INLEIDING

De voorjaarsbijeenkomst van de Nederlandse Vereniging voor Medische Microbiologie (NVMM) en de Nederlandse Vereniging voor Microbiologie (NVvM) in samenwerking met de Nederlandse Vereniging voor Medische Mycologie (NVMy) vindt plaats op dinsdag 1 en woensdag 2 april 2008 te Papendal.

Het onderwerp van de plenaire ochtendsessie is 'Systems Biology', een onderdeel van de wetenschap die zich bezighoudt met de studie van interacties binnen complexe biologische systemen. Het basisbegrip in 'Systems biology' is integratie en als dusdanig staat dit misschien wel voor wat wij met de Voorjaarsvergadering beogen: het samenbrengen van eenieder in Nederland die zich bezig houdt met microbiologie. Na de parallelsessies zal de dinsdagmiddag plenair worden afgerond met twee belangrijke momenten: de Kluyver-lezing die dit jaar zal worden gehouden door Jeff Errington en de uitreiking van de Kiem-prijzen.

De inmiddels bijna traditionele nieuwe formules die enkele jaren geleden werden geïntroduceerd worden alle gehandhaafd: thematische sessies die door leden zelf zijn voorgesteld, traditionele sessies die al jaren deel uitmaken van het programma, een groot feest na de postersessie en kosteloze deelname aan de bijeenkomst voor de jonge onderzoekers onder ons die hun werk presenteren. En niet te vergeten: de twee 'Voorjaarsdagen' worden voorafgegaan door een middag en avond voor artsen in opleiding tot arts-microbioloog, voor een toets en een aantal lezingen.

Al met al belooft het weer een wervelend programma te worden; wij wensen u allen een vruchtbare Voorjaarsvergadering 2008.

Het programma van het ochtendsymposium ziet er als volgt uit:

- **Vertical genomics in microorganisms, models for systems biology**
  B.M. Bakker, VU University Medical Centre, Amsterdam
- **Functional anatomy of a biofilm**
- *R. Kolter, Harvard Medical School, Boston, USA*
- **Mapping and integrating virus entry and endocytic pathways in human cells**
  L. Pelkmans, ETH Zürich, Zürich, Switserland
- **The bacterial pan genome and the world of non-coding RNA. A case study in Cyanobacteria**
  W.R. Hess, Freiburg University, Freiburg, Germany

Kluyver lecture:

- **A reproducible system for generating wall-less (L-form) bacteria: Implications for the evolution of cell proliferation**
  J. Errington, University of Newcastle, Newcastle, United Kingdom

Voorbereidingscommissie

Prof. dr. C.M.J.E. Vandenbroucke-Grauls, voorzitter
Dr. C.H.E. Boel
Prof. dr. S. Brul
Mw. Dr. B. Duim
Prof. dr. J.M.D. Galama
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Mw. Dr. A.M. Wensing
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De Wetenschappelijke Voorjaarsvergadering 2008 wordt georganiseerd door de Nederlandse Vereniging voor Medische Microbiologie (NVMM) en de Nederlandse Vereniging voor Microbiologie (NVvM) in samenwerking met de Nederlandse Vereniging voor Medische Mycologie (NVMy).

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Congress Care
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Dates
31 March, 1 & 2 April 2008

Venue
Hotel en Congrescentrum Papendal
Papendallaan 3
Arnhem
Tel. 026 - 483 79 11

Website
Please check www.congresscare.com for up-to-date program information and www.nvmm.nl or www.nvvm-online.nl for more information on the NVMM or NVvM.

Language
The language will be English during the scientific sessions, unless stated otherwise.

Accreditation
The 'Wetenschappelijke Voorjaarsvergadering 2008' will be accredited by the NVMM with 8 points for day 1 (Tuesday 1 April) and 5 points for day 2 (Wednesday 2 April).

Please note that in order to receive accreditation you need to supply the congress secretariat with your BIG-number. If the secretariat does not receive your BIG-number, you will NOT receive accreditation.

Name badges
All participants should wear their name badges throughout the conference.

Registration desk
The registration desk will be open on Monday, Tuesday and Wednesday during conference hours.

Poster session
Yakult Nederland sponsors the poster price for the best poster and the poster price ceremony with drinks. The price is € 250.
The poster price ceremony will be held on Tuesday April 1 at 22.00 hours. The winner has to be personally registered and present.

Young Investigator's Grant
The registration fee for presenting PhD-students and AIOS (oral or poster, presenting author only) will be waved.

Grant 'Antonie van Leeuwenhoek Stichting'
The 'Antonie van Leeuwenhoek Stichting' supports PhD-students and AIOS to attend the 'Wetenschappelijke Voorjaarsvergadering 2008' and therefore, they will sponsor the hotel costs (1 night) for presenting PhD-students and AIOS (poster or oral, presenting author only).
Please fill out on the registration form whether you would need a hotel room and please provide the congress secretariat with a written certification by the head of the department or employer stating you are a PhD-student or AIOS.

Dance Party 'Groot Microbiologie Feest'
The poster price ceremony will be followed by a dance party open for all participants.

Catering
Coffee/tea will be available at the exhibition during the breaks. The lunch will be served at the exhibition during the lunch break.

Hotel rooms
If you have reserved a hotel room in 'Hotel and Congress Center Papendal' you may collect the room key as of 13.00 hours at the front desk of the hotel. Please make sure to check out before 10.00 hours.

Hotel and Congress Center Papendal
All participants receive a route description together with their confirmation of registration. For more info please check www.papendal.nl.
Papendal taxi: the Papendal taxi will bring you from Central Railway Station Arnhem to Hotel and Congress Center Papendal for € 7,50 per person. If you would like to make use of this service, please call BTC taxi at 026-321 00 00 (mention the Papendal taxi). The taxi's can be found at the 'Sonsbeek' side of the railway station.
You have to pay at arrival at the hotel reception. The taxi driver will wait for a ticket you have to give him. At the end of congress you can order at the hotel reception a Papendal taxi to bring you to the railway station.

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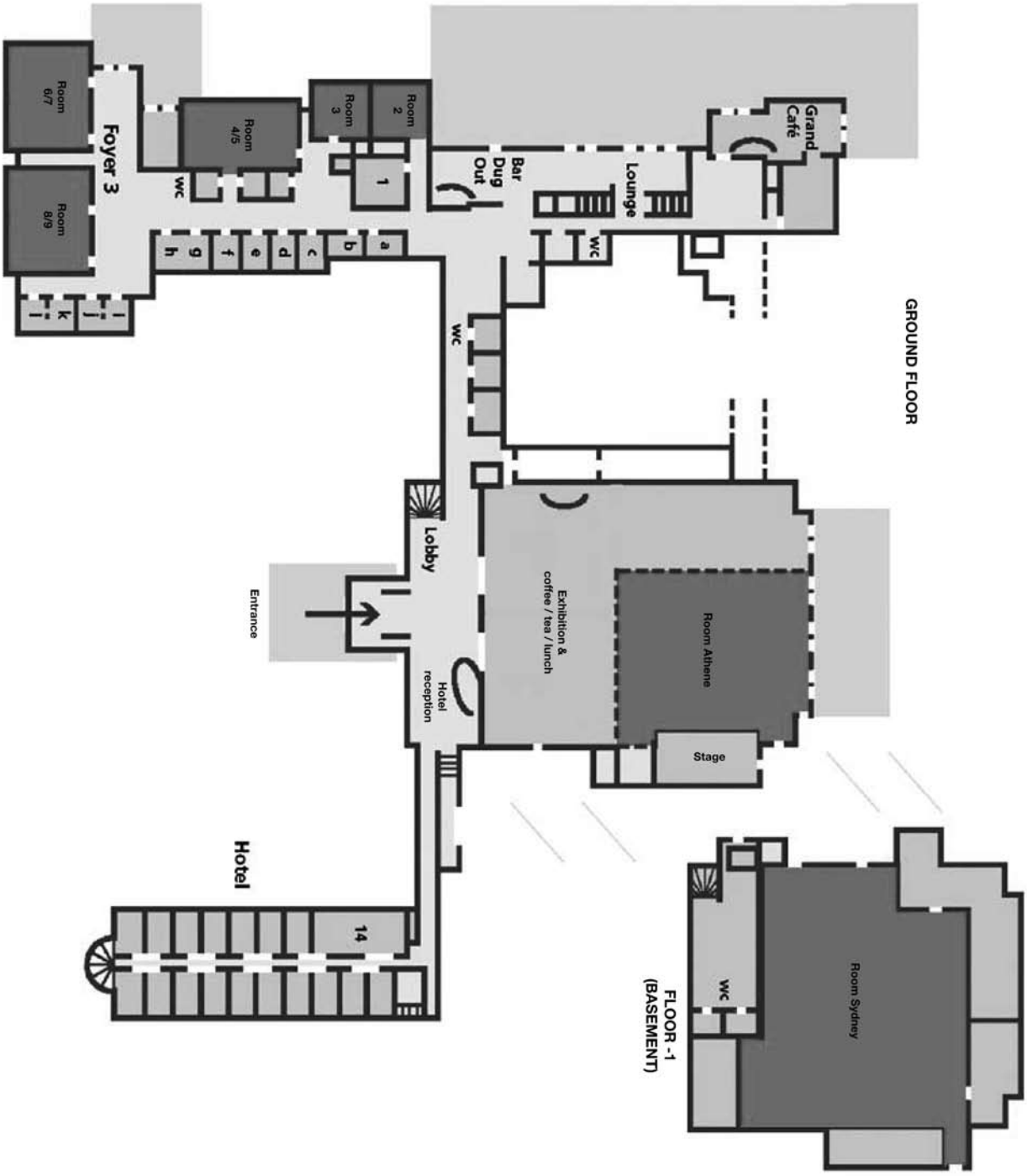
Sponsor poster price and drinks on Tuesday evening, April 1:

Yakult

FLOORPLAN CONGRESS CENTRE



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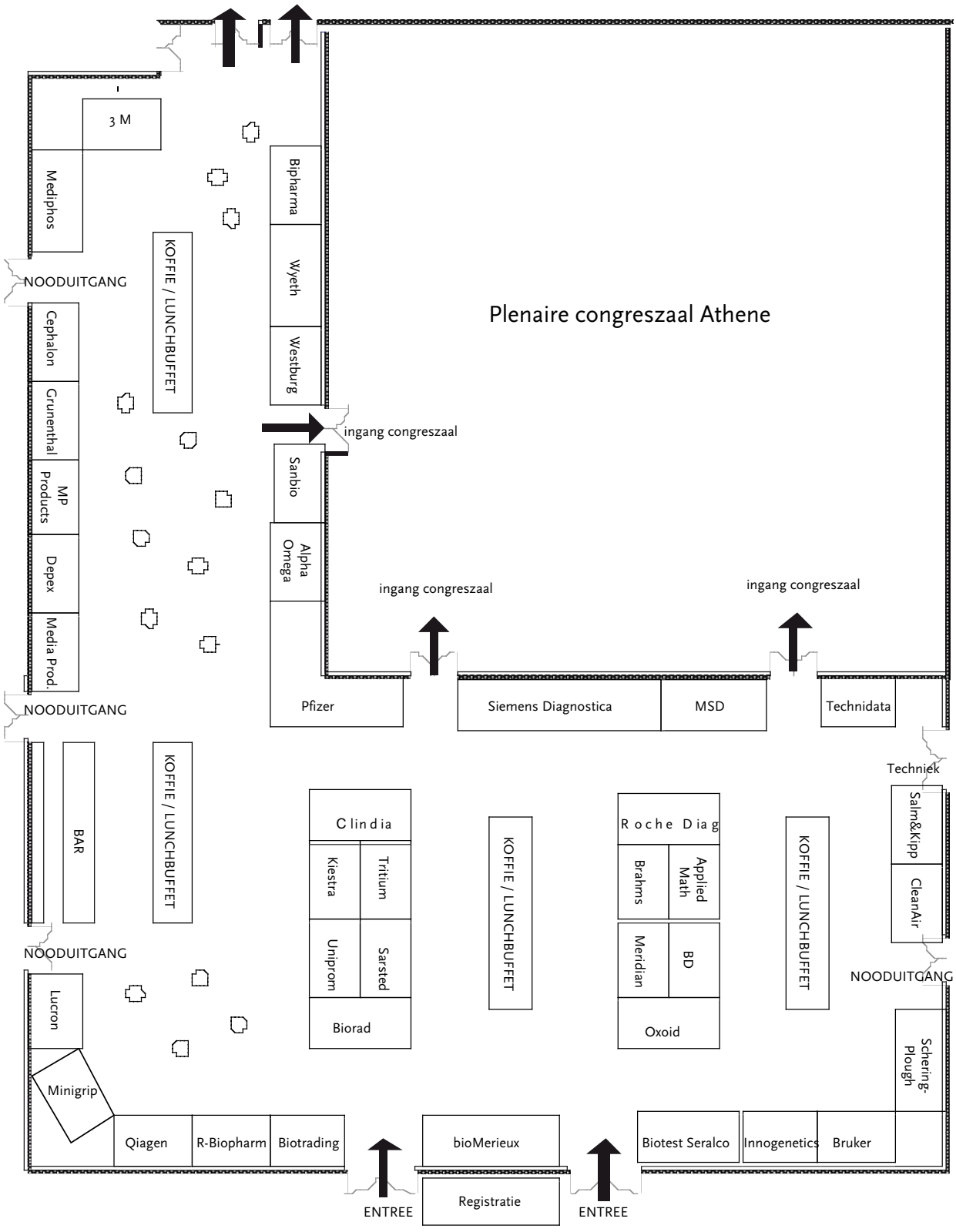

MONDAY MARCH 31 2008

Room Sydney		
12:00	Registration and lunch	
13:00 - 15:00	National Examination for medical microbiologists in training	
15:00 - 15:30	Coffee/tea	
15:30 – 16:15	*N. Hartwig*	
16:15 – 17:00	*A. Voss*	
17:00 – 18:30	Drinks	
18:30 – 20:30	Dinner	

TUESDAY APRIL 1 2008

Room Athene	**Plenary session 'Systems biology'**	
	Chairmen: C.M.J.E. Vandenbroucke-Grauls & H.A.B. Wösten	
09.30 – 10:15	*B.M. Bakker (Amsterdam)*	O001
	Vertical genomics in microorganisms, models for systems biology	
10:15 – 11:00	*R. Kolter (Boston, USA)*	O002
	Functional anatomy of a biofilm	
11:00 – 11:30	Coffee/tea	
11:30 – 12:15	*L. Pelkmans (Zürich, Switzerland)*	O003
	Mapping and integrating virus entry and endocytic pathways in human cells	
12:15 – 13:00	*W.R. Hess (Freiburg, Germany)*	O004
	The bacterial pan genome and the world of non-coding RNA. A case study in *Cyanobacteria*	
13:00 – 14:00	Lunch	
Room Athene	**Parallel session 'Outsourcing 1** (Nederlandstalige sessie)**'**	
	Chairman: G.J.H.M. Ruijs	
14:00 – 14:25	*G.J.H.M. Ruijs*	O005
	Outsourcing of outsorcering	
14:25 – 14:45	*R.W. Jansen*	O006
	Outsourcing van een klinisch-chemisch en hematologisch ziekenhuislaboratorium; ervaring uit de praktijk	
14:45 – 15:05	*Spreker namens Inspectie voor Volksgezondheid*	O007
15:05 – 15:30	*Discussie o.l.v. M. Buiting*	O008
15:30 – 16:00	Coffee/tea	
Room Sydney	**Parallel session 'WOGIZ'**	
	Chairman: J.W. Dorigo-Zetsma	
14:00 – 14:15	*A.J. Jacobi*	O011
	Oefening baart kunst	
14:15 – 14:30	*A. Timen*	O012
	Outbreakmanagement: wetenschap, praktijk of beide?	
14:30 – 15:00	*G.R. Westerhof*	O013
	Inspectieonderzoek naar de kwaliteit van het medisch-microbiologisch handelen in Nederland	
15:00 – 15:30	*M.R. Klein*	O014
	Het nieuwe BSL4-lab van het RIVM, alleen voor het allerengste!	
15:30 – 16:00	Coffee/tea	
Room 2	**Parallel session 'Pertussis outbreak in Rotterdam, fact or fiction'**	
	Chairman: P. Petit	
14:00 – 14:15	*H. Götz*	O015
	De Rotterdamse epidemie	
14:15 – 14:30	*J. Schellekens*	O016
	Diagnostiek van kinkhoest: state of the art	
14:30 – 14:45	*G. Berbers*	O017
	Invloed vaccinatie op diagnostiek	
14:45 – 15:00	*D. Notermans*	O018
	Betekenis van IgA bij kinkhoest	
15:00 – 15:15	*S. de Greeff*	O019
	Epidemiologie van kinkhoest	
15:15 – 15:30	*P. Schneeberger*	O020
	Consensusdiscussie 'wanneer is het kinkhoest'?	
15:30 – 16:00	Coffee/tea	
Room 3	**Parallel session 'Are the Netherlands ready for *Chlamydia trachomatis* screening?'**	
	Chairman: R.P. Verkooijen	
14:00 – 14:30	*I. Rours*	O021
	Consequences of *Chlamydia trachomatis* infection during pregnancy for the newborn	
14:30 – 15:00	*M. Postma*	O022
	Cost-effectiveness of widespread screening for *Chlamydia trachomatis* in the Netherlands; an update	

15:00 – 15:30	*E.L.M. Op de Coul*	O023
	CSI-Netherlands: a large scale internet-based *Chlamydia* screening implementation program	
15:30 – 16:00	Coffee/tea	

Room 4/5 **Parallel session 'A genomics approach to identify bacterial virulence factors'**

Chairmen: A. Camilli (Boston, USA) & P.W.M. Hermans

14:00 – 14:30	*A. Camilli (Boston, USA)*	O027
	Vibrio cholerae	
14:30 – 14:45	*H. Bootsma*	O028
	Identification of genes essential for *in vivo* survival of *Streptococcus pneumoniae* by genomic array footprinting	
14:45 – 15:00	*S. Rooijakkers*	O029
	Genomic clustering of *S. aureus* complement modulators	
15:00 – 15:15	*E.J.M. Stoop*	O030
	Forward genetic screen to identify mycobacterial genes involved in granuloma formation	
15:15 – 15:30	*M. Llamas*	O031
	Cell-surface signaling in *Pseudomonas aeruginosa*	
15:30 – 16:00	Coffee/tea	

Room 6/7 **Parallel session 'Microbial biofilms'**

Chairman: T. Abee

14:00 – 14:15	*T. Abee*	O032
	Introduction	
14.15 – 14:30	*B.P. Krom*	O033
	Biofilm formation of clinical isolates of *Enterococcus faecalis*: the role of culture heterogeneity	
14:30 – 14:45	*K. Hellingwerf*	O034
	Molecular systems biology of single-species biofilm formation	
14:45 – 15:00	*W. Crielaard*	O035
	Mixed species oral biofilms	
15:00 – 15:30	*M. Gohar (Guyancourt, France)*	O036
	Bacillus cereus biofilm formation	
15:30 – 16:00	Coffee/tea	

Room 8/9 **Parallel session 'Medical Mycology 1'**

Chairman: J. Meis

14:00 – 14:15	*G. Walther*	O038
	Launch of a curated ITS DNA barcode database as a new tool for the rapid diagnostics of dermatophytes	
14:15 – 14:30	*H. Ma (Birmingham, United Kingdom)*	O039
	Macrophage 'hijacking' by the human pathogen *Cryptococcus*	
14:30 – 14:45	*C.H.W. Klaassen*	O040
	Molecular typing of pathogenic fungi: and then what?	
14:45 – 15:00	*L.M.E. Vanhee (Ghent, Belgium)*	O041
	Rapid detection and quantification of *Aspergillus fumigatus* in air using solid-phase cytometry	
15:00 – 15:15	*M. Sudhadham*	O042
	Genotypes with different virulence in *Exophiala dermatitidis*	
15:15 – 15:30	*G.J. Wisselink*	O043
	Comparison of PCR-reverse Line Blot and real-time PCR for the detection of dermatophytes in clinical samples	
15:30 – 16:00	Coffee/tea	

Room Athene **Parallel session 'Outsourcing 2** (Nederlandstalige sessie)**'**

Chairman: G.J.H.M. Ruijs

16:00 – 16:20	*W.M. Verweij*	O044
	Professional outsourcen	
16:20 – 16:40	*R. Steenbergen*	O045
16:40 – 17:10	*A.C. Rodloff (Leipzig, Germany)*	O046
17:10 – 17:30	*Discussie o.l.v. M. Buiting*	O047

Room Sydney **Parallel session 'Cross-border dissemination of MRSA'**

Chairmen: E.E. Stobberingh & F.H. van Tiel

16:00 – 16:15	*R.H. Deurenberg*	O051
	Cross-border dissemination of MRSA in the Euregion Meuse-Rhine	
16:15 – 16:30	*A.W. Friedrich (Münster, Germany)*	O052
	Differences in the molecular epidemiology of MRSA in the Euregion Twente/Münsterland	
16:30 – 17:00	*M.J. Struelens (Brussels, Belgium)*	O053
	Cross-border dissemination of MRSA – the European perspective	
17:00 – 17:15	*E. van Duijkeren*	O054
	Transmission of methicillin-resistant *Staphylococcus intermedius* between animals and humans	
17:15 – 17:30	*L.M. Schouls*	O055
	Comparative typing of MRSA using Multiple-locus variable number tandem repeat analysis (MLVA), pulsed field gel electrophoresis (PFGE) and spa-sequence typing	

Room 2 **Parallel session 'The emergence of *Clostridium difficile* infections in humans and animals'**

Chairman: E.J. Kuijper

16:00 – 16:15	*D. Notermans*	O056
	The epidemiology of *Clostridium difficile* PCR-ribotype 027 in the Netherlands since 2005	

16:15 – 16:30	*A. Goorhuis*	O057
	Surveillance of *Clostridium difficile* associated disease in the Netherlands	
16:30 – 16:45	*J. Corver*	O058
	Regulation of toxin genes in *Clostridium difficile*	
16:45 – 17:00	*L.A.M.G. van Leengoed*	O059
	Clostridium difficile in animals: from stock to meat	
17:00 – 17:15	*R.F. de Boer*	O060
	Development and validation of a real-time PCR assay for detection of toxigenic *Clostridium difficile*, as part of a Dutch study on the epidemiology of gastro-enteritis	
17:15 – 17:30	*D. Bakker*	O061
	Characterization of *Clostridium difficile* ribotype 078 from human and animal origin	

Room 3 **Parallel session 'Human papillomavirus and cervical cancer: vaccination and future strategies for population-based screening'**

Chairman: W. Melchers

16:00 –16:30	*P.J.F. Snijders*	O062
	Implications of HPV testing in cervical screening	
16:30 – 17:00	*W. Quint*	O063
	Vaccination against HPV to prevent cervical cancer	
17:00 – 17:30	*Tj. Tijmstra*	O064
	Grenzen aan medische testen. Over ziekterisico's en gezondheidskansen	

Room 4/5 **Parallel session 'General Pathogenesis 1'**

Chairman: B. Zaat

16:00 – 16:15	*B.W. Bardoel*	O065
	Evasion of Toll like receptor 5 recognition by alkaline protease of *P. aeruginosa*	
16:15 – 16:30	*J.J.E. Bijlsma*	O066
	Streptococcus pneumoniae has a specific response to transcytosis: identification of an essential role for Mg^{2+} transport during translocation	
16:30 – 16:45	*L.E. Cron*	O067
	Putative Proteinase Maturation Protein A (PpmA) of *Streptococcus pneumoniae* contributes to pneumococcal adherence and colonization	
16:45 – 17:00	*J.J.G. Geurtsen*	O068
	Mycobaterial alpha-glucan modulates host immune responses via the interaction with DC-SIGN and Toll-like receptor 2	
17:00 – 17:15	*C. Vink*	O069
	The *Mycoplasma pneumoniae* MPN229 gene encodes a protein that selectively binds single-stranded DNA and stimulates RecA-mediated DNA strand exchange	
17:15 – 17:30	*A.J. King*	O070
	Comparative genomic profiling of Dutch clinical *Bordetella pertussis* isolates using DNA microarrays: identification of genes absent from epidemic strains	

Room 6/7 **Parallel session 'Single cell environmental microbiology'**

Chairmen: J. Leveau & H. Smidt

16:00 – 16:30	*J.U. Kreft (Birmingham, United Kingdom)*	O071
	Concepts and practice of individual-based microbial ecology	
16:30 – 17:00	*C. Ingham*	O072
	New petri dishes - microscale microbial culture chips	
17:00 – 17:15	*E.L. Lagendijk*	O073
	Improved red fluorescent protein mcherry used in visualization of *Pseudomonas* biofilms on abiotic and biotic surfaces	
17:15 – 17:30	*M. Emsermann*	O074
	Bacterial bioreporters for quantifying individuality	

Room 8/9 **Parallel session 'Medical Mycology 2'**

Chairman: S. de Hoog

16:00 – 16:15	*C. Ingham*	O076
	Rapid susceptibility testing of fungi	
16:15 – 16:30	*I.M. Curfs-Breuker*	O077
	The use of chromogenic media in medical mycology	
16:30 – 16:45	*M.J. Najafzadeh*	O078
	Species of *Fonsecaea* causing human chromoblastomycosis	
16:45 – 17:00	*W.W.J. van de Sande*	O079
	Efficacy of voriconazole and anidulafungin in combination in experimental invasive pulmonary aspergillosis in neutropenic rats	
17:00 – 17:15	*L.E. Jansen*	O080
	Invasive pulmonary aspergillosis as a presenting sign in a HIV-positive patient	
17:15 – 17:30	*E. Snelders*	O081
	Environmental *Aspergillus fumigatus* isolates are resistant to triazoles	

Room Athene **Plenary session**

Chairmen: S. Brul & C.M.J.E. Vandenbroucke-Grauls

17:30 – 17:45	Announcement Kiem price winner	
17:45 – 18:30	Kluyver lecture	O082
	J. Errington (Newcastle, United Kingdom)	
	A reproducible system for generating wall-less (L-form) bacteria: Implications for the evolution of cell proliferation	

Restaurant	**Conference dinner**	
18:30 – 20:30		
Room Sydney	**Poster session & drinks (sponsored by Yakult)**	
20:30 – 22:00		
20:30 – 21:15	Presentations odd poster numbers	
21:15 – 22:00	Presentations even poster numbers	
22:00	Presentation Yakult Poster Price	
As of 22:15	**Dance party "Groot microbiologie feest"**	

WEDNESDAY APRIL 2 2008

Room Athene	**Parallel session 'Medical Microbiology'**	
	Chairman: K. Hol	
09:00 – 09:15	*M. Damen*	O085
	Viral culture on tAN versus CaCo2 and HT-29 cell lines	
09:15 – 09:30	*A.J. C. van den Brule*	O086
	Rapid detection of dermatophytes in clinical specimens using an internally controlled duplex real-time PCR	
09:30 – 09:45	*M.H. Nabuurs-Franssen*	O087
	Validation of M.I.C. Evaluator strips following ISO guidelines	
09:45 – 10:00	*N. al Naiemi*	O088
	A practical aid for detection of Extended-Spectrum Beta-Lactamases in *Enterobacteriaceae*	
10:00 – 10:15	*M.J. Bruins*	O089
	Association between group A beta-haemolytic streptococci and vulvovaginitis in adult women: a case control study	
10:15 – 10:30	*R. de Boer*	O090
	Caenorhabditis elegans as a model system to study (anti-retroviral) drug-induced mitochondrial dysfunction	
10:30 – 11:00	Coffee/tea	

Room Sydney	**Parallel session 'WMDI: Molecular aspects of emerging infections'**	
	Chairmen: E.C.J. Claas & R. Schuurman	
09:00 – 09:30	*M. Panning (Bonn, Germany)*	O092
	Travelers and imported emerging infections: perspectives from the laboratory	
09:30 – 10:00	*V.J. Munster*	O093
	Diagnosing avian influenza	
10:00 – 10:15	*H. Vennema*	O094
	Molecular epidemiology of human gastro-enteritis viruses	
10:15 – 10:30	*M. Schutten*	O095
	Molecular diagnostics of infections with uncommon viral pathogens	
10:30 – 11:00	Coffee/tea	

Room 2	**Parallel session 'Sectie onderwijs** (Nederlandstalige sessie)**'**	
	Chairman: L. van Alphen	
09:00 – 09:30	*G. van Bellen*	O097
09:30 – 10:00	*G. van der Groen*	O098
	Filovirus haemorrhagic fever outbreaks: much ado about nothing?	
10:00 – 10:15	*R. Wijffels*	O099
	Biodiesel uit algen	
10:15 – 10:30	*F. Verhoeven*	O100
	The general public's beliefs about methicillin resistant *Staphylococcus aureus*: A mental models approach	
10:30 – 11:00	Coffee/tea	

Room 3	**Parallel session 'Oral microbiology'**	
	Chairmen: A. J. van Winkelhoff & W. Crielaard	
09:00 – 09:30	*B. Keijser*	O101
	Exploring the oral microbial universe using TNO's OC-chip	
09:30 – 10:00	*E.C.I. Veerman*	O102
	Effect of energy depletion on the sensitivity of *C. albicans* for antimicrobial peptides	
10:00 – 10:15	*D. Deng*	O103
	Stress responses in *Streptococcus mutans*	
10:15 – 10:30	*W.A. van der Reijden*	O104
	Transmission of *Tannerella forsythensis* in related families	
10:30 – 11:00	Coffee/tea	

Room 4/5	**Parallel session 'General Pathogenesis 2'**	
	Chairman: A. van Belkum	
09:00 – 09:15	*I. Jongerius*	O106
	Modulation of Innate and Adaptive immune responses by C3d binding molecules of *S. aureus*	
09:15 – 09:30	*A. van Diepen*	O107
	Proteome characterization of respiratory virus-infected host cells by 2-D DIGE and mass spectrometry	
09:30 – 09:45	*R.P.L. Louwen*	O108
	RFLP association of the *Campylobacter jejuni* genes Cj1522 and Cj1523 with the Guillain Barré syndrome. Do they function as a toxin?	
09:45 – 10:00	*K.E.M. Elberse*	O109
	Population structure assessment of *Streptococcus pneumoniae* strains isolated from patients with invasive disease in the pre-vaccination era using MLVA and sequence based surrogate serotyping	
10:00 – 10:15	*M. Emonts*	O110
	Transcriptome profiles of children suffering from meningococcal sepsis	

10:15 – 10:30	*A.C. Fluit*	O111
	The distinct epidemiology of CA-MRSA versus HA-MRSA may be explained by rRNA operon copy number	
10:30 – 11:00	Coffee/tea	

Room 6/7 **Parallel session 'Secretion mechanisms in pathogens'**

Chairman: C. van der Does

09:00 – 09:30	*P.J.J. Hooykaas*	O112
	Type IV secretion of proteins and DNA molecules by pathogens into eukaryotic cells	
09:30 – 10:00	*W. Bitter*	O113
	Type VII secretion - Mycobacteria show the way	
10:00 – 10:15	*G. Buist*	O115
	Secretion and binding of wall located virulence factors of *Staphylococcus aureus*	
10:15 – 10:30	*E. Pachulec*	O116
	The type IV DNA secretion system of *Neisseria gonorrhoeae*	
10:30 – 11:00	Coffee/tea	

Room 8/9 **Parallel session 'Werkgroep Oost & West 1: Lyme-borreliosis: clinical aspects, diagnostics, and epidemiology'**

Chairman: R.W. Vreede

09:00 – 09:30	*A. Hofhuis*	O117
	Epidemiology of erythema migrans and tick bites, and infection of ticks with *Borrelia*	
09:30 – 10:00	*J. Oksi, (Turku, Finland)*	O118
	Clinical pictures of disseminated Lyme-borreliosis	
10:00 – 10:30	*A. van Dam*	O119
	Diagnosis of Lyme-borreliosis: value of serological and molecular methods	
10:30 – 11:00	Coffee/tea	

Room Athene **Parallel session 'Clinical epidemiology'**

Chairman: W. Manson

11:00 – 11:15	*F. Hagen*	O120
	Where is the origin of the *Cryptococcus gattii* Vancouver Island outbreak?	
11:15 – 11:30	*J.A.M. Labout*	O121
	Interactions between respiratory pathogens during colonization in the first months of life. The Generation R Study	
11:30 – 11:45	*M.J.A. de Regt*	O122
	Environmental contamination: a risk factor for acquisition of CC17 ampicillin-resistant *Enterococcus faecium* (ARE)	
11:45 – 12:00	*G.H. van Ramshorst*	O123
	Complication registration under-estimates the incidence of superficial surgical site infections: a prospective cohort study	
12:00 – 12:15	*I.H.M. Friesema*	O124
	Differences in clinical presentation between norovirus genotypes in nursing homes	
12:15 – 12:30	*R.H.C.A. Deurenberg*	O125
	The *S. aureus* virulence factors collagen-adhesion and toxic shock syndrome toxin 1 can increase the discriminatory power of spa typing	
12:30 – 14:00	Lunch	

Room Sydney **Parallel session 'NWKV: Molecular diagnostics of viral respiratory infections'**

Chairman: A. Vossen

11:00 – 11:15	*E.C.J. Claas*	O126
	Where do we stand with molecular testing for RTI in the Netherlands?	
11:15 – 11:30	*R. Molenkamp*	O127
	Three tube pentaplex PCR assay for detection of viral respiratory pathogens	
11:30 - 11:45	*J. Gooskens*	O128
	Clinical evaluation of pediatric viral respiratory infections detected by real-time PCR	
11:45 – 12:00	*J. Schinkel*	O129
	Importance of human Bocavirus as a respiratory pathogen	
12:00 - 12:15	*M.M. van der Zalm*	O130
	Prevalence of the newly identified WU and KI polyomaviruses in children with and without respiratory symptoms	
12:15 - 12:30	*C.F.M. Linssen*	O131
	Presence of human metapneumo-virus in bronchoalveolar lavage fluid samples detected by means of RT-PCR	
12:30 – 14:00	Lunch	

Room 2 **Parallel session 'Modeling disinfection on surfaces and in biofilms in food technology'**

Chairman: R. Beumer

11:00 – 11:30	*G. Wirtanen (Helsinki, Finland)*	O132
	An overview of disinfection procedures in food microbiology, and their effects on micro-organisms (in biofilms)	
11:30 – 11:45	*J. Wijman*	O133
	Diversity in *Bacillus cereus* biofilm formation capacity and spore contents; implications for disinfection procedures	
11:45 – 12:00	*J.M. Straver*	O134
	Modelling microbial survivors in various disinfection processes for better results	

12:00 – 12:30	*J. Poulis*	O135
	Disinfectants from an international point of view: problems, solutions and future developments	
12:30 – 14:00	Lunch	

Room 3 **Parallel session 'SKMM: Meten is weten?'**

Chairman: G. van Doornum

11:00 - 11:30	*P. Verweij*	O137
11.30 - 12.00	*M.F. Peeters*	O138
	Serologie van bacteriële luchtweginfecties	
12:00 - 12:15	*J. Kerremans*	O139
	Detection of MRSA using a modified PCR-assay	
12:15 – 12:30	SKMM vergadering	
12:30 – 14:00	Lunch	

Room 4/5 **Parallel session 'General Pathogenesis 3'**

Chairman: B. Appelmelk

11:00 – 11:30	*M. Höök (Houston, USA)*	O141
	Biology of MSCRAMMs	
11:30 – 11:45	*A.P. Heikema*	O142
	Role of Siglec/sialic acid interaction in phagocytosis of *Campylobacter jejuni* in human monocytes	
11:45 – 12:00	*N.J.P. Smits*	O143
	Determination of growth dependent gene expression of *Staphylococcus aureus* with a newly developed whole genome microarray	
12:00 – 12:15	*A.C. Fluit*	O144
	Preliminary analysis of the whole genome sequence of a clinical ST30 MSSA isolate	
12:15 – 12:30	*K. Stol*	O145
	A novel *in vivo* otitis media model	
12:30 – 14:00	Lunch	

Room 6/7 **Parallel session 'Progress in Microbiology 1'**

Chairman: S. Brul

11:00 – 11:15	*W. Pusch*	O146
	Identification and classification of microorganisms by mass spectrometry fingerprinting	
11:15 – 11:30	*E.J. Gaasbeek*	O147
	Inhibition of natural transformation through an endogenous DNase in *Campylobacter jejuni*	
11:30 – 11:45	*A. Paauw*	O148
	Diversity of the *Enterobacter cloacae* complex determined by a mixed genome array and multi locus sequence analysis	
11:45 – 12:00	*A.T. Kovacs*	O149
	Response of *B. cereus* ATCC14579 to a challenge with the circular bacteriocin enterocin AS-48	
12:00 – 12:15	*T. Shen*	O150
	Freeze- thaw treatment represses growth metabolism related functions and induces the envelope stress response and cell wall biosynthesis pathways of *B. subtilis*	
12:15 – 12:30	*W. van Schaik*	O151
	Draft genome sequencing of two *Enterococcus faecium* strains by pyrosequencing technology reveals niche-specific gene acquisition	
12:30 – 14:00	Lunch	

Room 8/9 **Parallel session 'Werkgroep Oost & West 2 / ESBL: Historie en nieuwe ontwikkelingen'**

Chairman: J. Kluytmans

11:00 – 11:15	*M. Leverstein - van Hall*	O152
	ESBL: Historisch perspectief en epidemiologie	
11:15 – 11:30	*D. Mevius*	O153
	ESBLs in animals	
11:30 – 11:45	*J. Cohen Stuart*	O154
	The clinical impact of extended spectrum beta-lactamases (ESBL's)	
11:45 – 12:00	*N. al Naiemi*	O155
	Guideline of the Dutch Society for Medical Microbiology for screening and confirmation of extended-spectrum beta-lactamases in *Enterobacteriaceae*	
12:00 – 12:15	*J. Kluytmans*	O156
	ESBL en infectiepreventie	
12 :15 – 12 :30	*M. Leverstein - van Hall*	O157
	ISIS and ESBL	

Room Sydney **Ledenvergadering NVvM**

12:45 – 14:00

Room 2 **BBC-MMO vergadering**

12:45 – 14:00

Room 4/5 **Lunchbijeenkomst 'Registratie van Nascholing'**

13:00 – 14:00 Korte voorlichtingsbijeenkomst over alles wat met nascholingsactiviteiten te maken heeft, variërend van GAIA en achteraf accreditatie van gevolgde nascholing tot het bijhouden van uw eigen nascholing in uw persoonlijk dossier. Er is ruime mogelijkheid tot het stellen van vragen.

Room Athene **Parallel session 'Therapie van parasitaire infecties** (Nederlandstalige sessie)**'**

Chairman: T. Kortbeek

14:00 – 14:15	*R.W. Sauerwein*	O160
	Het gebruik van artemisinine in de perifere ziekenhuizen	
14:15 – 14:30	*T.A.M. Hekker*	O161
	The treatment of *Dientamoeba fragilis*	
14:30 – 14:45	*T. Mank*	O162
	Nitazoxanide voor Cryptosporidium	
14:45 – 15:00	*L.G. Visser*	O163
	Doxy of albendazol ipv DEC voor filariasis?	
15:00 – 15:15	*T. Kortbeek*	O164
	Toxocara: wel of niet behandelen	
15:15 – 15:30	*L. van Dommelen*	O165
	Evaluation of 5 different rapid tests for the detection of *Giardia lamblia* cysts and/or *Cryptosporidium parvum* oöcysts in faecal samples	
15:30 – 16:00	Coffee/tea	

Room Sydney **Parallel session 'The human microbiome/Typing of the human gastrointestinal microflora'**

Chairman: P. Savelkoul

14:00 – 14:30	*H. Smidt*	O168
	Bringing light into the tunnel – Composition and functionality of gut microbiota	
14:30 – 15:00	*J. Hugenholtz*	O169
	Functional biodiversity of mixed strain starters for production of fermented foods	
15:00 – 15:15	*D.J. Soeltan-Kaersenhout*	O170
	Terminal restriction fragment length polymorphism as a diagnostic tool in gastrointestinal disease: a preliminary study	
15:15 – 15:30	*I.H.M. Friesema*	O171
	National outbreak of shiga-toxin producing *Escherichia coli* O157	
15:30 – 16:00	Coffee/tea	

Room 2 **Parallel session 'Energy from Biomass'**

Chairman: G.J. Euverink

14:00 – 14:15	*J.S. Geelhoed*	O172
	Electron transfer in microbial fuel cells	
14:15 – 14:30	*E. Croese*	O173
	Microbial community in hydrogen producing microbial fuel cells	
14:30 – 14:45	*M.F. Temudo*	O174
	Microbial diversity in open mixed cultures fermentation	
14:45 – 15:00	*M. Verhaart*	O175
	Genome analysis and microarray analysis of the hydrogen producing thermophilic bacterium *Caldicellulosiruptor saccharolyticus*	
15:30 – 16:00	Coffee/tea	

Room 3 **Parallel session 'ICT: Standaardiseer de standaard** (Nederlandstalige sessie)**'**

Chairman: C.H.E. Boel

14:00 – 14:30	*R. Cornet*	O178
	SNOMED CT: 'De semantische standaard' van de toekomst?	
14:30 - 15:00	*A. Hamster & C.H.E. Boel*	O179
	IHE lab in Nederland	
15:00 - 15:30	*H.R.A. Streefkerk*	O180
	(Inter)national standardization, a contradiction in medical microbiology?	
15:30 – 16:00	Coffee/tea	

Room 4/5 **Parallel session 'General Pathogenesis 4'**

Chairman: O.P. Kuipers

14:00 – 14:15	*J. Bestebroer*	O181
	Staphylococcal superantigen-like protein 5 is a broad spectrum chemokine and anaphylatoxin inhibitor	
14:15 – 14:30	*P.J. Burghout*	O182
	Characterization of a pneumococcal competence-induced operon: link between DNA repair and carbon dioxide fixation?	
14:30 – 14:45	*W.T. Hendriksen*	O183
	Regulation of nitrogen metabolism in *Streptococcus pneumoniae* by CodY and GlnR: link between nutritional gene regulation and virulence	
14:45 – 15:00	*N.N. Driessen*	O184
	Role of phosphatidylinositol mannosides in the interaction of mycobacteria with human dendritic cells	
15:00 – 15:15	*A.P.A. Hendrickx*	O185
	EcbA and SgrA, two LPXTG surface proteins of *Enterococcus faecium* CC17 bind to components of the extra-cellular matrix	
15:15 – 15:30	*M. van Gent*	O186
	An investigation into the cause of the 1983-1987 whooping cough epidemic in the Netherlands	
15:30 – 16:00	Coffee/tea	

Room 6/7 **Parallel session 'Progress in Microbiology 2'**

Chairman: M. Zwietering

14:00 – 14:15	*F. Talarico Saia*	O187
	Characterization of the microbiota from a benzene-degrading, nitrate reducing bioreactor	

14:15 – 14:30	*A. van Mourik*	O188
	Regulation of the energy metabolism in *C. jejuni*	
14:30 – 14:45	*M. van der Voort*	O189
	Diversity in sporulation and germination of *Bacillus cereus* strains	
14:30 - 15:00	*M.W.J. van Passel*	O190
	The peculiar distribution of simple sequence repeats	
15:00 - 15:15	*K.M. Schwarz*	O191
	Systems biology of *Clostridium acetobutylicum* – understanding solvent production	
15:30 – 16:00	Coffee/tea	

Room 8/9	**Parallel session 'Carbapenemases'**	
	Chairman: Y.J. Debets	
14:00 – 14:30	*P. Nordmann (Le-Kremlin-Bicetre, France)*	O193
	Acquired Carbapenemases: a worldwide spread	
14:30 – 14:45	*N. al Naiemi*	O194
	First detection of transferable metallo-beta-lactamases in the Netherlands	
14:45 – 15:30	*M.J. Schwaber (Tel Aviv, Israel)*	O195
	Infection control at the national level: containment of an outbreak of carbapenem-resistant *Klebsiella pneumoniae* in Israeli hospitals	
15:30 – 16:00	Coffee/tea	

Room Athene	**Ledenvergadering NVMM**
16:00 – 18:00	

O011

Oefening baart kunst

A. Jacobi

Landelijk Coördinatie Infectieziektebestrijding, Centrum Infectieziektebestrijding, National Institute for Public Health and the Environment (RIVM), Bilthoven

Oefenen is in.

Wat voor het leger, politie en de brandweer al jaren een vast onderdeel is van de voorbereiding op calamiteiten is voor het veld van de gezondheidszorg en vooral de openbare gezondheidszorg een redelijk nieuw terrein. In de crisisbeheersing zijn de laatste jaren interessante technieken ontwikkeld om realistisch te oefenen. U herinnert wellicht het tv-programma 'Crisis'. In een gesimuleerde omgeving werden politici en bestuurders geconfronteerd met stevige dilemma's. De tv heeft ons daarmee een interessante inkijk laten nemen in de keuken van de crisisbeheersing en besliskunde door politici.

Crises gebeuren niet vaak genoeg om er echt ervaren in te worden. Je moet dus wel oefenen om ervaring op te doen om te merken hoe je zelf in een bepaalde situatie reageert. Een oefening hoeft niet altijd om een crisisachtige situatie te gaan. Oefeningen zijn er in vele soorten en maten en met een verschillend doel. Het Centrum voor Infectieziektebestrijding van het RIVM is actief met het ontwikkelen van oefeningen voor GGD'en. De komende tijd zal ook voor de microbiologische laboratoria een aantal modeloefeningen worden ontwikkeld. Een begeleidingsgroep met vertegenwoordigers vanuit de GGD, de OGZ-laboratoria en het RIVM bepalen de inhoud van deze modeloefeningen en zijn gebaseerd op de basis van de modelconvenanten tussen de GGD en de OGZ-laboratoria. De samenwerking met de afdeling infectieziektebestrijding van de GGD op het gebied van *outbreak*-management en de koppeling met het RIVM komen hierbij aan bod.

In de presentatie wordt ingegaan op diverse aspecten van effectief oefenen.

O012

Outbreakmanagement: wetenschap, praktijk of beide?

A. Timen

Landelijk Coördinatie Infectieziektebestrijding, National Institute for Public Health and the Environment (RIVM), Bilthoven

'One can think of the middle of the 20th century as the end of one of the most important social revolutions in history, the virtual elimination of the infectious disease as a significant factor in social life ...'

Sir MacFarlane Burnett, Natural history of infectious diseases

Terugblik

Een snelle terugblik in de infectieziektebestrijding laat ontwikkelingen zien die het optimisme van Burnett tegenspreken. De (perceptie van de) dreiging van infectieziekten is de afgelopen 10 tot 15 jaar eerder toegenomen. Landelijke crisissituaties deden zich voor als gevolg van veranderingen in de epidemiologie van bekende verwekkers (bijvoorbeeld de verheffing van meningokokken serogroep C) of als gevolg van de verspreiding van nieuwe verwekkers, zoals het A/H7N7-virus of het SARS-coronavirus. Bioterrorisme (met als voorbeeld de 'epidemie' van poederbrieven) speelde zowel in internationale context als in Nederland een belangrijke rol. Daarnaast heeft de – van oudsher ziekenhuisgebonden bacteriële resistentieproblematiek – steeds vaker gevolgen voor *public health* gehad. Zo is in 2005 in Nederland *Clostridium difficile* ribotype 027 voor het eerst aangetoond als oorzaak van moeilijk te bestrijden outbreaks in zorginstellingen. In 2006 werden MRSA-stammen bij varkensbedrijven voor het eerst gesignaleerd als probleem voor de volksgezondheid. Het moeilijk in te schatten risico van een grieppandemie en de onvoorspelbare genetische ontwikkelingen van de aviare influenzavirussen beïnvloeden nog steeds het dagelijkse werk van artsen infectieziekten en virologen. Zeer recent heeft in Nederland een grote uitbraak van Q-koorts plaatsgevonden die de kwetsbaarheid van Nederland voor dreigingen van zoönotische aard opnieuw heeft bevestigd.

***Evidence/knowledge based* adviseren**

In crisissituaties brengt het Outbreak Management Team (OMT), een professioneel advies over maatregelen uit aan het Bestuurlijk Afstemmingsoverleg (BAO), dat namens de minister van VWS besluit over de uitvoering.

De crisisadvisering in de infectieziektebestrijding wordt gekenmerkt door een grote mate van complexiteit en onderscheidt zich ten opzichte van andere dreigingen door de tijdsdruk waaronder alle relevante informatie moet worden verzameld, de vele onzekerheden en de verschillende percepties van de risico's door professionals, beleidsmakers, het publiek en de media.

Het OMT brengt een advies uit, gebaseerd op de stand van de medische wetenschap. De kracht van het bewijs wordt gewogen in het advies. In sommige gevallen kan niet worden gesproken over evidence-based handelen omdat de situatie zich nog niet eerder (op een grote schaal) heeft voorgedaan. De mening van de experts op dat deelgebied in het OMT geeft dan de doorslag.

Tijdens de presentatie zal worden ingegaan op een aantal voorbeelden van risico-inschatting en risicomanagement van de afgelopen jaren.

O013

Inspectieonderzoek naar de kwaliteit van het medisch-microbiologisch handelen in Nederland

De rol van het medisch-microbiologische laboratorium in de openbare gezondheidszorg

G.R. Westerhof

Inspectie voor de Gezondheidszorg, Utrecht

De medisch-microbiologische laboratoria (MML) vormen een essentiële schakel binnen de gezondheidszorg als geheel. Dit geldt voor zowel de individuele gezondheidszorg als de openbare gezondheidszorg. Daarmee zijn de MML ook een wezenlijk onderdeel in de structuur van de infectieziektebestrijding. Door de toenemende dreiging van uitbraken van infectieziekten worden steeds hogere eisen gesteld aan deze infrastructuur. De beroepsgroep heeft de afgelopen jaren zelf normen en eisen opgesteld voor het leveren van verantwoorde zorg. De koepel van de MML (Nederlandse Vereniging voor Medische Microbiologie) heeft deze normen en eisen vastgesteld.

De inspectie heeft onderzoek gedaan naar de kwaliteit van het microbiologisch handelen in Nederland, omdat er bij de inspectie onvoldoende inzicht is hoe het met deze kwaliteit is gesteld. Daartoe zijn eind 2006 vragenlijsten uitgestuurd naar alle toen bekende medisch-microbiologische laboratoria in Nederland. Met een respons van 100% is in het voorjaar van 2007 gestart met de analyse van de data. Op basis van de aangeleverde gegevens zijn individuele rapporten opgesteld en verstuurd. De MML kregen de gelegenheid op de rapporten te reageren om feitelijke onjuistheden te corrigeren en inmiddels zijn de meeste rapporten definitief vastgesteld. Op basis van alle individuele rapporten is er nu een geaggregeerd rapport in de maak.

Naast algemene onderwerpen in de vragenlijst zoals werkterrein, kwaliteitssysteem, veiligheid en rapportage en archivering is nadrukkelijk ook aandacht besteed aan de openbare gezondheidszorg. Deze presentatie gaat daarover.

Op het onderdeel openbare gezondheidszorg wordt over het algemeen redelijk gescoord. Specifieke zaken die hoog scoren zijn onder meer:

- Melding B-ziekten
- Melding van clusters door clinici.

Laag scoren onder meer de onderdelen:

- Epidemiologische analyse van laboratoriumgegevens
- Systematische analyse van resistentiegegevens
- Beveiliging opslag pathogenen.

De MML worden nu gevolgd in de uitvoering van het plan van aanpak.

De MML werken zelf al aan een kwaliteitsverbetering op grond van de professionele standaarden. Dit gebeurt veelal in de aanloop naar CCKL-accreditatie. Door mee te werken aan dit onderzoek van de inspectie hebben de MML bijgedragen aan de transparantie over de kwaliteit binnen deze laboratoria. Daarnaast draagt dit bij aan de positieversterking van een essentiële functie binnen zowel de individuele als de openbare gezondheidszorg.

O028

Identification of genes essential for *in vivo* survival of *Streptococcus pneumoniae* by genomic array footprinting

H.J. Bootsma

Laboratory of Pediatric Infectious Diseases, Radboud University Nijmegen Medical Centre, Nijmegen

Background: *Streptococcus pneumoniae* is a major cause of serious infections such as pneumonia and meningitis in both children and adults worldwide. The current capsular polysaccharide vaccine protects against only a fraction of the 90 known capsular serotypes. In addition, antibiotic resistance among circulating strains is rapidly increasing, highlighting the need for novel strategies for therapy or prevention. We have recently developed genomic array footprinting (GAF), a genome-wide negative-selection screen for conditionally essential genes of *S. pneumoniae*. Here, we used GAF to identify genes essential during infection of the host, in particular colonisation of the nasopharynx, since genes essential *in vivo* are considered prime targets for vaccine or antimicrobial design.

Methods: For GAF, microarrays are used to create footprints from random transposon mutant libraries before and after a particular challenge condition. Comparison of footprints will indicate which mutants have disappeared during challenge, and, consequently, identify conditionally essential genes. For the genome-wide colonisation screen, four TIGR4 mariner mutant libraries of 1,000-2,000 CFU were used as inoculum in a mouse colonisation model. At 0.5h, 24h, 48h, and 96h post infection, groups of 4 mice were sacrificed, and nasopharyngeal lavage (NPL) samples were collected to monitor selective disappearance of mutants by GAF. In addition, we used the 1,000 mutant library in a pneumonia model, and collected NPL and blood samples at 24 and 48h for GAF analysis. Finally, for validation of identified targets, individual mutants were generated and used individually in a mouse pneumonia co-infection model.

Results: In total, 363 genes were identified as being essential for pneumococcal colonisation of the nasopharynx (i.e., the corresponding mutants were negatively selected from the population), of which the majority (74%) were identified at two or more time-points. Importantly, we identified 66 genes that have previously been linked to virulence in studies using signature-tagged mutagenesis or other methods, such as *ply, pspA, lytB, hyl,* and *pavA*. The 297 novel genes identified were distributed among a variety of functional

categories, but were enriched for transporter (41), metabolic (28), and regulatory (21) genes, and, most prominently, those of putative or unknown function (149). Furthermore, we found a considerable overlap in mutants counter-selected from NPL when comparing the colonisation and pneumonia models 102 genes were identified in both infection models, 25 of which are known virulence genes. We are currently performing a genome-wide screen for pneumococcal genes essential for survival in the bloodstream using a mouse bacteremia model of infection.
To validate the contribution of the identified targets to pneumococcal virulence, we tested directed knockout mutants of nine genes predicted to encode surface-localised proteins in a mouse pneumonia co-infection model. While *in vitro* growth was comparable to wild-type, six mutants showed a significantly diminished ability to colonise the nasopharynx.
Conclusions: By applying GAF to murine infection models, we successfully identified several bacterial genes that are essential during colonisation, the initial stage of pneumococcal disease, and gained insight into infection kinetics of mutants by GAF analysis of samples collected at different time points.

