nederlandse vereniging voor Medische Microbiologie

NEDERLANDS TIJDSCHRIFT VOOR MEDISCHE MICROBIOLOGIE

Supplement bij veertiende jaargang, april 2006

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

in samenwerking met:

Microbiële Oecologie, Technische Microbiologie en Mycologie
Microbiële Pathogenese
Nederlandse Vereniging voor Medische Mycologie
Nederlandse Werkgroep Klinische Virologie Sectie Algemene Virologie
Sectie Levensmiddelenmicrobiologie
Secties Algemene en Moleculaire Microbiologie
Stichting Kwaliteitsbewaking
Medische Microbiologie
Werkgroep Epidemiologische Typeringen
Werkgroep Moleculaire Diagnostiek Infectieziekten
Werkgroepen Oost en West Medische Microbiologie

Papendal, 10 - 12 april 2006
Programma-overzicht
Abstracts
Auteursindex

14e Jaargang • april 2006 • Supplement

Advertentie

INLEIDING

De voorjaarsbijeenkomst van de Nederlandse Vereniging voor Medische Microbiologie (NVMM) en de Nederlandse Vereniging voor Microbiologie (NVvM) vindt in 2006 plaats op 11 en 12 april te Papendal.
Traditiegetrouw beginnen we met een plenaire sessie op dinsdagochtend met als thema: "Host-pathogen innate immune interactions". Onze kennis over het aspecifieke afweersysteem is de laatste jaren exponentieel toegenomen, tijd dus voor een state-of-the-art symposium. De oproep die we vorig jaar deden aan alle leden om mee te denken over mogelijke onderwerpen voor thematische sessies heeft heel veel reacties losgemaakt. Er werden maar liefst ruim 40 voorstellen ingediend. Hartelijk dank aan eenieder voor het enthousiasme en het meedenken. Het is jammer dat door de beperkingen van ruimte en tijd we niet alle voorstellen kunnen uitvoeren. De voorbereidingscommissie heeft haar best gedaan om te komen tot een gevarieerde en evenwichtige keuze van thematische sessies.
Sinds een aantal jaren zijn AIO's en promovendi die een voordracht of poster presenteren vrijgesteld van het betalen van inschrijving. Teneinde de deelname van de jonge microbiologische onderzoekers verder te stimuleren worden vanaf dit jaar de verblijfskosten (overnachting) voor AIO's en promovendi die hun werk presenteren, vergoed door de Stichting Antonie van Leeuwenhoek. De Stichting ondersteunt activiteiten die kennisuitwisseling op het gebied van de microbiologie bevorderen en wil hiermee participatie van jonge mensen aan de Voorjaarsvergadering vergroten. Hartelijk dank hiervoor!

Tijdens de afgelopen voorjaarsvergadering werd de postersessie voor het eerst in de Sydney-zaal gehouden, onder het genot van een drankje. Gezien de positieve reacties van zowel deelnemers als Yakult zal dit worden gecontinueerd. Om de interactie tussen de verschillende bloedgroepen van de NVvM en NVMM nog meer kans te geven zal aansluitend in dezelfde ruimte het eerste jaarlijkse *Groot Microbiologie Feest* worden gehouden. Vorig jaar werd op maandagmiddag een sessie georganiseerd voor de artsen in opleiding tot medisch microbioloog, waarbij zij eerst aan een toets deelnamen, gevolgd door cursorisch onderwijs. Deze sessie zal in 2006 opnieuw plaats vinden en wederom verwachten wij dat alle artsen in opleiding hieraan zullen deelnemen.

We wensen eenieder een geslaagde Voorjaarsvergadering 2006.

Het programma van het ochtendsymposium ziet er als volgt uit:

- **Host-pathogen innate immune interactions - Innate immunity of plants against fungi; arm race or balancing selection**
  P. De Wit, Wageningen University, Wageningen
- **Innate immunity, the Drosophila model**
  J.M. Reichhart, IBMC UPR 9022 CNRS, Strasbourg, France
- **Poxvirus immune evasion strategies are linked to host tropism**
  G. McFadden, University of Western Ontario, London, Canada
- **Bacterial innate immune evasion**
  J.A.G. van Strijp, University Medical Center Utrecht, Utrecht

Voorbereidingscommissie

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Mw. Dr. A. Vossen

De NVMM en de NVvM organiseren deze bijeenkomst in samenwerking met
Microbiële Oecologie, Technische Microbiologie en Mycologie
Microbiële Pathogenese
Nederlandse Vereniging voor Medische Mycologie
Nederlandse Werkgroep Klinische Virologie
Sectie Algemene Virologie
Sectie Levensmiddelenmicrobiologie
Secties Algemene en Moleculaire Microbiologie
Stichting Kwaliteitsbewaking
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Werkgroepen Oost en West Medische Microbiologie

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Dates
10 - 12 April 2006

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 2006' will be accredited by the NVMM with 5 points per day and maximal 10 points for the whole meeting.

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

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

Poster Session
Posters will be on display throughout the congress. The numbers on the poster boards correspond with the abstract numbers in the program/abstract book. Poster authors are requested to man their posters on Tuesday evening 11 April from 20:30 - 22:00 hours.

Poster price
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 11 April at 22:00 hours. The winner has to be personally registered and present.

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

Exhibition, lunch break, coffee/tea break
Coffee and tea will be available at all times at the exhibition. The lunch will be served at the exhibition during the lunch break.

Hotel rooms
If you have reserved a hotel room 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 en Congres Centrum 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 en Congrescentrum Papendal (EUR 6,50 per person). If you would like to use this service, please call 026-3210000 (mention the Papendal taxi).You have to pay at arrival at the hotel reception. 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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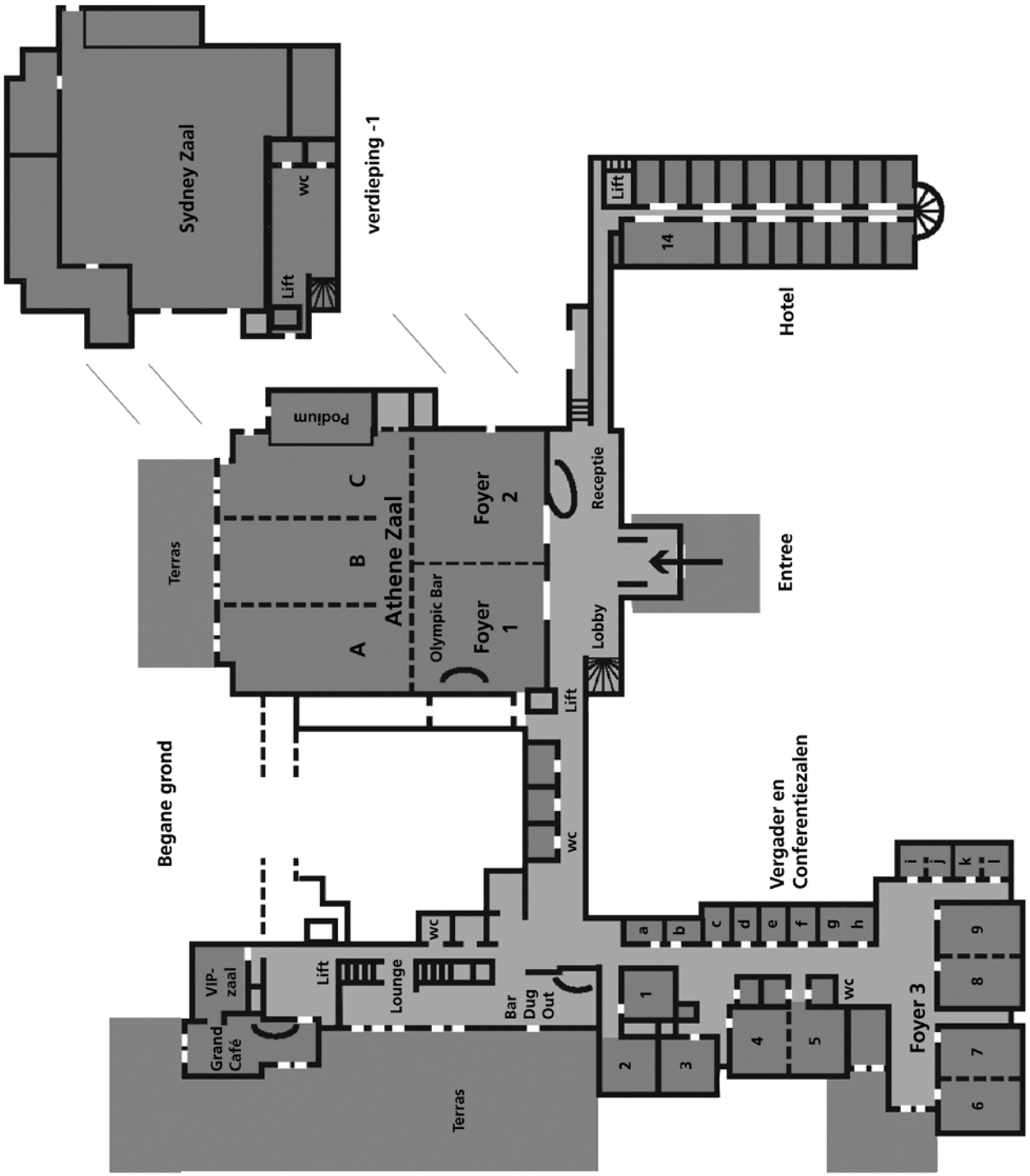
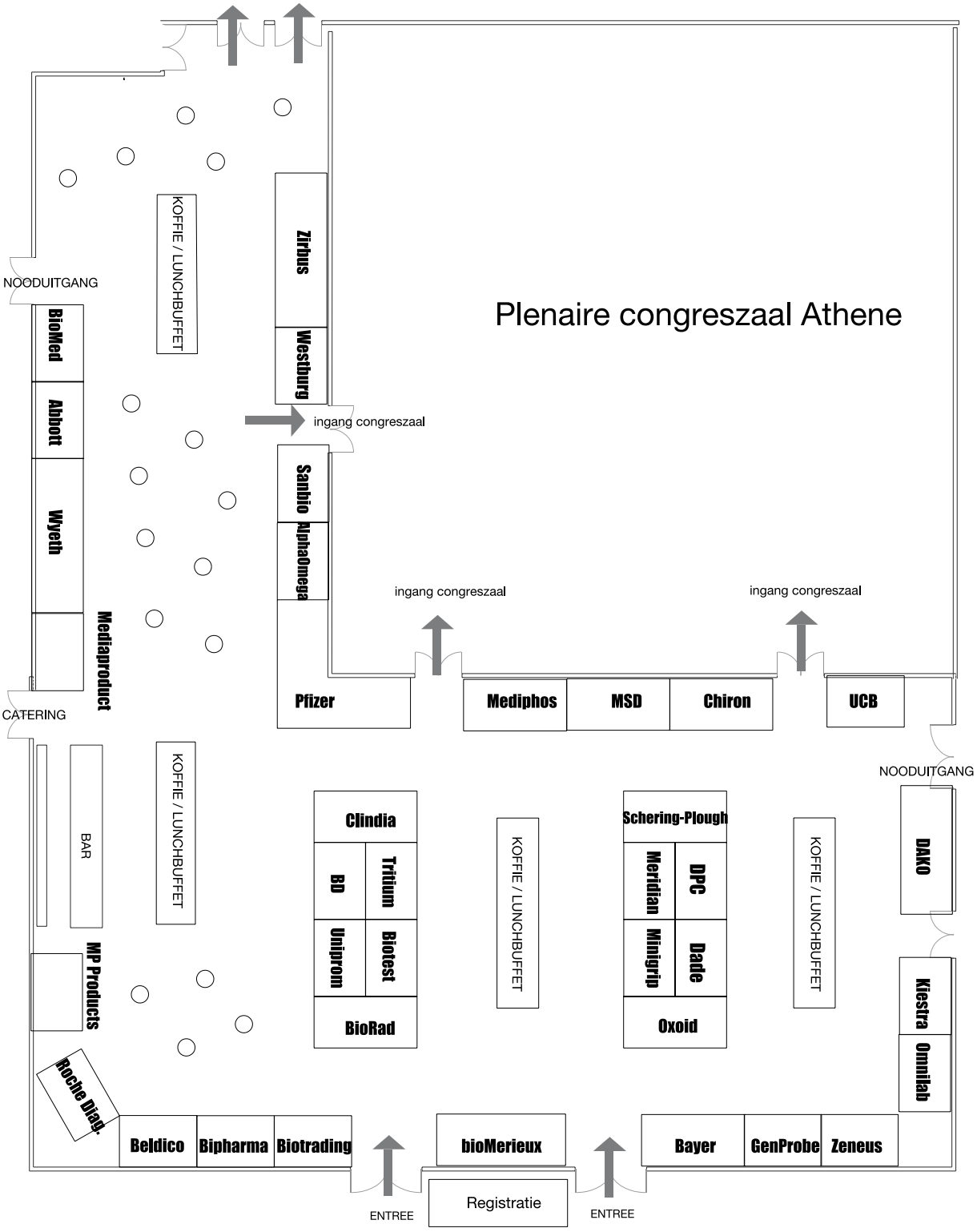
FLOORPLAN CONGRESS CENTRE



Floorplan exhibition



MONDAY 10 APRIL 2006

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	P.E. Verweij	
	Diagnostic approaches to invasive aspergillosis	
16:15 - 17:00	E.J. Kuijper	
	Recognition of *C. difficile* PCR ribotype O27	
17:00 - 17:15	Coffee/tea	
17:15 - 18:00	J.W. Mouton	
	The true interpretation of susceptibility tests	
18:30	Dinner	

TUESDAY 11 APRIL 2006

Congress introduction by W. Spaan

1 Room Athene B/C	Plenary session 'Host-pathogen innate immune interactions'	
	Chairmen: J. Verhoef & R. Laanbroek	
09:30 - 10:15	P. de Wit (Wageningen)	01.01
	Innate immunity of plants against fungi; arm race or balancing selection	
10:15 - 11:00	J.M. Reichhart (Strasbourg, France)	01.02
	Innate immunity, the Drosophila model	
11:00 - 11:30	Coffee/tea	
11:30 - 12:15	G. McFadden (London, Canada)	01.03
	Poxvirus immune evasion strategies are linked to host tropism	
12:15 - 13:00	J.A.G. van Strijp (Utrecht)	01.04
	Bacterial innate immune evasion	

Room Athene B/C **Lunch sponsorsymposium Schering-Plough**

13:00 - 14:00

2 Room 4/5 **Medical Mycology 1**

Chairman: S. de Hoog

14:00 - 14:15	M. Arabatzis	02.01
	Rapid detection and identification of six commonly encountered dermatophytes by multiplex real-time PCR	
14:15 - 14:30	M.A.S.H. Mennink-Kersten	02.02
	Detection of the surrogate marker (1,3)-beta-D-glucan in patients receiving intravenous amoxicillin-clavulanic acid	
14:30 - 14:45	M. Sudhadham	02.03
	Host shift in the neurotropic black yeast *Exophiala dermatitidis*: a steam bath colonizer emerging from the tropical rain forest	
14:45 - 15:00	A.H. Groll	02.04
	Site-directed antifungal pharmacokinetics and pharmacodynamics	
15:00 - 15:15	J.M. Harrak	02.05
	Exophiala species causing disease in cold-blooded animals	
15:15 - 15:30	M.T. Illnait	02.06
	Cryptococcosis in Cuba	
15:30 - 16:00	Coffee/tea	

2 Room 4/5 **Medical Mycology 2 (continued)**

Chairman: S. de Hoog

16:00 - 16:15	P.E. Verweij	02.07
	Kinetics of circulating glucan compared with galactofuranose-antigens in patients with invasive aspergillosis	
16:15 - 16:30	F. Hagen	02.08
	Where is the origin of the *Cryptococcus gattii* Vancouver Island outbreak?	
16:30 - 16:45	A.J. van Griethuysen	02.09
	A patient with unbearable headache	
16:45 - 17:00	W.W.J. van de Sande	02.10
	Melanin protects *Madurella mycetomatis* against itraconazole and ketoconazole, first-line treatment agents against mycetoma	
17:00 - 17:15	Ruo-yu Li	02.11
	Black yeast infections in China	
17:15 - 17:30	H. de Valk	02.12
	Colonization of Cystic Fibrosis patients with *Aspergillus fumigatus* is a recurrent phenomenon	
17:30 - 17:45	D.W. Warnock	02.13
	Epidemiologic issues in invasive fungal infections	

3 Room 2 **Therapie van parasitaire infecties in Nederland**

Voorzitter: T. Kortbeek

14:00 - 14:15	T. Kortbeek Congenitale toxoplasmose	03.01
14:15 - 14:30	T. Mank Therapie van Giardia	03.02
14:30 - 14:45	T. van Gool Behandeling van *Dientamoeba fragilis*	03.03
14:45 - 15:00	L. Visser Malariaprofylaxe en therapie	03.04
15:00 - 15:15	... Het toelaten en van de markt halen van geneesmiddelen	03.05
15:15 - 15:30	D. Haddad Treatment of *Dientamoeba fragilis* infection with paromomycin (Humatin®) in children: parasitological and clinical effectiveness	03.06
15:30 - 16:00	Coffee/tea	

4 Room Athene B/C **Infecties in zorginstellingen 1**

Voorzitter: G.J.H.M. Ruijs

14:00 - 14:05	Opening door de voorzitter	
14:05 - 14:25	R.van Balen Kenmerken van patiëntgroepen in zorginstellingen	04.01
14.25 - 14.30	Discussie	
14.30 - 15.00	C.J. Büla Nursing home infections: cause and consequences of functional impairment	04.02
15:00 - 15:05	Discussie	
15:05 - 15:25	J.J.A.H. Klein Breteler Financiering van zorginstellingen, nu en in de toekomst	04.03
15:25 - 15:30	Discussie	
15:30 - 16:00	Coffee/tea	

4 Room Athene B/C **Infecties in zorginstellingen 2**

Voorzitter: G.J.H.M. Ruijs

16:00 - 16:20	J.A.J.W. Kluytmans (Multi)Resistente micro-organismen in verpleeghuizen, van MRSA's tot ESBL's	04.04
16:20 - 16:25	Discussie	
16:25 - 16:40	M.J.H.M. Wolfhagen Diagnostiek van urineweginfecties bij verpleeghuispatiënten	04.05
16:40 - 17:00	P.B.M. Went Nieuwe richtlijn urineweginfecties in verpleeghuizen	04.06
17:00 - 17:05	Discussie	
17:05 - 17:20	A.C.M. Kroes Virusinfecties in verpleeghuizen: de waarde of noodzaak van (snel-)diagnostiek	04.07
17:20 - 17:35	H.J.M. Cools Influenza: implementatie van een dynamische richtlijn	04.08
17:35 - 17:40	Discussie	
17:40 - 17:45	Afsluiting door voorzitter	

5 Room 8/9 **Pathogenesis: Immune modulation**

Chairman: W. van Eden

14:00 - 14:30	A. Koets HSP protect against *M. paratuberculosis*: an example for other mycobacterial diseases	05.01
14:30 - 15:00	E. Wiertz Herpesvirusses avoid T cell recognition by inhibition of TAP in infected cells	05.02
15:00 - 15:15	J. Bestebroer Staphylococcal superantigen-like protein 5 (SSL5) inhibits PSGL-1-mediated processes under static and flow conditions and inhibits CXCR2-induced cell activation	05.03
15:15 - 15:30	I. Jongerius SCIN and CHIPS homologues are located on a new Immune Evasion Cluster in *S. aureus*	05.04
15:30 - 16:00	Coffee/tea	

5 Room 8/9 **Pathogenesis: Staphylococci**

Chairman: W. van Leeuwen

16:00 - 16:30	S.H.M. Rooijakkers Complement inhibition by *S. aureus*	05.05
16:30 - 16:45	H.F.L. Wertheim Clumping factor B is an essential bacterial factor for *Staphylococcus aureus* nasal colonization in humans	05.06
16:45 - 17:00	E. van Duijkeren Increasing prevalence of infections with methicillin-resistant staphylococci in animals	05.07
17:00 - 17:15	M.G.R. Hendrix Culture based and molecular prevalence of MRSA in the Twente-Achterhoek region	05.08
17:15 - 17:30	W.T.M. Jansen Novel variants of *Staphylococcus* Cassette Chromosomes excised by ccrA/B type 2 recombinases in *Staphylococcus aureus*	05.09

6 Room Sydney **Genomics studies & tools in food microbiology**

Chairman: S. Brul

14:00 - 14:30	R.C. Montijn Microbial genomics for the food processing industry: novel possibilities for controlling *Bacillus* spoilage	06.01

14:30 - 15:00	F.H.J. Schuren	06.02
	Genomotyping: a novel genomics based approach for controlling *Bacillus* spoilage	
15:00 - 15:30	S. Brul	06.03
	Bacterial spores in food processing; molecular detection, identification and process survival analysis	
15:30 - 16:00	Coffee/tea	

6 Room Sydney **Genomics studies & tools in food microbiology (continued)**

Chairman: S. Brul

16:00 - 16:30	L.M. Hornstra	06.05
	Spore germination of thermally injured *Bacillus subtilis* spores	
16:30 - 17:00	P. Vos	06.06
	Multi analyte molecular detection of food pathogens and spoilers	
17:00 - 17:30	H.J.M. Aarts	06.07
	Detection and identification of food borne pathogens by molecular methods	

7 Room 6 / 7 **Biofilms in the spotlight**

Chairman: M.J. Teixeira de Mattos

14:00 - 14:10	Biofilms: Introduction by the chair	
14:10 - 14:30	M.B. Melchior	07.01
	In vitro susceptibility of biofilm growing *Staphylococcus aureus* bovin-emastitis isolates	
14:30 - 14:50	W.J.B. van Wamel	07.02
	Condition dependent Esp expression and biofilm formation of *Enterococcus faecium*	
14:50 - 15:10	J.M. Key	07.03
	Blue light is an environmental regulator of *Escherichia coli* biofilm formation	
15:10 - 15:30	K.J. Hellingwerf	07.04
	Effects of phosphorelay perturbations and light on architecture, sporulation and spore resistance in biofilms of *Bacillus subtilis*	
15:30 - 16:00	Coffee/tea	

8 Room 3 **Sectie onderwijs NVvM (Nederlandstalige sessie)**

Voorzitter: L. van Alphen

14:00 - 14:10	Introductie door voorzitter	
14:10 - 14:30	K. Eijkemans en A. van Goor	08.01
	Introductie sectie onderwijs	
14:30 - 15:00	J. Laforet	08.02
	Rondom het MLO	
15:00 - 15:30	K. Breg	08.03
	Microbiologische practica voor middelbare scholieren	
15:30 - 16:00	Coffee/tea	

9 Room 6/7 **WOGIZ: Moleculaire epidemiologie en de openbare gezondheidszorg: toy or tool?**

Chairman: P. Schneeberger

16:00 - 16:25	H.L. Zaaijer	09.01
	Moleculaire typering van HBV in Nederland: toy & tool	
16:25 - 16:50	S.M. Bruisten	09.02
	Tracking hepatitis A virus within and among risk groups	
16:50 - 17:30	M. Šebek en G. de Vries	09.03
	Moleculaire technieken verleggen de grenzen van de tuberculosebestrijding!	

