak with *Campylobacter fetus* after consumption of unpasteurized sheep cheese

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Background: From May 2015 to October 2015 an unexpectedly high number (6) of invasive *Campylobacter fetus* subsp. *fetus* infections in human patients with underlying disease (n = 5) or septic abortion (n = 1) were reported in the province of Zeeland (n = 5) and the neighbouring province of Noord-Brabant (n = 1) in the Netherlands.

Methods: The patients were interviewed by de Public Health Service (GGD) to identify a potential common source. Cheese was purchased at a suspected sheep farm for microbiological investigation. Faecal samples were collected from two suspected sheep flocks and analysed for the presence of *C. fetus*. To support the source investigation, genome sequencing was used to investigate genetic relatedness of patient isolates, isolates from the suspected sheep flock of origin, and a set of Dutch *C. fetus* reference genomes from human and ruminant origin.

Results: Based on epidemiological information and patient questionnaires, we concluded that the pregnant patient from Noord-Brabant most probably acquired the infection abroad, whereas all patients in Zeeland consumed unripened sheep cheese from unpasteurized milk. For 4 of the patients, the product could be traced to one sheep farm, while for the 5th patient a second farm was indicated. Microbiological investigation of sheep faeces revealed the presence of *C. fetus* in sheep (8/67) from the first farm but not of the second (0/114). The SNP profiles irrevocably showed that 5 of the human isolates belonged to a distinct clone while the 6th isolate from the patient from Noord-Brabant belonged to a separate clone. The outbreak isolates generally differed between 0-7 SNPs in their core genome, while reference isolates differed by at least 26 SNPs. Genome sequencing of the sheep isolates identified isolates with identical genomes to the patient isolates, confirming the epidemiological source identification. The farmer was informed about the findings, the production of the unpasteurized cheese was prohibited by the Netherlands Food and Consumer Product Safety Authority (NVWA) and the farmer was forced by the NVWA to remove misleading messages from the internet page of the farm stating the positive effect of consumption of sheep cheese on health in particular for people suffering from cancer, asthma or rheumatoid arthritis.

Conclusions: The most probable scenario is that faecal contamination of milk from a single sheep farm resulted in *C. fetus* contaminated cheese, leading to an outbreak of invasive *C. fetus* infections after cheese consumption. The link between the 5th patient and the first farm remains to be resolved. This case shows the risk of consumption

of unpasteurized milk products and the severity of *C. fetus* infections in people with immunosuppressed health condition.

P120

Comparative genomics of completely nitrifying 'comammox' *Nitrospira*

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Nitrification is a two-step process where ammonia is oxidized to nitrate with nitrite as intermediate. Since the discovery of the first organisms capable of catalyzing this process by Winogradsky in 1890, it was believed that the two reactions are catalyzed by different functional groups: the ammonia-oxidizing bacteria and archaea and the nitrite-oxidizing bacteria. This paradigm of the biogeochemical nitrogen cycle was recently overturned by our discovery of members of the genus *Nitrospira* that could be shown to catalyze complete ammonia oxidation to nitrate (comammox).[1] By now at least two additional comammox *Nitrospira* were identified in metagenomic studies.[2,3]

In this study we performed comparative genomic analyses of *N. nitrosa* and *N. nitrificans*, the two comammox organisms present in our enrichment culture. Both encode all enzymes necessary for ammonia oxidation via nitrite to nitrate in their genomes, and were shown to indeed conserve energy by the complete oxidation of ammonia to nitrate. While the genes for ammonia monooxygenase (AMO), hydroxylamine dehydrogenase (HAO), and nitrite oxidoreductase (NXR) are highly conserved in both organisms, there are also distinct differences in copy numbers. While *N. nitrosa* has two AMO alpha subunits and two rather dissimilar copies of the NXR complex, there are a second HAO and four highly similar NXR complexes encoded in the *N. nitrificans* genome. Similarly, genome analyses of the respiratory chain revealed clear distinctions between the two comammox species. While the core gene sets encoding for respiratory complexes I to IV are present in both genomes, there is one less NADH:quinone oxidoreductase complex present in *N. nitrificans* and one of two canonical complex III copies was replaced by the so-called alternative complex III, which contains a molybdopterin-binding active subunit instead of a Rieske iron-sulfur protein. Additionally, only *N. nitrificans* contains a *bd*-type terminal oxidase, which apparently was lost by *N. nitrosa*. Furthermore, *N. nitrificans* appears to be better equipped to deal with oxygen stress, as it encodes a superoxide dismutase and katalase, which are absent in the *N. nitrosa* genome.

Another unexpected feature was revealed by phylogenetic analyses of the comammox AMO enzymes. These are phylogenetically distinct from currently identified AMOs, rendering recent acquisition by horizontal gene transfer from known ammonia-oxidizing microorganisms unlikely. We also found highly similar amoA sequences (encoding the AMO subunit A) in public sequence databases, which were apparently misclassified as methane monooxygenases.

In conclusion, this recognition of a novel *amoA* sequence group will lead to an improved understanding of the environmental abundance and distribution of ammonia-oxidizing microorganisms. Furthermore, detailed comparative genomic analyses of the comammox *Nitrospira* will give first indications of possible adaptation mechanisms that give them a competitive advantage over canonical ammonia and nitrite-oxidizing microorganisms.

References

1. Van Kessel MA, Speth DR, Albertsen M, et al. Complete nitrification by a single microorganism. Nature. 528:555-9.
2. Daims H, Lebedeva EV, Pjevac P, et al. Complete nitrification by Nitrospira bacteria. Nature. 2015;528:504-9.
3. Pinto AJ, Marcus DN, Ijaz UZ, et al. 2015. Metagenomic evidence for the presence of comammox Nitrospira-like bacteria in a drinking water system. mSphere 1:e00054-15.

P121

Dynamics of faecal carriage of ESBL-producing *Escherichia coli* in dairy cattle and farm employees

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Introduction: Although the presence of extended spectrum beta-lactamases producing *E. coli* (ESBL) in dairy cattle has been reported, little is known about its dynamics within herds. The purpose of this study was to determine the dynamics of faecal carriage of ESBLs in dairy cattle in different age groups and presence of ESBLs in people working or living on the farm. Such knowledge is important to identify control options.

Material & Methods: Initially (T0), 20 Dutch farms with a relatively high antimicrobial usage were selected and approximately 100 samples were taken from individual animals on each farm distributed over 4 age groups, being 0-8 wks, 8 wks-1 yr, 1-2 yrs and >2 yrs. Next, sampling on 10 farms with the highest ESBL prevalence was continued every 2 months for 1 year (T2 to T12). At each sampling, all new animals in the youngest age group, all animals that had moved to another group and all animals tested positive

previously were sampled. Human samples were collected at T4 and T12. Individual samples were cultured in Luria-Bertani broth supplemented with 1 mg/L cefotaxime and subsequently streaked onto MacConkey agar supplemented with 1 mg/L cefotaxime (MCC). In parallel, a dilution range was made of all samples, which were subsequently inoculated on MCC for quantitative analysis. ESBL suspected isolates were screened by PCR and sequencing analysis.

Results: At the start 8 farms were positive for ESBL, of which 3 showed a relatively high prevalence (10% to 28%), and 5 farms had a low prevalence (below 2.6%). These 8 positive farms were completed with 2 negative farms for studying the dynamics of ESBL carriage. The 3 farms with a relatively high prevalence in phase 1 continued to be the farms with the highest number of positive animals. At each sampling moment, a single ESBL variant was predominant, suggesting epidemic spread. At consecutive sampling moments these predominant variants changed suggesting low persistence and exposure of the animals to other ESBL types from potentially different sources. ESBL variants clustered mostly in young calves and adult animals, and within the same stable. The use of $1^{st}/2^{nd}$ generation cephalosporins or extended spectrum penicillins was identified as a risk factor with an OR of 5, however this could only explain 16% of all newly acquired ESBL positive samples. At T4 1 out of 38 human faecal samples was positive, and at T12 1 out of 25 human samples was positive. The ESBLs found in human samples could not be linked to the ESBLs found in cattle.

Conclusions: The ESBL prevalence on the dairy farms included in this study is relatively low.

The presence of ESBLs on the farms is suggested to be caused mainly by (re)introduction from not yet identified sources.

ESBLs found in employees could not be linked to ESBLs found in the animals.

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Commensal *Streptococcus pneumoniae* in Europe: capsular types distribution

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Objectives: The aim of our study was to assess the distribution of commensal *Streptococcus pneumoniae* serotypes and their association with antibiotic resistance and pneumococcal immunization status in healthy persons in nine European countries.

Methods: Strains were collected as a part of 'The Appropriateness of prescribing antibiotics in primary health care in Europe with respect to antibiotic resistance' (APRES) study as described by van Bijnen et al.[1] Shortly, general practitioners from Austria, Belgium, Croatia, France Hungary, Spain, Sweden, the Netherlands and the United Kingdom (20 per country) recruited 200 individuals each to provide nasal swabs. Bacterial identification was performed in the national microbiological laboratory in each participating country except for France. Putative *S. pneumoniae* isolates were sent to the microbiological laboratory of Maastricht University Medical Center (MUMC) in skimmed milk at -80°C for further analysis. Antibiotic resistance of the isolates was assessed with microdilution in accordance with EUCAST guidelines and EUCAST epidemiological cutoffs were used as resistance breakpoints. Determination of serotype by Capsular Sequence Typing (CST), was performed as described by Elberse et al.[2]

Results: 3% (n = 958) of the tested individuals were pneumococcal carriers. Capsular sequence types (CST) were assigned to 912 of these isolates and 46 isolates were not typable. 46 different CST were found. The most common serotype was CST 11A (n = 62, 6.4%) followed by CST 23F (n = 60, 6.2%) and CST 23A (n = 59, 6.1%).

Serotype 23F was the most frequent in Croatia (n = 15, 11.5%) and Sweden (n = 16, 14.2%), and serotype 6C was the most common in The Netherlands (n=11, 8.6%) and Spain (n = 11, 6.5%). Serotypes 3 (n = 6, 14.0%), 15A (n = 6, 12.8%), 11A (n = 18, 10.6%), 23A (n = 9, 7.8%) and 10A (n = 5, 13.9%) were the most prevalent in Austria, Belgium, France, Hungary and UK, respectively. Of all participating patients, 635 (65.7%) were colonized by non-vaccine serotypes, 174 (18.0%) carried PCV7 serotypes, 20 (2.1%) carried PCV10 minus PCV7 serotypes and 129 (13.3%) carried PCV13 minus PCV10 serotypes. The 4 serotypes with highest resistance to penicillin were 14,19A, 15A and 15C. The serotype most resistant to all tested antibiotics was 19A.