O029
Genomic clustering of *Staphylococcus aureus* complement modulators

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Complement activation is a crucial step in our innate immune defense against invading bacteria. Complement proteins can quickly recognise invading bacteria and subsequently label them for phagocytosis or kill them by direct lysis. In order to survive in the human host, *Staphylococcus aureus* has evolved a number of secreted proteins that interfere with several steps of the complement cascade. These proteins are able to block the complement system at various steps: C3 activation by C3 convertases (SCIN, SCIN-B, SCIN-C), C1q recognition of IgG (Staphylokinase), C3b-containing convertases (Efb and Ecb), C5 activation (SSL7) and C5a receptor activation (CHIPS). In the *S. aureus* genome, we find all these complement modulators to be clustered on genomic regions together with other immune evasion molecules. The genes for SCIN, CHIPS and SAK are clustered with staphylococcal enterotoxin A on the conserved 3' end of beta-hemolysin (hlb)-converting bacteriophages, representing the first immune evasion cluster (IEC). Recently we discovered a novel second IEC in *S. aureus* that encodes Efb and Ecb. Furthermore, the staphylococcal superantigen-like protein 7 (SSL7) belongs to a group of close relatives of the superantigens that are located on a separate gene cluster within a 19-kb region of the pathogenicity island SaPIn2. The localisation of several important virulence factors on mobile elements facilitates their spread among *S. aureus*, and will have a profound impact on staphylococcal pathophysiology. The combined action of this growing group of essential virulence factors ascertains efficient complement evasion.

O030
Forward genetic screen to identify mycobacterial genes involved in granuloma formation

E.J.M. Stoop[1], F. Hannes[1], C.M.J.E. Vandenbroucke-Grauls[1], G. Besra[2], B.J. Appelmelk[1], W. Bitter[1], A.M. van der Sar[1]
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Mycobacterium marinum, a close genetic relative of *Mycobacterium tuberculosis*, causes tuberculosis like disease in its natural host the zebrafish. We use the *M. marinum*/zebrafish embryo infection model to screen a library of randomly mutated *M. marinum* E11 to identify mycobacterial genes involved in granuloma formation. Because zebrafish embryos are translucent, we can monitor the aggregation of macrophages containing red fluorescent mycobacteria in real time. So far, we identified four mutants that failed to elicit efficient granuloma formation. Preliminary results show that at least one of these mutants, with a transposon in MM5053, a FadE33 homologue, is able to infect and grow normally in THP-1 cells. FadE33 is annotated as an Acyl-CoA dehydrogenase with a predicted function in lipid synthesis. However, no differences in lipid content were observed in TLC. The other three mutants have a transposon insertion in MM0200 (Rv3879c), MM5425 (Rv3879c) and MM5427 (Rv3864), respectively. Strikingly, though homologous to genes of the RD1 region, they all lie outside the extended RD1 region of *M. marinum*. Interestingly, these three mutants also have diminished ESAT-6 secretion as analysed by Western Blot. In conclusion, the zebrafish embryo model has an added value compared to macrophage screens in identifying bacterial genes involved in granuloma formation. Furthermore, we demonstrate that genes outside the extended RD1 locus are necessary for ESAT-6 secretion and granuloma formation.

O031
Cell-surface signaling in *Pseudomonas aeruginosa*

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Cell-surface signaling is a sophisticated regulatory mechanism used by gram-negative bacteria to sense signals

from outside the cell and transmit them into the cytoplasm. This regulatory system consists of an outer membrane-localised TonB-dependent receptor (TonB-dependent transducer), a cytoplasmic membrane-localised anti-sigma factor and an extracytoplasmic function (ECF) sigma factor. In addition, to be functional, this system needs to be energised by the TonB-ExbBD complex. The outer membrane receptor senses a specific extracellular signal and transduces this signal to the inner membrane protein, which in turn leads to the activation of the ECF sigma factor. The activated sigma factor directs RNA polymerase to the promoter region of gene(s) under control of the signaling system. The human opportunistic pathogen *Pseudomonas aeruginosa* contains in total thirteen surface signaling systems. Two of these systems are known to be involved in the production and uptake of the siderophore pyoverdine.[1,2] We have identified the regulons of eight novel *P. aeruginosa* signaling systems. For that, the ECF sigma factor of each regulatory system has been overexpressed and their target gene candidates have been identified using DNA microarray, proteomic analysis, and/or lacZ reporter construct. All eight ECF sigma factors control the production of at least one TonB-dependent transducer, and six of them control (metal) transport systems. Three of these signaling systems respond to the extracellular presence of the siderophores ferrichrome, ferrioxamine, and the *Mycobacterium* siderophores mycobactin and carboxymycobactin, respectively, and regulate the utilisation of these heterologous siderophores. Finally, we have identified two ECF factors that also regulate genes unrelated to iron incorporation: one of them regulates the expression of *P. aeruginosa* pyocins, whereas the other ECF factor induces the transcription of several potential virulence factors. The latter ECF sigma factor seems to be induced by a host signal.

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O033

Biofilm formation of clinical isolates of *Enterococcus faecalis*: the role of culture heterogeneity

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Biofilm formation is an increasing problem in medicine, due to the intrinsic resistance of micro-organisms in the biofilm mode of growth against the host immune system and antimicrobial therapy. In general, adhesion is an important step in biofilm formation and adhesion of *Enterococcus faecalis* has been a main focus of research in our group for many years. Adhesion is influenced, amongst others, by surface proteins, hydrophobicity and charge of both the substratum and the adhering micro-organism. An overview of factors influencing adhesion and biofilm formation such as Aggregating substances (Agg's), Enterococcal surface protein (Esp) and zeta potential distributions, will be discussed. Research on common lab strains as well as clinical isolates of *E. faecalis* will be presented.

Related publications:
- Merode AE van, Pothoven DC, Mei HC van der, Busscher HJ, Krom BP. Surface charge influences enterococcal prevalence in mixed-species biofilms. J Appl Microbiol 2007;102(5):1254-60.
- Merode AE van, Mei HC van der, Busscher HJ, Krom BP. Influence of culture heterogeneity in cell surface charge on adhesion and biofilm formation by *Enterococcus faecalis*. J Bacteriol 2006;188(7):2421-6.
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O035

Mixed species oral biofilms

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Dental plaque is a multi-species biofilm community from which many microbial species have been isolated. Therefore, instead of studying monocultures, it is more realistic to study the properties of oral pathogenic micro-organisms in the presence of other micro-organisms. Certainly since it is known that different micro-

organism may influence each others gene expression and virulence.
In our current studies we use a combined genomics, proteomics, biochemical and molecular biological approach to study the dynamic properties of multi-species oral biofilms.
We have shown that i) *Streptococcus mutans* and *Veillonella parvula* dual-species biofilms have a different acid production profile and a higher resistance to antimicrobials than their single-species counterparts, ii) this co-existence alters the physiology of *S. mutans*, iii) a high expression of *S. mutans* genes coding for stress-responsive proteins in these mixed biofilms, iv) the involvement of *S. mutans* ClpP in adaptation to several antimicrobials and v) the involvement of *S. mutans* PgdA in determining cell surface hydrophobicity and salivary agglutinin mediated biofilm formation.

O038

Launch of a curated ITS DNA barcode database as a new tool for the rapid diagnostics of dermatophytes

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Dermatophytes cause infections of the keratinised tissues of humans or animals. Species recognition by conventional methods, such as direct microscopy or culture study, is time-consuming and often hampered by the variable macro- and micromorphology of the fungi. DNA-based methods have the potential to speed up the identification and to increase its accuracy, provided that reliable reference data are available for sequence comparison. Current identification routines include BLAST searches against GenBank and other INSD databases. However, the significant proportion of misidentified species and/or the sometimes insufficient taxonomic sampling compromise the accuracy of the results. In order to develop a reliable and rapid routine identification tool for dermatophytes we are setting up a publicly accessible database of ITS sequences. The backbone of the database consists of sequences of unambiguously identified isolates that are deposited in public collections (= ITS DNA barcodes). These are supplemented by selected GenBank sequences for further covering the extant biodiversity of the group. The comprehensive taxon sampling enables us to assess the intra- and interspecific variability of the sequences and to develop 'validated DNA barcodes' (= consensus sequences of all DNA barcodes of the same species). The databases will allow the user to perform BLAST analyses for identifying unknown clinical isolates and Neighbor joining (NJ) analyses based on pairwise alignments. The output contains a NJ tree, showing the position of the unknown sequence, alignments with the most similar sequences from the database and furthermore, with the relevant validated barcode sequences. From the beginning, this database will greatly improve the reliability of the species identification compared to INSD databases. Future plans include the continuous extension of the database by sequences from underrepresented species and regions of the world.

O040

Molecular typing of pathogenic fungi: and then what?

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Molecular typing methods are increasingly being used to determine the relatedness between microbial isolates, including fungi. If the genotyping method that is being used has sufficient discriminatory power, isolates with identical fingerprints are believed to be clonally related. Entirely different fingerprints are indicative of the isolates being unrelated. In-between is a grey zone of interpretation where isolates could be related, or not. Molecular fingerprinting data are usually analysed using various available algorithms. Microsatellites are popular targets to determine if fungal isolates are clonally related or not. Microsatellites are especially interesting since they provide extremely high discriminatory power which is due to the inherent instability of these markers during DNA replication. Interpretation of microsatellite data is commonly done using either a categorical approach or the Euclidian distance parameter. Here, I will show that both algorithms oversimplify the relationships between different isolates which could lead to false conclusions. A novel algorithms is therefore needed to properly estimate the underlying relationships between a collection of isolates. Such algorithm should take the behaviour of individual markers and alleles into account.

O041

Rapid detection and quantification of *Aspergillus fumigatus* in air using solid-phase cytometry

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Aspergillus fumigatus is an ubiquitous fungus causing severe infections such as aspergilloma, allergic bronchopulmonary aspergillosis and invasive aspergillosis in immunocompromised patients. Monitoring of the number

of *A. fumigatus* spores in the air inhaled by these patients is crucial for infection control. In the present study, a new and rapid technique for the quantificatin of *A. fumigatus*, based on solid phase cytometry and immunofluoresent labelling, was developed. Air samples were collected by impaction on a water soluble polymer that was subsequentely dissolved. A part of the sample was filtered and microcolonies were allowed to form on the filter for 18 hours at 47°C. Subsequentely, labelling with a monoclonal anti-aspergillus antibody and tyramide signal amplification was used to detect the microcolonies with the aid of a solid phase cytometer (ChemScan *RDI*). The detected spots were microscopically validated using an epifluorescence microscope. The specificity and sensitivity of the assay were evaluated by testing pure cultures of 40 *A. fumigatus* strains, 12 other *Aspergillus* species, 14 different *Penicillium* species and 14 other filamentous fungi. All *A. fumigatus* strains yielded labelled microcolonies, which confirmed the sensitivity of the assay. Only *Rhizopus stolonifer* and *Paecilomyces varotii* were labelled with the antibody and were able to form microcolonies at 47°C. These fungi, however, could be discriminated from *A. fumigatus* based on morphology. Comparison with traditional culture-based methods indicated that our novel approach is a rapid and reliable alternative with a high dynamic range.

O043

Comparison of PCR-reverse line blot and real-time PCR for the detection of dermatophytes in clinical samples

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Objectives: In a previous study PCR-Reverse Line Blot (PCR-RLB) was compared with culture and the potassium hydroxide test (KOH) for the detection of dermatophytes. PCR-RLB showed to be more sensitive than culture and KOH. Drawbacks of the PCR-RLB are the laborious nature of the test, the difficult standardisation and the interpretation of weak results. Therefore a multiplex real-time PCR was developed. The aim of this study was to compare PCR-RLB analysis with multiplex real-time PCR for the detection of dermatophytes.
Methods: Both PCR-RLB and real-time PCR targeted the ITS1 region located between the genes coding for 18S and 5.8S rRNA. The RLB membrane harboured 13 different probes to identify and discriminate between 9 different dermatophyte species. Real-time PCR consisted of two multiplex assays. One assay targeted *Trychopython rubrum*, *Trychopython violaceum* and *Trychopython tonsurans*. The second targeted *Microsporum* spp., *Trychopython* interdigitale group and the whole group of dermatophytes. Phocine herpes virus-1 was used as internal control for the real-time assays. Samples were processed using QIAamp® DNA mini kit (Qiagen, Germany) with a separate pre-lysis step.
In total 100 clinical samples (52, 38, 10 respectively nail-, skin- and hair samples) were analysed retrospectively by real-time PCR and compared with PCR-RLB.
Results: Of the 100 samples, 60 were positive with the PCR-RLB (27 *T. rubrum*, 14 *T. interdigitale*, 6 *T. tonsurans*, 3 *T. violaceum*, 1 *Moraxella canis* and 9 *Trichophyton* spp.). All samples identified as *T. rubrum*, *T. interdigitale*, *T. tonsurans* and *T. violaceum* by the PCR-RLB were confirmed by the real-time PCR. The sample which tested positive for *M. canis* by PCR-RLB was identified as *Microsporum* spp. by real-time PCR.
The 9 samples which scored positive for *Trichophyton* spp. in the PCR-RLB yielded weak results. Of these 9 samples, real-time PCR identified 3 samples as *T. interdigitale*, 1 as *T. rubrum*, 1 as *T. tonsurans*, 1 as dermatophyte positive and 3 samples remained negative.
The real-time PCR detected 8 additional samples which scored negative with the PCR-RLB. Of these 8 samples real-time PCR identified 4 samples as *T. rubrum*, 3 as *T. interdigitale* and 1 as dermatophyte positive.
Conclusion: These data show that real-time PCR is a sensitive method for detection of the most prevalent dermatophytes in nail-, skin- and hair samples. Furthermore, real-time PCR is more standardised and less laborious than PCR-RLB, making it a useful tool in routine diagnostics.

O051

Cross-border dissemination of MRSA in the Euregion Meuse-Rhine

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Introduction: The Euregion Meuse-Rhine (EMR) consists of the border regions of Belgium, Germany and the Netherlands. Cross-border patient mobility and free access to healthcare facilities are important issues in the EMR, but concern is rising about the possible dissemination of methicillin-resistant *Staphylococcus aureus* (MRSA), since the prevalence of MRSA is different in the three countries (24%, 14%, and 2% in Belgium, Germany, and the Netherlands, respectively). The aim of the study was to investigate the dissemination of MRSA in the EMR and the emergence and possible spread of community-associated (CA) MRSA strains in hospitals in the EMR.
Methods: MRSA isolates (n=152; 63 from Dutch, 40 from Belgian and 49 from German hospitals), isolated between 1999 and 2004, were characterised using pulsed-field gel electrophoresis (PFGE), SCC*mec* typing and multilocus

sequence typing (MLST). In 2005 and 2006, 257 MRSA isolates (44 from Belgian, 93 from German and 121 from Dutch hospitals) were characterised by *spa* typing, together with the algorithm based upon repeat pattern (BURP) and SCC*mec* typing. The presence of Panton Valentine leukocidin (PVL) was determined by real-time PCR as a genetic marker for CA-MRSA.

Results: From 1999 to 2004, the dissemination of the Brazilian/Hungarian, the Iberian, the New York/Japan, the Southern Germany and the UK EMRSA-2/-6 clone was observed in the EMR. In 2005 and 2006, it was observed that the Dutch MRSA isolates had a more diverse genetic background compared to the Belgian and German MRSA isolates. The majority of the Dutch isolates were associated with the New York/Japan, the Pediatric, the UK EMRSA-2/-6, the ST30-MRSA-IV, and the Berlin clone, while the Belgian and German isolates were associated with the Berlin and the New York/Japan clone, respectively. Between 1999 and 2004, the prevalence of PVL was 1.3%, while in 2005 and 2006 the PVL-prevalence had increased to 5% These MRSA isolates had a diverse genetic background associated with sequence type (ST) 1, 8 and 80, and the majority harbored SCC*mec* type IV.

Conclusions

1. Between 1999 and 2004, MRSA clones from CC5 and 8 were disseminated in the EMR.
2. In 2005 and 2006, three additional MRSA lineages (CC22, 30 and 45) were disseminated in the EMR and the Dutch isolates had a more diverse genetic background compared to the Belgian and German isolates.
3. Different lineages of CA-MRSA have entered the hospital environment in the EMR.
4. Cross-border surveillance of antibiotic-resistant micro-organisms is an important requisite to facilitate cross-border healthcare.

O054

Transmission of methicillin-resistant *Staphylococcus intermedius* between animals and humans

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Introduction: *Staphylococcus intermedius* is a commensal and a pathogen in dogs and cats, but is rarely isolated from humans. However, *S. intermedius* in humans has been associated with dog bite wounds, bacteraemia, pneumonia and ear infections. In the Netherlands, the prevalence of canine and feline infections with methicillin-resistant *S. intermedius* (MRSI) is increasing and therefore also the risk of their (anthropo-) zoonotic transmission.

Methods: At Utrecht University, MRSI were cultured from samples from infected surgical wounds of five dogs and one cat which had undergone surgery at the same veterinary clinic (clinic A). In order to identify the source, samples were taken from the noses of the surgeon and six nurses and from the nose and coat of two healthy dogs living at the clinic. In addition, 22 environmental samples were taken from several sites at the clinic. *S. intermedius* was identified in these samples using standard techniques. Antimicrobial susceptibilities were determined by an agar diffusion method. The mecA gene was detected by PCR. The isolates were genotyped by PFGE using SmaI as restriction enzyme. Four epidemiologically unrelated MRSI isolates from patients at other veterinary clinics were also included.

Results: MRSI was cultured from the nose of the surgeon, three nurses, one healthy dog and four environmental samples. The isolates were resistant against ampicillin, amoxicillin with clavulanic acid, cephalexin, ceftiofur, ceftazidime, enrofloxacin, gentamicin, kanamycin, chloramphenicol, lincomycin, clindamycin, tetracycline and trimethoprim/sulphamethoxazole and susceptible to fusidic acid and rifampicin. The isolates from the six animals (patients) showed the same pattern. All isolates were mecA positive by PCR. The PFGE profiles from the MRSI isolates from clinic A were all indistinguishable and differed from the profiles of the isolates from other clinics.

Conclusions: Together, these data strongly suggest transmission of MRSI between animals and humans. The surgeon and/or the nurses appear to have been the source for at least some of the patients. To our knowledge, this is the first report on the transmission of MRSI between humans and animals. People working at veterinary clinics should be aware of this risk for their own, their pets and their patients' sake. Although *S. intermedius* rarely causes disease in humans, its prevalence may be underestimated because *S. intermedius* may be misidentified as *Staphylococcus aureus* because it is coagulase and DNAse positive.

O055

Comparative typing of MRSA using multiple-locus variable number tandem repeat analysis (MLVA), pulsed field gel electrophoresis (PFGE) and spa-sequence typing

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Staphylococcus aureus is an opportunistic bacterial species causing a wide range of hospital and community acquired

infections. In particular infections caused by methicillin-resistant *S. aureus* (MRSA) pose a major problem in health care settings. In the Netherlands, the restricted use of antibiotics and a 'search and destroy' policy have kept incidence at a low level. However, in recent years there seems to be a slow increase in incidence and the finding that there is an animal reservoir has increased the interest further. As a result, there is considerable interest in molecular typing to study the epidemiology of MRSA.

S. aureus seems have a relatively stable core genome resulting in a predominantly clonal genomic background. Increased virulence and antibiotic resistance is acquired through lateral transfer of DNA. The clonal nature of *S. aureus* background enables the identification of certain lineages through molecular typing. A plethora of typing techniques have been used to characterise *S. aureus,* but in recent years PFGE, MLST and spa-sequence typing have become the most widely used techniques. The, until recently, most frequently used technique, PFGE, is a band based technique which is difficult to standardise and unsuitable for (inter)national molecular typing databases. The sequence based MLST is a robust technique yielding unambiguous results that is extremely well suited for electronic data exchange. However, MLST is both labor intensive and expensive. Spa-sequence typing is rapidly becoming the new molecular typing standard for *S. aureus.* However, spa-sequence typing uses only a single genomic locus for typing which may result in insufficient discriminatory power. For this reason, we developed a MLVA and compared typing capabilities with those of PFGE and spa-sequence typing.

For MLVA, the number of repeats was determined by amplifying the VNTRs of 8 different VNTR loci, including spa, in 2 multiplex PCRs. The fluorescently labeled PCR fragments were size on an automated DNA sequencer and the number of repeats of each locus was combined to create an 8-number numerical MLVA profile which was used for clustering. Approximately 1680 *S. aureus* strains (predominantly MRSA) isolated from humans were included in the study. Although MLVA yielded many different genotypes most types could be assigned to one of 11 large complexes which were made up of single-locus MLVA variants. There was 52% congruence between MLVA and spa-sequence typing and 33% congruence between MLVA and PFGE. Congruence between spa-sequence typing and PFGE amounted 40%. Sensitive *S. aureus* strains (MSSA) were present among all MLVA clusters and no MSSA-specific MLVA types were found. MRSA strains belonging to the PFGE non-typeable class, often referred to as pig strains all belonged to single MLVA complex, confirming the clonal nature of this type of strains.

MLVA appears to be a method that is suitable for molecular typing of *S. aureus*. Its simplicity, robustness, relatively low costs and unambiguous data make it an alternative for MLST. The method is superior to PFGE and its data are easier to use in clustering than those obtained by spa-sequence typing.

O056

The epidemiology of *Clostridium difficile* PCR-ribotype 027 in The Netherlands since 2005

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Introduction: In 2005, outbreaks of *Clostridium difficile* associated diarrhoea (CDAD) with the virulent PCR ribotype 027/toxinotype III were first reported in the Netherlands. *C. difficile* PCR ribotype 027 was already causing considerable problems in hospitals in the United States, Canada and England over the preceding years. Several investigations have shown an increase in morbidity and mortality and an increased relapsing rate for this type. Studies on risk factors at individual patient level showed an association with fluoroquinolones.

Methods: The Centre for Infectious Disease Control at the National Institute for Public Health and the Environment (RIVM) set up surveillance for CDAD in collaboration with the Leiden University Medical Centre (LUMC). Hospitals can send isolates or toxin positive faeces samples for typing, if they suspect type 027 based on an increased CDAD incidence or a severe clinical picture. From hospitals with an outbreak with or transmission of type 027, information is collected, including monthly CDAD incidence.

Results: Between February 2005 until half August 2007, 1886 samples have been typed, coming from 75 healthcare institutions and laboratories. In 418 cases (22%), PCR ribotype 027 was found. PCR ribotypes 001 (18%), 014 (7%) and 078 (8%) were also frequently found. To this date, in 23 of in total of 97 Dutch hospitals, type 027 has been detected. In 13 of the hospitals, the introduction of 027 caused an increased incidence of CDAD, two of which occurred since December, 2006. Ribotype 027 has also been detected in ten nursing homes. In eight of 11 hospitals where ribotype 027 was detected in 2005 or 2006 and outbreak occurred, no ribotype 027 has been detected since April, 2007. Two hospitals that had the epidemic well under control for a long time were faced with a new increase in incidence. In most of the hospitals where outbreaks occurred in 2005, the incidence decreased substantially in 2006.

Conclusion: The spread of PCR ribotype 027 in the Netherlands is still continuing. Outbreaks in new institutions are limited compared to previous hospital epidemics in

2005. Ribotype 027 caused new outbreaks in hospitals that appeared to have controlled the situation. In addition to the hospitals, type 027 has been detected in ten nursing homes. In other European countries, type 027 is more frequently recognised and this type has been detected in 15 countries. For control of CDAD, a multidisciplinary approach of medical microbiologists, hospital hygienists, infectiologists and nursing home physicians is important.

O057

Surveillance of *Clostridium difficile* associated disease in the Netherlands

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Background: Two years after the first outbreaks of severe *Clostridium difficile* associated disease (CDAD) in the Netherlands, caused by the hypervirulent PCR-ribotype 027, a national surveillance was undertaken by the National Reference Laboratory at the LUMC. In 2006, interest was raised in another PCR-ribotype, Type 078. This type has been found as the predominant type in cattle and recent findings indicate that type 078 is also frequently found in meat products.
Methods: Microbiologists were requested to send strains isolated from patients with a severe course of CDAD or from health-care facilities where an increased incidence of CDAD was noticed. A standardised clinical questionnaire was used to collect demographic, clinical and epidemiological data on each patient. Strains were characterised at the reference laboratory by PCR ribotyping, toxinotyping, presence of toxin genes and antimicrobial susceptibility patterns.
Results: Since February 2005, isolates from 1486 patients were investigated. Types 027 and 078 were found in 285 (19.2%) and in 123 (8.3%) of patients, respectively. Both types contained binary toxin, toxin A and toxin B genes as well as a deletion in *TcdC*. Type 027 and 078 strains were toxinotype III and V, respectively. Type 027 isolates were resistant to erythromycin and ciprofloxacin but susceptible to clindamycin and metronidazole. Type 078 isolates were resistant to ciprofloxacin and susceptible to metronidazole and vancomycin. Susceptibility to erythromycin and clindamycin varied. Of 1486 requests for questionnaires 617 (41.5%) were received; 128 (20.7%) from type 027 patients, 46 (7.5%) from type 078 patients and 443 (71.8%) from non-027/non-078 patients (other types). Compared to other types, type 027 was associated with age ($p<0.0005$)), use of fluoroquinolones (OR 3.00, 95% CI 1.49-3.90) and 2nd generation cephalosporins. (OR 6.35, 95% CI 2.15-18.8). Regarding outcome, type 027 was associated with the most severe disease (OR 3.60, 95% CI 1.68-8.23), the highest mortality of 13.1% (OR 6.88, 95% CI 1.43-32.9) and recurrence rate of 25.3% (trend: OR 2.26, 95% CI 0.94-5.41). Type 078, compared to type 027 was most often community associated (27.3% vs 8.3%, OR 3.75, 95% CI 1.89-7.44) and disease severity (35.6%) was an intermediate between type 027 (40.7%, not significant) and other types (25.5%, trend: OR 2.65, 95% CI 0.71-9.89). Attributable mortality was similar in type 027 patients (4.1%) and type 078 patients (4.4%).
Conclusions: Type 027 continues to be present causing severe CDAD. Type 078 is a new emerging strain with similar virulence factors as type 027, and a disease severity between that of type 027 and other types.

O058

Regulation of toxin genes in *Clostridium difficile*

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Clostridium difficile is the main cause of nosocomial diarrhoea. Since the appearance of the hypervirulent ribotype 027, the number of CDAD (C*lostridium difficile* associated diarrhea) patients has increased dramatically. Not only the number of patients has risen, also the severity of the symptoms and complications of the disease has increased. In recent years, numerous countries have reported outbreaks caused by the 027 ribotype, with a steep increase in the morbidity and the mortality.
Well known virulence factors for *C. difficile* are the presence of the two large clostridial toxins, TcdA and TcdB and the binary toxin. In addition, high virulence is associated with deleterious mutations in the gene coding for the negative regulator of toxin expression, TcdC. The epidemic ribotype 027 possesses all these virulence factors and has been shown to be a hyperexpressor of TcdA and TcdB.
Toxin expression is positively regulated by the sigma factor TcdR, which is essential for recognition of tcdA and tcdB promoters by the RNA polymerase. TcdC has been described to act as an anti-sigma factor, which means that it prevents toxin promoter recognition by the RNA polymerase/TcdR complex, and thus is responsible for inhibition of transcription of the toxin genes.
Since TcdC is the natural molecule to inhibit toxin production by *C. difficile*, it is important to know how this is established. This knowledge may lead to the development of drugs that are able to stop toxin synthesis in patients. Although the function of TcdC is known, the mechanism by which it displays its action is unknown. We set out to unravel the mechanism by which TcdC inhibits the recognition of toxin promoters by the RNA polymerase/

TcdR complex, including an analysis of the biochemical characteristics of TcdC.
Recombinant TcdR, TcdC and TcdC mutants were expressed in *Escherichia coli* and purified using standard techniques. Activity of the recombinant proteins was tested in an *in vitro* RNA transcription reaction. Mutants were generated that lacked the hydrophobic N-terminus, the putative dimerisation domain, or deletions described to occur in ribotypes 027 (18 bp deletion) and 078 (39 bp deletion). All the mutants were tested for their ability to form dimers, a TcdC feature previously described, of which it is unknown whether it is important for function. In addition, mutants of TcdC were tested for their ability to inhibit RNA transcription in an *in vitro* RNA transcription assay. In addition, using the mutants of TcdC we plan to map the domain of TcdC that interacts with TcdR.
Results obtained with the mutant TcdC molecules will be shown, and possible implications for drug development will be discussed.

O059

Clostridium difficile in animals: from stock to meat

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Humans exchange intensively microbiological and non-microbiological materials with their environment. Animals are part of this environment, and provide us companionship (pets), recreation (zoo animals) and food (stock animals). Since ancient times, people are aware that animals can cause diseases in humans. It is suggested that most religious food laws have found their origin in this awareness. Clostridia infections occur in humans and in animals. In fact, *Clostridium* spp. are increasingly a cause of neonatal mortality in swine husbandry. For example, *Clostridium perfringens* is a common cause for hemorrhagic enteritis in neonatal pigs.[1] Most cases of gastroenteritis in pigs, however, are not confirmed by microbiological examination. This is not favourable from a veterinary and a public health point of view. There are an increasing number of reports on the occurrence of *Clostridium difficile* in animals, including dogs, swine, veal, close in presence to humans [for examples Refs.2, 3]. Recently, as the cause of persistent diarrhoea in a few farms was more closely inspected, we found *C. difficile* PCR ribotype 078 toxinotype V in Dutch piglets, that were genetically identical to species found in hospitalised human patients.[4-6] *C. difficile* PCR ribotype 078, and, in particular, toxinotype V, has caused severe outbreaks with high morbidity and mortality in human populations. At this moment there is no evidence, however, whether this bacterium can be transmitted from humans to animals (anthropozoonosis) or from animals to humans (zoonosis). It is thus not clear that *C. difficile* in animals has played any role in past *C. difficile*-associated disease outbreaks in (hospitalised) humans. This paper will discuss the potential public health risk of *C. difficile* in piglets and will discuss some feasible hygienic measures in swine production to reduce infections risk for the animal and for humans.
The herd size in commercial pig farming increases rapidly. The production efficacy and logistic advantages of large farms coincides with an increase of animal contacts, as sorting animals on size and sex is common. Farm workers play an important role in contacts between neonatal piglets. During the first three days of life all piglets are at least handled twice by farm workers for ear tagging, iron injection, coccidiosis prevention and in many cases for one or two preventive antibiotic treatments. Also teeth clipping and castration of male piglets is still practised on many farms. The handling of pigs by farm workers, who do not wear gloves, nor disinfect their hands between handling of litters, is a risk for spreading neonatal infections between litters. Moreover, the idea that spreading infections lead to immunity is the reason that many farmers still collect diarrhoea of neonatal piglets and feed this to pregnant sows. These working procedures promote the prevalence of carriers of various bacterial diseases and are a solid basis for settling endemic bacterial infections. Hygiene procedures of all-in all-out suggest that a group of pigs is kept together and moved together to a clean disinfected environment, or to slaughter. However, regrouping of slow growing pigs and poor hygiene of farm workers completely neutralise the advantages of all-in all-out. This results in a high prevalence of infected pigs with or without symptoms. Farm and animal hygiene procedures that are designed to minimise spread of endemic infection are still uncommon in pig farming. In contrast, the number of SPF-herds (specific-pathogen-free) with breeding stock, that was caesarean-derived and raised under isolated conditions, is increasing. These SPF-farms are characterised by strict external biosecurity regulations and introduction of swine pathogens is often successful.
Until now we have identified three sow herds in the Netherlands with a high incidence of *C. difficile*-associated neonatal diarrhoea. The lethality from neonatal diarrhoea in theses farms is usually low, but morbidity can go up to 100%. Piglets with CDAD show orange stained diarrhoea. Piglets with characteristic symptoms were euthanised and dissected. Characteristic is the colonic oedema and in severe cases presence of volcano like lesions in the colon. From the gut content we isolated *C. difficile* that was further characterised as *C. difficile* PCR ribotype 078 toxinotype V. CDAD in these herds is very persistent and occurring

almost weekly in newly born piglets from 1 to 4 days of age. Recovered pigs have some growth retardation.

The three farms identified with neonatal CDAD had a long history of outbreaks with *C. perfringens* type C (CPC) in neonatal piglets. Sows, vaccinated against CPC, protect their offspring by antibodies in their milk. However, an increasing number of herds face a CPC strain that produce a b_2 toxin that is not included in registered vaccines. Routine treatment of all born piglets with amoxicillin to prevent CPC was practised on these farms for several years. In addition, sows were treated with trimethoprim-sulfomethoxazole several days before and after farrowing. When CDAD was diagnosed, preventive medications were stopped, but CDAD is still quite common on these farms. Most likely we underestimate the number of herds infected with *C. difficile* PCR ribotype 078 toxinotype V. Until now we only have isolated *C. difficile* from diseased piglets or form healthy piglets that were housed in contact with the diseased piglets. The sensitivity of our isolation procedure needs to be improved in order to monitor the infection route and to assess infection risk of exposed animals and humans. On these three farms all pigs as well as all farm workers were exposed to *C. difficile* PCR ribotype 078 toxinotype V. The manure of the piglets is collected in basins and spread upon agricultural land or pastures. Ventilation canals of the farms spread barn dust that may contain *C. difficile*.

Farm workers are most exposed to *C. difficile*, especially those who work in farrowing compartments that house neonatal piglets. No reports were made about higher incidence of pseudomembranous colitis of farm workers. Basic hygiene procedures to prevent or reduce transmission of *C. difficile* from pig to pig and from pig to humans need to part of the standard operating procedures on pig farms. Educational level of farm workers needs much improvement with respect to hygiene concepts.

When resistant pathogens like *C. difficile* PCR ribotype 078 toxinotype V are amplified in pig herds, inevitably they contaminate the environment by air, or by manure or by meat or meat products. In Canada *C. difficile* was isolated from ground meat.[2] What route includes most risk to human health is not (quantitatively) assessed. Prevention of spread of *C. difficile* and other potential human pathogens should start with omitting and reducing risk factors for colonisation of *C. difficile* in the pig gut. It includes prudent and restricted or banned use of antibiotics in pigs. This should not be restricted to fluoroquinolones and cephalosporines, but to all antibiotics that may induce Clostridia overgrowth in the large intestine.[7] Although the use of antibiotics as growth promoter is banned the therapeutic and preventive use of antibiotics is not declined.[8,9] Increase of antimicrobial resistance in all food-producing animals is a serious thread to human health. Operating procedures with respect to use of antimicrobial drugs should be part of the license to produce.

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O060

Development and validation of a real-time PCR assay for detection of toxigenic *Clostridium difficile*, as part of a Dutch study on the epidemiology of gastro-enteritis

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Objectives: *Clostridium difficile* is the etiologic agent responsible for human diseases ranging from mild diarrhea to severe pseudomembranous colitis, which are

collectively referred to as *C. difficile* associated disease (CDAD). The cell cytotoxicity (CT) assay is considered the gold standard for detection of toxigenic *C. difficile*. However, this method is laborious and time-consuming (turn-around time: >48 hours). Rapid diagnosis of CDAD is important, since it may result in early treatment and help prevent nosocomial transmission. Therefore, a real-time PCR based assay was developed and validated. Also, this assay will be used as a screening tool for a gastroenteritis study (GEops Study). This study will commence in May 2008 in 6 hospitals in the Netherlands. Its primary goal is to assess the incidence, etiology and course of patients hospitalised for gastroenteritis.

Methods: An assay targeting the tcdA and tcdB genes was developed. Stools were processed with the easyMAG specific A stool protocol (bioMérieux). The phocine herpes virus-1 was used as internal control. The selectivity of the assay was validated with a panel of well characterised *C. difficile* strains and clinical isolates (n=50) and non-*C difficile* strains (n=43). The analytical sensitivity was assessed by dilution series (n=3), spiked in a homogenous faecal matrix. Also, the assay was compared with the CT assay in a clinical validation performed on stool samples (n=163) of patients suspected of CDAD.

Results: The screening assay proved to be specific for *C. difficile* as no cross-reaction was observed. The assay was capable of detecting approximately 1560 CFU/g of stool with a 100% hit rate. Sixteen PCR positive and 139 PCR negative samples were in complete concordance with the CT assay. The PCR was positive in 8 additional samples which remained negative in the CT assay. One of these 8 samples could be confirmed as truly positive by CT in a consecutive sample of the same patient. In comparison with the gold standard (CT) PCR showed 100% sensitivity, 95% specificity, 67% positive predictive value, and 100% negative predictive value. PCR inhibition was observed in less than 1% of all 163 screened stool samples.

Conclusions:

1. This in-house real-time PCR assay offers a rapid and sensitive method for the first screening for CDAD.
2. The assay will be used as a rapid screening tool for the detection of *C. difficile* in the GEops Study that will commence in May 2008.

O061

Characterization of *Clostridium difficile* ribotype 078 from human and animal origin

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Background: A recent study demonstrates that *Clostridium difficile* PCR-ribotype 078 (Type 078) is the predominant ribotype in cattle. Type 078 seems to be an emerging type in human disease, with an increase from 5.6% of all submitted isolates to the national reference laboratory in 2006 to 10% in 2007. To characterise human and animal type 078 isolates in more detail, we applied 'multi locus variable tandem repeat analysis' (MLVA) and investigated the isolates for the presence of resistance genes (ermB) and genes encoding enterotoxin A (tcdA), cytotoxin B (tcdB) and binary toxins (cdtA, cdtB). Additionally, we sequenced a part of the negative regulator (tcdC) of the pathogenesis locus and determined the susceptibility pattern of 51 *C. difficile* both human (n=43) and pig (n=8) type 078 isolates. The isolates originated from human diarrhoeal patients (n=60) from the year 2005, 2006 and 2007 and from diarrhoeal pigs (n=10).

Methods: The presence of the toxin and ermB genes was established by conventional PCR using previously described primers. Sequencing of the tcdC gene was performed by cycle sequencing and analysis. Susceptibility to moxifloxacin, ciprofloxacin, clindamycin and erythromycin was investigated using E-test method using the most recent CLSI breakpoints (2007). MLVA was applied on 19 human and 10 pig isolates. Genetic relationships were determined by clustering isolates according to MLVA type using the summed absolute distance as the coefficient for calculating the minimum-spanning tree.

Results: All 51 strains tested positive for tcdA, tcdB, cdtA and cdtB. The ermB gene was present in 50% of pig and 7% of human isolates (average percentage 14%). All 51 characterised isolates contained two point mutations at location 183 bp and 184 bp and had a 39 bp deletion in the tcdC gene. Resistance for moxifloxacin, ciprofloxacin, clindamycin and erythromycin was found in 12, 88, 6 and 75% of the isolates, respectively. MLVA revealed that, with the exception of one human isolate, all (97%) tested human and pig type 078 isolates were genetically related (single locus variants (SLV's) with a summed tandem repeat difference (STRD) ≤10). Two genetically highly related (clonal) clusters were found (SLV's with a STRD ≤2), containing both human (n=7) and pig (n=6) isolates.

Conclusions:

3. Toxin profiles (tcdA, tcdB, cdtA and cdtB) of human and pig isolates were identical;
4. Most of the isolates (66%) highly resistant to clindamycin and erythromycin are ermB positive.
5. The point mutation at location 184 bp introduces a stopcodon in the tcdC gene leading to a non-functional tcdC gene.
6. In total, 97% of human and pig type 078 isolates were genetically related. Two genetically highly related (clonal) clusters containing both human and pig isolates suggesting zoonotic transmission.
7. *C. difficile* type 078 isolates have the same virulence markers as the highly virulent *C. difficile* ribotype 027.

O062

Implications of HPV testing in cervical screening

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In the Dutch organised cervical screening program 750,000 women between 30 and 60 years of age are invited annually for a Pap smear (with a 5 year screening interval). Almost 480,000 of them attend. Two percent of them will receive a repeat advice and 0.8% a referral advice for colposcopy. Current drawbacks are substantial numbers of false negative (an estimated 3,500 CIN2+ lesions remain undetected by cytology) and false positive smears (11,000 women have repeated cytology or are referred to detect 4,500 CIN2+ lesions). Moreover, half of the cervical cancers detected in the 30-60 age group involve women who did not have a prior screening history and the estimation is that 4,500 CIN2+ lesions remain undetected in the 20-25% of women that do not respond to the invitation for screening (i.e. non-responders). We investigated whether high-risk HPV (hrHPV) testing can offer improvement in any of these problematic areas.

Firstly, a prospective, randomised trial was initiated to evaluate the effectiveness of hrHPV testing as an adjunct screening tool in a large scale population-based primary screening setting. This POBASCAM (Population based screening Amsterdam) trial compares the yield of CIN3+ among 44,102 women in a primary screening program by either cytology testing alone (control arm) or cytology and HPV testing (intervention arm), using a GP5+/6+ PCR enzyme immunoassay (EIA). Interim analyses up to 18 months of follow-up revealed that the sensitivity and negative predictive value of HPV testing for CIN3+ is superior to that of cytology. Analysis of about 17,000 women who reached already the second screening round (after 5 years) revealed that the number of CIN3+ lesions detected during the baseline round was substantially higher in the intervention than in the control group, although the total number of CIN3+ over two screening rounds did not differ between groups. Therefore, hrHPV testing leads to earlier detection of clinically relevant CIN3+, permitting extension of the screening interval. We also performed a study on non-responder women. Aiming at improving the coverage 2,546 of these women were offered a package for taking self-sampled vaginal specimens at home to be sent to the laboratory for hrHPV testing. Another 284 non-responder women received instead an extra recall for cytology (control group). Active response was higher in the self-sampling than in the control group (34.2% vs 17.6%; p<0.001). HrHPV positive self-sampling responders were less likely to have a prior screening history than screening participants (p<0.001), indicating that they are regular non-responders. The hrHPV prevalence was similar (8.0% vs 6.8%; p=0.11), but the CIN2+ yield higher in self-sampling responders compared to screening participants (OR=2.93, 95% CI 1.48-5.80; p=0.0013). The costs per CIN2+ lesion detected via self-sampling were in the same range as those calculated for conventional cytological screening. Thus, offering self-sampling for hrHPV testing in non-responders is an attractive adjunct to effectively increase population coverage of screening without the adverse effect of markedly increased costs per detected CIN2+ lesion.

Together, these data are in favour of implementing HPV testing as screening tool in a new era of cervical cancer prevention that will be faced in the near future.

O064

Grenzen aan medische testen. Over ziekterisico's en gezondheidskansen

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Bij screeningsprogramma's worden diagnostische procedures aangeboden aan mensen die op dat moment nog geen klachten hebben. Uitgangspunt is dat vroege ontdekking van afwijkingen een gezondheidswinst kan opleveren: het terugdringen van sterfte, het verminderen of uitstellen van ziekte, het bevorderen van de kwaliteit van leven. Screening kent ook nadelen en bezwaren en vraagt daardoor steeds om een zorgvuldige afweging van de voors en tegens. Vroege opsporing van afwijkingen betekent ook dat onbezorgde levensjaren worden ingeleverd, soms tegen een slechts geringe kans op een langer leven. Screening is een vorm van 'technische preventie' (t.o. 'leefstijlpreventie'). Bij het publiek ontmoet deze vorm van preventie veel bijval. 'Hoe eerder erbij, hoe beter' is het adagium. Onderzoek heeft aangetoond dat er bij de bevolking nogal naïeve opvattingen bestaan over de zinvolheid en toepasbaarheid van screeningsprogramma's.

Werd er aanvankelijk vooral onderzoek gedaan naar beginnende afwijkingen, tegenwoordig betreft screening steeds vaker het in kaart brengen van risico's op het krijgen van ziekte. De moderne geneeskunde ontwikkelt steeds meer diagnostische procedures waarmee het voor een bepaalde persoon geldende risico op naderend onheil (ziekte, sterfte, aangeboren afwijking) kan worden gekwantificeerd. Met dergelijke diagnostiek wordt het voor het collectief geldende, algemene risico voor de betreffende persoon geïndividualiseerd. De uitkomst maakt het mogelijk om een voorspelling te doen over iemands gezondheidsverloop, maar kan ook tot een aanzienlijke wijziging in iemands leefsituatie leiden. Want kennis maakt verantwoordelijk, een verantwoordelijkheid waaraan

maar moeilijk valt te ontsnappen, want je kunt nou eenmaal niet ophouden met te weten wat je al weet.
De medisch-technische mogelijkheden voor vroege opsporing en risico-inventarisatie zullen een steeds grotere vlucht nemen, waardoor mensen steeds vaker met ziekterisico's en gezondheidskansen zullen worden geconfronteerd. Met name de moderne DNA-technologie zal hieraan een belangrijke bijdrage leveren. Het leren omgaan met risicokennis wordt daarom een steeds belangrijker aspect van onze samenleving. Gebleken is dat mensen maar moeilijk met kansen en risico's om kunnen gaan en rationaliteit hierbij nogal eens zwicht voor emoties.
Waar liggen de grenzen van het screeningsaanbod? In de westerse cultuur wordt veel waarde gehecht aan het autonomieprincipe: de mens wordt beschouwd als een vrij individu dat autonoom beslissingen neemt. Maar er is ook het collectieve belang. Wie bepaalt welke 'kansbiedende' diagnostische opties aan mensen moeten/mogen worden aangeboden? En is het, met de ontwikkelingen op het gebied van het internet, wel mogelijk om mensen dit soort kennis te onthouden? Dit soort vragen zullen de komende tijd nog veel aan de orde komen in de gezondheidszorg.

O065

Evasion of Toll like receptor 5 recognition by alkaline protease of *Pseudomons aeruginosa*

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Introduction: Toll-like receptors (TLRs) recognise various conserved molecules of micro-organisms. Activation of these receptors is a crucial step in antimicrobial defense. The ligand of TLR5 is flagellin, which is the major component of the bacterial flagellum. Flagellin polymerises to form long filaments, which are essential for the motility and virulence of flagellated bacteria. TLR5 recognises a conserved and functionally essential part of monomeric flagellin.
Bacteria secrete proteins that interfere with different pathways of the innate immune system, thereby they evade recognition and killing. In this study, we identified and characterised a protein secreted by *Pseudomonas aeruginosa* as an inhibitor of TLR5 activation.
Materials and methods: Human embryonic kidney (HEK) cells transfected with TLR5 were incubated with bacterial supernatant and stimulated with flagellin. IL-8 production of HEK-TLR5 cells was measured as readout for TLR5 activation. Recombinant AprA was expressed and isolated from *Escherichia coli*. Flagellin and flagellar filaments were isolated from *P. aeruginosa*.
Results: We screened supernatants of several bacteria for TLR inhibitors and identified that *P. aeruginosa* inhibits TLR5 activation. After fractionation of the supernatant by ion-exchange chromatography and gel filtration the protein of interest was identified by mass spectrometry as alkaline protease (AprA) of *P. aeruginosa*. AprA is a zinc-metalloproteasis that forms a gene cluster together with its inhibitor AprI and AprD, AprE and AprF, which are necessary for the secretion of AprA.
Recombinant AprA isolated from *E. coli* also inhibits TLR5 activation, while AprI blocked this effect. To identify the mechanism of action, we tested the effect of AprA on TLR5 and flagellin. HEK-TLR5 cells were still functional after incubation with AprA, while flagellin was cleaved by alkaline protease. Monomeric flagellin of *P. aeruginosa* and *Salmonella typhimurium* were both cleaved in the same way according to SDS-PAGE. Interestingly, flagellar filaments were not susceptible to degradation by AprA.
Conclusion: AprA of *P. aeruginosa* is an inhibitor of TLR5 activation and thereby the first bacterial inhibitor described to date with such a function. AprA cleaves flagellin, however flagella are not affected. In this way, bacteria preserve their motility, while they evade recognition via TLR5.

O066

Streptococcus pneumoniae has a specific response to transcytosis: identification of an essential role for Mg^{2+} transport during translocation

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The integrity of body compartments is maintained in part by boundaries of epithelial and endothelial cells. Thus, to become invasive, *Streptococcus pneumoniae* has to cross these barriers, which occurs through a specific and receptor-mediated process called transcytosis, pivotal for the pathogenesis of the bacterium. Some *S. pneumoniae* strains are more likely to cause invasive disease than others, which implies that there are specific genetic determinants that predispose for transcytosis. However, little is known about the bacterial factors that are needed for the translocation of eukaryotic cell layers. To identify *S. pneumoniae* genes needed for transcytosis, we established a transwell system using Human Brain Microvascular Endothelial Cells (HBMEC). In this system, cells are grown into a tight monolayer on a membrane with a pore size that allows for the passage of bacteria but not eukaryotic cells. The bacteria are co-incubated with the cells in the upper compartment of this system, which enables them to adhere and invade the cells. The ability to translocate is assessed by following the appearance of bacteria in the lower compartment over time.

A significant stress encountered by *S. pneumoniae* within the endothelial cell vesicles is bound to be Mg2+ limitation. Analysis of the published *S. pneumoniae* genomes revealed the presence of several genes that putatively encode Mg2+ transporters and a mutant was generated in a homologue. *In vitro* growth experiments showed that this mutant has a severe growth defect, which could be restored by the addition of an excess of Mg2+ but not by other ions. Thus, this gene, now designated as mgtA, encodes the main Mg2+ transporter of *S. pneumoniae* under *in vitro* growth conditions. Expression of mgtA under control of a heterologous promoter restored growth of the mutant to wildtype levels, indicating that the observed effects are solely due to deletion of the gene. Adhesion of the mgtA mutant to HBMEC was about one log lower than that of the parent strain and, consequently, invasion was slightly lower than that of the parent strain. However, the percentage of adherent mgtA mutants that invaded and survived in the HBMEC was comparable to the parent strain, indicating that the mutant is not impaired in these respects. Strikingly, when the transcytosis of both strains was compared, the mutant failed to reach the lower compartment, whereas the parent strain did, indicating that mgtA is essential for the translocation of the cell layer. Introduction of the plasmid containing mgtA in the mutant restored its ability to translocate the cell layer.

Conclusion: These data show that *S. pneumoniae* has an active response to transcytosis and that Mg2+ plays an important role in this process.

O067

Putative proteinase maturation protein A (PpmA) of *Streptococcus pneumoniae* contributes to pneumococcal adherence and colonization

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An important first step towards pneumococcal disease is the colonisation of the nasopharynx of the respiratory tract. This process is mediated by adherence of *Streptococcus pneumoniae* to nasopharyngeal epithelial cells. Putative proteinase maturation protein A (PpmA) is an abundantly expressed, conserved, and surface-exposed lipoprotein. To examine the contribution of PpmA to pneumococcal virulence, ppmA knockout mutants of *S. pneumoniae* strains D39, NCTC10319, TIGR4, and ATCC6319 (serotypes 2, 35A, 4, and 19F, respectively) were tested in *in vitro* adherence and internalisation assays and a murine model of colonisation. Interestingly, DppmA mutants of all strains except D39 revealed a significant decrease in adherence to the human pharyngeal epithelial cell line Detroit 562 *in vitro*. Expression of PpmA appeared to have a negative effect on the uptake of D39 by J744 professional macrophages as a significant increase in the amount of internalised D39DppmA was found compared to the D39 wild type. However, no difference in uptake of NCTC10319 wild type and DppmA mutant by J774 cells was observed. In addition, ppmA mutants of D39 and NCTC10319 were found to have a significantly higher association to human PMNs than their respective wild types when examined by flow cytometry. *In vivo* experiments performed in both single and mixed mouse colonisation models showed that there was a statistically significant reduction of bacterial load in the nasopharynx of D39DppmA compared to its wild type over a period of 192 hours. *In vivo* results of the NCTC10319fjppmA and NCTC10319 showed a similar trend, however this was not significant. These data suggest that the reduced bacterial load in the nasopharynx of mice is due to the impaired ability of ppmA mutants to adhere to the nasopharyngeal epithelium.

Conclusion: We have shown that PpmA contributes to pneumococcal virulence, particularly at the early stages of infection, namely colonisation.

O068

Mycobaterial alpha-glucan modulates host immune responses via the interaction with DC-SIGN and Toll-like receptor 2

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Capsules are protective polysaccharide structures on the surfaces of many bacteria. They display a tremendous structural diversity and have been shown to be critically important for the successful colonisation of the host. An important group of bacteria that express a capsule are the mycobacteria, which include the human pathogens *Mycobacterium tuberculosis* and *Mycobacterium leprae*.

The interaction between mycobacteria and the host immune system is very complex. Mycobacteria express immunomodulatory factors that influence the host immune response. Major efforts have been put in identifying these factors and in trying to understand the underlying mechanisms. However, up till now, most research has been focused on cell-wall (glyco) lipids, whereas only minor attention has been paid to the capsular constituents.

In this study, we investigated the role of capsular polysaccharides in the *Mycobacterium*-host interaction. We focused on an alpha-1,4 glucose polymer known as alpha-glucan, which is the major capsular polysaccharide of mycobacteria. Recently, alpha-glucan was shown

to modulate immune responses by suppressing IL-12 production by dendritic cells (DCs). Although this effect could clearly be ascribed to the glucan, the underlying mechanism remained unexplained. The goal of this project was to unravel this mechanism by identifying the host alpha-glucan receptors and investigating their role in glucan-mediated immunomodulation. For this, we screened the glucan-binding capacity of various lectins using lectin-Fc constructs and found that alpha-glucan specifically interacts with the C-type lectin DC-SIGN. This lectin was previously shown to be important for the binding to and modulation of DCs by mycobacteria. To see whether alpha-glucan could also bind to DC-SIGN on the cell surface, we incubated fluorescently-labelled polystyrene beads coated either with alpha-glucan or with human serum albumin as a control together with Raji cells, Raji cells expressing DC-SIGN, or DCs and analysed binding of the beads by flow cytometry. The results showed that the glucan-coated beads specifically bound to both the DCs and the Raji cells expressing DC-SIGN, but not to the Raji cells alone, and that this interaction could be abrogated by pre-incubating with mannan or with antibodies that block DC-SIGN. These results clearly demonstrate that mycobacterial alpha-glucan binds to DC-SIGN and that this interaction may be important for its immunomodulatory activity.