10 Room 3 **Diagnostics**

Chairman: F. Verduyn - Lunel

16:00 - 16:15	S.B. de Bast	10.01
	Application of a rapid immunochro-matography assay during an outbreak of *Clostridium difficile* associated diarrhoea	
16:15 - 16:30	E. Pinelli	10.02
	Detection of specific IgG1 and IgG4 antibody response for the immunodi-agnosis of cystic echinococcosis	
16:30 - 16:45	C.H. Krause	10.03
	Diagnosis of Mumps by IgM-ELISA in Scotland - An assay comparison	
16:45 - 17:00	H.F.M. Willemse	10.04
	Use of Raman spectroscopy for the identification of *Burkholderia* spp.	
17:00 - 17:15	A. Bart	10.05
	Cutaneous leishmaniasis in Dutch military personnel in Afghanistan: correlation between *L. major* genotype, clinical picture and deployment area	
17:15 - 17:30	D. Vastert-Koop	10.06
	Diagnosis of *Cryptosporidium parvum* with microscopy, striptest, ELISA and real time PCR	

Room Athene B/C **Plenary session**

Chairman: W. Spaan

17:45 - 18:15	News
18:30 - 20:30	Dinner

Postersession and Presentation Yakult Poster Price

20:30 - 22:00	Posterpresentations

Drinks and poster price are sponsored by Yakult

22:00	Presentation Yakult Poster Price
22:15	Dance Party '*Groot Microbiologie Feest*'

WEDNESDAY 12 APRIL 2006

Room 3 Breakfast symposium Sanofi Pasteur MSD

07:30 - 08:45 Nieuwe Vaccins!!

Varicella, herpes zoster, rotavirus en HPV Van ziektebeeld tot vaccin
- H. Rumke: Varicella
- J. Lange: Herpes zoster
- N. Hartwig: Rotavirus
- H. Nijman: HPV

11 Room 4/5 SKMM: Kwaliteit (Nederlandstalige sessie)

Voorzitter: G.J.J. van Doornum

09:00 - 09:30	L. van Lieshout	11.01
	Microscopie in de parasitologische diagnostiek - kerntaak of specialistenwerk?	
09:30 - 10:00	J. Mouton	11.02
	Interpretatie van gevoeligheidsbepalingen	
10:00 - 10:15	E.J. Kuijper & R. van den Berg	11.03
	Laboratoriumdiagnostiek van *Clostridium difficile*-geassocieerde diarree	
10:15 - 10:30	Vergadering SKMM	

12 Room 2 Oral Microbiology in 2006

Chairman: A.J. van Winkelhoff

09:00 - 09:30	M.A. Curtis	12.01
	Structural analysis of a novel anionic polysaccharide in the oral pathogen *Porphyromonas gingivalis*	
09:30 - 10:00	J.M. ten Cate	12.02
	Oral biofilms: models for drug testing	
10:00 - 10:15	A. Bart	12.03
	Bacterial biota in the oropharynx	
10:15 - 10:30	W. Crielaard	12.04
	Interaction of Streptococcus mutants with *Veillonella parvula* grown in dual species biofilm	

13 Room 3 Moleculaire diagnostiek van virale infecties bij beenmerg transplantatie patiënten (Nederlandstalige sessie)

Voorzitter: R. Schuurman

09:00 - 09:30	...	13.01
	Diagnostiek, monitoring en behandeling van EBV reactivaties na beenmerg transplantatie	
09:30 - 10:00	J.J. Boelens	13.02
	Klinische betekenis van virusinfecties bij HSCT in kinderen	
10:00 - 10:15	L. Kroes	13.03
	De betekenis van adenovirus-infecties voor ontvangers van stamceltransplantaten	
10:15 - 10:30	A. Lankester	13.04
	Klinische relevantie van HSV-1 drug resistentie na beenmerg transplantatie	

5 Room 8/9 Pathogenesis: General

Chairman: P.W.M. Hermans

09:00 - 09:15	W. Bitter	05.10
	A specific secretion system mediates PPE protein transport in Mycobacteria and is required for virulence	
09:15 - 09:30	W.T. Hendriksen	05.11
	CodY contributes to colonization of *Streptococcus pneumoniae*	
09:30 - 09:45	J. Stoof	05.12
	Metal-responsive regulation and role in iron acquisition of the two *Helicobacter mustelae* TonB orthologs	
09:45 - 10:00	Y. Pannekoek	05.13
	Hfq mediated riboregulation in *Neisseria meningitidis*	
10:00 - 10:15	A.P.A. Hendrickx	05.14
	Identification of putative surface exposed proteins specific for hospital adapted vancomycin-resistant *Enterococcus faecium*	
10:15 - 10:30	N.D. van Burgel	05.15
	Infections of complement resistant and complement sensitive *Borrelia burgdorferi* sl in Wildtype and C3 deficient mice	
10:30 - 11:00	Coffee/tea	

5 Room 8/9 Pathogenesis: Vaccines

Chairman: S. Weling-Wester

11:00 - 11:15	P.J. Haas	05.16
	Identifying conformational epitopes for human-IgG within the CHIPS protein	
11:15 - 11:30	S. van Selm	05.17
	Nasal immunization with pneumococcal proteins displayed on a *Lactococcus lactis*-based carrier provides protection against fatal pneumonia	
11:30 - 11:45	P. van der Ley	05.18
	Improvement of LPS-containing vaccines by modification of lipid A biosynthesis in *Neisseria meningitidis* and *Bordetella pertussis*	
11:45 - 12:00	A. Riezebos-Brilman	05.19
	A comparative study on the immunotherapeutic efficacy of recombinant Semliki Forest virus and recombinant adenovirus	
12:00 - 12:15	E. de Wit	05.20
	Influenza vaccines for pandemic preparedness; current developments and future opportunities	
12:15 - 12:30	K. Stittelaar	05.21
	Intervention strategies against smallpox	
12:30 - 14:00	Lunch	

5 Room 8/9 **Pathogenesis: Antimicrobial peptides**

Chairman: H. Haagsman

14:00 - 14:30	P.S. Hiemstra Antimicrobial peptides: the magic bullets of innate immunity	05.23
14:30 - 14:45	B. Zaat Autolysis products protect *Streptococcus pneumoniae* against cationic antimicrobial peptides	05.24
14:45 - 15:00	E.C.I. Veerman Candidacidal effects of LL-37 and histatin 5	05.25
15:00 - 15:15	A. van Dijk Localization and antimicrobial activity of chicken gallinacin-6	05.26
15:15 - 15:30	E.J.A. Veldhuizen *Salmonella typhimurium* causes upregulation of porcine β-defensins in a porcine intestinal cell line	05.27
15:30 - 16:00	Coffee/tea	

6 Room Sydney **Spore formers: ultimate survivors! - their formation and properties**

Chairman: M. Zwietering

09:00 - 09:30	J. Dijksterhuis Fungal spores as survival capsules in time and space	06.08
09:30 - 10:00	T. Abee Global regulation of survival strategies of the bacterial spore former *B. cereus*	06.09
10:00 - 10:15	T. Shen Mode-of-action of High Pressure Low Temperature induced damage to *Bacillus subtilis* in the IceI-IceII domain	06.10
10:15 - 10:30	H. Wösten Transport of mRNA and proteins from a fungal mycelium to sporeforming structures?	06.11
10:30 - 11:00	Coffee/tea	

6 Room Sydney **Progress in Microbiology**

Chairman: H.V. Westerhoff

11:00 - 11:15	G. Roeselers Diversity of phototrophic bacteria in microbial mats in Arctic hot springs (Greenland)	06.12
11:15 - 11:30	M.J. Foti Diversity of sulfate reducing bacteria in soda lakes	06.13
11:30 - 11:45	A. Wegkamp Metabolic engineering of folate biosynthesis in *Lactobacillus plantarum*	06.14
11:45 - 12:00	R. Orij Measuring yeasts intracellular pH upon sorbic acid stress *in vivo*	06.15
12:00 - 12:15	J. Postmus Modeling the response of yeast glycolysis to temperature changes	06.16
12:15 - 12:30	Discussion	
12:30 - 14:00	Lunch	

6 Room Sydney **Systems biology for micro organisms and vice versa**

Chairman: H.V. Westerhoff

14:00 - 14:10	H.V. Westerhoff SYSMO and the ten commandments of microbial systems biology	06.18
14:10 - 14:45	P. Michels Towards new drugs for African sleeping sickness by systems biology and structure-based discovery	06.19
14:45 - 15:00	J. Teixeira de Mattos A systems biology model for the adaptation of *S. cerevisiae* to heat stress	06.20
15:00 - 15:15	D. Molenaar The logic of growth	06.21
15:15 - 15:30	S. Rossell Unravelling the complexity of flux regulation	06.22

14 Room 4/5 **Molecular analysis and genomics-based approaches to reveal biodiversity and individual strain performance in complex microbial ecosystems**

Chairmen: L. De Vuyst & E. Smid

09:00 - 09:30	B. Teusink A genome-scale model of *Lactobacillus plantarum* WCFS1: useful for omics data integration and exploring metabolic capacities	14.01
09:30 - 10:00	G. Huys Elucidation of biodiversity and population dynamics in complex microbial ecosystems found in food fermentations and in the intestinal tract	14.02
10:00 - 10:15	R. van der Meulen Metabolite target analysis and population dynamics of sourdough fermentation processes	14.03
10:15 - 10:30	L.M. Hebben-Serrano Role of thioredoxin reductase (trxB1) in oxidative stress response of *Lactobacillus plantarum* WCFS1	14.04
10:30 - 11:00	Coffee/tea	

15 Room Athene B/C **Evolutionary genetics and population biology of bacteria**

Chairman: R. Willems

09:00 - 09:30	L.M. Schouls Molecular typing of bacterial pathogens reveals a spectrum from clonal to panmictic population structures	15.01
09:30 - 09:45	H.L. Leavis Phylogenomic analysis of *Enterococcus faecium* using mixed whole genome microarray technology discerns a globally dispersed hospital clade	15.02

09:45 - 10:00	X.W. Huijsdens	15.03
	Non-typeable methicillin-resistant *Staphylococcus aureus* form a clonal cluster which seems to be related to pig farmers and pigs	
10:00 - 10:15	E.M. Stam-Bolink	15.05
	Spread of a persistent methicillin-resistant *Staphylococcus aureus* ST80 clone in the community of the northern part of The Netherlands	
10:30 - 11:00	Coffee/tea	

16 Room Athene B/C **Werkgroep Oost / West: Prikaccidenten 1 (Nederlandstalige sessie)**

Voorzitter: E.A.P.M. Thewessen

11:00 - 11:30	P.T.L. van Wijk en P.M. Schneeberger	16.01
	Landelijke enquête: verschillen in interpretatie van risico's en aanpak	
11:30 - 12:00	H.L. Zaaijer	16.02
	Risico-inschatting en consequenties: HIV, HBV en HCV	
12:00 - 12:30	Plenaire discussie	
12:30 - 14:00	Lunch	

21 Room Athene B/C **Werkgroep Oost/West: Prikaccidenten 2 (Nederlandstalige sessie)**

Voorzitter: R.W. Vreede

14:00 - 14:30	J.J.A. van Boven	21.01
	Het belang van de hulpverlener en het recht van de patiënt	
14:30 - 15:00	G.J.B. Sonder	21.02
	Ervaringen met PEP: start van de behandeling en follow-up	
15:00 - 15:30	R.A. de Man	21.03
	Nieuwe behandelingsmogelijkheden van vroege hepatitis-C-virusinfectie	

17 Room 2 **Drug resistance**

Chairman: R. Anthony

11:00 - 11:20	S. Gillespie	17.01
	Bacterial fitness and drug resistance	
11:20 - 11:40	H. Grundman	17.02
	International aspects of antimicrobial resistance in opportunistic bacterial pathogens	
11:40 - 11:55	A. van Belkum	17.03
	Identification of drug resistance in the microbiological laboratory	
11:55 - 12:10	I. Bergval	17.04
	Mutator strains	
12:10 - 12:25	I. Willemsen	17.05
	Determinants of Inappropriate (IA) use of antibioticx identified in prevalence surveys	
12:30 - 14:00	Lunch	

18 Room 6/7 **HIV: pathogenesis and resistance**

Chairmen: C. Boucher & M. Nijhuis

11:00 - 11:30	A. Osterhaus	18.01
	HIV CTL activity and vaccine development	
11:30 - 11:45	I. Schellens	18.02
	The presence of the protective HLA-B27 allele results in increased responsiveness of HIV-1 specific CTL restricted by HLA-A2	
11:45 - 12:00	N.M. van Maarseveen	18.03
	HIV-1 variants with multiple protease mutations can persist because loss of single resistance mutations reduces replicative capacity and blocks evolution to wild type	
12:00 - 12:15	M.C.D.G. Huigen	18.04
	A novel and rare amino acid substitution E40F in HIV-1 reverse transcriptase (RT) increases zidovudine (AZT) resistance and decrease replication capacity	
12:15 - 12:30	V.V. Ganusov	18.05
	Estimating the costs and benefits of CTL escape mutations in SIV/HIV infection	
12:30 - 14:00	Lunch	

19 Room 3 **Actinomyces in biotechnology, medicine and ecology**

Chairman: L. Dijkhuizen

11:00 - 11:20	E. Takano	19.01
	What is the role of γ-butyrolactones in *Streptomyces coelicolor* A3(2)?	
11:20 - 11:40	G. van Wezel	19.02
	A novel nutrient sensory system that controls central metabolism, morpho-genesis and antibiotic production in streptomycetes	
11:40 - 11:55	R. van der Geize	19.03
	Engineering the steroid catabolic pathway of *Rhodococcus*: inactivation of multiple gene homologues	
11:55 - 12:10	E.E.E. Noens	19.04
	Members of the SALP family play a role in peptidoglycan assembly and degradation of sporulation-specific cell division	
12:30 - 14:00	Lunch	

20 Room 4/5 **Clinical epidemiology**

Chairman: E. van de Vorm

11:00 - 11:15	N. Al Naiemi	20.01
	A CTX-M Extended-Spectrum β-Lactamase in *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*	
11:15 - 11:30	T.I.I. van der Kooi	20.02
	Clostridium difficile PCR ribotype 027 toxinotype III in The Netherlands	

11:30 - 11:45	E.A.E. Verhoef	20.03
	Increase in patients with impetigo caused by a *Staphylococcus aureus* clone intermediate resistant to fusiic acid	
11:45 - 12:00	A. Hofhuis	20.04
	Investigation of an outbreak of *Salmonella typhimurium* DT104 in The Netherlands, September-November 2005	
12:00 - 12:15	M.A. Leverstein - van Hall	20.05
	Strong increase in integron prevalence in intestinal flora of young children due to cotrimoxazole use	
12:15 - 12:30	J.W.B. van der Giessen	20.06
	Update of *Echinococcus multilocularis* in The Netherlands: evidence of increasing presence in the southern border area in The Netherlands	
12:30 - 14:00	Lunch	

Room Athene B/C **Lunch sponsor symposium Chiron**

12:40 - 13:40 Cubicin® (daptomycine): the class of 2006
- *in vitro* and preclinical data Cubicin®
- clinical and safety profile Cubicin®
- discussion

Room Sydney **Business meeting NVvM**

12:45 - 14:00

22 Room 4/5 **Werkgroep Epidemiologische Typering (WET): Genome analysis to trace virulence factors**

Chairmen: L. Dijkshoorn & P. Savelkoul

14:00 - 14:30	J. Boekhorst	22.01
	Comparative genome analysis in the study of host-microbe interactions	
14:30 - 15:00	J.Green	22.02
	Searching raw genome sequences for putative virulence factors	
15:00 - 15:30	A. van Belkum	22.03
	Pathotyping in clinical microbiology	

23 Room 6/7 **NWKV**

Chairman: J.M.D. Galama

14:00 - 14:15	H.C. Gelderblom	23.01
	Detection of hepatitis C virus RNA by transcription-mediated amplification in PCR negative samples during antiviral treatment	
14:15 - 14:30	M.P.D. Deege	23.02
	Epstein-Barr virus as a possible pathogen in interstitial lung abnormalities	
14:30 - 14:45	J. Gooskens	23.03
	Fatal cases of influenza-associated encephalopathy in The Netherlands	
14:45 - 15:00	R.P. Schade	23.04
	Herpes zoster caused by wild-type varicella zoster virus in a vaccinated patient with immunosuppression	
15:00 - 15:15	J. Schinkel	23.05
	Identification of a fourth human parechovirus serotype	
15:15 - 15:30	Discussion	

24 Room 3 **(Inter)nationale ICT ontwikkelingen in de zorg**

Chairman: C.H.E. Boel

14:00 - 14:35	G. Freriks	24.01
	Zorg van de toekomst en ICT van de toekomst	
14:35 - 15:10	E. Sanders	24.02
	IHE, Intergratie uw zorg?	
15:10 - 15:30	B. Schijvenaars	24.03
	Scientific intelligence	
15:30 - 16:00	Coffee/tea	

Room Athene B/C **Business Meeting NVMM**

16:00 - 18:00

01.01

Innate immunity of plants against fungi; arms race or balancing selection

P.J.G.M. de Wit, M. Bolton, O. Boras, S. Gabriëls, J. van 't Klooster, I. Stulemeijer, J. Vossen, P. van Esse. E. Fradin, U. Ellendorff, I. Stergiopoulos, M. Joosten, B. Thomma.
Wageningen University, Laboratory of Phytopathology, Wageningen

Avr genes are supposed to have virulence functions in the absence of the corresponding resistance (*R)* gene. We have cloned four Avr and four Ecp genes of the tomato pathogen *Cladosporium fulvum* that all encode cysteine-rich peptides secreted by the fungus during infection of tomato leaves. Recognition of Avr and Ecp proteins is mediated by Cf proteins and leads to an innate immune or hypersensitive response (HR), co-ordinated death of a few host cells at the site of penetration by the pathogen. *C. fulvum* avoids recognition by its host by various mechanisms including: loss of Avr genes or point mutations, frame shift mutations or transposon insertions in Avr genes. Avrs are supposed to interact with a virulence target in the host that is sensed by Cf proteins that subsequently trigger an HR. Although all Avr and Ecp proteins are supposed to represent virulence functions, deletion of single genes do not significantly reduce virulence of the fungus. For two Avr proteins we have indications for their biological function. Avr4 is a chitin-binding protein that protects the fungus against basic plant chitinases. Avr4 proteins encoded by virulent alleles in strains of *C. fulvum* are no longer recognised by Cf-4 plants, but still bind to chitin, suggesting that chitin-binding by Avr4 could represent a defensive virulence function. The Avr2 peptide is secreted by *C. fulvum* into the apoplast of tomato leaves and, in the presence of the tomato extracellular, membrane-anchored Cf2 protein, triggers the HR that also requires the extracellular tomato cysteine protease Rcr3. Avr2 binds and inhibits Rcr3, and the Rcr3-Avr2 complex is subsequently recognized by the Cf-2 protein.