Conclusions: This study has shown the distribution of pneumococcal serotypes among carriers in nine European countries. Despite the presence of serotypes 19A in PCV-13, this serotype is still frequent. Serotype 15A is one of the most resistant serotypes and could therefore be considered for a future vaccine.

References

Van Bijnen EM, den Heijer CD, Paget WJ, et al. The appropriateness of prescribing antibiotics in the community in Europe: study design. BMC Infect Dis. 2011;293.

Elberse KE, van de Pol I, Witteveen S, et al. Population structure of invasive Streptococcus pneumoniae in The Netherlands in the pre-vaccination era assessed by MLVA and capsular sequence typing. PLoS One. 2011;6:e20390.

P123

Risk factors for sporadic *cryptosporidiosis* in the Netherlands, a mid-term analysis of an ongoing case-control study, 2013-2015

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Background: In 2012, an increase in cryptosporidiosis occurred in the Netherlands. *Cryptosporidium hominis* was found to be the predominant species. However, no single source for the increase was identified. In April 2013, we began a three year population based case-control study to identify risk factors for sporadic cryptosporidiosis in collaboration with 17 Dutch microbiology laboratories.
Methods: A case was defined as a *Cryptosporidium* laboratory confirmation (microscopy or PCR). The collaborating laboratories sent positive samples to the RIVM Center for Infectious Disease Research, Diagnosis and Screening for *C. hominis* and *C. parvum* speciation by RT-PCR. The laboratories also posted a letter to the general practitioner (GP) containing the positive *Cryptosporidium* result and a letter addressed to the patient with the study questionnaire. Only samples from cases that returned a questionnaire to RIVM were speciated. A questionnaire was posted to controls selected from the population register and frequency matched on age. We excluded cases that travelled outside the Netherlands and controls with diarrhoeal illness within two weeks of completing the questionnaire. We analysed case and control questionnaire data collected during the first two study years. We performed single variable analysis and variables with a P-value≤0.10 were included in a multivariable logistic regression model.
Results: We obtained speciation data for 295 cases in the first year; 240 were *C. parvum* (81%) and 55 were *C. hominis (19 %)*; in the second year we obtained speciation data for 156 cases; 145 were *C. parvum* (93%) and 11 were *C. hominis (7%)*. In the first year analysis, we included 192 cases (response 52%; 61% female; median age 24 years (range 1-95)) and 587 controls (response 32%; 56% female; median age 21 years (range 0.5-84)). Cases were more likely than controls to swim in rivers or lakes (aOR 4.9; 95% CI:2.0-12), to be exposed to cattle (aOR 3.8; 95% CI:2.0-7.0), to eat barbequed foods (aOR 3.8; 95%CI:2.4-6.1) and to have household members with diarrhoeal illness (aOR 1.8; 95%CI:1.1-3.0). Cases were less likely to play in a sandbox compared to controls (aOR 0.4; 95% CI:0.2-0.7). In the second year analysis, we included 164 cases (response 51%; 55% female; median age 31 years (range 1-82)) and 514 controls (response 30%; 58% female; median age 30 years (range 1-79)). Cases were more likely than controls to have eaten unusual or out of the ordinary food (aOR 6.1; 95% CI:2.4-15), to have had contact with someone who had diarrhoeal illness who was not a household member (aOR 3.0; 95% CI:1.2-7.5) and to have had diarrhoeal illness among household members (aOR 2.8; 95% CI:1.3-5.8).
Conclusion: This study identified *C. parvum* as the predominant species in the first two years of the study. Preliminary results of the risk factors show exposure to cattle, swimming in rivers or lakes, diarrhoeal illness among non-household and household members and consuming barbeque or unusual food as possible risk factors for cryptosporidiosis. Information about potential risks of *Cryptosporidium* exposure during outdoor recreational activities, consuming food and improvements in hand-hygiene within households could prevent future infections.

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Comparison of 2 molecular assays, 2 immunoassays and culture for the diagnosis of *Clostridium difficile*

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Introduction: Clostridium difficile is a major cause of health-care associated diarrhea, mainly induced by the use of broadspectrum antibiotics. Current diagnostics in our institute is based on culture and toxin detection using an immunoassay from Biomerieux on a Mini Vidas system. Since we wanted to improve the turn-around time and sensitivity and specificity of our screening, we validated 2 molecular assay and 2 immunoassay compared those with our current diagnostic strategy.
Methods: We set up a multiplex real-time PCR assay detecting TcdA and TcdB based on assays described in literature. We compared this assay with the Clostridium difficile assay from EntericBio, which is a real-time PCR assay that is not preceded by a DNA isolation. Next to these molecular assays we also validated a Glutamate Dehydrogen (GDH) and toxine immunoassay from Diasorin on the Liaison XL. Both the molecular assays and the immunoassays were compared to the routine diagnostics in a clinical validation on 174 feces samples
Results: Of the 174 collected feces samples 41 were not tested in the immunoassays on the Liaison XL because inadequate material. 132 samples were negative in all performed assays and 42 samples were positive in 1 or more assays. Comparison between GDH immunoassay and culture showed 103 negative results, 14 positive results and 16 discrepancies, of which 15 were positive in the immunoassay and negative by culture and 1 vice versa. Comparison of the 2 toxine immunoassays showed 114 negative results, 7 positive results and 7 discrepancies. Of the 7 discrepancies 5 were positive in the Liaison

assay and negative in the Mini Vidas assay, however 3 of these were negative in culture, GDH immunoassay and the toxin PCRs and are therefore likely false-positive. Of the 2 discrepant samples that were positive in the Mini Vidas assay and negative in the Liaison also 1 samples was negative in all other assays. Comparison between the 2 molecular assays showed 153 negative results, 15 positive results and 6 discrepancies that were all positive in the in house PCR and negative in the EntericBio assay.

Conclusion: Overall the molecular assays showed more positive results than the toxine immunoassays. This could be explained by a higher sensitivity but might also mean that the toxins aren't actually expressed and the presence of the genes alone is of no clinical importance without the expression of the toxins. To calculated the sensitivity en specificity of the different assays we considered samples that were positive in either the culture or the GDH immunoassay ánd in either one of the toxine immunoassay as true positive. Both the in house PCR and the GDH immunoassay than have a sensitivity of 100%, but the specificities are, with respectively 94 and 85%, lower than that of the current diagnostics (which is 96%). When the GDH immunoassay is combined with the toxin assay on the Liaison the specificity is increased to 100%, this lowers the sensitivity to 82%, but this is still higher than that of the current diagnostics (which is 67%).

P125

Natural strain improvement of *Bacillus* sp. by food-grade DNA transfer

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Introduction: Strain improvement of industrial microorganisms and designing starter cultures starter cultures for the production of fermented foods and beverages with novel properties requires gene technology methods.[1] However, the use of ingredients in the food industry is regulated and the use of genetically modified organisms (GMOs) in food and feed is strictly forbidden in Europe. Thus, the current classical strain improvement involve only non-recombinant strategies (UV radiation, chemical treatment) resulting in many unwanted modifications. In order to engineer novel strains with industrially desired features, a new set of genetic tools is needed. It is well known that bacterial genetic modifications occur in nature via horizontal gene transfer mechanisms like *competence, conjugation or phage transduction*. These natural events provide a potential means of new tools for genome editing (Kuipers O.P., 2015).

Goal: *Bacillus subtilis* is a Gram-positive, naturally competent, soil-dwelling bacterium that it is classed by the U.S. Food and Drug Administration as "Generally Recognized as Safe" and commonly used for food fermentation processes. In the work within the BeBasic program we are focused on food grade DNA transfer for natural strain improvement of *Bacillus sp.*

Methods: In order to obtain the goal, the efficiency of natural transformation was studied and the importance of the size of transformed donor DNA and the level of homology to the recipients' genome, were investigated. Thus, for the transformation experiments, we used supercoiled and fragmented genetic material. To vary the size of DNA, methods like sonication and needle-shearing were optimized for donor-genome fragmentation. Transferred DNA was obtained from eight different *Bacillus sp.* strains, which showed differences in homology towards *B. subtilis* 168
in range of 30-100%. For the identification of the linear DNA fragments, acquired by the competence machinery and integrated to *B. subtilis* 168 genome, easy-screened selectable marker, conditioning tryptophan auxotrophy, was used.

Results: During transformation experiments we could observe differences in transformation efficiencies with distinct DNA sizes (sheared genomic DNA was even 10^2 greater than supercoiled). Additionally it was observed that *B. subtilis* 168 is able to up-take and efficiently integrate DNA fragments with homology even lower than 60% with fragments that can be larger than 20 kb.

Reference:

1. Johansen E, Øregaard G, Sørensen KI, Derkx PMF. 2015. Modern approaches for isolation, selection and improvement of bacterial strains for fermentation applications. In: W. Holzapfel (ed.). Advances in Fermented Foods and Beverages. Elsevier, pp.227-248.

P126

Detection of identical carbapenemase-producing bacteria in three patients hospitalized at different time periods in two intensive care units in Romania

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Introduction: the prevalence of carbapenemase-producing bacteria (CPB) in Europe is increasing. In Romania up to 50% of all *Klebsiella pneumoniae* isolates are carbapenem resistant. Recently a Dutch patient (patient 1) was hospitalized in a Dutch intensive care unit (ICU) after three days of hospitalization in an ICU in Bukarest, Romania. He was colonized with 7 different CPB. Several months later, two Romanian patients (patient 2 and 3) were transferred to another ICU in The Netherlands. Patient 3 was hospitalized in the same Romanian ICU as our Dutch patient and patient 2 in another ICU. These two patients

were colonized with several CPB as well. Because some of the cultured isolates of the 3 patients appeared identical, we compared the strains at a molecular level to identify possible clonal spread of CPB in the Romanian ICU's.

Methods: we used the Amplified Fragment Length Polymorphism (AFLP) method to compare the different CPE of the three patients. Patient 1 was colonized with two different carbapenemase-producing *Acinetobacter baumannii* isolates (OXA-23 gene positive), a carbapenemase-producing *Enterobacter cloacae/asburiae* (OXA-48 gene positive), 3 different carbapenemase-producing *K. pneumoniae* isolates (OXA-48 gene positive) and a carbapenemase-producing *Providencia stuartii* (NDM-1 gene positive). Patient 2 was colonized with 2 different carbapenemase-producing *A. baumannii* isolates (OXA-23 gene positive) and a carbapenemase-producing *K. pneumoniae* (OXA-48 gene positive). Patient 3 was colonized with 2 different carbapenemase-producing *A. baumannii* isolates (OXA-23 gene positive), a carbapenemase-producing *K. pneumoniae* (OXA-48 gene positive), a carbapenemase-producing *P. stuartii* (NDM-1 gene positive) and a carbapenemase-producing *Pseudomonas aeruginosa* (VIM gene positive).