Alpha-glucan is not unique to mycobacteria and similar molecules can be found in a number of species including some fungi. Interestingly, alpha-glucan from the fungus *Pseudallescheria boydii* has been reported to induce the secretion of pro-inflammatory cytokines in a mechanism involving toll-like receptor 2 (TLR2). To test whether mycobacterial alpha-glucan can also activate TLR2, we incubated TLR2-transfected HEK293 cells with alpha-glucan and measured the TLR2-dependent production of IL-8. Interestingly, our results showed that mycobacterial alpha-glucan, like the fungal glucan, can activate TLR2. Thus, besides binding to DC-SIGN, alpha-glucan can also bind and activate TLR2. Exactly how this interaction occurs and what influence it has on the host immune-response is currently unknown. Our current experiments are aimed at providing insight into these important questions.

O069

The *Mycoplasma pneumoniae* MPN229 gene encodes a protein that selectively binds single-stranded DNA and stimulates RecA-mediated DNA strand exchange

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Mycoplasma pneumoniae has previously been characterised as a micro-organism that is genetically highly stable. In spite of this genetic stability, homologous DNA recombination has been hypothesised to lie at the basis of antigenic variation of the major surface protein, P1, of *M. pneumoniae*. In order to identify the proteins that may be involved in homologous DNA recombination in *M. pneumoniae*, we set out to characterise the MPN229 open reading frame (ORF), which bears sequence similarity to the gene encoding the single-stranded DNA-binding (SSB) protein of *Escherichia coli*. The MPN229 ORF has the capacity to encode a 166-amino acid protein with a calculated molecular mass of 18.4 kDa. The amino acid sequence of this protein (Mpn SSB) is most closely related to that of the protein predicted to be encoded by the MG091 gene from *Mycoplasma genitalium* (61% identity). The MPN229 ORF was cloned, and Mpn SSB was expressed in *E. coli* and purified. The Mpn SSB protein was found to: i) exist primarily as dimer in solution, ii) selectively bind single-stranded DNA in a divalent cation- and DNA substrate sequence-independent manner, and iii) stimulate *E. coli* RecA-promoted DNA strand exchange.

We conclude that Mpn SSB represents the *M. pneumoniae* counterpart of the *E. coli* SSB protein. The results from this study will pave the way for unraveling the DNA recombinatorial pathways in *M. pneumoniae*.

O070

Comparative genomic profiling of Dutch clinical *Bordetella pertussis* isolates using DNA microarrays: identification of genes absent from epidemic strains

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Introduction: *Bordetella pertussis* causes whooping cough or pertussis in humans and is particularly severe in infants. Despite vaccination, whooping cough remains a public health problem. Since the 1990s, a significant increase in the *B. pertussis* incidence was observed in many countries. Also in the Netherlands, an increase in *B. pertussis* has been observed since 1996. Several causes for the re-emergence have been suggested, there is evidence that pathogen adaptation also plays a role. Numerous studies have demonstrated that the *B. pertussis* population has changed since the start of vaccination. In several countries, the *B. pertussis* population was found to be variable since polymorphisms in surface proteins were found. In the Netherlands, we observed that strains with a particular allele of the ptx promoter (ptxP) i.e. ptxP3 have expanded in the Dutch *B. pertussis* population. The increase in frequency coincided the increase in *B. pertussis* notifications in the Netherlands. We aimed to use microarrays to identify additional changes in strains associated with the Dutch epidemic.

Methods: We developed an oligonucleotide-based (70-mers) microarray representing 93% of the gene repertoire of the Tohama I strain of *B. pertussis*. Microarray-based comparative genome hybridisation (CGH) was used to investigate the gene contents of 43 Dutch *B. pertussis* strains.
Results: Of the 3,581 genes spotted on the microarray, 93 (2.6%) were found to be absent in at least one of the *B. pertussis* strains tested. The absent genes were clustered in 8 different loci. All Dutch *B. pertussis* isolates carrying the ptxP3 allele (epidemic strains) had lost 18 genes, located in one locus, compared to *B. pertussis* strains with the ptxP1 allele (pre-epidemic strains).
Conclusion: The most prominent characteristic of the epidemic strains was a genomic deletion removing about 23,000 bp. Although the relevance of this deletion is still unknown, our results provide 'proof of principle' as we were able to identify an epidemic strain.

O072
New petri dishes – microscale microbial culture chips

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A microbial growth format has been created that allows up to a million individual samples to be cultured. The base of this culture chip is a thin strip of porous aluminium oxide (PAO) which allows microbial cells to grow on the upper surface. PAO is an excellent material for culturing micro-organisms; it is flat, inert and uniquely porous. The walls of the chip which segregate samples are created by laminating the PAO with a acrylic film. The acrylic film is selectively removed by reactive ion etching. The result is to create culture areas as small as 7x7 µm on the base, with walls 10 µm high. The PAO chips can be scanned by microscopy. Individual strains can be isolated by a micromanipulator.
The PAO chips are versatile and have been used for viable counting and various examples of high-throughput screening. Environmental samples have been screened on the basis of metabolism of an organic phosphate dye by oligotrophic bacteria. This experiment cultured the bacteria on the same aliquot of Rhine water that the microorganisms were isolated from. Out of 22 isolates, 6 previously uncultured bacteria were recovered and typed by sequencing PCR products derived from 16S rRNA genes. This suggests the chips may have a role in phenotyping and isolating previously uncultured microorganisms.

Reference

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O074
Bacterial bioreporters to quantify individuality

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Traditionally, bacteria are investigated as populations, and their activities described and understood only as population averages. This approach greatly overlooks findings which show that bacterial individuality exists and to a large extent contributes to population structure and activities. We will present a novel GFP-based bacterial bioreporter that allows us to quantify the life history of individual bacteria in terms of ancestral reproductive success. We describe its construction, characterisation, and experimental boundaries. The utility of the bioreporter in environmental microbiology will be demonstrated by its application to the phyllosphere, or plant leaf surface, a unique bacterial habitat. To show that single-cell analysis can offer new and complementary data to population-based measurements, the bioreporter will be used to study the effects of abiotic factors (e.g. water availability or UV radiation) and biotic factors (e.g. competitors for nutrients or herbivorous damage to the plant) on bacterial life on the leaf surface at the micrometer scale.

O076
Rapid susceptibility testing of fungi

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Pathogenic fungi often grow slowly. Fungal susceptibility testing by culture is therefore time consuming. We have developed a culture method by which microorganisms are grown on a porous ceramic support (porous aluminium oxide, or PAO). The material is inert and biocompatible. Planar strips of PAO allows microorganisms to be grown on the upper surface and supplied with nutrients and test compounds from beneath. The resulting microcolonies can be stained with a fluorogenic dye and imaged by microscopy. Data capture by digital camera and processing with publically available software (ImageJ) allows quantification. The effect of an antibiotic or drug on growth can be measured. A few rounds of division are required to see the effect of an antimicrobial agent.
Previously, we have used this technique to perform rapid antibiotic sensitivity testing in bacteria.[1] A similar approach has been applied to clinical isolates of yeasts (*Candida* species) and filamentous fungi (primarily *Trichophyton* species). For *Candida*, a result can be obtained with 4 to 7h culture depending on the antifungal agent. In 74 assays, in 65 cases the assignment by PAO (sensitive [S], resistant [R], intermediate [I]) was identical to the E-test.

Where differences occurred these were single category disagreements (i.e. never a mismatch of S against R). Testing clinical isolates of *Trichophyton* spp. was possible within 16 hr.
The method is simple, low cost and versatile and can be adapted to new species and antimicrobials. Additionally, the potential for PAO to be microengineered[2] suggests further applications in clinical microbiology can be developed.

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O077

The use of chromogenic media in medical mycology

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Purpose: Because of rising incidence of candidiasis, the high morbidity and mortality in immunosuppressed patients with systemic fungal infections and the variation in antifungal susceptibility of non-Candida albicans species, the need for rapid differentiation and identification of yeasts in mixed cultures is desirable. Several chromogenic media have been developed; on these media the yeast colonies have strongly contrasting colours produced by reactions of species-specific enzymes with proprietary chromogenic substrates.
The purpose of this study was to compare CandiSelect 4 (Bio-Rad, CS4) and Chromogenic Candida agar (Oxoid, CCA) with CHROMagar Candida (ITK Diagnostics B.V., CHROM).
Methods: 400 clinical samples (sputa, BAL, swabs of throat, nose, ear and mouth, mouthwashes) were cultured on CS4, CCA and CHROM, incubated at 30°C and daily examined, according to the manufacturers instructions, for 7 days. During examination special attention was paid to clear difference and intensity of the colours and the capability to suppress the growth of bacterias. For yeast identification the germ tube test and Auxacolor 2® were used. The results of CS4 and CCA were compared to CHROM.
Results:
Compared to CHROM:
- equal score on CS4 in 354 and on CCA in 350 cases
- lower score on CS4 in 16 and on CCA in 24 cases
- better score on CS4 in 25 cases and on CCA in 21 cases.

Findings on CS4 and CCA:
- colony colours specific for *Candida albicans* on CS4 and CCA were identified as *C. albicans*. Of all other colony colours no strain was identified as *C. albicans*.
- the examination of similar colours is subjective
- poor discrimination between light blue and light blue with turquoise centre on CS4 after an incubation time of 5-≥7 days
- sporadic or no growth of resistant gram-negative rods.

Findings on CS4:
- *C. albicans* colour is the clearest and most discriminating of all chromogenic media
- making difference between the colours of *Candida tropicalis* and *Candida krusei* is difficult
- the *C. tropicalis* colour is identified as *Saccharomyces cerevisiae* in 4% of all cases
- discriminating ability is lost when medium is exposed to light ≥8 hours.

Findings on CCA:
- the *C. tropicalis* colour is identified as *S. cerevisiae* in 5% of all cases
- yellow/beige colonies are difficult to observe
- no large discrimination between green and light blue colonies on CCA after an incubation time of 5-≥7 days.

Conclusions: CS4 has the widest differentiating ability and a discriminating colour for *Candida glabrata*. On CS4 and CCA the colours of *C. albicans* are unique to *C. albican*s, blue and green colony colours are undistinguishable after a long incubation time and the capability to suppress the growth of bacteria.

O078

Separation of species of *Fonsecaea* on the basis of genealogical concordance phylogenetic species recognition (GCPSR)

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A multilocus sequence typing (MLST**)** system based on sequence analysis of the ribosomal internal transcribed spacers (ITS), β-tubulin (BT2) and actin (ACT) regions was performed in 38 strains morphologically identified as *Fonsecaea* and originating from clinical and environmental sources in Central and South America, Africa, East Asia and Europe. Elongation factor 1-alpha (EF1) could not be amplified. Based on the sequences of ITS, strains of *Fonsecaea* were classified into three major clades: a group representing *Fonsecaea pedrosoi,* a group representing *Fonsecaea monophora*, and an intermediate group. The same grouping was found with ACT and BT2.

The two major, clinically relevant *Fonsecaea* species can be separated based on sequence data of the ITS, ACT and BT2 regions. Most environmental strains with *Fonsecaea*-like appearance belong to separate species, in part remote from the core of *Fonsecaea*.

O079

Efficacy of voriconazole and anidulafungin in combination in experimental invasive pulmonary aspergillosis in neutropenic rats

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Introduction: At present voriconazole is being used as drug of first choice in patients with invasive pulmonary aspergillosis (IPA). As in advanced stage of IPA, voriconazole is not always fully effective or tolerated, there is still a need to further improve the outcome of treatment. Improvement of the therapeutic efficacy of voriconazole could be achieved by adding another antifungal agent, such as the echinocandin anidulafungin to the treatment schedule. In the present study, we investigate in a model of aerogenic left-sided IPA in transiently neutropenic rats, developed in our laboratory, if combining voriconazole and anidulafungin in the treatment of advanced IPA would result in higher therapeutic outcome. After administration, voriconazole is metabolised by hepatic P450 isoenzymes and converted into a N-oxide which has no antifungal activity. When studying therapeutic efficacy of voriconazole in rodents one should take into account that this process develops faster than in humans, resulting in more rapid clearance of the antifungal agent. Therefore, for experimental studies in rodents compensation for the rapid metabolisation of voriconazole is necessary.
Methods: To achieve human pharmacokinetically equivalent dosages for voriconazole and anidulafungin in rats adjustment of the dosage regimen was explored. For voriconazole, administered intraperitoneally, an increment dosage schedule was applied and for anidulafungin, administered subcutaneously, an uploading dose was applied. AUCs at each dosage interval were determined. Toxic side effects if present of both treatment regimens were assessed by measuring the hepatic and renal functions. Therapeutic efficacy of voriconazole was determined after administration at 16h (early infection), 24h and 72h (advanced infection) after fungal inoculation. The effect of addition of anidulafungin to voriconazole was investigated in advanced stage of IPA (treatment start 72h). Parameters for therapeutic efficacy were rat survival, and quantitative fungal burden by determining the galactomannan (a fungal cell wall polysaccharide) concentration in lung and serum.
Results: By adjustment of the dosage regimen of voriconazole and anidulafungin AUC values similar as those in humans were achieved in our rats. In these dosage schedules both antifungal agents were well tolerated by the rats, without effects on renal and hepatic functions. Voriconazole showed excellent efficacy in early infections (100% survival). Voriconazole was less efficacious in advanced IPA, resulting in 50% rat survival, however a decrease of galactomannan concentrations in serum was still found in surviving animals. Anidulafungin administered in advanced IPA resulted only in 22% rat survival and no decrease in galactomannan concentrations in serum. Addition of anidulafungin to voriconazole did not significantly increase therapeutic efficacy of voriconazole alone in advanced IPA, resulting in 67% rat survival and a decrease in galactomannan concentration in serum.
Conclusion: Voriconazole and anidulafungin are therapeutically effective in the treatment of advanced stage IPA, combining both agents does not significantly improve therapeutic outcome.

O080

Invasive pulmonary aspergillosis as a presenting sign in a HIV-positive patient

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A 43-year-old man with a history of polycystic kidney disease presented at the emergency department with dyspnoea, vomiting, diarrhoea, hypotension, leukopenia ($0{,}4 \times 10^9$/L), thrombopenia (21×10^9/L), acute renal insufficiency and pulmonary infiltration on X-ray. With a presumed diagnosis of sepsis, he was admitted to the ICU where he was mechanically ventilated. Cultures of blood, urine and sputum were taken and intravenous therapy with ceftriaxon 2dd 1 gram and ciprofloxacin 2dd 400 mg was started. Because he had risk factors for HIV-infection a combined HIV-antigen/antibody test was performed. This test was positive. Direct immunofluorescence with monoclonal antibodies to *Pneumocystis jiroveci* on bronchoalveolar fluid was negative. Because of his low CD4 cell count (10×10^6/L) cotrimoxazol was started in a prophylactic dose of 480 mg daily. Blood cultures grew *Salmonella enteritidis* that was sensitive to ciprofloxacin but resistant to ceftriaxon, thus ceftriaxon was stopped and therapy with ciprofloxacin was continued. His leukocytes and thrombocytes returned to normal and his renal function improved, but his pulmonary function deteriorated and

ARDS was suspected. Cultures of sputum and bronchoalveolar fluid showed large quantities of Aspergillus fumigatus. Voriconazole 300 mg twice daily was added to the ciprofloxacin. Serum was taken for galactomannan detection that turned out to be very high 5,4 (serum/ratio ≥0,5 is positive). The patient gradually improved and two weeks later highly active antiretroviral therapy (HAART) consisting of lopinavir/ritonavir, abacavir and lamivudine was started. Drug levels were frequently monitored leading to dosing adjustments because of interactions between voriconazole and lopinavir/ritonavir. During follow-up the pulmonary HRCT-scan revealed multiple caverns, tree-in-bud phenomenon with peribronchial consolidation and nodules with a distinct air crescent sign further strengthening our diagnosis of pulmonary aspergillosis. During therapy galactomannan levels decreased and are now negative. Six months later HIV-RNA levels decreased to undetectable levels (<40 copies/ml), CD4 cells increased to $260x10^6$/L and the patient is doing well.

Conclusion: Invasive pulmonary aspergillosis (IPA) is most frequently found in patients who are severely immunocompromised and have a prolonged period of neutropenia. In HIV-positive patients invasive aspergillosis is rare and usually diagnosed at end-stage disease or postmortem. Morbidity and mortality due to invasive aspergillosis is usually high. In this case we present a successful recovery of IPA in a newly diagnosed HIV-patient who was treated with voriconazole and HAART.

O081

Environmental *Aspergillus fumigatus* isolates are resistant to triazoles

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Introduction: Recently the emergence of triazole-resistance was reported in clinical *Aspergillus fumigatus* isolates. It is unclear if azole resistance arises during therapy or that it is due to other causes of azole exposure. We investigated the possibility that azole-resistance is present in environmental *A. fumigatus* isolates.

Methods: Soil, leaves, seeds and compost samples were obtained from local plant nurseries and a local garden centre. Soil was also sampled in the surroundings of the Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands. In addition. *A. fumigatus* isolates from the hospital indoor environment present in our fungus culture collection were also tested for resistance. All samples were cultured on Sabouraud agar plates with and without itraconazole. Azole-resistant *A. fumigatus* isolates were matched with representative azole-sensitive control isolates. Phenotypic susceptibility profiles (CLSI reference method) and molecular resistance mechanisms (cyp51A gene) were determined as well as molecular strain identification. Genotypical analysis was performed to determine if the environmental isolates clustered with clinical isolates.

Results: *A. fumigatus* was cultured from 49 of 79 environmental samples, with 10 exhibiting an azole resistant phenotype. Resistant isolates were also cultured from soil from flower beds (6 isolates), seeds (1), compost from plant nursery (1) and compost from the garden centre (2). Five of 248 isolates from the hospital indoor environment were azole-resistant: patient rooms (3) and hospital water (2). A tandem repeat and a L98H substitution were present in 13 of the 15 resistant isolates. This resistance mechanism was previously described to be the dominant change in triazole-resistant clinical isolates. Genotypical analysis showed that all isolates are unique but genetically clustered, 13 of the 15 environmental resistant isolates were clustered together in one clade. These environmental isolates also showed clustering with azole resistant clinical isolates.

Conclusion: Azole-resistant *A. fumigatus* is present in the environment. Since azole-resistant *A. fumigatus* was cultured only from the environment that had been manipulated by humans, a link with exposure to azole fungicides seems plausible. Molecular analysis of the azole resistant environmental isolates may suggest that patients acquire azole resistant aspergillus disease by inhaling resistant conidia from their environment.

O082

A reproducible system for generating wall-less (L-form) bacteria: implications for the evolution of cell proliferation

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The cell wall is an essential structure for most bacteria. It is a tough outer shell that protects the cell from damage and osmotic lysis, and it is the target of our best antibiotics. L-form strains are wall-deficient derivatives of common bacteria that have been studied for decades. However, they are difficult to generate and typically require passage for many generations on osmotically protective media, in the presence of antibiotics that kill walled forms. Despite their potential importance for understanding antibiotic resistance and pathogenesis, little is known about the generation of L-forms, their genetic basis or means of propagation. We have now developed a controlled system for generating L-forms in the highly tractable model bacterium *Bacillus subtilis*. We show that we can delete genes essential for synthesis of the major structures of the cell wall, supporting the idea that L-form bacteria do not have residual wall synthesis. We also show

that propagation of these cells does not require the normal FtsZ-dependent division machine. Their remarkable mode of proliferation has interesting implications for possible modes of proliferation of early forms of cellular life on earth.

O085

Viral culture on tAN versus CaCo2 and HT-29 cell lines

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Introduction: At the Laboratory of the Municipial Health Service of Amsterdam viral cultures of faeces are routinely performed on tAN (tertiar monkey kidney cells), RD-C (human rhabdomyosarcoma cells) and HFL (human fetal lungfibroblasts) cells and cultures of respiratory materials on tAN, HFL and R-HELA (rhinovirus sensitised Henriette Lacks) cells. The production of tAN is uncertain and the quality of these cells is unstable. Therefore the performance of 2 other cellines, CaCo2 (human Caucasian colon adenocarcinoma) and HT-29 (human colon adenocarcinoma), was compared with the performance of tAN in the routine setting.

Methods: The study is ongoing, results on faecal samples are presented.

All 1251 faeces samples sent to our laboratory for viral culture from June 2005 till September 2007 were analysed on the routine cell lines as well as CaCo2 and HT-29 cell lines. Cell lines were judged on CPE two times per week. If CPE was observed confirmation was performed with monoclonal antibodies and typing. Performance of tAN was compared to CaCo2 and HT-29 for the isolation of adenovirus, parechovirus, coxsackie B and coxsackie A/echovirus.

Results: In 862 samples (69%) no virus was isolated. 107 (9%) adenoviruses were isolated, 55% on tAN, 47% on CaCo2, 94% on HT-29 and 96% on CaCo2/HT-29. 136 echo/coxsackie A viruses were isolated, 71% on tAN, 87% on CaCo2, 83% on HT-29 and 92% on CaCo2/HT-29. 41 coxsackie B viruses were isolated, 90% on tAN, 83% on CaCo2, 95% on HT-29 and 95% on CaCo2/HT-29. 38 parecho viruses were isolated, 55% on tAN, 42% on CaCo2, 50% on HT-29 and 82% on CaCo2/HT-29.

Conclusion: For viral culture on faeces the combination of CaCo2 and HT-29 cell lines is superior to tAN for all virus groups studied. These cell lines seem therefore promising in routine diagnosis.

O086

Rapid detection of dermatophytes in clinical specimens using an internally controlled duplex realtime PCR

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Dermatophytes are fungi able to superficially infect skin, hair and nails. Three genera can be distinguished, i.e. *Trichophyton*, *Microsporum* and *Epidermophyton*, of which *Trichophyton rubrum* and *Trichophyton interdigitale* are the most frequently occuring species. Conventional diagnostics is performed using culture and fluorescence microscopy, which can take up to several weeks. This study aimed to develop a rapid and sensitive realtime PCR assay to detect all dermatophytes and to subtype the most commonly species in clinical specimens.

Unique pan-dermatophyte primers and probe were selected targeting the 18S-28S ribosomal DNA. Using these primers and Taqman probe chemistry a realtime PCR was optimised using an ABI7000 platform. This assay was internally controlled with spiked Phocine Herpesvirus. Sensitivities and specificities were determined of this duplex realtime PCR, resulting in a detection limit of 2-10 genome equivalents of *T. rubrum*. In addition, a separate duplex PCR was developed enabling the identification of *T. rubrum* and *T. interdigitale* using differently labeled Taqman probes. Finally, both duplex PCRs were clinically evaluated in a prospectively collected group of nails and skin samples (n=100). Results were compared to conventional culture and microscopy. Most common species detected were *T. rubrum* and *T. interdigitale*. In addition, PCR detected more dermatophyte positive samples as compared to culture and microscopy (55% vs. 25% and 40%). All culture positive samples were also found to be positive using PCR, and identical species were identified.

In conclusion, a sensitive and rapid Taqman based duplex PCR was developed enabling the detection of all dermatophytes in clinical specimens. At present the diagnostic value of this PCR is being evaluated either as a prescreening or replacement of culture.

O087

Validation of M.I.C.Evaluator strips following ISO guidelines

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Introduction: Oxoid has developed a new system for determining the MIC. These M.I.C. Evaluators (MICE strips) provide a gradient of antibiotic stabilised on a polymer strip covering 15 doubling dilutions. The aim of the study was to evaluate these MICE strips vs the MIC reference method (ISO 20776-1) according to ISO guidelines (ISO 20776-2).

Methods: Following the ISO protocol, during daily clinical laboratory work 300 consecutive gram-negative strains (fresh isolates) were collected. Identification was performed following standard procedures. From each strain, the MICs

were determined following ISO guidelines (ISO 20776-1) and instructions of the MICE manufacturer, respectively. For the MICE, an inoculum of 0.5 McFarland was streaked on a Mueller-Hinton agar plate. The MICE strips were put on the plate and incubated for 16-20h. The MIC values were read following instruction by two independent lab technicians. The following MICE strips were evaluated: gentamicin 256-0.016 mg/L (Ge 256), gentamicin 1024-0.064 mg/L GE024), amoxicillin 256-0.016 mg/L (AM), cefotaxime 32-0.002 mg/L (Cef32), cefotaxime 256-0.016 mg/L (Cef256), levofloxacin 32-0.002 mg/L (LE), imipenem 32-0.002 mg/L (IM), ciprofloxacin 32-0.002 mg/L (CI), amoxicillin/clavulanic acid 256 – 0.016 mg/L (AC). For the interpretation of the MIC and MICE the EUCAST breakpoints were used. Discrepancy analysis was performed following the ISO guideline.

Results: The isolates collected belonged to the following species *Citrobacter* species (n=15), *Enterobacter* spp (n=35), *Escherichia coli* (n=80), *Hafnia alveii* (n=1), *Klebsiella* spp (n=84), *Morganella morganii* (n=10), *Pantoea agglomerans* (n=1), *Proteus* species (n=67), *Providencia rettgeri* (n=1), *Serratia marcescens* (n=6). 63% of the strains were susceptible for the different antimicrobial agents. The category agreement after discrepancy analysis was:
GE256 99%, GE1024 99%, AM 96%, cef32 99%, cef256 99%, LE 98%, IM 99%, CI 98%
and AC 89% (caused by a minor discrepancy of 11%). Very major discrepancy was found only for CI 1%, Cef32 0.7% and Cef 256 0.3%; and none for the other antimicrobial agents. There was no major discrepancy was for any antimicrobial agents.

Conclusion: The new MICE strips to determine MICs demonstrated an excellent a performance for the interpretive category determinations of SIR of the antimicrobial agents tested.

O088

A practical aid for detection of extended-spectrum beta-lactamases in *Enterobacteriaceae*

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Introduction: The detection of Extended-Spectrum Beta-Lactamases (ESBLs) is of special importance, because this resistance genes present serious implications for therapy. Currently, genotypic characterisation of ESBL genes is considered the gold standard for ESBL identification. However, the detection of ESBL genes with PCR and sequencing is complex and does not provide information about their expression. In this study, we evaluated the performance of the combination of the molecular hyplex® ESBL ID (h-ES-ID) test and the ESBL screening agar (ESA) in the identification of ESBL-producing *Enterobacteriaceae* isolates.

Methods: The h-ES-ID is a multiplex-PCR-ELISA for the direct detection of beta-lactamase-producing bacteria. In the presence of relevant DNA, specific amplification products of the blaTEM, bla SHV, bla CTX-M (except CTX-M-8) and bla OXA-1 group are synthesised and subsequently visualised with the h-ES-ID hybridisation modules. The ESA is a MacConkey agar with third generation cephalosporins, AmpC beta-lactamase inhibitor and vancomycin. We examined a total of 149 clinical Enterobacteriaceae isolates, 61 of them had been previously characterised as ESBL-producers with PCR and sequencing. The other 88 isolates were ESBL negative.

Results: The sensitivity, specificity and duration of test of h-ES-ID and ESA were 98% (60/61), 57% (50/88), 3 hours, 100% (61/61), 93% (82/88) and 18 hours respectively. With the combination of h-ES-ID and ESA, all the ESBL-positive and ESBL-negative isolates were detected correctly. CTX-M ESBL producers were detected within 3 hours with h-ES-ID. The low specificity of h-ES-ID was mainly due to TEM-1 producers among *Escherichia coli* isolates. These isolates were correctly identified with the ESA..

Conclusion: The combination of h-ES-ID and ESA is a quick, effective, and easy to use method for ESBL detection, especially of CTX-M ESBLs, which are the most prevalent ESBLs worldwide. This combination of tests provides useful early therapeutic guidance by the rapid identification of ESBL-positive isolates.

O089

Association between group A beta-haemolytic *Streptococci* and vulvovaginitis in adult women: a case control study

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Introduction: Vulvovaginitis including fluor vaginalis accounts for approximately 250,000 visits in the Netherlands to general practitioners (GPs) each year. Guidelines for managing vaginal discharge mention *Candida albicans*, *Trichomonas vaginalis*, bacterial vaginosis, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* as the usual causes. Culturing for other agents is not recommended, unless symptoms recur or persist despite treatment. Lately, we noted a high isolation rate of non-group B (A, C, F, G) beta-haemolytic *Streptococci* from fluor vaginalis samples from women with recurrent vulvovaginitis in our region. Group A *Streptococci* are known as a cause of vulvovaginitis in children, but evidence of infection in adult women is limited to a few

case reports and two methodologically flawed studies lacking well-defined control groups. The significance of group C, F and G *Streptococci* in vaginal flora is unclear. To investigate the association between non-group B beta-haemolytic *Streptococci* and vulvovaginitis in adult women, we conducted a case control study.

Methods: Cases (n=1010) were women consulting their GP with an abnormal non-bloody vaginal discharge with or without itch, irritation, redness or pain from whom a fluor sample was cultured. Controls (n=206) were asymptomatic women consulting their GP to have a smear taken for the cervical cancer screening programme, who consented to having a vaginal swab taken. Additionally, asymptomatic volunteers among hospital personnel submitted a self-obtained vaginal swab. Ages of cases as well as controls ranged from 30 to 60 years.

Results: Non-group B *Streptococci* were isolated from 86 (8.5%) cases and from 6 (2.9%) controls (OR 3.1, 95% CI 1.3-7.2; p<0.01). The significant difference was caused by group A *Streptococci*, that were isolated from 49 (4.9%) cases and not from any of the controls (p<0.01). Isolation rates of group C, F and G *Streptococci* from cases were low (1.2, 0.1 and 2.4% respectively) and did not differ statistically from those from controls (1.0, 0.0 and 1.9% respectively).

Conclusion: Group A beta-haemolytic *Streptococci* are associated with persistent vaginal discharge in women aged between 30 and 60 and should be diagnosed and reported as a pathogen in vulvovaginitis. The role of other non-group B *Streptococci* requires more study because of the low numbers isolated. For adequate management of vaginal discharge culturing is necessary if initial treatment fails. Guidelines should be adapted to this effect.

O090

Caenorhabditis elegans as a model system to study (anti-retroviral) drug-induced mitochondrial dysfunction

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Introduction: Mitochondrial dysfunction is the underlying cause of a wide range of human diseases. In recent years, it has become increasingly clear that mitochondrial dysfunction can also be a direct consequence of adverse effects of therapeutic drugs. The best studied example comprises the antiviral drugs used to treat HIV-1 infection, which have been shown to inhibit the mitochondrial DNA polymerase and deplete cells of mitochondrial DNA (mtDNA), thus initiating adverse effects. Many studies have addressed mitochondrial toxicity as a cause of these side effects, but the exact mechanism by which it is caused remains unknown. In my project, *Caenorhabditis elegans* is used as a model organism to study these side effects. The project aims at i) dissecting the molecular mechanism underlying drug-induced mitochondrial dysfunction, ii) identifying markers which can predict the onset of mitochondrial dysfunction at an early stage and iii) identify compounds that can alleviate mitochondrial dysfunction.

Methods: To quantify mitochondrial DNA, real-time PCR using the applied Biosystems 7300 real-time PCR system is used. Mitochondrial morphology is studied in a *C. elegans* strain with muscle specific expression of mitochondrially localised GFP [1] using fluorescent microscopy (Axiovert 40, Carl Zeiss). Quinon levels are measured by HPLC detection on a reversed phase Lichrosorb (Chrompack, Bergen op Zoom, the Netherlands) 10 RP 18 column (size 4.6 mm, internal diameter, X 250 mm) which is calibrated with a 1:1 methanol/ethanol solution. Fractions were detected using a LKB 2151 variable wavelength monitor.

Results: Several assays have been set up to be able to study the effect(s) of a number of anti-retroviral drugs on different aspects of mitochondrial function, in the nematode *C. elegans*. Using quantitative PCR, we have been able to show that there is a decline in mtDNA copies in *C. elegans* when they are cultured in the presence of some anti-retroviral drugs, in a concentration-dependent manner. Using fluorescence microscopy, we have been able to show that there is a disruption in the mitochondrial network, when worms are cultured in the presence of anti-retroviral drugs. Respiratory chain function has been studied by quinon analysis. Finally, early results indicate that treatment of worms with antiretroviral drugs can result in a decreased lifespan. Interestingly, the observed effects are not necessarily coupled (for instance mtDNA depletion does not have to correlate with changed mitochondrial morphology and vice versa).

Conclusions: In *C. elegans* anti-retroviral drug treatment results in mitochondrial dysfunction, indicated by:

- mtDNA depletion
- aberrant mitochondrial morphology
- decreased lifespan
- a shift in the ratio of reduced:oxidised ubiquinon.
- Several of the observed effects are similar to the effects observed in patients on anti-retroviral therapy, indicating that *C. elegans* is a suitable model organism to study drug induced mitochondrial dysfunction.

Reference

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O093

Molecular diagnosis of avian influenza A viruses

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Wild birds are the reservoir for low pathogenic avian influenza (LPAI) viruses in nature. In contrast to LPAI viruses, highly pathogenic avian influenza (HPAI) viruses of the H5 and H7 subtypes constitute a continuous concern from the public, veterinary and wildlife health perspectives. The emergence of HPAI viruses is primarily the result of large-scale poultry husbandry. The last decade has seen a marked increase in the size and frequency of HPAI outbreaks in poultry all over the world. The ongoing HPAI H5N1 outbreak that started around 1997 is unprecedented in scale and geographical spread and has resulted in virus transmission to a wide variety of mammalian species including humans, and multiple introductions in wild birds.
Classical diagnosis of influenza A viruses relies on virus culture and subsequent characterisation of the influenza A virus isolate by serology. However, the development of molecular diagnostic tools allows more rapid detection of influenza A viruses, and more safe detection in the case of HPAI viruses with a broad host-range. Sophisticated real-time reverse transcriptase-PCR (RRT-PCR) systems have become widely in use in diagnostic laboratories, including the diagnostic centre of the department of Virology at Erasmus MC. The detection of any influenza A virus from any host, including Asian lineage H5N1 viruses is based on detection of highly conserved sequences in the matrix (M) gene segment. In addition to the generic M RRT-PCR assay, hemagglutinin (HA) subtype-specific RRT-PCR assays were designed for the human H1 and H3 and the avian H5 and H7 subtypes.
Conclusion: We conclude that RRT-PCR assays are useful for influenza A virus detection in humans using the generic M-assay in combination with the subtype-specific H1, H3, and H5 assays, or for surveillance in birds using the generic M RRT-PCR in combination with the subtype specific H5 and H7 assays. Because both human and avian influenza A viruses change rapidly, RRT-PCR assays may need to be updated frequently.

O098

Filovirus haemorrhagic fever outbreaks: much ado about nothing?

G. van der Groen

Marburg virus (MARV) and Ebola virus (EBOV) are members of the Filoviridae which cause outbreaks of severe hemorrhagic fever (HF), mainly in Africa.[1]
MARV was discovered first in Marburg an der Lahn, Germany in 1967 and EBOV in Sudan and in the Democratic Republic Congo (DRC) in 1976. DRC being so far the country with the largest number of HF cases.
The damage on the local level of some of the filovirus outbreaks has been devastating at times, but was marginal on the international level, despite the considerable media attention these outbreaks received. Since 1967, approximately 2160 cases (Case fatality rate (CFR) 71.8%) due to MARV and EBOV, have been diagnosed, compared with 40 million AIDS cases (CFR 100%) due to HIV-1 since 1981!
In 2007, MARV nucleic acid was found for the first time in a limited number of bats, captured in a mine in northeastern DRC, which was associated with a HF outbreak in 1998- 2000. EBOV nucleic acid was found in 2005 in Gabon. In both cases, it was impossible to isolate live virus.
Combine the high CFR (71.8%) of filovirus infections in Africa with the airborne transmission of EBOV Reston in a quarantine facility for monkeys in Reston, near Washington DC, USA, in 1989, and you get the kind of horror scenario that fuels the interest of media, public and bio- terrorists!
According to Ken Alibek,[2] MARV and EBOV have been weaponised by the Russians during the cold war period between them and the USA.
We should remain vigilant since it is not excluded that new filoviruses still hide in nature and/or in laboratories, which combines the pathogenecity of EBOVZaire with the aerogenic spread of EBOV Reston Virus X?[3]

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O100

The general public's beliefs about methicillin resistant *Staphylococcus aureus*: a mental models approach

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is becoming an increasing public health threat. Risk communication strategies should create public awareness in order to prevent misconceptions leading

to non-compliance with infection control measures. Therefore, risk communication should be matched to the general public's beliefs about MRSA. These beliefs were determined in this study.

Methods: The mental models approach was applied. Based on the literature, a conceptual model was created which was used as an interview scheme to elicit (in)correct public beliefs (n=17). These beliefs served as items in a a close-ended questionnaire (n=239) in order to estimate the prevalence of these beliefs among the general public.

Results: Although the majority of the general public (62%) had heard of MRSA via television (90%) and/ or newspaper (31%), great misconceptions exist. e.g., 26% of the public thought MRSA is a muscular disease, and 9% viewed MRSA as a synonym for repetitive strain injury.

After revealing the respondents that MRSA is also known as the hospital bacterium, they appeared to hold correct beliefs concerning risk factors and consequences of MRSA: 72% believed that MRSA may cause infections, 59% was convinced hospitalised MRSA-carriers are treated in isolation, that a weakened immune system increases one's risk of getting MRSA-infections (75%), and 62% was aware that MRSA can also be found outside the hospital. Respondents were less aware about prevention, reservoir, treatment, and transmission of MRSA: 70% incorrectly assumed vaccination would prevent MRSA-colonisation. While 39% thought MRSA can be found in the blood, only 16% assumed MRSA lives on the skin, and 64% of the sample was unaware that most antibiotics are ineffective against MRSA. 7% supposed MRSA is transmitted by insects, and 56% did not know whether MRSA could be found at cattle or not.

47% of the respondents believed that MRSA is a serious risk for society, although MRSA was not experienced as a personal health threat.

Conclusions:

1. Although the Dutch general public recognises the well-known risk factors and consequences of MRSA and is slightly aware of its threat to society, many misconceptions exist.
2. Important facts, like MRSA-prevalence among cattle and the presence of MRSA on the skin, are unfamiliar to the general public.
3. Risk communication should show attention to these misconceptions in order to raise public awareness and prevent non-compliance with control measures.

O102

Effect of energy depletion on the sensitivity of *Candida albicans* for antimicrobial peptides

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Saliva contains several peptides with candidacidal activity, including histatins, defensins and cathelicidins. Inhibitors of the energy metabolism, such as sodium azide and valinomycin, increase the yeast cells resistance against the killing action of these peptides. In this study the Histatin 5-mediated killing of the opportunistic yeast *Candida albicans* was used as a model system to comprehensively investigate the molecular basis underlying this phenomenon. Using confocal and electron microscopy it was demonstrated that the energy poison azide reversibly blocked the entry of Histatin 5 at the level of the yeast's cell wall. Azide treatment hardly induced depolarisation of the yeast cell's membrane potential, excluding it as a cause of the lowered sensitivity. In contrast, the diminished sensitivity to Histatin 5 of energy-depleted *C. albicans* was restored by increasing the fluidity of the membrane using the membrane fluidiser benzyl alcohol. Furthermore, rigidification of the membrane by incubation at low temperature, or in the presence of the membrane rigidifyer DMSO, increased the resistance against Histatin 5, while not affecting the cell's energy charge. In line, azide induced alterations in the physical state of the interior of the lipid bilayer. These data demonstrate that changes in the physical state of the membrane underly the increased resistance to antimicrobial peptides.

O103

Stress responses in *Streptococcus mutans*

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In the oral cavity a balanced physiological response is essential for the cariogenic bacterium *Streptococcus mutans*, to survive various types of external challenges. In this presentation, the role of two genes, vicK and clpP, both functional in the adaptive and stress response of *S. mutans* is discussed. VicK is a sensor protein and part of a two-component regulatory system. ClpP is a serine protease, functional in the turnover of cellular proteins. By constructing vicK and clpP promoter Green Fluorescent Protein reporter strains, we were able to show that vicK is triggered by oxidative stress and that ClpP is involved in the 'general' stress response. These findings were further substantiated by comparing the physiology of vicK and clpP mutant strains with that of the wild type strain. The vicK mutant strain was shown to be more sensitive to oxidative stress. The clpP mutant strain was more sensitive all of the stresses tested. Moreover, we found an increased resistance to toxic levels of hydrogen peroxide and chlorhexidine after pre-incubation with sub-lethal

levels of the corresponding compounds in the wild type strain but not in the clpP knockout. These experiments indicate that the clpP gene also involved in the adaptive response of *S. mutans*.

O104

Transmission of *Tannerella forsythensis* in related families

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Background: The periodontal pathogen *Tannerella forsythensis* is strongly associated with aggressive forms of periodontitis. Carriership in periodontal healthy subjects reaches levels of almost 48% in the Netherlands. The genetic variation of this pathogen is, however, unknown and there are only few data upon associations between clinical status and *T. forsythensis*-genotypes.
Objectives: To obtain more insight in the clonality and transmission of *T. forsythensis*, we developed an amplified fragment length polymorphism analysis and applied them on isolates from related inhibitants (spouses and/or siblings of spouses) of a tea estate on Western Java, Indonesia.
Methods: An amplified fragment length polymorphism technique (AFLP), based on restriction enzymes MseI and PstI, was developed to observe whole-genome variation of *T. forsythensis*. The AFLP method was optimised to observe sufficient variation and validated on isolates from twenty-seven individual non-linked subjects with periodontitis. In addition, intra- and interexperimental variation was determined. 178 *T. forsythensis* isolates from 72 subjects (one to four isolates per subject) according to 39 married couples were used to determine transmission of the pathogen between couples and/or siblings. These couples were clustered into 17 family trees.
Results: The intra-isolate homology was >96% when a single strain was processed five times in a single experiment. This intra-isolate homology between independent experiments was 78%. Taking this into account, we found that in only 6 out of 72 subjects two AFLP-genotypes were observed. One of the subjects was carrying two genotypes. Each genotype was identical in two separate non-related subjects.
Conclusions: AFLP is a valuable tool to determine clonality of *T. forsythensis* within and between healthy subjects. In an Indonesian population, only 8.3% of the subjects carried more than one genotype. Transmission of *T. forsythensis* between spouses or siblings was not observed. Only one subject carried two genotypes that could be recovered in two different unrelated subjects.

O106

Modulation of innate and adaptive immune responses by C3d binding molecules of *Staphylococcus aureus*

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Introduction: Extracellular fibrinogen binding protein (Efb) and extracellular complement binding protein (Ecb) of *Staphylococcus aureus* are small excreted complement modulators that specifically bind to the C3d part of C3. Thereby, Efb and Ecb target C3b containing convertases and thus block C3b deposition via the alternative pathway (AP) and C5a/C5b-9 formation via all pathways. Recently, we demonstrated that Ecb is a potent complement inhibitor since it completely inhibits neutrophil influx *in vivo*. We are currently preparing efb and ecb knock-out strains to study the role of these proteins in *S. aureus* pathogenesis *in vivo*. Because C3d plays and essential role in the adaptive immune response by stimulating antigen presentation via binding to complement receptor 2 (CR2) on antigen presenting cells, we hypothesise that Efb and Ecb could also modulate adaptive immunity.
Methods: Efb and Ecb were cloned and expressed in *Escherichia coli* as His-tagged proteins and purified by nickel affinity chromatography. B-cells were incubated with human C3d in the presence of Efb/Ecb and C3d binding was detected using anti-C3d antibodies and flow cytometry.
Results: Efb, Efb-C (the C3d binding part of Efb) and Ecb completely block binding of C3d to B-cells, indicating that they also modulate adaptive immune responses. Inhibition was detected on several B-cell lines using several antibodies. Efb and Efb-C both inhibit the C3d binding to B-cells which indicates that the C3d binding site of Efb is responsible for the inhibition. Future studies will be performed to study whether Efb and Ecb also inhibit B-cell proliferation.
Conclusion: Efb and Ecb inhibit binding of C3d to CR2 on B-cells. Future investigations will include B-cell proliferation assays and Biacore studies with purified CR2.

O107

Proteome characterization of respiratory virus-infected host cells by 2-D DIGE and mass spectrometry

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Respiratory virus infections are among the primary causes of morbidity and mortality in humans and animals. Especially infants and young children as well as the elderly are very susceptible to severe and potentially

lethal infection with respiratory viruses. Influenza virus, respiratory syncytial virus, parainfluenza and human metapneumovirus are major causes of severe respiratory illness in humans. Only against influenza classical vaccines are available but these need to be improved. In this study we aim to gain detailed insight into the molecular pathogenesis of respiratory virus infections by studying the protein expression profiles in infected lung epithelial cells.

Type II alveolar A549 human epithelial cells were infected *in vitro* with a set of respiratory viruses (RSV, hMPV, PIV and Measles virus) using both live and UV-inactivated virus particles. Cells were harvested at different time points after infection and processed for proteomics analysis. Protein expression in these samples was analysed by 2-dimensional difference gel electrophoresis (2-D DIGE), a technique that is based upon the labeling of samples with 3 different fluorescent CyDyes allowing the analysis of 3 different samples on a single 2-D gel. Using special software, protein expression in the different samples was quantitatively analysed. Protein expression in samples derived from respiratory virus infected cells was compared to mock infected cells to define protein spots that are differentially expressed due to infection. The use of live and UV-inactivated virus particles allowed differentiation between actively induced changes in protein expression by the viruses and passively induced changes due to virus adherence and/or uptake.

A total of 33 2-D gels were scanned and analysed resulting in the detection of 1890 protein spots per gel. Several comparisons and statistical analyses were performed to identify protein spots that were differentially expressed due to respiratory virus infections in these A549 cells. Per virus and per timepoint 5-22 spots showed at least 2-fold, statistically significant altered expression of which on average 65% showed down-regulation and 35% up-regulation compared to mock infected cells. In addition, RSV and hMPV infected cells showed more induced changes in protein expression than the PIV and Measles virus infected cells. In total 286 spots were found to be of interest and were picked from a preparative 2-D gel and were processed for protein identification by mass spectrometry. We were able to identify the proteins in 246 spots resulting in the identification of 160 different gene products of which most are involved in cell structure, biosynthesis, stress, protein modification, metabolism and transport suggesting the involvement of these proteins and corresponding cellular pathways in respiratory virus infection of A549 cells.

By proteomic analysis we have monitored protein expression levels induced by *in vitro* respiratory virus infection of A549 cells at different time points after infection to get more insight into the interaction between the viruses and the host cells and to explore differences in induced protein expression profiles between different respiratory viruses. A better understanding of these events is required for the development of new strategies for treatment and prevention of these infectious diseases.

O108

RFLP association of the *Campylobacter jejuni* genes Cj1522 and Cj1523 with the Guillain Barré syndrome. Do they function as a toxin?

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Introduction: *Campylobacter jejuni* is known to be associated with the Guillain Barré syndrome (GBS). The molecular mimicry hypothesis between the peripheral nerve and *C. jejuni* lipo-oligosaccharide (LOS) is currently the most important explanation for this post-infectious complication. The mechanism behind triggering the molecular mimicry and the found antibody isotype switch are until now unsolved. A toxin can possibly be involved in generating this isotype switch. Therefore we searched the *Campylobacter* genome for genes with toxin homology. The hypothetical gene Cj1523 in particular, showed homology with different toxins, i.e. Botulinum, Edema factor and Vacuolating toxin A. This homology became stronger when Cj1522 and Cj1523 were combined in the protein blast analysis. Cj1523 is found to be part of a gene cluster with an unknown function. We hypothesise that Cj1523 is functioning as a toxin, possibly influencing cellular processes important for survival of *C. jejuni* in the host.

Methods: For blast and fingerprinting analysis at protein level with Cj1523 the Swissprot database was used in Expasy. The PCR-RFLP technique was used to analyse Cj1522 and Cj1523 by testing 26 GBS and 154 enteritis associated strains matched on age and gender. The Cj1523 gene from 13 GBS and 19 enteritis associated strains was sequenced. A knock out (KO) in Cj1523 was created in 3 GBS associated strains and the 11168 reference strain by knock out mutagenesis. The invasion capacity of wild type and 1523KO strains was tested using the Caco2 cell line. For cytotoxicity measurement of wild type and 1523KO strains the LDH and neutral red assays were used. A growth rate experiment was setup to test possible differences between the wild type and 1523KO strains.

Results: Blast and fingerprint analysis showed homology with different toxin motifs for Cj1523. PCR-RFLP analysis resulted in 5 RFLP types for Cj1522 and 3 RFLP types for Cj1523. RFLP type 1 of Cj1522 is associated with GBS ($p=0.017$). RFLP type 1 of Cj1523 shows a trend towards an

association with GBS (p=0.069). RFLP type 1 of Cj1522 and Cj1523 together are strongly associated with GBS (p=0.001). Sequencing and alignment of Cj1523 showed clustering of 7 out of the 13 GBS associated strains. No differences in invasion capacity between wild type and 1523KO strains were found which was also true for the growth rate experiment. The LDH and neutral red assay showed a clear difference in generating cell damage, between wild type and 1523KO after overnight incubation on different cell lines.

Conclusion: Polymorphisms in the genes Cj1522 and Cj1523, sequencing and alignment confirmed an association with GBS. No differences in invasion between wild type and 1523KO were found, suggesting that Cj1523 is not involved in the invasion process of *C. jejuni*. The differences in cell damage caused at different cell lines between wild type and 1523KO suggests a toxic function for the Cj1523 gene. Elucidating the function of this gene (cluster) may contribute in better understanding the pathogenic processes underlying the Guillain Barré syndrome.

O109

Population structure assessment of *Streptococcus pneumoniae* strains isolated from patients with invasive disease in the pre-vaccination era using MLVA and sequence based surrogate serotyping

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The introduction of nationwide pneumococcal vaccination may lead to the selection of nonvaccine serotypes of the pneumococcus. Apart from this serotype, replacement new variants may emerge that have expanded their genetic repertoire through recombination. Such changes may have considerable consequences for future vaccination strategies. To monitor alterations in the pneumococcal population simple and reliable tools for characterisation are required. The assessment of the serotypes of pneumococcal strains is necessary to monitor serotype replacement. Analysis of the genetic make up of circulating strains is required to monitor for the emergence of variants evolved through genetic recombination and leading to phenomena such as capsule switching.

We developed a surrogate serotyping assay in which we determine the partial sequence of the protein-tyrosine phosphatase gene (wzh). The wzh gene is one of the regulatory genes of the locus responsible for the synthesis and export of capsular polysaccharides. This method is rapid and specific and enables to identify most of the 90 serotypes of the pneumococcus.

To determine the genetic background of pneumococcal strains we designed a MLVA (Multiple-Locus Variable number tandem repeats Analysis) based on 8 BOX elements. The 8 BOX loci are amplified in two multiplex PCRs and the fluorescently labeled fragments are sised on an automated DNA sequencer to accurately determine the number of repeats present per BOX element. The composite of the number of repeats of the BOX loci make up a numerical profile that is used for identification and clustering.

We used MLVA and wzh-based sequence typing to create a snapshot of the Dutch pneumococcal population causing invasive disease before the introduction of the pneumococcal conjugate vaccine in the Netherlands in 2006. More than 1200 clinical isolates collected and serotyped by the Netherlands Reference Laboratory for Bacterial Meningitis were included in the study. This collection represents approximately 25% of all cases from Dutch patients with invasive pneumococcal disease in the Netherlands in 2004 and 2005.

MLVA demonstrated that strains belonging to some serotypes (e.g. 1 and 4) had a relatively high genetic diversity whilst other serotypes (e.g. 9V) had a very homogeneous genetic background. The wzh-base sequence typing was successful in discriminating most serosubtypes. However, some serosubtypes such as 18B and 18C could not be discriminated using this method.

MLVA and wzh-based sequence typing appear to be valuable tools to determine the population structure of *S. pneumoniae* strains and will be used to monitor the effects of pneumococcal vaccination in the Netherlands.

O110

Transcriptome profiles of children suffering from meningococcal sepsis

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Objective: Meningococcal sepsis remains an important cause of childhood morbidity and mortality. It is widely appreciated that it is a complex disease, but most studies only assess the kinetics of a limited number of molecules. We conducted a microarray-based transcriptome study to analyse the kinetics of RNA expression during the course of meningococcal sepsis.

Patients and methods: In this prospective case-control study, six children with (suspected) meningococcal sepsis and age, sex and ethnically matched controls were included. Blood was drawn for RNA isolation from lymphocytes, monocytes and granulocytes, as well as from whole blood on admission to the PICU and during the course of the disease. Affymetrix microarray technology was used to assess RNA expression profiles.

Results: On admission LCN2, LTF and IL1R2 expression were most increased (48-, 40- and 18-fold, respectively) when compared to controls, while KLRB1 and MME expression was decreased (8.5- and 7.4-fold, respectively). Expression profiles in lymfocytes were mostly decreased when compared to controls, while expression in monocytes, and whole blood overall showed increased expression. Besides expected differential expression in pro- and anti-inflammatory pathways, we observed increased expression of pathways involved in oxidative stress response, insulin receptor pathway, apoptosis, and protein ubiquitination pathway. The expression patterns differed significantly between the cellular subtypes.

Conclusion: RNA expression patterns revealed a complex multiple pathway involvement in the pathogenesis of meningococcal sepsis with differential expression between leukocyte subtypes.

O111

The distinct epidemiology of CA-MRSA versus HA-MRSA may be explained by rRNA operon copy number

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Background: CA-MRSA are genetically and epidemiologically different from HA-MRSA.

The mechanism behind the enhanced fitness of CA-MRSA in the community is largely unknown. In *Escherichia coli*, variation in rRNA operon copy number affects fitness. Our aim is to study whether variation in rRNA operon copy number explains the distinct epidemiology of CA-MRSA versus HA-MRSA.

Methods: A total of 304 *Staphylococcus aureus* belonged to at least 70 different MLST types. The number of rRNA copies was determined by a copy-number specific PCR, real-time PCR and southern blotting. Growth rates were determined in an automated OD420-580 nm reader using different media.

Results: Eleven out of 165 MSSA isolates and 66 out of 139 MRSA isolates contained 5 instead of 6 rRNA copies (Chi-Square Test, p<0.0001, OR: 0.079, 95% CI: 0.039-0.159). Of the 11 MSSA isolates that contained 5 copies, 5 had a unique MLST type (single locus variants). Furthermore, within the MRSA cluster, 47 out of 69 HA-MRSA and 14 out of 48 CA-MRSA had 5 instead of 6 copies (Chi-Square Test, p<0.0001, OR: 5.2, 95% CI: 2.3-11.6). *In vitro* growth rates of MSSA and MRSA with 6 copies were clearly enhanced as compared to isolates that contained 5 copies. On the other hand, serial exposure to linezolid *in vitro* induced increased resistance accompanied by the loss of an rRNA operon.