01.02

Innate immunity of insects

J.M. Reichhart
Strasbourg, France

Drosophila mounts a potent host defence when challenged by various microorganisms. Molecular and genetic analyses of this defence have now provided a global picture of the mechanisms by which this insect senses infection, discriminates between various classes of microorganisms and induces the production of effector molecules, among which antimicrobial peptides are prominent. A major result in these studies was the discovery that most of the genes involved in the Drosophila host defence are similar to genes implicated in the mammalian innate immune response. Recent progress in research on Drosophila immune defence and the newly discovered similarities or differences between Drosophila defence mechanisms and mammalian innate immunity will be discussed.

01.03

Poxvirus immune evasion strategies are linked to host tropism

G. McFadden
Robarts Research Institute, London, Canada

Despite the eradication of smallpox as an extant human disease a quarter of a century ago, there remains considerable fear that variola virus, or other related pathogenic poxviruses like monkeypox, could emerge and spread in the human population again. Although remarkable advances have been made in our understanding in the molecular events of poxvirus infections, we are still mostly ignorant about why most poxvirus infections of vertebrate hosts usually exhibit strict species specificity, or how zoonotic poxvirus infections occur when poxviruses occasionally leap into novel host species. Unlike many other viruses, poxvirus tropism appears to be regulated not at the level of specific host receptors, but rather at intracellular events downstream of virus binding and entry. This seminar summarizes our current understanding of poxvirus tropism and host range, with specific emphasis on the prospects for exploiting host-restricted poxvirus vectors for vaccines or gene therapy and developing host-targeted oncolytic viral therapies for human cancers. Our lab has studied one particular poxvirus, myxoma virus, which exhibits strict species specificity for the rabbit. Targeted knockout analysis of specific myxoma virus genes has revealed new clues about the viral and host determinants of tropism and host range.

01.04

Bacterial innate immune evasion

J.A.G. van Strijp
UMC Utrecht, Eijkman-Winkler Institute, Dept. of Experimental Microbiology, Utrecht

Upon entering the human body, bacteria are confronted with the sophisticated innate defense mechanisms of the

human host. From work in recent years it has become obvious that a new and growing family of small and excreted proteins can counteract the antibacterial effects of innate immunity. These highly selective proteins pick out crucial elements of our immune system and inhibit their function. In *Staphylococcus aureus* these proteins act on specific cellular receptors, on antimicrobial peptides and especially on the complement system. The combined action of this growing group of essential virulence factors ascertains efficient innate immune evasion. In a relatively short period of time we and others have identified an impressive amount of highly specific innate immune evasion molecules in a single microorganism. This is probably just the tip of the iceberg. If we can document the complete armory of innate immune evasion mechanisms in *S. aureus*, this will indirectly provide an increasing insight into the fundaments of bacterial pathophysiology in general. Furthermore, it will provide insight into our own innate immune system and open the way to develop smart and specific anti-inflammatory compounds.

Selected papers:

Trends Microbiol 2005, 13:596-601.
Nat Immunol 2005, 6:920-7.
J Exp Med 2004, 199:687-95.

02.01

Rapid detection and identification of commonly encountered dermatophytes by multiplex real-time PCR

A.M.M Arabatzis[1], L.E.S. Bruijnesteijn van Coppenraet[1], S. de Hoog[2], R. Summerbell[2], S. Lavrijsen[3], E.M.H. van der Raaij-Helmer[3], K. Templeton[1], A. Velegraki[4], E.J. Kuijper[1]
[1]Leiden University Medical Center, Department of Medical Microbiology, Leiden, [2]Centraalbureau voor Schimmelcultures, Utrecht, [3]Leiden University Medical Center, Department of Dermatology, Leiden, [4]Medical School, University of Athens, Department of Medical Microbiology, Athens, Greece

Introduction: Current diagnosis of dermatophyte infections based on direct microscopy and cultures is slow and has low sensitivity, especially in infections of hair and nails. In addition, the identification procedure of the isolates is slow and requires great expertise. The objective of the study was to develop a rapid real-time PCR assay for routine diagnosis of dermatophytes and concurrent species identification.
Methods: Two assays were designed and optimised, one for detecting the *Trichophyton mentagrophytes* species complex, *Trichophyton tonsurans* and *Trichophyton violaceum* that was based on amplification of ITS1 region and a second one for detecting the *Trichophyton rubrum* species complex, *Microsporum canis* and *Microsporum audouinii* that was based on amplification of the ITS2 region. The assay was performed using Taqman and minor groove binding probes carrying different fluorophores to discriminate targets. Phocine herpes virus (PhHV) was used as internal control. Sensitivity was tested by serial DNA dilutions and specificity was tested on a panel of 36 different fungal species including all dermatophytes, (non pathogenic) dermatophytoids, skin yeasts and bacteria. The proposed real-time PCR protocol was evaluated by testing blind 92 clinical specimens (67 patients), collected prospectively from suspicious skin-nail-hair lesions over a 6 months period.
Results: The system correctly identified the aforementioned dermatophyte species from pure culture. The analytical sensitivity of both assays was 0.1 pg, corresponding to 2.5 genomes per sample. The method detected all the microscopy and/or culture positive samples (40), correctly identifying all the species (*T. rubrum, T. mentagrophytes, M. audouinii, T. violaceum*) grown in culture (29). It also detected 7 additional positive samples that were negative by microscopy/culture and identified 2 mixed infections, both by *T. rubrum* and *T. mentagrophytes*. Using culture as gold standard, the sensitivity and specificity of real-time PCR was 100%.
Conclusions: The proposed real-time PCR assay has a high sensitivity, enables accurate diagnosis of six commonly encountered dermatophyte species and it could be potentially incorporated in the clinical laboratory routine diagnostic methodology.

02.02

Detection of the surrogate marker (1,3)-beta-D-glucan in patients receiving intravenous amoxicillin-clavulanic acid

M.A.S.H. Mennink, D. Ruegebrink, A. Warris, P.E. Verweij
UMC St Radboud, Medical Microbiology, Nijmegen,

Background: The fungal component 1,3-beta-D-glucan (BG) is increasingly used to diagnose invasive aspergillosis (IA) and other fungal infections in immunocompromised patients. We observed reactivity in serum samples of 2 hematology patients during treatment with intravenous amoxicillin-clavulanic acid (AMC). Samples were negative once treatment had been discontinued. Neither patient had evidence for invasive fungal disease. We aimed to find the cause for this false reactivity.
Methods: Using the BG assay (Fungitell, Associates of Cape Cod), we tested 10 serum samples from 6 hematology patients without evidence for invasive fungal disease that were treated with intravenous AMC. Furthermore, the AMC batches used for treating these patients were also tested for BG reactivity. In addition, the serum of 2 patients was tested before and after completing *i.v.* administration of AMC. The results were compared with BG reactivity in sera from patients treated with ceftazidime and healthy blood donors.

Results: BG was detected in 9 of 10 serum samples. The level of mean reactivity (1339 ± 1798 pg/ml) was significantly higher than found in serum of 10 patients treated with ceftazidime (17.7 ± 26.5 pg/ml) (p=0.002) and healthy blood donors (8.0 ± 13.8 pg/ml) (p=0.001). The serum of two patients tested before *i.v.* administration of AMC was negative but levels of 805 and 446 pg/ml, resp., were detected after completing the infusion. Ten batches of AMC infusion fluid used during this period were found positive for BG (9414 ± 7774 pg/g antibiotic) as opposed to 4 batches of ceftazidime (10 ± 21 pg/g antibiotic) (p=0.004). The serum of patients treated with AMC also contained significantly higher levels of galactofuranose-antigens (Platelia Aspergillus ELISA, BioRad) compared with those of ceftazidime treated patients and healthy blood donors (p=0.003 and p=0.009, resp.).
Conclusions: These results are highly suggestive of cross-reactivity of the BG assay with AMC. Physicians should be aware of the possibility of false positive BG in patients treated with this antibacterial agent. The presence of two different fungal components in AMC strongly supports a fungal origin.

02.03

Host shift in the neurotropic black yeast *Exophiala dermatitidis*: a steam bath colonizer emerging from the tropical rain forest

M. Sudhadham[1,2], P. Sihanonth[2], B.G. van den Ende[1], S. de Hoog[1]
[1]*Centraalbureau voor Schimmelcultures, Utrecht;* [2]*Chulalongkorn University, Department of Microbiology, Bangkok, Thailand*

Objective: The black yeast *Exophiala dermatitidis* is an uncommon etiologic agent of fatal infections of the central nervous system in otherwise healthy, mainly adolescent patients in East Asia. The route of infection is still a mystery. The steam bath apparently provides a novel environmental opportunity for this fungus, but its natural niche is still unknown. Two preponderant ITS rDNA genotypes are known, which might be used as markers in population dynamic processes. It is our aim to reveal the natural niche and to establish whether the transition to the human-dominated environment may be accompanied by natural selection and/or evolutionary adaptation to the new habitat.
Methods: Strains were isolated by pre-incubation in Raulin's solution, and subsequently on Erythritol-Chloramphenicol Agar (ECA) at 40°C. Strains were purified with Tween 0.1%. The rDNA ITS region was sequenced for most strains, and elongation factor 1α for a selection of strains. Genotype-specific assays were developed using Single-Strand Confirmation Polymorphism (SSCP), by restriction analysis (RFLP) and by applying selective primers. dDNA homology was performed spectrophotometrically. Animal experiments were performed by intravenous injection into BALB/c mice.
Results: The species was recovered in small but significant amounts in the faeces of fruit-eating tropical animals, and on tropical fruits. The human-dominated niche is known to be the public steam bath. Genotype detection was enhanced by the use of specific primers and SSCP. The distribution of genotypes in environmental niches is very different from that of intestinal and cerebral strains in humans. Virulence of strains tested in the animal model proved to be strain-dependent.
Conclusion: The preponderance of one ITS genotype cannot be explained by differences in invasive potential, as virulence proved to be strain-dependent in the animal model. The existence of two separate species rather than one was excluded by sequencing of elongation factor 1α and by nDNA hybridization. The phenomenon therefore must be explained by population dynamics, such as founder effects.

02.04

Site-directed antifungal pharmacokinetics and pharmacodynamics

A.H. Groll[1], D. Mickiene[1], R.Petraitiene[1], V.Petraitis[1], T.J. Walsh[2]
[1]*Infectious Disease Research Program, Center for Bone Marrow Transplantation and Department of Pediatric Hematology/ Oncology, University Children's Hospital, Münster, Germany;* [2]*Immunocompromised Host Section, National Cancer Institute, Bethesda, MD, U.S.A.*

Polyene lipid formulations (amphotericin B colloidal dispersion [ABCD], amphotericin B lipid complex [ABLC], unilamellar liposomal AMB [LAMB]) and multilamellar liposomal nystatin [LNYS] have different pharmacokinetics than deoxycholate amphotericin B (DAMB), which may result in differences in antifungal activity at different sites. We therefore investigated the pharmacokinetics and pharmacodynamics of five polyene formulations in tissue sites that are common targets of fungal infections at standard dosages (DAMB, 1 mg/kg; ABCD, ABLC and LAMB, 5 mg/kg; and LNYS, 2.5 mg/kg BID and 5 mg/kg QD).
Using a model *Candida albicans* meningoencephalitis, we were able to demonstrate that the four amphotericin B formulations possess different activity against experimental *Candida albicans* infection of the Central Nervous System. DAMB and LAMB achieved the greatest antifungal efficacy at this site, and this activity was concentration- and time dependent as reflected by a strong correlation between Cmax/MIC, AUC/MIC and Ttau > MIC and antifungal efficacy. Both LNYS regimen were less effective as DAMB and LAMB in the brain ($p<0.01$). As compared to DAMB, LAMB and LNYS at 2.5 mg/kg BID ($p<0.05$),

rabbits receiving LNYS at 5 mg/kg QD had significantly decreased survival due to severe CNS-candidiasis with occurrence of generalized seizures. Both dosage regimens of LNYS produced mean brain tissue levels that were below the MIC of the infecting isolate at 0.5 and 12 hours post dose. The clinical failure of the QD regimen correlated with a shorter mean Ttau > MIC in plasma as compared to the BID regimen.

In a kidney target model of hematogenous invasive candidiasis, only treatment with DAMB ($p<0.001$) and LAMB ($p<0.01$) significantly reduced the residual fungal burden. There was a trend towards improved tissue clearance with DAMB when all active treatment cohorts were compared ($p=0.0882$ by ANOVA). This finding coincided with a higher renal clearance and % recovery of AMB in urine ($p<0.05$) after administration of DAMB, but not with tissue concentrations at peak and trough and plasma concentration-derived pharmacodynamic parameters or nephrotoxicity. The two dosage regimens of LNYS had similar efficacy as DAMB and LAMB on the fungal burden. Antifungal efficacy of LNYS appeared to correlate with Cmax/MIC, Ttau tissue/MIC and exposure of NYS in urine.

We also investigated the comparative intrapulmonary disposition of the four AMB formulations in lung tissue, epithelial lining fluid, and pulmonary alveolar macrophages in uninfected animals. At 24 h after the last of eight daily doses, concentrations of AMB in lung tissue and PAMs were highest in ABLC-treated animals, exceeding concurrent plasma levels 70- and 375-fold, respectively. Drug concentrations in ELF were generally much lower than those achieved in lung tissue and PAMs. Among the different cohorts, highest ELF concentrations were found in LAMB-treated animals. While the disposition of ABCD was overall not fundamentally different to that of DAMB, ABLC showed prominent accumulation in lung tissue and PAMs and LAMB achieved highest concentrations in ELF. The impact of these findings is unclear, since no differences in antifungal efficacy were noted in a persistently granulocytopenic rabbit model of invasive pulmonary aspergillosis.

These experimental data demonstrate markedly different disposition patterns of antifungal polyene formulations that have impact upon their antifungal efficacy in tissue sites that are common targets of opportunistic fungal infections.

02.05

Exophiala species causing disease in cold-blooded animals

J.M. Harrak[1], K.S. Cruz[2], G. S. de Hoog[1]

[1]*Centraalbureau voor Schimmelcultures, Utrecht,* [2]*Universidade do Estado do Amazonas, Manaus, Brazil*

Drinking water appears to be an unexpected habitat of many melanized fungal species. Among these are several *Exophiala* spp., members of *Chaetothyriales,* a fungal order containing numerous members known from human disease, such as *Exophiala dermatitidis* and *Cladophialophora bantiana.* Based on ribosomal ITS sequences, waterborne *Exophiala* strains cluster with isolates from cold-blooded animals: several species of fish, turtles, crabs, sea horses and frogs. ITS analysis distributes these strains over 10 clusters, among which three clusters represent as yet undescribed *Exophiala* species. Also the sympodial species *Veronaea botryose,* which is morphologically very different from the annallidic *Exophiala* species, is found between the waterborne species. A comparison of the entire order *Chaetothyriales* using SSU rDNA operon shows that the waterborne species form a consistent clade within the parsimony tree. Cardinal growth temperatures of those species were also established. A correlation is observed between the maximum growth temperature and the source of isolation and the natural habitat of the host; fungi growth with maxima below 30°C are found causing disease in ocean animals, while those with maximum growth temperatures around 33°C cause epidemics in cold blooded animals living in shallow tidal zones in the subtropics. A striking example is an emerging crab disease in mangroves along the east coast of Brazil. Histopathological studies show that the infection leads to dissemination with enormous fungal loads, internal organs being entirely invaded. Remarkably, some waterborne *Exophiala* species have occasionally been isolated from human skin disorders, particularly in elderly patients with diabetes known to have relatively low body temperatures in their extremities. In general, thermophilic *Exophiala* species seem to have a preference for humans, while mesophilic species are predominantly found in cold blooded animals.

These findings are in contrast with waterborne melanized fungi belonging to the order *Leotiales,* for example *Cadophora malorum*. These fungi are supposed to be plant endophytes and have never been found to be involved in animal disease.

02.06

Cryptococcosis in Cuba

M.T. Illnait, G.F. Martinez, C.M. Fernandez, I.C. Valdes, M.R. Perurena, M. Torres

Institute of Tropical Medicine (IPK), National Reference Laboratory of Mycology, Havana, Cuba

The incidence of cryptococcosis has increased substantially worldwide in the last 20 years, being closely related to the AIDS pandemic and Cuba has not been an exception.

Objectives: 1) To describe the clinical-and laboratory findings in Cuban patients with cryptococcosis prior to the widespread use of HAART. 2) To identify species, varieties and serotypes of *Cryptococcus* strains isolated

from clinical sources. A total of 83 patients (72 with AIDS and 11 HIV negative) were studied with mycological evidence of cryptococcosis (1997 through 2002). In both groups, the frequency of clinical signs and symptoms were similar except for neurological signs, which prevailed in AIDS patients. Curiously, the HIV- negative patients did not had an obvious predisposing illness and they had a normal CD4/CD8 ratio. *Cryptococcus neoformans* infection was found to be the initial AIDS - defining illness in 45.8% of the AIDS patients. Thirty three percent (7 of 21) of the HIV + infected patients died in the first 2 weeks of diagnosis. A fatal outcome, related to treatment failure was associated ($p<0.001$) with abnormal mental status, convulsions, and low glucose concentration in CSF. A total of 76 *C. neoformans* strains belonging to the collection of the National Reference Laboratory of Mycology at the IPK were studied. These strains were isolated in our institution from CSF of AIDS and non-AIDS patients from 1988 through to 1997 and one strain was isolated from a Cheetah at the National Cuban Zoo. The identification of *C. neoformans* strains was established by conventional procedures. To determine the biovariety, two methods were used. The capability to grow on CGB medium and the D-proline assimilation test. Serotyping studies were carried out using the Crypto Check agglutination test (Iatron Labs Inc, Tokyo). Remarkably all Cuban strains isolated from humans (56 from AIDS patients and 20 from non AIDS patients) were *C. neoformans* var *neoformans* serotype A (var *grubii*) and the only veterinary isolate from a cheetah was serotype B but we are not sure that it is an autochthonous strain because the animal came from South Africa.