Results: AFLP showed that all three patients were colonized with the same 2 different carbapenemase-producing *A. baumannii* isolates (OXA-23 gene positive). Patient 1 and 2 shared an identical *K. pneumoniae* isolate. Patient 1 and 3 shared an identical *P. stuartii* isolate.

Conclusion: three patients hospitalized in two different ICU's in Bukarest, Romania, at different time periods were colonized with several identical carbapenemase-producing bacteria. Patient 1 was colonized with 7 different CPB after a hospital stay of only 3 days. According to these findings we assume that the CPB prevalence is high in Romanian hospitals and there is significant clonal spreading of CPB between the hospitals. This has serious implications for management of patients who are transferred to Dutch hospitals from hospitals in Romania, but probably also from hospitals in surrounding countries. Important considerations are infection control measurements, empiric antibiotic therapy in case of infections and considering discontinuation of selective bowel decontamination in the ICU.

P127

Direct molecular detection and identification of fungi and yeasts in clinical specimens by IS-pro: proof of concept study

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Introduction: Immunocompromised patients are at risk to develop fungal and yeast infections. Mortality caused by these infections is high, while fungal infections are difficult to diagnose. Traditional culturing techniques are insensitive and may take weeks to become positive. Indirect (antigen detection) tests such as galactomannan detect only a selection of fungi and yeasts. Molecular techniques to diagnose fungal infections are upcoming, but have shown mixed results: some studies show superior performance of polymerase chain reaction (PCR) compared to antigen detection assays, whereas others show the opposite. In analogy with the IS-pro technique for detection of bacterial infections, we developed primers with the aim to detect and identify fungi and yeasts directly in clinical specimens. We here describe the proof of concept of this approach on a range of clinically relevant fungi and yeasts.

Methods: We developed primers that cover a part of the 18S and 5.8S regions that encode ribosomal RNA and the internal transcribed spacer 1 (ITS1) region. Different species can be discriminated by length variation of the ITS1 region. We used 3 group-specific fluorescently labeled forward primers to detect filamentous fungi, 6 to detect yeasts and 4 to detect zygomycetes. Four allround non-labeled reverse primers were developed. All primers could be combined in a single PCR reaction. We isolated DNA (NucliSENS easyMAG, Biomérieux, Marcy l'Etoile, France) from 9 different cultured yeasts (including several *Candida* species and two different *Cryptococcus neoformans* species), 11 filamentous fungi (including several *Aspergillus* species, a *Fusarium oxysporum* and several dermatophytes) and 3 different Zygomycetes (*Rhizomucor pussilus*, *Absidia corymbifera*, *Rhizopus oryzae*). Automated capillary gel electrophoresis was performed after PCR to identify species specific length polymorphisms (ABI PRISM 3500, Applied Biosystems, Foster City, CA, USA).

Results: From all 23 fungi and yeast species tested, different lengths of peaks were visible with IS-pro. Profiles were different for different species. In most isolates more than one product was visible, corresponding to multiple ITS1 alleles with varying length per isolate. The color of peaks sorted the isolates into one of the three groups of filamentous fungi, yeasts or zygomycetes.

Conclusions: The developed primers detected all 23 different and clinically important fungi and yeasts. Importantly, the different species showed different length polymorphism patterns. Together with different peak colors, this method facilitates the identification of fungal and yeast isolates and directly sorts all species into the clinically relevant groups of yeasts, filamentous fungi and zygomycetes. This approach holds great promise for clinical application as a universal fungal detection approach.

P128

Predictors of positive broad-range 16S ribosomal DNA polymerase chain reaction of culture-negative samples

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Introduction: 16S ribosomal DNA (rDNA) polymerase chain reaction (16S PCR) can play an important role in the identification and detection of bacterial species in direct specimens that remain culture negative. However, estimated sensitivity rates in culture-negative samples are low, even in the presence of a positive Gram-stain result. In the current study, predictors of positive 16S PCR for the detection of bacterial species in clinical samples are evaluated.

Methods: Data on all culture-negative 16S PCR samples in direct specimens performed at the Radboudumc (Nijmegen, the Netherlands) between the period of October 2012 and December 2015 were collected. Three potential predictors were examined: Gram-stain result, sample type and origin of the clinical sample. For the assessment of sample type, the samples were classified into two categories: solid or soft tissues and bodily fluids. In order to determine the sample origin, all samples were categorized into one of the following groups: musculoskeletal, cardiovascular, neurological, pulmonary, abdominal, lymphatic, ocular, dermatological or gynaecological. Univariate analysis was performed using Pearson $\chi 2$ statistics and Fisher's exact tests. Furthermore, multivariate logistic regression analysis was applied to further analyze potential independent predictors for positive 16S PCR results. In addition, clinical relevance of all positive samples was assessed by comparing 16S PCR outcomes with the clinical course and additional microbiological and laboratory results.

Results: A total of 171 16S PCR's were performed on culture-negative samples, of which 9.4% (n = 16) had a positive PCR result. Gram-staining was performed in 111 samples, resulting in 19 positive and 92 negative Gram-stains. In univariate analysis, samples with positive Gram-stains, tested significantly more often positive with 16S PCR than negative Gram-staining samples: 26.3% versus 7.3%, respectively (p = 0.031). Furthermore, significant more fluid samples had a positive 16S PCR outcome compared to tissue samples: 15.4% versus 4.3%, respectively (p = 0.013). None of the musculoskeletal samples (n = 60) showed a positive 16S outcome, whereas non-musculoskeletal samples (n = 111) tested positive in 14.4% (p = 0.002). No significant differences were found for other sample origins. In our logistic regression model, positive Gram-stain results and fluid samples were identified as independent predictors of positive 16S PCR results (p = 0.048 and p = 0.041, respectively). The adjusted odds ratios for positive Gram-staining and fluid samples were 3.8 (95% CI 1.0 - 14.3) and 5.2 (95% CI 1.1-25.7), respectively. Eighty-eight percent (n = 14) of the positive 16S PCR samples were found to be clinically relevant.

Conclusion: Our results confirm previously published data that 16S PCR has limited additional value for detecting bacteria in clinical culture-negative samples. We found that both positive Gram-staining and fluid samples are independent predictors for a positive 16S PCR. A micro-organism identified by 16S PCR was found to be clinically relevant in a majority of cases; i.e. the detected microorganism most likely was the cause of the patients' clinical condition. These results can aid in clinical decision making of when to perform a 16S PCR.

P129

Characterisation of the replicative helicase and primase activities of the Gram positive pathogen *Clostridium difficile*

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Clostridium difficile is the main cause of health-care associated diarrhea. Limited treatment options and reports of reduced susceptibility to current treatment necessitate the development of novel antimicrobials. DNA replication is an essential and conserved process in all domains of life and may therefore serve as a target for the development of new antimicrobial therapeutics.

Compared to its well characterized relative, *Bacillus subtilis*, knowledge of the molecular biology and genetics of *Clostridium difficile* is still in its infancy. There is no direct evidence which proteins are involved in DNA replication in *Clostridium difficile*.

Here, we identified several *C. difficile* genes with homology to *B. subtilis* replication initiation proteins and characterized the replicative helicase (CD3657) and primase (CD1454) proteins using biochemical assays. Binding of ATP to helicase is essential for its interaction with the putative loader protein CD3654. Helicase activity could only be demonstrated *in vitro* in the presence of primase. Triplet specificity of primase is atypical for Firmicutes, and is modulated by helicase. Our data suggest that critical aspects of *C. difficile* DNA replication differ from the Gram positive model *Bacillus subtilis*.

P130

Monitoring the presence of Shiga-toxin producing *E. coli* in food in the Netherlands

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focuses on the inspection of food and feed and the detection of diverse (pathogenic) microorganisms to monitor food safety. One of the target organisms for inspections is Shiga-toxin producing *E. coli* (STEC), a group of food-borne pathogens which is nowadays considered to be a major public health problem worldwide. Symptoms of STEC infection can vary from mild diarrhea to severe conditions, such as hemorrhagic colitis, and the life threatening condition hemolytic uremic syndrome (HUS). The policy of the NVWA is aimed at not allowing any STEC in products of high risk, such as ready-to-eat food products. Whereas for low risk food products, which for instance are products that will be heated properly, the NVWA policy does not allow STEC with characteristics that have previously been linked to severe disease. These features are specific serotypes, such as the well-known serotypes O157 and O104:H4, in combination with the presence of factors enhancing attachment of STEC to intestinal cells.

The detection of STEC in food consists of two parts, which are 1) the screening of the enrichments of food products for the presence of the Shiga-toxin encoding *stx* genes by real-time PCR and 2) the subsequent isolation of STEC colonies. As not all positive screenings result in an isolation of STEC, the results of both parts are described here.

The food inspection on STEC in 2014 was performed on a total of 2596 samples. The majority of products that were screened were meat products, in total 1968 samples (76%). In total, 193 meat samples were found positive for STEC in the screening (9.8 % positives). For beef 12.3% of the samples (1093 samples) were positive, whereas for pork only 3% of the samples (605) were positive. For sheep and exotic meat 19.8% and 31.7% out of only 79 and 81 samples were positive, respectively. For all samples of non-animal origin only 13 samples were positive in the screening, these 13 belonged to herbs (3 samples), vegetables (3) and sprouts (7).

The isolation of STEC was successful for 65 samples (31.5% of screening positives), of which 64 were meat samples and 1 was from endive (vegetable). The isolates from meat can be further specified into 45 isolates from beef, 10 from sheep, 2 from pork, and 7 from exotic meat. Surprisingly none of the isolates found belonged to the serotype O157. The most found serotypes were O113 and O174, with 12 and 7 isolates, respectively. Both these serotypes have been described to be able to cause serious disease, including HUS.

In conclusion, the 2014 inspection on STEC indicates that next to beef, also sheep and exotic meat are sources of STEC, however, more samples need to be tested. Moreover, prevalence of the main disease causing serotype O157 appears to be low in food in the Netherlands.