Conclusions: There is a significant overrepresentation of 6 rRNA copies in MSSA and CA-MRSA as compared to MRSA and HA-MRSA, respectively. Based on their increased *in vitro* growth rates, isolates with 6 rRNA copies may be more fit which renders them more successful in the community. On the other hand, 5 rRNA copies may facilitate resistance development, which would promote survival in the hospital environment. Thus, variation in rRNA operon copy number can explain the distinct epidemiology of CA-MRSA versus HA-MRSA.

O112

Type IV secretion of proteins and DNA molecules by pathogens into eukaryotic cells

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Many pathogenic bacteria use either a type3 (TTSS) or a type4 (TFSS) secretion system to translocate effector proteins directly into target cells during infection. In this presentation, the focus shall be on the TFSS, which is now known to be evolutionary related to the cell machineries that are involved in bacterial conjugation and thus in the spread of antibiotic resistance genes in bacterial populations.

The prototype of the TFSS is the system used by the soil bacterium *Agrobacterium tumefaciens* to deliver a segment of oncogenic DNA into plant host cells. *A. tumefaciens* is the only known bacterium which thus uses genetic modification to infect its natural hosts, higher plants. Infection leads to the formation of plant tumours called crown galls as the transferred DNA (T-DNA) embraces genes which encode enzymes involved in the production of plant growth factors. The T-DNA is transferred as a single stranded DNA molecule, which is reminiscent of the process of conjugation and indeed the *Agrobacterium* virulence system is capable of transferring mobilizable plasmids to other bacteria. What is remarkable of the *Agrobacterium* system though is that it apparently has acquired the ability for 'transkingdom conjugation', not only to plant cells, but also to yeast and fungal cells.

The TFSS of *A. tumefaciens* is made up of a set of 11 different VirB proteins encoded by the *virB* operon and its presence in the bacterium is visible as a thin T-pilus on its surface. A similar *virB* operon has been discovered in the genomes of several animal and human pathogens

including *Bordetella pertussis*, which uses it for the secretion of pertussis toxin. Also *Brucella* spp, *Bartonella* spp, *Anaplasma phagocytophilum* and *Helicobacter pylori* have a TFSS that is important for virulence and known now to be involved in the secretion of effector proteins into the infected human cells. Similarly, also the *Agrobacterium* TFSS delivers several effector proteins in the plant host cells independently of the T-DNA to boost transformation. Some of these *Agrobacterium* effector proteins protect the T-DNA during its journey to the cell nucleus and other help to direct the T-DNA into the nucleus. In general, effector proteins may also be involved in inducing or interfering with signalling pathways in the eukayotic cells. In order to combat the disease caused by human pathogens which rely for infection on delivery of effector proteins by a TFSS maybe either inhibitors of TFSS or of specific cell signalling pathways that are activated by effector proteins can be developed as 'antibiotics' in the future.

The effector proteins which are secreted in the host cells are characterised by an arginine rich signal in their C-termini. Coupling of this signal to heterologous proteins can drive their transfer to host cells and this may be used for 'protein therapy'of eukaryotic cells. The T-DNA is transferred to plant cells as a nucleoprotein complex with a protein called VirD2 covalently bound to its 5'end. The VirD2 protein has a TFSS secretion signal and therefore the T-DNA may be transferred by piggybacking on this protein. In fact TFSS therefore seem protein delivery systems that have been adapted to accommodate the transfer of proteins with DNA linked to them allowing the evolution of conjugation systems.

O113

Type VII secretion – Mycobacteria show the way

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Mycobacteria have unique, highly impermeable cell walls that protect them against desiccation and other environmental or host-derived stresses. However, these bacteria have to transport proteins across this barrier, such as proteins that are designed to be functional in the culture supernatant but also proteins that are displayed on the bacterial cell surface, as single proteins or as part of surface appendages. Genome analysis shows that mycobacteria do not have functional homologues of type I to type VI secretion systems, and therefore a novel secretion system was to be expected. Recent evidence shows that these bacteria have indeed developed a novel and specialised secretion system, which is called type VII secretion, for the transport of extracellular proteins across this hydrophobic wall. Strikingly, up to five of these transport systems can be present in mycobacteria. Two of these systems, ESX-1 and ESX-5, are studied in more detail. Variants of this pathway are present in various gram-positive bacteria.

The ESX-1 secretion system of *Mycobacteria tuberculosis* is the paradigm for type VII secretion. This system plays an important role in virulence and is in fact the major discriminatory factor of the tuberculosis vaccine strain *Mycobacterium bovis* BCG. The secretion signal for ESX-1 has been located in the C-terminal tail of one of the substrates, CFP-10. This signal is not conserved in the other substrates, and, because all substrates seem to be dependent on each other for secretion, this suggests that clusters of proteins are secreted via ESX-1.

The ESX-5 secretion system is specific for the slow-growing mycobacteria, which includes all the major pathogens of this family. ESX-5 of *Mycobacterium marinum* is responsible for the secretion of a large number of different proteins, including many PE and PPE proteins. The PE and PPE families are unique for the mycobacteria and are highly expanded in pathogenic species, suggesting a role in virulence. Mutation of ESX-5 results in a dramatic shift in the cytokine response of infected macrophages. [Abdallah *et al.*, in preparation]

O115

Secretion and binding of wall located virulence factors of *Staphylococcus aureus*

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A state-of-the-art roadmap of the *Staphylococcus aureus* secretome has been generated on basis of available genome sequences, proteomics data and bioinformatics. The secretome map includes both protein transport pathways and the extracytoplasmic proteins. This has resulted in overviews of the exported virulence factors, pathways for protein transport, signals for cellular protein retention or secretion, and the exoproteomes of different *S. aureus* isolates. For this purpose, several prediction tools for determination of the presence of a signal peptide or transmembrane domains were combined in the *in silico* pipeline 'Locapred'. The dataset of the predicted secreted proteins of various sequenced *S. aureus* strains were compared within 'Locapred' to discriminate between the core and variant secretomes. While the core secretome seems to be largely employed for general house-keeping functions, necessary to thrive in particular niches provided by the human host,

the variant secretome seems to contain the 'gadgets' that *S. aureus* needs to conquer these well-protected niches.
Available literature data combined with computer searches using MEME and BLAST were used to predict the covalently and non-covalently cell wall-bound proteins. Consensus sequences for the putative wall-binding domains of the *S. aureus* proteins have been generated and added to the prediction pipeline. At least 5 different groups of non-covalently wall-bound proteins were distinguished. Comparison of the protein sequences of the predicted covalently and non-covalently wall-bound proteins showed that some of these are truncated or absent in some of the strains and the length of the covalently bound proteins was found to vary among the strains.
The group of non-covalently wall-bound proteins, containing so-called LysM motifs, has been shown to specifically bind peptidoglycan. In different studies the LysM-containing proteins of *S. aureus* have been shown to be involved in virulence and most of them are antigenic. Using specific antibodies directed against LysM containing proteins of *S. aureus*, it could be demonstrated that these proteins bind in a non-covalent manner to *S. aureus*.
To verify the above predictions the proteins of the wall-fraction were isolated and identified through a proteomics approach. Although quite some cytoplasmic and membrane proteins were present, several of the predicted wall-bound proteins of the different groups were identified. By rebinding of the extracted wall proteins to purified wall-fragments, specific non-covalently bound wall proteins could be identified. Six strongly bound proteins were identified which surprisingly did not belong to the groups of predicted non-covalently wall-bound proteins. All proteins are predicted to be secreted and share a high pI, contain a high number of Lys and Asn residues and most of them are involved in adhesion to human tissue.
The identified non-covalent wall-bound virulence factors can potentially be used in a vaccine against *S. aureus*, using non-pathogenic carrier cells.

O116

The type IV DNA secretion system of *Neisseria gonorrhoeae*

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The human pathogen *Neisseria gonorrhoeae* causes the sexually transmitted disease-gonorrhea. Most gonococcal infections are localised in the genital tract but in untreated cases the bacterium can produce disseminated gonococcal infection (DGI). In recent years, antibiotic resistance in *Neisseria* has spread rapidly. Conjugal DNA transport via type IV secretion systems is one of the main causes responsible for this spread, but the mechanism that governs conjugal DNA transport is still unknown.[1-3]
Approximately 80% of *N. gonorrhoeae* strains contain a horizontally acquired 57 kb genetic element, the Gonococcal Genetic Island (GGI). The GGI contains at least three operons. Two operons encode homologs of the F-plasmid type IV conjugation system of *Escherichia coli*. One smaller operon encodes homologs of a relaxase (TraI) and a coupling protein (TraD), while the larger operon encodes proteins with homology to the Mating Pair Formation (MPF) components of the F-plasmid system. The third large operon encodes proteins with putative functions in DNA processing. Based on similarities with the F-plasmid conjugation system, it was proposed that the GGI encodes a type IV DNA conjugation machinery.
We have set out to study the DNA secretion mechanism of *N. gonorrhoeae,* and we determined the minimal composition of the type IV DNA secretion machinery. To characterise the DNA transfer genes in the GGI, insertion-duplication mutagenesis was used to create polar and non-polar mutations in genes of the GGI. The mutants were tested for their ability to secrete DNA by using a fluorescence-based DNA secretion assay
Type IV secretion systems contain a pilin subunit, which is assembled in a pilus like structure involved in attachment to the acceptor cell. In several systems, the pilin subunit is processed by a peptidase which circularises the pilin protein before it is assembled into the pilus. The GGI contains both a pilin homologue (TraA) and a peptidase (TrbI). Remarkably, neither deletion of TraA, nor of TrbI, has any effect on DNA secretion. Using advanced BLAST searches, we found that our laboratory strain MS11A strain contains a frameshift mutation, resulting in a truncation of 8 amino acids. DNA secretion experiments demonstrated that the strain with full length TraA does not secrete any DNA. Deletion of TrbI in the strain with full length TraA (which abolishes correct assembly of TraA) shows similar DNA secretion levels as observed for the strain with the truncated TraA. Thus non-functional TraA results in DNA secretion by the proteins encoded within the GGI. Remarkably, further experiments with strains containing the full length TraA showed an increase of at least 10^4 in the conjugation frequency in filter plate assays. This demonstrated that full length TraA might be used for conjugation, while non-functional TraA results in DNA secretion. Finally, to check whether the truncated TraA is also found within the population, clinical isolates were tested for the presence of the full length TraA. Approximately 17% of the clinical isolates contained the truncated version. No relation was observed between any of the characteristics of the clinical isolates and the absence or presence of functional TraA.

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O117

Epidemiology of erythema migrans and tick bites, and infection of ticks with *Borrelia*

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Since Lyme disease is not notifiable in the Netherlands, retrospective studies have been carried out to determine the occurrence of tick bites, erythema migrans and Lyme disease. In 1995, 2002 and 2006, all general practitioners in the Netherlands (approximately 8000) were asked to complete a postal questionnaire on tick bites and erythema migrans case-patients seen in the previous year. Based on these survey responses, the incidence of erythema migrans consultations was estimated at 39 per 100,000 population in 1994, which doubled to 74 per 100,000 in 2001, and tripled to 103 per 100,000 in 2005. The incidence of tick bite consultations increased from 191 per 100,000 in 1994 to 372 per 100,000 in 2001, and continued to increase to 446 per 100,000 in 2005. The largest increase in tick bites and erythema migrans was seen in the south and northeast of the country, and several locations along the coast in the west. Statistical analysis of ecological risk factors by local government showed that the increase in tick bites and erythema migrans was associated with increases in tourism, places with horse riding facilities, and new forest in urban regions. Annual counts of hospital admissions, obtained from a database of the Dutch National Medical Register, coincided geographically with locations where physicians were consulted for tick bites and erythema migrans. The estimated annual number of hospital admissions for Lyme disease increased gradually from 118 patients in 1991 to 233 patients in 2002. From thereon, the number of patients increased strongly to 517 patients in 2006. To study the tick density and the dynamics of *Borrelia* infection in ticks in different habitats, ticks were collected from the field by dragging a blanket in four habitat areas in the Netherlands: dunes, heather, forest, and a city park. Results from 2000 to 2004 showed that the lowest tick density was observed in the heather area the highest tick density was found in the dune area. Tick densities and infection rates of ticks varied between years and types of vegetation, 0.8% to 11.5% of the collected ticks were infected with *Borrelia burgdorferi* sensu lato. In addition, other potentially pathogenic micro-organisms like *Anaplasma/Ehrlichia* (1%-15%), *Rickettsia* (5%–60%), and *Babesia* (0%-1%) were also found, which poses an increasing but unknown threat for public health. To gain more insight in the infection risk of *Borrelia* and other tick borne pathogens, a prospective study started in 2007: the National Tick bites study. The study design included approximately 160 general practices throughout the Netherlands. Patients with an erythema migrans or a tick bite are enrolled into the study to gain insight in the risk of transmission of tick-borne micro-organisms as detected in the collected ticks from these patients to humans and to observe their serological and clinical outcomes. As data collection is ongoing in 2008, only preliminary results will be presented.

O120

Where is the origin of the *Cryptococcus gattii* Vancouver Island outbreak?

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The pathogenic basidiomycetous yeast *Cryptococcus gattii* may cause a life-threatening disease of the central nervous system, lungs and skin in humans and animals. *C. gattii* is found mainly in tropical and sub-tropical regions of South America, Africa, Asia and Australia where it is endemic. Recently, a cryptococcosis outbreak in both humans and animals occurred on Vancouver Island (British Columbia, Canada) (Kidd *et al.*, PNAS 101, 2004). This outbreak was shown to be caused by a rare genotype of *C. gattii* (AFLP6A or RAPD VGIIa). The objective of this study was to find the origin of the outbreak isolates.

A selection of thirty-four *C. gattii* outbreak isolates and ninety *C. gattii* reference strains were analysed by AFLP. The six different AFLP fingerprint analyses were carried out in duplicate. Reproducible marker fragments were used for population genetic analysis. In addition, polymorphic fragments from the AFLP analyses were used to develop a multilocus sequence typing (MLST) approach.

Fraser *et al.* (Nature 437, 2005) suggested that the Vancouver Island outbreak isolates originated from Australia. However, our results based on AFLP and MLST analyses show that the outbreak isolates originated from South America. South American isolates were found to be ancestral to Australian and Asian isolates as well.

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O121

Interactions between respiratory pathogens during colonization in the first months of life. The Generation R study.

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Introduction: The nasopharyngeal cavity forms a dynamic ecosystem that is variably colonised by different pathogenic and commensal bacteria. Competitive and cooperative microbe-microbe as well as microbe-host-microbe interactions may play an important role in the microbial colonisation dynamics of the nasopharynx.

Methods. We investigated in a population-based prospective cohort study of 1079 infants the microbial associations between *Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae* and *Moraxella catarrhalis* during colonisation in the first 14 months of life.

Results. In consecutive 1.5 months, 6 months and 14 months samples, we observed an increasing carriage rate of *S. pneumoniae* (8.3%, 31.3% and 44.5%, respectively), *H. influenzae* (7.2%, 23.8% and 31.7%, respectively) and *M. catarrhalis* (11.8%, 29.9% and 29.7%, respectively), whereas the carriage rate of *S. aureus* decreased (53.0%, 20.4% and 14.5%, respectively). Regression models showed a negative association between carriage of *S. pneumoniae* and *S. aureus* at 1.5 months (adjusted odds ratio [aOR] 0.40, 95% CI 0.18-0.89) and 14 months (aOR 0.65, 0.39-1.08) of age. The negative association was primarily observed between non-vaccine type (NVT) pneumococci and *S. aureus*. We observed a positive association between carriage of *S. pneumoniae* and *H. influenzae* at 1.5 months (aOR 3.33, 1.17-9.47), 6 months (aOR 1.39, 0.85-2.29) and 14 months (aOR 1.77, 1.13-2.77) of age. This association was present for both vaccine type (VT) and NVT pneumococci. Finally, no association was observed between *S. pneumoniae* and *M. catarrhalis* carriage.

Conclusion: In summary, our data show a negative association between pneumococcal carriage and carriage of *S. aureus*, in particular between NVT pneumococci and *S. aureus*, and a positive association between pneumococcal carriage and carriage of *H. influenzae* in early infancy.

O122

Environmental contamination: a risk factor for acquisition of CC17 ampicillin-resistant *Enterococcus faecium* (ARE)

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Objectives: The last decade colonisation and infections with ARE increased in our hospital, a trend seen worldwide. Based upon MLST, epidemic and most invasive ampicillin resistant isolates cluster in clonal complex 17 (CC17). Patient-to-patient transfer via hands of healthcare workers is considered an important route in the spread of ARE. In addition, environmental contamination may contribute to ARE acquisition. In this study we quantified ARE colonisation and environmental contamination rates on a haematology ward where patient colonisation is endemic in order to better understand the role of environmental contamination in ARE epidemiology.

Methods: Between March 5th and May 25th 2007, all admissions on a 20-beds haematology ward were screened for rectal ARE-carriage <24 hrs after admission and <48 hrs before discharge. Subsequently, the environment of ARE-carriers was screened for ARE at 8 predetermined sites (blood pressure cuff, over-bed table, television remote control, bed rails, inside handle bathroom door, soap dispenser, toilet seat, control panel infusion pump) once weekly and after discharge until all swabs were negative. Swabs, enriched in enterococcal broth, were cultured on enterococcosel agar plates with ampicillin (16µg/ml). All ARE isolates were typed with MLVA.

Results: Of 72 admissions, 64 (89%) were screened for ARE on admission, of which 14 (22%) were colonised. Of 35 ARE negative admissions that were screened before discharge, 9 acquired ARE: an acquisition rate of 26%. The mean colonisation pressure was 38% (range: 14-69%). From 18 colonised patients, 412 environmental swabs were taken, of which 98 (24%) were ARE positive. Sites most often contaminated were the toilet seat (43%), over-bed table (38%) and television remote control (31%). Genotyping revealed presence of 3 circulating CC17 strains (MT1, MT159 and vancomycin-resistant MT287). In 96% of admissions the rectal strain was concordant with the environmental strains. MT159 was found predominantly: in 16 (70%) colonised patients and 73% of the positive environmental cultures.

Conclusion: Endemicity of ARE colonisation on our haematology ward is characterised by high admission (22%), high acquisition (26%) and high environmental contamination rates (43%,38%,31%) of CC17 ARE. The frequently occurring environmental contamination may act as an additional source for cross-transmission, propagating ARE directly or via contaminated health care workers.

Infection prevention measures for ARE (or VRE) should not only target on direct patient-to-patient transmission, but also on environmental hygiene.

O123

Complication registration underestimates the incidence of superficial surgical site infections: a prospective cohort study

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Introduction: The registration of surgical complications has become increasingly important in recent years. This has been underlined by the introduction of the National Surgical Complication Registration (Landelijke Heelkundige Complicatie Registratie) and the introduction of performance indicators by the Inspectorate (Inspectie voor de Gezondheidszorg) and by the patient safety programme of the Ministry of Health. Surgical site infections (SSI) are amongst the most frequent complications of surgery and are associated with high morbidity. We performed an incidence study through daily inspection by a trained team. Comparing these results with results of two existing methods of registration allowed us to assess the reliability of these methods.

Methods: Adult surgical patients who underwent conventional abdominal operations in our hospital during the period May 1-October 31 2007 were invited to take part in this prospective cohort study. The abdominal wounds of included patients were inspected and photographed daily (including weekends) from postoperative day 2 onward to check for presence of superficial SSI according to the criteria of the Centers for Disease Control and Prevention. After 21 days of clinical observation or at discharge, patients were asked to keep a diary until postoperative day 30. After this period an outpatient visit or telephone check-up took place. During the research period complications were reported according to custom in the plenary registration and the local complication registration in the electronic patient file. Eventually, the data reported in the plenary and local complication registration were compared with collected data.

Results: 243 patients were followed during the first 30 postoperative days. The following operations were included: kidney transplants (21%), colorectal surgery (14%), oesophagus resections (12%), stomach-small intestines surgery (10%), pancreas operations (10%) and other (33%). Superficial wound infections were diagnosed in 64 patients (26,3%). Mean diagnosis of infection was at postoperative day 10 (range: 3-26). The majority of these infections (84%) were diagnosed in hospital (n=54), 10 patients developed a wound infection after discharge. In total, 23/54 were registered in the plenary complication registration (43%), versus 21/54 in the local complication registration (39%). In 13/54 cases of wound infection, data in both registration systems overlapped. Nearly half of all superficial wound infections (47%) were not reported in any of the existing registration systems.

Conclusion: Despite the emphasis our clinic puts on registration of complications by both plenary complication and local complication registration, superficial wound infections appear to be underreported in both systems.

O124

Differences in clinical presentation between norovirus genotypes in nursing homes

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Introduction: Noroviruses are the most common cause of gastroenteritis both in the community and in outbreaks. In healthcare settings, outbreaks of norovirus are predominated by genotype II.4 (GII.4) strains. Periodically, new variants of GII.4 emerge, causing a temporary increase of outbreaks. Whether different norovirus strains also show differences in illness severity has not been investigated yet. In the present study, the relationship between symptoms and genotype was studied during norovirus outbreaks in nursing homes. It offers the opportunity to search for factors that may contribute to the succesful spread of GII.4 noroviruses.

Methods: Data of 49 nursing homes which were monitored prospectively for norovirus outbreaks in the winter seasons of 2005/2006 and/or 2006/2007 were used. All participating homes were asked to report a (suspected) norovirus outbreak as soon as possible to the local health service, and send in stool samples from 5 to 10 patients to the RIVM. The collected stool samples were tested for norovirus, and at least one positive sample per outbreak was genotyped. Symptoms and duration of illness were registered during norovirus outbreaks using a patient questionnaire.

Results: Data on symptoms and duration of illness were available for 465 residents and 174 staff members from 28 outbreaks. GII.4 was responsible for 21 outbreaks (75%): 7 outbreaks of GII.4 variant 2004, 10 outbreaks of GII.4 2006a, and 4 outbreaks of GII.4 2006b. In outbreaks caused by GII.4, residents vomited more often (58%) than in outbreaks with other genotypes (42%). They also had more often complaints of nausea (50% versus 35%), abdominal cramps (24% versus 9%), fever (19% versus

7%), and mucus in stool (7% versus 0%). Comparison within GII.4, the GII.4 2004 outbreaks showed higher percentages of nausea, stomach ache, and fever than outbreaks with GII.4 2006a. Differences in duration of illness between genotypes were not found. Attack rates of residents seemed to be higher in GII.4 outbreaks compared to other genotypes, and were highest for GII.4 2004. However, these differences were not statistically significant. In staff, no clear differences were found for the different genotypes.

Conclusion: Norovirus GII.4 was found to be related to more symptomatic disease, including increased vomiting, in residents and, to a lesser extent, attack rates of residents than other norovirus genotypes.

O125

The *Staphylococcus aureus* virulence factors collagen-adhesion and toxic shock syndrome toxin 1 can increase the discriminatory power of spa typing

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Introduction: To investigate the epidemiology of *Staphylococcus aureus*, spa typing, together with the algorithm based upon repeat pattern (BURP), is used. However, spa typing sometimes lacks discriminatory power. It has been suggested that decreasing the 'cost' setting can solve this, but a disadvantage is that the number of singletons will increase. The aim of this study was to investigated if the lineage-specific virulence factors collagen-adhesion (CNA) and toxic shock syndrome toxin 1 (TSST-1) could increase the discriminatory power of spa typing.

Methods: MSSA (n=180) and MRSA (n=175) strains were isolated between 1999 and 2006. The isolates were characterised by spa typing, and analysed with BURP. In addition the isolates were analysed for the presence of CNA and TSST-1 using a real-time PCR assay.

Results: A correlation was observed between the presence of CNA and *S. aureus* isolates from lineage clonal complex (CC)1, 12, 22, 30, 45, 51, and 239, and between TSST-1 and CC30. BURP analyses showed that the heterogeneous MSSA spa-CC 012 (78 isolates) consisted mainly of MSSA isolates from CC7 (23 isolates), 15 (13 isolates) and 30 (26 isolates). However, with CNA and TSST-1 as lineage-specific markers for CC30, this CC could be distinguished from CC7 and 15.

Conclusions:

1. CNA is a useful genetic marker for *S. aureus* clones from lineage CC1, 12, 22, 30, 45, 51, and 239, while TSST-1 is a useful marker for CC30. Therefore, CNA and TSST-1 could be used to increase the discriminatory power of spa typing.
2. Further research on other lineage-specific virulence factors using micro-arrays is needed to select additional genetic markers for *S. aureus* lineages to increase the discriminatory power of spa typing.

O127

Three tube pentaplex PCR assay for detection of viral respiratory pathogens

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Molecular diagnosis of respiratory viral infections would greatly benefit from the possibility of simultaneously detecting multiple targets. There are at least 15 different viruses that can infect the upper or lower respiratory tract. These pathogens may cause a wide range of very similar respiratory tract symptoms ranging from mild cold to severe pneumonia. As correct identification of the pathogen involved may influence disease management, fast and reliable diagnosis of the causative agent is desirable. We present here a three-tube multiplex PCR to simultaneously detect fifteen respiratory viruses (influenzaviruses A&B, adenovirus, enterovirus, parechovirus, rhinovirus metapneumovirus, respiratory syncitial viruses A&B, group I&II coronaviruses, and parainfluenzaviruses 1-4), and an internal control. Analytical sensitivity of multiplex reactions was compared to that of the corresponding individual single-target PCRs by analyzing synthetic DNA in different concentrations. The effects of the presence of multiple targets on the sensitivity of the assay was also analysed with synthetic DNA. The sensitivity of the multiplex assay was found to be similar to the sensitivity of the individual assays. In addition, multiple targets present in the PCR did not influence the overall analytical sensitivity of the assay.

Clinical evaluation was performed on randomly selected samples from young children hospitalised with respiratory symptoms either from a summer or winter period. Samples were analysed by multiplex and single-target PCRs. Good correlation was found between the multiplex assay and the individual PCRs, although for some targets the sensitivity appeared to be slightly lower for the multiplex assay in the clinical evaluation.

O128

Clinical evaluation of pediatric viral respiratory infections detected by real-time PCR

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Objective: To study the epidemiology, clinical presentation and outcome for specific respiratory viruses detected by multiplex real-time PCR among children with acute respiratory tract infection (ARTI) presenting at the hospital.
Material and methods: Medical records were reviewed of children aged 0-17 years, visiting the hospital during the 2005-06 and 2006-07 winter seasons (April through November), with signs of ARTI and sampled within 48 hours of presentation (single specimen for each patient). Routine multiplex real-time PCR included detection of respiratory syncytial virus, human rhinovirus, influenzavirus A and B, parainfluenzavirus 1-4, human metapneumovirus, adenovirus and coronavirus 229E, NL63 and OC43. Additional PCR was performed for human bocavirus, coronavirus HKU1, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* on specimens during a single winter season (2006-2007).
Results and conclusions: PCR yielded respiratory viruses in a high percentage of children with ARTI presenting at the hospital (224/274; 81.8%). Most common respiratory viruses detected included respiratory syncytial virus (25.2%) and human rhinovirus (19.3%), followed by influenzavirus (5.8%), parainfluenzavirus (2.6%), human metapneumovirus (2.6%), adenovirus, (2.2%), coronavirus (1.5%) and mixed infections (22.6%). Clinical ARTI symptoms at presentation could not significantly differentiate between respiratory viral infections, although some clinical variables were more associated with specific viruses. Clinical important outcome differences were observed between different respiratory viruses. PCR yielded an additional etiological role for human bocavirus (6.8%) and coronavirus HKU1 (0.7%), however not for *M. pneumoniae* (0%) or *C. pneumoniae* (0%), indicating human bocavirus should be included in routine respiratory virus multiplex PCR.

O129

Importance of human Bocavirus as a respiratory pathogen

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Since the first description of human Bocavirus (HBoV) in 2005 by Allander *et al.*, a large number of studies have been published on the presence of HBoV in respiratory tract samples, all indicating that HBoV is a common viral agent, especially in young children. In addition, the presence of HBoV in other samples (blood & faeces) has also been reported. Although few studies have addressed the causality between the presence of HBoV and respiratory symptoms, evidence that HBoV causes respiratory disease is substantial. However, as HBoV is often co-detected with other respiratory pathogens, sometimes in low quantities, the presence of HBoV in a respiratory sample does not indicate causality in each case. In the presentation the current knowledge of HBoV will be summarised.

O130

Prevalence of the newly identified WU and KI polyomaviruses in children with and without respiratory symptoms

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Introduction: Recently, two new polyomaviruses were identified in respiratory samples, KI (KIPyV) and WU (WUV). The clinical relevance of these viruses has not yet been determined. The aim of this study was to investigate the prevalence and pathogenicity of the WU and KI polyomavirus in children.
Methods: In a prospective longitudinal study 18 healthy children aged 0-7 years were followed for respiratory symptoms during 13 subsequent episodes of two weeks, covering a six-month winter period. Halfway each episode nose and throat swabs were collected regardless of any symptoms. Real-time polymerase chain reaction (rtPCR) was used to detect WUV and KIPyV. In addition, a broad panel of PCRs was used to exclude other respiratory pathogens. Episodes were defined 'asymptomatic' if no symptoms of any respiratory tract illness were present one week prior to one week after sampling.
Results: WUV was found in 18/217 samples (10%); 16/155 (10%) samples originate from symptomatic episodes and 2/62 (3%) from asymptomatic episodes. It was detected in 9 different children (50% of the children). KIPyV was found in 6/217 samples (3%); 5/155 (3%) samples were from symptomatic episodes and 1/62 (2%) from an asymptomatic episode. It was detected in 3 different children (17%). WUV and KIPyV were detected in 9% (9/105) of samples that were previously negative for any other respiratory pathogen, increasing the diagnostic yield from 52% to 56%. Five WUV and 2 KIPyV were detected in symptomatic samples in which previously no virus was detected (n=69),

increasing the diagnostic yield in these samples from 55% to 60%. In the asymptomatic samples, previously negative for respiratory pathogens (n=36), one sample was positive for WUV and one sample for KIPyV resulting in a total diagnostic yield of 45% in these samples.
Conclusions: In this unique patient-control study, WUV and KIPyV are frequently found in children with respiratory symptoms and seem to be responsible for some of the symptomatic episodes. In addition, WUV virus is found more frequently in the study population than KIPyV.

O131

Presence of human metapneumovirus in bronchoalveolar lavage fluid samples detected by means of RT-PCR

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Introduction: Human metapneumovirus (hMPV) is a paramyxovirus causing symptoms of respiratory tract infection comparable to those of respiratory syncytial virus. The virus has been shown to play a causative role in pneumonia in infants, the elderly and immunocompromised patients. So far, analysis of bronchoalveolar lavage fluid (BALF) samples obtained from patients with haematological malignancies often do not result in the identification of a causative infectious organism. To investigate the potential role of hMPV in these patients we analysed a cohort of BALF samples of patients with haematological malignancy for the presence of hMPV by means of reverse transcriptase polymerase chain reaction (RT-PCR).
Materials and method: This study was conducted in the intensive care unit (ICU) and the haematology-oncology ward of the University Hospital Maastricht, a 750-bed hospital. All consecutive BALF samples obtained in the period April 1999 until June 2006 from patients with a haematological malignancy suspected of pulmonary infection were eligible for inclusion. Data on BALF total cell count, differential cell count, quantitative bacterial culture and detection of viruses, mycobacteria and fungi was noted. BALF samples were retrieved from -80°C storage. All samples were analysed by RT-PCR targeting the nucleoprotein gene of hMPV.
Results: A total of 117 BALF samples from 95 patients (82 patients admitted to the haematology-oncology ward, 15 admitted to the ICU) were included. RNA of hMPV was detected in 7 out of 117 (6%) BALF samples of 5 patients (3 patients admitted to the haematology-oncology ward, 2 ICU patients). In 2 out of 5 hMPV positive patients the underlying disease was non-Hodgkin lymphoma, the other three patients suffered from multiple myeloma, myelodysplastic syndrome and mantle cell lymphoma. In one patient, four BALF samples retrieved within 1 month were available. The first three BALF samples were hMPV PCR positive, the fourth (collected 1 month after the first) was PCR negative. No other infectious agents were detected in the hMPV-positive BALF samples. Neither total cell count nor differential cell count were significantly differenced between the hMPV positive and -negative group.
Conclusions: In 6% of BALF samples collected from adult patients with a haematological malignancy suspected of a pulmonary infection, hMPV RNA was detected. hMPV thus may be considered as the causative agent of pulmonary infection in patients with a haematological malignancy when analysis for other infectious agents is negative.

O133

Diversity in *Bacillus cereus* biofilm formation capacity and spore contents; implications for disinfection procedures

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Biofilm formation by food pathogens causes serious problems in food industry. Establishment of pathogen biofilms on food processing equipment can cause equipment failure and recontamination of products, thereby affecting both food quality and safety. The occurrence of stress-resistant cells and spores in biofilms may have a large impact on the efficiency of cleaning and disinfection procedures. Therefore, the impact of *Bacillus cereus* strain diversity on biofilm forming capacity and concomitant spore production was assessed. A prescreening with 56 strains of *B. cereus*, including the two sequenced strains ATCC 14579 and ATCC 10987, was used to assess diversity in biofilm formation and to define conditions for massive biofilm formation. Subsequently, specific strains were selected to be analysed in more detail including enterotoxic and emetic strains, and psychrotolerant variants. Biofilm production could be maximised by modulation of incubation time, temperature and medium. Notably, some strains showed rapid biofilm formation within the first 24 h, followed by active dispersion in the next 24 h. Thick biofilms of *B. cereus* developed at the air-liquid interface of stainless steel coupons while in submerged systems the amount of biofilm formed was much lower. This suggests that *B. cereus* biofilms develop in industrial systems that are partly filled during operation, or where residual liquid has remained after a production cycle. Moreover, spore counts were found to be higher in the biofilm than in suspension and constituted up to 90% of the total biofilm counts. This indicates that *B. cereus* biofilms can act as a nidus for spore formation, and subsequently boost their spores into food production environments. Based on these results, a number of recommendations associated with processing equipment design and disinfection procedures will be discussed.

O134

Modelling microbial survivors in various disinfection processes for better results

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Appropriate cleaning and disinfection processes in food industry are fundamental to assure the microbial safety and quality of our foods. Cleaning should remove undesired (product) substances from equipment, whereafter disinfection should eliminate (or substantially reduce) undesired micro-organisms present.

Most general purpose disinfectants are formulated to reduce bacterial population by at least 5 log orders within 5 minutes in suspension (Holah, 1995). Test standards define a number of indicator organisms (e.g. *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecium*) as well as interfering substances to be tested (e.g. 0.03 or 0.3% bovine serum albumin solution to simulate organic matter in clean and dirty disinfection conditions respectively).

Under industrial conditions, micro-organisms that remain after cleaning will be surface attached and will remain surface attached during the disinfection process (Holah, 1995). This attachment (and additional biofilm formation) can drastically higher the concentration of disinfectant needed, as for instance exemplified by Luppens *et al.* (2002) Moreover, concentrations optimised for indicator organisms might not be the most optimal conditions to reduce the relevant micro-organisms for industry, i.e. pathogens (such as *Salmonella* and *Listeria monocytogenes*) and product-specific spoilage flora (e.g. lactobacilli).

Insight in the relation between disinfectant efficacy and disinfectant type, concentration, contact time, temperature, (state of the) microorganism and interfering substances is needed. Lambert (1998) suggested that a modelling approach could be used to predict efficacy of disinfectants. The objectives of this presentation are to explore the possibilities and impossibilities in modelling the efficacy of disinfection and to illustrate how modelling can be used to integrate existing knowledge and to guide further experimentation in order to get better results.

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O138

Serological diagnosis of bacterial respiratory tract infections

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Microbiological assays are done to identify the etiologic agent causing infection. Ideally, the identification of the pathogen is in the early stage of disease, early enough to be useful in patient management.

Diagnostic studies for specific bacterial agents of respiratory tract infection

PATHOGENS	AVAILABLE ASSAYS
Streptococcus pneumoniae * }	gram-stain
Haemophilus influenzae }	+
Moraxella catarrhalis }	(blood)cultures
Staphylococcus aureus	
Gram-negative rods	* Urinary antigen test for *S. pneumoniae* available
Mycobacterium species }	Acid-fast stain/culture/PCR
Nocardia species }	
Legionella species	Urinary antigen detection/ culture/PCR /**serology**
Mycoplasma pneumoniae	**Serology**/PCR/culture
Chlamydia psittaci	**Serology**
Chlamydophila pneumoniae	**Serology**/(PCR)
Coxiella burnetii	**Serology**/PCR/culture
Bordetella species	**Serology**/PCR/culture
Francisella tularensis	**Serology**/(PCR) (culture)

Diagnostic methods for 'atypical' pathogens are mostly serological.

Legionella pneumophila. Indirect immunofluorescence assays, microagglutination tests and ELISA assays are available. ELISA's are preferred nowadays because they are most sensitive. Seroconversion may take several weeks. Approximately 25-40% of patient seroconvert within the first week after onset of symptoms.[1]

Mycoplasma pneumoniae. Since no reference standard is generally accepted, serological assays for *M. pneumoniae* have not been evaluated on a broad scale. Beersma *et al.* evaluated 12 commercially available serologic assays and

the complement fixation test (CFT) by using *M. pneumoniae* DNA detection by real-time PCR as the 'gold standard'. The best ROC area under the curve was found for CFT.[2]
Chlamydia psittaci. Complement fixation is mostly used. The CF is only genus specific and does not distinguish *C. psittaci* from *Chlamydia trachomatis* or *Chlamydia pneumoniae*, both of which are common pathogens.
Coxiella burnetii. The diagnosis of Q-fever is almost always confirmed serologically using CF and phase II antigen.
Bordetella pertussis. Most patients with pertussis are diagnosed with serology because they consult the physician late in disease. Measurement of IgG antibodies to pertussis toxin is the most sensitive and specific immunoassay.[3]

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O139

Detection of MRSA using a modified PCR-assay

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Methods: The objective of this study was to evaluate the test characteristics of a modified BD-GeneOhm MRSA-assay on individual and pooled samples in a setting of low MRSA-revalence. The results of the PCR-assay were compared with culture results from selective phenol-red mannitol broth subcultured after 48h. Sensitivity, specificity, and positive and negative predictive values were calculated.
Results: For individual testing, 581 samples from 201 persons were collected; 18 (3.2%) were MRSA culture positive. 510 broths from 174 persons were combined in 106 pools after overnight incubation; 8 pools (7.5%) contained one or more MRSA culture positive specimens. There were no inhibited PCR tests. The combined sensitivity of individual and pooled specimens was 92% (95% CI 73-99), the specificity was 98% (95% CI 96-99) and the positive and negative predictive values were 63% and 99.7%, respectively.
Conclusion: Our modified procedure gives satisfactory results and the pooling of broths reduces costs.

O142

Role of Siglec/sialic acid interaction in phagocytosis of *Campylobacter jejuni* in human monocytes

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Introduction: Siglecs are sialic acid-binding immunoglobulin-like lectins expressed on cells of the immune system. Tyrosine-based inhibition motifs (ITIMs) in the cytoplasmic tail of a number of Siglecs are involved in cellular inhibition upon ligand binding. Sialylated lipooligosaccharides (Sia LOS), present on the surface of *Campylobacter jejuni* strains, are bound by Siglecs. Interaction between *C. jejuni* Sia LOS and Siglecs on phagocytic cells could possibly limit or delay the activation of an immune response, thereby prolonging survival of the pathogen.
Methods: Binding to Siglecs was tested in three ways: 1) Guillain-Barré associated *C. jejuni* strains were tested for Siglec binding in ELISA; 2) Siglec mediated adherence was demonstrated using Siglec transfected CHO cells; 3) Phagocytosis was assessed in a monocytic cell-line, positive for expression of Siglec-3,- 5 and -7, using a gentamicin exclusion assay.
Results: The ELISA showed that *C. jejuni* with Sia LOS specifically bind to several members of the Siglec family that are expressed on monocytes and macrophages. Sia negative fjcstII mutants were unable to bind Siglecs. In the adhesion assay, a *C. jejuni* strain positive for Siglec-1 binding was unable to adhere to wt CHO cells but did adhere to Siglec-1 transfected CHO cells. A fjcstII mutant of the same *C. jejuni* strain was unable to bind to the Siglec-1 transfected CHO-cells. Phagocytosis of a Siglec-7 binding strain was 2-fold higher than cstII knockout phagocytosis.
Conclusions: *C. jejuni* is able to bind to several Siglec structures present on a range of immune related cells. Although Siglecs are involved in *C. jejuni* adherence in a Sia dependent way, phagocytosis of *C. jejuni* by human monocytes is not inhibited by Siglec binding.

O143

Determination of growth dependent gene expression of *Staphylococcus aureus* with a newly developed whole genome microarray

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Background: Survival and pathogenesis of *Staphylococcus aureus* in the host requires the ability to respond to changes. However, during infection these response mechanisms are poorly understood. The aim was to validate a new microarray and to identify gene expression over time during growth in synthetic medium.
Methods: A whole genome Agilent microarray was developed based on 9 publicly available strains, using MSSA476 as main strain. Expression of MSSA476 during 5 standard growth curves was examined in 7 samples from early log till stationary phase.
Results: Validation of the microarray revealed that the array covers 100% of the coding regions of MSSA476 and 99.4% of the remaining genomes. PCA plots of the growth curve experiment clearly showed time dependency with a clustering during log phase and stationary phase, resp. At the 4h time point (late log growth), the PCA plot showed a division with 2 points clustering near the stationary phase and 3 points close to the log phase.
K-means clustering showed around 150 genes that were down-regulated and roughly 110 genes that were up-regulated over time. About 40 genes increased up to mid-log and then decreased.
Fibrinogen binding proteins of *S. aureus* are necessary for colonisation and virulence. During bacterial growth these genes are differentially expressed. ClfA was expressed throughout growth, while clfB was only expressed in early log phase. Genes involved in innate immunity, like spa and scn were also differentially expressed. Spa was expressed in early log phase and decreased quickly over time. Scn was expressed at a high level throughout time, with a peak in late log phase.
Conclusion: The newly developed whole genome microarray was validated for the expression of *S. aureus* genes. The time-dependent clustering in the PCA plots shows that expression at the level of the bacteria is dependent on the growth phase. In addition, expression of individual genes showed different profiles, even for gene products with similar functions. The expression profiles of all genes from *S. aureus* in a standard growth curve provides essential information for the study of host-pathogen interactions.

O144
Preliminary analysis of the whole genome sequence of a clinical ST30 MSSA isolate

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Background: The whole genome sequences of approximately a dozen *Staphylococcus aureus* isolates are publicly available. The whole genome sequence of a ST30 isolate was not available, although ST30 is considered the founder of clonal complex 30 (CC30), which includes EMRSA 16. The aim of this study was to sequence the whole genome of a clinical ST30 MSSA isolate and compare its genome with sequences from other strains.
Methods: The whole genome of ST30 MSSA strain S0158 was sequenced using a Genome Sequencer FLX system with 454 sequencing technology (Roche). Data analysis was performed using Kodon and BaSys programs. The sequence was compared with that of MRSA252, an EMRSA-16 which belongs to ST36 and CC30.
Results: A total of 45 contigs >1 kb (range 1071-480500 bp) were obtained. Total genome coverage was 23.5 times. The size of the genome of S0158 is approximately 2.8 Mb compared to 2.9 Mb for MRSA252. The sequences of both strains are largely identical. Also the phages present in both strains closely resemble each other. This indicates that these phages may not be exchanged as easily as sometimes suspected. But differences in νSaα encoding mainly exotoxins and putative lipoproteins and νSaβ encoding a serine protease cluster and several enterotoxins are observed. Both elements in S0158 appear to lack the modification enzyme of the restriction modification system. In addition, strain S0158 lacked a number of other sequences compared to MRSA252. These sequences include the 53 kb SCCmec, at least 12 insertion sequences/transposases elsewhere in the genome, and Tn554. However, S0158 contained a SaPI1-like element containing the tst-1 gene encoding toxic shock syndrome toxin, which is usually absent in ST30 isolates. Both S0158 and MRSA252 encode several serine-aspartate (SD) repeat proteins like SdrC, bone sialoprotein binding protein, clumping factor ClfA and fibrinogen and keratin-10 binding protein (clfB). In addition, S0158 encodes SdrD another SD repeat protein.
Conclusion: The genomes of strain ST30 MSSA S0158 are largely similar, although S0158 lacks a number of important features compared with MRSA252 related to resistance and insertion sequences, some additional genes are present in S0158 such as tst-1.

O145
A novel *in vivo* otitis media model

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Introduction: Otitis media (OM) is one of the most frequent diseases in childhood. It is the most common reason for children to visit a physician, to receive antibiotics, or to undergo surgery. While OM management has no universal standard yet and the costs for general health care are expanding, vaccine development against OM is of interest. Our department aims to design a new, non-invasive

animal model for OM and subsequent antigen screening of the three major pathogens causing OM: *Streptococcus pneumoniae*, *Moraxella catarrhalis* and non-typeable *Haemophilus influenzae*.

In contrast to previous transtympanic OM animal models, Tonnaer *et al*. designed a new infection method in rats via the nasopharynx, comparable to the humane route of infection leading to middle ear infection. To further optimalise this technique, the study has been extended to mice, while a better understanding of mouse immunology and the possibility to generate knockouts and transgenics will be of particular interest for vaccine development.

Methods: A non-invasive method to represent the physiological route of middle ear infection was established, using a pressure cabin. To verify this new procedure female BALB/C mice (6 weeks old) were inoculated intranasal under general anaesthesia using Evans blue dye or bacterial suspension of one of the pathogens mentioned above. Mice were placed in back position in the pressure cabin and pressure was elevated stepwise after regaining consciousness and the first swallowing movement. Blue staining of the tympanic membrane and optimum pressure were determined using otomicroscopy. The course of infection was monitored using otomicroscopy, bacterial culture and histology at respectively 1, 48, 96 and 144 hours. For bacterial culture blood, nose lavage and homogenates of the right ear and both lungs, were extracted after termination of the experiment, using 5 mice each time point. The left ear was stored on formaldehyde for latter histological screening.

Results:

1. Bilateral blue staining of either the tympanic membrane or middle ear cavity was established using a pressure increase of 40kPa, without mechanical damage of the tympanic membrane afterwards.
2. A dose of 5´106 CFU per 10uL volume resulted in the most optimal condition to obtain OM caused by *S. pneumoniae*.
3. Kinetic studies and histological screening showed a maximum of disease at 4 days after infection with *S. pneumoniae*.

Conclusion: Our laboratory succeeded to develop a new, non-invasive otitis media model in mice, which will support multivalent vaccine development. A better insight in OM pathogenesis will be created by thorough investigation of bacterial pathogens and immune responses. In future research, the applicability of this method will be evaluated for non-typeable *H. influenzae* and *M. catarrhalis*.

O146

Identification and classification of microorganisms by mass spectrometry fingerprinting

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Introduction: Fast and secure identification of microorganisms is an essential requirement in medical healthcare. Currently, mainly methods based on morphological investigation and tests regarding biochemical capabilities are used in routine. Unfortunately, these methods have limitations in resolution, applicability and reliability. Frequently, they end up in genus determination. Modern molecular methods which have a higher resolution power generally are too laborious and costly for high-throughput routine usage. MALDI-TOF mass spectrometry fingerprinting has been shown to be well suitable for the identification of microorganisms. The utilisation of this technology in combination with dedicated bioinformatic tools for the reliable detection of clinical relevant bacteria and fungi on the species as well as the subspecies level will be reported.

Methods: Microorganisms were cultured on solid media and applied to a MALDI sample target, directly or after a short inactivation/extraction protocol. After addition of matrix, linear profile spectra were acquired using a microflex MALDI-TOF MS instrument in the range of 2000 to 20000 Dalton. Based on the acquired profile spectra an according reference data base of more than 1500 different microorganism species was created using the MALDI BioTyper software, extracting species- and subspecies-relevant information. This database could be used for analysis of microorganism profile spectra with dedicated software algorithms contained in the BioTyper software, i.e. pattern matching, weighted pattern matching, principle component analysis and correlation analysis.

Results: MALDI fingerprinting combined with the standard pattern matching algorithm against the reference database was found to be a very robust method for identification of bacteria without any further pre-assessment. Simple sample preparation by direct smear of bacteria onto the target plate resulted in mass spectra of good quality in many cases. For more difficult samples, e.g. microorganisms with robust cell walls like yeast, a short extraction protocol increased the quality of results, significantly. Identification was possible on the genus and mostly also on the species level without any fine-adjustment. Further algorithms, e.g. a weighted pattern matching approach which applies the selective analysis of differentiating features in closely related groups, were able to resolve closely related species as well as subtypes

of bacteria. The technology was applicable to a very broad range of clinically relevant microorganisms and gave excellent results compared to standard methods.
Conclusions: The results indicate that MALDI-TOF MS fingerprinting in combination with the MALDI BioTyper software is a highly suitable method for secure species and subspecies identification in routine, high-throughput analysis of clinical samples.

O147

Inhibition of natural transformation through an endogenous DNase in *Campylobacter jejuni*

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One of the most reported causes of acute gastroenteritis in humans worldwide is *Campylobacter jejuni*. Multilocus sequence typing (MLST) indicates that *C. jejuni* has a weakly clonal population structure and that horizontal gene transfer (HGT) is common in this species. HGT is one of the mechanisms involved in the generation of genetic diversity in bacteria and natural transformation is one of the processes by which HGT takes place. Previously we have shown that differences in natural transformability between *C. jejuni* strains correlate with diversity, suggesting that a deficiency in natural transformation contributes to the preservation of stable *C. jejuni* clones in certain lineages. The aim of the present study was to investigate the occurrence of non-transformability in the natural competent species *C. jejuni*. Therefore, a genomics survey by means of microarray-based comparative genome hybridisation was performed with chromosomal DNA isolated from 6 natural transformable and 20 non-transformable *C. jejuni* strains. A gene encoding a putative DNase was present in more than half of the non-transformable strains but absent in nearly all natural transformable strains. The predicted DNase activity of the gene product and its inhibitory effect on natural transformation was demonstrated by using a DNase-negative knock-out mutant and a DNase-complemented *C. jejuni* strain. These results show that in a subset of non-transformable *C. jejuni* strains a DNase is responsible for the inhibition of natural transformation through its ability to hydrolyse DNA.

O148

Diversity of the *Enterobacter cloacae* complex determined by a mixed genome array and multi locus sequence analysis

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Background: The population structure of the *Enterobacter cloacae* complex (ECC) is unknown. Sequencing a fragment of hsp60 or rpoB was proposed to distinguish different (sub)species. The aim of this study was to determine the genetic relationship between human specific ECC isolates using a mixed genome array (MGA) and multi locus sequence analysis (MLSA).
Methods: All isolates were identified as *E. cloacae* by the Phoenix system (BD). 8 clinical *E. cloacae* isolates were used to construct the MGA. 180 slides were hybridised with 153 non-repeat clinical isolates, 3 environmental isolates and 2 ATCC strains. Reproducibility was assayed by testing 18 isolates in duplicate or triplicate. Hsp60 and rpoB fragments of all isolates were sequenced. Fragments of fusA, gyrB, leuS, pyrG, and rplB were sequenced for a subset of 50 isolates selected for diversity after comparative analysis of rpoB and hsp60 sequences and MGA data.
Results: Principal component analysis (PCA) of the MGA data divided isolates into two genetically distinct groups. The same broad distinction was obtained from the MLSA data for 50 isolates using 6 gene fragments. The isolates could be subdivided into 8 clusters by MGA. Group I MGA isolates (cluster1-3) appeared more closely related using MLSA than by MGA. Cluster I isolates were identified as *Enterobacter hormaechei* based on rpoB sequences. MGA and MLSA data for group II isolates were not completely congruent. Cluster 5 was one distinct cluster in MLSA and identified as *Enterobacter asburiae*. Cluster 4 was a separate cluster in MLSA and not identified, as were the isolates from cluster 6. Isolates from cluster 7 and 8 were mixed in MLSA, but nevertheless fell into completely distinct groups previously named either *Enterobacter ludwigii* or *E. cloacae*. One cluster 7 isolate identified as *E. hormaechei* and another as *E. asburiae* by rpoB, were clearly distinct both by MGA and MLSA.
Conclusions: The MGA approach is a new tool to determine (sub)species differences based on the presence and absence of a large number of DNA fragments. The ECC consists of several clonal and heterogenic (sub)species, which have to be redefined, because of disagreements with known or proposed nomenclature.

O149

Response of *Bacillus cereus* ATCC14579 to a challenge with the circular bacteriocin enterocin AS-48

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Bacteriocins present one of our last resorts against bacteria that are resistant against most of the commercially available antibiotics. Enterocin AS-48 is a broad-spectrum antimicrobial peptide produced by *Enterococcus faecalis*, beloning to Class II of enterococal bacteriocins. This 70-residue cyclic peptide makes pores with a size of appr. 0.7 nm in the bacterial cytoplasmic membrane, thereby disrupting the proton motive force and causing cell death. This feature of AS-48 makes if a great interest as a food preservative and alternative antibiotic and therefore it is of high relevance to know how the target bacteria react on bactericin treatment. AS-48 was succesfully applied against *Bacillus cereus*, a rod-shaped, gram-positive spore-forming bacterium and an important food born human pathogen.

We have performed transcriptome analysis comparing *B. cereus* ATCC14579 cells treated with AS-48 to untreated cells. Most of the at least 2-fold upregulated genes encode membrane-associated proteins. The BC0406-BC0409 operon, coding for genes involved in arginine metabolism was the only one found to be downregulated.

The BC4206-BC4207 operon was found to be the most upregulated target in our experiments. BC4206 codes for a PadR type transcriptional regulator, while BC4207 codes for a hypothetical membrane protein. The operon structure and genes are conserved in *B. cereus* and *Bacillus thuringiensis* species, but are not present in *Bacillus anthracis* and *Bacillus subtilis*. Using real-time qPCR, we have shown that these genes are upregulated if we treated the cells with AS-48, but not upon nisin treatment.

To get more insight into the possible function of these genes in *B. cereus* cells treated with AS-48, we have introduced these genes into *B. cereus*. Overproduction of the PadR regulator, BC4206 resulted in enhanced sensitivity for AS-48, in line with a previously suggested role of PadR regulators functioning as repressors of target genes. Upon overexpression of BC4207 in *B. cereus*, we observed an increased resistance against AS-48. Expression of BC4207 in *B. subtilis* 168, which lacks this operon also showed increased resistance against AS-48. Thus, this membrane protein is involved in the resistance mechanism of *B. cereus* cells against AS-48. The possible function of these genes will be further discussed.