02.07

Kinetics of circulating glucan compared with galacto-furanose-antigens in patients with invasive aspergillosis

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Background: Several recent studies have compared the beta-1,3-Glucan (BG) assay with the Platelia Aspergillus (PA) ELISA for diagnosis of invasive aspergillosis (IA). However, no study has been published in which the kinetics of BG and Galactofuranose (Galf)-antigens are compared. We retrospectively analyzed prospectively collected consecutive serum samples from patients with probable or proven IA.
Methods: 170 serum samples were collected from 10 patients with IA, i.e. 5 patients with proven and 5 patients with probable IA based on the EORTC/MSG consensus definitions. These serum samples included series that had consequently negative galf-antigen tests and series that show conversion from negative to positive circulating antigen. All samples were tested in duplicate with the Fungitell BG assay (Associates of Cape Cod) and results were compared with the galf-antigen assay (PA ELISA, BioRad).
Results: Results were compared using a cut-off of 1.0 ng/ml galactomannan (GM) for the PA ELISA and a cut-off of 60 pg/ml BG for the Fungitell assay. Circulating BG was detected on days -13, 0, +2, +4 and +32 compared with circulating galf-antigens in the 5 patients with proven IA. In 4 patients with probable IA, circulating BG was detected on days -6, -11, 0 and +4 compared with circulating galf-antigens. In one patient with probable IA and persistent negative PA ELISA serum reactivity, the Fungitell assay also showed no reactivity.
Conclusions: Circulating BG was detected in 3 of 10 patients earlier than (Galf)-antigens, but later in 4 of 10. This variability might imply that monitoring of both markers simultaneously is required in high risk patients.

02.08

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., 2004). This outbreak was shown to be caused by a rare genotype of *C. gattii* (AFLP6A or RAPD VGIIa) using Amplified Fragment Length Polymorphism (AFLP) and sequence analyses. 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 analyzed by AFLP. The AFLP fingerprint analyses were carried out with six different primer combinations 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. (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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02.09

A patient with unbearable headache

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A 56-year-old, previously healthy woman presented at the outpatient clinic of neurology with since several weeks progressive headache localized behind the left eye. Since one-week she experienced double vision. Neurological examination revealed paralysis of the left abducens nerve, ptosis of the left eye and decreased sensation of the left side of her face. MRI of the brain showed a mass at the skull base with bony destruction and intracranial perineural extension. CT scan of the sinuses showed opacification of the left sphenoid sinus with destruction of the sphenoid walls to the sinus maxillaris and the temporal lobe. Biopsy of the fossa pterygopalatina showed a necrotic infection with hyphal elements consistent with *Aspergillus*. From a second biopsy specimen *Aspergillus fumigatus* was cultured. Circulating galactomannan was not detected in serum. Because of the extent and the localisation of the mass near the carotid artery, surgical removal was not feasible. Therapy was first started with liposomal amphotericin B and was switched to voriconazol after fungal identification. Despite this therapy, the lesion progressed and caspofungin and recombinant human granulocyte colony-stimulating factor were added. Eventually, because there was no effect, the therapy was stopped and patient died soon afterwards. Autopsy showed an extensive fungal abscess of the skull base with extension in the left temporal lobe and recent bleeding at the skull base. *A. fumigatus* could still be cultured from tissue obtained at autopsy. Amphotericin B, itraconazole, voriconazole and caspofungin showed *in vitro* activity against the *A. fumigatus* isolates, and no difference of MIC was found between pre- and post treatment isolates.

Conclusion: We present a case of an immunocompetent woman with an invasive sphenoid sinus *A. fumigatus* infection. Surgical treatment was not possible and the patient died despite antifungal therapy.

02.10

Melanin protects *Madurella mycetomatis* against itraconazole and ketoconazole, first-line treatment agents against mycetoma

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The ability of certain microbes to produce melanin has been linked to virulence and pathogenicity for their respective animal or plant hosts. The aim of this study was to determine the pathway used by *Madurella mycetomatis* to form its melanin. Furthermore, we wanted to know if melanin protects the fungus against the host immune system or antifungal agents used to treat mycetoma infections.

Fungal melanin can be formed via three different pathways, the DHN-, the DOPA-pathway and the pheo-pathway. By using inhibitors specific for these pathways we could establish that *M. mycetomatis* uses the DHN- and Pheo-pathways to produce melanin.

Melanin has been known to protect fungi like *Cryptococcus neoformans* and *Aspergillus* spp to oxidants and even antifungal agents. From our experiments it appeared that melanin is an agent that blocks the chemical reduction of TNB into DTNB by permanganate.

Futhermore, by using the recently published YeastOne Sensitrek method for *M. mycetomatis* MICs were determined with or without supplementation of melanin. Supplementation of *M. mycetomatis* DHN-melanin resulted in an increase in MIC with 5 two-fold dilution steps for the azoles itraconazole and ketoconazole. In short, a 16 times more concentrated solution was needed to prevent fungal growth. This means that about 60% of the strains considered susceptible to these tests appeared resistant after melanin-supplementation. This is worrying since both itraconzole and ketoconazole are antifungals routinely given to patients to prevent recurrent infections. No increase in MIC was found for the azoles fluconazole and voriconazole and the polyene amphotericin B. MIC shifts under the influence of melanin have not been described yet. What has been described so far for fungal species like *Cryptococcus neoformans* is that non-melanised cells are killed faster than melanised cells with amphotericin B.

Since itraconazole and ketoconazole are the drugs used in the clinic in preventing recurrent infections after surgery it should be noted that these drugs might not be the best

choice. Other drugs like amphotericin B and voriconazole should be considered.

02.11

Black fungal infections in China

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The black fungi are a large and heterogenous group of filamentous moulds with dark colored colonies and cell walls. They could produce brown melanin or melanin-like pigment in the cell wall of their hyphae or conidia, or both. Clinically, black fungi could cause the following diseases: chromoblastomycosis; phaeohyphomycosis; eumycotic mycetoma; onychomycosis; tinea nigra; black piedra and mycotic keratitis. Chromoblastomycosis is a distinctive infection of the skin and subcutaneous tissues caused by several dematiaceous moulds. It is the most common black fungal infection in China. The causative agents were *Cladophialophora carrionii* (64.7%), *Fonsecaea pedrosoi* (24.6%), *Phialophora verrucosa* (2.4%) and *Fonsecaea compacta* (0.8%). Phaeohyphomycosis is a generic term to be applied to any mycosis involving a dematiaceous fungus. The pathogenic agents of phaeohyphomycosis are widespread in the environment include soil, wood, decomposing plant matter as well as polluted water. Until 1998, 60 genera, 109 species were reported to be human pathogenic. The causative agents in China mainly included: *Exophiala* spp. (most common), C*haetomium* spp., *Bipolaris spinifera, Alternaria alternata, Veronaea botryosa, Ochroconis gallopavum, Curvularia clavata, Phialophora verrucosa, Hendersonula toruloidea*. The treatment of black fungal infection is still difficult. In summary, chromoblastomycosis is the major type of dematiaceous fungal infection in China. Phaeohyphomycosis is increasing in immunocompromised patients and the management of it still challengeable. The classification and identification of dematiaceous fungi will depend on the combination of morphological, physiological, molecular biological as well as other supplementary methods.

02.12

Colonization of cystic fibrosis patients with *Aspergillus fumigatus* is a recurrent phenomenon

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Introduction: *Aspergillus fumigatus* strains often colonize the respiratory tract of Cystic Fibrosis (CF) patients. Previous low discriminatory molecular typing assays suggested the majority of sequential cultured isolates to be of the same genotype. We used a novel high-resolution fingerprinting assay to analyze multiple *A. fumigatus* strains from CF patients.
Methods: We collected *A. fumigatus* strains from nine patients. From 6 patients each, two isolates were collected with a one year interval. From 3 patients, isolates were collected over a period of 3 to 4 years (3, 16 and 13 isolates respectively). All strains were analyzed using the STRAf (Short Tandem Repeats of *A. fumigatus*) assay.
Results: From 6 patients, all intrapatient, isolates were of different genotypes. One patient with two isolates was colonized by the same strain over a period of one year. From the patient with 16 isolates, 13 different genotypes were found; two types were isolated more than once within a 5 months period. The patient with 13 isolates harbored four unique isolates and 3 clusters of 3 isolates were from the same type and succeeded each other during the last year.
Conclusion: Over a long period of time, different genotypes of *A. fumigatus* were found in most of the examined CF-patients. If the same genotype was found more than once, this only occurred in a short time period. Airway colonization of CF patients with *A. fumigatus* is appear to be a recurrent event. To substantiate this further more isolates from more CF patients should be analyzed.

02.13

Epidemiologic issues in invasive fungal infections

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The last two decades have seen unprecedented changes in the pattern of serious fungal infections among immunocompromised individuals, including hematopoietic stem cell transplant (HSCT) and solid organ transplant (SOT) recipients, persons with hematological malignancies, and persons living with AIDS. These infections are being seen in ever increasing numbers, largely because of the increasing size of the population at risk. Although gains have been made in the treatment and prevention of some fungal infections, medical developments and changing health care practices have contributed to the emergence of new pathogens, and new at-risk groups.
Sentinel and population-based surveillance programs have documented significant changes in the rates of invasive candidiasis (IC) among different groups of immunocompromised patients, with the emergence of non-albicans *Candida* species, particularly *Candida glabrata*, as important pathogens. More cases of IC now occur among critical care patients than are diagnosed among neutropenic cancer patients or HSCT recipients.

Surveillance programs have also demonstrated that rates of drug resistance among bloodstream isolates of *Candida* species remain low and have not changed over time.
In the USA, population-based surveillance, conducted between 1992 and 2000, documented the declining incidence of cryptococcosis among persons with AIDS. In contrast, surveillance programs in developing countries with large HIV epidemics have demonstrated that the burden of this disease is increasing.
Although still few in number, sentinel surveillance programs have begun to provide important information about the incidence of invasive aspergillosis (IA) and other serious mould infections after HSCT. In an interim report from the TransNet sentinel surveillance program, the aggregate cumulative incidence of IA at 19 transplant centers throughout the USA, between March 2001 and December 2002, ranged from 0.5% after autologous HSCT, to 3.9% after transplantation from an unrelated donor. Of note, wide variations in incidence were seen between the different centers. Many factors could account for these differences, including variations in rates of follow-up, and variations in diagnostic methods and practices between sites. Transplant-related factors (e.g., donor type, stem cell type, transplantation conditioning, and post-transplantation immunosuppression) and host-related factors (e.g., underlying disease prompting transplant, comorbid conditions) may also impact the incidence of IA at individual centers.
In addition to IA, a number of other mould infections have been seen after HSCT. Between March 2001 and December 2003, IA, zygomycosis and fusariosis accounted for 68%, 8%, and 6%, respectively, of the identified mould infections reported to the TransNet program. Although information is limited, data from this program appear to support recent anecdotal reports that the incidence of zygomycosis is increasing. Compared with IA, prior use of voriconazole appears to be an independent predictor for zygomycosis.

03.06

Treatment of *Dientamoeba fragilis* infection with paromomycin (Humatin®) in children: Parasitological and clinical effectiveness

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Dientamoeba fragilis is a commonly observed protozoan parasite associated with gastrointestinal symptoms. Effective treatment in symptomatic patients often results in alleviation of complaints. However, there is no consensus which antiparasitic drug is most effective for treatment of *D. fragilis*.
In the present study, treatment with paromomycin (25-35 mg/kg/day for seven days) was studied in children with *D. fragilis* infection. The children were asked to keep a diary in which they recorded, on a daily basis, abdominal pain, other gastrointestinal symptoms and non-gastrointestinal symptoms (fever and headache). The primary endpoint was parasitological effectiveness, the secondary endpoint clinical effectiveness.
Twenty-one children (11 male and 10 female, age 4-18 years) were enrolled. In 20 out of 21 (95%) children no *D. fragilis* was observed in faeces samples taken directly after end of treatment. Two and three weeks after therapy 4 (19%) and 7 (33%) patients showed recurrence of *D. fragilis*, respectively. A significant reduction of abdominal pain (mean reduction from 6 to 3 in a range of 0-12) and non-gastrointestinal symptoms (mean reduction from 0.44 to 0.16 in a range of 0-2) was observed. A non-significant reduction of other gastrointestinal complaints was observed.
Conclusion: This study suggests that paromomycin is a highly effective drug for dientamoebiasis. The high recurrence of *D. fragilis* in feces is not understood but can be a result of reinfection or inadequate dosaging. Most children showed clinical benefit of paromomycin in this uncontrolled study.

05.03

Staphylococcal superantigen-like protein 5 (SSL5) inhibits PSGL-1-mediated processes under static and flow conditions and inhibits CXCR2-induced cell activation

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Introduction: *Staphylococcus aureus* carries up to 11 staphylococcal superantigen-like proteins (SSLs) on pathogenicity island SaPIn2. SSLs show structural homology to staphylococcal and streptococcal superantigens, but have no superantigenic activity. Recently it was observed that the C-terminal domain of SSLs is homologous to Chemotaxis Inhibitory Protein of *S. aureus* ($CHIPS_{31-121}$) which inhibits C5a-induced neutrophil responses. Currently, we are investigating possible immunomodulatory properties of SSLs. Here we describe interference of P-selectin glycoprotein ligand 1 (PSGL-1)-mediated processes and inhibition of CXCR2-induced stimulation of neutrophils by SSL5.
Methods: SSL5 was cloned and expressed from *S. aureus* strain NCTC 8325. A screening assay for surface-expressed receptors of leukocytes was performed to identify PSGL-1 as the receptor for SSL5. Subsequently, several competition

experiments with SSL5 and P-selectin-Fc chimera or antibodies directed against PSGL-1 were performed with human neutrophils. Functional role for SSL5 was investigated through rolling experiments with neutrophils under flow on a P-selectin surface. Influence of SSL5 on chemokine signaling was investigated in neutrophils and U937 cells through a calcium mobilization assay.

Results: SSL5 inhibited binding of anti-PSGL-1 and P-selectin-Fc to neutrophils by 90% and 60%, respectively. SSL5 also strongly blocked adhesion and rolling of neutrophils to immobilized P-selectin under static and flow conditions comparably to anti-PSGL-1. Finally, SSL5 specifically inhibited the ? and Neutrophilα-interleukin-8 (IL-8), growth-regulated oncogene alpha (GRO Activating Protein 2 (NAP2)-induced calcium mobilization in neutrophils and U937 cells expressing the CXCR2.

Conclusions:

1) SSL5 binds PSGL-1 and interferes with PSGL-1-mediated binding to P-selectin under static and flow conditions.
2) SSL5 inhibits CXCR2-induced cell activation.
3) SSL5 is an important immunomodulatory protein of *Staphylococcus aureus*.

05.04

SCIN and CHIPS homologues are located on a new Immune Evasion Cluster in *S. aureus*

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Introduction: Staphylococcal Complement Inhibitor (SCIN) and Chemotaxis Inhibitory Protein of Staphylococci (CHIPS) are crucial for the staphylococcal defense against our innate immune system. SCIN and CHIPS are located on the first Immune Evasion Cluster (IEC) in *Staphylococcus aureus*. A BLAST search revealed four unknown staphylococcal proteins with high sequence homology to SCIN or CHIPS. SCIN-B and SCIN-C, sharing 45% and 48% homology with SCIN, also function as complement inhibitors. The CHIPS-like proteins, Formylated Peptide Receptor Like-1 (FPRL-1) Inhibitory protein (FLIPr) (28% homology) and FLIPr-like (77% homology with FLIPr) prevent activation of FPRL-1 or FPR&FPRL-1 respectively. In this study we describe the unique location of SCIN and CHIPS homologues on a new Immune Evasion Cluster (IEC2) in *S. aureus*.

Methods: Database analyses were performed to characterize IEC2. The prevalence of SCIN-B, SCIN-C, FLIPr, FLIPr-like and neighboring genes in 90 clinical *S. aureus* strains was determined by PCR. For functional analyses, we cloned and expressed the protein with 33% homology to Extracellular Fibrinogen Binding protein (Efb) (Efb-like) in *Escherichia coli*. Efb-like was tested in whole blood phagocytosis assays using FITC-labeled *S. aureus*. Calcium mobilization was performed to study effects on C5a production.

Results: We describe a new Immune Evasion Cluster (IEC2) in *S. aureus* that carries the genes for SCIN-B (46% of strains) or SCIN-C (33%), FLIPr (79%) or FLIPr-like (4%), Alpha-toxin (100%), Efb (86% C3b modulator), Efb-like (58%), an unknown membrane protein (90%) and 3 putative exotoxins (93%). Efb-like was identified as a potent immune evasion molecule since it effectively prevents phagocytosis in whole blood. Since Efb-like prevents generation of C5a, it is a new complement modulator.

Conclusion:

1) The immune evasion molecules SCIN-B, SCIN-C, FLIPr and FLIPr-like are located on a new Immune Evasion Cluster (IEC2) in *S. aureus*.
2) On this IEC2 we also found the genes for Alpha-toxin, Efb, 4 unknown proteins and Efb-like.
3) Efb-like represents a new complement modulator in *S. aureus*.

05.05

Complement inhibition by *S. aureus*

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The complement system plays a pivotal role in host defense but also contributes to tissue injury in several diseases. Here we describe the discovery of Staphylococcal Complement Inhibitor (SCIN), an excreted 9.8 kD protein that blocks human complement by specific interaction with C3 convertases (C4b2a and C3bBb). The assembly of C3 convertases (C4b2a and C3bBb) is a prerequisite for complement activation. The convertases catalyze C3b deposition on activator surfaces. SCIN binds and stabilizes C3 convertases, interfering with C3b deposition via the classical, lectin and alternative complement pathway. This leads to a dramatic decrease in phagocytosis and killing of *Staphylococcus aureus* by human neutrophils. As a highly active and small soluble protein that acts exclusively on surfaces, SCIN is a promising anti-inflammatory molecule. Furthermore, we address the role of other staphylococcal proteins that modulate the complement system.

05.06

Clumping factor B is an essential bacterial factor for *Staphylococcus aureus* nasal colonization in humans

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Background: *Staphylococcus aureus* persistently colonizes the vestibulum nasi in a significant fraction of humans. The mechanism whereby the bacterium establishes resident populations is unknown and has only been studied *in vitro* studies and in small rodents.

Methods: We define the role of the staphyloccal cytokeratin-binding protein clumping factor B (ClfB) in the colonization process by artificial inoculation of human volunteers with a wild type strain and its single locus ClfB knock out mutant. Persistence was followed up by quantitative nasal culture and bacterial genotyping for 28 days.

Results: Our results show that the mutant strain is eliminated from the vestibulum nasi of volunteers significantly faster than the wild type: median of 8 days versus 3 days (p=0.0174). The number of *S. aureus* CFUs after inoculation were always higher for the wild type strain. The load was statistically significantly higher at days 7 and 21. After 1 week, the average numbers of CFUs (log CFU) per culture were higher for the wild type strains (0,85 CFU versus 0,26 CFU; p=0,022).

Conclusion: Here we present the first 'in homo' data showing that the ClfB protein by itself is already a prime determinant of nasal *S. aureus* nasal carriage.

05.07

Increasing prevalence of infections with methicillin-resistant *Staphylococci* in animals

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In 2003, we described the first isolation of a methicillin-resistant *Staphylococcus aureus* (MRSA) from animal origin in the Netherlands.[1]

In the period from 2003 to 2006, we found five new unrelated cases of MRSA infections in household pets and horses. These MRSA were cultured from two horses with arthritis, a dog with a wound infection, a cat with cystitis and a cat with a wound infection. In addition, two dogs had infections with methicillin-resistant *Staphylococcus intermedius* (MRSI) and one dog was colonized with MRSI. Isolates suspected of being MRSA or MRSI were tested for the presence of the *mecA* gene by PCR. *MecA*-positive strains were further analyzed using pulsed-field gel electrophoresis (PFGE),[2] multilocus sequence typing (MLST) and were screened for Panton-Valentine leukocidin (PVL) genes by PCR.[3] Characterization of the staphylococcal chromosome cassette *mec*(SCC*mec*) was performed by PCR. The MRSA isolates had PFGE type 6b (n=2), 113 (n=2) and 379 (n=1) and MLST ST8 (n=2) or ST45 (n=3). All MRSA isolates had ccr type 2 and SCC*mec* type IV. The MRSI strains had SCC*mec* type III (n=2) and V (n=1). None of the MRSA or MRSI isolates were PVL-positive. In conclusion, the prevalence of MRSA and MRSI infection seem to increase in animals in the Netherlands. These MRSA strains in animals were all of SCC*mec* type IV. The SCC*mec* type IV is typically found in community acquired MRSA strains.

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05.08

Culture based and molecular prevalence of MRSA in the Twente-Achterhoek region

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Introduction: Recently several PCRs came available for the detection of MRSA in clinical specimen. Although very promising, these PCRs were only evaluated in centers with a high MRSA prevalence. We tested 2 of these PCR-systems in our centre with a low MRSA-prevalence (< 0,5%), also incorporating a culture based pre-enrichment step.