P131

Added value of Hepatitis E virus diagnostics in hepatitis screening serology in primary and secondary care

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Introduction: Hepatitis E virus (HEV) genotype 3 is endemic in Europe and an underdiagnosed and emerging public health issue in the Netherlands, with a seroprevalence of 29%. HEV genotype 3 is known to be a porcine zoonosis, however, the transmission route still needs to be unraveled. We investigated the added value of HEV diagnostics in the hepatitis screening serology in our laboratory, performing diagnostic services for primary and secondary care.

Material & Methods: In a 2-year time period (May 2013 -May 2015), sera of 2521 patients with symptoms of acute hepatitis, including elevated alanine aminotransferase (ALT) levels, were screened for hepatitis A, B, C and E virus, Epstein-Barr Virus (EBV) and Cytomegalovirus (CMV) using commercially available enzyme linked immunosorbent assays (ELISAs). In patients tested positive for HEV IgM, supplementary testing was performed using HEV RNA PCR, followed by HEV genotyping when the level of viremia was sufficient (≥2 log IU/ml).

Results: In 235 of the 2521 patients (9.3%), a viral cause of the acute hepatitis was indicated by positive serology. Acute HAV was diagnosed in three out of 235 positive patients (1%), HBV in 34 (15%), HCV in 10 (4%), EBV in 69 (29%) and CMV in 42 (18%). 78 patients (33%) tested positive for HEV IgM. Subsequently, 38 patients tested positive for HEV RNA of which 31 had sufficient levels of viremia (≥2 log IU/ml) for HEV genotyping. All typing results were genotype 3. The majority of samples of patients with acute HEV were referred by medical specialist (68%), general practitioners (GPs) referred the remaining 32%. Our analysis showed that in particular male patients above the age of 50 years are at risk for an acute HEV infection. HEV-seroprevalence in our study was 24%, suggesting that clinical asymptomatic HEV-infections do occur frequently.

Conclusions: HEV genotype 3 is the most frequently diagnosed cause of acute hepatitis in patients seen by both medical specialist and GPs in our region, almost 13 times more than an acute HAV infection. In investigating new hepatitis patients, physicians should always include HEV diagnostics. These results warrant addition of HEV diagnostics in the standard diagnostic workup for acute viral hepatitis in endemic countries of HEV genotype 3.

P132

ESX-1 secretion facilitates mycobacterial crossing of the blood-brain barrier

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Introduction: Tuberculous meningitis (TBM), the devastating extra-pulmonary form of tuberculosis, is the most commonly found type of meningitis in developing countries in young children. Improving diagnosis and treatment remains a challenge and is not possible without a better understanding of both host and bacterial factors involved in early pathogenesis.

Several important virulence factors are shared by *Mycobacterium tuberculosis* and *Mycobacterium marinum,* of which the ESX-1 locus is probably the best studied. This locus encodes a virulence secretion system and is required for phagosomal escape, macrophage recruitment and subsequent dissemination. Here, we specifically study its role in tuberculous meningitis pathogenesis.

Methods: Transgenic zebrafish embryos, with fluorescently labelled blood vessels, infected with fluorescently labelled mycobacteria were used to analyse migration of *M. marinum* across the blood-brain barrier (BBB). Migration in embryos depleted of their phagocytic pool using pu.1 morpholino and Clodronate filled liposomes was compared to migration in controls. Both groups were infected with WT *M. marinum* or an isogenic strain mutated in (parts of) the ESX-1 locus at 4 days post fertilization (dpf), one day after the formation of the BBB. Confocal microscopy and electron microscopy at 5 days post infection (dpi) was used to study BBB crossing of bacteria in great detail.

Results: Confocal analysis showed that both WT and ESX-1 lacking mycobacteria can cross the BBB within macrophages in a physiological situation. In addition, where WT *M. marinum* seem to be able to employ macrophage independent migration mechanisms to pass the blood-brain barrier, ESX-1 mutant *M. marinum* seem to lack this ability. For, infection of a macrophage depleted zebrafish embryo with an ESX-1 mutant *M. marinum* strain led to uncontrolled replication of bacteria within the blood vessels, indicating that growth of this mutated bacterial strain is not restricted by the absence of macrophages. Interestingly, this replication solely took place in the vessel lumen and outgrowth in brain tissue only occurred when overgrowth led to bursting of vessel walls. This suggests that the ESX-1 locus plays an important role in crossing an endothelial cell layer such as the blood-brain barrier in a macrophage independent manner.

Conclusion: 1. Both WT and ESX-1 lacking *M. marinum* can cross the BBB within macrophages acting as a Trojan Horse.

2. WT *M. marinum* is able to use macrophage independent migration mechanisms.

3. ESX-1 secretion mutants of *M. marinum* are unable to cross the BBB independently of macrophages, suggesting a role for ESX-1 secretion in this process.

P134

An experimental *Staphylococcus aureus* decolonisation and inoculation model in Macaca Mulatta

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Introduction: *Staphylococcus aureus* (*S. aureus*) nasal carriage in humans is a risk factor for the development of *S. aureus* infections. As nasal colonization experiments with *S. aureus* in humans for obvious reasons are difficult to perform, the field needs realistic colonization models in order to develop new decolonization procedures. Although there are several animal models for *S. aureus* nasal colonization around, these models have their restriction, as most of the species used are no natural hosts of *S. aureus.* Recent data showed nose carriage of *S. aureus* in rhesus macaques (*Macaca mulatta*). Therefore, we setup a pilot study for *S. aureus* decontamination and inoculation in rhesus macaques, in order to investigate the usefulness of this model.

Methods: We have determined the *S. aureus* nasal carrier status of 40 rhesus macaques. Only persistent carriers were included in the study. The rhesus macaques were decontaminated with mupirocin once daily or mupirocin plus trimethoprim/sulfadiazine intramuscularly once daily for five days. In a follow-up period of 10 weeks we examined if recolonisation in nose, throat and rectum with *S. aureus* occurred. Rhesus macaques, which were negative for *S. aureus* in the nose after the follow-up period, were selected for inoculation with the human *S. aureus* strain 8325. Therefore we have applied 10*7 colony forming units (CFU) in each nostril. Over a period of 5 weeks 4 swabs on the three carrier sites were taken and cultured for *S. aureus* to determine survival of the human strain in the nose of rhesus macaques.

Results: 20 rhesus macaques were selected as persistent carriers of *S. aureus.* 10 animals received mupirocin only and 10 mupirocin plus trimethoprim/sulfadiazine. At the start of the decontaminating therapy (t = 0), 18 animals were positive in the nose of which 3 were solitary nose carriers. 11/18 were also positive in the throat and 4/18

animals were positive in throat and rectum. 1 animal (A) was negative on all carrier sites and 1 animal (B) was positive in throat and rectum but negative in the nose at the start of the therapy. A total of 13 animals, including A and B, were negative for *S. aureus* in the nose after the follow-up period. 7 of them received the treatment with mupirocin and the others received the combination treatment. The 13 animals were inoculated with the human *S. aureus* strain. In 6 out of 11 animals survival of the human strain was seen for at least 5 days but less than 14 days.

Conclusion: The effectivity of the decontamination with mupirocin and the combination treatment with mupirocin plus trimethoprim/sulfadiazine is similar.

The human *S. aureus strain* 8325 is capable of surviving in the nose of rhesus macaques for at least 5 days but less than 14 days.

P135

SOAP typing: universal cloud-based bacterial strain typing for real-time infection control

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Introduction: Bacterial strain typing is essential for the process of tracking and tracing the spread of bacterial strains. Current typing methods do not provide the speed and universal applicability that is needed for this situation. Moreover, lack of standardization presents a major pitfall in national and international comparison of epidemiological data. Here we demonstrate a universal cloud-based bacterial strain typing approach that can be applied in any laboratory within a day.

Methods: For this, we chose a modernized amplified fragment length polymorphism (AFLP) approach. AFLP has the advantage of being applicable to any bacterial species, but the main drawback is low inter-laboratory comparability of resulting data. To overcome this, we optimized and standardized all steps of the process and built an automated software pipeline for fully standardized data-analysis. First, we developed a one-step DNA isolation approach that can be performed within 10 minutes. Secondly, we standardized the restriction/ligation step and PCR with bulk-produced mastermixes optimized for performance, speed and stability. Finally, fragment analysis was performed by capillary gel electrophoresis, resulting in digital profiles. These profiles were uploaded to the cloud-based analysis algorithm that performed all steps of profile analysis, regularized resulting data and stored all information in the cloud server. We coined the technique Standardized One-day Automated Polymorphism (SOAP) typing. We tested SOAP typing for its universal application on 50 different bacterial species using identical protocols. To determine the resolution of SOAP typing, we took 14 different strains from a Clostridium-difficile outbreak and compared SOAP typing to NGS based typing. Finally, to test inter-laboratory reproducibility of SOAP typing, we performed the full procedure in two different laboratories on a panel of strains from three different bacterial species.

Results: SOAP typing resulted in high-quality profiles in 50 different bacterial species using the same protocol and mastermix. Similarity of profiles was higher for species from the same genus than for more distantly related species. Interestingly a number of *Klebsiella* isolates that were classified by culture as *Klebsiella oxytoca*, clustered separately. These isolates were identified as *Klebsiella michiganensis*, a rare species that is difficult to discriminate from *K.oxytoca* by traditional technques. The epidemiological relations of the strains from the *C. difficile* outbreak were identical when analysed by either NGS or SOAP typing. Strains that were identical with SOAP typing showed at most a single SNP variation in the NGS based analysis. Reproducibility of SOAP typing was very good between laboratories for all three species that were tested. Strains showed almost identical profiles between the two laboratories. Detected variation was almost entirely attributable to low-level background noise that could easily be adjusted for by the software. Software parameters may be further optimized with additional testing.

Conclusion:

SOAP typing can be performed in a single-day and is universally applicable to bacterial species.

SOAP typing shows the same resolution as NGS-based typing.

SOAP typing is highly reproducible between laboratories.

SOAP typing may enable real-time epidemiologic monitoring not only for local hospital infection control, but also on a national and global level.

P136

Characterization of the *Clostridium difficile* HtrA-like foldase/protease

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Introduction: *Clostridium difficile* has emerged as an important gut pathogen in the past decade. *C. difficile* infection symptoms range from mild diarrhea to potentially fatal pseudomembranous colitis. The two main virulence factors are toxins A and B, but other virulence factors are likely to play a role in establishment of the disease.