O150

Freeze-thaw treatment represses growth metabolism related functions and induces the envelope stress response and cell wall biosynthesis pathways of *Bacillus subtilis*

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Introduction: None-thermal preservation processes are an attractive alternative for the treatment of processed foods. Shen *et al.* (2005) reported in model systems that pressurisation of a frozen suspension of *Bacillus subtilis* resulted in up to 5-log cycle reductions of viable counts. Membrane damage was suggested as the prime mode-of-action (Shen *et al.*, 2008) of either FT (freeze-and-thaw) or HPLT (high pressure and low temperature) treatment. Here, a genome-wide microarray analysis of survivors was performed in order to infer mechanisms of FT induced damages and get indications for the mechanisms involved in damage repair reactions.

Methods: One portion of a suspension of mid-exponential (OD600nm=0.6) *B. subtilis* cells was collected and used as control. Another portion was put in a -80fflC freezer and stored for 7 days. After rapid thawing in a 37°C water bath, the suspensions were diluted (OD600nm=0.3) and incubated at 37fflC until their OD600nm level doubled (ca. 56 min). During that time a total of 7 samples were collected for analysis. Five replicates were prepared. The microarray data was analysed statistically to reveal the DEGs (differential expressed genes) with a false discovery rate <0.05. Significantly regulated groups were determined using T-profiler (Boorsma *et al.*, 2005; TerBeek *et al.*, 2008).

Results: Up to 2423 and 2099 DEGs (>50%) were found during 10-20 min of recovery (t2 and t3). The number of DEGs decreased to 369 (9%) after 50 min (t6). No significant difference was found afterwards. Genes responsible for cell wall biosynthesis and adaptation to atypical conditions were significantly induced.

Regulons such as SigW SigX, PerR, Rok,Xpf, and YvrH were found up-regulated significantly, while CcpA, CodY, PurR, Fur, ComA, PyrR, SigA, SigD, StrCon, and TnrA were strongly depressed.

Conclusions:

1. An FT treatment induced severe damage in *B. subtilis* cells exemplified by the large number of DEGs between FT and control upon recovery. Upon resuming growth, the FT cells were still characterised by a slow doubling time (ca. 56 min). For comparison, an untreated normal cell doubled every 32 min under our experimental conditions. A new (pseudo) steady state in gene-expression was established after 50 min. However, significant difference between FT and control still existed.

2. FT repressed the major central metabolic pathways and processes related to cell growth. Unexpectedly, both the stringent response and the general stress response were repressed. Only the cell envelope stress response genes and cell wall biosynthesis pathways were induced.
3. These results call for further study to identify the genes that have a causal link with cellular repair of FT damage. In addition, a microarray experiment of HPLT treated *B. subtilis* will be performed.

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O151

Draft genome sequencing of two *Enterococcus faecium* strains by pyrosequencing technology reveals niche-specific gene acquisition

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Objectives: *Enterococcus faecium* has recently become an important cause of nosocomial infections. The rapid rise in *E. faecium* infections can be explained by the acquisition of antibiotic resistance mechanisms combined with the evolution of a hospital-associated *E. faecium* clade, termed Clonal Complex 17 (CC17). To reveal the genetic diversity in *E. faecium* strains from different environmental niches, we sequenced the genomes of two *E. faecium* strains from different clonal complexes (CC22 and CC17, respectively) using pyrosequencing technology.

Methods: The strains studied were E1071 (CC22, a vancomycin-resistant isolate that was picked up during routine hospital surveillance) and E1162 (CC17, a vancomycin-sensitive bloodstream isolate). The genomes of these two *E. faecium* strains were sequenced by Keygene (Wageningen, the Netherlands) using the GS FLX sequencer (454 Life Sciences, Branford CT, USA), with 20-fold coverage of the genome. Sequences were assembled using Newbler Assembler (454 Life Sciences). The draft genomes were annotated using the TIGR Annotation Engine and the encoded proteins were compared using PROMPT (Protein Mapping and Comparison Tool; http://webclu.bio.wzw.tum.de/prompt).

Results: The total number of sequence that could be assembled into contigs was 2.7 Mb for both strains, suggesting all but complete coverage of the genome. Automated and manually curated annotation of the draft genome sequences resulted in the prediction of 2716 and 2697 proteins for E1071 and E1162, respectively. More than 2200 proteins were more than 95% identical on the amino acid level between the two strains. Approximately 10% of the proteins were unique to each strain. Using PROMPT the predicted proteins from the genomes of these two strains and the publicly available *E. faecium* DO strain (CC17) were compared. This revealed that the CC17 strains had genomic islands with functions in sugar uptake and metabolism that were absent in E1071. Two (partial) prophages were also specific for the CC17 strains. The presence of the TcrB copper resistance system and a specific point mutation in the vanX gene in strain E1071 strongly suggest that this strain originates from pigs.

Conclusion: Rapid draft genome sequencing has revealed the acquisition of several niche-specific genes in *E. faecium* and identified several genes and genetic elements that may be unique for CC17. The genome sequences contribute to ongoing functional studies into *E. faecium* in our laboratory.

O153

Extended spectrum beta-lactamases (ESBLs) in animals

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A potentially important reservoir for transferable beta-lactamases is animal husbandry, a reservoir with high antibiotic consumption and as a result high selection pressure. Companion animals (pets and horses) are described as a potential reservoir as well.[1]

The first detection of an ESBL in an animal was reported in Japan in 1988, from a laboratory dog infected by a FEC-1-producing *Escherichia coli*.[2] Broad-spectrum SHV-1, TEM-1 and OXA beta-lactamases have been frequently described in *E. coli* and *Salmonella enterica* from animals and food of animal origin in Spain, Germany, USA and UK. TEM-1 was the most common variant among these isolates, but the occurrence of ESBLs among isolates from food producing animals has recently increase as reflect the number of publications on ESBLs in animal derived bacteria during the last years.[1]

In the UK CTX-M-14 is described to occur in calves and to persist on a cattle farm in *E. coli*,[3,4] but the most prevalent CTX-M variant among human isolates is CTX-M-15 (90%) -not yet detected in bacteria from animals in this country, but nonetheless identified in several *Salmonella* strains from humans, whereas CTX-M-3 and CTX-M-9 are rarer.

A study performed in 2001-2002 on amoxicillin-resistant *Salmonella* isolated from poultry, poultry products and human patients in the Netherlands revealed that the

TEM-52 variant was the most common ESBL detected in this bacterial collection.[5] In particular, TEM-52-producing salmonellae of the Blockley, Virchow, Typhimurium and Paratyphi B d-Tartrate positive serotypes were identified from poultry, and strains of the Thompson, London, Enteriditis and Blockley serotypes were identified from human patients. The dispersal of TEM-52 different *Salmonella* serotypes strongly indicated that a single clone was not circulating in Dutch poultry, nor that there was a direct association of the human cases to food consumption. However, the frequent occurrence of this ESBL in food products is of concern for the future diffusion of such resistance. *Salmonella* seems to be a common reservoir for this ESBL, since in France in 2002-2003 the TEM-52 was detected in *Salmonella typhimurium*, *Salmonella enteriditis*, *Salmonella panama*, *Salmonella blockley* human isolates but only one case had a suspected link to food consumption, but reservoirs in other bacterial species may exist as well.[6]

The increasing number of reports of ESBL positive *E. coli* and *Salmonella* in animals indicates widespread horizontal transfer in the animal reservoir and the occurrence of a potential reservoir from which transfer of ESBLs can occur to human pathogens.

Linkage to other resistance genes and co-selection will play an important role in the epidemiology of bacteria of animal sources.[7,8] In animal husbandry a high selection pressure by mass medication with antibiotics exists, which may contribute to the occurrence and dissemination in animals.

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O154

The clinical impact of extended spectrum beta-lactamases (ESBLs)

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Infections involving ESBL-producing micro-organisms are associated with a delay in effective antibiotic therapy and increased mortality. Furthermore, preventive hygienic measures may be warranted for patients colonised or infected with ESBL-producing micro-organisms, because these organisms are associated with epidemics, multi-resistance, and increased costs.

Carbapenem antibiotics are the mainstay of treatment for infections due to ESBL producing micro-organisms. The plasmids harboring ESBL genes frequently contain additional genes conferring resistance to other antibiotic classes. This results in high frequencies of co-resistance (quinolon resistance 40-60% co-trimoxazole resistance 40-60% and aminoglycoside resistance 40-60%), making these classes unsuitable as empiric antibiotic choices in case of ESBL production.

Whether infections with ESBL producing micro-organisms can be treated with beta-lactamase inhibitor combinations (piperacillin/tazobactam, amoxicillin/clavulanic acid), is a matter of debate. Despite reported successes, these combinations should be avoided in serious infections. Some experts believe that uncomplicated urinary tract infections, caused by an ESBL producing micro-organism, may be treated with amoxicillin/clavulanic acid, if susceptible *in vitro*. However, there is limited clinical data to recommend this and more clinical data is required to solve this issue.

Finally, to prevent selection of carbapenemase producing micro-organisms due to copious use of carbapenems for combating the ever increasing ESBL problem, unorthodox combinations of clavulanic acid and cephalosporins (third and fourth generation) deserve evaluation as alternatives to carbapenem antibiotics. Similarly, clinical response to antibiotics such as tigecycline and colistin, with good in vitro activity against ESBL producers, need to be evaluated.

O155

Guideline of the Dutch society for medical microbiology for screening and confirmation of extended-spectrum beta-lactamases in *Enterobacteriaceae*

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Standardisation of the extended-spectrum beta-lactamases (ESBLs) detection methods is essential to get a real insight in the epidemiology and prevalence of these resistance genes. The complexity of ESBL-detection and the lack of uniform guidelines may lead to underestimation of this serious issue. The aim of this guideline is to standardise ESBL-detection in *Enterobacteriaceae* in Dutch laboratories for clinical microbiology. This guideline is developed, reviewed and updated through open and wide consultation process, using the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), the Clinical and Laboratory Standards Institute (CLSI), the Health Protection Agency-British Society for Antimicrobial Chemotherapy (HPA-BSAC), the Swedish Reference Group for Antibiotics (SRGA), the European Antimicrobial Resistance Surveillance System (EARSS) and literature interpreted by the Dutch, European and American ESBLs experts. The ESBL detection strategy is to use an indicator cephalosporin to screen for likely ESBL-producer and then to seek for clavulanate/cephalosporin synergy. Recommended methods for ESBL-screening are broth dilution, agar dilution, disk diffusion and automated systems. The working party recommends a MIC >1 mg/L for cefotaxime and ceftazidime as the ESBL-screening breakpoint. *Enterobacteriaceae* isolate resistant to cefotaxime and/or ceftazidime in the screening test should be subject to the confirmation test. The combination disk diffusion test or, alternatively, the ESBL E-test, are recommended as a confirmation method in this guideline. Due to the potential masking of ESBL-detection by AmpC beta-lactamases, the *Enterobacteriaceae* have been divided into two groups, with each a specific strategy for ESBL screening and confirmation. For all confirmed ESBL-producing isolates, the reported antibiogram should be adapted. The isolate should be considered resistant to all penicillins, cephalosporins (except cefoxitin) and aztreonam, including beta-lactamase inhibitor combinations. This is the first guideline which harmonised the European (HPA-BSAC, SRGA and EUCAST) and the American (CLSI) ESBL-screening breakpoints.

O157

ISIS and ESBL

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Antimicrobial resistance among nosocomial pathogens poses a serious threat to public health. The emergence of the extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* is a striking example. Infections with ESBL-positive strains are associated with a higher morbidity and mortality, an increased length of stay and increased hospital costs. Since hardly any new antibiotic classes for these often multiresitant strains are to be expected in the coming years, the importance of infection control is increasing. According to the 'Guidelines for the Prevention of Antimicrobial Resistance in Hospitals' (formulated in 1997 by a joint committee of the SHEA and IDSA) infection control programs should contain three elements to prevent or slow down antimicrobial resistance development and dissemination of highly resistant micro-organisms: 1) active surveillance of resistance, 2) active and effective infection control programs to minimise secondary spread of resistance; and 3) effective programs of antimicrobial stewardship.

The first item, surveillance, is a critically important component of any control program, allowing detection of newly emerging resistance traits, monitoring epidemiologic trends, and measuring the effectiveness of interventions. Obviously, a regional (or better nationwide) approach offers the optimal chance for a successful public health response to the antibiotic resistance epidemic. In the epidemiology of highly resistant microorganisms (HRMO), the dynamics of patient traffic between health care institutions play an important role. Outbreaks and/or endemicity of HRMO in one health care institution (HCI) will influence the occurrence of HRMO in another institution, either through patient transfer or through dissemination in the community, or through both processes.

In June 2007, the director of the Centre of Infectious Disease Control (CIb) appointed a team with the assignment to develop, in collaboration with the NVMM and the SWAB, a nationwide resistance surveillance system (ISIS-AR) covering (at least) all pathogens mentioned in the WIP guideline on HRMO. Focus of the new system is the monthly monitoring of susceptibility results from cultures ordered as part of routine clinical care, accompanied by all relevant epidemiological data present in the laboratory information systems (e.g. date of birth, specimen, ward, institution, screening/clinical). Anonymised data are coded according to the semantic standard of the NVMM. In the

second half of 2007 a database was built and by February 2008 pilot studies with two laboratories were successfully completed. Policies to monitor the quality of the data in the database are now being developed. Additionally, in cooperation with a NVMM working group, an interactive website is being built as feedback module for the participating laboratories. The aim of 2008 is to include data of least another 8 of the 38 laboratories that have committed themselves to the surveillance program.
Next to surveillance and quality improvement of the participating laboratories, this database is an important tool for epidemiological studies. The first study using this database will be on the prevalence of ESBLs in the different HCIs and their catchment populations. As part of this study, participating laboratories will be asked to store part of their isolates with a positive ESBL screentest according to the NVMM guideline. Subsequently, these isolates will be genotyped and their ESBLs will be characterised.
The result of this study will provide 1) an evaluation of the database, 2) knowledge on the epidemiology of ESBL-positive *Enterobacteriaceae* among clinical isolates in the nosocomial setting, nursing homes and community in the Netherlands, and 3) an evaluation of the NVMM guideline on ESBL detection.

O160

The use of artemisinins for malaria treatment in Dutch peripheral hospitals

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The current international standard for malaria treatment in endemic countries is based on artemisin combination therapy (ACT). The high efficacy combined with the excellent safety profile as well as the absence of clear evidence of resistance have made this drug the cornerstone of malaria treatment policy. National guidelines for ACT combinations differ however between countries and several regimens are still under investigation.
Artemisinin derivatives are available in several formulations with different properties e.g. artesunate, arteether, dihydro-artemisinin. Artmisinin should not be used in the first trimester and with caution in later trimesters of pregnancy.
Registration in the Netherlands is limited to artemether/lumefantrine (Riamet®) tablets (only >12 years and >35kg) and artemotil *i.m* (Artecef®) (only <16y, can be used in patients >16 years with their approval).
Every year 200-300 cases of malaria are registered in the Netherlands. The first choice for uncomplicated malaria is either atovaquon/proguanil (Malarone®) or Riamet® as excellent alternative. Artemotil *i.m*. can be used for more severe presentation of uncomplicated malaria requiring hospital admission or for classical severe malaria as defined by WHO classification. The classical treatment for the latter group is quinine, which is still effective in combination with a second anti-malaria drug. However, it has a narrow therapeutic range and many side effects. From limited number of trials in endemic countries, it appears that there is no difference in efficacy for treatment of severe malaria between the two drugs.
Artemisinins clearly show added value to the portfolio of anti-malarial drugs. Compared to other drugs they have a simple and short treatment schedule, show quick recovery and parasite clearance and are highly effective and well tolerated. Availability of an *i.v.* preparation that can better ensure therapeutic blood levels than the *i.m*. preparation, would be highly beneficial.

O161

The treatment of *Dientamoeba fragilis*

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Dientamoeba fragilis is an intestinal protozoa, once classified with the amoebae, but now with the flagellates. *Dientamoeba* occurs in a trophozoite stage only and does not encyst. Diagnosis relies on the detection of trophozoites in multiple, freshly passed, warm stools, or in freshly passed stools that have been preserved using fixative, e.g., sodium acetate-acetic acid-formalin (SAF), like in triple feces tests (TFT). Trophozoites measure 7-12 µm in diameter, but can vary within a wider range (3.5-22 µm). They are sometimes uninucleate but mostly binucleate, with an extranuclear mitotic spindle between the nuclei, only to be seen in permanent stained preparations.
They live in the large intestine under anaerobic or microaerophilic conditions.
D. fragilis has been known as a possible pathogen since the beginning of the 19th century. The mode of transmission is unknown. Because of the low resistance of trophozoites to unfavorable environmental conditions and failure to infect humans with cultured trophozoites *Enterobius vermicularis* as a helminth vector has been assumed, but could not be proven. Numerous studies have shown that a large proportion of patients with dientamoebiasis were co-infected with other gastrointestinal protozoan parasites.
Clinical symptoms may vary from IBS-like symptoms to travelers' diarrhea, chronic or acute.
Specific treatment is indicated in symptomatic patients with *D. fragilis* when no other pathogenic organisms are present. There is no consensus about the drug of choice. We will discuss several treatment regimens with paromomycin, clioquinol, tetracycline, oxytetracycline,

metronidazole or other nitroimidazole preparations and erthromycine.

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O163

Albendazole and doxycycline as possible alternatives to diethylcarbamazine for treatment of filariasis

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The primary aim of treating filariasis is to kill the macrofilaria (lymphatic filariasis and loiasis) and to reduce microfilaraemia with the least possible side effects following the release of bacterial and parasitic components that trigger inflammation. The macrofilaria are responsible for the pathology in lymphatic filariasis (*Wucherria bancrofti*, *Brugia malayi*) and loaiasis; the microfilaria for the pathology in onchocerciasis (*Onchocerca volvulus*).

Diethylcarbamazine (DEC), the first line treatment of many forms of filariasis, is often not available in the Netherlands. In addition, the macro- and microfilaricidal activity of DEC can induce severe local and systemic inflammatory responses. Therefore, we reviewed the literature for the efficacy and safety of albendazole and doxycycline as treatment alternatives.

W. bancrofti, B. malayi

Diethylcarbamazine (DEC) has a fair macrofilaricidal and strong microfilaricidal activity. In a comparative study albendazole (400 mg bid, 21 days) had similar activity on microfilaraemia as DEC.[1] At this dosage, albendazole may also be toxic to macrofilaria. This can lead to severe local inflammatory responses. Most randomised controlled trials have been performed with single dose treatment regimens. Unfortunately, single dose albendazole (400 mg) did not show microfilaricidal activity in comparison to placebo, even when combined with ivermectine (0.200 mg/kg) or DEC (6 mg/kg).[2] Treatment with a high single dose ivermectine (0.400 mg/kg) had a more pronounced microfilaricidal activity, but no activity against macrofilaria.

Eight- and 6-week courses of doxycycline (200 mg, daily) have led to significant reductions in microfilaraemia.[3] Doxycycline kills *Wolbachia* endobacteria which are vital for larval development, and adult-worm fertility and viability. In addition, by depleting bacterial antigens doxycycline also reduces the infammatory response of other macrofilaricidal drugs. Sequential treatment with doxycycline followed by a 3-week course of albendazole has not been studied.

In conclusion, an 8-week course of doxycycline, 200 mg daily is an alternative treatment for lymphatic filariasis, with follow-up after 12 months with ultrasound, antigen-detection and microfilarial count.

Onchocerca volvulus

The microfilaricidal activity of ivermectine (0.150 mg/kg) greatly reduces the skin manifestations of onchercciasis. But, ivermectine does not have macrofilaricidal or permanent sterilizing effects on the adult female filariae, making 3- to 6-monthly therapy necessary.[4] The addition of a 6-week course of doxycycline (200 mg daily) significantly enhances ivermectin-induced suppression of microfilaridermia upto 18 months, by long-term sterilisation and death of adult female filariae.[5]

In conclusion, the initial treatment with ivermectine should be followed by an 8-week course of doxycycline, 200 mg daily.

Loa Loa

DEC is curative in approximately 60% of patients who acquire loiasis as long-term visitors to an endemic area. There are no trials comparing DEC with albendazole. However, albendazole, 400 mg bid for 21 days, may be useful for the treatment of symptomatic loiasis as it has been shown to slowly decrease microfilaraemia and may be macrofilaricidal.[6]

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O165

Evaluation of 5 different rapid tests for the detection of *Giardia lamblia* cysts and/or *Cryptosporidium parvum* oöcysts in faecal samples

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Rationale: In most Dutch laboratories, the diagnosis of giardiasis (GL) or cryptosporidiosis (CP) is made by microscopy of faecal samples. RT-PCR is an upcoming method for the detection of parasites in faeces. Both techniques are labour intensive and expensive, therefore, the need is felt for alternative diagnostic methods without these drawbacks.

Objective: Evaluation of 5 rapid tests for the detection of GL and/or CP antigen: Xpect Giardia/Cryptosporidium (Remel Inc., Lenexa, KS, U.S.A.), Crypto-Strip, Giardia-Strip and Crypto/Giardia-Strip (Coris Bioconcept, Gembloux, Belgium) and the ImmunoCard Stat! Crypto/Giardia (Meridian Bioscience Inc., Cincinnati OH, U.S.A.).

Methods: All liquid and mushy faecal samples sent to our laboratory for parasitic and/or bacteriologic analysis from June 2007 until December 2007 were collected and frozen at -20 °C. Standardised triple faecal test microscopy was performed on all samples and modified Ziehl Neelsen staining was added if cryptosporidiosis was suspected. GL and CP were detected by PCR using the method of Verweij *et al*. (JCM Mar. 2004 p.1220-1223). All rapid tests were performed according to the manufacturers instructions. Microscopy was used as the gold standard for detection of GL. Microscopy, PCR and the rapid tests were used as gold standard for the detection of CP (optimal test result).

Results: GL microscopy was positive in 18/45 samples. Sensitivities and specificities of the Giardia-Strip, Crypto/Giardia-Strip, Immunocard Stat Crypto/Giardia and Xpect Giardia/Cryptosporidium were 72% and 100%, 78% and 100%, 89% and 100% and 89% and 96%, respectively, for detecting GL antigen. 4/45 samples were regarded as CP positive. Sensitivities and specificities of the Crypto-Strip, Crypto/Giardia-Strip, Immunocard Stat Crypto/Giardia and Xpect Giardia/Cryptosporidium were 25% and 100%, 75% and 100%, 25% and 100% and 50% and 100%, respectively, for detecting CP antigen. When taking into account the combined performances of the Crypto/Giardia-Strip, Immunocard Stat Crypto/Giardia and Xpect Giardia/Cryptosporidium, the GL and/or CP result was predicted correctly in 89%, 82% and 84%, respectively, of the cases. The differences in performance can be explained by the high failure rates with the Immunocard Stat Crypto/Giardia and Xpect Giardia/Cryptosporidium tests. Regarding the ease of use, the unfixated faecal samples used for the Immunocard Stat Crypto/Giardia and Xpect Giardia/Cryptosporidium needed to be diluted 1:4 before use, which makes their procedures more labour intensive compared with the other rapid tests. Discrepant or missing data are being processed and evaluated.

Conclusion: Overall performance was highest for the Crypto/Giardia-Strip. Sensitivities of the Immunocard Stat Crypto/Giardia and Xpect Giardia/Cryptosporidium for detecting GL antigen were significantly higher compared with the other rapid tests. Preliminary results for the CP rapid tests suggest a higher sensitivity for the Crypto/Giardia-Strip.

O170

Terminal restriction fragment length polymorphism as a diagnostic tool in gastrointestinal disease: a preliminary study

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Introduction: Terminal restriction fragment length polymorphism (T-RFLP) has been utilised as a typing method to examine microbial community structure in human faecal samples. Adherence of microbes to the gastrointestinal mucosa rather than luminal presence however is a prerequisite for development of intestinal disease.

To this date, the T-RFLP method has not yet been evaluated for the possibility to obtain a rapid, broad view of the (adherent) microbial composition of the human intestine and its dynamics in diseased states. This evaluation might result in a screening tool for abnormalities in the adherent gastrointestinal microflora by way of analysing changes in terminal restriction fragment (TR-F) profiles. This study was done to obtain evidence on the performance of the T-RFLP method.

Method: The technique involves amplification of the 16S rRNA gene with two differently labeled primers, digesting the amplicon with restriction endonucleases, and retrieving the molecular weight of the labeled terminal restriction fragments (the outer ends of the amplicon) in an automated DNA sequence apparatus. The labeling of both the forward and reverse primers might lead to a more accurate identification of species within a community profile since the presence of two peaks instead of one confirm the presence of a certain species.

Firstly, cultures of three common intestinal habitants of the human gut were subjected to T-RFLP analyses, alone and in mixtures, to determine if competition in the PCR between species has an effect on the T-RFLP profiles.

Secondly, mucosal biopsies from five colon locations per patient were gathered from 20 patients for analysis by T-RFLP. The three aforementioned species were quantified in the samples by quantative real-time PCR to determine if adequate amounts were present for detection by T-RFLP.

Results: It was shown that the T-RFLP method produces consistent, reproducible profiles. The patient samples showed little to no intrapatient varation in T-RFLP profile compared to interpatient variation.

Conclusion: For reasons of simplicity, similarity between T-RF sizes of different genera should be omitted or reduced to a minimum by way of careful restriction enzym selection. Furthermore, a database containing DNA sequences of the adherent human intestinal microbial community, combined with adequate computer software capable of performing meta-analyses of forward and reverse peak presence, is essential for obtaining rapid T-RFLP profile results.

O171

National outbreak of shiga-toxin producing *Escherichia coli* O157

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Introduction: Early in October 2007, an increase in notifications of human cases infected with Shiga toxin (Stx)-producing *Escherichia coli* (STEC) O157 was seen in the Netherlands. The cases were distributed across the whole country, with a concentration of the cases in the western part. Are the cases related and what is the source of this increase?

Methods: An epidemiological survey was performed to identify the source(s) of the outbreak, using the standard questionnaire which is part of the enhanced surveillance of laboratory-confirmed STEC patients in the Netherlands, extended with a more extensive questionnaire for more detailed information. Patient isolates were confirmed as O157 and typed with pulsed-field gel electrophoresis (PFGE) using Xba-I as primary restriction enzyme. PFGE patterns were compared to a database containing over 850 other *E. coli* O157 PFGE patterns isolated in the Netherlands since 1997.

Results: STEC O157 strains were isolated that contained both stx1 and stx2 genes in 41 cases. Subtyping of these isolates by PFGE showed an identical pattern not previously observed in the Netherlands for 39 cases. Both other isolates showed closely related patterns. The onset of illness for the cases was between mid-September and mid-October. 29% of the cases were aged between 20 and 29 years. A case to case comparison within the cases of 2007 was performed which revealed raw vegetables as the possible source of the outbreak. Municipal health services went back to the outbreak cases with the more extensive questionnaire which then pointed towards pre-packaged shredded lettuce purchased at several supermarket chains as the possible source. Simultaneously, an outbreak with an identical PFGE pattern occurred in Iceland with a strong lead to a Dutch shredding company for fresh vegetables.

Environmental investigations, done by the Dutch Food and Safety Authority, did not yield microbiologic evidence for a source. Furthermore, several vegetable growers were visited and samples were taken, also without positive results.
Conclusion: A nation-wide outbreak of STEC O157 occurred in the period mid-September and mid-October, linked with a simultaneous outbreak in Iceland. In total, 41 Dutch cases were identified by PFGE. In the Netherlands, meat is the most common food source of STEC O157. However, the source of this outbreak was most likely pre-packaged shredded lettuce, which could not be confirmed with microbiologic evidence.

O172

Electron transfer in microbial fuel cells

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In microbial fuel cells, microorganisms generate electricity from biodegradable materials. In the absence of oxygen and other electron acceptors, a number of microorganisms is able to transfer the electrons liberated in the degradation of organic materials to an electrode. When this electron accepting electrode (=anode) is connected via an electrical circuit to a cathode where electrons are consumed, a current is generated.
In general, a microbial fuel cell consists of an anaerobic anode compartment and a cathode compartment separated by a proton-permeable membrane. Microorganisms convert the substrate to CO_2, protons and electrons. The electrons travel through the electrical circuit to the cathode where they, together with the protons that move through the membrane, are used to convert O_2 or another electron acceptor. Typical current densities that have been obtained are 1-3 $A \cdot m^{-2}$ anode surface area. Currently, several technological constraints still exist, e.g. cell design and materials properties, that limit the current output of the microbial fuel cell. However, ultimately, current production is determined by the rate at which micro-organisms transfer electrons to the anode. The ability to transfer electrons to the anode is not a property common to all anaerobic microorganisms as it requires exocellular transfer of electrons. Several different mechanisms have been described for exocellular electron transfer which include the use of electron shuttle compounds, which may be derived from the environment or be produced by the microorganisms itself. Electrons may also be conveyed directly via c-type cytochromes and, recently, the use of conductive pili structures has been proposed as a mechanism for direct electron transfer.
We have tested pure cultures in a microbial fuel for their ability to generate electricity and studied the current production in response to the anode potential and the effect of electron shuttle compounds. The biofilms that developed on the anode were investigated using electron microscopy. In addition, using mixed inoculum, microbial consortia were enriched on the anode and the microbial community analysed.
Our research aims to increase the insight in the process of microbial electron transfer to electrodes and the mechanism or mechanisms used by different microorganisms.

O173

Microbial community in hydrogen producing microbial fuel cells

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The Microbial Electrolysis Cell (MEC) is based on the principle of the Microbial Fuel Cell (MFC). The MFC consists of an anode and a cathode compartment, separated by an ion exchange membrane and connected to an electrical circuit. In the anaerobic anode compartment, bacteria are present that are able to oxidise organic substrates and transfer the released electrons to the electrode. Protons that are produced in the oxidation of organic substrates move through the membrane to the cathode compartment where they, together with electrons, are consumed at the cathode for e.g. the reduction of oxygen. In the MEC a small voltage is applied to the system to provide the energy required for the production of hydrogen gas by combining protons and electrons at the cathode.
The bacteria used in the anodic compartment are able to oxidise different organic compounds including organic material from wastewater. By using organic substrates from wastewater in this process, the wastewater can be purified and energy can be generated in the form of hydrogen gas which can be used as a green energy source.
Recent experiments have shown that also the use of microorganisms in the cathode as catalyst for hydrogen production is possible.
The efficiency of hydrogen production in an MEC is for an important part dependent on the rate at which bacteria transfer electrons to the anode and consume electrons at the cathode. Knowledge about the microbial community and the electron transfer between electrode and micro-organism in the MEC is therefore essential. However, at the moment very little is known about these microbial communities. Our research focuses on the characterisation of the microbial communities in the MEC using molecular

techniques and understanding the function of individual species and the way they transfer electrons to and from the electrodes. This kind of knowledge will be of great importance for optimizing and understanding the MEC.

O174

Microbial diversity in open mixed cultures fermentation

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Mixed culture biotechnology may become an attractive option to convert waste streams into valuable products. It has been proposed that in mixed cultures fermentation (MCF) systems the operational conditions will determine which catabolic product allows the more efficient growth, and will therefore dominate. Fermentation by open mixed culture has been studied, aiming at controlling the conversion of organic compounds present in a waste stream into a known and stable range of fatty acids and/or solvents.

The MCF process was investigated at different environmental pH values (4-8.5) and with three carbon sources (glucose, glycerol and xylose). Depending on the operational conditions imposed, different fermentation products were formed. To evaluate whether the different operational results were related to changes in the microbial community, the population composition from each steady state was assessed by denaturating gradient gel electrophoresis (DGGE) analysis of the 16S rRNA gene fragments. Using this approach we were able to investigate the link between operational characteristics and microbial composition, as well as the dynamics in both variables.

The environmental pH had a determinant effect on the microbial population at extreme values; whereas at intermediate-pH values the product spectrum seemed to be sensitive to variations in the microbial population. The fermentation of different substrates enabled different populations to establish, and in the simultaneous presence of two substrates mixotrophic microorganisms (capable of degrading both substrates) were found to overgrow the originally dominant specialists.

In some cases, a clear shift in the product spectrum was not associated with a shift in the microbial population, and sometimes it was. At this stage the study of the microbial composition is an interesting tool to investigate mixed culture processes. It allows for microbial diversity analysis and identification of dominant metabolic groups in an ecosystem. However, the analyses performed do not provide a direct relationship between the process characteristics (associated to a metabolic response) and the microbial population present.

O175

Genome analysis and microarray analysis of the hydrogen producing thermophilic bacterium *Caldicellulosiruptor saccharolyticus*

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Hydrogen is becoming a realistic option for the sustainable energy economy, provided that production occurs from renewable primary energy sources and not from fossil fuels. In addition to thermochemical technologies, fermentative hydrogen production is gaining a lot of interest. For thermodynamic reasons fermentative hydrogen production is easier at elevated temperatures and comparison of various hydrogen-producing anaerobes shows that thermophilic bacteria and archaea indeed have better hydrogen yields and produce less other reduced end products. Among the different thermophilic saccharolytic hydrogen producers that are available to date, *Caldicellulosiruptor saccharolyticus* has excellent characteristics for efficient hydrogen production. It grows optimally at 70ffiC, it can grow on many different carbohydrates (including cellulose, xylan and pectin) and it produces high levels of hydrogen. Moreover, it can simultaneously convert C6- and C5-sugars, the main constituents of commonly used biomass feedstocks. For these reasons *C. saccharolyticus* is one of the key microorganisms of the dark fermentation within the EU-KP6 program HYVOLUTION. For the same reason this organism was selected by the DOE for a genome sequencing project, which was completed in May 2007. In the current presentation we describe the results of the genome annotation and of a genome-wide transcriptional analysis of glucose/xylose utilisation.

O178

SNOMED CT: 'De semantische standaard' van de toekomst?

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Steeds meer informatie wordt in de computer vastgelegd. Dankzij netwerken kan deze informatie ook worden uitgewisseld tussen verschillende systemen. Nu de *technische* interoperabiliteit is gerealiseerd dankzij diverse standaarden als TCP/IP en HL7 is de tijd gekomen om te komen tot *semantische* interoperabiliteit. Dit houdt in dat de inhoud van een bericht niet alleen door mensen, maar ook door machines kan worden begrepen.

Een terminologiestelsel dat een steeds grotere rol vervult in het realiseren van semantische interoperabiliteit is SNOMED CT. SNOMED CT is een internationaal terminologiestelsel

dat de basis kan vormen voor een gestandaardiseerde en gestructureerde vastlegging van patiëntgegevens en voor het gebruiken van die gegevens voor zorg, beslissingondersteuning, en evaluatie van zorg.
SNOMED CT onderscheidt zich op een aantal wijzen van andere veelgebruikte terminologiestelsels. Ten eerste richt het zich op de zorg als geheel, en is dus niet specifiek voor één specialisme bestemd. Dit maakt SNOMED CT bij uitstek geschikt voor interdisciplinaire informatie-uitwisseling.
Ten tweede is de structuur van SNOMED CT uitgebreider dan de meeste terminologiestelsels. Het is gebaseerd op concepten, dat het mogelijk maakt verschillende beschrijvingen voor één concept te specificeren. Bovendien is de betekenis (semantiek) van de concepten formeel expliciet gemaakt. Daardoor kunnen gegevens op verschillende manieren gezocht en geordend worden, in tegenstelling tot systemen met een vaste hiërarchische indeling.
In deze presentatie zal worden ingegaan op het waarom, wat en hoe van SNOMED CT.
Hierbij zal ook een link worden gelegd naar 'De Semantische Standaard Verrichtingen in de Medische Microbiologie en Medische Immunologie'.

O179
IHE-lab in Nederland

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'Integrating the Healthcare Enterprise' is een internationale organisatie die zich richt op het oplossen van veel voorkomende integratievraagstukken die ontstaan wanneer klinische informatie tussen gezondheidszorgsystemen moet worden uitgewisseld. IHE bestaat sinds eind jaren '90 en is begonnen in het radiologiedomein. Het unieke aan IHE t.o.v. andere soortgelijke organisaties is dat IHE een initiatief is waar gebruikers én leveranciers gezamenlijk oplossingen definiëren op basis van bestaande communicatiestandaarden. Met andere woorden, IHE is geen 'Standards Developing Organisation' zoals bijvoorbeeld HL7 of DICOM. De oplossingen die IHE aandraagt, vertalen zich in concrete aanwijzingen hoe bestaande communicatiestandaarden moeten worden gebruikt om een concreet (door gebruikers gevoeld) probleem op te lossen. Sinds haar oprichting in 1998 is IHE sterk uitgebreid en actief geworden in andere domeinen zoals cardiologie, radiotherapie en ook in het laboratoriumdomein.
Sind medio 2007 is er binnen de Nederlandse tak van IHE een werkgroep 'Lab' opgezet. Deze werkgroep, waarin gebruikers (o.a. vertegenwoordigd door de NVMM en de NVKC) en leveranciers (o.a. ZIS, LIS en HIS) zijn vertegenwoordigd, is bezig om voor de Nederlandse situatie probleemgebieden (use-cases) in kaart te brengen waar gestandaardiseerde oplossingen mogelijkheden bieden tot verbetering van de kwaliteit en kwantiteit van de laboratoriumwerkzaamheden. Denk daarbij bijvoorbeeld aan use-cases die betrekking hebben op het doen van een (elektronische) aanvraag door een huisarts, of aan het leveren van lab-uitslagen aan elektronische patiëntendossier.
Vanuit deze laatste invalshoek is ook het NICTIZ sterk betrokken bij de IHE-'Lab'-werkgroep en geïnteresseerd in de uitkomsten ervan om deze in te passen in de landelijke ontwikkelingen op het gebied van het nationale elektronische patiëntendossier.
In deze presentatie zult u meer te weten komen van het IHE-'Lab'-initiatief en een indruk krijgen op welke (probleem)gebieden IHE reeds oplossingen aandraagt.

O181
Staphylococcal superantigen-like protein 5 is a broad spectrum chemokine and anaphylatoxin inhibitor

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Background: *Staphylococcus aureus* secretes several virulence factors that interfere with host-cell functions. *Staphylococcal* superantigen-like proteins (SSLs) are a family of 14 newly identified exotoxins encoded on pathogenicity island SaPI2 and immune evasion cluster 2 present in all sequenced strains. SSLs show structural homology to superantigens, but do not exhibit superantigenic activity. Latest evidence suggests though that SSLs play a role in immune evasion by other means. Recently, we demonstrated that SSL5 binds to P-selectin glycoprotein ligand 1 (PSGL-1) dependently of sialyl lewis X (sLex) and inhibits P-selectin-dependent neutrophil rolling. Here we describe interference of chemokine- and anaphylatoxin-induced cell stimulation by SSL5.
Methods: Calcium mobilisation and actin polymerisation experiments were performed to investigate the influence of SSL5 on activation of human neutrophils and monocytes by chemoattractants. Competition experiments were performed with SSL5 and antibodies directed against several GPCRs. Human embryonic kidney (HEK) cells were transfected with G protein-coupled receptors (GPCRs). Binding experiments with SSL5 and biotin-labeled chemokines were performed on U937 cells. Importance of sialic acid residues was examined by treating neutrophils with neuraminidase or preincubating SSL5 with sLex.
Results: SSL5 potently and specifically inhibited leukocyte activation by anaphylatoxins and all classes

of chemokines, but not by other chemoattractants. Competition experiments demonstrated that SSL5 targeted chemokine and anaphylatoxin receptors. In addition, transfection studies showed that SSL5 not only bound the N-termini of chemokine and anaphylatoxin receptors but of all GPCRs tested. Ligand specificity is exerted by the nature of the ligand; SSL5 only inhibits stimuli of protein nature that act at least through the N-terminal region of the target GPCR, in contrast to lipid and peptide ligands that activate GPCRs independent of the N-terminus. In case of chemokines, SSL5 has a second mechanism of action. SSL5 sequestered chemokines from the chemokine receptors as increased binding of chemokines to cells was observed independent of chemokine receptors. Treatment of cells with neuraminidase abolished SSL5 binding to cells and eliminated its inhibitory effect on stimulus-induced cell activation and chemokine binding. The same results were observed when SSL5 was loaded with sLex.

Conclusions:

1. In addition to binding to PSGL-1, SSL5 targets GPCRs and inhibits cell activation by chemokines and anaphylatoxins.
2. SSL5 effects are dependent of glycoproteins on the cell surface.
3. SSL5 is an important immunomodulatory protein of *Staphylococcus aureus*.

O182

Characterization of a pneumococcal competence-induced operon: link between DNA repair and carbon dioxide fixation?

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Introduction: The respiratory tract pathogen *Streptococcus pneumoniae* can acquire novel genetic elements, such as antibiotic resistance and virulence factors, from its surroundings via natural genetic transformation. In this gram-positive bacterium, competence for genetic transformation is a transient physiological state during which most genes required for the synthesis of a DNA uptake and recombination machinery are being expressed. Interestingly, it has recently become clear that the same regulatory pathway is also implicated in processes that are not related to uptake of genetic material, such as response to (antibiotic) stress and the formation of pneumococcal biofilms.

Method: In a first step to understand the competence regulon, we applied a novel negative selection strategy called genomic array footprinting (GAF) to identify all pneumococcal genes that are essential for genetic transformation. Genome-wide mariner transposon mutant libraries in *S. pneumoniae* were challenged by transformation with an antibiotic resistance cassette and growth in the presence of the corresponding antibiotic. Mutants defective in transformation did not acquire the antibiotic resistant cassette, disappeared from the mutant pool, and were, consequently, identified by GAF. Novel transformation genes and operons were further characterised by directed KO mutagenesis and various *in vitro* assays.

Results: The GAF screen yielded 9 putative novel transformation genes. After characterisation of each gene, only the one coding for the competence-induced putative DNA repair protein RadA was identified as essential for efficient transformation. Since RadA is not implicated in transformation with replicating plasmid, it is considered to have a role in recombination of donor DNA with chromosomal markers. The radA mutant displayed an increased sensitivity to treatment with the DNA damaging agent methyl methanesulfonate, and, hence, RadA has a DNA repair function. The radA gene is the third gene of a five-gene competence-induced operon. The other four genes in the operon have unknown or putative functions in pyrimidine synthesis and metabolism, and knockout mutants are not affected for genetic transformation. The metabolic gene is a putative carbonic anhydrase, which converts carbon dioxide (CO_2) to carbonate, which is required in several metabolic pathways. A KO mutant in the carbonic anhydrase genes was shown to be defective in growth at low CO_2 concentrations, whereas it has wild-type growth rates in a 5% CO_2-enriched environment. How the presence of both DNA repair and CO2 fixation enzymes in a single competence-induced operon relate is currently under investigation.

Conclusions:

1. Application of GAF technology resulted in the identification of the RadA DNA repair protein, which appeared to be essential for efficient recombination of donor DNA into the pneumococcal genome.
2. In addition to its role in genetic transformation, RadA is implicated in DNA repair.
3. None of the other four genes in the competence-induced radA operon are implicated in transformation, but seem to have unrelated functions. This suggests, that competence does not only drive different aspects of the pneumococcal life cycle, but that the different aspects of the competence regulon are also genetically intermingled.

O183

Regulation of nitrogen metabolism in *Streptococcus pneumoniae* by CodY and GlnR: link between nutritional gene regulation and virulence

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Introduction: For bacteria, regulation of nitrogen metabolism is of utmost importance for survival in various environments. In the human pathogen *Streptococcus pneumoniae*, CodY and GlnR are transcriptional regulators involved in nitrogen metabolism. Both regulators have been extensively studied in *Bacillus subtilis, Lactococcus lactis*, but not in *Streptococcus pneumoniae*. In this study, we examined the role of CodY and GlnR in gene regulation and experimental virulence.

Materials and methods: We used various assays to investigate the role of these regulators in the pneumococcal life cycle, such as targeted mutagenesis, microarray analysis, 2D difference gel electrophoresis (2D DIGE), electrophoretic mobility shift assays, DNAse footprinting, adherence assays to human pharyngeal epithelial Detroit 562 cells and mouse infection models.

Results: The CodY-regulon appeared to consist predominantly of genes involved in amino acid metabolism, but also several other cellular processes, such as carbon metabolism and iron uptake, most of which were found to be under direct control of CodY. By mutating DNA predicted to represent the CodY-box based on the L. lactis consensus, we demonstrated that this sequence is indeed required for *in vitro* DNA-binding to target promoters. Similar to *L. lactis*, DNA-binding of CodY was enhanced in the presence of branched chain amino acids, but not by GTP. We observed in experimental mouse models that codY is transcribed in the murine nasopharynx and lungs, and is specifically required for colonisation. This finding was underscored by the diminished ability of the codY-mutant to adhere to nasopharyngeal cells *in vitro*. Furthermore, we found that pcpA, activated by CodY, is required for adherence to nasopharyngeal cells, suggesting a direct link between nutritional regulation and adherence.

To assess the contribution of the GlnR-regulon to virulence, D39 wild-type and mutants lacking genes of this regulon were tested in an *in vitro* adherence assay and murine infection models. All mutants, except fjglnR, were attenuated in adherence to human pharyngeal cells, suggesting contribution of these genes to adherence during colonisation of humans. During murine colonisation only the fjglnA and glnP-glnA double mutant (fjglnAP) were attenuated, in contrast to fjglnP, indicating that the effect is caused by the lack of GlnA expression. In our pneumonia model, only fjglnP and fjglnAP showed a significantly reduced number of bacteria in the lungs and in blood, indicating that GlnP is required for survival in the lungs and possibly for dissemination to the blood. In intravenously infected mice, glnP and glnA are individually dispensable for survival in the blood, whereas fjglnAP was avirulent. Finally, transcriptome analysis of the fjglnAP showed that many genes involved in amino acid metabolism were upregulated. Interestingly, many of these upregulated genes belong to the CodY regulon. This signifies the importance of glutamine/glutamate uptake and synthesis for full bacterial fitness and virulence.

Conclusion: These observations illustrate that nutritional regulators play an important role in pneumococcal pathogenesis by regulating genes required for bacterial survival at specific sites in the host.

O184

Role of phosphatidylinositol mannosides in the interaction of mycobacteria with human dendritic cells

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Mycobacterium tuberculosis persists, often for a lifetime, in the human host. One mechanism of persistence is the ability of the pathogen to modulate the immune response. Dendritic cells (DCs) are professional antigen presenting cells and are pivotal in determining the nature of host immune response. We therefore initiated studies to understand which bacterial surface antigen(s) determine the interaction between mycobacteria and DCs. Previously, we have shown that, in complete contrast with the current dogma, the mannose caps of the surface glycolipid lipoarabinomannan (ManLAM) do not dominate mycobacterium-host interaction. However, the binding to DCs could still be blocked by mannan, which suggests other mannosylated ligands are present in the mycobacterial cell wall. Mannosylated glycolipids with similar structures to ManLAM are phosphatidylinositol mannosides (PIMs). PIMs consist of a glycosylated phosphatidylinositol (GPI) anchor which can be mannosylated with one to six mannosyl residues (denoted as PIM1 to PIM6). Furthermore, the α1,2-linkage between the two and three terminal mannosyl residues of PIM5 and PIM6 respectively, is identical to the linkage between the

mannosyl residues of the mannose cap on ManLAM. This led us to hypothesise that PIM5 and PIM6 are a potential second group of glycolipids acting as immunomodulators via DC-SIGN. The enzyme catalyzing the transfer of the fifth mannose to PIM4 to produce PIM5 is PimE. We used *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG) as a model for *M. tuberculosis* and tested a BCG *pimE* knockout (Δ*BCG_1220*) for its abilities to bind to DC-SIGN and for its induction of cytokine secretion by human DCs. To overcome the possibility of redundancy in DC-SIGN-binding ligands in the mycobacterial cell wall, also a double knockout in BCG was created which is not able to synthesise PIM5, PIM6 and the mannose cap on LAM. Although cell lysates from the knockout strains probed on SDS-PAGE / immunoblot with a DC-SIGN-Fc construct showed lack of binding at the position of the PIMs, FACS experiments showed no significant reduction in binding of the BCG knockouts to DCs nor to Raji-cells expressing DC-SIGN compared to wild-type BCG. Also ELISA measurements on IL-10 and IL-12p40 secretion by LPS-activated DCs incubated with BCG, did not indicate differences between the immune response on BCG wild-type and on the knockout strains.

Conclusion: We conclude, that evidently the role of PIM5 and PIM6 in binding to DC-SIGN and their effect in modulating the immune response via DC-SIGN is limited and hence, another as yet unknown ligand determines the binding of mycobacteria to DCs.

O185

EcbA and SgrA, Two LPXTG surface proteins of *Enterococcus faecium* CC17 bind to components of the extracellular matrix

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Background: The incidence of infections caused by *Enterococcus faecium* has dramatically increased in hospitals world wide. Nosocomial outbreak associated and most clinical isolates cluster together by multilocus sequence typing in a hospital-selected genogroup, designated CC17. Recently, ecbA and sgrA (also known as orf2430 and orf2351) were found to be specifically enriched in CC17 *E. faecium* strains (Hendrickx *et al.*, 2007). The objective was to functionally characterise EcbA and SgrA, which are potential targets for immunotherapy to prevent and treat CC17 *E. faecium* infections.

Methods: Both ecbA and sgrA were cloned, expressed as 6xHis fusion proteins (rEcbA and rSgrA) in *Escherichia coli* and purified using affinity chromatography. Binding of rEcbA and rSgrA to collagen types I to V, fibronectin, fibrinogen, and BSA (negative control) was assessed by ELISA and Far Western blot. Reverse transcriptase (RT) PCR was used to detect mRNA transcripts of ecbA and sgrA. Transmission electron microscopy (TEM) was used to determine association of the LPXTG surface proteins with the cell wall.

Results: Purified rEcbA and rSgrA bound to immobilised collagen type V and fibrinogen, respectively, in a concentration dependent and saturable manner and not to fibronectin, collagen types I, III and IV or BSA. Far Western blotting indicated that rSgrA bound to the alpha, bèta and gamma subunits of fibrinogen and rEcbA to the alpha subunit of collagen type V. Using RT-PCR, mRNA transcripts of sgrA were detected in all phases of growth at 37ffiC, whereas for ecbA only in mid to late log cultures. TEM demonstrated association of EcbA and SgrA with the cell wall.

Conclusion: We showed that ecbA and sgrA are expressed at mRNA level, encode LPXTG surface proteins, and mediate adherence to collagen type V and fibrinogen respectively. This makes EcbA and SgrA, together with Acm, members of a new MSCRAMM (microbial surface component recognizing adhesive macromolecules) family in *E. faecium*. The ability to adhere to fibrinogen and collagen type V may contribute to enhanced pathogenesis of CC17 *E. faecium* in hospital-related infections, which could explain its recent emergence as nosocomial pathogen.

O186

An investigation into the cause of the 1983-1987 whooping cough epidemic in the Netherlands

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Introduction: Whooping cough, caused by the gram-negative bacteria *Bordetella pertussis*, is a disease of the upper respiratory tract and most severe for unvaccinated infants. Despite more than 50 years of vaccination, whooping cough is still an endemic infectious disease with regular epidemic outbreaks. In the last 20 years, two epidemics were observed in the Netherlands. The first occurred in the period 1983 to 1987, while the second started in 1996 and is still ongoing. The cause of the first epidemic is contentious. At the time it was suggested to be an artifact caused by changes in diagnostic procedures. An alternative explanation is that the epidemic was caused by a reduction in the vaccine dose, which was implemented to reduce side effects. The aim of this study was to elucidate the cause of the epidemic by identifying changes in the pathogen population.

Methods: 252 *B. pertussis* clinical isolates, selected from the period 1965-1992, were serotyped and genotyped. Genotyping was focused on the identification of alleles for three important virulence factors of *B. pertussis*; pertussis toxin (Ptx), pertactin (Prn) and tracheal colonisation factor (TcfA). Further, strains were typed by Multiple-Locus Variable-number tandem repeat Analysis (MLVA).
Results: The analysed period could be divided in three periods: 1965-1979 (79 isolates), 1980-1988 (141 isolates) and 1989-1992 (32 isolates), where the second period represented the epidemic period. Serotype 3 strains dominated the first period (78%) and third period (70%), while serotype 2 strains dominated in the intermediate, epidemic period (71%). No changes were observed in the frequencies of ptx and tcfA alleles. However, the prn1 allele (also found in the vaccine strains) was gradually replaced by two novel (non-vaccine type) alleles prn2 and prn3. New MLVA types appeared and dominated the epidemic period but disappeared when the epidemic subsided.
Conclusion: The *B. pertussis* population in the epidemic period was distinct from the pre- and post-epidemic periods, suggesting that the lowering of the vaccine dose had a significant effect on host immunity. These observations, together with the increased hospitalisations observed in the epidemic period, suggest that the increased notifications were not a surveillance artifact, but reflected a true epidemic caused by decreasing the vaccine dose.

O187

Characterization of the microbiota from a benzene-degrading, nitrate reducing bioreactor

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Benzene is an industrial compound that is used to produce various chemicals, drugs, and fuel oils. Due to its high toxicity and solubility compared to other monoaromatic hydrocarbons, much attention is focused on biodegradation of benzene, especially in the absence of molecular oxygen. At TNO a benzene-degrading, nitrate-reducing bioreactor has been obtained and operated stable for more than four years. However, the microbiota have not been characterised yet. The aim of this study was to get insight into microbial community of this bioreactor by using classical and molecular approaches. Suspensed cells and biofilm cells from glass wall of the bioreactor were serially diluted (from 10-1 until 10-10) in flasks with benzene (20 µM), acetate (10mM) or benzoate (500 µM). Nitrate (5 mM) was used as electron acceptor. Also a dilution series with benzene (20 µM) and oxygen (5% on the headspace) was prepared. The cultures were grown on AW-1 sulfate medium (Wolterink *et al.*, 2002). The microbiota from the bioreactor and from the highest positive dilutions using nitrate as acceptor electron (10-1 for benzene; 10-10 for benzoate and acetate) was analysed by denaturing gradient gel electrophoresis (DGGE).
Degradation of benzene occurred with nitrate (rate 2 µM/day) and oxygen (rate of 3.3 µM/day) in the flasks inoculated with biofilm and suspensed cells. Also benzoate and acetate were degraded at rate of 23 µM/day and 1 mM/day, respectively.
The DGGE patterns of the samples from the bioreactor were complex even though the bioreactor was stable for four years. In the cultures grown on benzene and nitrate at least four bands were observed and the patterns were similar between biofilm and suspensed cells (data not shown). One distinct band was observed in all cultures (acetate, benzoate, and benzene), but the others were present only in the flasks with benzene. One of them was favoured by benzene addition and probably corresponds to the microorganism responsible for benzene degradation. In the culture growing with benzoate three distinct bands were obtained, two of them were also present in the culture growing with acetate, but one of them was observed only in the culture with benzoate, indicating that this microorganism could be responsible for benzoate degradation. The sequencing of the dominant bands is under present investigation.