Methods: A total of 152 swabs obtained from 152 patients with chronic wounds (diabetic ulcers and pressure sores) were included. After standard (not MRSA specific) bacteriological culture bacteria still adherent to the swabs were suspended in 1ml saline and 100 µl of this saline was transferred to a pre-enrichment broth containing

75 mgr/liter aztreonam and 5 μg/liter ceftizoxim and incubated for > 15 hours at 370°C. DNA was extracted (Magna-Pure Roche) from both the saline and the broth and subjected to a real-time multiplex PCR assay based on the Huletsky protocol (Québec, Canada). DNA extracted from the broth was also subjected to the MRSA-test from HAIN (Germany). Two swabs obtained from two patients with a MRSA infected wound were included as positive controls. PCR positive samples were subjected to extensive culture procedures.

Results: The 2 MRSA-positive swabs were positive in all tests. None of the 152 swabs were MRSA-positive on standard culture. However, the Huletski-PCR showed that 4/152 saline samples and 25/152 broth samples were clearly MRSA-positive (Ct values < 40). By using the HAIN-test on the broth samples, 7/152 samples proved to be MRSA-positive, these 7 samples were also positive in the Huletsky-PCR. After extensive culturing from 17/25 Huletsky-PCR positive samples a MRSA-strain could be recovered.

Conclusions: Molecular detection provides a very powerful method for the detection of MRSA, especially when a pre-enrichment broth is part of the procedure. Disturbing however is the high MRSA prevalence when using these strategies. Based on these result, the MRSA prevalence in our region is between 5 and 15%, so at least 10 times higher than expected.

05.09

Novel variants of *Staphylococcus* Cassette Chromosomes excised by ccrA/B type 2 recombinases in *Staphylococcus aureus*

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Background: Staphylococcal Cassette Chromosome mec (SCCmec) type IV is associated with virulent community-acquired MRSA and frequent horizontal transfer among *Staphylococci*. To gain insight into the mechanism of transfer, we studied the by ccrA/B type 2 recombinase mediated excision of SCCmec II and IV.

Methods: A ccrA/B type 2 plasmid was constructed, in which the ccrA/B genes were placed under control of a constitutive phage-repressor promoter. The plasmid was transduced into different MRSA strains, including MR108, MW2, Ca05 (JCSC1968), JCSC1978, Mu50 and N315, and a set of strains that demonstrated the *in vivo* transfer of SCCmec IV (MSSA wkz1, MRSA wkz2, and *Staphylococcus epidermidis* O7.1 (Wielders et al. Lancet 2001:357:1674). Excision of SCC elements was determined by PCR and sequencing.

Results: In wild-type MRSA and *S. epidermidis* strains containing SCCmec IV, but not in SCCmec II MRSA strains, excision of the cassette was observed. Introduction of the ccrA/B type 2 plasmid in the different strains yielded excision of SCCmec II and multiple excision variants of SCCmec IV. Sequencing of the alternatively excised products in several SCCmec IV wild-type strains and clones identified a 100 bp shortened SCCmec variant and novel excision products in MRSA wkz2 not present in SCCmec donor O7.1. Sequencing showed a 5,877 bp, conserved SCC-like element that lacks mecA and ccrA/B recombinases, which was also present in MR108. Excision of the SCC-like element in wild type *S. aureus* was dependent on the presence of SCCmec. The element could be excised separately or as part of a novel composite cassette together with SCCmec.

Conclusions: Four SCC excision variants were identified in SCCmec IV strains, including the *in vivo* formation of a new composite SCCmec cassette with a SCC-like element. SCCmec II was only excised in the presence of the ccrA/B 2 plasmid. The variety in SCC excisions may increase horizontal transfer and genetic plasticity in SCCmec IV MRSA strains.

05.10

A specific secretion system mediates PPE protein transport in *Mycobacteria* and is required for virulence

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The *Mycobacterium tuberculosis* genome contains two large gene families, the so-called PE and PPE genes, that are unique to mycobacteria. Here we report that one of the PPE proteins, i.e. PPE41, is secreted both *in vitro* and in macrophages. Furthermore, we have identified that PPE41 is secreted by a novel secretion system, which is homologous to the mycobacterial ESAT-6 secretion system. This secretion system, designated ESX-5, is conserved in pathogenic mycobacteria, but is not present in the environmental species *Mycobacterium smegmatis*. However, introduction of the entire ESX-5 region in *M. smegmatis* resulted in efficient secretion of heterologously expressed PPE41, which shows that ESX-5 is necessary and sufficient for PPE transport. ESX-5 secretion mutants showed reduced spreading of mycobacteria to uninfected macrophages, which shows that ESX-5 secreted substrates play an important role in virulence.

05.11

CodY contributes to colonization of *Streptococcus pneumoniae*

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CodY is pleiotropic transcriptional regulatory protein that is involved in nitrogen metabolism. In *Lactobacillus lactis* and *Bacillus subtilis*, CodY regulates the expression of various enzymes, e.g. oligopeptide permeases, proteases and genes involved in genetic competence (*B. subtilis*).
For transcriptional profiling, total RNA of D39 Δ*codY* and the parental strain isolated at two distinct optical culture densities was applied to amplicon-based DNA microarrays. For conformational purposes, proteome expression analysis was performed using 2D DIGE gel-electrophoresis. To investigate whether the lack of CodY has an effect on adherence to nasopharyngeal epithelial cells, an *in vitro* adherence assay using Detroit 562 cells was performed. The *codY*-mutant was used in a murine model to assess the contribution of the *CodY* to virulence. Mice were infected with either D39 Δ*codY* or the wild type strain in three different animal models, i.e. colonization, pneumonia and sepsis. The putative DNA-binding box of *L. lactis* was used for an *in silico* search in the genome sequence of strain R6.
The transcriptional pattern of the Δ*codY* differed substantially from the wild type pattern. Among the genes displaying increased expression in the D39 Δ*codY* strain are genes involved in amino acid metabolism (i.e. *amiA*, *amiD* and *amiE*), nucleic acid synthesis and metal binding. In addition, 2D DIGE gel-electrophoresis confirmed several of these putative targets, such as the *ami*-operon. Using murine animal models, we showed that the *codY*-mutant has a significant reduction in colonization of the nasopharynx ($p < 0.05$). The colonization data correlated with the *in vitro* adherence assay using the *codY*-mutant in a *cps*-negative genetic background (p=0.001). No statistical differences between *codY*-mutant and wild type were found in either a pneumonia or sepsis model of infection. *In silico* use of the *CodY* binding box of *L. lactis* resulted in 27 putative DNA-binding boxes in *Streptococcus pneumoniae*. Among these putative targets are *aliA*, *gdhA*, and *liv*, all genes identified by microarray and 2D DIGE.
In conclusion, regulation of CodY is essential for efficient pneumococcal colonization.

05.12

Metal-responsive regulation and role in iron acquisition of the two *Helicobacter mustelae* TonB orthologs

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Introduction: *Helicobacter mustelae* is a gastric pathogen of ferrets. Like almost all organisms, *Helicobacter* species require iron for growth. However, iron-sequestration by mucosal surfaces is a non-specific defense host mechanism against bacterial pathogens, and conversely iron acquisition is considered an important bacterial virulence factor. Iron-transport in Gram-negative pathogens is energized via the TonB-ExbB-ExbD complex. The *H. mustelae* genome sequence contains two genes encoding TonB orthologs. In this study we have characterized the regulation and function of the TonB orthologs of *H. mustelae*.
Methods: Isogenic tonB mutants were created in *H. mustelae* strain ATCC 43772 by insertional mutagenesis. The wild-type strain and tonB mutants were plated under iron-limited conditions, and hemin, hemoglobin or ferric citrate were supplemented as sole iron source. The growth promotion zone was measured after 48h. Regulation of *tonB1* and *tonB2* expression was assessed by Northern hybridization.
Results: Homology searches of the *H. mustelae* genome sequence allowed the identification of two TonB orthologs, tentatively named *tonB1* and *tonB2*. Wild-type *H. mustelae* was able to utilize $FeCl_3$, ferric citrate, hemoglobin and hemin as sole iron source. A *tonB1* mutant was unable to grow with hemin as sole iron source, but was not affected in growth on the other tested iron sources. In contrast, mutation of the *tonB2* gene resulted in reduced growth with ferric citrate and hemoglobin. Transcription of *tonB1* was iron- and nickel-repressed, whereas transcription of *tonB2* was not affected by iron or nickel.
Conclusions: The two TonB orthologs of *H. mustelae* have differential roles in iron acquisition, with TonB1 functioning in hemin uptake. Interestingly, hemin and hemoglobin uptake seem to require different TonB orthologs. The TonB orthologs of *H. mustelae* are also differentially regulated, which allows for additional finetuning of iron uptake. This adds another dimension to the intricate process of adaptation of *Helicobacter* species to the conditions occuring in the gastric mucosa.

05.13

Hfq mediated riboregulation in *Neisseria meningitidis*

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The small non-coding RNA (sRNA) chaperone Hfq emerges as an important regulatory factor of a riboregulated network controlling bacterial physiology such as

stress resistance and virulence. Hfq mediates basepairing between sRNAs and complementary sequences present in target mRNAs and thus controls gene expression at the posttranscriptional level.
A *hfq* homolog is present in the available sequenced genomes of *Neisseria meningitidis*, but its riboregulated network is unknown. The aim of our study is to unravel this network to identify novel, Hfq dependent, meningococcal virulence factors, being potentially new targets for intervention and diagnostics.
A *hfq* knock-out of N. meningitidis strain H44/76 was constructed. This knock-out strain is highly sensitive to exposure to UV light compared to the wild type strain. In addition, the mutant is severely hampered in growth in rich media, and does not grow at all under conditions of iron limitation. Expression of *hfq* in trans in the knock-out strain restored growth. Preliminary analysis of proteins subjected to Hfq regulation, assessed by the comparison of protein profiles of the knock-out strain and the wild type strain and peptide mass finger prints, resulted in the identification of meningococcal components involved in i) iron-acquisition (major ferric iron binding protein, *fbpA*), ii) nitrogen sensing (*glnD*), iii) protection of the cells against damage by free radicals (putative oxido-reductases such as *sucA* and NMB1796), and components involved in the assembly of pili and a variety of outer membrane proteins with unknown functions.
The reduced growth rate and tolerance for stress conditions of the *hfq* knock-out strain and the identification of Hfq regulated genes that encode for components involved in adaptation to the environment and adherence, strongly indicates that meningococcal Hfq is involved in the regulation of the response to environmental stress and thereby contributes to the virulence of the bacteria.

05.14

Identification of putative surface exposed proteins specific for hospital adapted vancomycin-resistant *Enterococcus faecium*

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Introduction: The incidence of infections caused by vancomycin-resistant *Enterococcus faecium* (Efm) has dramatically increased in hospitals world wide. The majority of clinical relevant Efm cluster together by multilocus sequence typing (MLST) in a hospital-adapted genogroup, designated clonal complex-17 (CC17). Due to their multi-resistant nature, infections with CC17 Efm are difficult to treat. The objective of the current study was to identify cell surface proteins (CSP) that are found enriched in CC17 Efm and that may serve as targets for immunotherapy to prevent and treat infections with these bacteria.
Methods: Two approaches were followed to identify CC17 enriched surface proteins; (1) CSP of multiple Efm strains from CC17 and non-CC17 were covalently labelled with biotin to detect differences in CSP expression, (2) the genome of Efm DO (which belongs to CC17), was searched for genes encoding CSP containing the LPXTG cell-wall anchor motif. Using PCR a set of 100 Efm isolates belonging to CC17 and other complexes was screened for the presence of these CSP genes.
Results: Biotin labelling of CSP of CC17 and non-CC17 isolates revealed at least one CSP unique for CC17. The genome search revealed 16 putative CSP genes and PCR screening of 100 CC17 and non-CC17 isolates identified 5 CSP genes, which were predominantly found in CC17. Negative PCRs were confirmed with Southern hybridization. Clustering based on the presence and absence of the CSP genes showed a comparable grouping as MLST suggesting that the clinical relevant strains of CC17 have an unique profile of putative CSP.
Conclusions: (1) Using biotin labelling one CSP was identified, which is unique for and highly expressed in CC17. (2) PCR screening identified 5 putative CSP genes enriched in CC17 and highly homologous to microbial surface components recognizing adhesive macromolecules. These putative CSP could be potential targets for new treatments to combat the emergence of CC17 Efm. (3) The distinct CSP profile of CC17 Efm may enhance its pathogenic potential thus contribute to its success in the hospital environment.

05.15

Infections of complement resistant and complement sensitive *Borrelia burgdorferi* sl in Wildtype and C3 deficient mice.

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Of the different species within *Borrelia burgdorferi* sensu lato, *B. burgdorferi* sensu stricto, *Borrelia afzelii* and a subgroup of *Borrelia garninii* strains show resistance to complement, whereas other *B. garinii* strains and *Borrelia valaisiana* strains are sensitive. This resistance is due to binding host factor H through CRASPs (Complement Regulatory Aquired Surface Proteins). We evaluated in a murine model whether absence of complement would influence infectivity and pathogenicity of complement-sensitive and -resistant *Borreliae*.
Five groups of 3 mice deficient in complement component C3 (C3KO) and syngeneic C57Bl/6 control mice were challenged with a pathogenic *B. burgdorferi* ss. strain

or with 5 different *B. afzelii*, *B. valasiana*, complement sensitive *B. garinii* and complement resistant *B. garinii* strains of unknown infectivity. Mice were sacrificed two weeks postinoculation and quantitive PCR (qPCR), culture, histopathology and immunofluorescence (IF) was done on heart, joint, brain, bladder and skin.
All mice challenged with *B. burgdorferi* ss. and *B. afzelii* showed high loads in qPCR in heart, bladder and skin. Higher, though not significantly different loads were seen in C3KO mice. In joints of C3KO mice challenged with *B. burgdorferi* ss. loads were significantly higher compared to WT (844 vs 0 spirochetes/ 1000 mouse cells, $p < 0.05$). Mice challenged with complement-resistant *B. garinii* had low loads in WT and C3KO mice, not consistently present in all organs.
In culture, *B. burgdorferi* ss. grew from all organs within 2 weeks. *B. afzelii* and *B. garinii* complement resistant strains grew after 4-6 weeks, and only from heart tissue.
Histopathology showed a direct relationship of borrelial load and inflammation. Only *B. burgdorferi* ss. and *B. afzelii* spirochetes were detected by IF in the tissues. No *Borreliae* were detected in complement-sensitive *B. garinii* and *B. valaisiana* challenged mice by any technique.
Complement-susceptible strains did not become infective in C3KO mice. Loads of strains that showed some degree of complement-resistance were marginally, but generally not significantly higher in C3KO mice.

05.16

Identifying conformational epitopes for human-IgG within the CHIPS protein

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Background: Chemotaxis Inhibitory Protein of *Staphylococcus aureus* is a very potent inhibitor of the C5a-receptor (C5aR) and formylated peptide receptor (FPR). The C5aR is described as an important target in the control and treatment of a large variety of inflammatory diseases like ischemia-reperfusion injury, auto-inflammatory diseases and sepsis. Since *S. aureus* is a common bacterium, everybody encountered the CHIPS protein and possesses anti-CHIPS antibodies. These antibodies interfere with CHIPS activity *in vivo* and induce adverse reactions upon intravenous administration of CHIPS. Separating the C5aR blocking properties from the immune reactive properties is an essential step in creating a CHIPS derived anti-inflammatory drug.
Methods: Using The Ph.D.7 and -C7C phage libraries (New England Biolabs), we identified conformational epitopes for human IgG within the CHIPS31-113 protein (the part of CHIPS responsible for C5aR inhibition). The Ph.D. libraries contain phages expressing 7 mer random peptides. These phages are selected for binding to affinity purified human-α-CHIPS31-113-IgG. After selection the phages were amplified and used for additional selection rounds. Following four rounds of selection with increasingly stringent washing steps the selected phages are sequenced to find consensus sequences representing epitopes for human-IgG. These epitopes can than be mapped onto the structure of the CHIPS molecule.
Results: After four rounds of selection against affinity purified anti CHIPS human IgG we sequenced 48 clones. 4 different consensus sequences were mapped onto the surface of the CHIPS31-121 structure.
Discussion: These data show that random peptide phage display is a very powerful method to identify conformational epitopes within a protein. Currently we are confirming the epitopes of the affinity purified anti CHIPS human IgG using synthetic peptides mimicking the found epitope sequence. Depletion of the anti CHIPS IgG stock for antibodies directed against these epitopes could provide an indication of remaining epitopes present.
Identification of the epitopes can lead to further understanding of the immune reactive properties and the pathogenesis of *S. aureus* infections

05.17

Nasal immunization with pneumococcal proteins displayed on a *Lactococcus lactis*-based carrier provides protection against fatal pneumonia

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Introduction: *Streptococcus pneumoniae* is a major cause of infections as pneumonia, meningitis, sepsis and otitis media in both children and adults worldwide. The available vaccines are based on capsular polysaccharides, and although very effective, they protect only against a limited to number of the 90 serotypes that are known to exist. The development of a protein vaccine that is effective against invasive pneumococcal disease and affordable to produce, will have major benefits in both developing and developed countries.
Methods: A protein-based nasal vaccine against *S. pneumoniae* was constructed, in which three pneumococcal proteins were displayed on the surface of non-living, non recombinant *L. lactis*. The pneumococcal proteins IgA1 protease (IgA1p), putative proteinase maturation protein A (PpmA) and streptococcal lipoprotein A (SlrA) were bound to the cell wall of pre-treated *L. lactis*, designated

as GEM (Gram-positive Enhancer Matrix), by means of a peptidoglycan binding domain. The vaccine was evaluated for immunogenicity and protective efficacy in an intranasal challenge murine model for pneumococcal pneumonia. Adjuvant properties of the GEM particles were studied *in vitro* by dendritic cell maturation and TNF-alpha production.

Results: The immune-stimulating potential of the GEM-based pneumococcal vaccine is very high. Nasal immunisation results in an immune-protective response against invasive pneumococcal disease, and antibody responses were induced at systemic and local levels. The adjuvant capacity of the GEM particles was demonstrated by their ability to maturate dendritic cells and induce the production of TNF-alpha.

Conclusion: We conclude that intranasal immunization with the trivalent pneumococcal vaccine without additional adjuvants showed significant protection against fatal pneumococcal pneumonia in mice. The display technology, in which *Lactococcus*-based particles act as both carrier and mucosal adjuvant, has great potential to develop a broadly applicable mucosal *S. pneumoniae* vaccine.

05.18

Improvement of LPS-containing vaccines by modification of lipid A biosynthesis in *Neisseria meningitidis* and *Bordetella pertussis*

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Lipopolysaccharide (LPS), a major constituent of the outer membrane, is present in several bacterial vaccines in significant amounts. As such, it can have a potential role as both immunogen and adjuvant. However, its endotoxic activity also causes significant reactogenicity which may limit widespread acceptance of these vaccines. Both the endotoxic and adjuvant activity of LPS are largely determined by the specific acylation pattern of the lipid A moiety, the membrane-anchoring part of LPS. Therefore, modification of the lipid A biosynthetic pathway might provide a means to obtain improved LPS-containing vaccines. In the case of *Neisseria meningitidis*, we have identified and mutated the genes encoding the acyltransferases involved in lipid A biosynthesis. The resulting altered acylation pattern might offer the possibility to create novel LPS species with altered biological activity, more suitable for inclusion in meningococcal outer membrane vesicle vaccines. This led to the unexpected discovery that an *N. meningitidis lpxA* mutant is viable without LPS. Despite the complete lack of LPS, hardly any defects were observed in the assembly of the major integral outer membrane proteins. Still, the immunogenicity of outer membrane preparations of this LPS-deficient mutant turned out to be very poor, but could be restored by adding either wildtype LPS or less toxic LPS of specifically constructed meningococcal lipid A mutants having five instead of six fatty acyl chains. Especially the penta-acylated *lpxL1* mutant displayed reduced toxicity as measured by cytokine induction, but normal adjuvant activity. In the case of *Bordetella pertussis*, whole-cell vaccines contain a different penta-acylated LPS which significantly contributes to reactogenicity. Mutants in the *lpxL* homologues of *B. pertussis* could not be isolated, presumably because they are not viable. However, additional possibilities for lipid A modification in this organism are provided by the *pagL* and *pagP* genes, which encode outer membrane enzymes capable of deacylation and acylation, respectively, of fully synthesized LPS. We have investigated the effect of these modifications on the biological activity and immunogenicity of both purified *B. pertussis* LPS and whole cells.