Conserved high-temperature requirement A (HtrA)-like proteases have been shown to have a role in bacterial

protein homeostasis and quality control. This affects the functionality of virulence factors and the resistance of bacteria to (host-induced) environmental stresses. Previously, we have found that the *C. difficile* 630 genome encodes a single HtrA-like protease (CD3284; HtrA). We hypothesized that knocking out *htrA* would result in a severe attenuated strain. However the *htrA* knockout showed enhanced virulence in the Golden Syrian hamster model of acute *C. difficile* infection and microarray data analysis showed a pleiotropic effect of *htrA* on the transcriptome of *C. difficile*, including upregulation of the toxin A gene. In addition, the *htrA* mutant showed reduced spore formation and adherence to colonic cells.[1]

The hypothesis of this study is that HtrA is not involved in the *C. difficile* stress response.

Methods: We characterized the biochemical properties of HtrA, including oligomerization through gel filtration experiments, in vitro protease and foldase activity of recombinant HtrA and we analyzed expression of htrA in C. difficile in time, using Western blot.

Results: We found that recombinant HtrA is a monomer, in contrast to other HtrAs in literature. It oligomerizes when exposed to casein, a model substrate. HtrA also displays proteolytic activity (towards casein) and is able to refold unfolded citrate synthase in vitro.

Finally, surprisingly, we found that HtrA is highly expressed in both the logarithmic and stationary growth phases of *C. difficile*.

Conclusions: Although we have shown that *C. difficile* HtrA displays some of the characteristics of other HtrAs, such as proteolytic activity and foldase activity, we conclude from our experiments that HtrA is probably not involved in the stress response in *C. difficile*. This is based on the fact that it is constitutively expressed and that we found no effects of the knockout when it was exposed to stresses.

References

1. Bakker D, Buckley AM, de Jong A, et al. The HtrA-like protease CD3284 modulates virulence of Clostridium difficile. Infect Immun. 2014;82:4222-32.

P137

Comparison of four real-time PCR assays and one Point-Of-Care assay for the detection of viral and bacterial respiratory pathogens

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Introduction: Molecular assays are well recognised as the accepted means for diagnosis of respiratory infections caused by viral and atypical bacterial pathogens. Etiological diagnosis may be important for individual patient management, infection control, and epidemiology. Various commercial assays are available with different characteristics concerning the different targets included, sensitivity, specificity, rapidity, and the ability to use the assay for single specimen and point of care testing. These characteristics and the available resources determine which assay is suitable for a laboratory.

Methods: Twenty-four respiratory specimens from patients with clinical symptoms of respiratory tract infection that previously tested positive for different viral pathogens using the RealAccurate kit 2.0 (Pathofinder) were included, as well as 4 negative specimens. The 24 positive specimens contained 40 viral and 14 bacterial targets. The specimens were stored at -40°C for a maximum of two years. Specimens that were positive for a single viral pathogen were pooled with other positive specimens to produce a 1:4 dilution to reduce costs and to have sufficient material for later discrepancy analysis. EasyMag was used to extract DNA and RNA (bioMérieux) from all specimens except for the FilmArray Respiratory Panel, which includes the nucleic acid extraction. Performance of the following assays was compared: FilmArray Respiratory Panel (bioMérieux), Respiratory Multiwell system (Argene), RealAccurate Respiratory Quadruplex (Pathofinder), Resp'one and Resp'easy (Diagenode).

Results: Resp'one is only tested for Influenza A and B, RSV, Metapneumovirus, and Legionella pneumophila. All four negative specimens tested negative with all assays. Influenza A (number of positive specimens, n = 5) was detected five times with all assays except with Argene (four) and Resp'one (four). Influenza B (n = 2) was detected in two specimens with all assays except for Resp'one with one positive. Parainfluenza 1 (n = 4) was detected four times with bioMérieux and Argene and two times with Pathofinder en Diagenode. Parainfluenza 2 (n = 2) was positive two times with bioMérieux and once with Resp'easy, Pathofinder and Argene. Parainfluenza 3 (n = 2) tested two times positive with the Argene and Resp'easy asay and two times negative with bioMérieux and Pathofinder. Parainfluenza 4 (n = 2) was detected two times with all assays and Pathofinder detected no positives. Coronavirus (n = 8) tested positive with Pathofinder, bioMerieux, Argene and Resp'easy, seven, six, five, and six times, respectively. RSV (n = 4) was detected with Resp'easy, Resp'one, bioMérieux, Pathofinder and Argene, four, four, three, two and one time, respectively. Adenovirus (n = 4) was detected four times by bioMérieux and Argene, and two times by Pathofinder and Resp'easy. Resp'easy, bioMérieux, Pathofinder, Resp'one and Argene detected Metapneumovirus (n = 6) six, five, five, four and two times, respectively. Rhinovirus/enterovirus target (n = 10) was detected by bioMérieux, Argene, Resp'easy and Pathofinder, ten, seven, six and four times, respec-

tively. Pathofinder, Argene and Resp'one detected all four *L. pneumophila* specimens. All five *M. pneumoniae* and *C. pneumoniae* were detected by Pathofinder, Argene and bioMérieux.

Conclusion: The most viral pathogens were detected by Resp'easy from Diagenode and FilmArray Respiratory Panel from bioMerieux and showed a good overall agreement.

All bacterial pathogens were detected by the assays that included these targets.

BAMA-O01

Functional characterization of actin homolog MreB in the anaerobic ammonium oxidizing bacterium *Kuenenia stuttgartiensis*

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Anammox bacteria combine ammonium and nitrite to form dinitrogen 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 genes 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 peptidoglycan cell wall synthesis. 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 and advanced (cryo) transmission electron microscopy techniques.

BAMA-O02

A novel nitrite reductase: a 60-heme-containing heterododecameric protein complex in the anaerobic ammonium oxidizing bacterium *Kuenenia stuttgartiensis*

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Anaerobic ammonium oxidizing (anammox) bacteria couple the oxidation of ammonium to the reduction of nitrite and produce dinitrogen gas in the absence of oxygen. It has been estimated that anammox bacteria are responsible for the production of approximately 50% of the dinitrogen gas in the atmosphere, making them key players in the global nitrogen cycle. The anammox process has already been successfully applied in an environmental-friendly and cost-effective technology for nitrogen removal from wastewater.

Anammox bacteria reduce nitrite to nitric oxide, then combine this intermediate with ammonium to form hydrazine, which is then oxidized to dinitrogen gas. In the first step, nitrite is reduced to nitric oxide by two different enzyme families: (1) cytochrome cd_1-type (NirS) and (2) copper-dependent (NirK) nitrite reductases. Although genomic data on *K. stuttgartiensis* identified an open reading frame coding for a cd_1-type nitrite reductase, transcriptome and proteome analyses have shown very low expression levels of NirS. Based on protein sequence analyses and high expression levels in both RNA and protein, a hydroxylamine oxidoreductase (HAO)-like protein (encoded by kustc0457 and kustc0458 gene) was suggested as the enzyme catalyzing nitrite reduction in.

Planktonic cells of *K. stuttgartiensis* were harvested from a membrane bioreactor and used to prepare cell-free extracts. Kustc0457-8 was purified to homogeneity following a three-step liquid chromatography protocol using anion exchange followed by hydroxyapatite columns. With the use of MALDI-TOF mass spectrometry, the isolated protein was determined to be the product of the kustc0457-8 gene cluster. Elucidation of the crystal structure of the purified native protein revealed an $\alpha_6\beta_6$ heterododecameric complex formed by non-covalent interactions with a molecular mass of ~ 480 kDa. The protein complex contains 60 heme groups, which is the highest number ever found in a protein complex. The enzyme showed very low oxidizing activity during *in vitro* spectroscopic assays; however, using methylviologen as electron donor, nitrite reduction was observed at appreciable rates. Altogether, this study identified a highly expressed HAO homolog of the anammox bacterium *K. stuttgartiensis* as the dedicated nitrite reductase, suggesting that it could catalyze the first step of the anammox pathway.

BAMA-O03

Identification and characterization of fungal components involved in immune evasion

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There are different mechanisms for pathogens to evade the immune system. One of these mechanisms is the secretion

of compounds that bind immune receptors blocking their activation. The *A niger* D15#26 mutant strain, and to a lesser extent the wild-type strain, was shown to release small molecules (<3 kDa) that interact with immune receptors. The D15#26 strain contains multiple mutations amongst which a mutation in the *laeA* gene. LaeA is a regulator of secondary metabolites and strains with a l*aeA* deletion or mutation have altered production of secondary metabolites. In this report we show that production of the immune-active compounds is related to the *laeA* mutation. Deletion of this gene in a wild-type strain resulted in the release of the immune-active compounds. Additionally, it was shown by filtering experiments that these molecules have a size smaller than 1 kDa. They were partly purified by hydrophobic exchange chromatography on a Sep-Pak C18 column and eluted with aqueous methanol.
Another mechanism for *Aspergillus* species to evade the immune system is via melanin that protects the fungus against reactive oxygen species. To be able to investigate the role of melanin in the interaction of *Aspergillus* with neutrophils, knock out strains of DHN-melanin and pyomelanin were created in *Aspergillus fumigatus* KU80 strain.

BAMA-O04

Microbicidal effect of LL-37 and teicoplanin in combination against *Staphylococcus aureus* and *Staphylococcus epidermidis*

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Bacterial infections that occur after implantation of biomaterials are a growing concern in modern medicine. In order to treat such infections, novel antimicrobial strategies are desired. The aim of this study therefore was to investigate the combined effect of the conventional antibiotic teicoplanin and the human cathelicidin LL-37 against *Staphylococcus epidermidis* and *Staphylococcus aureus,* the most common pathogens in biomaterial associated infections (BAI). LL-37 is a cationic antimicrobial peptide (CAMP) with an amphipathic character. These properties allow the peptide to interact with the bacterial cytoplasmic membrane via electrostatic interaction, subsequently causing lysis and bacterial cell death.Checkerboard assays were used to determine the interaction between LL-37 and teicoplanin. All assays were performed in RPMI 1640 medium. A fractional bactericidal index (FBCI) was calculated, which is based on the increase or reduction of the lethal concentrations of two agents in combination compared to the lethal concentrations of the single agents. The assessment of synergy (FBCI ≤0,5), antagonism (FBCI >4) or no interaction (FBCI >0,5-4) was done for the microbicidal effect of two agents in combination against *S. epidermidis* and *S. aureus*.The experiments showed that LL-37 combined with teicoplanin had a synergistic microbicidal effect (FBCI = 0,03) against *S. aureus*. However, no synergy was observed against *S. epidermidis*. Also, *S. aureus* showed a higher level of resistance against LL-37 (MBC_{RPMI} = 90 μM) compared to *S. epidermidis* (MBC_{RPMI} = 6,25 μM). Explanations for these differences were sought in the level of alanyl esterification of the teichoic acids in the bacterial cell wall (regulated by the *dlt*ABCD operon) or in a difference in the level of lysinylation of phosphatidylglycerol in the bacterial cytoplasmic membrane (regulated by the *mprF* gene). Both of these changes increase repulsion of CAMPs due to an elevated positive net surface charge of the bacterial cell. Mutant *S. aureus* strains that contained deletions in *dlt*ABCD and *mprF* were therefore tested for their susceptibilities. Resistance against LL-37 was severely reduced in both deletion mutants. The synergistic effect of LL-37 combined with teicoplanin was abolished in the experiments with the *dlt* deletion mutant. However, the synergistic microbicidal effect was still present against the *mprF* deletion mutant. Experiments with *S. aureus* mutant strains show that the high resistance of *S. aureus* against LL-37 can be circumvented by using LL-37 combined with teicoplanin. A high level of alanylation may be a prerequisite for synergy between teicoplanin and LL-37 since synergy was abolished in the *dlt* deletion mutant. These results are supported by the fact that LL-37 and teicoplanin still synergized against the *mprF* deletion mutant. These findings contribute to the understanding of interactions between CAMPs and glycopeptides, and might contribute to improved treatment of BAI and other types of infection.