O188

Regulation of the energy metabolism in *Campylobacter jejuni*

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An important adaptation mechanism for bacteria to survive in different environments is their ability to regulate their energy metabolism. To maximise energy generation, bacteria have evolved multiple metabolic pathways regulated through complex regulatory mechanisms. The human gut pathogen *Campylobacter jejuni* changes its energy metabolism in response to oxygen availability, allowing it to thrive in aerobic external environments and under the oxygen-starved conditions present in the host. In the absence of oxygen, less rewarding electron acceptors such as nitrate, fumarate, TMAO or DMSO are used. Although many conserved genes involved in the energy metabolism are present in *C. jejuni*, all known bacterial transcriptional regulators involved in energy metabolism are absence. In this study we found a *C. jejuni* response regulator to play an important role in the energy

metabolism in this organism. Micro-array analysis and realtime RT-PCR results allowed us to identify a number of genes regulated by this regulator. Furthermore, our results indicate that this response regulator is activated during oxygen limitation in the presence of nitrate and that it is important for colonisation/persistence of the host.

O189

Diversity in sporulation and germination of *Bacillus cereus* strains

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Four strains of *Bacillus cereus* were studied for their sporulation and germination behaviour. These four strains were the enterotoxin producing *B. cereus* ATCC 14579 and ATCC 10987, the emetic toxin producing PAL25 (AH187) and the psychrotolerant strain *Bacillus weihenstephanensis* KBAB4. Diversity between the strains was observed in growth, sporulation and germination. The four strains were grown and subsequently sporulated at 30 °C in nutrient broth supplemented with maltose and sporulation elements. The biggest difference in onset of sporulation was observed between the strains PAL25 and ATCC 14579. For PAL25, the first spores were observed 2h after the end of growth, whereas for ATCC 14579 the first spores were detected 8h after the end of growth. Moreover, the total time for sporulation also differed between the four strains, sporulation for PAL25 and ATCC 10987 was completed in 2h, whereas for ATCC 14579 and KBAB4 sporulation was completed only after 7h. This shows the emetic strain to complete sporulation 4h after the end of growth, whereas the enterotoxic strains required at least 8 to 15h. The strains studied showed sporulation efficiencies ranging from 90 to 95%. In silico analysis of the available genomes revealed the number of nutrient receptor encoding operons to be different for the four strains. For *B. cereus* PAL25 eight operons encoding nutrient receptors were identified, for *B. cereus* ATCC 14579 seven, and for *B. cereus* ATCC 10987 and *B. weihenstephanensis* KBAB4 only six of these operons were identified. Phylogenetic analysis showed that within this group for all four strains at least one specific nutrient receptor was present. Moreover, for *B. cereus* PAL25 and *B. weihenstephanensis* KBAB4 one of their nutrient receptors is encoded on a plasmid. A putative G promoter binding site could be identified in front of all nutrient receptor encoding operons, and a quantitative PCR showed expression of these operons in the different strains at the start of sporulation. In germination experiments at 30°C it could be observed that the *B. cereus* ATCC 14579 strain germinated in response to the largest amount of nutrients, including the amino acids L-alanine (1 mM), L-cysteine (1mM), L-threonine (1mM) and L-glutamine (1mM), whereas for the other three strains no clear response to amino acids could be monitored. Notably, all strains germinated in response to a combination of the nutrients inosine (2.5 mM) and L-alanine (5 mM). Until now, no link could be established between the nutrient receptors that could be identified and the specific nutrient-induced germination properties, indicating that next to nutrient receptors other factors of the spore are of great importance in nutrient-triggered germination of *B. cereus* strains. Therefore, future research will be aimed at determining the properties for the different strains of the outer part of the spores and of the enzymes degrading these outer parts.

O190

The peculiar distribution of simple sequence repeats

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Introduction: The surge of available sequence data has paved the way for large-scale systematic analyses into mutational patterns. Motifs such as simple sequence repeats (SSRs) have received ample attention for their epidemiological implications (as 'microsatellites'), but also for their adaptive relevance, and their prevalence has therefore been analysed in numerous microbial genomes. SSRs are mutational hotspots, and their disruptive ability largely explains their relative underrepresentation in protein coding regions. But SSRs are also associated with the phenomenon of phase variation; depending on the exact length of the repeat in the gene and its effect on the reading frame, genes can be switched on or off. By exploring genome-wide distribution patterns of SSRs within coding regions, we can examine the evolutionary consequences of the selective pressures that have acted on these disruptive repeats.
Methods: First we analysed the location of SSRs in phase-variable genes in *Neisseria meningitidis*. Next we extended the analysis to all coding regions in a large number of sequenced genomes of bacteria and archaea. Sequence data were obtained from NCBI, and motif searches were carried out with perl scripts.
Results: The location of both mononucleotide and oligo-nucleotide repeats in known phase-variable genes are biased towards the 5' end of genes. Furthermore, in genome-wide analyses of protein coding regions, we found that homopolymeric tracts are in fact universally biased towards the 5' end of genes. Also, we showed that this location bias becomes more pervasive with an increased repeat length. Finally, when discriminating between

essential and non-essential genes, we observed that, although essential genes display a similar repeat location bias, there are fewer essential genes that contain repeats.
Conclusion: Motif analyses clearly show a pervasive and universal bias of sequence repeats towards the 5' end of protein coding genes. We suggest that this location bias arose to minimise the costs associated with transcribing and translating non-functional genes. This suggests that pseudogenes, which are traditionally seen as selectively neutral sequences, need in fact not be evolving in a strictly neutral manner.

O191

Systems biology of *Clostridium acetobutylicum* understanding solvent production

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Clostridium acetobutylicum, the non-pathogenic model organism of clostridia, is able to fundamentally change its metabolism from the production of organic acids (acetate, butyrate) to solvents (acetone, butanol, ethanol) dependent on the culture conditions. This characteristic has already been used in large scale fermentation processes. The involved metabolites and enzymes are well-established, however, little is known about the regulation of the metabolic shift, the characteristics of key-regulatory elements as well as bottlenecks of the metabolism. Goal of the Systems Biology of Microorganisms (´SysMo`) collaborative project ´COSMIC` (*Clostridium acetobutylicum* Systems Microbiology) is to increase the knowledge of this clostridial metabolism and its regulatory patterns and to establish it as a paradigm of the clostridial systems biology. The project is carried out in close collaboration with research groups in Rostock, Ulm, Göttingen, and Nottingham. The focus will be on the key regulatory and metabolic events that occur during the exposure to stress conditions in general and butanol stress, in particular. The dynamic, quantitative data obtained will be used to mathematically model the various interactions at the cellular level. Therefore, *C. acetobutylicum*, grown in a well-known phosphate limited chemostat under defined conditions guaranteeing the direct control of the metabolic shift, will be analysed by different state-of-the-art methods. This includes DNA-microarrays, proteomics and metabolomics using LC-MS-NMR and LC-FTMS. Currently, research focuses on the analysis of the oscillation of the culture`s redox potential under solvent producing conditions. A net change of the redox potential of 10 mV up to 18 mV depending on the dilution rate (0.055 h-1 to 0.21 h-1) could be observed. As the dilution rate defines the growth rate and cells` need of metabolites, especially energy and reduction equivalents, this effect will give information about so far unknown regulation patterns.

O194

First detection of transferable metallo-beta-lactamases in the Netherlands

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Introduction: Metallo-beta-lactamases (MBLs) are of special importance due to their role in microbial resistance to beta-lactam antimicrobial agents, especially carbapenems. The prevalence of *Pseudomonas aeruginosa* strains that produce MBLs is increasing worldwide. Currently, nothing is known on the prevalence of these strains in the Netherlands. We determined the prevalence and the type(s) of MBLs produced by strains of *Pseudomonas* species isolated at the VU University Medical Centre, in Amsterdam
Methods: During the first 7 months of 2007, the susceptibility profiles of 650 isolates from 329 patients were analysed. Isolates with decreased susceptibility to imipenem- and/or meropenem were tested for the production of MBL with MBL E-test. Site of infection/ colonisation, and location of the patient in the hospital at the moment of strain isolation were recorded. DNA fingerprinting of the strains was performed with amplified fragment length polymorphism analysis (AFLP) to determine clonal relations. Transfer of resistance was investigated with conjugation experiments. The presence of VIM MBL genes was determined by PCR with generic primers . Further molecular characterisation of resistance genes confirmed the resistance pattern.
Results: Of 329 patients, 12 (3.6%) carried a MBL-positive *Pseudomonas* strain. Six strains were associated with clinical infection. The urinary tract was the most common site of infection. Epidemiological and molecular analysis showed no indications for a clonal outbreak in our hospital. Results of the conjugation experiment showed that these resistance genes were transferable. VIM PCR confirmed the production of VIM MBL in *Pseudomonas* isolates of two patients.
Conclusion: To the best of our knowledge, this is the first report that describes infections caused by MBL-producing *Pseudomonas* spp. strains in the Netherlands. Although the prevalence of metallo-beta-lactamases is yet low, these results signal the need for development of accurate diagnostic tests by clinical laboratories to detect the presence of these resistance genes, so that specific isolation precautions can be taken to control their dissemination.

P01

Genotype distribution in acute and chronic hepatitis B in the Netherlands

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Objective: To obtain hepatitis B virus (HBV) genotype distributions of acute and chronic patients in the Netherlands and to study dissemination of HBV.
Methods: Blood samples and epidemiological data from notified acute and chronic HBV patients were collected in 2005/2006. Of the S-gene 648 bp was sequenced and analysed phylogenetically and for clustering.
Results: Via the Dutch mandatory reporting system we identified 225 acute and 944 chronic HBV positives. Reasons for HBV serologic testing was having clinical symptoms (82%) in acute patients and contact tracing (38%) or pregnancy screening (15%) in chronic carriers. Blood samples were available from 117 acute (52% of notified) and 137 chronic HBV patients (15% of notified). Acute HBV was transmitted by sexual contact; 33% by homosexual men (MSM) and 27% heterosexual. For chronic HBV the most important route was vertical transmission (58%); these people contracted HBV in a high endemic country. Genotypes A-F were found. Genotype A was significantly more present in acute (68%) than in chronic HBV patients (30%, $p<0.001$) and was found mostly in MSM. Genotype D was more prevalent in chronic than in acute HBV patients (38% versus 22%, $p=0.003$). Cluster analysis on sequences of acute patients identified a large genotype A cluster with many identical strains, from Dutch MSM. In chronic HBV patients more subclusters were found and less identical strains.
Conclusion: Genotype A was most common in acute HBV patients, mainly in Dutch MSM whereas in chronic HBV patients more sequence diversity and mainly genotype D was found, mostly in immigrants. The differences in genotype distribution between acute and chronic HBV infections probably reflect the differences in risk groups in the Netherlands.

P02

Methicillin-resistant *Staphylococcus intermedius* (MRSI) infections in dogs and cats: zoonotic potential and environmental contamination

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Introduction: *Staphylococcus intermedius* is a commensal and a pathogen in dogs and cats. Starting in 2006, we observed a clear increase in the number of isolations of methicillin-resistant *S. intermedius* (MRSI) from submitted routine samples from pet animals. MRSI was found in a variety of clinical conditions like otitis externa, (post-operative) wound infections and skin infections. In this study the risk of transmission of MRSI to humans and the environmental contamination was assessed through follow-up of MRSI-infected pets.
Methods: Fifteen MRSI positive patients (13 dogs and 2 cats) were selected from routine submissions received by the Veterinary Microbiological Diagnostic Centre (VMDC) of Utrecht University. The households of these pets were visited and environmental (dust) samples were taken as well as samples from the nose of the humans and other pet animals present. The 10 veterinary clinics that had been visited by one of the MRSI-positive cases were also studied. At two of these clinics MRSI had been isolated from humans (n=4) at the time the clinical case visited the clinic. Environmental samples and nose samples from personnel and from their pets if they were routinely brought along to the clinic, were collected. The samples were analysed for the presence of *S. intermedius* by using standard techniques and the mecA gene was detected by PCR.
Results: At present, 19/35 environmental household samples were positive for MRSI, 2/4 of the household contact pets (one cat and one dog) were positive for MRSI and none of the 11 human samples were positive. In all cases the persistent shedding of the index case was confirmed. Analyses of 6/10 clinics are complete; 13/83 environmental samples were positive for MRSI. In none of the 4 contact animals (1 dog and 3 cats) MRSI was detected. None of the sampled humans (n=66) was found positive for MRSI.
Conclusions: These data show that MRSI-positive clinical cases spread MRSI into the environment both in households and in the veterinary clinics they visited. Transmission from patients (animals) to healthy contact animals may occur. No MRSI positive human cases were identified. These results, together with the four MRSI-positive human cases in two of the veterinary clinics found before, demonstrate that MRSI can be found in the nares of exposed humans and household pets and that they readily contaminate the household and clinic environment. This implicates that the environment in the clinic and the household are potential sources of MRSI-infection.

P03

Changes in the population structure of *Staphylococcus aureus* isolates from ICU patients in the Netherlands between 1996 and 2006

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Introduction: The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) is increasing in the Netherlands. This increase might be due to a genetic macro-evolution in methicillin-susceptible *S. aureus* (MSSA) isolates. MRSA originated through the transfer of the mobile resistant element Staphylococcal Cassette Chromosome mec (SCCmec) into MSSA. We studied the changes in the population structure of *S. aureus* and the macro-evolutions of MSSA among isolates from ICU patients.

Methods: *S. aureus* isolates (n=856) from ICU patients in the Netherlands were isolated between 1996 and 2006 within a large resistance surveillance program. They were yearly collected from January until July in two university hospitals and twelve general hospitals. Only one isolate per patient was included in the study. The genetic background of the isolates was determined with spa typing and the algorithm Based Upon Repeat Pattern (BURP).

Results: A total of 287 spa types were observed, 58 of them were identified new spa types and three new spa repeats were found among the new spa types. Among the *S. aureus* isolates, fourteen spa-clonal complexes were found. Ten isolates could not be clustered and were marked as singletons. Three of the singletons were classified as ST109. Seventy isolates were excluded from the analyses. A genetic background common to MRSA clones, e.g. CC1, CC5, CC8, CC22, CC30 and CC45 was observed among 46% of the isolates (n=393). The remaining isolates were associated with CC7, CC15, CC25, CC26, CC51, CC97 and CC101.

Conclusions:

1. Almost half of the MSSA isolates had a genetic background common to MRSA clones.
2. The genetic background of the MSSA isolates was comparable with an earlier study in the Netherlands, where the genetic background of MSSA isolates of patients from general practitioners was determined.
3. Several clonal complexes not related to MRSA clones were prevalent in the hospitals.
5. Further studies to the changes over time will be performed.

P04

***Spa* typing of *Staphylococcus aureus*, a comparison with PCR fingerprinting**

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Introduction: In our lab, typing of *Staphylococcus aureus* is performed with PCR fingerprinting. Generally, suitable results are generated within one experiment. However, inter-experiment differences are observed and faint bands cause difficulties with interpretation. To allow the construction of a database, a method with minimal inter-experiment variation is required. Sequence based typing methods meet this condition. We implemented *spa* typing of *S. aureus* and compared the results with our routine PCR-fingerprinting method in 27 local outbreak investigations.

Methods: For PCR fingerprinting, DNA was isolated from *S. aureus* cultures using a MagNAPure Compact (Roche). PCR was performed with ERIC1 – ERIC2 primers using Ready-To-Go PCR Beads (Amersham) and PCR products were analysed on agarose gels. The scanned photographs were further analysed with Bionumerics software (Applied Maths).

Spa PCR was performed on crude lysates and PCR products were analysed using an ABI 3130 sequence analyser (Applied Biosystems). *Spa* types were assigned and analysed with the *spa* typing plugin of bionumerics.

Results: Duplicate *spa* typing of 64 strains resulted in identical results for each strain. In 24 out of 27 outbreak investigations, similar groups of identical strains were identified with *spa* typing and PCR fingerprinting. Cluster analysis showed differences in the calculated similarity between strains based on PCR-fingerprinting and *spa* typing results. In 3 out of 27 outbreak investigations, a different *spa* type was assigned to strains with an identical PCR-fingerprinting pattern or vice versa. The *spa* typing results fitted well with the epidemiological information of the outbreaks.

Conclusions: *Spa* typing was robust and no inter-experiment variation of results was observed, allowing the construction of a database. The discriminatory power of *spa* typing and PCR fingerprinting was similar and appropriate for molecular epidemiological studies of *S. aureus* strains in local outbreaks. The observed differences between *spa* typing and PCR fingerprinting in calculated similarity can be expected, considering the fact that *spa* typing is a single locus typing method, whereas various regions of the genome are involved in PCR-fingerprinting. The reproducibility of *spa* typing, combined with the possibilities for automation, renders *spa* typing an attractive typing method for diagnostic laboratories with a sequence facility.

P05

Staphylococcus aureus prevalence and transmission dynamics on different European Intensive Care Units

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Background: Approximately 30% of all patients admitted to an intensive care unit (ICU) acquire an infection and *Staphylococcus aureus* is one of the main pathogens in these infections. Colonisation with *S. aureus* is an important risk factor for developing an infection. In this study we have assessed the number of acquisitions of *S. aureus* on 6 ICUs in 6 European countries to determine optimal ICU-setting and infection control measures to reduce acquisition of *S. aureus* on ICUs.

Methods: Six ICUs participated in the study, of which one treated all patients in single rooms, one isolated patients after acquisition of resistant pathogens and 4 did not isolate patients. During a three month period all patients admitted to the ICUs were swabbed on admission, twice weekly and on discharge. Samples were cultured and processed using the same study protocol in all centres: 24h culturing in TSB enrichment broth with 5 mg/l aztreonam, followed by 24h culture on BA- and MSA plate. Patient information was obtained. The principal investigator obtained relevant information on all ICUs.

Results: A total of 629 patients were admitted to the ICUs during the study period, of whom 224 (36%) were found to be colonised by *S. aureus* at least once during their ICU stay. Of these 45 patients (20%) acquired the colonisation during their ICU stay. The percentage of patients acquiring *S. aureus* was assessed per centre and differed strongly (4.3% to 15.4%). Treatment of all patients in single rooms was the main factor in the reduction of the number of acquisitions of *S. aureus* (p= 0.07). The level of hand hygiene, nurse-bed ratio, length of stay, antibiotic pressure, and colonisation pressure did not significantly correlate with a lower number of acquisitions of *S. aureus* in this study, even when MRSA and MSSA were assessed separately.

Conclusions: Of all infection control measures, treatment of all patients in single rooms on an ICU had the highest impact. As hand hygiene compliance and health care worker workload have been shown to have a high impact, but are also difficult to improve, treatment of patients in single rooms should be considered as future standard.

P06

Interpretation of DiversiLab Typing Data requires an accurate cutoff for strain discrimination

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Introduction: For routine molecular typing in medical microbiology laboratories typing methods are needed that are highly reproducible, technically easy to perform and rapid. The method should allow differentiation of unrelated and related strains and is preferably applicable for typing of all bacterial species that contribute to hospital-acquired infections. We evaluated a commercial available molecular typing system, the DiversilabTM System (Biomérieux) for differentiation of strains of *Staphylococcus aureus* and compared it with spa typing and PCR fingerprinting using ERIC primers.

Methods: The strains consisted of 10 *S. aureus* isolates, all obtained in the same hospital, that were sent to the Public Health Laboratory for typing between 2003 and 2007. Repetitive extragenic palindromic (REP-PCR) sequences were amplified using the DiversilabTM kit. PCR fragments were analysed on an Agilent® 2100 Bioanalyser and fingerprints were analysed in the supplied web-based software. Spa typing was performed according to a slightly modified RIDOM procedure. PCR fingerprinting was performed with ERIC1 and ERIC2 primers. Data were analysed with BioNumerics software.

Results: Spa typing and PCR fingerprinting identified 5 different types, that belonged to two clusters consisting of 3 strains, one cluster with 2 strains and 2 unrelated strains. With the DiversiLab typing, concordant results were obtained when the cutoff for similarity was set at 98%. A cutoff at 95% identified 3 different REP-PCR types and was less discriminative.

Conclusion: The DiversiLab system is robust and can be performed for typing in routine laboratories. However, the cutoff for strain discrimination has to be established for each species that is typed. A prerequisite for the use of this system is the availability of a database with REP-PCR and epidemiological data, from unrelated strains, outbreak strains, well defined reference strains and prospective clinical isolates.

P07

Coagulase-negative staphylococcal carriage among NICU personnel

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Introduction: Coagulase-negative staphylococci (CoNS) are a major cause of late-onset sepsis in neonatal intensive care units (NICU). Since these microorganisms are part of the normal skin flora, we determined differences between CoNS isolated from the hands of NICU personnel and a control group from the general population. Furtermore, we investigated the presence of clonality of CoNS among personnel and the effect of a period of absence from the NICU.

Methods: This study was performed from June to September 2005 at the NICU of the Erasmus MC. Every two weeks and directly after vacation, we cultured CoNS from the thumbs of nurses and physicians using phenol-mannitol salt agar plates. The control group consisted of people at the non-medical setting of the Erasmus University Rotterdam. These were cultured only once. The CoNS blood isolates from the sample period were collected as well.

Species identification was performed for all isolates. Antibiotic susceptibility patterns of personnel and control isolates were determined, as well as presence of mecA and icaA genes. Personnel and blood isolates were genotyped by restricted fragment end labeling.

Results: Sixty-nine personnel members were included. We analysed 51 isolates before and 80 isolates after vacation, as well as186 control and 29 blood isolates. The pre-vacation, post-vacation and control isolates consisted of S. warneri (32-47%), *Staphylococcus epidermidis* (30-36%), *Staphylococcus haemolyticus* (11-14%), and other species (8-19%). The blood isolates consisted of *S. epidermidis* (90%), *S. haemolyticus* (7%) and other species (3%). Oxacillin, gentamicin and penicillin resistance, multiresistance and mecA carriage were significantly higher in the personnel groups than in the control group. Before vacation, gentamicin resistance, and mecA carriage were significantly higher than after vacation. Carriage of icaA was significantly higher in the before vacation group compared to the controls (all $p<0.05$).

Genotyping showed great heterogeneity in personnel isolates. Only 7 (23%) health care workers carried strongly related isolates before and after vacation. Among the blood isolates three large groups of closely related strains, comprising a total of 21 strains (72%), we found. Of the 29 blood isolates, 26 (90%) were closely related to personnel isolates.

Conclusions:

1. CoNS on the hands of NICU personnel differ greatly from CoNS on the hands of the general population.
2. There was no clonality in CoNS carriage among NICU personnel.
3. Blood isolates are closely related to strains on the hands of NICU personnel.
4. Other CoNS are found on the skin after a period of absence from the NICU.

P08

Antibiotic resistance characteristics of CoNS isolated from neonates in the first weeks of life

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Introduction: Coagulase-negative staphylococci (CoNS) are a major cause of late-onset sepsis in neonatal intensive care units (NICU). Furthermore, CoNS skin isolates from NICU personnel are more antibiotic resistant than those of the general population. Since neonates are exposed to both CoNS from the mother as well as the NICU personnel, their skin and intestine flora may change during admission at the NICU. Therefore, we studied the CoNS colonisation of the skin and intestine of neonates during their admission at the NICU and determined antibiotic resistance characteristics of these CoNS.

Methods: This study was performed from November 2006 to March 2007 at the NICU of the Erasmus MC, Rotterdam. Forty-one neonates with an expected admission of at least two weeks were included within 24 hours after birth. We isolated CoNS from skin and feces of infants at 1, 2, 3, 7, 14 and 21 days after birth. Furthermore, we isolated skin CoNS of the mother once in the first three days after birth and after 1, 2, and 3 weeks. Antibiotic susceptibility patterns and the presence of the mecA gene of all isolates were determined

Results: CoNS isolates from feces were generally more antibiotic resistant and mecA positive than other isolates. Penicillin, gentamicin and cefoxitin resistance, as well as multiresistance and mecA carriage of CoNS from skin and feces from neonates were high (40-100%) during the entire sample period. Antibiotic resistance and mecA carriage were consistently low (0 – 27%) in maternal isolates, except for penicillin resistance (15-57%). We compared the CoNS skin isolates at 2 days with those at 7 days. Levofloxacin, gentamicin, erythromycin and cefoxitin resistance, as well as multiresistance and mecA carriage was significantly higher at 7 days among the skin isolates. Among the feces isolates, levofloxacin, co-trimoxazol and erythromycin resistance were significantly higher at 7 days (all $p<0.05$).

Conclusions:

1. Antibiotic resistance and mecA carriage in CoNS on the skin of infants rise during admission.

2. Antibiotic resistance and mecA carriage in CoNS in the intestine of infants is generally high from birth on.
3. Antibiotic resistance and mecA carriage in CoNS on the skin of mothers is low in comparison to the infant isolates.

P09

Endemic *Clostridium difficile* associated disease. Interim results of a case control study at the Leiden University Medical Center

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Background: After emerging outbreaks of *Clostridium difficile* associated disease (CDAD) in Canada and the United States, outbreaks of CDAD were also reported in the Netherlands.
Methods: A multi-centre case-control study was initiated to investigate the national incidence of CDAD and to identify risk factors for the disease in the epidemic and the endemic situation. From July 2006 through December 2007 all hospitalised CDAD patients at the Leiden University Medical Centre (LUMC) were included; results from these patients are presented here. CDAD patients were defined as patients with diarrhoea and a toxin test positive for *C. difficile*. We compared demographic and clinical data among cases (n=43) and both controls without diarrhoea (group 1, n=43) and controls with diarrhoea but with a toxin test negative for *C. difficile* (group 2, n=30). A logistic regression method was used to identify independent risk factors for CDAD.
Results: In the endemic situation at the LUMC, the CDAD incidence was 2.1 per 10.000 inpatient-days. Cases were more likely to have received antibiotics. Controls in group 2 however, received a larger number of antibiotics and a larger number of defined daily doses (DDDs). In comparison with group 1, independent risk factors for CDAD included age >65 years, hospitalisation in the preceeding 3 months and the use of 2nd generation cephalosporins. In comparison with group 2, risk factors for CDAD included age >65 years, hospitalisation in the preceding 3 months and antibiotic treatment. A trend towards a higher risk associated with the use of 2nd generation cephalosporins was found in comparison with group 2.
Conclusions: The results of this study suggest that hospitalisation in the preceding 3 months, older age and possibly the use of 2nd generation cephalosporins are independent risk factors for CDAD in an endemic situation at the LUMC. The number and duration of prescribed antibiotics or DDDs did not influence the risk of CDAD in this situation.

P10

Genetic diversity of methicillin-resistant *Staphylococcus aureus* in a Dutch university hospital between 2002 and 2006

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Introduction: The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in the Netherlands is still low (circa 2%), but an increase in prevalence has been observed during recent years. This may be due to increased cross-border healthcare, which is especially important for our university hospital (azM) situated near the borders of three countries (Belgium, Germany and the Netherlands with a MRSA prevalence of 24%, 14%, and 2% respectively), or may be due to an increase of the prevalence of community-associated (CA) MRSA. The aim of this study was to test this hypothesis by characterizing MRSA isolates cultured in the azM during recent years.
Methods: MRSA isolates (n=175), isolated between January 2002 and December 2006, were characterised by spa typing, together with the algorithm Based Upon Repeat Pattern (BURP), and typing of the resistance element Staphylococcal Cassette Chromosome mec (SCCmec). The first isolate of each patient was analysed, bringing the number of isolates per year to 32, 29, 35, 39, and 40, respectively. The presence of Panton Valentine leukocidin (PVL), as a genetic marker for CA-MRSA, was determined by real-time PCR.
Results: Between 2002 and 2005, the Pediatric clone was the most prevalent MRSA clone in the azM. However, the spa type of the Pediatric clone changed from t002 to t447 during these years. Besides the Pediatric clone, other endemic MRSA clones, such as the Iberian clone, the New York/Japan clone, the Southern Germany clone, and the UK EMRSA-3 clone were observed sporadically. From 2005, the genetic background of the MRSA clones became more diverse and during 2006, the Pediatric clone was only sporadically observed. From 2005, the Berlin clone, the New York/Japan clone, the UK EMRSA-2/-6 clone, and the UK EMRSA-15 clone were increasingly observed. Besides these endemic MRSA clones, several other MRSA clones, such as the Brazilian/Hungarian clone, were observed sporadically during the study period. Four of the MRSA strains, isolated in 2005 and 2006, harbored PVL and SCCmec type IV, and belonged to the USA300 clone (n=1) and the European CA-MRSA clone (n=3).
Conclusions:
1. MRSA isolates associated with the New York/Japan clone, the Pediatric clone, the Southern Germany clone,

the UK EMRSA-3 clone, and the UK EMRSA-15 clone have not been described before in the Netherlands. These MRSA clones could have been imported into the Netherlands through cross-border healthcare.
2. The increase of the MRSA prevalence in the Netherlands is probably due to the introduction of new MRSA clones into the Netherlands.
3. The low prevalence of SCCmec type IV- and PVL-positive MRSA isolates suggests that the prevalence of CA-MRSA is low in the azM.

P11

Evaluation and implementation of a chromogenic agar medium for the detection of salmonella in stool in routine laboratory diagnostics

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Introduction: Each year our laboratory processes more than 5,000 stool samples from patients with gastroenteritis for culture of *Salmonella* species. On conventional agar media recognition of *Salmonella* is generally based on lactose fermentation and H2S production. Specificity is low and lactose-fermenting salmonellae may go undetected. We evaluated which chromogenic *Salmonella* agar medium would improve the sensitivity and specificity of our *Salmonella* stool culture protocol and thereby decrease workload and costs.
Methods: In the first phase of the study, we tested BBL CHROMagar *Salmonella* (BBL; Becton Dickinson, the Netherlands), SM ID2 (SM; bioMérieux, France) and Oxoid *Salmonella* Chromogenic Medium (OX; Oxoid, United Kingdom) with 34 *Salmonella* strains and 19 non-*Salmonella* strains. Secondly, we prospectively analysed the two deemed most appropriate media with stool samples submitted from general practice patients with gastroenteritis. The chromogenic media were processed alongside the conventional media *Salmonella-Shigella* agar (SS), Xylose-Lysine-Desoxycholate agar (XLD) and Hektoen-Enteric agar (HE) according to the current stool culture protocol. This included subculturing after broth enrichment and presumptive identification of colonies suspected of being *Salmonella* using conventional biochemical tests.
Results: With stock isolates, sensitivities of BBL, SM and OX were 100%, 94% and 85%, respectively after both 24h and 48h of incubation. Specificities were 84%, 90% and 74% after 24h and 68%, 79% and 53% after 48h, respectively. Based on this, we continued the study with BBL and SM. Of 1339 stool samples, 32 (2.4%) were *Salmonella* positive on at least one medium. After direct plating and enrichment sensitivities for SS, XLD, HE, BBL and SM were 72, 97, 84, 88 and 78%, specificities were 79, 80, 81, 96 and 96%, respectively. On SM more overgrowth of faecal flora was found compared to BBL. XLD and BBL together yielded a sensitivity of 100%. The higher specificities of the chromogenic media reduced the required amount of presumptive identification considerably.
Conclusion: Of the chromogenic media tested, we found BBL the most appropriate substitute for conventional *Salmonella* media. We expect a combination with XLD to give the best results for *Salmonella* and to provide optimal isolation of Shigella. Adaptation of the culture protocol to this effect will result in a substantial decrease of costs of *Salmonella* diagnosis in our laboratory.

P12

A case of fatal, undetected *Haemophilus parainfluenzae* endocarditis: the role of the bloodculture system

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Objectives: We report a fatal case of *Haemophilus parainfluenzae* endocarditis. The causative strain was cultured from the mitral valve, but not detected in our bloodculture system. Therefore we evaluated growth of this, and other, *H. parainfluenzae* strains in the BacT/ALERT 3D™ bloodculture system (BioMérieux) and compared it to another bloodculture system, the BacTec 9240™ (Becton Dickinson). To evaluate whether observed differences in growth of H. parainfluenzae between these two bloodculture systems was a nationwide phenomenon, data from the Dutch Infection Surveillance Information System (ISIS) were used. In this surveillance system, 17 medical microbiology laboratories participate, covering 36% of the country.
Methods: Four clinical *H. parainfluenzae* strains, including the patient's strain, were tested in the two bloodculture systems. Each bloodculture bottle, filled with 10 ml of healthy donor blood, was inoculated with 10 and 100 cfu/ml of the different *H. parainfluenzae* strains. Time to detection was compared between the two bloodculture systems.
Bloodculture data from ISIS laboratories, collected between 1994 until September 2007, were obtained. Ten of 17 participating laboratories rely on a BacTec™ system and seven on a BacT/ALERT™ system. The number of patients with *Staphylococcus aureus* and *Escherichia coli* bacteremia was used as a denominator value to normalise the number of patient with *H. parainfluenzae* bacteremia diagnosed in the laboratories using the two different bloodculture systems.
Results: One of four *H. parainfluenzae* strains tested was detected by the BacT/ALERT™ 3D system at 100 cfu/ml

blood. The BacTec™ 9240 system detected all *H. parainfluenzae* strains in both low and high concentrations after two to five days. If growth was detected in both systems, time to positivity was lower in the BacT/ALERT™ system.
Using cases of *S. aureus* and *E. coli* bacteremias as a denominator value (6169 cases in ten labs using BacTec™, and 6975 cases in seven labs using BacT/ALERT™) *H. parainfluenzae* was significantly less often diagnosed in the seven laboratories relying on BacT/ALERT™ for blood-cultures: 24 patients were diagnosed with H. parainfluenzae bacteremia in labs using BacTec™ versus 13 patients in BacT/ALERT™ labs (24/6169 versus 13/6975, p<0.0001).
Conclusion: The BacT/ALERT™ 3D is less sensitive in recovering *H. parainfluenzae* than the BacTec™ 9240 system. Blood culture negative endocarditis in hospitals using the BacT/ALERT™ could be due to *H. parainfluenzae*.

P13

Discrimination between MRSA and MRSE in clinical samples by relative quantification of the mecA gene and the SA422 gene

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Introduction: Simultaneous detection of a *Staphylococcus aureus*-specific PCR and a mecA-specific PCR is a cheap, rapid and sensitive method to identify methicillin mesistant *S. aureus* (MRSA). The major drawback of this approach is the high number of false-positives caused by the simultaneous presence of a methicillin-sensitive *S. aureus* (MSSA) and a methicillin-resistant *Staphylococcus epidermidis* (MRSE) in a clinical sample. Relative quantification (Ct values) of both PCR products however gives a possibility to study the diagnostic applicability on direct clinical samples after overnight enrichment in selective broth.
Methods: MRSA-culture swabs were inoculated in Phenol Mannitol Broth (PMB) with 75 µg/l Aztreonam and 5 µg/l ceftizoxim and incubated for 16 hours at 37°C. After incubation, DNA was extracted with the X-tractor Gene (Corbett Life Science) and amplified in an ABI 7500 PRISM thermocycler (Applied Biosystems). Positive MRSA controls and an internal amplification control were used in the real-time PCR.
Results: A total of 112 clinical samples that were positive for both MecA and *S. aureus* PCR were studied retrospectively. The mean difference in Cycle threshold (Ct) value of the MecA and *S. aureus* PCR was 3.4 (SD:7.9 Ct, range -15.1 to 23.1). Of these 112 samples, 26 were culture-positive for MRSA. No culture positive samples were negative by PCR. In the samples containing MRSA the Ct values between the MecA and *S. aureus* varied only between -1.82 to 2.24, with one unexplained and highly aberrant exception (Ct difference 13.5). The mean difference in Ct value of the other 25 culture positive samples was 0,49 (SD:0,82 Ct, range -1,82 to 2,24) independent for the simultaneous presence of MRSE and/or MSSA in the sample. Based upon these results, cut-off points of -2,0 and 3,0 Ct for samples to be considered 'MRSA positive by PCR' result in a 96% sensitivity (25/26). With these cut-off values, 66 of 86 culture negative samples (77%) could have been be identified as MSSA+MRSE combinations directly.
Conclusion: The use of the difference in Ct value between the MecA and *S. aureus* PCR can be used to increase the specificity of MRSA detection. The fact that there is no increase in the differences between Ct values when MRSA+MSSA and MRSA+MRSE strains are present can be explained by differences in growth rate. *S. epidermidis* grows relatively slow under standard culture conditions and antibiotics inhibit growth of MSSA in the PMB broth.
In general, this procedure seems promising as a rapid and cheap screening alternative for the presence of MRSA in clinical samples.

P14

The (non-)sense of Lyme serology; a prospective clinical study on the serological diagnosis of Lyme disease in Twente-Achterhoek

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Introduction: Lyme disease or Lyme borreliosis is a multisystem disease involving many organs and is caused by the tick-borne spirochete *Borrelia burgdorferi* (sensu lato).
For the development of diagnostic tools, the heterogeneity of the causative agents must be considered. Although serological tests in the so-called two-test approach are advised to apply in diagnosing a patient, the results are not always easy to interpret in relation to the clinical presentation of the individual patient.
Aim: To investigate the diagnostic performance of 4 different commercially available serological EIAs used for the detection of circulating *Borrelia* antibodies in a prospective manner in the region Twente-Achterhoek (approximately 6000 investigations/year).
Methods: Sera from patients were collected prospectively from April until October 2007. The consulted physicians were asked to fill in a questionnaire regarding the complaints of the patients and to give an estimate of the pre-test chance of Lyme disease on a scale from 1 (less likely) to 10 (most likely). The different IgG EIAs marketed for the detection of *Borrelia* antibodies are common

available EIAs in the Netherlands (the exact names of the firms are available on request). Positive sera were analysed in the recomBlot *Borrelia* IgG (Mediphos Group, Renkum, NL). All assays were performed according to the manufacturers instructions. Statistical analysis was performed using SPSS 15.0 software.

Results: Three thousand and forty patients participated in the study (max:3040 sera). From 11,2% (n=339) a completed questionnaire was available. The initial results are shown in *table 1*.

Crosstabulations between the EIAs showed that EIA 2 and EIA 3 corresponded very well with each other (k=0.7); less than 9.4 % of the serological IgG data were discrepant. Most striking was the poor correspondence between EIA 1 and all other 3; over 35.5% of all IgG positive results by EIA 1 were not diagnosed positive with either other one.

RecomBlot analysis after the initial screening assay with EIA 1 showed that 48% of the samples were considered true positive, with EIA 2, 81,1%, with EIA 3, 82,6% and with EIA 4 57,6% were considered true positive respectively.

Studying the pre-test probality of Lyme borreliosis in relation to the IgG results of the 4 different EIAs all showed a dose response correlation. When the pre-test probability of Lyme was high (a score between 7 and 10) EIA 1 showed an 27,9% IgG reactivity, whereas EIA 2 showed 20,5% IgG positivity, EIA 3 42,4% and EIA 4 23,3% respectively. A low pre-test probability (a score between 1 and 3) the IgG reactivities were 14,3%, 11,1%, 15,4% and 9,2%.

Conclusions:

1. Detection rate of IgG antibodies varies between 10,7 and 17,9% using 4 different EIAs irrespective of the clinical presentation
2. The best results, defined as RecomBlot positive, are obtained by EIA 2 and EIA3
3. The clinical association between IgG EIA positivity is not clear cut; the value of additional assays i.e. PCR on skin biopsy and/or culture of the microorganism from CSF, synovial fluid of EDTA blood are subject for further study in this respect.

Table 1. shows the initial serological IgG results tested by the 4 different Eias

EIA	1 N; %	2 N; %	3 N; %	4 N; %
Positive	543; 17,9	329; 10,9	276; 10,7	361; 16,9
Equivocal	106; 3,5	100; 3,3	85; 3,3	77: 3,6
Negative	2391; 78,7	2585; 85,8	2225; 86,0	1702; 79,5

N: absolute number; %: percentage of total

P15

Evaluation of the Corbett X-tractor Gene for automated nucleic acid extraction in a laboratory for clinical microbiology

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Introduction: The X-tractor Gene (Corbett Life science, Brisbane, Australia) is a fully-automated 96-well nucleic acid extraction system. The X-tractor Gene can be used with different nucleic acid extraction kits form different suppliers. We evaluated the X-tractor Gene for use in a medical microbiological laboratory in a general hospital.

Methods: Samples from different origin were extracted with the Corbett core DNA extraction kit with 180 µl input volume and 150 µl elution volume. As a reference, manual extraction with the Qiagen spin column mini kit as a reference method (200 µl input and 100 µl elution). DNA amplification by real-time PCR in an ABI 7500 thermocycler (Applied Biosystems). Samples were tested with both methods without storage for longer than 24 hrs at 4°C, unless stated otherwise. Samples positive with one method but negative with the other were tested again from a stored (-20°C) stock and evaluated for potential contamination.

Results: A total of 35 positive samples and 10 negative samples with internal controls were included in this study. One internal control was negative after manual extraction due to inhibition of the PCR. In general, Cycle threshold (Ct) values hardly differed between the two methods. In the study period, no suspected of contamination due to X-tractor Gene DNA extraction was noticed (data not shown). The reproducibility of the Ct value of internal controls was somewhat better with X-tractor Gene DNA (Standard deviation 0,3 Ct) than manually isolated DNA (0,7 Ct).

Conclusion: The X-tractor Gene is suitable for extraction of DNA from different types of micro-organisms out of medically relevant sample types. The yield is comparable to the manual reference method in all cases. A fixed amount of internal control is equally efficient extracted from different clinical sample types. No evidence of inhibition or contamination was observed.

P16

Development of an internally controlled PCR assay for broad range detection of bacteria in platelet concentrates

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Introduction: At the moment, bacterial contamination of blood products is the most common microbiological

risk of transfusion. The prevalence of contamination of cellular blood products is approximately 1 in 3000 donations. The risk is greatest for platelet concentrates (PC) as they are stored at 22°C under constant agitation to preserve function and vitality. These conditions make PCs an excellent growth medium for bacteria. In the Netherlands, screening of PCs for the presence of bacteria is done by automated culturing with the BacT/Alert culturing system (Biomerieux). Although the system is sensitive, in theory it can detect 1 colony forming unit (CFU) per 5 to 10 ml PC, its use is restricted by long assay times. Slow growing bacteria or low bacterial loads are not always detected by the system. An alternative test that is both sensitive and rapid is desirable. Therefore, a real-time PCR assay based on the 16s rRNA gene was developed as alternative for culturing of PCs.

Methods: A previously developed 16S rRNA primer set was used, to which two primers were added to detect all bacteria that are relevant for bacterial contamination in PCs. A recombinant lambda phage internal control (IC) was developed to control DNA isolation and amplification. The IC DNA was packaged into bacteriophage particles making the IC target inaccessible to nucleases. This makes it a suitable control for monitoring lysis of pathogens during DNA isolation. The lambda phage IC contains the primer sites of the 16s rRNA assay, but a different probe sequence. This makes it possible to perform both tests in one reaction by making use of different fluorophores. The length of the IC target is 20 bp longer than the bacterial target thereby favouring the amplification of the bacterial target. Two model bacteria, *Staphylococcus aureus* and *Escherichia coli* were used to determine the sensitivity of the assay in plasma and PCs. Twenty five different bacterial strains were used to demonstrate the specificity of the assay in plasma.

Results: Thirty three CFU/ml of *E. coli* and 72 CFU/ml of *Staphylococcus epidermidis* could be detected in plasma, and 97 CFU/ml of *S. epidermidis* in PCs. Addition of the IC had no effect on the sensitivity of the assay. The assay detected all bacteria relevant for bacterial contamination of PCs.

Conclusions:

1 A real-time PCR test, based on the 16S rRNA gene was developed to screen PCs for bacterial contamination. A recombinant lambda phage IC was successfully used as a control to monitor both DNA isolation and amplification.
2 Because of the short turn around time of the real-time PCR assay it can be used as alternative for the BacT/Alert, especially when PCs are tested for bacterial contamination shortly before transfusion.

P17

Shiga toxin-producing *Escherichia coli* carrying stx2f as an emerging cause of diarrhea in the Netherlands

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Objectives: Shiga toxin-producing *Escherichia coli* (STEC) that carry the stx2f gene are considered to be host adapted to pigeons, and therefore of limited clinical relevance to humans. In fact, to date only 6 cases have been documented in the recent literature (1990-2007), and with the exception of a single case, all have been linked with uncomplicated diarrhoea. Due to this low incidence, routine diagnostic procedures have not been adapted to detect this variant. In this study we investigated the possible role of stx2f carrying STEC in aetiology of diarrhoea.

Methods: A total of 2155 stool specimens, for which routine screening for gastrointestinal pathogens was requested, were analysed between September 2006 and March 2007. DNA was extracted from the specimens using the NucliSENS easyMAG specific A protocol. STEC were detected by internally controlled multiplex real-time PCR (Schuurman *et al.* J Microbiol Meth 70:406-415) adapted for detection of the stx1c, stx1d, and stx2f genes. Positive specimens were partially subtyped using the singleplex real-time PCRs to identify stx1, stx1c, stx1d, stx2f, and the group containing stx2, stx2c, stx2d, stx2e, and stx2g (stx2cdeg). Isolation of STEC strains was performed from stx2f positive specimens by culture on sorbitol MacConkey agar and colony screening by PCR

Results: A total of 37 specimens showed STEC specific amplification signals. Subtyping was successful in 35 of 37 specimens, and revealed stx2f (n=4) as the third most prevalent genotype after stx2cdeg (n=13) and stx1 (n=6). Other genotypes detected included stx1+stx1c (n=2), stx1c (n=2), stx1+stx2cdeg (n=1), and stx1/stx1c+stx2cdeg (n=1). Patients positive for stx2f had uncomplicated diarrhoea (n=2) and bloody diarrhoea (BD) (n=2), although the BD may also be explained by other factors in both these patients. Isolation of an STEC strain was successful in 2 of 4 stx2f-positive specimens, and yielded an O63:H6 serotype, eae gene (intimin) positive in both cases. Both strains were also shown to be highly related based on pulsed field gel electrophoresis, although no epidemiological link was apparent between both patients.

Conclusions: STEC that carry the stx2f gene were the third most prevalent genotype in the Netherlands during the study. Our results suggest that stx2f-carrying STEC may be an overlooked cause of diarrhoea. Diagnostic screening strategies will need to be adapted to detect this variant in the routine diagnostic laboratory.

P18

Development and clinical evaluation of a new real time RT PCR for the detection of norovirus type I and II in faecal samples

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As many diarrhea outbreaks appear to be norovirus (NoV) related and this virus is not cultivable, a norovirus RT PCR was developed and clinically evaluated with faecal samples. Since database analyses showed that published norovirus RT PCRs did not cover all known DNA sequence variants, a new realtime RT-PCR was developed targeting the ORF 1 and 2 junction region. Separate PCRs were developed for norovirus type I and II. Equine arteritis virus (EAV) was used as an internal control (J Clin Virol 2005:33(4):306-11). The NoV PCR assays appeared to be highly specific after in silico analyses and testing with different viral and bacterial gastrointestinal micro-organisms. Either identical or higher sensitivities were obtained when testing known NoV positive samples. However, duplex testing of NoV I and NoV II in one single reaction was not possible without notable loss of sensitivity in spite of optimisation attempts. Fortunately, the EAV internal control could be efficiently combined with the norovirus II PCR without loss of sensitivity.

These NoV assays were clinically evaluated on 100 consecutive not solid faecal samples and 93 samples for which norovirus was specifically asked for (April 2006-May 2007). Faecal samples were suspended in 0.6% NaCl and centrifuged at low speed. RNA extractions were performed from the obtained fluids with the easyMag extractor (Biomerieux). From the 100 consecutive samples 7 (7%) were positive for norovirus II. From the samples for which norovirus was specifically asked for 33 (36%) out of 93 were positive for norovirus II of which most were obtained in December and January 2006. 10 of these 33 positive samples were related to outbreaks and as a standard policy also send to the Dutch National institute for Public Health and Environment. These were all confirmed. None of the samples tested were positive for NoV type I.

Conclusion: Sensitive and specific real-time PCR assays for NoV I and II in combination with an internal control, were developed. These assays proved to be clinically valuable in testing faecal samples.

P19

Novel combination of two sample preparation methods for extraction of bacterial DNA from whole blood

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Introduction: Molecular pathogen diagnostics becomes more and more of interest in the field of infectious diseases. Conventional culture techniques are time-consuming and therefore causing a delay in the diagnosis of the patient. Alternative techniques based on nucleic acid amplification offer a shorter turn-around-time, a possible reduced influence of antibiotic use and the ability to identify fastidious and non-culturable organisms. A determining factor in the quality of performance of real-time polymerase chain reaction (PCR) is the type of sample material. Clinical specimens such as whole blood and blood cultures contain a lot of factors negatively influencing the reaction. The elimination of human DNA and other PCR inhibitors could enhance the effectiveness of the DNA isolation resulting in a more optimal performance of PCR. Therefore in this study, we evaluated a unique combination of two sample preparation methods for the detection of bacterial DNA from whole blood.

Methods: MolYsis Basic (MolZym GmbH & Co. KG, Bremen, Germany) is a blood DNA removal kit providing a pre-treatment for whole blood samples. The kit enables the removal of human DNA and bacterial cell wall degradation prior to DNA isolation. In this study, MolYsis Basic is used in combination with the MagSi-DNA Isolation Kit (MagnaMedics Diagnostics B.V., Maastricht, the Netherlands) for detection of methicillin-resistant *Staphylococcus aureus* in whole blood samples. The level of detection after application of both reagents was compared with the level of detection after a more conventional DNA isolation method with the MagNA Pure instrument (MagNA Pure LC Microbiology Kit MGrade, Roche Diagnostics GmbH, Mannheim, Germany). The principle of both DNA isolation methods is magnetic separation. The presence of methicillin-resistant *S. aureus* DNA was determined by a real-time PCR assay for the detection of three methicillin-resistant *S. aureus* target genes.

Results: The detection level of the multiplex methicillin-resistant *S. aureus* assay after pre-treatment with MolYsis Basic and isolation with the MagSi-DNA protocol was 102 colony forming units (CFU) per PCR. Conventional DNA isolation with the MagNA Pure instrument without pre-analytical sample processing could only detect methicillin-resistant *S. aureus* at a concentration of 103 CFU per PCR.

Conclusion: Our data concerning DNA extraction with pre-treatment show an up to 10-fold increase in sensitivity compared to conventional DNA isolation with the MagNA

Pure instrument. These results suggest that an additional step involving the elimination of human DNA and other PCR inhibitors combined with the new MagSi-DNA extraction could be a powerful tool to improve the molecular analysis of bacteria from whole-blood.

P20

Development and evaluation of SYBR Green real time PCR for detection of pathogenic *Leptospira* species in clinical materials

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Introduction: Leptospirosis is a zoonotic disease caused by pathogenic bacteria of the genus *Leptospira*. Several conventional and real-time PCR's for the diagnosis of leptospirosis have been described for the rapid detection of leptospires in serum, blood and tissue in the last decade. However, the investigation for amplification of all pathogenic leptospires and the evaluation in clinical samples has been done insufficiently. One of the previously described PCRs uses two primers pairs, i.e. G1/G2 and B64I/B64II that target on the secY and flaB gene, respectively. We have adapted the G1/G2-based PCR to make it suitable for SYBR Green real-time PCR and enabling the amplification of all pathogenic leptospires.
Methods: To determine the analytical sensitivity and specificity, DNA (extracted with QIAamp® DNA mini kit) from 59 strains belonging to seventeen pathogenic, non-pathogenic and intermediate *Leptospira* species, as well as 55 other micro-organisms were analysed. The reproducibility and repeatability of the assay was performed by testing DNA from twenty PCR positive and twenty PCR negative kidney tissues from rodents. The evaluation in clinical samples was performed on blood samples from 71 patients with suspected leptospirosis.
A new primers pair SecY-IVF and SecY-IV was selected via alignment of over ninety partial and complete secY leptospiral DNA sequences (unpublished data) taking into account standard parameters for real-time PCR. Real-time PCR was performed on iQTM5 Multicolour Real-Time PCR Detection System (Bio-Rad) using the DNA-binding dye technique (SYBR Green).
Results: Determination of critical control parameters: Slight effect on changes in MgCl2 concentration (range 3.0-4.0 mM per reaction), primer concentration (range 0.2-0.4 µM per reaction), annealing temperature, the incubation time as well as the elongation time appeared not critical on melting temperature and cycle number.
Analytical specificity: All the pathogenic Leptospira gave a positive reaction. All saprophytic Leptospira and other organisms were negative.
Analytical sensitivity (detection limit): The sensitivity of DNA template of strains with a high, intermediate and low detection level was 1, 1.2 and 1.5 leptospires per reaction respectively. In spiked serum, blood and kidney tissue the sensitivity was 10, 20 and 20 leptospires per reaction respectively.
Reproducibility and repeatability: Twenty PCR positive and 20 PCR negative rodent kidney tissues were at least twice tested blindly by two different persons. We found the reproducibility and repeatability 100%.
Preliminary estimation of diagnostic specificity and sensitivity: PCR was performed on blood samples from 71 patients suspected for leptospirosis and compared with culturing as gold standard. 10 patients were positive by culture and 12 by PCR. 7 patients were positive both by PCR and culture. Based on this comparison, the diagnostic sensitivity and the specificity are 70% and 91.8%, respectively.
Conclusion: In conclusion, we described SYBR Green real-time PCR as simple and quick method for early detection of pathogenic leptospira, the assay has been evaluated against the gold standard and was found to have comparable sensitivity and specificity.

P21

Development and validation of a rapid molecular screening panel for the detection of *Clostridium difficile* ribotype 027

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Objectives: *Clostridium difficile* is the etiologic agent responsible for human diseases ranging from mild diarrhea to severe pseudomembranous colitis, which are collectively referred to as *C. difficile* associated disease (CDAD). Since 2002, an epidemic *C. difficile* clone characterised as PCR ribotype 027, North American pulsed-field type I, and restriction-endonuclease analysis group type B is associated with higher morbidity and mortality rates, and increased antimicrobial resistance. Therefore, rapid identification methods for *C. difficile* ribotype 027 isolates are needed, since this ribotype requires modified treatment and hygienic measures. In this study real-time PCR based typing assays for *C. difficile* ribotype 027 were developed and validated.
Methods: We developed typing assays targeting the tcdC gene (Δ117 and Δ330_347) and the binairy toxin gene (CDTb). Stools were processed with the easyMAG specific A stool protocol (bioMérieux). The selectivity of the assays was validated with a panel of well *University C. difficile* strains and clinical isolates (n=50, of which 9 were ribotype 027) and non-*C. difficile* strains (n=43).