05.19

A comparative study on the immunotherapeutic efficacy of recombinant Semliki Forest virus and recombinant adenovirus

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Introduction: Viral vectors are being developed for immunotherapy of cancer and infectious diseases. We are developing an immunization strategy against Human Papillomavirus (HPV)-induced cervical cancer based on an alphavirus vector, i.e. Semliki Forest virus. In the present study we compare the efficacy of recombinant SFV (rSFV) with recombinant adenovirus (rAd).

Methods: Mice were immunized and boosted with rSFV expressing a fusion protein of the HPV proteins E6 and E7 (SFV-enhE6,7) or rAd encoding the same fusion product. Cytotoxic T cells precursors (pCTLs) induced upon immunization were determined with E7-specific MHC class I tetramers. CTL activity was measured by standard 51Cr-release assay. The therapeutic efficacy was determined in tumour treatment experiments. To unravel the observed differences between the vectors, T cell depletion, and gene expression experiments were conducted.

Results: Immunization with SFV-enhE6,7 resulted not only in 2-fold higher pCTL frequencies and significantly higher levels of CTL activity, but also in a significantly superior therapeutic effect requiring 100-1000-fold lower doses compared to Ad-enhE6,7 immunization. The difference in activity could not be ascribed to different effectors induced. Yet, while a priming immunization with rAd completely

abrogated gene expression of a booster injection, rSFV priming did not.

Conclusion:

1. The SFV vector system proved significantly more immunogenic than the Ad vector system.
2. Together with the high level of biosafety of the SFV vector and the absence of pre-existing neutralizing antibodies in humans against this virus, it seems justified to consider clinical evaluation of SFV-enhE6,7 in cervical cancer patients.

05.20

Influenza vaccines for pandemic preparedness; current developments and future opportunities

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Current influenza vaccines, for which the antigens are produced in embryonated chicken eggs, can result in a significant reduction of influenza-related morbidity and mortality. Nevertheless, there is a demand for vaccines with a higher efficacy and that can be produced more rapidly and flexibly in response to an epidemic or a pandemic. Current developments in the field of influenza vaccine preparation include novel vaccine production technologies, reverse genetics technology for the generation of vaccine strains, novel adjuvants for the improvement of vaccine immunogenicity and alternative routes of antigen administration. These current developments and future opportunities will be discussed in the context of the 2003 outbreak of H7N7 influenza virus in The Netherlands. During the outbreak 89 people were infected with this H7N7 virus and one veterinarian died as a result of the infection. A vaccine was developed to protect against H7N7 influenza A virus based on a low pathogenic, avian influenza A virus of the H7 subtype. The efficacy of a classical non-adjuvanted subunit vaccine and an ISCOM-adjuvanted vaccine was compared in a mouse model.

05.21

Intervention strategies against smallpox

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Following a worldwide vaccination program the World Health Organization (WHO) declared smallpox to be eradicated in May 1980. Soon thereafter, general vaccination against smallpox was discontinued. Variola virus, the etiological agent of smallpox, is now ranked high on the list of biological agents that may be used as a bioweapon because infection with this virus results in approximately 30% mortality and to date the vast majority of the population lacks protective immunity. In addition, there are growing concerns from the observation that other mammalian poxviruses, like cowpox virus and monkeypox virus (MPXV), may now cross the species barrier to humans more easily. Several countries are now stockpiling smallpox vaccine, but the use of classical smallpox vaccines is associated with serious adverse events and current plans do not envisage mass vaccination with traditional smallpox vaccines until after an outbreak has been detected. Efficacy testing of new intervention strategies in experimental animals, in comparison with the use of traditional smallpox vaccines, will form an essential part of the data required to register new intervention strategies against smallpox. To this end animal models that mimic the natural infection of variola virus in humans are particularly important. MPXV infection of macaques resembles smallpox and this model can be used for the evaluation of new candidate smallpox vaccines such as modified vaccinia virus Ankara (MVA; Stittelaar et al. J Virol 2005;79:7845-51). We directly compared the efficacy of MVA, alone and in combination with classical VV based vaccines. MVA-based smallpox vaccine protected macaques against lethal respiratory challenge with monkeypox virus and is therefore an important candidate for prophylaxis against smallpox. In another study in the same model we directly compared the efficacy of post exposure smallpox vaccination versus that of antiviral treatment with acyclic nucleotides in the same macaque-MPXV model (Stittelaar et al. Nature 2006;9 February). Antiviral treatment, initiated 24 hours after lethal intratracheal MPXV infection, with either cidofovir, (HPMPC, Vistide®) or with a related acyclic nucleoside phosphonate analogue, [HPMPO–DAPy], resulted in significantly reduced mortality and reduced numbers of cutaneous monkeypox lesions. In contrast, when macaques were vaccinated 24 hours after the same MPXV infection, with a standard human dose of the classical smallpox vaccine, no significant reduction of mortality was observed. These data show that adequate preparedness for a biological threat involving smallpox should include the possibility to treat exposed individuals with antiviral compounds such as cidofovir or other selective anti-poxvirus drugs.

05.23

Antimicrobial peptides: the magic bullets of innate immunity

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The innate immune system forms an effective barrier against pathogenic micro-organisms. Antimicrobial peptides (AMPs) are effector molecules of the innate immune system that provide protection against microbial challenge by their broad-spectrum antimicrobial activity against bacteria, fungi and viruses. Most AMPs are cationic peptides (3.5-5 kDa) that act by disrupting microbial membrane structures. In humans these peptides are mainly produced by neutrophils and epithelial cells. Recent studies have demonstrated that these peptides not only act as endogenous antibiotics, but also contribute to the regulation of immunity, inflammation and wound repair. Through this range of activities AMPs may use various mechanisms to protect the host from infections. Whether excessive production and/or uncontrolled activity of these peptides also contributes to inflammatory disorders is incompletely understood.

Our studies have focussed on the regulation of AMPs expression in the lung, and on the effect of two classes of human AMPs (defensins and cathelicidins) on human airway epithelial cells, smooth muscle cells and granulocytes. Based on our studies on the cathelicidin LL-37, these activities will be highlighted and the host receptors involved will be discussed. Finally the implications of these findings for the development of new antibiotic drugs for the treatment of infections will be discussed.

05.24

Autolysis products protect *Streptococcus pneumoniae* against cationic antimicrobial peptides

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Background: *Streptococcus pneumoniae* colonizing the nasopharynx may cause severe disease such as otitis media, pneumonia, meningitis and sepsis. By producting the CibAB 2-component bacteriocin, *S. pneumoniae* is considered to initiate LytA autolysin-mediated lysis of siblings (heterolysis). Heterolysis would yield DNA, nutrients, and release virulence factors like pneumolysin. We considered a novel biological function for pneumococcal heterolysis. The airway epithelium and immune cells recruited to infected sites produce cationic antimicrobial peptides. We hypothesized that heterolysis might serve to protect against such peptides. The capsule might also contribute to such protection. Methods. We tested the influence of lysates of *S. pneumoniae* D39, its isogenic capsule locus deletion mutant D39Δcps, strain RX-1 (an other capsule-deficient D39 derivative) and RX-1 lytA, on the activity of Human Neutrophil Peptide 1-3 (HNP) and of I29K, a novel synthetic microbicidal peptide, in microdilution assays. Standardized autolysates were prepared after 25h of incubation of pneumococci in 10 mM phosphate buffer.

Results: In absence of any autolysate, > 99.9% of D39 and D39Δcps were killed by 7.5 μM HNP within 120-240 min, indicating that capsule did not influence susceptibility. I29K killed > 99.9% of D39, D39Δcps and RX-1 at 15 μM, but Rx-1 lytA required 30 μM I29K. The autolysates of D39, D39Δcps and RX-1 fully inhibited cidal activity of the peptides at the above concentrations. Autolysate of RX-1 lytA reduced the activity of I29K and HNP to a lesser extent. Autolysates also inhibited peptide activity against Escherichia coli, indicating a scavenger function.

Conclusion: Non-capsule components released from pneumococci lysed at least in part in a LytA-dependent process, contributed to protection of non-lysed pneumococci against cationic antimicrobial peptides. This may be an important biological function for heterolysis of pneumococci.

05.26

Localization and antimicrobial activity of chicken gallinacin-6

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Introduction: Defensins, small cationic peptides with broad spectrum antimicrobial activity, are important effectors of innate immunity. Here we report the expression pattern and antimicrobial properties of chicken β-defensin, gallinacin-6 (Gal-6).

Methods: Tissue distribution of Gal-6 mRNA was investigated by RT-PCR. Analysis of the Gal-6 promoter region was performed with MatInspector and JASPAR software. Clustal W alignment of Gal-6 with avian and mammalian β-defensins was used to deduce the mature Gal-6 peptide sequence. Synthetic Gal-6 (sGal-6) was made by Fmoc chemistry with random disulfide bridge cyclization, refolded in tris buffer and purified by reverse phase HPLC. Recombinant Gal-6 (rGal-6) was expressed in HEK293 cells and subsequently purified by affinity chromatography. The antimicrobial properties of sGal-6 and rGal-6 against bacteria en yeasts were investigated by broth microdilution assays and kill-curve studies.

Results: High levels of Gal-6 mRNA were found in esophagus and crop tissue. Promoter analysis of the Gal-6 upstream region revealed putative transcription binding sites for nuclear factor kappa beta (NF-kB), activator protein-1 (AP-1), nuclear factor interleukin-6 (NF-IL6). Clustal W alignment of Gal-6 with other β-defensins indicated a 41 amino acid mature peptide. SGal-6 and rGal-6 were bactericidal against Gram-negative and Gram-positive bacteria, but fungistatic against yeasts. SGal-6 treatment of *Clostridium perfringens* at 1x and 4x the minimal inhibitory concentration (MIC) resulted in a 5 LOG unit decrease within 60 min, whereas a 0.5 LOG decrease was observed for MIC values of rGal-6.

Conclusions:

1) Gal-6 mRNA is highly expressed in crop tissue, independent of breed, but variably expressed in young animals.
2) Gal-6 may be regulated via NF-kB and AP-1 pathways.
3) Synthetic and recombinant Gal-6 are bactericidal and fungistatic.
4) Kill-curve studies indicate a fast killing mechanism for sGal-6 and a slow killing mechanism for rGal-6.

05.27

Salmonella typhimurium causes upregulation of porcine β-defensins in a porcine intestinal cell line

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Introduction: Beta-defensins are small antimicrobial peptides that are part of the innate immune system of animals and plants. In the intestine, they are produced and secreted by epithelial cells, and for some defensins, the expression can be induced upon a bacterial trigger. In this study a porcine Ileal epithelial cell line (IPI) was used to detect whether porcine defensins 1 and 2 (pBD1&2) are inducible upon bacterial infection.

Results: Baseline pBD expression increased in IPI cells during growth in DMEM medium/10% FCS. To optimize the infection experiments, cells were grown in synthetic medium in which the baseline pBD production remained constant over the time course of the experiment. *Salmonella typhimurium* infection of IPI cells resulted in elevated levels of pBD2 mRNA after 6 h and longer while PBD1 mRNA was elevated after 24 h. In addition, elevated levels of IL-8 were observed upon infection. Similar infections of IPI cells using *Staphylococcus aureus*, *Arcobacter* and *Salmonella enteritidis* had similar (but slightly lower) effects on secreted IL-8 levels. However, no effect on pBD expression was observed. These results suggest that the regulation of expression of porcine defensins by bacteria in IPI cells is species-dependent.

06.01

Microbial genomics for the food processing industry: novel possibilities for controlling _Bacillus_ spoilage

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Introduction: Microbial food spoilage is even nowadays a serious problem for food processing. Combating spoilage in most cases is based on a black box approach, mostly based on a worst case scenario. What is lacking from our point of view is a better understanding of the behavior of the spoilage micro organism under specific environmental conditions. Genomics technology now enables a novel approach for opening up this black box and tries to understand the behavior of spoilage micro-organisms. As an example the response of *Bacillus subtilis* cells towards sublethal and lethal heat exposure is studied in more detail.

Methods: Vegetative *B. subtilis* cells were exposed to sublethal and lethal temperatures. Metabolism was quenched immediately and RNA was isolated. Labeled RNA was hybridized to *B. subtilis* microarrays and obtained data were analyzed by Principal Component Analysis and other bioinformatics tools.

Results: Not surprisingly analysis of obtained data shows that *B. subtilis* cells strongly respond to exposure to (sub)lethal temperatures. This response however is shown to be different for even slightly differing temperatures. Even exposure to different lethal temperatures results in a specific responses at the gene expression level. Next to showing these results the up- and down regulation of specific metabolic processes under these conditions will be discussed.

Conclusions: Directly studying the response of spoilage micro-organisms on preservation treatments such as heating enables novel possibilities for preservation approaches. As an example a novel preservation strategy at lower temperatures might be possible based on the results presented here resulting in considerable energy savings and an improved food product quality. In the forthcoming years microbial genomics will result in a much better understanding of specific spoilage causing bacterial strains and thereby in improved possibilities for spoilage control.

06.02

Genomotyping: a novel genomics based approach for controlling _Bacillus_ spoilage

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Introduction: Microbial food spoilage is even nowadays a serious problem for food processing. Historically, specific species of spoilage organisms were indicated as the source

of this spoilage without paying attention to differences in spoilage potential between individual strains. Detailed characterization of the strains causing spoilage was and is often difficult or impossible due to a lack of methods enabling sufficient resolution to distinguish between closely related strains. This research was focused on developing a novel genomics based approach called genomotyping enabling very detailed and specific discrimination between closely related strains of spoilage organisms.

Methods: *Bacillus* spoilage organisms were studied by using a Smart Man Genomics approach. Genomic DNA of relevant strains of *Bacillus* species (*Bacillus subtilis*, *Bacillus coagulans*, *Bacillus pumilis*, *Bacillus licheniformis*, *Bacillus sporothermodurans* and *Bacillus cereus*) was used to construct genomic libraries which were spotted on microarrays. Genomic DNA of about 50 *Bacillus* strains was hybridized to these microarrays containing over 6000 spots and obtained hybridization results were analyzed.

Results: Analysis of obtained data resulted in selections of biomarkers (DNA fragments) enabling the following distinctions: 1) Unique markers for specific strains within one species: 2) Markers specific for one species; 3) Markers representing multiple but not all species; 4) Markers present in all species studied. DNA sequences of representative markers of all these groups have been determined and will be presented. Also data on analysis of novel strains will be presented.

Conclusions: Genomotyping enables both discrimination between closely related strains of one species and multiple species in one analysis. When based on Smart Man Genomics this is a generic, flexible and cost efficient approach for studying microbial spoilage. In the forthcoming years genomotyping may result in a much better understanding of specific spoilage causing bacterial strains and thereby in improved possibilities for spoilage control.

06.03

Bacterial spores in food processing; molecular detection, identification and process survival analysis

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In the food processing industry extremely heat-resistant bacterial endospores create problems due to their ability to survive classical food preservation treatments (see among others: Kort et al. AEM 2005:71:3556-64). The spores are able to subsequently germinate and form actively growing vegetative cells that spoil foods and cause disease. With genomic library micro-array hybridisation we identified a number of unique genome fragements that correlated with specific strains and their thermal stress resistance. These genome fragments were converted into probes for the Check-Points DNA-chip platform in order to perform spore detection assays in real foods and food ingredients. In addition, we showed with the sequenced Bacillus subtilis laboratory strain that sporulation in the presence of a cocktail of calcium, magnesium, iron, manganese and potassium promotes thermal resistance of developing spores. This observation correlated with an increased expression during sporulation of genes encoding small acid soluble spore proteins known to be important in the protection of spores against a variety of stresses (see Oomes and Brul. IFSET 2004;5:307-16; O'Brien et al., 2006, proteomics manuscript in preparation). Degradation patterns of specific molecular markers upon spore thermal injury were found to be predictive for the outgrowth behaviour of B. subtilis spores. This finding was quantitatively measured and patented (Keijser, 2005). Furthermore, the molecular program that forms the basis of spore germination has been analysed using genome-wide expression analysis. Here we observed that genes involved in DNA-repair were transiently expressed in germinating wild-type spores (Keijser et al., 2005, paper in preparation). Of the genes that were specifically expressed during spore germination individual knock-out mutants were generated. Some of these mutants showed a significantly delayed outgrowth but none were completely perturbed in germination and outgrowth. Implications of this for the inhibition of germination-specific damage repair processes through mild combination preservation techniques are discussed.

06.05

Spore germination of thermally injured *Bacillus subtilis* spores

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Over the years, the food industry has developed safe food preservation methods to ensure healthy, safe and tasty food products. Gradually, consumers demands have changed to fresh-like foods were a minimum level of processing has been applied to maintain positive aspects of fresh food without compromising food safety. Unfortunately, mild processing provides opportunities for pathogenic and spoilage organisms, in particular spore forming organisms as the spores are

generally not destroyed by mild food processing. Once present in the food, germination and outgrowth of the spore may result into toxin producing vegetative cells. As better understanding of spore germination may provide new tools to the industry in the battle against spores, we have considered this process in more detail. Germination and outgrowth of *Bacillus subtilis* 168 spores was studied in detail by transcriptional analysis during germination. This analysis revealed successive genetic processes occurring during spore germination and outgrowth. Identified processes include re-initiation of metabolism, active spore repair, initiation of DNA replication, chromosomal segregation followed by cell growth and septum formation. Furthermore, germination of spores surviving a thermal (food processing) treatment was studied and germination and outgrowth diverges from spores germinated under ambient conditions. Most striking is the increase in lag time before the spore is able to resume growth. Within this prolonged time frame, the injured spore is apparently able to overcome the damage caused by the thermal treatment, indicating that perhaps spore repair mechanisms may play a role in this. Moreover, heat injured spores were shown to be more sensitive towards environmental stress conditions such as high salt concentration or aberrant pH values. Finally, this knowledge can provide the food industry with crucial information concerning the development of innovative food preservation techniques.

06.06

Multi analyte molecular detection of food pathogens and spoilers

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Food and ingredients may be contaminated with a number of pathogenic and spoilage micro-organisms. Until recently, such micro-organisms could only be detected using traditional microbiological methods. The disadvantage of these methods is that they are laborious and time consuming, generally taking several days before the test result is known. In addition, for each micro-organism a separate test is required. Often the food products are already delivered to the customer when the test results become known, requiring costly recall actions in case pathogens or spoilage organisms are detected.

Nowadays traditional microbiological test methods become more and more replaced by molecular detection methods, such as PCR or real-time PCR. The advantage of these methods is that they are fast and sensitive, however, like in traditional microbiology a separate test for each micro-organism is still required. Check-Points has developed a new detection system enabling multiplex detection of many micro-organisms in a single test. This new concept combines a proprietary multiplex amplification method with detection on a diagnostic microarray platform. Several examples will be presented showing detection of a range of pathogens and spoilers, and demonstrating the power of this new system.

06.07

Detection and identification of foodborne pathogens by molecular methods

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Introduction: Various techniques aiming at the morphological, biochemical or immunological properties of the organism are available to detect and/or identify foodborne pathogens. Nowadays however, molecular detection and identification methods are becoming increasingly popular. In our laboratory we are using various PCR assays for the detection of foodborne pathogens and recently we have developed a multiplex PCR for the detection and identification of *Salmonella paratyphi-B* variant Java. Furthermore, we have developed and implemented a micro-array platform (van Hoek et al. 2005) for the detection of antibiotic resistance (AR) genes.

Methods: A multiplex PCR was developed based on: a primerset specific for *Salmonella* (Aabo et al., 1992): a primerset able to discriminate between tartrate positive and negative strains (Malorny et al., 2003) and a primerset specific for paratyphi B. In total 232 *Salmonella* strains belonging to various serotypes were analysed. Approximately 200 *Salmonella* strains and 200 Lactic Acid Bacteria strains (non pathogenic bacteria also associated with food) were analysed by microarray analysis according to van Hoek et al. (2005).