BAMA-O05

Human monoclonal antibody against the staphylococcal complement inhibitor protein SCIN for the specific detection of *Staphylococcus aureus*

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Introduction: *Staphylococcus aureus* can cause a wide variety of infections and can provide a serious public health burden particularly within health care settings. *S. aureus* is able to adapt to many different environments which causes the high efficiency in building resistance to new antibiotics. Faster detection of *S. aureus* could result in a more specific and direct treatment, preventing resistance development. Therefore novel treatment options such as immune therapy and faster diagnostics are needed. Human monoclonal antibodies (HuMAbs) are both a

possible treatment option and a diagnostic tool due to their relatively straightforward synthesis, long history of safe use and high specificity.

Methods and Results: Patients with epidermal bullosa (EB) have been shown to elicit high humoral responses against *S. aureus* due to the high challenge and carriage with diverse *S. aureus* strain types. A random screen and selection for binding of HuMAbs from various EB donors in an optimized whole-cell *S. aureus* binding assay resulted in several HuMAbs that specifically hybridized with *S. aureus*. Western detection of cell and supernatant fractions of *S. aureus* Newman*fjspafjsbi* using one of the HuMAbs resulted in the identification of a 10 kDa protein. Immunoprecipitation using the HuMAb and subsequent mass spectrometry analysis resulted in the identification of multiple possible target proteins. One of these proteins is the staphylococcal complement inhibitor (SCIN) which has a mass of 9.8 kDa for the mature secreted protein. A protein extract of an *S. aureus* strain lacking phage 13, which encodes SCIN, did not hybridise with the HuMAb. SCIN, which was expressed and secreted from *Lactococcus lactis,* could be detected by the HuMAb thereby proving to be the target protein. Using side directed, deletion and fusion derivatives of SCIN in an ELISA approach it was determined that the HuMAb binds to the C-terminal 15 amino acid domain of SCIN. This HuMAb was succesfully labeled with irdye CW800 for direct detection. Blast analyses showed that the complete SCIN is only expressed by *S. aureus* strains. In a western analysis on various Staphylococcal strains expressing proteins with slight homology to SCIN the HuMAb did not hybridise while 8 sequenced *S. aureus* strains, known to express SCIN, did hybridise. Out of 24 randomly selected clinical strains 23 could be detected by the HuMAb.

Conclusion: These results show that the isolated HuMAb recognises the *S. aureus* SCIN protein most likely at its C-terminus. The CW800 labelled HuMAb was succesfully used to specifically detect only *S. aureus* strains expressing SCIN. From this and earlier research it is clear that most human associated *S. aureus* strains express SCIN. Therefore this HuMAb has the potenty to be used to specifically target and detect human associated *S. aureus* strains in infections.

BAMA-O06

Mycoplasma genitalium; prevalence of azithromycin resistance and development of genotyping in clinical samples

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Introduction: Of all 2.5 million reported cases each year of NGU (non-gonococcal urethritis) in the USA, 15-20% are caused by *Mycoplasma genitalium*. Besides urethritis in men, cervicitis and pelvic inflammatory disease can occur in women. Possible complications during pregnancy include premature birth and spontaneous abortion. There are no official protocols in the Netherlands regarding diagnosis or treatment of this pathogene. Azithromycin is currently the recommended treatment for both *Mycoplasma genitalium* and *Chlamydia*. This study was undertaken to gain insight in the current status of antibiotic resistance and to explore methods of genotyping of *Mycoplasma genitalium* in clinical samples.

Materials: Patient samples used included swabs of the urethra, cervix, rectum and throat and urine samples. These samples originated from the STD clinic and other hospital departments as well as general practitioners in the Rotterdam area.

Methods: PCR detection was carried out using the in house qPCR for detection of *Mycoplasma genitalium* at the Maasstad Ziekenhuis.

For the typing of *Mycoplasma genitalium,* High resolution melting-point (HRM) analysis was used, combined with sequencing with various primers.

The azithromycin resistance study focused on the detection of azithromycin susceptibility decreasing mutations located on the 23S gene. To detect these mutations, part of the 23S gene was sequenced.

Results: PCR screening resulted in 110 samples positive for Mycoplasma genitalium amongst 100 unique patients. Investigation into the azithromycin resistance resulted in the following: 42% of the patients PCR positive for *Mycoplasma genitalium* carried a mutated strain. Mutation 2071A>G occurred in 11% of studied cases and 2072A>G, in 30% of all cases. Strikingly, the rarer 2071A>G mutated strains were mostly collected in the STD clinic. These patients are a higher risk group and more prone to reoccurring visits.

One primer set used for HRM allowed discrimination between different samples and provided comparable results regarding single patients. Regrettably the target site included a repeat region of which the repeat number varied within strains, possibly caused by gene regulation. When using HRM to make distinction between samples based on single nucleotide polymorphisms in a +/-200bp long DNA product this repeat region makes HRM a less reliable method.

Conclusion: A reliable typing method using HRM is not yet available for *Mycoplasma genitalium*, and to make HRM applicable, more research is required. We suggest to first rule out the possibility of a mutated *Mycoplasma* strain present, to minimize the risk of treating *Mycoplasma genitalium* and *Chlamydia* inadequately.

BAMA-O07

The role of IL-1 family members on Aspergillus fumigatus-induced ROS production and LC3-associated phagocytosis

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Introduction: *Aspergillus fumigatus* is an opportunistic pathogenic fungus that can cause invasive asprgillosis in immunocompromised hosts as a result of secondary immunodeficiency. However a primary immunodeficiency, chronic granulomatous disease (CGD), results in a defective production of reactive oxygen species (ROS), and is associated with an increased susceptibility to aspergillosis. These CGD patients have defective microbial killing since ROS is required for LC3 associated phagocytosis (LAP), which is required for fungal killing. Previously it was demonstrated that treatment with the IL-1 receptor antagonist can restore LAP in these patients, through mechanisms that remain unexplained. We therefore want to investigate how IL-1 cytokines influence *A. fumigatus*-induced ROS and phagocytosis.
Methods: Peripheral blood mononuclear cells (PBMCs) from healthy individuals were stimulated with IL-1 cytokines and *A. fumigatus*, and induction of ROS was measured by luminol-dependent chemiluminescence assay. Live cell imaging was performed to assess the effect of IL-1 cytokines on phagocytosis of *A. fumigatus*.
Results: Pre-stimulation of PBMCs for 24 hours with IL-1β significantly increased *Aspergillus*-induced ROS production, whereas stimulation with its antagonist IL-1Ra leads to reduced ROS production. Additionally, preliminary analyses of live cell imaging indicate that IL-1 cytokines alter the phagocytic activity of macrophages.
Conclusion: Collectively our data illustrates that IL-1 cytokines modulate ROS production upon stimulation with *A. fumigatus*. Furthermore, phagocytic activity seems to be altered by IL-1 cytokines, though this has to be further investigated. These results implicate a role for IL-1 cytokines in modulating ROS production and phagocytosis, thereby highlighting the potential for targeting these cytokines in immunotherapy.

BAMA-O08

Interactions of *Mycobacterium marinum* with *Candida albicans*

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Mycobacteria are a leading cause of infectious disease worldwide. Their habitat is diverse and shared with many other microbes, including the opportunistic fungal pathogen *Candida albicans*. Both Mycobacteria and *C. albicans* can use humans as a host. *C. albicans* is able to interact with many pathogenic bacteria, both chemically and physically. Some cases even report *C. albicans* helping *S. aureus* infect the human body by attracting macrophages. This aspect is of importance as *Mycobacterium tuberculosis* and also Non-Tuberculous-Mycobacteria such as *Mycobacterium marinum* use human macrophages to thrive in. The question therefore rises whether *C. albicans* plays a role in uptake of Mycobacteria by macrophages. In this study both a chemical and physical interaction between *C. albicans* and *M. marinum* was studied, along with a phagocytosis assay using J774 murine macrophages. Biofilm samples from the liquid air interface of a co-culture showed that both pathogens are able to live in close proximity. Adjacent plating of both microbes resulted in hyphal formations of *C. albicans* projecting towards *M. marinum* after seven days. Kinetic growth assays with spent medium from *M. marinum* suggested a growth-inducing effect on *C. albicans* for three day old spent medium. *M. marinum* showed strong physical adherence to *C. albicans* hyphae when tested using a bioflux and live automated microscopy. However, *M. marinum* adherence to *C. albicans* hyphae did not affect phagocytosis of *M. marinum* by macrophages. In the presence of the detergent Tween-80, less adhesion was observed. Washing with Tween-80 after adhesion showed mild dissociation of *M. marinum* from *C. albicans* hyphae. No adherence of *M. marinum* to *C. albicans'* less hydrophobic yeast cells was observed. It is concluded that at least part of the adhesion is caused by the hydrophobic nature of both *C. albicans* hyphae and *M. marinum*. Both microbes are able to coexist and no direct link with immunoactivation or deactivation has been found.