The analytical sensitivity was assessed by a dilution series (n=1), spiked in a homogenous faecal matrix. Also, the assays were used in a clinical validation performed on stool samples (n=163) of patients suspected of CDAD. Samples were pre-screened for toxigenic *C. difficile* with a real-time PCR targeting the tcdA and tcdB genes. To confirm our typing assays, ribotyping PCR was performed at Leiden University Medical Centre (LUMC) on all cultured isolates.

Results: The combined results of the three typing assays proved to be specific for *C. difficile* ribotype 027, as no cross-reaction was observed. The assays were capable of detecting approximately 3x10^3, 3x10^3, and 3x10^5 CFU/g of stool with a 100% hit rate with the Δ117, Δ330_347, and CDTb PCR respectively. Of the 163 clinical stool samples tested, 24 were positive for *C. difficile* in the screening PCR (14.7%). By our typing assays 2 of the 24 *C. difficile* PCR positive samples w ere identified as *C. difficile* ribotype 027 (1.2%). Ribotyping PCR performed on cultured isolates from the same patients confirmed these 2 samples to contain ribotype 027.

Conclusion: In combination with a *C. difficile* screening PCR, these in-house real-time PCR typing assays offer a rapid and sensitive detection method for *C. difficile* ribotype 027 directly from stool specimens.

P22

Development and validation of a real-time PCR assay for detection of *Yersinia enterocolitica*, as part of a Dutch study on the epidemiology of gastroenteritis

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Objectives: *Yersinia enterocolitica* is an important human enteroinvasive pathogen with global distribution. Most commonly, yersiniosis is associated with self-limiting gastroenteritis.

However, rapid diagnosis of yersiniosis is important, since it may result in early treatment of secondary, post infectious sequelae, like acute and chronic arthritis.

The gold standard for the detection of *Y. enterocolitica* in stool samples is a selective overnight culture followed by biochemical testing, which is a laborious and time-consuming method. In May 2008, a study in 6 Dutch hospitals will commence to asses the incidence, etiology and course of patients hospitalised for gastroenteritis (GEops study). As part of this study, and to facilitate rapid diagnosis, a real-time PCR assay was developed and validated for the detection of *Y. enterocolitica*.

Methods: Real-time PCR assays targeting the ail, ystA and ystB genes were developed. DNA isolation from samples was performed with the easyMAG specific A stool protocol (bioMérieux). As internal control, phocine herpes virus-1 was used. The selectivity of the assay was validated with a panel of well-characterised *Y. enterocolitica* isolates, biotypes 1A, 1B, 2, 3, 4 and 5 (n=61), a panel of *Yersinia* spp. isolates (n=31) and a panel of non-*Yersinia* strains (n=35). Analytical sensitivity was assessed by dilution series (n=2), spiked in two different faecal matrices, one of liquid consistency and one of fixed consistency. Also, a clinical validation was performed on stool samples routinely screened for bacterial and parasitic enteric pathogens (n=188).

Results: The assay proved to be specific for *Y. enterocolitica*, as no cross reaction was observed. The ystA and ail assays, were capable of detecting approximately 4400 CFU per gram of stool (for both faecal matrices). The ystB assay was capable of detecting approximately 3520 CFU per gram of stool (for both faecal matrices).

All forty isolates of biotypes 1B (n=2), 2 (n=1), 3 (n=20), 4 (n=15) and 5 (n=2), scored positive in the real-time PCR for the targets ail and ystA. Of the 21 biotype 1A isolates, 20 scored positive for the ystB target. One biotype 1A isolate scored positive for the ail target and negative for the ystB target.

Y. enterocolitica DNA was not detected with these real-time PCR assays in any of the 188 clinical samples. PCR inhibition was observed in less than 1% of these samples.

Conclusion: Although all clinical samples were negative, sensitivity experiments prove the assay to be a sensitive method for the screening of *Y. enterocolitica* in stool samples. The assay will be used as a rapid screening tool for the detection of *Y. enterocolitica* in the GEops study.

P23

Rapid profiling of the human intestinal microbiome

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While the human commensal microbiota is well known to be an important factor in health and disease, its exact role and the ways in which it influences our wellbeing remains elusive. One of the major obstacles in gaining a better understanding is the analysis of the microbiome. New insights have recently been gained with high throughput sequence analysis but, although valuable, these methods are not suited for large scale screening. Here we present a straightforward assay for rapid profiling of the human microbiota.

This method is based on two features of the bacterial genome: the length of the interspace (IS) region between

the 16s and the 23s rDNA and specific primer sequences by which Firmicuta and Bacteroidetes, the two major phyla in the human colon, can be discriminated. The IS region is conserved within a species, but varies between species. Thus bacteria can in principle be identified by the length of their IS region. Using the specific primer sequences, unknown species, constituting more than 60% of the intestinal microbiome, can be sorted into their phylum. The primers were combined with non-specific reverse primers in a double label multiplex PCR. Size and color sorting of fragments was performed in an ABI 3130XL sequencer.
For validation purposes, an in silico profile of 10 bacteria was constructed based on known sequence information and tested with the cultured bacteria. Furthermore, 7 cultured species of *Bacteroides* and 7 cultured species of *Clostridium*, both prominent genera in the human gut, were tested in different combinations to evaluate inter-species competition in the PCR reaction.
Then, colonic mucosal biopsies from 20 patients were analysed using this method. Intra- and interpatient composition of colonic microbiota was analysed in 5 biopsies per patient, from coecum, flexura hepatica, flexura lienalis, sigmoid and rectum.
All cultured bacteria showed a profile identical to the in silico profile. All species of *Bacteroides* and *Clostridium* tested showed comparable efficiency in the PCR reaction. Subsequently, excellent bacterial profiles were obtained for the clinical samples. Each patient had a unique bacterial profile, with only a few fragments being identical between patients. These fragments corresponded to well known colonic bacteria such as *Faecalibacterium prausnitzii* and *Bacteroides thetaiotaomicron*. Patterns obtained from the different sites of the colon of a single patient were almost identical.
With obtained patterns being identical to expected profiles based on sequence data and no observed competition in the PCR reaction, the method seemed ideally suited for the characterisation of the complex human microbiome. Indeed, analysis of clinical samples showed excellent performance. With this method, profiles of the human colonic microbiota can be obtained in a straightforward and reliable fashion. The method is universal, fast, reproducible and ideally suited for analysis of large sample sets.

P24

Effect of lower inoculum on MIC broth microdilution: a comparison with the ISO reference method

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Objectives: Rapid, accurate and reproducible determination of the MIC value is important for adequate patient care, for local and international resistance surveillance. For epidemiological studies of susceptibility and for comparison of new and existing antimicrobial agents. The ISO reference method (ISO 20776-1) prescribes an inoculum of 2-8x10^5. The rationale behind the lower limit is not fully clear. In addition, some MIC devices do use lower inocula. For instance, the (former) guideline of the BSAC stated 10^5 /ml. The aim of this study was to evaluate the effect of a lower inoculum size on the MIC determination using the ISO protocol (ISO 20776-2).
Method: During daily clinical laboratory work 300 consecutive gram-negative strains (fresh isolates) were collected. Identification was performed following standard procedures. From each strain, the MICs were determined with both the 2-8x10^5 inoculum (ISO 20776-1) and with a 5-10x10^4 inoculum. MIC values were read independently by two lab technicians. For the interpretation of the MICs the EUCAST breakpoints were used. The following antimicrobial agents were tested: amoxicillin (AM), cefotaxime (Cef), ciprofloxacin (CI), amoxicillin/clavulanic acid (AC). The MIC values of the lower inoculum size was compared to the reference method following ISO guideline (ISO 20776-2).
Results: The isolates collected belonged to the following species *Citrobacter* spp (n=15), *Enterobacter* spp (n=35), *Escherichia coli* (n=80), *Hafnia alveii* (n=1), *Klebsiella* spp (n=84), *Morganella morganii* (n=10), *Pantoea agglomerans* (n=1), *Proteus* spp (n=67), *Providencia rettgeri* (n=1), *Serratia marcescens* (n=6). Forty-five percent of the strains were susceptible for the different antimicrobial agents. Following ISO guidelines the essential agreement after discrepancy analysis was: AM 99%, Cef 98%, CI 98%, AC 98% and the category agreement was: AM 96%, Cef 99%, CI 97.%, AC 92%, respectively. The Very Major Discrepancy was found only for Cef 0.3%. Discrepancy analyses with the M.I.C.E. found the same MIC as the 5-10x10^4 inoculum. A Major discrepancy was only found for Cef 0.3%. Minor discrepancies were found for AM 4%, Cef 0.6%, CI 3.3%, AC 8.3%.
Conclusion: In this study, a lower inoculum size (5-10^4) did not influence the outcome of the MIC determination; there was a very good category agreement and essential agreement between the different inoculum sizes for the antimicrobial agents tested compared to the reference method.

P25

Evaluation of the Cica-Beta-Test for phenotypic identification of extended-spectrum beta-lactamases

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Introduction: Bacterial strains producing an extended-spectrum beta-lactamase (ESBL) are becoming a major concern for antimicrobial treatment and infection control.

Laboratory detection and identification of ESBLs is complex and usually time consuming. The Cica-Beta-Test is a chromogenic test that directly measures β-lactamase activity. Through the use of specific inhibitors it can differentiate the different types of β-lactamases involved. We evaluated this test because it could significantly reduce ESBL detection time in routine diagnostics.

Methods: The Cica-Beta-Test measures hydrolysis of the chromogenic oxyimino-cephalosporin HMRZ-86 with and without specific inhibitors of extended-spectrum, metallo- and AmpC beta-lactamases. Beta-lactamase activity induces a colour change from yellow to red. We examined a total of 80 clinical gram-negative isolates, of which 40 had been previously characterised as ESBL-producers with phenotypic and molecular analysis. The other 40 isolates were ESBL negative. Among the strains were 10 ESBL positive and 10 ESBL negative *Enterobacter* spp. Strains were grown overnight with a cefpodoxime disc to promote beta-lactamase expression. Colonies adjacent to the disc were then tested with the Cica-beta-Test according to the instruction manual.

Results: The overall sensitivity and specificity of the Cica-Beta-Test for detection of ESBL production in this study were respectively 78 % (31/40) and 100 % (40/40). In *Enterobacter* spp. 6 out of the 10 ESBL-positive strains were detected, no false positive results were recorded.

Conclusions: The necessity to grow bacteria in the presence of a cefpodoxime disc annihilates the reduction in detection time. For accurate interpretation of the results, the test should be read in conjugation with antibiogram data. Although the Cica-Beta-Test did not always detect the ESBL production correctly, it showed high specificity in this study.

P26

Development and validation of an automated 16S rDNA assay for the detection of bacterial DNA in clinical samples

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Objectives: Since 2002, the department of Research and Development of the Laboratory for Infectious Diseases started to analyse clinical specimens on request for the presence of bacterial DNA. The assay consists of a manual DNA extraction method, followed by conventional broad-range PCR. PCR positive products are subsequently sequenced to identify the organism that was present in the clinical specimen (ref. Schuurman *et al.*, JCM 2004:42(2):734). However, the assay is very laborious and time consuming. To decrease hands on time (HOT) the extraction and detection procedure were automated. This study describes the validation of an automated 16S rDNA assay (A) for the detection of bacterial DNA in clinical samples. Method A was compared with our conventional detection method (C).

Methods: Prior to DNA extraction, samples were treated with mechanical disruption for both methods. DNA extraction was performed with the manual Boom method (C) or with the automated easyMAG specific A protocol (bioMérieux) (A). Conventional (C) and real-time (A) PCR were performed with universal primers (probe), which amplified/detected the whole 16S rRNA gene. All positive PCR products were subsequently sequenced. The analytical sensitivity of method A was assessed by dilution series of different model organisms (n=3), spiked in phosphate buffered saline. Also, both methods were compared in a validation performed on clinical samples (n= 89).

Results: Analytical sensitivity of both methods were comparable, with limits of detection of approximately 400-40 CFU/PCR, 1450-145 CFU/PCR, and 800-80 CFU/PCR for gram- negative (*Escherichia coli)* and gram-positive organisms (*Staphylococcus aureus* and *Staphylococcus pneumoniae*) respectively. Thirty-eight positive and 46 negative samples were in complete concordance for both methods (94.4%). Method A detected 4 additional clinical significant positive samples (*Staphylococcus intermedius*, *Stenotrophomonas maltophila*, *Streptococcus pneumoniae* and a mixed sequence). Method C yielded 1 additional positive result (*S. aureus*), which also was regarded clinical significant. PCR inhibition was detected in 10% of all samples for method A in comparison with 49% for method C. Also, HOT for obtaining a PCR result decreased (˜50%) when method A was applied.

Conclusions: With regards to sensitivity both methods were comparable in detecting bacterial DNA from clinical samples. However, when method A was applied HOT was reduced considerably, PCR results were easier to interpret, and inhibition rates decreased dramatically.

P27

Evaluation of six commercially available Epstein-Barr virus enzyme immunoassays

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Introduction: Six commercially available enzyme immunoassays (Serion, Novitec, Biotest, Medac, Diasorin and Novagnost) were evalued for the serologic detection of Epstein-Barr virus (EBV) viral capsid antigen (VCA) immunoglobulin M (IgM), VCA IgG and EBV nuclear antigen (EBNA) IgG.

Methods: Serum samples from 75 immonocompetent patients were tested with the 6 different commercial

assays. 11 samples were derived from patients with an acute primary EBV infection, 24 samples came from patients with known past EBV infections, 18 samples were from patients without prior EBV infection and 16 samples were from patients with an acute primary CMV infection. In addition we included 6 samples that were inconclusive (IgM anti-VCA positive and IgG anti-EBNA positive) in our current assay.

Results: Sensitivity for detecting a recent infection with EBV, based on a positive test for IgM anti-VCA ranged from 75% to 100% using cut-offs according to the manufacturers instructions. Using ROC-analysis we found that the assay had an area under the curve ranging from 0,887 to 0,969. When assuming a 95% specificity, sensitivity dropped for most assays. Medac, Novagnost and Serion had the highest sensitivity (88%, 75% and 85% respectively) at a 95% specificity level. The 6 samples that were inconclusive (IgM anti-VCA positive and IgG anti-EBNA positive) in our current assay yielded widely divergent results in most assays. However, the Medac ELISA tested all these samples negative for IgM anti-VCA antibodies.

Conclusions: All tested assays perform reasonably to well. The Medac, Novagnost and Serion IgM assays have the highest sensitivity for detecting a recent infection, with the Medac assay having the highest specificity.

P28

Performance of the VIDAS CDAB test compared to cell culture for detection of *Clostridium* toxine A and B in routine diagnosis

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Introduction: At the Laboratory of the Municipial Health Service Amsterdam incubation of faeces on Vero (African green monkey kidney) cells is used for detection of toxine from *Clostridium difficile*. This test needs to be incubated over night and is very time costly. Results are not immediately available. We therefore evaluated the VIDAS® *Clostridium difficile* toxin A and B test (CDAB).

Methods: Fourty-nine faecal samples sent to or laboratory for routine diagnosis on *Clostridium difficile* and frozen at -20°C were (re)tested in cell culture and in the CDAB test (Biomerieux). Furthermore, 37 faecal samples from the Laboratory of Medical Microbiology, Reinier de Graaf Gasthuis, Delft, and 14 samples from the Regional Laboratory for Microbiology, Groningen, were tested in both assays. The samples from Delft and Groningen were mainly positive samples.

Results: Results of cell culture versus CDAB test are depicted in the *table*. Discrepant and equivocal results were mainly observed in samples which were low positive in cell culture.

CELL CULTURE	VIDAS		
	positive	equivalent	negative
positive	18	5	12
negative	0	1	58
Not int.	1	1	4

Conclusion : The CDAB test is easy to perform, fast and very specific. Sensitivity compared to cell culture is 66%. The discrepant results need to be investigated further in another assay and the results need to be analysed per measured titre in cell culture.

P29

Occurrence of qnr genes in *Salmonella* in the Netherlands

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Introduction: Nowadays variants of qnr genes are isolated from clinically important *Enterobacteriaceae* worldwide, including *Salmonella enterica*. In the Netherlands, the presence of qnrA1 was first detected in a multidrug-resistant *Enterobacter cloacae* originating from a large hospital outbreak in Utrecht. Data on the occurrence of qnr genes in other *Enterobacteriaceae* in the Netherlands are lacking. The aim of the study was to detect and characterise qnr genes in a selection of Dutch *Salmonella* isolates and the possible linkage of qnr genes to ESBLs.

Methods: Our strain collection encompasses more than fifteen thousand *Salmonella* isolates, isolated from humans and animals in the period 1999-2006. Thirty-nine of these isolates showed a typical phenotype being low-level resistant to ciprofloxacin (MICs 0.25-1 mg/L) but still susceptible to nalidixic acid (MICs:8-16 mg/L). All isolates showing this typical phenotype were screened for the presence of qnrA, qnrB and qnrS genes by PCR. In addition all isolates was screened for mutations in the QRDR region and for the presence of the AAC(6')-Ib-cr gene. On all qnrS1-positive *Salmonella corvallis* isolates PFGE were performed according to the CIDC Pulsenet protocol. The first isolates with multidrug-resistant ESBL and qnr suspected isolates were identified in 2007 with a miniaturised microarray. The ESBL and qnr genes were characterised by PCR and sequencing. Detection and size determination of plasmids was performed by S1 nuclease treatment followed by PFGE. The detected plasmids will be further characterised.

Results: Thirty four of the 39 isolates with the typical low-level resistant ciprofloxacin phenotype were qnr-positive. All strains originated from humans except for

one qnrB2-positive *Salmonella bredeney*, which was isolated from a Dutch broiler chicken. No mutations were detected in the QRDR region in these isolates.

PFGE analysis of the 25 qnrS1 positive Dutch *S. corvallis* isolates reveal a high similarity with previously published Danish PFGE patterns of qnrS1 positive *S. corvallis* isolates.

All 3 multidrug-resistant *Salmonella* harboured a large variety of resistance genes including qnrB, blaTEM-1, blaSHV-12 and blaCTX-M-15.

Conclusions:

1. QnrB and qnrS genes are present in low-level ciprofloxacin resistant Dutch *Salmonella* isolates.
2. In 2007, we found the first multidrug-resistant *Salmonella* carrying both ESBL and qnr genes.

P30

In vivo transfer of a class 1 integron containing gene cassettes dfrA1-aadA1

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Introduction: Antimicrobial resistance and, in particular, multi drug resistance (MDR) is an increasing problem worldwide. MDR encoded by gene cassettes on integrons is considered to be involved in the transfer of MDR between bacterial populations. Therefore, exposure of humans to MDR bacteria via the food chain is not only a potential health risk through food borne infections with MDR food borne pathogens but also because integrons can transfer horizontally from bacteria from food-producing animals to human pathogens.To determine the *in vivo* transfer of a class 1 integron containing gene cassettes dfrA1-aadA1 from *Salmonella* to *Escherichia coli* with and without the influence of doxycycline or trimethoprim/sulphamethoxazole.

Methods: Thirty four day old chickens colonised with *E. coli* K12 the groups were divided into 3 groups of 10 animals and placed into an isolators. On day 11 the chickens were inoculated orally with 10 4 CFU *Salmonella typhimurium* containing a class 1 integron with gene cassettes dfrA1-aadA1. Two days after infection with *Salmonella*, one group was treated orally with doxycycline, one group orally with trimethoprim/sulphamethoxazole en one group was not treated (control group). *E. coli* K12, *S. typhimurium* and the transconjugants were isolated from cloacae samples on selective MacConkey agar plates. The transfer of the integron was confirmed by plasmid characterisation, PCR and PFGE.

Results: *E.coli* K12, *S. typhimurium* and transconjugants were isolated from all chickens. In all three groups, the class 1 integron transferred almost immediately after inoculation with *S. typhimurium*, the two antibiotics used had neither a negative nor a positive effect on the transfer rate of the integron. Moreover, the antimicrobial resistance profiles of the transconjugants show that in addition to the transfer of resistance genes associated with the integron, resistance genes encoding for ampicillin and tetracycline present in the donor strain were transferred as well. Remarkably, the transconjugants were fitter than the native *E. coli* K12.

Conclusions: Our data demonstrate that *in vivo* a class 1 integron containing gene cassettes dfrA1-aadA1 transferred readily from *S. typhimurium* to *E. coli* K12, with or without selective pressure of antibiotics. In addition to the integron containing resistance gene cassettes other resistance genes and genes affecting the fitness of the organism were transferred as well.

P31

Identification and functional analysis of genes involved in resistance of _Streptococcus pneumoniae_ D39 to bacitracin, nisin and LL-37

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Streptococcus pneumoniae is a major human pathogen that causes pneumonia, otitis media, meningitis, bacteriemia. Resistance to commonly used antibiotics is on the rise in *S. pneumoniae*. To investigate if there is a common defense mechanism of *S. pneumoniae* transcriptome analysis was performed with three distinct antimicrobial peptides (AMPs): bacitracin, nisin and LL-37. These AMPs differ in their biochemical structure but all three act on the bacterial cell wall and/or membrane. For the transcriptome analysis *S. pneumoniae* D39 was exposed for 15 and 30 minutes to an amount of the AMPs that killed 10% of the bacteria. The transcript levels of genes involved in many processes such as regulation, transport, fatty acids synthesis, phosphotransferase systems had changed significantly. Several highly upregulated genes were chosen for further analysis: sp0385-0387, encoding for an ABC transporter and the two component system number three (TCS03), sp1714-1715, encoding a GntR family regulator and an ABC transporter, sp0785-0787, encoding a RND like protein and an ABC transporter, sp0912-0913, encoding an ABC transporter. Measuring their promoter activity in B-galactosidase assays confirmed the transcriptome analysis. Subsequently, mutants of these genes were made and the Minimum Inhibitory Concentrations (MICs) for bacitracin, nisin, LL-37, ethidium bromide, Hoechst 33342, gramicidin, lincomycin, clindamycin

and daunomycin were established. This demonstrated that the sp0912-0913 ABC transporter is involved in the resistance to bacitracin, nisin and lincomycin. Another ABC transporter, sp0785-0787 is involved in the resistance to lincomycin, clindamycin and LL-37. Interestingly, the GntR like regulator (sp1714) and/or the sp1715 ABC transporter protect against bacitracin and Hoechst 33342. The influence of deleting the GntR like regulator on the expression of sp0385-0387, sp0785-0787, sp0912-0913, and sp1714-1715 was studied. This showed that the sp0785-0787 and sp1714-1715 are negatively affected by the GntR like regulator. These data's show that resistance to the three AMPs in S. pneumoniae is mediated by several ABC transporters. In addition, a GntR type regulator was identified that regulates two of these transporters. These results give insight into the transcriptional stress response of S. pneumoniae to structurally different AMPs, which will enable finding common features of the molecular defense mechanisms against various antimicrobial substances in this organism.

P32

Epidemiology and molecular screening of ESBL producing *Enterobacteriaceae* in a Dutch hospital

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Introduction: Over the last five years, resistance against third-generation cephalosporins has increased exponentially in the Netherlands. Until 2002, ESBL-producing micro-organisms were virtually absent in our hospital. Nowadays approximately 6-7% of clinical *Escherichia coli*, *Enterobacter cloacae* and *Klebsiella pneumoniae* isolates carry plasmids encoding the ESBL phenotype. In analogy to our MRSA policy, we actively search for ESBL positive micro-organisms to contain their further spread. To enable rapid screening, we developed a real-time PCR test to detect the CTX-M gene in *Enterobacteriaceae* with an increased MIC to third-generation cephalosporins. Theoretically, the benefit of this molecular screening would be a two days shortening in the time needed to detect an ESBL.

Methods: We performed a retrospective analysis of the epidemiology of ESBL producing *E. coli*, *E. cloacae* and *K. pneumoniae* strains in our hospital. Subsequently, all ESBL producing strains were screened for the presence of TEM, SHV and CTX-M genes by a classic multiplex PCR. A real-time PCR, detecting all 5 CTX-M groups, was then developed and used to enable rapid, weekly screening for ESBLs on our intensive care unit (ICU).

Results: In 2002, less than 1% of our clinical *E. coli* isolates was ESBL positive. This number rose steadily to 6% in 2007. For *E. cloacae* and *K. pneumoniae*, these numbers were 1.3% in 2002 and 7% in 2007 and 1% in 2002 and 4.4% in 2007, respectively.

Molecular analyses showed that approximately 60% of the ESBL-producing strains in our hospital carried the CTX-M gene, with the relative contribution of CTX-M increasing over time. We therefore developed a real-time PCR to detect the presence of this gene. This PCR was then used in an ongoing weekly screening for ESBL producing *Enterobacteriaceae* in our ICU.

Conclusion: The occurrence of ESBL producing *Enterobacteriaceae* in our hospital has increased from approximately 1% in 2002 to 7% in 2007. Sixty percent of these bacteria carried the CTX-M gene. Molecular screening for this gene in clinical isolates from the ICU speeds up the detection of more than 60% of ESBL producing *Enterobacteriaceae* in our hospital with two days. Parameters to evaluate the additional value of this newly developed strategy are the reduction of secundary cases and the time needed to control an ESBL outbreak.

P33

Extended-spectrum beta-lactamase producing *Enterobacteriaceae* in horses

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Introduction: In human medicine, the prevalence of infections with extended-spectrum beta-lactamase (ESBL) producing *Enterobacterieaceae* is increasing. In veterinary medicine, third and fourth generation cephalosporins like ceftiofur, cefquinome, cefovecin and cefaperazone are also used for the treatment of infections. However, data on ESBL producing *Enterobacteriaceae* in animals, especially in companion animals, is limited. The objective of the present study was to investigate if ESBL-producing *Enterobacteriaceae* are present in clinical samples of companion animals, like dogs, cats and horses.

Methods: Isolates from the strain collection and samples from patients submitted to the Veterinary Microbiological Diagnostic Centre were selected based on resistance to ampicillin and cephalexin or ceftiofur. A total of 142 isolates (117 *Escherichia coli* isolates, 18 *Proteus mirabilis* isolates and 7 *Klebsiella* spp.) originating from clinical infections from horses, dogs and cats were included. The isolates were screened for ESBL production by a disk diffusion method using discs with amoxicillin with clavulanic

acid, ceftazidime, cefepime, cefotaxim and aztreonam. A confirmatory test using ceftazidime or cefepime alone and in combination with clavulanate was performed on all isolates positive in the screening test. In all isolates positive in the screening and confirmation tests the ESBLs were characterised using a commercial miniaturised micro array (CLONDIAG Chip Technologies GmbH).

Results: Fifteen isolates (2 *Klebsiella* spp. and 13 *E. coli*) were positive in both phenotypic screening and confirmation tests. Fourteen of these were confirmed as ESBL positive by the micro array. All positive isolates were from horses and carried a gene of the blaCTX-M-1- family. In addition, 4 isolates carried a gene of the blaCTX-M-2 -family, 2 isolates carried SHV type beta-lactamase genes and 13 TEM type beta-lactamases. All genes will be further characterised by PCR and sequencing.

Conclusions: The prevalence of ESBL's in the equine isolates was higher than expected. Horses may be a potential source of ESBL's producing *Enterobacteriaceae*.

P34

Characterization of beta-lactamase genes in cefotaxim-resistant *Salmonella* and *Escherichia coli* isolated from Dutch poultry

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Introduction: In the last three years a rapid increase has been observed in cefotaxim-resistance in commensal *Escherichia coli* isolated from broilers and a simultaneous increase in cefotaxim-resistance in *Salmonella enterica* isolated from poultry sources in the Netherlands. This indicates a horizontal transmission between *E. coli* and *S. enterica* in the gastrointestinal tract of poultry. The objective of this study is to characterise the beta- lactamase genes and plasmids in cefotaxim-resistant *E. coli* and *S. enterica* strains isolated from Dutch poultry.

Methods: In the Dutch antimicrobial resistance surveillance program in animals in 2006, 359 *S. enterica* strains were isolated from various poultry sources, and 157 *E. coli* strains were isolated from broiler faeces for susceptibility testing. From this collection a selection of 23 *S. enterica* and 23 *E. coli* strains was made based on the cephalosporin resistance phenotype. According to the EUCAST epidemiological cut-off values, all isolates in the selection were cefotaxim resistant and the majority was also resistant to ceftazidime. Beta-lactamase genes were characterised by miniaturised microarray, PCR, DNA-sequencing and plasmids were characterised.

Results: In total 9 different beta-lactamase genes (CTXM-1, CTXM-2, TEM-1, TEM-135, TEM-20, TEM-52, ACC-1/2, CMY-2 and SHV-2) were detected. In *E. coli* the predominant beta lactamase genes were CTXM-1(48%) and TEM-1 (30%). The latter gene was always accompanied by CTXM-1, CTXM-2, CMY-2 or a SHV-2 gene. In *Salmonella* CTXM-1 and TEM-52 were predominant (both 30%). CMY-2 , TEM-135 and SHV-2 were only detected in *E. coli*. ACC-1/2 were only detected in *S. enterica*. The other beta lactamases (CTXM-1, CTXM-2, TEM-1, TEM-20 and TEM-52) were detected in both genera.

Conclusion: This study shows the occurrence of a variation of beta lactamase genes resulting in cephalosporin resistant *S. enterica* and *E. coli*, isolated from Dutch poultry sources. As many of the strains harbour a beta lactamase gene that occurs in both genera, horizontal transmission of genes is very likely to exist. This is of great concern because of the possible transfer of these genes not only between animal species but also from animal to human species.

P35

Phylogeny of the pyrrolnitrin biosynthetic loci prnA and prnD: high levels of homology detected among diverse soil-derived pyrrolnitrin-producing bacteria

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Bacterial strains isolated from diverse bulk and rhizosphere soils were tested for their capacity to produce the antimicrobial metabolite pyrrolnitrin (Prn) by Thin Layer Chromatography (TLC). A total of 20 strains, divided over the genera *Burkholderia*, *Pseudomonas* and *Serratia*, were found to produce Prn. The pyrrolnitrin producers of the potato rhizosphere, mostly identified as *Pseudomonas* and *Serratia* species, showed strong *in vitro* antagonistic activity towards the phytopathogenic fungus Rhizoctonia solani AG3, whereas growth inhibition of Fusarium moniliforme was more frequently detected for *Burkholderia* strains retrieved from sugar cane planted in Brazil. The pyrrolnitrin biosynthetic loci prnA and prnD, required for the production of pyrrolnitrin from tryptophan, were detected in the genomes of all Prn-producers by PCR-hybridisation procedures. While Prn producers were shown to be genotypically diverse as evidenced by whole-genome BOX-PCR fingerprinting, restriction length fragment polymorphism (RFLP) analyses of the prnA and prnD biosynthetic loci indicated a low degree of heterogeneity in these genes, with the exception of polymorphisms observed within a few *Burkholderia* isolates. Sequencing of the prnA, prnD and 16S ribosomal RNA genes of the Prn producers revealed high levels of homology within the former genes as compared to the latter. Heterogeneities in the Prn biosynthetic loci were more evident within the

Burkholderia species, whereas strains belonging to the genera *Pseudomonas* and *Serratia* shared higher levels of sequence homology. Moreover, we obtained evidence for the contention that the rate of divergence of the prnA locus differs from that of the prnD locus, the former being more conserved than the latter. The high degree of nucleotide sequence homology in the Prn biosynthetic loci of divergent bacterial lineages is an indication for the postulate that this operon may be part of the horizontal gene pool. Analysis of the sequence of the regions flanking the Prn biosynthetic locus is shedding light on this intriguing question.

P36

Prevalence of extended-spectrum-lactamase among *Escherichia coli* and *Klebsiella pneumoniae* isolates from Intensive Care Units in the Netherlands 1998-2005

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Introduction: Outbreaks of extended-spectrum β-lactamase (ESBL)-producing strains on Intensive Care Units (ICUs), are associated with prolonged hospital admissions, delay of adequate antimicrobial therapy, and increased mortality. Therefore, reliable and rapid detection of these strains is important.

In this study, the prevalence of ESBL among *Escherichia coli* and *Klebsiella pneumoniae* isolates from 14 Dutch ICUs, participating in a national surveillance programme, was determined over an 8-year period.

Methods: Unique *E. coli* (n=1267) and *K. pneumoniae* isolates (n=402) from ICU patients were collected between 1998 and 2005. Minimal inhibitory concentrations (MICs) of broad-spectrum penicillins with and without β-lactamase inhibitors, cephalosporins, aminoglycosides, co-trimoxazol and fluoroquinolones were determined by broth microdilution method with Mueller-Hinton II cation adjusted broth according to the Clinical Laboratory Standards Institute (CLSI) guidelines. In total, 65 *E. coli* and 35 *K. pneumoniae* isolates with a MIC ≥2 mg/L for ceftazidime and/or cefotaxime were putative for ESBL production and hence selected. ESBL production was demonstrated by the double disk diffusion test (DDDT), as described by Jarlier *et al.*, and the combination disk diffusion test (CDDT), according to the guidelines of the Dutch Society for Medical Microbiology.

In addition, the presence of TEM and/or SHV genes was determined by PCR according to the method of Nyberg *et al.*

Results: Of the 65 *E. coli* isolates, 12 (18.5%) were positive with the DDDT and 9 (14%) with the CDDT. For the 35 *K. pneumoniae* isolates, the figures were 27 (77%) and 22 (63%), respectively. The overall ESBL prevalence among *E. coli* isolates was 0.9 % with the DDDT and 0.7% with the CDDT. For *K. pneumoniae*, the percentages were 6.7% and 5.5%, respectively. Seven *E. coli* and 20 *K. pneumoniae* isolates were positive, with both DDDT and CDDT, and were also PCR positive. In addition, 16 *E. coli* and 8 *K. pneumoniae* isolates were only positive with PCR, 5 *E. coli* and 2 *K. pneumoniae* isolates were positive with DDDT and/or CDDT, and were PCR negative.

Conclusions:

1. With both phenotypic tests, the overall ESBL prevalence among *E. coli* isolates from 14 Dutch ICUs was significantly lower compared to that of *K. pneumoniae* isolates ($p<0.001$)
2. Further investigation regarding the presence of CTX-M β-lactamase, and characterisation of the TEM/SHV genes with sequencing, are mandatory.

P37

Identification of parameters specific for borderline oxacillin-resistant *Staphylococcus aureus*

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Introduction: In addition to the methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* groups (MSSA and MRSA, respectively), a third group has been described, comprising borderline oxacillin-resistant *S. aureus* (BORSA). This variant (MIC ≥4 mg/ml of oxacillin) was previously shown to be associated with surgical wound and skin infections. To date, very few studies have described BORSA. Moreover, by using fast molecular tests, BORSA are currently identified as MSSA, since they lack the MRSA-specific mecA gene. Yet, BORSA was suggested to be an intermediate in the evolution towards MRSA. The risk of BORSA infection may therefore be underestimated. Thus, we set out to characterise BORSA specific parameters to design a rapid test for BORSA identification.

Methods: In the current study, we identified 14 BORSA strains from a collection *S. aureus* samples taken during 2006 from 656 healthy persons and 617 GP patients without infection-related complaints. MIC values for oxacillin were determined by microdilution test. Genetic backgrounds were determined by spa typing. Plasmids were isolated by lysostaphin treatment and alkaline lysis miniprep, and subsequently analysed by HindIII digestion and agarose gel electrophoresis. Colony sizes were determined by the ImageJ digital image analysis program.

Results: Approximately two-thirds of all hospital-associated (HA-) BORSA were previously shown to belong to the multilocus sequence typing (MLST) clonal complex (CC)25.

In our community population, one BORSA was identified as sequence type (ST)26, being the only isolate out of 14 to fit the CC25 profile. The genetic background of the remaining isolates was diverse and comprised both MRSA- and non-MRSA-associated CCs. Only 3 out of 14 isolates yielded BORSA-specific pBW15/pBORa53-like plasmids, contrary to the two-thirds of the HA-BORSA described in previous studies. The pBW15-like plasmids were suggested to be BORSA-specific. However, we isolated a pBW15-like plasmid from 1 out of 16 MSSA isolates of the same study population. Interestingly, all 14 BORSA isolates had a small colony phenotype. The colony diameters were approximately half of those of the 16 MSSA and 13 MRSA control isolates.

Conclusion: From this study it appears that community-acquired (CA-) BORSA are more genetically diverse than HA-BORSA from previous studies. Moreover, the pBW15/pBORa53 plasmids are less frequent in the community and appears not to be BORSA-specific. Other parameters, such as beta-lactamase activity and cell wall synthesis gene polymorfism will be addressed in future studies.

P38

The prevalence of resistance to disinfectants and heavy metals of *Staphylococcus aureus* and *Escherichia coli* from healthy volunteers

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Introduction: Global use of disinfectants and heavy metals (DHMs) is increasing both by consumer use, in industry, food-production and in the environment. Likely as a direct consequence, recent international studies report high or increasing prevalence to these antimicrobial compounds. However, almost no data are available regarding DHM resistance prevalence in the Netherlands. Additionally, studies are showing cross- or co-resistance between DHMs and antibiotics. As selection for DHM resistance is expected to occur mostly outside the hospital, there is a risk that this also leads to selection for antibiotic resistance in the open community. The aim of this study was therefore to obtain data regarding the prevalence of DHM resistance in Dutch isolates by either phenotypic or genotypic methods.

Materials and methods: Isolates of *Staphylococcus aureus* (n=138) and *Escherichia coli* (n=73) obtained from healthy volunteers were screened for their susceptibility to heavy metals zinc and copper. Analysis were performed by agar dilution methods according to NCCLS. *S. aureus* ATCC 29213 and *E.coli* JM109 were used as reference strains with known MIC values for both copper and zinc.

Results: The *S. aureus* isolates showed MIC values for Zn2+ ranging from 0.5 to 5 mM and for Cu2+ ranging from 4 to 6 mM. For *E. coli* MIC values between 5 and 8 mM for Cu2+ and between 2 and 4 mM for Zn2+. Comparison of these results with data from a Danish study from veterinary isolates showed that most data were in agreement regarding the found MIC data for both compounds. For *S. aureus* however the majority of the isolates showed 2 to 3-fold higher MIC values for Zn2+, whereas for Cu2+ the Dutch *E. coli* isolates showed 2 to 4-fold lower MIC values.

Conclusions: To our knowledge, this study has generated the first data regarding susceptibility of Dutch isolates of *E. coli* and *S. aureus* to heavy metals such as zinc and copper. All data show very low-level resistance comparable to data from a Danish prevalence study on veterinary isolates. Only for *S. aureus* isolates susceptibility for Zn2+ was higher than data reported in another European study. Future work will focus on testing susceptibilities to other heavy metals as well as genotypic determinations of presence of DHM resistance genes. Furthermore, isolates from clinical and veterinary sources will be tested as well.

P39

Prevalence and determinants of veterinary methicillin-resistant *Staphylococcus aureus* in humans working or living on pig farms in the Netherlands

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Results have not yet been published; therefore it is of high importance that they are strictly confidential until the moment of presentation at the 'Wetenschappelijke Voorjaarsvergadering 2008'.

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) has recently emerged as a zoonosis with an extensive reservoir in pigs. The MRSA strains in the animal reservoir are almost all non-typable (NT) with pulsed field gel electrophoresis, the standard typing method in the national MRSA surveillance. Further analysis revealed that these

strains are genetically related to each other. The primary objective of this study was to estimate the prevalence and several determinants of nasal MRSA-carriership in persons working or living on pig farms.

Methods: Fifty randomly selected pig farms in the Netherlands were visited. Pig farmers, household members and employees were interviewed about their working activities and behavioral factors that could influence the MRSA-risk. A second questionnaire collected general data of the pig farms, for example type of pigs and number of pigs kept. Presence of MRSA was determined in all humans, a selection of pigs and dust samples. Data analysis was performed using multivariate multilevel logistic regression modeling.

Results: Complete data were obtained from 232 individuals, of whom 50 persons were pig farmers, 169 were household members and 13 were employees. Preliminary analyses show that of the individuals working in the sty and therefore in intensive contact with pigs, 29% (95% confidence interval (CI) 20-38%) was MRSA-carrier, versus 12% (3-29%) MRSA-carriage with medium contact with pigs and 2% (0,5-6,4%) carriage in persons who lived on the pig farm but without contact with pigs. In the multivariate multilevel analyses working in the sty and presence of sows on the farm were strongly and significantly associated with human MRSA-carriage. When differentiating between pig types for presence of MRSA, farms with finisher pigs seemed to have a significantly higher risk of MRSA in pigs. As expected, there were strong correlations between animal, human and dust MRSA presence.

Conclusions:

1. Prevalence of MRSA in pig farmers and their household members is significantly higher than in the normal Dutch population.
2. Working in the pig sty is an important risk factor for human carriage with animal MRSA, contact with live pigs seems relevant. Further evidence for this hypothesis is the significant effect of presence of sows on human MRSA-carriage, as well as the higher risk of finisher pigs on pig MRSA-carriage. Therefore, it is assumed that contact with pigs in terms of physical handling is more important for the interspecies-spread of MRSA than the proportion of positive pigs.
3. These results can be used for future interventions in order to decrease the spread of NT-MRSA.

P40

In vitro antibacterial activities of daptomycin, linezolid, oxacillin, and vancomycin against clinical isolates of borderline oxacillin resistant *Staphyloccoccus aureus*

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Introduction: In addition to the methicillin sensitive (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) also borderline oxacillin-resistant *S. aureus* (BORSA) has been described. BORSA isolates are defined as *S. aureus* with an increased MIC for flucloxacillin of ≥4 µg/mL and the absence of the mecA gene. At present, limited information is available regarding the clinical relevance and the optimal antibiotic therapy. The antibacterial activities of agents currently used for treating staphylococcal infections were determined for BORSA isolates and compared to those of *S. aureus* ATCC 29213.

Methods: *S. aureus* ATCC 29213 and two clinical isolates of BORSA were used in all experiments. The *in vitro* activities of daptomycin, linezolid, oxacillin and vancomycin were determined by using time-kill methodology. Overnight cultures were diluted and incubated for another 3 hours at 37°C to reach log-phase and diluted again to yield a final inoculum of approximately 1´106 CFU/mL. Antibiotics were added in a concentrations ranging from 0.5 to 8 times the MIC. Samples were taken every 2 hours from 0 to 10 hours and at 24 hours after addition of the antibiotics. CFU counts were obtained using a spiral plater and a colony counter. Each experiment was performed at least in triplicate.

By calculating the logarithm of the ratio of the numbers of CFU/mL in the control growth and those in the presence of antibiotics over time, and by subsequently plotting the corresponding AUC against the concentration, a dose-effect curve was constructed. The Hill equation with variable slope was fitted to the data to calculate Emax, EC50 and the Hill coefficients. Potency ratios were derived from parallel line shift comparisons and/or changes in slope.

Results: In the presence of linezolid, the dose-effect curve of the BORSA strain showed a shift to the left compared to that of the ATCC strain. With oxacillin a shift to the right was observed. The efficacy of daptomycin decreased as well. As a consequence the dose-effect relationship is in favour of linezolid for the BORSA strain. In contrast, oxacillin and daptomycin showed the best dose-effect relationship for the ATCC strain.

For the ATCC strain The Hill coefficient was <1 for the concentration-dependent drug daptomycin and >2.2 for the concentration-independent drugs. Linezolid demonstrated a more concentration- dependent effect against the BORSA strain.

Conclusion: These prelimiairy data strongly suggest that, out of all three antibiotics that were evaluated in this study, linezolid is the most potential therapeutical option for treatment of infections with BORSA strains. More BORSA strains will be included in future analyses to support these findings.

P41

Bacterial endophytes of rice molecular analysis of communities across ten cultivars

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Plant-microbe interactions can result in a plethora of phenomena varying from pathogenesis to the increase of crops yield. The endophytic occurrence of bacteria might be related to deterministic factors or be the result of stochastic events. Among the deterministic factors, the plant genotype may play a key role in endophytic community composition. The present study investigates, by a culture-independent approach (PCR-DGGE), the effect of rice cultivar on the composition of endophytic bacterial communities. While the total bacterial and *Alphaproteobacteria* communities were responsive to alterations in plant genotype, *Betaproteobacteria*, *Pseudomonas* and *Actinobacteria* were mostly influenced by other factors, and distinct communities were observed among individual plants. Additionally, a comparison between isolation and culture-independent approaches was made to study the endophytic communities of one selected cultivar, denoted APO. Intriguingly, a higher diversity was found among isolates than among clones obtained from DNA directly extracted from the endophytic community. The isolates obtained were classified as members of the *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Firmicutes* and *Actinobacteria*, while the clones were mainly affiliated with the Bacilli. Considering the biases that are intrinsic in both methodologies, a polyphasic approach is required to extensively explore the endophytic bacterial communities that are associated with rice plants.

P42

Selection of bacterial populations in the mycosphere of *Laccaria proxima* – Is type III secretion involved?

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The bacterial communities in the *Laccaria proxima* mycosphere (soil from beneath the fruiting bodies) and the corresponding bulk soil were compared by cultivation-dependent and cultivation-independent methods. To assess the distribution of type III secretion systems (TTSS), a PCR-based system for the broad detection of a highly conserved gene involved in TTSS, i.e. hrcR, was developed and used to assay the bacteria (culturable and non-culturable fraction) from the *L. proxima* mycosphere and surrounding bulk soil. PCR-DGGE based on the 16S ribosomal RNA gene showed the selection of presumably mycosphere-specific bacterial groups in the mycosphere of *L. proxima* compared to the bulk soil in three sampling years. Moreover, plate counts revealed that the numbers of culturable heterotrophic bacteria were increased in the mycosphere as compared to the bulk soil. Strikingly, the percentage of randomly-picked isolates that carried the hrcR gene showed a significant increase, from 2.8 in the bulk soil to 13.4 in the mycosphere soil. The bacterial group most frequently isolated from the mycosphere was a hrcR-positive organism related to *Pseudomonas fluorescens* (CFU count: 8.0x105/g dry mycosphere soil). This organism was, together with another hrcR-positive bacterium identified as *Burkholderia terrae* (CFU: 5.1x104/g dry mycosphere soil), not found in the bulk soil.. PCR-DGGE analyses based on the developed TTSS detection showed a similar selection of TTSS in the total bacterial community of the mycosphere. Sequence analyses of a hrcR clone library from mycosphere and bulk soil, showed the selection of two types of *Pseudomonas* TTSS in the mycosphere. One of these sequences was similar to the hrcR gene of the *P. fluoresens* isolate. Thus, different bacteria are enriched in the L. proxima mycosphere and TTSS can be involved in interactions with the fungal host.

P43

The selection of *Sphingomonas* spp. in the mycosphere of *Laccaria proxima* and *Russula emetica*

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The effect of the dense hyphal network directly underneath ectomycorrhizal mushrooms on the bacterial community of soil is a rather unexploited field. A few studies have shown that, next to the well described rhizosphere effect, a mycosphere effect on soil microbes also exists, i.e. there is specific selection of bacteria underneath the mushroom foot. In this study, we describe the selection of specific sphingomonads in the mycosphere of the ectomycorrhizal fungi *Laccaria proxima* and *Russula emetica*. To do so, we used a *Sphingomonas*-specific PCR-DGGE system previously designed by Leys *et al.*, complemented with clone library analyses. Both analysis showed a clear selective effect of the mycosphere on the *Sphingomonas* community. The effect was especially prevalent in the mycosphere of *R. emetica*. Strikingly, similar fungi from different sampling locations showed similar DGGE patterns, while corresponding bulk soil derived patterns differed from each other. The mycospheres of *L. proxima* and *R. emetica*

did not have overlapping community structure, indicating that different fungi select for different sphingomonads. Excision of specific bands from DGGE and clone library analyses showed that major groups in both the mycosphere and the bulk soil did not cluster with known bacteria from the database, indicating new groups of sphingomonads present in these environments. A cultured Sphingomonas type typical for the *L. proxima* mycosphere, denoted HB44, closely resembled *Sphingomonas echinoides*.

P44

Development of a web-based learning tool to enhance health care workers' knowledge, attitude, and risk perception about safe work practices concerning methicillin-resistant *Staphylococcus aureus*

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Introduction: Currently, infection control protocols are more content-driven than user-oriented. In order to be effective, the protocol has to provide reliable, adequate information, and should enable health care workers (HCWs) to make the right decisions. Here, web-based learning tools can be an effective instrument. Our objective was to develop a web-based learning tool based on the available national methicillin-resistant *Staphylococcus aureus* (MRSA) policy, to enhance HCWs' knowledge, attitude, and risk perception concerning safe work practices.

Methods: A validated questionnaire and scenario-based tests were used to determine HCWs' key questions about safe work practices regarding MRSA. Next, the questions were categorised into groups by the Card Sort Method and analysed by WebSort software in order to provide a user-centered search structure for the web-based learning tool. The study was conducted among physicians, nurses, and domestic staff in four hospitals in Germany and the Netherlands.

Results: The results of 276 questionnaires showed inadequate levels of knowledge (97% of respondents) and risk perception (24%) about safe work practices, and negative attitudes (63%) toward complying with these practices. The 28 additional interviews demonstrated that lack of knowledge is mainly related to being uninformed about transmission routes of MRSA (14%), resulting in an inadequate perception of the personal risk to obtain and transmit MRSA (10%). A negative attitude is caused by HCWs questioning the usefulness of adhering to safe work practices (14%). These factors lead to non-compliance with safe work practices. HCWs seem to favor a more personal and social approach of safe work practice documents, stressing personal risks and the rationale behind applying the practices. In sum, 167 key questions were found about which the knowledge, the attitudes or the risk perceptions were unsatisfactory. The web-based learning tool should address these questions. The Card Sort Method (n=10) resulted in ten categories in which the key questions can be grouped. HCWs are best served with a web-based system in which they can actively search these questions in their own language both via a search engine and a table of contents representing HCWs' terminology and information needs.

Conclusions:

1. The applied mixed-methods design provides a powerful approach to analyse HCWs' key questions concerning safe work practices, and provides us with a search structure for a user-centered web-based learning tool.
2. HCWs' involvement in the development of the tool might encourage compliance with safe work practices, which remains subject for further investigation.

P45

Stress! An investigation of emotional and information needs of three types of methicillin-resistant *Staphylococcus aureus*-carriers in the home situation

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Introduction: This study's objective was to analyse emotional and information needs of methicillin-resistant *Staphylococcus aureus* (MRSA)-carriers in the home situation, in order to optimise patient education.

Methods: Semi-structured in-depth interviews were conducted with carriers of three different MRSA-types (n=22): community-acquired (CA-MRSA; n=6), veterinary-acquired (VA-MRSA; n=8) and hospital-associated (HA-MRSA; n=8), based on cognitive and emotional coping strategies. A focusgroup with representants of the three MRSA-types (n=6) was performed to validate the interview results. Critical Incident Theory (CIT) was used to detect stress incidents caused by MRSA-colonisation.

Results: MRSA-colonisation was particularly perceived as stressful by CA- and HA-MRSA carriers. Of the 826 reported stress events, 295 (36%) were mentioned by CA-MRSA carriers and 3 of 40 (41%) by HA-MRSA

carriers. They experienced stress mainly because of feeling stigmatised and a lack of knowledge, leading to emotional problems and information needs (174 and 233 of 505 needs citations, respectively). Used strategies for emotional coping were seeking social support with family, friends, and colleagues. Information needs were met by actively searching for background and practical information.
VA-MRSA carriers reported relatively less stress events compared to CA- and HA-MRSA carriers (191 of 826; 23%). Their coping strategies could be characterised by denial, suppression, and escapism.
In order to fulfil information needs, all carriers actively searched for information on the internet (51 of 164 citations on information resources), although the internet was perceived as an unreliable source providing ambiguous information. To fulfil emotional needs, CA-MRSA-carriers consulted the public health department, and HA-MRSA-carriers contacted the microbiologist, whose information was valued as insufficient to handle their complaints.
Conclusions:

1. MRSA-colonisation turned out to be a stressful event for CA- and HA-MRSA-carriers, and therefore they had much more emotional and information needs compared to VA-MRSA carriers.
2. Therefore, the provided patient education should depend on the type of MRSA . CA- and HA-MRSA-carriers have to be addressed more personally, e.g., by a personal conversation with a health care provider so that emotional needs are met. VA-MRSA-carriers' needs can be met by providing them facts about MRSA.

P46

Single feature polymorphisms using affymetrix microarray analysis reveals substantial differences between pig-related ST398 MRSA isolates

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Introduction: In the last years, it has become evident that in pig and calf farms large numbers of animals are carriers of ST398 MRSA. Nowadays, in the Netherlands most cases of MRSA in hospitals are related to farming. Although al isolates belong to sequence type 398, they contain either SCCmec type IV or V. To obtain better insight in the clonality of the ST398 MRSA lineage, the Affymetrix *Staphylococcus aureus* microarray was used to look for differences between 4 isolates from different clinical backgrounds.
Methods: S385 is ST398 SCCmec type V from a patient with endocarditis. The other 3 isolates are SCCmec type IV and were isolated from the same farm from the nose of a pig, the nose of the farmer, and from a case of exudative epidermitis in a pig, respectively. All isolates were analysed in triplo on an Affymetrix *S. aureus* microarray.
Results: Between the 3 isolates from the same farm, only a few single feature polymorphisms (SFP) were found. Therefore, these were considered together and referred to as S452. Between S385 and S452 over 2000 SFP were found of which more than 1500 were located in genes. S385 contained 23 genes not present in S452, while 35 genes were missing in S385 but present in S452. Remarkably, almost all of the genes present only in S385 and all genes present only in S452 were SCCmec, phage or pathogenicity island related.
Conclusion: Single feature polymorphism detection is a useful approach to analyse differences between closely related isolates. 58 genes were found to be present in S385 or S452 only. A major drawback of this approach is that the information on the microarray is limited to 4 sequenced genomes, which may explain why only minimal differences were found between the 3 isolates from the same farm but with different clinical backgrounds.

P47

The expression of formate dehydrogenases and hydrogenases in *Syntrophobacter fumaroxidans* in different growth phases and at different pH

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Under anaerobic conditions, the complex organic matter degradation process can not be completed by one bacterium. Instead, specialised syntrophic microorganisms play their own specific role. Propionate is an important intermediate in anaerobic degradation and is used as substrate by *Syntrophobacter fumaroxidans*. *S. fumaroxidans* is a non-motile, non-spore-forming, gram-negative propionate degrading delta-proteobacterium, which was isolated from a culture enriched from anaerobic granular sludge. It can oxidise propionate syntrophically in co-culture with the hydrogen- and formate- utilizing *Methanospirillum hungateii* and *Methanobacterium formicicum*, but it is also able to oxidise propionate and a few other organic compounds in pure culture with sulfate or fumarate as the electron acceptor.
S. fumaroxidans' genome, sequenced by DOE Joint Genome Institute, contains four formate dehydrogenases (FDH) coding gene clusters. FDH-1, FDH-2 and FDH-4 each contain a SeCys residue in the amino acid sequence whereas FDH-3 contains a conventional Cystein. *S. fumaroxidans*' genome also encodes six putative hydrogenase (H2ase) gene clusters: three [NiFe]-H2ases, two [NiFeSe]-H2ases and one [FeFe]-H2ase. The differences in expression of [NiFe] and [NiFeSe] H2ase may be regulated by pH.