Results: 71 out of 72 *Salmonella paratyphi-B* variant Java strains and 14 out of 15 *Salmonella paratyphi-B* strains were identified correctly. All other *Salmonella* serotypes were distinguishable from the paratyphi-B strains with the exception of strains belonging to *Salmonella abony*, *Salmonella wagenia*, *Salmonella* I 4,12:b:- and *Salmonella* I 4,(5),12:b:. The microarray data concerning the presence or absence of AR genes were mostly but not always in concordance with the available phenotypic data. Occasionally genes were detected that had never been described for the type of strain under investigation.

Conclusions: 1) The developed multiplex test is a good alternative to the laborious tartrate test necessary to distinguish between *Salmonella paratyphi-B* and *Salmonella paratyphi-B* variant Java. 2) The developed microarray is suitable for the detection of AR genes both in *Salmonella* and Lactic Acid Bacteria.

06.08

Fungal spores as survival capsules in time and space

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The variety of fungal spores is bewildering, but there main function is distribution. They can serve for propagation of the fungus from adverse locations towards better conditions. These spores often are produced in large numbers and are transported through air, water or by the action of living organisms. Spores also can serve for dispersion in 'time' by literally waiting for better times. These spores often have thick cell walls and are not dispersed. These spores show constitutive dormancy, that is a metabolic block that is released only after special triggers. Further, these spores can be highly resistant to many stressors and exhibit different very specialised features during dormancy and germination as in case of the fungus *Talaromyces macrosporus*. Communication between spores or spore compartments (in case of multi-cellular spores) may serve a fine-tuning of the rather stochastic process of distribution. Examples of such processes are discussed with the fungi *Penicillium paneum* and *Fusarium culmorum*. The apparatus of spore dispersal is highly specialised and may be prone to quick devaluation when not extensively used, as is discussed with the fungus *Rhizopus oligosporus*.

06.09

Global regulation of survival strategies of the bacterial spore former *Bacillus cereus*

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Bacillus cereus is a common cause of food-borne disease that thrives in many different ecological niches. For the control of this pathogen, it is especially relevant to know which mechanisms it can utilize to sustain growth in the many environments that it can inhabit. We aimed to assess global regulation in *B. cereus* highlighting the role of a range of sigma factors, including the general stress sigma factor σ^B, the early sporulation sigma factor σ^H, and a number of selected extra-cytoplasmic-function (ECF) sigma factors, and the catabolite control protein CcpA in the performance of *B. cereus* under various growth and stress conditions, relevant in the processing and preservation of foods.
Using *B. cereus* ATCC 14579 and targeted sigma factor- and *ccpA*-deletion mutants, the impact of these regulators and their regulons on *B. cereus* growth performance, stress response, sporulation efficiency and surface behaviour, including swarming and biofilm formation, were assessed. In addition, proteomics and gene profiling, employing *B. cereus* whole genome ORF-based micro-arrays, are used to further identify key elements in *B. cereus* eco-physiology and virulence that may affect its performance and survival in industrial settings. This approach showed an involvement of σ^B in stress response, roles for σ^H and an ECF sigma factor in sporulation and biofilm formation, and regulatory roles of CcpA in key metabolic pathways, biofilm formation, and sporulation.

06.10

Mode-of-action of high pressure low temperature induced damage to *Bacillus subtilis* in the IceI-IceII domain

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The damages on *Bacillus subtilis* vegetative cells induced by subzero temperatures and pressures up to 250MPa in buffer solution (i.e. in the area of IceI-IceIII phase transitions) was studied by means of flowcytometry in combination with membrane permeability and viability probes: PI (propidium iodide) and cFDA (carboxyfluorescein diacetate). The growth of single cells was traced by measuring the optical density and light scatter of the growth medium. Bacterial cells showed high heterogeneity in stress resistance to the treatment. Treated cells displayed a distribution into four populations characteristically by green (cFDA) and red (PI) fluorescent intensity: high green/low red; high green/high red; low green/high red; and low green/low red. Single cells from C in TSB (trypcase soy broth). Each population were sorted and incubated at 25 Very few cells from the high red populations were found to grow after 50 days. A number of wells gated from the low red populations showed positive growth after 2.5-20 days, while the lag time of untreated cells was only around 0.7 day under the same growth condition. The lag time of the cells treated with different conditions does not differ significantly. Untreated cells sporulate immediately after reaching the maximum growth, which is less than 2 days' incubation. Finally, on the one hand, most cells treated by either freezing or HPLT generally show slower growth rate. These cells did not sporulate even 25 days after the onset of growth. On the other hand, cells sorted both from populations of high green/low red and low green/low red resulted in a similar number of positive wells and lag time.
Conclusions: 1) Plasma membrane damage seemed to be the first mode-of-action of HPLT on the bacteria. 2)

Damage mechanisms other than membrane integrity and intracellular enzyme (esterase) activity exist, which caused lethal damages to the cell. These results call for further study on the mechanisms of HPLT induced damages to the bacterial vegetative cells.

06.11

Transport of mRNA and proteins from a fungal mycelium to spore-forming aerial structures?

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Filamentous fungi form mycelia consisting of hyphae that extend at their apices while branching subapically. The cytoplasm of these mycelia is continuous. Previously, we have shown that cytosolic GFP streams through the vegetative mycelium of *Aspergillus niger*. We here assessed whether GFP also streams from the vegetative mycelium into aerial structures of this fungus. *A. niger* was transformed with constructs encompassing *GFP* with or without a nuclear targetting signal and expressed from the glucoamylase (*glaA*) promoter or that of the glyceraldehyde triphosphate dehydrogenase (*gpdA*) gene. Nuclei of the vegetative mycelium of transformants expressing the nuclear targetted reporter gene from either promoter were highly fluorescent when colonies were grown on maltose. In contrast, nuclei of the spore producing conidiophores were only weakly fluorescent. Yet, conidiophores do have the capacity to produce nuclear targetted GFP as was shown using the mannitol dehydrogenase (*mtdA*) promoter. Both the vegetative mycelium and the aerial structures were fluorescent when the cytosolic version of the reporter was expressed from the *gpdA* or *glaA* promoter. Fluorescence was restricted to the aerial structures in case of the *mtdA* promoter. We can thus conclude that *glaA* and *gpdA* are only lowly expressed in the aerial structures. However, cytosolic GFP that results from the activity of the promoters of these genes in the vegetative mycelium are transported into the aerial structure. It is thus tempting to speculate that a wide variety of cytosolic proteins in conidiophores are imported from the vegetative mycelium rather than being expressed in this aerial structure.

06.12

Diversity of phototrophic bacteria in microbial mats in Arctic hot springs (Greenland)

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We investigated the genotypic diversity of oxygenic and anoxygenic phototrophic microorganisms in microbial mat samples collected from hot springs at three different localities on the east coast of Greenland. These hot spring microbial mats form unique Arctic ecosystems that have never been studied in detail before. Specific oligonucleotide primers for cyanobacteria, purple sulfur bacteria, green sulfur bacteria, and Choroflexus-like green non-sulfur bacteria were used for the selective PCR amplification of 16S rRNA gene fragments. Amplification products were separated by denaturing gradient gel electrophoresis (DGGE) and sequenced. In addition, several cyanobacteria were isolated from the mat samples, and classified morphologically and by 16S rRNA-based methods. The cyanobacterial 16S rRNA sequences obtained from the denaturing gradient gels represented a diverse, polyphyletic collection of cyanobacteria. Ribosomal RNA gene sequences obtained from the cyanobacterial isolates showed high similarity to some DGGE derived sequences. Our results show that the cyanobacterial community composition in the samples was different for each sampling site. Different layers of the heterogeneous mats often contained distinct and different communities of cyanobacteria. We observed a relationship between the cyanobacterial community composition and the insitu temperature of different mat parts. A remarkable low diversity was observed among the green and purple sulfur bacteria and the Chloroflexus type bacteria as compared to the diversity of these bacteria in similar mat ecosystems. The low diversity of anoxygenic phototrophs is possibly related to the photochemical conditions within the mats resulting from the Arctic light regime.

06.13

Diversity of sulfate reducing bacteria in soda lakes

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Soda lakes are naturally occurring highly alkaline and saline environments. The pH is kept constant at a pH of up to 10.5 by the high buffering capacity of sodium carbonate/bicarbonate, which are the major anions in solution. Soda lakes are ubiquitous in locations with an arid climate and the right geochemical conditions. Well known soda lakes are located in California (USA), Kenya, Egypt and Central Asia. Although the sulfur cycle is one of the most active element cycles in soda lakes, little is known about the sulfate reducing bacteria (SRB) in this habitat.
In this study we investigate the SRB diversity in 11 sediment samples from soda lakes of the Kulunda Steppe in South-East Siberia. For this purpose denaturing gradient gel electrophoresis (DGGE) of the *dsr* (dissimilatory sulfite reductase) gene, encoding the key enzyme in the sulfate

reduction, was applied. The DGGE profiles showed a relatively high SRB diversity and similar profiles for lakes with similar salinity and pH. Individual DGGE bands were sequenced and phylogenetic analysis was performed. Firstly, the closest relatives were determined using the BLAST search tool, subsequently the sequences were imported into the ARB software program, manually aligned and added into an existing tree. All our sequences grouped with those from Deltaproteobacteria and in particular with sequences of *Desulfonatronum hydrogenovorans* and *Desulfonatronum lacustre*, two low salt-tolerant alkaliphilic sulfate reducers isolated from soda lakes.
These results give for the first time insight in an important functional group of microorganisms in soda lakes, which is essential for a better understanding of the sulfur cycle in these ecosystems.

06.14

Metabolic engineering of folate biosynthesis in *Lactobacillus plantarum*

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Many plants and micro-organisms have the ability to synthesise folate, whereas animals lack this ability. Folate is an essential cofactor for the synthesis of DNA, RNA and certain amino acids. Folate-deficiency in humans is often associated with anaemia, neural tube defects and cardiovascular diseases. A number of folate related deficiencies in humans can be overcome by uplifting the folate consumption levels. One way of doing this is to produce fermented food products with elevated folate levels. As an alternative for traditional fortification, this could be achieved by using folate (over)producing lactic acid bacteria in the fermentation process.
Here we present a metabolic engineering strategy that yields a *Lactobacillus plantarum* strain producing elevated folate pools. The genes involved in folate biosynthesis in *L. plantarum* were identified and cloned on a vector and subsequently transferred to the host strain *L. plantarum* WCFS1. The transformed *L. plantarum* strains were found to produce high folate levels (10 mg/l) upon the supplementation of *para*-aminobenzoic acid (a building block in the folate biosynthesis). In the folate overproducing strain the transcriptome and metabolome were compared to that of the wild type using DNA-microarrays and deferential LC-MS, respectively. The transcriptome and metabolome data will subsequently be projected on metabolic pathway maps specifically designed for *L. plantarum* WCFS1. The transcription profiling coupled with the metabolic map will facilitate the formulation of improved metabolic engineering strategies to further increase folate levels in the host strain. In addition, this approach will lead to a better understanding of regulatory aspects of folate biosynthesis in *L. plantarum*.

06.15

Measuring yeasts intracellular pH upon sorbic acid stress *in vivo*

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Sorbic acid, a weak organic, is the most widespread food preservative used in the industry. Yeast and other fungi are, to a certain extent, able adapt to this acid and resume growth in the presence of the highest concentrations allowed in foods. This can result in product spoilage and thus create substantial economic losses. Quite a lot is known with respect to the end point of the yeasts' response to sorbic acid stress, i.e. when growth is resumed, from genome-wide transcript analyses and studies with yeast knockout mutants. However currently we still do not know why the cells initially arrest growth upon the weak-acid challenge. Also the molecular physiological events that occur during the adaptation phase and finally lead to a resumption of growth are poorly understood. Thus, to understand the mechanisms of growth limitation and adaptation we perform time-resolved studies of yeast cells exposed to sorbic acid in an integrated way. That is, we perform analyses at the level of gene expression, protein composition, and cellular metabolism. By calculating energy generating capacity, we try to map the cost and benefit of the various aspects of the stress response towards weak acids. In practice this means we determine metabolic fluxes, ATP/ADP ratios and ultimately try to construct a mathematical model of the response to the stress. Currently we are working on an experiment to measure intracellular pH upon sorbate stress *in vivo*. We use a pH sensitive GFP originally developed in mammals called a 'pHluorin' (Miesenböck et al., 1998). This GFP has been constructed in such a way that it can monitor the pH of its environment without disturbing cell function. We targeted this GFP to different compartments of the cell using different targeting sequences. The first results show that the system works in yeast and online pH measurements without applying a stress are possible.

06.16

Modeling the response of yeast glycolysis to temperature changes

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Organisms are able to respond to their environment to maintain homeostasis. A well-studied environmental parameter is temperature variation, which exerts a complex combination of effects on the cell. We have studied the relation of growth rate and temperature of *Saccharomyces cerevisiae* under well-defined conditions and focused on the effect on glycolysis. We selected a temperature from the supra-optimal side of the temperature growth rate graph and shifted a culture from the reference temperature to a higher temperature (38°C) to examine quantitatively the effects on glycolytic flux during adaptation. We observed a sevenfold increase in glycolytic flux. We want to find out how this flux increment is regulated; is it regulated metabolically, through temperature effects on the glycolytic enzyme reaction rates, through variations in metabolite concentrations, or is it regulated hierarchically, at the level of mRNA levels, protein levels, through the modulation of the expression alternative iso-enzymes. We will use regulation analysis to determine this for all enzymes in the glycolytic pathway. The principles of this approach are outlined in the current poster.

06.20

A systems biology model for the adaptation of *S. cerevisiae* to heat stress

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The physiological response of microorganisms to changes in their environment can be regarded as the net resultant of the induced events at the genetic, enzymatic and metabolic level. Therefore, a full understanding of the cell's response demands a quantitative integration of these events. Here, we will present an analysis of the responses of *Saccharomyces cerevisiae* to heat stress at the enzymatic and metabolic level. Experimentally, it was shown that an increase in temperature of the growth medium from 28°C to 37, 39, 40, 41, 42, or 43°C resulted initially in an increased specific uptake rate of glucose and a redistribution of catabolic and anabolic fluxes. Further, it was accompanied by a net synthesis of intracellular trehalose, ATP, ADP and AMP and for all temperature shifts above 37°C, a lag phase in the growth rate was observed, followed by a lower, pseudo-steady state growth rate. During this lag period the most dramatic changes with respect to glucose consumption and trehalose synthesis occurred. In addition, these alterations in catabolism coincided with activation of the pkc1 pathway (as measured by the degree of phosphorylation of slt2p). It was demonstrated that activation of this socalled cell integrity pathway is triggered by a change in the relative osmolarity due to trehalose accumulation.[1] Subsequently, a mathematical model was constructed that is based on known enzyme kinetics of (clustered) major steps in metabolism (including temperature dependence), on the need for energy conservation (ATP synthesis) and consumption (ATP hydrolysis) by the cell, and finally on cell growth itself and temperature-dependent protein turnover upon a temperature upshift. Thus, the model describes the events that take place upon perturbation of steady state growth by heat stress. The model takes into account the bioenergetic demands (maintenance energy) that accompany such perturbation as well as energy consuming processes that are involved in cell growth and protein turnover that is specific for proliferation at increased temperatures. Less pronounced branches like trehalose accumulation are considered as well. It will be shown that specific anabolic and catabolic rates as predicted by the model fit well the experimental data described above.

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06.21

The logic of growth

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It can be reasoned that with sufficient supply of nutrients optimization of growth rate is an important driving force in the evolution of microorganisms. The properties of such organisms may thus be predicted to a certain degree with optimization techniques. We use very simple models of cell growth to show that observed growth-rate dependent phenomena in microorganisms are reproduced when maximizing the growth rate in these models. For example, it is shown that at high growth rates the ribosomal content of cells should increase proportionally with the growth rate, as is observed experimentally. Moreover, at low growth rate the optimal regulation of the ribosomal content deviates from linearity. This deviation was also observed experimentally and was interpreted as sub-optimal behaviour

with the purpose of supplying a surplus of ribosomes in an environment with fluctuating nutrient concentrations, enabling cells to start growing quickly when nutrient concentrations increase. In contrast, our models suggest that the experimentally observed regulation is optimal with respect to growth rate.
The second phenomenon we studied was the use of metabolically efficient and inefficient pathways in micro-organisms. When given the choice of two alternative metabolic pathways, one with high metabolic efficiency but using many enzymes, and one with low metabolic efficiency but using less enzymes, our models predict that it is optimal to use the metabolically efficient pathway at low growth rates and the inefficient pathway at high growth rates. Such behaviour is observed, for example, in lactic acid bacteria that use mixed acid fermentation at low growth rates and homolactic fermentation at high growth rates, or in yeast that start producing alcohol aerobically at high growth rates.
Our models are characterized by a highly simplified description of a complete growing system and include some known physical and biochemical constraints. We show that such simple models can help in understanding growth-rate dependent regulation. They may be used as a starting point for more detailed descriptions in systems biology.

06.22

Unravelling the complexity of flux regulation

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An important question is to what extent metabolic fluxes are regulated by gene-expression or by metabolic regulation. There are two distinct aspects to this question: (i) the local regulation of the fluxes through the individual steps in the pathway, and (ii) the influence of such local regulation upon the pathway's flux. We developed regulation analysis so as to address the former aspect, for all steps in a pathway. We demonstrate the new method for the issue of how *Saccharomyces cerevisiae* regulates the fluxes through its individual glycolytic and fermentative enzymes when confronted with nutrient starvation. Regulation was dissected quantitatively into: (i) changes in maximum enzyme activity (V_{max} – called hierarchical regulation), and (ii) changes in the interaction of the enzyme with the rest of metabolism (called metabolic regulation). Within a single pathway, the regulation of the fluxes through individual steps varied from fully hierarchical to exclusively metabolic. Existing paradigms of flux regulation (such as single- and multi-site modulation, and exclusively metabolic regulation) were tested for the first time for a complete pathway, and falsified for a major pathway in an important model organism. We propose a subtler mechanism of flux regulation, with different roles for different enzymes, i.e. 'leader', 'follower' or 'conservative', the latter attempting to hold back the change in flux. This study makes the subtlety, so typical for biological systems, tractable experimentally, and invites reformulation of the questions concerning the drives and constraints governing metabolic flux regulation.

07.01

In vitro susceptibility of Biofilm growing _Staphylococcus aureus_ bovine mastitis isolates

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Biofilm growing *Staphylococcus aureus* are related to chronic infections in human medicine since the 1990s, while in bovine mastitis *S. aureus* is well known for its therapy resistance in chronic infections. Biofilms are a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface. Several *in vitro* studies have shown that bacteria growing in biofim can become 10-1000 times more resistant to the effects of antimicrobial agents compared to planktonic, free floating bacteria of the same strain. Current antibiotic susceptibility testing according to CLSI guidelines show that, in general, *S. aureus* mastitis isolates have good susceptibility for most therapeutic agents, however therapy results, especially in more chronic cases, are often disappointing.
Objective of this research was to test susceptibility of several *S. aureus* mastitis strains growing in Biofilm, for a panel of 10 antimicrobials in cation adjusted Mueller-Hinton broth and milk, in order to compare these results with outcomes of clinical trials.
Results show that several several *S. aureus* therapeutics have very poor efficacy against Biofilm growing bacteria, despite their good efficacy against planktonic bacteria.

07.02

Growth condition dependent esp expression and Biofilm formation of _Enterococcus faecium_ (Efm)

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Background: Hospital outbreaks of vancomycin-resistant Efm are primarily caused by strains belonging to MLST-based clonal complex 17 (CC17), which is characterized by the presence the Efm variant of enterococcal surface protein (Esp). Esp of *Enterococcus faecalis* (homologous up to 90% with Efm Esp) is involved in initial adherence and biofilm formation. To explore the role of Esp in the pathogenesis of Efm, Esp expression was studied under different growth conditions and the role of cell surface associated Esp in primary attachment and biofilm formation was assessed.