BAMA-P01

Metagenomics: the bioinformatics bottleneck

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In recent years, metagenomic analysis has become routine. Not fifteen years ago a fully sequenced genome would guarantee a high impact publication, whereas today, multiple sequences in one publication are not uncommon. Next-generation sequencing methods have made obtaining high quality reads of increasing length available to many labs. These technological advances are especially useful for microbial ecologists, who study environmental samples that often reveal an intense increase in diversity when sequenced than could be cultured in the lab. However, between sequencing and biological interpretation there

exists a black box for many biologists. This black box is the bioinformatics bottleneck. Metagenome sequencing produces incredible amounts of data: many gigabytes for a single environmental sample is not unheard of. Ensuring that these data produces high-quality information for biological analysis is therefore a daunting task that becomes increasingly difficult with the increasing size of datasets. Many microbial ecologists are unaware of these challenges and rely on bioinformaticians to do this work for them. In my Master's project, I focus on clearing this black box. Using metagenome data obtained from brewery wastewater treatment sludge, I aim to produce reliable information about microbial diversity and functionality using cutting-edge bioinformatic approaches. Furthermore, I aim to create pipelines that will enable researchers with little to no knowledge of bioinformatics to produce reliable information with their own data. In my presentation for the BAMA symposium, I will walk through the general steps of metagenome analysis, common biases in different approaches and focus on the most powerful methods currently available to facilitate genome extraction from metadata. The presentation will conclude with the results from the example dataset: a complex microbial community (hypothetically) capable of completely metabolizing organic compounds to carbon dioxide and elemental nitrogen.

BAMA-P02

Copper tolerant ecotypes of *Heliscus lugdunensis* differ in their ecological function and growth

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Introduction: Leaf litter from riparian zones is considered the main energy source for low order streams. Aquatic hyphomycetes decompose leaf litter and, in this way, play an important ecological role in the energy transfer from riparian plant litter to stream invertebrates and higher trophic levels as well as in nutrient cycling. Metal pollution caused by industrial activities, particularly mining, poses risks to the functionality and health of aquatic ecosystems. In response to high metal concentrations, some species of aquatic hyphomycetes have evolved tolerances by adapting life history traits such as growth and sporulation. Several studies reported that metal tolerance in aquatic hyphomycetes varies according to the pollution levels at their isolation site, suggesting the possibility for genetic adaptation. Whereas previous research has been mainly focusing on the effects of metal exposure on interspecies diversity of aquatic hyphomycetes, stress responses at intraspecies level remain largely unexplored. This raises the question of how in particular metal tolerant strains of aquatic fungal species differ in their ecological function in regard to litter decomposition and if they differentiate into ecotypes. *Heliscus lugdunensis* is a ubiquitous aquatic hyphomycete commonly found in different aquatic environments, including metal polluted sites.

Methods: The aim of this study was to explore whether strains of *H. lugdunensis* are expressed as ecotypes with distinct physiological and functional signatures in response to metal stress. Investigations were made on reproductive output, metabolic activity and decomposition activity as well as growth inhibition and growth rates of strains of *H. lugdunensis* isolated from reference streams, a moderately polluted stream and a highly polluted stream contaminated with copper (Cu).

Results: When exposed to Cu, strains of *H. lugdunensis* differed significantly in their litter decomposition and reproductive activity (sporulation) as well as mycelial growth, corresponding to the Cu concentrations at their isolation site. Strains isolated from sites with high Cu concentrations induced the highest litter decomposition or invested most in growth.

Conclusion: The results of this work suggest that Cu contamination may have led to evolved adaptations within species that resulted in ecotypes. The exact mechanisms allowing different ecotypes to cope with metal stress deserves further investigation as well as investigations on genetic differences between strains. Our findings are anticipated to foster our understanding of metal tolerance in aquatic hyphomycetes as they elevate the importance of the fundamental ecological role of fungal ecotypes as they maintain ecological functions (decomposition rates) under pollution conditions and therefore might minimize the metal pollution effects in the environment.

BAMA-P03

The microbiome of the gastrointestinal tract of *Phoca Vitulina* and *Halichoerus grypus*

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The main purpose of this study is to detect the bacterial composition of the microbiome of the gastrointestinal tract of harbour seals (*Phoca Vitulina)* and grey seals (*Halichoerus grypus)* by analyzing the 16S ribosomal

ribonucleic acid (16S rRNA) gene using next-generation sequencing.. With the obtained data assessment of the effect of rehabilitation on the composition of the bacterial microbiome is possible for both seal species.
For this study, we obtained 469 rectal ESwabs from 102 aforementioned seals sheltered in the seal rehabilitation centre Pieterburen. The rectal ESwabs were taken on eight different moments of the rehabilitation process of the seals. These moments include: at the day of arrival in the rehabilitation centre, eight and fifteen days after arrival, before receiving antibiotic treatment, during antibiotic treatment, seven days after receiving antibiotic treatment, when released into the North Sea, and after death of a seal. Because the rectal ESwabs were received at these different moments, a difference in the composition of the microbiome is expected. The results and conclusion of this study will be generated the next few weeks, and will be available for the BAMA symposium.

BAMA-P04

Co-culturing of the methanogen *Methanosarcina barkeri* and the methanotroph *Methylosinus sporium*

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Wetland ecosystem are considered to be the biggest natural source of methane emissions, contributing to 20% of the global methane emissions. Current thawing of permafrost leads to an increase in potential methane emitting wetland area. In these ecosystems methane cycling is controlled by microbial methanogenesis and methanotrophy. A better understanding of the microbial processes is needed to predict methane feedback from permafrost. In this study a bioreactor was constructed in which a methanogenic archaeon, *Methanosarcina barkeri*, and a methanotrophic bacterium, *Methylosinus sporium* could be cultured in a pre-designed common medium with acetate as methanogenic substrate. After 63 days of incubation clusters containing the two species were observed using FISH microscopy. The genome of *Methylosinus sporium* has been sequenced using Illumina MiSeq technology to allow subsequent transcriptome studies in response to e.g. temperature changes. This novel co-culture allows further research on the interactions of both organisms and their responses to environmental changes. Therefore activity and transcriptome analyses will be used to link environmental conditions to the properties of metabolic functions of these microorganisms.

BAMA-P05

The effect of Pulsed Xenon Ultraviolet Light (PX-UV) on the survival of *Klebsiella pneumonia* ST258 in the environment, an *in vitro* experiment

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Introduction: The introduction of Pulsed Xenon Ultraviolet Light (PX-UV) for disinfection of the environment in healthcare settings is relatively new. PX-UV causes irreversible cellular damage of the bacterial DNA preventing further cell replication. The objective of our study was to determine the influence of PX-UV after variable time-intervals on the survival time of *Klebsiella pneumoniae* Sequence Type (ST) 258, a pandemic strain.
Methods: An *in vitro* experiment was performed. Cover glasses were inoculated with 10 µL of a 0.5 McFarland *K.pneumoniae* ST258 in sterile saline and subsequently kept at room temperature. Bacterial survival on the glasses was determined hourly during the first day, and daily during the following 6 days. Glasses were placed into 2 mL brain-heart infusion (BHI) broth, and vortexed for 30 seconds. Sheep Blood Agar plates (SBA) were inoculated with 20 µL of a 10-fold dilution series of the BHI-broth, and the remaining BHI-broth was filtered over a sterile 0.45 µm cellulose Millipore filter, that was placed on a SBA. The number of viable bacteria on the glasses was estimated by counting the number of CFU on the SBA after overnight incubation at 35-37°C.The detection limit of the assay was 111 CFU/mL. Eight series were performed. In 4 series, PX-UV (REV Desinfectie Robots, Tiel, Netherlands) was applied during 5 minutes, immediately after inoculation of the glasses. In another 4 series, PX-UV was applied during 5 minutes after a time period of 3.5 hours. SPSS was used to analyse the reproducibility of the separate series and create survival curves (t = 0h and t = 3.5h).
Results: Application of PX-UV directly after inoculation, resulted in a 1.9 log reduction in bacterial counts. When applied after a period of 3.5 hours, a 4.7 log reduction in bacterial counts was observed. The difference in reduction was statistically significant.
Conclusions: Our study showed that PX-UV effectively reduces bacterial counts in the environment. However, the effect was much stronger after 3.5 hours. This is probably due to the evaporation of water, exposing the bacteria to the direct effect of UV-light. The PX-UV is a promising technique to control environmental contamination with multi-resistant microorganisms.

BAMA-P06

Survival of Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* on inanimate surface, in four different suspension fluids: an in vitro experiment

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Introduction: The role of environmental contamination in the transmission of Enterobacteriaceae is increasingly recognized. However, factors influencing the duration of survival in the environment have not yet been extensively studied. We evaluated the ability of ESBL-producing *E. coli* to survive over time on a dry inanimate surface, and assessed differences in survival between four different suspending media.

Methods: Two ESBL-producing *E. coli* strains (sequence type (ST) 131 and ST10) were used for this in vitro experiment. An 1 McFarland suspension of each strain was 1:1 diluted in sterile water, sterile saline, sheep blood or artificial urine. Cover glasses (18x18mm) where inoculated with 20 µL of the dilution and subsequently kept at room temperature. Bacterial survival on the glasses was determined hourly during the first day, once daily during the following 6 days, and from day 7, once weekly up to 100 days. To determine the number of viable bacteria for each time interval, cover glasses were placed into 2 mL brain-heart infusion (BHI) broth and vortexed until the inoculum was completely homogenized. Sheep blood agar (SBA) plates were inoculated with 20 µL of a 10-fold dilution series of the BHI broth. After overnight incubation at 35-37°C, we counted the number of colony-forming units (CFU) on the SBA plates, and used the results to estimate the number of viable bacteria on each glass. When the colony count of the dilutions were <30 CFU/plate, the remaining BHI-broth was filtered over a sterile 0.45 µm Millipore filter that was placed on a SBA plate, and proceeded to incubate and to count as described above. The detection limit of the assay was 56 CFU/mL. The experiment was repeated six times per strain for the suspension fluids sterile water, sterile saline, and sheep blood; and eight times per strain in artificial urine.

Results: We found no evidence of a difference in survival between the two sequence types, and results of both strains were analyzed together. Viable bacteria could be detected up to 71 days in sheep blood and sterile saline, and up to 64 days in water. Survival was significantly different between all 4 suspending media ($p < 0{,}05$), whereby the number of viable bacteria was consistently highest in sheep blood. Differences in survival were evident the first day after inoculation, and for isolates suspended in artificial urine no growth was observed after day 1.

Conclusion: Our study shows that ESBL-producing *E. coli* can survive on inanimate surfaces for extended periods. The type of suspension material was an important determinant of survival time. The enhanced survival in the presence of blood underpins the importance of environmental cleaning for effective infection control.