The gene expression level in different growth phases also may be different; normally it was measured in late log phase, early and mid stages were followed in this research. *S. fumaroxidans* was cultivated in pure cultures with hydrogen-fumarate and formate-fumarate at various pHs (6.0-8.0). Growth curves were made according to the OD, GC and HPLC measurements. However, gene expression experiments to measure pH influence are still in process. Furthermore cells were harvested in early, mid and end log phases, and gene expression levels were measured. Preliminary results suggest that the expression of FDH-1 is ten times lower in hydrogen-fumarate grown cells compared to formate-fumarate grown cells. Hydrogenase expression, however, seems similar in both growth substrates.

P48

Expression analysis of formate dehydrogenases and hydrogenases in *Syntrophobacter fumaroxidans* with quantitative PCR

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Syntrophic consortia of propionate oxidizing bacteria and methanogenic archaea are crucial for high rate wastewater treatment performance by the Upflow Anaerobic Sludge Bed (UASB) reactor. To keep anaerobic propionate oxidation energetically favourable, interspecies electron transfer, either as hydrogen or formate, is essential. We study the hydrogen and formate transfer between *Syntrophobacter fumaroxidans* (propionate oxidiser) and *Methanospirillum hungatei* (methanogen).

Genome analysis of *S. fumaroxidans* revealed the presence of four distinct gene clusters coding for formate dehydrogenases and six distinct gene clusters coding for hydrogenases, including five [NiFe]-hydrogenases and one [FeFe]-hydrogenase. Primers were designed, specific for each formate dehydrogenase and hydrogenase gene cluster and tested with quantitative PCR. During syntrophic growth with *M. hungatei*, the expression level of FDH-4 compared to the expression level in axenic growth. This strongly suggests that the FDH-4 of *S. fumaroxidans* plays an important role during syntrophic growth.

P49

MBL-associated serine protease 2 modulation by *Staphylococcus aureus*

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Introduction: MBL-associated serine protease 2 (MASP-2) is a key component of the lectin pathway and has recently been shown to activate the coagulation system. The LP triggers activation of the complement system by different carbohydrate recognition molecules: Mannose Binding Lectin (MBL) and ficolins. Binding of MBL and ficolin to microbes results in activation of MASPs. Four different MASPs were described, but only MASP-2 is known to activate the complement system by cleavage of C4 and C2 resulting in the formation of C3 convertases (C4b2a). The C3 convertases cover microbes with C3b molecules which is crucial for phagocytosis and activation of C5. The coagulation system, is an important part of hemostasis. Both the intrinsic and extrinsic pathways lead to cleavage of factor X into active form Factor Xa, which in turn activates prothrombin into thrombin. The role of thrombin is to convert fibrinogen into fibrin, the main compound of clots. Like Factor Xa, MASP-2 can activate prothrombin thereby inducing the formation of fibrin clots. *Staphylococcus aureus* is well-known for the excretion of numerous small molecules that specifically block critical steps of the innate immune defence. Since MASP-2 is a key player in both the innate immune defence and the coagulation system, we investigated the presence of specific MASP-2 modulators in *S. aureus* supernatant.

Methods: MASP-2 ELISA. Recombinant MASP-2 (2 CCP and serine protease domains) was cloned and expressed in *Escherichia coli* and purified as described earlier (Ambrus *et al.*, 2003). MASP2-coated microtiter wells were incubated with purified C4 at 37°C in the presence of staphylococcal supernatant. MASP-2-mediated deposition of C4b was detected using specific antibodies.

MASP-2 binding assays. 1) MBL-MASP complexes was captured by incubating GlcNAc-beads with IgG depleted human serum in 1M NaCl at 4°C. 2)Recombinant MASP-2 was immobilised to sepharose beads. 1+2) After washing, beads were incubated with staphylococcal supernatant at 4°C. The captured proteins were eluted with EDTA or 2M NaCL respectively and subjected to SDS-PAGE and silver-staining. Specific proteins were analysed by Liquid Chromatography Mass Spectrometry (LC-MS) and identified by using the Mascot database.

Results: Functional analyses of forty *S. aureus* supernatants in a LP ELISA led to the identification of at least three different supernatants that could block LP-mediated C3b deposition by 50%. These supernatants also prevented C4b deposition by the LP as well as C4b deposition by rMASP-2. 'Fishing' experiments in *S. aureus* supernatant with MBL-MASP complexes and rMASP2 immobilised on beads revealed two MASP-2 binding proteins in *S. aureus*: the 19kD form of staphylococcal nuclease (Snase) and the 12,5 kD form of extracellular fibrinogen binding protein (Efb).

Conclusion: Functional analyses revealed that *S. aureus* strains excrete specific LP inhibitors that block MASP-2 activity. Staphylococcal nuclease and Efb were purified from *S. aureus* supernatant as MASP-2 binding molecules. Further research will unravel the underlying mechanisms of these MASP-2 binding proteins. Identification of bacterial MASP-2 inhibitors will increase our understanding of the role of the Lectin pathway and the coagulation system in our defence against bacteria.

P50

Extended-spectrum beta-lactamases screening agar with AmpC inhibition

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Introduction: The serious increase in the prevalence of Extended-Spectrum Beta-Lactamases (ESBLs) worldwide creates a need for effective screening methods to detect these resistance genes. The currently used screening agars have low specificity, mainly due to growth of isolates of species with inducible AmpC beta-lactamases. In this study, we evaluated an ESBL Screening Agar (ESA) which inhibits growth of AmpC-producing isolates and *Enterococci*, and compared it with the commercially available BLSE agar (AES Laboratory, France) for selective isolation and presumptive identification of ESBL-producing Enterobacteriaceae.
Methods: The ESA consists of two MacConkey Agars containing either ceftazidime 1.0 mg/l or cefotaxime 1.0 mg/l + cloxacilline 400 mg/l + vancomycine 64 mg/l. The BLSE agar is a commercial double-plate agar (MacConkey + ceftazidime 2 mg/l and Drigalski + cefotaxime 1,5 mg/l). The agars were evaluated with 208 *Enterobacteriaceae* isolates, 70 of them had been previously genotypically characterised as ESBL-producers. The other 138 isolates were ESBL negative. The ESA was further evaluated with 100 clinical specimens. The clinical specimens were futher characterised for ESBL production with ESBL combined disc test and ESBL Etest.
Results: The sensitivity and specificity of the ESA and the BLSE agar tested with the 208 *Enterobacteriaceae* isolates were 100% (70/70) and 84,7% (117/138), and 100% (70/70) and 57,2% (79/138) respectively. Isolates of species with inducible AmpC were most commonly the false positives on BLSE agar. The ESA detected all 5 ESBL-positive clinical specimens correctly, 4 specimens were false positive. The sensitivity and specificity of the ESA in the clinical specimens in this study were 100% and 95,7% respectively.
Conclusion: The specificity of ESA for screening of ESBL-producing strains was significantly better than the specificity of BLSE agar; the better performance of the ESA was mainly due to less false positive results due to Amp-C-producing strains. The ESA performed well also when inoculated directly with clinical specimens. We conclude that ESA is a sensitive and convenient method to directly screen for ESBL-producing organisms in clinical specimens.

P51

A molecular diagnostic approach for MRSA hospital outbreaks providing all necessary data of patients and contacts within the first 24 hours

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During MRSA hospital outbreaks a rapid and accurate detection of patients infected or colonised with the outbreaking strain is crucial. However, the standard culture method for detecting MRSA is time-consuming, taking several days. Therefore, recently, several MRSA PCR detection methods are being developed to accelerate the MRSA tracing.
We designed a procedure combining two MRSA PCR detection methods, to manage all MRSA outbreaks within 24 hours, using the in house MecA/Martineau PCR developed by Van Hannen, Antonius Ziekenhuis, Nieuwegein and the commercially available IDI MRSA PCR (based on the Huletsky primers).
After an overnight culture incubation step, the MecA/Martineau PCR is used as a first step. Positive results are confirmed in the more specific Huletsky PCR.
Since the introduction of our '24 hours all-in' procedure we were able to manage six MRSA outbreaks in three different hospitals. Sixty-six out of 617 clinical samples (10.7%) appeared to be positive in the MecA/Martineau screening PCR and thus needed further processing with the IDI MRSA PCR. Of these samples 24.2% (16/66) were also positive in this Huletsky PCR. In these remaining 16 samples MRSA could be cultured in 11.
Conclusion: This approach combines the advantage of a high throughput, which is essential in outbreak situations, with a superior sensitivity and specificity through the combination of PCR's targeting different parts of the genome and is therefore able to give an exact overview of the spread of MRSA within 24 hours after receiving the samples.

P52

Age-related genotypic and phenotypic differences in *Moraxella catarrhalis* isolates from children and adults presenting with respiratory disease in 2001-2002

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Introduction: *Moraxella catarrhalis* causes respiratory tract-related disease in both children and adults. Little is known regarding the population biology or the differential incidence of vaccine candidate genes in isolates infecting these different age groups.

In this study, screening methodologies were utilised to identify genotypic and phenotypic differences in a global selection of *M. catarrhalis* isolates infecting both children and adults presenting with respiratory disease. The study provides valuable information regarding the pathogenic mechanisms associated with *M. catarrhalis* infection in these 2 age groups, as well as providing data for future vaccine design.

Methods: Bacterial isolates comprised 195 worldwide *M. catarrhalis* isolates cultured from children and adults presenting with respiratory disease in the years 2001-2002. Parameters compared between the two age groups included vaccine candidate gene prevalence, gene expression and differences in phenotype. Methods used for detection of gene prevalence were polymerase chain reaction (PCR) and PCR restriction fragment length polymorphism (PCR RFLP). Gene expression was determined by ELISA, differences in phenotype were studied by autoagglutination, biofilm and complement resistance assays.

Results: Significant differences in the prevalence of *uspA2* and *uspA2H* virulence-associated genes, and the distribution of lipooligosaccharide (LOS) types, were observed ($p<0.0001$ and $p=0.01$, respectively). A significant decrease in the prevalence of isolates expressing the hemagglutinin (Hag) protein was observed in adult isolates ($p=0.002$). Both *uspA2H* and LOS type B were associated with 16S rRNA type 1 isolates only, and 2 surrogate 16S rRNA lineage markers were identified. Phenotypically, a significant difference in biofilm formation ($p<0.001$), but not autoagglutination or serum resistance was observed.

Conclusion: An attempt has been made to map genotypic and phenotypic differences occurring in *M. catarrhalis* isolates infecting both children and adults. The results show that isolates infecting children tend to possess a greater biofilm forming capacity, and that differences in the prevalence and expression of several outer membrane-associated virulence genes exist between worldwide *M. catarrhalis* isolates associated with children and adults, possibly as a consequence of immune evasion. Novel genetic markers that distinguish between 16S rRNA type 1, and types 2 and 3 isolates (rRNA types associated with the two major *M. catarrhalis* lineages, as well as between upper and lower respiratory tract infections) have also been identified. From our data, it appears that UspA2, UspA2H, UspA1 and Hag alone may be unsuitable vaccine candidates for *M. catarrhalis*, unless an age-related vaccine is required. However, a multivalent vaccine comprising immunogenic epitopes from two or more of these proteins could possibly be effective.

P53

The first *Salmonella enterica* serotype *Typhi* with extended-spectrum beta-lactamase

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Introduction: *Salmonella enterica* serotype *Typhi*, the etiologic agent of typhoid fever, is responsible for endemic or epidemic cases of typhoid fever in tropical and subtropical countries. *S. enterica* serotype *Typhi* strain was cultured from blood and feces samples of a 54 year-old man who was admitted to Waterland hospital in Purmerend, the Netherlands, from 30 November until 7 December 2007, because of fever and diarrhea. Two weeks previously the patient had been in the Philippines for two months. The strain was resistant to third generation cephalosporins but sensitive to amoxicillin/clavulanate. Because ESBL-production in *S. enterica* serotype *Typhi* is of major worldwide concern, we investigated whether resistance to the third generation cephalosporins was due to production of extended-spectrum beta-lactamases (ESBL).

Methods: The strain was identified as *S. enterica* serotype *Typhi* with the Vitek-2 system, slide agglutination method, (partial)16S sequencing and amplified fragment length polymorphism analysis. Susceptibility testing and ESBL detection were done with the Vitek-2 system, E-test, ESBL-E- test, ESBL-combined disk test and PCR amplification of the TEM, SHV and CTX-M genes.

Results: The phenotypic and genotypic methods confirmed the identification of the strain as *S. enterica* serotype *Typhi* and the production of ESBL. The ceftzidime MIC was 48 mg/l and cefotaxime MIC was 6 mg/l. The strain was also resistant to aminoglycosides and trimethoprim/sulfamethazole.

Conclusion: To the best of our knowledge, this is the first report of an ESBL-producing *S. enterica* serotype *Typhi* strain from the Philippines. Three cases of infection

by an ESBL-producing strain of *S. enterica* serotype *Paratyphi A* have been reported from Nepal. The spread of ESBL-production among *Typhi* and *Paratyphi* is very worrisome and warrants further surveillance and accurate detection of this resistance mechanism.

P54

An increase in prevalence of SCCmec types IV and V MRSA in the Netherlands over an 11-year period

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major pathogen causing hospital-acquired (mainly SCCmec types I, II, and III) infections worldwide. In recent years, community-associated MRSA (CA-MRSA) is becoming a serious threat since CA-MRSA could spread into hospitals. CA-MRSA mainly causes primary skin infections, such as furunculosis and abscesses. Many of the CA-MRSA isolates carry the virulence factor Panton-Valentine leucocidin (PVL) and staphylococcal cassette chromosome mec (SCCmec) type IV or V.

The aim of this study was to determine the prevalence of SCCmec types IV and V MRSA in the Netherlands during an eleven year period (1996-2006).

Methods: From the years 1996, 2001, and 2006 one hundred MRSA strains per year were randomly chosen from the Dutch MRSA surveillance database and were characterised by SCCmec multiplex PCR as described by Kondo *et al*. All isolates were tested for the presence of the mecA gene, a *S. aureus* specific DNA fragment (Martineau), and the pvl genes.

Results: The percentage of SCCmec types IV and V assigned to the MRSA isolates from 1996, 2001 and 2006 were: 42% and 0%, 50% and 15%, 63% and 18%, respectively. The percentage of PVL positive isolates, often associated with CA-MRSA, were 13% (1996), 11% (2001), and 17% (2006). Surprisingly, 90% (37/41) of the PVL positive isolates carried the SCCmec type IV.

Conclusion: During the eleven year period an increase in SCCmec types IV and V is shown, but not all of these MRSA isolates were PVL positive. Therefore, differentiation between hospital-acquired and community-associated MRSA cannot be based on SCCmec typing alone. Our results suggest that a shift in MRSA isolates with SCCmec types I, II, or III to SCCmec types IV or V has occurred in Dutch hospitals in recent years.

P55

Campylobacter jejuni infections and anti-ganglioside antibodies in Guillain-Barré syndrome in Bangladesh

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Introduction: The Guillain-Barré syndrome (GBS) is an acute neuropathy, and immune-mediated flaccid paralysis frequently associated with *Campylobacter* infections. GBS is considered to be a post infectious disease since approximately two-third of patients report some form of preceding infectious illness. *Campylobacter jejuni* has recently been identified as a major cause of antecendent infections in GBS patients. Patients with GBS frequently have antibody reactivity against neural ganglioside such as GM1, GM2, GD1a, and GQ1b. Although a huge number of investigations have been done on GBS in developed countries, there is a paucity of reports on GBS in developing world like Bangladesh. The purpose of this study was to determine antibodies against *C. jejuni* and anti-ganglioside (GM1, GD1a and GQ1b) in serum of GBS patients, family controls (FC) and other neurological diseases (OND).

Methods: In a prospective study, we enrolled 100 patients fulfilling NINCDS criteria for GBS
admitted between July 2006 and June 2007 from Dhaka Medical College Hospital and Bangabandhu Sheikh Mujib Medical University, Bangladesh. Detailed clinical histories were obtained, electrophysiological studies were performed to determine GBS subtype, ELISA was performed against *C. jejuni* and anti-gangliosides and microbiological studies were also performed.

Results: Ninety-seven GBS sera were available for study from a total of 100 GBS patients. The highest number of cases occurred in October (n=13), February (n=14) and March (n=13). Data analysis suggested that the highest number of GBS was found in the youngest age-group (median 19.5). Male/female ratio was 3.6:1. Antecedent events were recorded in 69% of patients, the most frequent events being gastroenteritis (37%) and upper respiratory tract infection (19%). Among 58 GBS patients who had an EMG performed, 62% had acute motor axonal neuropathy (AMAN), 26% acute inflammatory demyelinating polyneuropathy (AIDP) and 12% acute motor sensory axonal neuropathy (AMSAN). There was a strong seasonal distribution of

AMAN cases in January to March. Fifty-seven percent of 97 GBS patients had serological evidence of recent *C. jejuni* infection as compared with 10% of FC, and 2 % of OND (p-value <0.001). Ninety-two percent of AMAN patients and 43% of AIDP patients were seropositive. Anti-gangliosides antibodies were more frequent in GBS patients compared with controls (56 % vs 6% of FC and 2 % of OND, p-value <0.001). The subgroup of GBS patients with anti-ganglioside antibodies suffered more severe neuropathy with predominantly distribution of weakness, and but no cranial nerves or sensory disturbance. Ten patients had positive stool cultures for *C. jejuni*.

Conclusion: This project is the first initiative to characterise the cause of antecedent infections, anti-ganglioside antibody response, and clinical and electrophysiological features of GBS in Bangladesh. Severe, axonal, and pure motor forms appear to be the most frequently observed GBS variants in Bangladesh. GBS is most frequently preceded by gastroenteritis and most affects the youngest age-group. *C. jejuni* is the predominant preceding infection and associated with a severe, axonal, and pure motor forms. The presence of anti-ganglioside antibodies was significantly associated with *C. jejuni* infections in GBS patients in Bangladesh.

P56

Viral culture and two molecular assays for the detection of enterovirus in cerebrospinal fluid samples

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Enteroviruses (EV) are a frequent cause of infection, especially in children. Most infants and young children will experience mild gastrointestinal or upper respiratory signs, but some will develop meningitis. It is not always easy to differentiate clinically viral meningitis from bacterial meningitis, a very serious and potentially life-threatening disease. Therefore, the suspicion of meningitis has always been a major reason for hospitalisation and immediate start of antibiotics. There is a need for rapid and reliable diagnostic tests. In this study we compared the classical viral culture assay, a real-time, TaqMan reverse transcriptase PCR (TqM) assay and the GeneXpert EV assay (GX), a fully automated and rapid real-time PCR assay, for the diagnosis of EV in cerebrospinal fluid (CSF) specimens.

Methods: CSF samples from 155 children (age group 0-16 years) were tested for the presence of EV RNA using the GX and TqM assays (GX and TqM) in the period from May till December 2007. Ninety-three of these samples were also tested by viral culture.

Results: From the 93 samples tested by culture, only 5 EV were isolated. One of these samples was negative in both molecular assays, and 15 and 18 were positive with the GX and TqM assay, respectively, increasing the diagnostic yield from 5.4% to 16.1% and 19.4%, respectively. From the 155 samples tested with the TqM assay, 26 were positive for EV RNA. Four of these samples were negative and one gave an invalid result in the GX assay. No additional positives were found with the GX assay.

Conclusions:

1 Viral culture has a much lower sensitivity and has a longer turn around time than both molecular assays.
2 The TqM PCR seems to be a more sensitive assay for the detection of EV RNA from CSF samples than the GX test. However, the GX assay has several advantages over the TqM assay. Each patient sample is individually analysed and the diagnosis of EV infection can be completed in only 2.5 hours. Because of the easier performance, GX test allows providing results even in the evening, at night or in week-ends. Molecular techniques may reduce the length of hospitalisation of the patient and the use of unnecessary antibiotic therapy.

P57

A higher prevalence of antibiotic resistance of indigenous *Staphylococcus aureus* isolates among health care workers compared to healthy volunteers?

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Background: Worldwide antimicrobial resistance is an increasing problem. It is generally accepted that this increase is due to increased antimicrobial use and exposure during the last decades. One can hypothesise that healthcare workers (HCW) are more often exposed to antibiotics as compared to the open population. Therefore, in this study the prevalence of antimicrobial resistance was compared between *Staphylococcus aureus* isolated from volunteers in the open population and healthcare workers.

Methods: During 2005, a random sample of 4000 inhabitants of a large city in the south of the Netherlands was taken from the Municipal Administration. Each person received an envelop by mail containing study instructions, an informed consent, a questionnaire, a nasal swab for taking a sample from the anterior nare, and material for returning the swab to the laboratory. In addition, in 2006 at the annual conference of general practitioners (GPs) of the Dutch College of General Practitioners (NHG) nasal swabs were taken from a random sample of GPs. The swabs were analysed for the presence of *S. aureus* using selective

media. The susceptibility to commonly used antibiotics was determined, including penicillin, methicillin, macrolides, tetracycline, fusidic acid and mupirocin. The presence of MRSA was confirmed using a real-time PCR. The nature of the observed MRSA clones was investigated using spa and SCCmec typing.

Results: In total, 2369 swabs out of the 4000 were received, (i.e. a spontaneous response rate of 60%) from the open population and 395 from the GPs at the NHG-conference. *S. aureus* strains were isolated in 28% of the volunteers and 33% of the GPs. Resistance percentages were similar for both populations i.e. for penicillin, macrolides and tetracycline 71%, 5%, and 3%, respectively. The prevalence of resistance to fusidic acid was low (2%) and no mupirocin resistant strains were detected. Methicillin resistance was found only in 11 strains (1.5%) in the open population, two of which carrying the mecA gene. These two MRSA isolates were associated with the New York/Japan clone and the Berlin clone.

Conclusion: The spontaneous response rate of 60% was high and the percentage *S. aureus* isolated was as one should expect. The carriage rate of *S. aureus* was only slightly higher in general practitioners. The HCW tested in the present study showed a similar prevalence of resistant *S. aureus* isolates as compared to the open population. Thus, HCW do not provide a reservoir of antibiotic resistant micro-organisms and resistance genes. The observed MRSA isolates did not have a genetic background commonly observed among community associated MRSA strains.

P58

Heterogeneity of *Aggregatibacter actinomycetemcomitans* serotype E strains identified by real-time PCR and 16S rRNA sequence analysis

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Aggregatibacter actinomycetemcomitans is a gram-negative pathogenic rod that is involved in periodontal bone loss of humans. For diagnostic purposes, *A. actinomycetemcomitans* can be cultivated or detected by (real-time) PCR.

From subgingival plaque samples of 103 patients with severe periodontitis the presence and numbers of *A. actinomycetemcomitans* were determined by culture on selective TSBV media and specific Real-Time PCR. One subject carried an *A. actinomycetemcomitans* that was cultured from the periodontal pocket that remained negative in the real-time PCR. This strain was identified on biochemical characteristics (API-ZYM and catalase-positive reaction), gram-staining and a PCR directed to the species-specific leukotoxin A gene of *A. actinomycetemcomitans*. To confirm the identification, a 478 bp sequence of the 16S rRNA was compared to the NCBI databank. It revealed a 100% similarity to sequences from *A. actinomycetemcomitans* serotype E, whereas other serotypes displayed less than 98% similarity. A serotype specific PCR confirmed the presence of the serotype E gene cluster in this strain. To elucidate the presence of the primer and probe sequences of the real-time PCR on the 16S rRNA genome, we have sequenced the 16S rRNA genome using universal primers to yield 1330 bp sequences of this strain, from 17 unrelated serotype e wild-type strains and 16 reference strains (serotype a-f).

From the 17 serotype e wild-type strains, only two displayed a positive reaction in the real-time PCR. The real-time PCR-negative strains harbor a conserved mismatch on the 3'-side of the primer-binding site of the real-time PCR. The probe-binding sites of the non-reacting serotype e strains display multiple mismatches throughout the sequence. Using AFLP analysis, this group displayed a clonal pattern, including references strains from various geographical origins.

Conclusion: The conserved mismatches and the clonal appearance suggest a distinct phylogenetic group within the population of A. actinomycetemcomitans serotype E. Since a type IV pilus gene homolog is required for natural transformation in *A. actinomycetemcomitans*, as observed by knockouts of a naturally transformable strain previously, the role of a functional type IV pilus-like gene cluster in clonal wild-type serotype E strains will be discussed.

P59

Evaluation of the specificity of a selective chromogenic agar medium (chromID ESBL, BioMerieux) for the screening of ESBL-producing *Enterobacteriaceae*

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Introduction: ESBL-producing *Enterobacteriaceae* are recognised worldwide as nosocomial pathogens of major importance. In accordance to the published BRMO guideline patients suspected of carrying ESBL-producing organisms are subjected to a surveillance culture procedure. This implies that patient specimens for surveillance screening need to be cultured on media promoting and selecting growth of ESBL-positive isolates. Recently, selective antibiotic-containing chromogenic media have been made available for the rapid detection of ESBL-producing organisms. The advantage of these media is detection and presumptive identification of ESBL-producing Enterobacteriaceae directly from clinical

specimens. Growth followed by specific colorisation is only possible if organisms are in possession of β-lactamase enzymes capable of hydrolyzing the in the agar present selective antibiotics. The drawback of this antibiotic selection is the existence of clinically important organisms also capable of expressing broad-spectrum β-lactamase enzymes (AmpC) other than ESBL-enzymes and which are therefore expected also to grow on this selective agar medium.

Objectives: As the prevalence of ESBL-positive organisms is low in our hospital we decided to focus on evaluating the specificity of the chromIDTM ESBL (BioMérieux) by screening patient specimens preferably containing gram-negative organisms.

Methods: To include only specimens containing gram-negative organisms expressing chromosomally encoded and/or plasmid encoded β-lactamases, specimens were cultured in duplicate. However, the duplicate specimen culture was performed after the results of the identification and susceptibility results of the organisms from the initial culture had been determined. After overnight incubation, the chromIDTM media were visually controlled for growth. Subsequently, the color of the colonies was recorded as well as the results of the presumptive identification by colorisation were compared to the results obtained by the initial non-selective culture media. Furthermore, the resistance mechanism with respect to the nature of the beta-lactamase(s) expressed was deduced from the VITEK 2 susceptibility results.

Results: Of the 44 specimens 29 specimens contained initially *Escherichia coli* (n=14), *Klebsiella pneumoniae* (n=10), *Klebsiella oxytoca* (n=2) and *Proteus mirabilis* (n=3) isolates. Of these 29, only 9 grew on the ChromIDTM ESBL plates. Four i.e. 2 *E. coli* and 2 *K. pneumoniae* were confirmed as ESBL-positive, the remaining 5 were ESBL-negative. Three out of five grew only as small pale colonies, which are not suspect for ESBL. Of the 15 specimens containing initially organisms capable of expressing chromosomal β-lactamases at high levels, 8 grew from which 5 were due to derepression of the AmpC β-lactamase.

Conclusion: In the present limited evaluation it is found that organisms as *Enterobacter cloacae, Serratia marcescens* and *Citrobacter* spp. also grow on chromIDTM media. Growth with specific colorisation is only shown in the organisms with a derepressed AmpC β-lactamase. The organisms not expressing AmpC at high levels do not grow or grow with colony colors not suspect for ESBL-producing organisms. The four isolates positive for ESBL did grow on the chromIDTM with specific colony colorisation. However, the number of ESBL-positive isolates is too low to give reliable data with respect to the sensitivity of the selective medium used and merits further evaluation of these media in outbreak situations.

P60

The emergence of a community-associated methicillin-resistant *Staphylococcus aureus* from Southeast Asia in the Netherlands

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The aim of this study was to evaluate the molecular and epidemiologic features of methicillin- resistant *Staphylococcus aureus* (MRSA) isolates with the Dutch PFGE cluster type 50 and 50a. In the 1990's the epidemiology of MRSA has changed when serious, sometimes even fatal, infections, began to occur among healthy persons with a community-onset and without the hospital-associated risk factors. A community associated MRSA (CA-MRSA) strain which has caused severe infections in Asia has emerged in the Netherlands.

Methods: MRSA strains were sent to the RIVM for typing for the national surveillance program. At the same time hospitals sent a questionnaire with epidemiological data. A subset was created of all MRSA strains from the period 2002-2007 which belonged to cluster 50 (n=76). All of the strains harboured the Panton-Valentine leucocidin (pvl) genes and the mecA gene. For molecular characterisation pulsed- field gel electrophoresis (PFGE) and staphylococcal protein A (spa) typing was performed. From 5 strains with the PFGE cluster type 50(a) multilocus sequence typing (MLST) and SCCmec typing was performed.

Results: Within MRSA cluster 50 three different spa types were found, t437 (n=71), t2517 (n=3) and t441 (n=2), respectively. All spa types are closely related. All 5 strains subjected to MLST yielded sequence type (ST) 59 and belonged to SCCmec type V. All the non-related strains were different by PFGE – and spa typing. The epidemiological data of 11 (14%) cluster 50(a) strains revealed a link with Southeast Asia (SEA). I.e. strains were isolated by adopted children or traveling. Strains with the same characteristics have been described previously as a predominant CA-MRSA clone in SEA.

Conclusion: This study has revealed that spread of a well known invasive MRSA clone has occurred from SEA to the Netherlands. This has probably by adoption of children and traveling from SEA. This clone has caused epidemics in several hospitals and families in the Netherlands.

P61

Abiotrophia defectiva infection of a total hip arthroplasty diagnosed by 16S r RNA sequencing

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Introduction: *Abiotrophia defectiva* is a fastidious gram-positive bacterium that is known for its pleiomorphic coccobacillar forms and that may appear as streptococci. Previously, it was referred to as part of the nutritionally variant streptococci. Nowadays, it is the single species of the *Abiotrophia* genus. It is a resident of human oral cavity and urogenital and intestinal tracts and accounts for approximately 5% of cases of bacterial endocarditis. There have been some sporadic reports of *A. defectiva* as infective agent of osteoarticular infections and we describe the first case of a total hip arthroplasty infection caused by *A. defectiva*.

Methods: A 71-year-old woman was admitted to our hospital for replacement of her left hip arthoplasty that she had undergone 2 years earlier in another hospital. She presented with a history of increasing pain in her left upper leg and knee. She had no fever. Progressive osteolysis near the prothesis was shown on consecutive radiographs and confirmed by scintigraphy. She was diagnosed with an arthroplasty infection.

Cultures of bone fragments and of pus near the prothesis showed only growth in broth media and on the plate used to detect anaerobes which among others contains horse blood and cysteine. Gram-stain revealed a pleiomorphic gram-positive coccobacillus that could not be identified by biochemical techniques. 16S rRNA sequencing was performed and showed 99% homology with A. defectiva sequences. Retrospectively the gram-stain matched the species.

Conclusion: Since its pleiomorphic appearance and relatively infrequent incidence, *A. defectiva* is difficult to identify. It mimics gram-positive rods and therefore might have a place in algorithms used to identify this group of bacteria. Standard biochemical techniques often fail to identify this micro-organism. 16 S rRNA sequencing offers a good methodology to recognise this bacterium. The proposed affinity of *A. defectiva* for avascular tissue is underlined by this first report of a hip arthoplasty infection. Considering the increasing use of foreign body material in modern medicine and the growing us of molecular diagnostics, one might expect a rising incidence of *A. defectiva* associated infections.

P63

Analysis of Tn1546 elements within vancomycin-resistant *Enterococcus faecium* isolates from Saudi Arabia

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Enterococci are found in both environment and hospital settings, frequently expressing glycopeptide/high-level aminoglycoside resistance. In recent years, a clonal complex of *Enterococcus faecium* isolates (CC17) has become more prevalent in facilitating global nosocomial-related infection. Currently, no data is available regarding the position of clinical *E. faecium* isolates from the Middle East within the global context. Therefore, we investigated 34 Saudi Arabian VanA type vancomycin resistant *E. faecium* isolates with respect to genotype, antibiotic susceptibility patterns, the presence of enterococcal surface protein (esp) and hyaluronidase (hyl) genes, characterisation of Tn1546 elements, and transformation efficiencies. PFGE and MLST analysis revealed the presence of 31 and 6 different genotypes, respectively. Further, three new ST types were determined. Ninety seven percent (33/34) of isolates were associated with clonal complex 17, with all isolates being resistant to ampicillin and sensitive to linezolid. The esp and hyl genes were found in 44% (15/34) and 53% (18/34) of isolates, respectively. Further, a significant negative correlation was observed between esp and hyl positivity (Pearson r=-0.47 (-0.70 to -0.15)), as well as an association between PFGE groups and esp/hyl positivity. Tn1546 analysis revealed that the isolates belonged to 5 different groups, including 2 new groupings. Taken together, our results help place vancomycin resistant VanA *E. faecium* from Saudi Arabia within the global context of clonal complex 17 isolates.

P64

Expression analysis of alveolar macrophages during phagocytosis of *Streptococcus suis*

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Streptococcus suis is the causative agent of severe infections in pigs. The typical symptoms include meningitis, septicemia, and arthritis. Pigs, especially young ones, often do not survive an infection. The pathogenesis of *S. suis* infections is rarely understood. Sows carry the bacteria symptomless on their tonsils, and pass them on to their piglets. The piglets cannot cope with the infection due to their immature immune system, and get sick.

S. suis is strongly encapsulated, 35 different capsular serotypes exist. Capsule is one of the first virulence factors described for *S. suis*. It has been shown that acapsular mutants of a virulent strain, are completely avirulent in two different porcine infection models. During infection, capsule synthesis is strongly regulated, indicating the importance of capsule during infection. *In vitro*, it was shown that an acapsular mutant was phagocytosed by alveolar macrophages 100-1000 times more efficient than its parent wild type strain. Therefore, we focused on the interaction between *S. suis* and porcine alveolar macrophages to study the host response to *S. suis* and to phagocytosis of bacteria.

Freshly isolated alveolar macrophages were incubated with either wild type *S. suis* or an isogenic acapsular mutant in a time course experiment. Seven independent experiments were performed. At all timepoints significant differences were found in phagocytosis between the wild type and the acapsular mutant ($p<0,05$). RNA was extracted from the macrophages for expression analysis. Macrophages that had not been in contact with bacteria were also included as a negative control. Gene expression was studied using two independent techniques. RNA was hybridised to a porcine whole genome Affymetrix array. Besides, expression of several well known innate immune genes was determined using qPCRs.

The array data showed that within the first fifteen minutes of incubation no large expression differences existed in time between the bacteria with and without capsule, whereas phagocytosis of the acapsular mutant took place within five minutes. After 60 minutes of incubation with bacteria, macrophages showed a different expression pattern compared to the previous timepoints. Several innate immune genes were strongly induced, such as IL-1-beta, TNF-alfa, and MIP-2-alfa. After clearing the bacteria, the macrophages obviously start producing pro-inflammatory cytokines in order to control the infection. These data were confirmed using qPCRs. In conclusion these data indicate that macrophages do not need additional gene regulation and probably delaying gene expression in order to phagocytose bacteria efficiently.

P65

Genomic analyses of a *Bordetella pertussis* lineage associated with the resurgence of pertussis

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Bordetella pertussis is the causative agent of pertussis or whooping cough in humans and is re-emerging worldwide despite vaccination. In many countries antigenic divergence has been observed between vaccine strains and clinical isolates. Recently, we found polymorphisms in the promoter for pertussis toxin (Ptx) that divides the *B. pertussis* strains predominating for the last twenty years into two lineages designated P1 and P3. The P3 strains were first observed in 1994, gradually increased in frequency and nearly completely replaced the P1 strains in the late 90s. The resurgence of pertussis was associated with the increase in frequency of P3 strains, suggesting that changes in Ptx production have increased the fitness of *B. pertussis*. Here, we applied the power of whole-genome sequencing to identify additional adaptations of *B. pertussis* that may play a role in the fitness of the P3 lineage.

We use the 454 sequencing technology to sequence four recently isolated *B. pertussis* strains, two P1 and two P3 strains. These strains were compared with the already sequenced strain Tohama I (Parkhill *et al.* 2003) and seven regions of difference (RDs) were identified. Compared with Tohama, the recent isolates harbour 43 additional genes which are homologues to genes of *Bordetella bronchiseptica* and *Bordetella parapertussis*. A large fraction of these genes appeared to be involved in metabolism. Some of the genes code for iron transport en exported proteins, suggesting they may be important for virulence. In addition, 30 genes were found to be unique for the Tohama strain. Differences in gene content were also observed when the P1 and P3 were compared. One RD, comprised of 18 genes, was unique for P1 strains while a second RD, comprised of 19 genes, was only found in P3 strains. In addition to the RDs about 200 single nucleotide polymorphisms (SNPs) have been identified in the four strains compared to Tohama, including some novel SNPs in known virulence genes. An interesting SNP was found in the bvg locus, which plays a central role in the regulation of *B. pertussis* virulence.

In conclusion, the two lineages could be distinguished by the absence or presence of several gene clusters and by SNPs. Further research is focussed on the role of these loci in the ecology of pertussis.

P66

Genomic diversity within USA300

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Background: Meticillin-resistant strains of *Staphylococcus aureus* (MRSA) are the most common cause of nosocomial infections. In the last decade, the incidence of MRSA in the community, so called community-associated MRSA (CA-MRSA), has greatly increased, starting in the US.

CA-MRSA are associated with unusually invasive disease, including severe septicaemia, necrotising pneumonia, and necrotising fasciitis. Nowadays, CA-MRSA infections in the US are predominantly caused by a single epidemic clone, USA 300. The last years, this clone also emerged in Europe. Based on various different genotyping schemes, PFGE, MLST, Spa and SCCmec typing, USA300 are considered to be highly clonal, a result of rapid clonal expansion without significant genomic diversification. However, the level genetic variability has not been studied in great detail yet. The aim of this study was to assess the level of genomic diversity in a collection of 14 USA 300 strains from Chicago.

Methods: 14 consecutive USA300 strains were isolated from patients with serious invasive infections admitted at Cook County Hospital Chicago. DNA of these isolates was hybridised to a multistrain PCR product *S. aureus* micro array in collaboration with the University of London, UK.

Results: Microarray hybridisation revealed that at least 84 of 2802 (3%) genes (which is a subset of the 3626 genes on the array) that are represented on the array were variable present among the 14 USA300 strains. Of these, 40 could be assigned to functional categories, while for 43 no presumed function could be assigned. Most genes (22) are presumed to be involved in metabolism and belong to the clusters of orthologous genes (COGs) E, G, P, H, C, and Q. Five genes belong to COGs V, T, and O, (cellular processes and signaling), and four to COGs J and L (information storage and processing). In the most cases only one or two strains differ in gene content. One strain lacked the entire beta-lactamase gene cluster, blaZ, blaRI, and blaI.

Conclusion: Our findings indicate a considerably genetic heterogeneity within the USA 300 clone, which is in contrast with the existing idea that all USA300 clones are genetically highly conserved. Whether or not this also relates to differences in pathogenicity or epidemicity remains to be investigated.

P67

Bactericidal factors in medical grade honey

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Introduction: Catheter-related bloodstream infections are a serious problem in critically ill patients. Skin colonisation at catheter insertion sites is associated with bloodstream infections. Despite regular disinfection, catheter insertion sites are often colonised. Local application of antibiotics and antiseptics reduces skin colonisation and associated catheter-related infections, but large scale prophylactic use is discouraged due to the risk for resistance development. An antimicrobial preparation not associated with resistance development is honey. We have recently demonstrated that medical grade honey significantly reduces skin colonisation in healthy volunteers, and therefore honey offers prospect to reduce colonisation at catheter insertion sites. Antimicrobial factors described in honey are i) osmotic stress due to its high sugar content, ii) the presence of glucose-oxidase which produces hydrogen peroxide, iii) its low pH and iv) additional as yet unidentified bactericidal compounds. We study the activity of Revamil medical grade honey which is produced in greenhouses under standardised conditions. The microbicidal activity of this honey has high batch-to-batch reproduciblility. The aim of the current investigation was to elucidate the relative contibution of the different antimicrobial factors to the bactericidal activity against different bacterial species.

Methods: We used a liquid bactericidal assay to quantitatively assess the contribution of sugars, hydrogen peroxide, the low pH and cationic components to the bactericidal activity of medical grade honey against several gram-positive and -negative bacteria. In addition, we tested whether enrichment of honey with the cationic antimicrobial peptide BP2 increased its microbicidal potential.

Results: *Bacillus subtilis* was killed very rapidly (within 5 min) by 10% honey. This activity did not involve sugars, hydrogen peroxide or the low pH. Addition of anionic polyanethole-sulphonate, which neutralises cationic components, completely abrogated the rapid bactericidal activity, but honey still retained its bactericidal activity after 24 hours of incubation. Methicillin-resistant *Staphylococcus aureus* (MRSA) was also killed by 10% honey, but required 24 hours of incubation. This bactericidal activity was largely dependent on hydrogen peroxide production in honey. Bactericidal activity against *Escherichia coli* and *Pseudomonas aeruginosa* was largely dependent on hydrogen peroxide and sugars, but additional, as yet unknown factors were also involved.

Enrichment of honey with 75 µM of the potent synthetic antimicrobial peptide BP2 resulted in rapid bactericidal activity against various bacterial species, including MRSA, within 2h of incubation.

Conclusions: The bactericidal factors in medical grade honey, being its high sugar concentration, hydrogen peroxide production and as yet unidentified cationic as well as non-cationic components, differentially contribute to bactericidal activity against different bacterial species. *B. subtilis* is rapidly killed by (an) endogenous cationic antimicrobial component(s) in honey. Other species are killed within a period of 2-24 hrs, depending on the concentration of honey. Rapid bactericidal activity of honey against various bacteria including MRSA, can be enhanced by enrichment of honey with exogenous antimicrobial peptides, such as BP2.

P68

Differential responses of periodontal ligament and gingival fibroblasts to infection with *Porphyromonas gingivalis*

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Porphyromonas gingivalis is an oral pathogen highly implicated in the pathogenesis of periodontitis. Periodontitis is a chronic inflammatory disease characterised by destruction of tooth-supporting tissues and the alveolar bone. Cytokines induced by *P. gingivalis* infection are major players in the pathogenesis of periodontitis that disturb normal osteoclast activity. Gingival fibroblasts (GF) and periodontal ligament fibroblasts (PDLF) are functionally different types of fibroblasts residing in the soft connective tissues of the tooth. GF are located sub-epithelial, and PDLF form the ligament connecting teeth to the alveolar bone. Several studies have indicated osteoblast-like properties of PDLF. Hence, these two types of fibroblasts may play different roles in periodontitis.

The aim of the present study was to investigate responses of GF and PDLF to infection with *P. gingivalis* with regard to several cytokines and receptors implicated in osteoclast-regulation.

Primary human GF and PDLF from 6 healthy individuals were challenged with live *P. gingivalis* for 6h.. Levels of gene expression interleukin (IL)-1, -6, -8 and osteoprotegerin (OPG) by real-time PCR.

P. gingivalis was able to induce very strong IL-6 gene expression implicating osteoclast activation, and IL-8 gene expression implicating chemotaxis of osteoclast precursors. Although overall responses of individuals were heterogeneous, they could be divided into two groups. One one hand gingiva-responders, showing a approximately 3 times higher response in GF than in PDL. On the other hand PDL-responders, showing a app. 30-fold higher response in PDLF than in GF.

Furthermore, *P. gingivalis* infection had differential effects on expression of OPG, an inhibitor of osteoclast formation. Prior to infection OPG expression levels differed greatly between individuals and apperared higher in GF than in PDLF After infection with *P. gingivalis*, differences in baseline OPG expression in GF and PDLF diminished to a similar level, suggesting that PDLF might initiate a rescue-response to prevent bone resorption.

Conclusion: Gingival and PDL fibroblasts respond differently to infection with *P. gingivalis*, and might therefore play a different role in *P. gingivalis*-induced periodontitis.

P69

The GlcNAc-epimerase EpsC has an important role in *Porphyromonas gingivalis* capsule biosynthesis

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Periodontitis is an oral inflammatory disease that can lead to severe bone-loss of the teeth supporting tissues, eventually leading to tooth loss. *Porphyromonas gingivalis* is a gram-negative obligate anaerobe that is strongly associated with severe periodontitis. Previous reports showed an association of *P. gingivalis* capsular polysaccharide (CPS) with virulence. Six capsular serotypes are described (K1-K6) of which K1 CPS was found to be more immunostimulatory than the other serotypes. A genetic locus (PG0106-PG0120) of thirteen genes was found to play a crucial role in CPS biosynthesis. The GlcNAc-epimerase encoding gene epsC is the most downstream gene in this locus. This gene was shown to be essential for survival in *Listeria monocytogenes*. Here we show by insertional inactivation that epsC is not essential for an encapsulated *P. gingivalis* strain. The epsC mutant still synthesises CPS, as seen by microscopic examination, but the recognition by the K1 polyclonal antiserum has been lost in the mutant. Furthermore, the epsC mutant autoaggregates and has a very dry appearance as usually seen in non-encapsulated *P. gingivalis* strains.

Our findings suggest that EpsC is an important player in the biosynthesis of CPS, as it seems to be significantly changed, but the role in virulence is to be elucidated. Interesting herein is the ambiguous role of CPS in phagocytosis on the one side and adherence on mucous membranes on the other.

P70

Pathogenesis of biomaterial-associated infection: immunohistochemical evidence for bacterial presence in tissue surrounding catheters of ICU patients

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Introduction: Biomaterial-associated infections (BAI) are generally caused by biofilm-forming bacteria, most often *Staphylococcus epidermidis*, considered to colonise the biomaterial-surface. In mouse experimental BAI however, *S. epidermidis* predominantly colonises the tissue surrounding subcutaneously implanted biomaterials.[1-3] To assess whether peri-biomaterial tissue might also

be a niche for bacteria potentially causing biomaterial-associated infections in humans, we retrieved catheters and surrounding tissue from deceased ICU patients. Nine of 35 (26%) peri-catheter tissue samples tested were highly culture positive. The corresponding catheter segments were culture negative or yielded only low numbers of bacteria. Bacteria cultured from different sites of the catheter and surrounding tissues almost all were coagulase-negative staphylococci (predominantly *S. epidermidis*) and *Enterococcus faecalis*.[4]

Aim: To investigate the histology of, and presence and localisation of bacteria within the tissue surrounding the catheters by microscopy.

Approach: Segments of tissue including catheter were fixed and embedded in plastic. Sections of tissue with the cathether still in place were deplastified, autoclaved and incubated with anti-lipoteichoid acid monoclonal antibodies, wich recognised most of the bacterial species cultured from the patients. Subsequently, sections were incubated with horse radish peroxidase-conjugated secondary antibody, followed by 3,3-DiAmino Benzidine tetrachloride-staining, and counterstaining with hematoxilin. Alternatively, the anti-LTA antibodies were fluorescently labelled using a Zenon-Alexa Fuor 488 mouse IgG1 labeling kit.

Results: Sections of the tissue surrounding catheters showed fibrin depositions directly adjacent to the catheter, extravasations of erythrocytes indicating small hematomas and a mild aspecific acute inflammatory response characterised by the presence of sparse neutrophils. In several cases there was an additional early reparative response of ingrowth of young (swollen) fibroblasts. Immunolabeling with anti-LTA antibodies showed positive staining of coccoid structures with the size of bacteria (1-2 µm diameter) either intracytoplasmatic in inflammatory cells or in the interstitium. This was confirmed by immunofluorescence microscopy using fluorescently labeled anti-LTA antibodies.

Conclusions: The (fluoresecently labelled) anti-LTA antibody allowed specific detection of bacteria within the tissue surrounding catheters, and may be more widely applicable to identify gram-positive bacteria in histology. Our observation show that bacteria are localised within tissue surrounding catheters, either intercellularly or associated with host phagocytes. Apparently, tissue surrounding biomedical devices forms a niche for bacteria in humans. This is an as yet non-recognised element in the pathogenesis of catheter-associated infections, with possible consequences for strategies of prevention and treatment of such infections.

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P71

Human adenovirus 36 is not a major cause of increased body weight or obesity in the Netherlands

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Background: In previous research, human adenovirus 36 (Adv36) infection has been shown to increase adiposity in chickens, mice and non-human primates. Adenovirus 36 DNA was directly detected in adipose tissues in these animal trials.[1] Seropositivity for antibodies against Adv36 has also been shown to have a statistically significant correlation to obesity in humans in the USA, present in 30% of obese humans and 11% of nonobese humans.[2]

Objectives: 1) To determine the prevalence of Adv36 antibodies in various groups in the Netherlands including non obese and obese humans and to calculate whether there is a correlation between seropositivity and Body Mass Index (BMI). 2) To determine if Adv36 genome is present in adipose tissue of obese humans.

Methods: 481 serum samples of 123 blood donors, 130 selected obese patients (BMI range 27-40), 50 overweight (BMI >25) and 50 lean (BMI <21.5) discordant twins and 128 health-care students were analysed for Adv36 antibodies using a serum neutralisation assay. Titers for antibodies were determined. As according to Atkinson, the cut off titer used to determine positivity was 1:8.[2] Adenovirus PCR was used to determine if adenovirus DNA was present in 31 morbidly obese surgical patients.

Results: An overall Adv36 seroprevalence of about 5% was found in 481 persons. All positive Adv36 titers were within the range 8-64 with the exception of one very high titer (1024) found in a lean person. BMI in Adv36 seropositive humans was not significantly different than in seronegative humans. No adenovirus DNA could be found using PCR on adipose tissue of 31 morbidly obese surgical patients.

Conclusions: In the Netherlands no significant correlation was found between Adv36 and body mass index. There is no indication that Adv36 DNA is present in visceral adipose tissue of morbidly obese Dutch patients.

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P72

The echinocandin caspofungin impairs innate immune mechanism against *Candida parapsilosis*

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Caspofungin, an echinocandin, inhibits fungal cell wall beta-glucan synthesis. Because phagocytic killing of *Candida* species by human neutrophils and monocytes/macrophages is the major host defense in serious fungal infections, it is important to study phagocytosis in the presence of caspofungin. The aim of our work was to investigate the effect of pretreatment of *Candida parapsilosis* with caspofungin on phagocytic mechanisms (opsonisation, oxidative burst, phagocytosis and killing). *C. parapsilosis* in the presence of caspofungin at concentrations above the MIC were more difficult to opsonise and to phagocytose. *C. parapsilosis* exposed to concentrations of caspofungin below and above the MIC was more difficult to kill. Caspofungin-treated *C. parapsilosis* impaired the oxidative burst. Caspofungin treatment of *C. parapsilosis* alters the capacity of PMNs to phagocytose and kill the organism. This may allow *C. parapsilosis* to persist in tissues.

P73

Validation of automated blood culture systems in a routine laboratory

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Background: In order to validate a new automated blood culture system, the proposed new system, the BacT/ALERT (BioMrieux, Marcy l'Etoile, France), was compared to the old system, BACTEC 9240 (BT, BD Diagnostic Systems, Sparks, MD, USA).

Methods: A set critical requirements for the new system was agreed up on in advance: 1) Both systems should be able to detect common pathogens causing bloodstream infections: *Escherichia coli* (EC), *Pseudomonas aeruginosa* (PA), *Staphylococcus aureus* (SAU) and *Streptococcus pneumoniae* (SP). 2) Both systems should be able to detect the quantity of bacteria expected in case of a bacteraemia. The pathogens were cultured overnight in brain-heart-infusion. An aliquot containing 0.5 McFarland of pathogens was then further serially diluted by using human blood, obtained through the local blood bank. Standard aerobic, anaerobic and paediatric blood culture bottles from both systems were inoculated with the different dilutions and aliquots were plated on standard media and Colony Forming Units (CFU)/ml was calculated. The time-to-positivity (TP) in both systems was recorded and compared.

Results: Both systems were able to detect all pathogens in all dilutions. Both systems were able to detect up to 1 CFU/ml pathogens. Median TPs:

PATHOGENS/HR	BACTEC	BACT/ALERT
Escherichia coli	9.07	11.28
Pseudomonas aeruginosa	11.54	13.68
Staphylococcus aureus	10.03	13.32
Streptococcus pneumoniae	12	15.36

Discussion/conclusion: The reported amount of CFU/ml in case of bacteraemia is between 1 to 10 CFU/ml (Magadia, Weinstein; IDCoNA 2001). Both systems were able to detect this critical threshold and the common pathogens associated with bacteraemia. Although the BacT/ALERT was slower to detect the pathogens, this difference of a few hours was not considered an important factor since TP is less critical in a routine laboratory with normal office hours. Therefore, the BacT/ALERT system was implemented in our laboratory.

P74

A novel, universal, automated biosensor system for rapid, nucleic acid based detection of emerging diseases

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Introduction: More than 1400 known pathogenic organisms are potential threats to human health, and newly emerging threats are likely to increase this number exponentially. To address the needs of human healthcare surveillance as well as requirements in other fields (e.g. animal health and food supply), new robust and automated systems are required to rapidly deliver solid answers.

Method: The Ibis T5000 biosensor system is a novel pathogen detection system, which uses broad range primers for amplification of nucleotide target sequences followed by time-of-flight (TOF) mass spectrometry (MS) analysis. Based on this MS information the system derives

base composition signatures to identify most organisms present in a sample without priory knowledge about the causative infectious agent(s).

Results: An eight primer set up panel targeting different influenza virus core segments detected and correctly identified 92 mammalian and avian influenza isolates, representing 30 different H and N types, including 29 avian H5N1 isolates. Further, the validation of the assay showed correct identification of 656 clinical respiratory samples with sensitivity and specificity better than 97%.

Conclusion: Rapid reverse transcription PCR/MS analysis can be used to simultaneously identify influenza viruses and moreover monitor global spread and emerge of novel viral genotypes (Sampath *et al*. PLoS ONE, 2007 May 30;2(5)). The broad applicability of this technology was also demonstrated recently in an alpha virus study where 35 of 36 mosquito extracts were determined unambiguously (Eshoo *et al*.Virology 2007 Jul 24). In summary the described method promises high throughput capabilities well suited for routine survey and detection of unanticipated, potentially unknown and newly emerging infectious diseases.

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