BAMA-P07

Synthesis of silver nanoparticles in *Colocasia esculenta* root extract and determination of their antimicrobial activity

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Introduction: The research in field of biotechnology has contributed to silver nanoparticles being used in varous antibacterial or antifungal agents.

Silver nanoparticles are particles of silver oxide. They cause the release of Potassium ions (K+) from bacteria thus disturbing the osmotic balance in the cytoplasm and further leading to its lysis.

The plant *Colocasia esculenta* belongs to family *Araceae* which is one of the staple foods in the tropical regions. This plant is known to have various antibacterial, antifungal, antioxidant and anticancerous properties.

Hence by combining their properties we tried to aim at the green synthesis of silver nanoparticles and hypothesizing their use as a broad spectrum antimicrobial agent.

Methods: The following methods were used:

Synthesis of nanoparticles by $AgNO_3$ and root extract of *Colocasia esclucenta*.

Testing of antimicrobial activity by Disc diffusion assay under the guidelines of Clinical and Laboratory Standards Institute (CSLI).

Quantitative determination by MIC by broth dilution method under the guidelines of CSLI.

Test microrganisms used : *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*.

Results: *Antimicrobial activity:* The antimicrobial activity was determined by the disc diffusion assay. The particles showed activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. No activity was observed against *E. coli*.

Determination of minimum inhibitory concentration: Broth dilution method according to the CLSI guidelines was performed to determine the minimum inhibitory concentration (MIC). The MIC obtained was 3.125mg/ml against Gram negative bacteria *P. aeruginosa* and Gram positive bacteria *S. aureus*. As the compound was turbid, only visually inspecting the tubes for the presence of growth was not possible. Hence, growth from the tubes was streak plated on plates of sterile nutrient agar and examined.

Conclusion: The nanoparticles showed inhibition of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Hence it can be concluded from the study that the silver nanoparticles synthesized from the root extract of *Colocasia esculenta* show a broad spectrum of antibacterial activity.

BAMA-P08

Cynodon dactylon: antimicrobial potential of crude extract as valuable medicinal plant

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Cynodon dactylon (Poacae) is a well known traditional plant used as a folk remedy in treatment of many symptoms and diseases like cramps, measles, tumors, wounds, warts, fever and rheumatic affections. In this study, the antimicrobial activity of the plant crude extract from three different extraction (hot and cold aqueous extraction, methanol extraction) was investigated against some of the gram positive bacteria (Staphylococcus epidermidis, Bacillus cereus) and gram negative bacteria (Escherichia coli, Pseudomonas aeroginosa, Salmonella typhi, Shigella dysenteriae) using disc diffusion method. Amoxicillin was taken as positive control. The diameter of the clear zone of inhibition surrounding the disc was measured. The aqueous extract of Cynodon dactylon had antimicrobial activity against all the test organisms indicating broad spectrum activity of the extract for both gram positive and gram negative bacteria. No clear zone formed with methanol extract. It can be concluded that aqueous extract of whole plant of Cynodon dactylon may be considered as an antibacterial agent and can be used to source antibiotic substances for possible treatment of bacterial infections.

BAMA-P09

Draft genome of _Morganella morganii_ isolated from the gut of the cabbage root fly larva

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Severe crop losses in *Brassica* plants of up to 50% are accounted to damage caused by the cabbage root fly *Delia radicum*. The defense mechanism of these plants, using toxic compounds such as isothiocyanates, does not seem to affect the larvae. The gut microbiome could be involved in the detoxification of the compound, therefore it was investigated. Genome sequencing provides a powerful tool to get an understanding of the microorganisms colonizing the gut of the root fly larvae. A genome of an isolate from the gut belonging to the species *Morganella morganii* was sequenced. The 16S rRNA sequence closely resembles 16S rRNA sequences of bacteria belonging to the family *Enterobacteriaceae*, of which many are colonizing gut systems. In the annotated genome, a multidrug efflux pump was found that may play a role in excreting the isothiocyanates, making the isolates resistant to them. Additionally, the genome was compared to other *Morganella* genomes. In conclusion, the genome of the isolated *Morganella morganii* elucidates the resistance mechanisms of microorganisms living in the gut of root fly larvae.

BAMA-P10

Characterization of _Pseudomonas aeruginosa_ from clinical isolates from animals

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Background: *Pseudomonas aeruginosa* is a gram negative rod which can cause opportunistic infections in humans, animals and plants. *P. aeruginosa* is intrinsically resistant to multiple classes of antibiotics and can acquire additional resistance genes from other organisms but also trough (point)mutation. (Multi) Resistant *P. aeruginosa* is often isolated from animal samples. Little is known about the phenotypic and genotypic characteristics of *P. aeruginosa* of clinical isolates from animals. The purpose of this study was to determine susceptibility profiles and to determine the genetic diversity of the strains by genotyping.
Material & Methods: In total 113 presumptive *P. aeruginosa* isolates, obtained from clinical samples from the Veterinary Microbiological Diagnostics Center (VMDC), were included which were isolated from dogs (99) but also from cats (6), horses (6) and rabbits (2). Species determination was confirmed by MALDI-TOF, susceptibility testing was performed by broth microdilution using a standardized panel of 24 clinically relevant antibiotics. In addition, a ChromID CarbaSmart agar was used to quickly detect carbapenemase activity. Genotypic characterization was performed by screening for most the common (plasmid-mediated) ESBL and carbapenemase genes by PCR (CTX-M, TEM, SHV, CMY-2, NDM, KPC, VIM, IMP and OXA). Multi-Locus Sequence Typing was performed to determine the sequence types which referred to the pubMLST database for *Pseudomonas aeruginosa*.
Results: In total, 109 isolates were confirmed to be *P. aeruginosa* by MALDI-TOF. The remaining four non-pseudomonas species were excluded for further analysis. Based on Minimal Inhibitory Concentration data, all isolates were classified as multi drug resistant because they were resistant to more than three antibiotic classes. There were 33 unique resistance profiles but also five frequent profiles. The frequent profiles consisted of 5, 7, 10, 14 and 16 isolates respectively, all resistant to 19

antimicrobials or more. 58.7% of the isolates were phenotypically positive in the carbapenemase-quick test, but tested all negative for the most common beta-lactamase genes by PCR. This may indicate the presence of intrinsic resistance determinants. 53 new sequence types (ST) were identified by MLST. Several previously described high risk clones in human, were also identified (ST244, ST274 and ST277). Interestingly, 6 isolates from cats were screened, of which 4 belonged to ST1320 and all 4 showed the same resistance pattern, whereas the strains were epidemiologically unrelated.

Conclusions: 1: The *P. aeruginosa* isolates screened showed a high level of antibiotic resistances. 2: The population of *P. aeruginosa* is genetically highly diverse, showing 53 new ST's and ST's that have been found in humans. 3: No common beta-lactamase genes were identified.

BAMA-P11

Investigation of transmission of potential pathogenic bacteria via working uniforms of health care workers within the UMCG using molecular typing methods

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Introduction: Hospitals are constantly trying to reduce the dissemination of antibiotic resistant bacteria. In patients these infections can pose a serious threat to their health while facilitating the process of spreading these bacteria to fellow patients. Therefore it is crucial to detect these infections early on and reduce the transmission of these bacteria. This project focussed on determining if bacterial species can spread in the UMCG via working uniforms. The analysis process was used to construct an educational framework for medical students.

Methods: Health care professionals of a department of the UMCG were asked to complete a questionnaire containing questions about their profession, workplace and duration of wearing the work related clothing. Work related clothing of all participants was sampled with RODAC plates on the back collar, inside of their right pocket and between the second and third button on their chest. After growth all colonies were analysed by determining the species via MALDI-TOF. Statistical analyses were carried out to determine the differences in the presence of potentially pathogenic species based on duration of uniform worn, profession of persons wearing it and the site of sampling. Antibiotic pefusion disk assays, Spa typing and sequencing were used to determine whether bacteria were methicillin or multiple resistant. The educational framework included the making of movies and pictures to facilitate the learning process.

Results: A total of 66 healthcare professionals were included in this study with 81.8% of the participants being female. Most of the nurses (96.7%) and 100.0% of the ward cleaners, nurses in training, and nutritional providers said that they had worn the uniform for 1 day only whereas 73% of the specialists and physicians had worn it for a period >2 days. Most professionals believed that work uniforms are a means for transmission of pathogens (94%). Most professionals believed their clothes to be moderately contaminated, giving their uniforms' a score of 5 to 7 out of 10 (10: highly contaminated).

In total 5 *S. aureus* strains were identified. In all cases the strains were found in the right pocket of the uniforms. The professionals carried multiple different items in the pockets of their uniforms. None of the samples resulted in being multiple resistant according antiobiotic pefusion disk assay. This correlated with the molecular typing which showed that none of the strains carried the *mecA* gene. Spa typing resulted in the identification of two identical Spa types.

Discussion and Conclusions: The presence of *Acinetobacter iwoffii, Pseudomonas spp., Pantoea agglomerans* on the uniforms was highly relevant as it potentially poses a threat to immunocompromised children in the ward. Such findings would suggest the need for more regular de-contamination of the work uniforms of the health professionals.

While MALDI-TOF analysis seemed moderately reliable it was the quickest approach for species determination. Complemented by antibiotic perfusion disk assays, a general overview of transmission can be provided. Based on the Spa-typing results transmission could have occured between health care workers. The analysis process has been successfully used by first year medical students.

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***Candida albicans* & *Clostridium difficile*: a love-hate relationship**

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The facultative anaerobic polymorphic fungus *Candida albicans* and strict anaerobic Gram-negative bacterium *Clostridium difficile* are two opportunistic microbes residing in the human gut. While *C. albicans* overgrowth is more prevalent in patients treated for *Clostridium difficile* infection, the nature of the interactions between these two microbes has never been studied. In this study the chemical and physical interactions between *C. albicans* and *C. difficile* were investigated. *C. difficile* was able to grow in *C. albicans* biofilm under aerobic, normally toxic,

conditions. *C. difficile* showed a strong hyphal inhibiting effect on *C. albicans* and was able to converting hyphae back into the yeast and pseudohyphal morphology, thus acting on a virulence factor of *C. albicans*. P-cresol is a product of fermentation of *C. difficile* and was shown to be the active component of spent medium inducing this morphological effect. *C. difficile* and *C. albicans* appear to have a love-hate relationship; *C. albicans* allows growth of *C. difficile* under toxic conditions while *C. difficile* inhibits virulence of C. *albicans*.

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