Methods: Esp-expression of 7 Efm strains (6 esp+, 1 esp-) grown at 37°C and 21°C and under aerobic and anaerobic conditions was studied by Western blot using polyclonal antibodies raised against recombinant N-terminal domain of Esp (rN-Esp). Cell surface localisation of Esp was assayed by flowcytometry using the same polyclonal antibodies. Primary attachment to polystyrene and biofilm development was determined.
Results: Esp-expression measured by western blotting differed substantially among strains and was increased at 37°C relative to 21°C under aerobic growth conditions. Under anaerobic conditions at 37°C, Esp expression was further elevated in 3/6 Esp+ strains. Using flowcytometry Esp appeared to be expressed on the cell surface with highest expression in cells grown anaerobically at 37°C. The amount of surface Esp-expression correlated with initial adherence to polystyrene (R2=0.7146) and biofilm formation (R2=0.7535). Polystyrene adherence was competitively inhibited by soluble rN-Esp.
Conclusions: Esp-expression on the surface of Efm is (a) heterogeneous, (b) growth condition dependent and (c) quantitatively correlated with initial adherence and biofilm formation. These data indicate that Efm is able to sense and respond to changing environmental conditions which might play an important role in the pathogenesis of this bacterium and in host-specific adaptation.

08.01
Stand van zaken sectie onderwijs
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Binnen de sectie Onderwijs zijn werkgroepen samengesteld rond de thema's Voeding, Milieu en Gezondheid met als doel de onderwijsactiviteiten en onderwijsmethoden op het terrein van de microbiologie in Nederland in kaart te brengen. De acties tot nu toe en de plannen voor de nabije toekomst worden uiteengezet. Daarbij zal onder andere ter sprake komen: i) de voortgang van de activiteiten in de werkgroepen en ii) de opzet van een website voor alle onderwijsinformatie.

08.02
Rondom het Middelbaar Laboratorium Onderwijs
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Het Middelbaar laboratoriumonderwijs (MLO) wordt beïnvloed door externe factoren. Het is de taak van de ROC's om in samenspraak met de laboratoria goede analisten af te leveren. Het MLO is afhankelijk van de toestroom, voornamelijk VMBO-leerlingen. In het VMBO is de laatste jaren veel veranderd. Hoe is de aansluiting VMBO-MLO wat betreft profielen en inhoud van de exacte vakken. En hoe gaan MLO's om met de VMBO-leerlingen van tegenwoordig?
Daarnaast komt er in opdracht van het ministerie van OCW een competentiegerichte kwalificatiestructuur in plaats van eindtermen. Hierbij zijn de leer- en burgerschapscompetenties geïntegreerd met de beroepscompetenties zodat er een evenwichtige opleiding ontstaat zodat een afgestudeerde MLO-analist gekwalificeerd is voor een beroep, voor de doorstroom naar het HBO en voor de maatschappelijke inpassing.

08.03
Microbiologische practica voor middelbare scholieren
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De VWO-campus van de Wageningen Universiteit streeft ernaar om universitaire kennis en materialen beschikbaar te stellen aan de tweede fase van het VWO. Daartoe organiseert VWO-campus uiteenlopende activiteiten, waaronder microbiologische experimenten voor leerlingen van 4, 5 of 6 VWO. Veel van deze experimenten zijn geschikt om op de middelbare school uit te voeren. De experimenten zijn in een leuk en modern jasje gestoken. Wat vinden leerlingen daar nu eigenlijk van, om bijvoorbeeld bezig te zijn met schimmels tijdens het practicum 'Op heterdaad betrapt!'?

09.01
Moleculaire typering van hepatis-B-virus in Nederland: toy & tool
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De laatste jaren staat in Nederland de moleculaire typering van hepatis-B-stammen (HBV) in de belangstelling.[1-3] De drie genoemde studies leveren interessante inzichten op: elke risicogroep heeft zijn eigen HBV-subtype; een 'Jan Modaal HBV-stam' bestaat niet; en HBV-stammen aangetroffen bij de verondersteld 'risico-vrije' bloeddonors blijken afkomstig uit alle risicogroepen. De vraag is echter of de studies het antwoord op de voorafgestelde vragen leverden. De doelstelling van de drie studies was respectievelijk:
- Inzicht verwerven in verspreiding van HBV in verschillende risicogroepen.
- Vanwege gemiste HBV-infectie: hoe variabel is HBsAg onder geïnfecteerde donors? Reduceert het Nederlandse risicogroepenbeleid het voorkomen van HBV onder low-risk, ongevaccineerde personen zoals bloeddonors?
- Meer inzicht verkrijgen in effectiviteit van de vaccinatiecampagne bij risicogroepen.

De eerste twee studies concluderen, nogal somber, respectievelijk:
1) The analysis indicates that the Dutch prevention strategy fails to stop transmission of hepatitis B from persistently infected individuals originating from hepatitis B endemic countries. 2) It must be realised that, after 20 years of vaccination of at-risk groups, HBV still circulates in the at-risk groups and Dutch blood donors acquire the HBV strains involved.
Kan de vorderende, landelijke typering van acute HBV-isolaten [3] nuttig licht op deze zaak werpen? Of is deze exercitie symptomatisch voor een steeds ingewikkelder, mogelijk inefficiënt risicogroepenbeleid?

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09.02

Tracking hepatitis A virus within and among risk groups

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In the Netherlands the incidence of infection with hepatitis A virus (HAV) is decreasing due to high hygienic standards and vaccination of targeted risk groups. As a consequence the susceptibility to HAV is increasing with a potential of larger outbreaks and higher morbidity among adults. In Amsterdam, we identified an association of groups at risk for HAV infection and (sub)genotypes of the virus. Travel to Morocco resulted mainly in infections of the 1B genotype, whereas among men having sex with men (MSM) exclusively genotype 1A was found.[1] During four years, with the cooperation of 8 GGDs, we established a national HAV database containing epidemiological data combined with phylogenetic analysis. It reinforced our findings that the correlation risk to genotype was valid all over the Netherlands.[2,3] Moreover, in other European countries the same MSM strains were identified during outbreaks, suggesting endemic transmission among MSM. Travel associated introductions generally appeared in small clusters, disappearing soon after introduction. This suggests that travel related HAV introductions are effectively recognized and stopped.
Among drug users a major outbreak occurred in Rotterdam in spring 2004. With molecular epidemiology all cases with the genotype 3A outbreak strain could be distinguished from sporadic cases with other genotypes. This was helpful to assess the efficacy of the mass vaccination campaign.[4] For public health purposes it is of major importance to know the period of infectivity. We therefore followed 27 acute HAV patients for 26 weeks, measuring viral load in blood and faeces. We found a significant correlation of HAV and ALT levels in serum. However, the acute load in faeces was not related to genotype, nor to duration of excretion. High loads in faeces were found for 81 days, suggesting that transmission may still occur when adhering to the current policy of 2 weeks of infectiousness after symptoms.
HAV typing is of use to aid establishig epidemiological links. Thus the national HAV database should be kept updated.

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09.03

Moleculaire epidemiologie en tuberculosebestrijding, toy and tool

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Achtergrond: In 1994 ging de *Mycobacterium tuberculosis* DNA en resistentiesurveillance van start, een co-project van KNCV Tuberculosefonds en het Rijksinstituut voor Volksgezondheid en Milieu (RIVM) in samenwerking met de GGD'en. Het doel is de transmissie van de *M. tuberculosis*-stammen in kaart te brengen en daarmee inzicht te krijgen in de verspreiding van de verschillende stammen onder de bevolkingsgroepen in Nederland; het traceren van bronnen en hun contacten en van onvermoede latente tuberculoseinfecties (LTBI).
Methode: Nadat op het RIVM onderscheid is gemaakt tussen materiaal van patiënten met een uniek (d.w.z. niet eerder gevonden) DNA-fingerprintpatroon en 100% identieke patronen (de zogenaamde clusters), meldt de verpleegkundig consulent surveillance de geclusterde patiënten aan de afdeling tuberculosebestrijding van de GGD'en. De sociaalverpleegkundigen trachten vervolgens epidemiologische verbanden aan te tonen samen met de sociaalverpleegkundigen van GGD'en waar andere patiënten in de clusters zijn gevonden aan de hand van de sociale anamnese van de patiënt(en). Over iedere geclusterde patiënt worden de bevindingen

teruggerapporteerd middels een enquêteformulier. Met de aldus verkregen informatie wordt inzicht verkregen in het optreden van transmissie en daarmee de bruikbaarheid van de DNA-fingerprintsurveillance voor het contactonderzoek en de tuberculosebestrijding en -bewaking. Daarnaast draagt de DNA-surveillance in belangrijke mate bij aan het traceren van (laboratorium-) kruiscontaminaties.

Resultaten: 11 jaar DNA-fingerprintsurveillance hebben geleid tot meer inzicht in de transmissie van de verschillende *M. tuberculosis*-stammen in Nederland. Epidemiologische verbanden, verdeling van epi-verbanden naar nationaliteit, nosocomiale transmissie en regio-overschrijdende clusters, geven informatie over transmissiepatronen van tuberculose binnen risico- en bevolkingsgroepen in Nederland.Zowel lokaal als regionaal zijn epidemiologische verbanden gevonden die niet eerder werden vermoed. Dit is van groot belang o.a. bij het aantonen van transmissie bij moeilijk bereikbare groepen en evaluatie van de effectiviteit van de screening van risicogroepen. Bij een afnemende incidentie van tuberculose in Nederland zal TBC zich steeds meer terugtrekken in risicogroepen voor tuberculose, zoals drugsverslaafden, daklozen en illegalen. Deze groepen zijn moeilijk bereikbaar en contacten zijn lastig te identificeren. Hoewel tot nu toe nog beheersbaar is ook het bewaken van MDR-tuberculose van belang. Door trendanalyse van clusters en hun kenmerken van de transmissie van tuberculose zal DNA-fingerprintsurveillance:

- de mate van recente transmissie in Nederland in kaart brengen
- als dijkbewaking fungeren
- het optreden van clustering van (resistente) tuberculose signaleren
- inzicht geven in risicogroepen en risicosituaties en de effectiviteit van de bestrijding
- bijdragen aan outbreakmanagement op zowel regionaal als landelijk niveau.

Ook internationaal wordt DNA-fingerprint toegepast, zij het niet gekoppeld aan een gedegen nationaal tuberculosenetwerk zoals wij dat in Nederland kennen. Vaak wordt het gebruikt bij (grote) 'outbreaks', bijvoorbeeld in gevangenissen en onder bevolkingsgroepen met een hoge infectiedruk om bijvoorbeeld onderscheid te kunnen maken dus re-activaties en re-infecties. Vermeldenswaardig is dat er binnen de EU een grensoverschrijdende databank ontwikkeld wordt voor clustering van MDR-tuberculosepatienten. Daarmee hopen wij tijdig zicht te krijgen op de transmissie van MDR-TB in Europa.

10.01

Application of a rapid immunochromatography assay during an outbreak of *Clostridium difficile* associated diarrhoea

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Recently, a real-time PCR was compared with toxin detection by a rapid immunochromatography assay (ICA) for the diagnosis of *Clostridium difficile* associated diarrhoea (CDAD). A sensitivity, specificity, positive predictive value, and negative predictive value were found for the ICA of 91, 97, 70, and 99%. Because the ICA is easy to perform and gives results within 15 minutes, we applied this rapid test during an outbreak of CDAD due to a toxinotype III strain in a medium large hospital (350 beds) in Harderwijk. During the epidemic, clinicians were advised to confirm a negative ICA result in patients with suspected CDAD by repeating the test, preferably within 48 hours. Retrospectively, we investigated the value of a second (or third) specimen on the microbiological diagnosis of CDAD. From April to October 2005, 235 patients suspected for CDAD were investigated. In 50 patients a positive ICA was found; 43 (86%) patients had positive results for *C. difficile* toxin on the first sample. In 131 patients with a negative ICA on the first specimen, the test was repeated within 7 days; three of these patients (2%) had a positive result on a second sample. Additionally, in one patient a third sample became positive which was obtained within seven days after the first and second negative specimen. Three patients had a positive result on a second or third specimen obtained more than seven days after the first sample (mean 24 days). Considering a time interval of more than seven days between consecutive faecal samples as a new episode of disease, the cumulative sensitivity of a first, second and third specimen was 92%, 98% and 100%, respectively. Overall the negative predictive value of a first faecal sample was 97%. These results suggest a limited value of ICA on a second or third faecal specimen in the exclusion of CDAD during an epidemic.

10.02

Detection of specific IgG1 and IgG4 antibody response for the immunodiagnosis of cystic echinococcosis

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Cystic echinococcosis is caused by the larval stage of *Echinococcus granulosus*, the small tapeworm of dogs. Humans become infected by ingesting the parasite's eggs that are shed with the feces of infected dogs. Hepatic involvement can result in abdominal pain and biliary duct obstruction. Pulmonary involvement can produce chest pain, cough, and hemoptysis.
The diagnosis of cystic echinococcosis relies in most cases on clinical findings and imaging procedures combined with serology. Serological tests with high specificity and sensitivity are therefore essential. For this reason we evaluated the reactivity of IgG1 and IgG4 antibodies to *E. granulosus* cyst fluid antigens using the immunoblotting procedure. Sixty five serum samples from patients with proven echinococcosis were evaluated. For determination of specificity, sera from 87 patients with other parasitic and bacterial infections were studied. Antigens of 7, 14 and 24 kDa were recognized by IgG1 and IgG4 antibodies from patients with cystic echinococcosis (CE) and not by patients with other infections. Evaluation of the specificity and sensitivity of the IgG1 in combination with the IgG4 immunoblots resulted in 99% specificity and 95% sensitivity. The immunoblots were also used for follow up of the immune response of treated CE patients. Results indicate that patients with good response to treatment showed decreased IgG1 and/or IgG4 response. The immunoblots here described have a higher specificity than the ELISA technique that is used in our laboratory for routine serological diagnostic of cystic echinococcosis. Due to the high sensitivity of the ELISA, we propose the use of the ELISA technique for screening purposes and the IgG1 and IgG4 immunoblots to confirm the positive ELISA results. The combined results from the *Echinococcus*-IgG1 and IgG4 are not only useful to serologically confirm an *Echinococcus* infection but it provides also additional information in follow up studies of treated patients.

10.03
Diagnosis of mumps by IgM-ELISA in Scotland - an assay comparison

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Introduction: Since the end of November 2003, Scotland has seen an outbreak of mumps with over 4000 confirmed cases in the last 2 years. The incidence of mumps continues to be high, particularly in adolescents who were either unvaccinated or given a single dose of mumps/measles/rubella vaccine. Laboratory diagnosis is usually requested on patients with symptoms. Therefore prompt diagnosis on acute samples is required. Here, the performance of 5 different commercial IgM assays was assessed.
Methods: The Specialist Virology Laboratory (SVC), Edinburgh, distributed a serum panel to all Scottish laboratories that perform mumps diagnosis by IgM-ELISA. The panel consisted of 45 true positive (TP) and 11 true negative (TN) serum samples. The TP samples had been confirmed by virus isolation on a parotid duct swab and/or a 4-fold rise by complement fixation testing (CFT) on a pair of serum samples. Date of sample collection after onset of symptoms (AOS) was recorded. The TN samples consisted of sera from patients with other infections. Five different commercial IgM-ELISAs were performed blindly: ELISAs Enzygnost® Antiparotitis-Virus/IgM (Dade Behring), Mumps IgM (Human), Mumps IgM capture EIA (Microimmune Ltd), Mumps ELISA IgG/IgM (Virotech), and Enzywell Mumps IgM (Diesse). Results were all reported to SVC.
Results: Sensitivity ranged from 51% to 24%; with the Microimmune assay being the most sensitive. Assays performed better on samples taken > 10 days AOS, although even then mumps-IgM was not detected by all assays. The Virotech assay was the only assay that reported mumps-IgM in all serum samples > 10 days AOS. Specificity was about 82% for most assays.
Conclusion: The anti-mumps-IgM assays assessed are insensitive and inappropriate for use in acute specimens. This could be due to the fact that development of anti-mumps-IgM is delayed or suppressed in partially immune individuals. For acute diagnosis laboratories should be developing the means to direct detection by virus culture or PCR.

10.04
Use of Raman spectroscopy for the identification of *Burkholderia* spp.

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The *Burkholderia cepacia* complex (BCC) is a group of at least nine closely related species. In CF patients pulmonary BCC infections have a considerable impact on clinical outcome and may lead to a fatal decline in lung function known as cepacia syndrome. Members of the BCC are well recognized as nosocomial and outbreak related pathogens and are able to colonize CF patients. Species identification of BCC organisms can be obtained with phenotypic methods such as automated commercial systems. Identification using these systems is doubtful, so confirmatory identification with expensive and laborious molecular methods is required. A powerful and inexpensive tool for the rapid identification of micro-

organisms is Raman spectroscopy. Vibrational spectra obtained with this technique are highly specific and reflect the overall molecular composition of a sample. They can serve as spectroscopic fingerprints and enable the accurate identification of microorganisms.[1] To evaluate the usefulness of Raman spectroscopy for the diagnosis of *Burkholderia* spp, a panel of 50 well-characterized clinical strains is used. The complete analysis was performed three times with independent samples. The first goal in this study is to discriminate between *Burkholderia* spp and related organisms. Results show that 94% of the strains were identified correctly at this level.

A second goal is to discriminate within the BCC to identify *Burkholderia cenocepacia* since this is the most prevalent and virulent species in the complex. This goal is met with a 77.8% accuracy. Rates of correct identification of *B. cenocepacia* by automated identification systems such as the BD Phoenix (Becton Dickinson) and the VITEK2 (bioMerieux) were 71% and 38% respectively.[2]

These first results indicate that Raman spectroscopy is an accurate and reproducible method to identify microorganisms frequently found in CF patients and to discriminate clinical relevant species within the *B. cepacia* complex.

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10.05

Cutaneous leishmaniasis in Dutch military personnel in Afghanistan: correlation between *L. major* genotype, clinical picture and deployment area

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Introduction: In 2005, several members of the Netherlands Armed Forces contracted cutaneous leishmaniasis during deployment to Afghanistan. Diagnosis of *Leishmania* infection was confirmed with classical diagnostic methods. PCR and sequence analysis were applied to determine the *Leishmania* species, the presence of one or more genotypes, and to gain more insight in epidemiology.

Methods: *Leishmania* DNA samples were obtained from 61 military personnel returning from different parts of Afghanistan. The mini-exon repeat sequence was PCR amplified from DNA isolated from biopsies of cutaneous leishmaniasis suspected skin lesions. PCR products were sequenced and analyzed with CodonCode Aligner 1.4.4 and ClustalW.

Results: Sequences analysis showed that *Leishmania major* was the causative agent of cutaneous leishmaniasis in all patients. Three different genotypes were observed. One genotype was exclusively found among 57 patients that served in North Afghanistan (Mazar-e-Sharif). Two other genotypes were found in four patients that were deployed to South Afghanistan (Kandahar). Clinical symptomatology was different between cases of cutaneous leishmaniasis from North and South Afghanistan. Cases from North Afghanistan showed extensive lesions and nodular lymphangitis, whereas the four cases from South Afghanistan showed a mild clinical picture with small single lesions and no nodular lymphangitis.

Conclusion: 1) *L. major* was the causative agent of cutaneous leishmaniasis in Dutch military personnel on deployment to North and South Afghanistan, 2) Three different *L. major* genotypes were observed. One genotype was exclusively found in patients from North Afghanistan. The clinical picture of these patients differed from that of patients from South Afghanistan.

10.06

Diagnosis of *Cryptosporidium parvum* with microscopy, striptest, ELISA and real time PCR

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Cryptosporidium parvum remains largely underdiagnosed in current routine diagnostic procedures in microbiology laboratories. We compared four different diagnostic methods for the detection of *C. parvum* in feces in both acute and chronic diarrhea.

Microscopic examination (Auramin stain confirmed by Kinyoun stain), Crypto-strip (Coris Bioconcept), ELISA (Novitec Cryptosporidium ELISA) and real time PCR for the detection of *C. parvum* were compared. During the summer of 2005, 515 feces were included. 154 watery specimens from acute diarrhea were sent for bacteriological examination and 361 triple feces test (TFT)-samples, representing a more chronic form of diarrhea, were sent to the parasitology department.

Using real time PCR as the gold standard, the positive predictive values of microscopy, Crypto-strip and ELISA were 100%, 85% and 99% respectively. The sensitivities of microscopic detection, Crypto-strip and ELISA were 37%,

78% and 71% respectively, while the specificities of the 3 methods were never lower than 98%.
Remarkably, the majority of the positive *Cryptosporidum* samples were not found in watery, as described in all textbooks, but rather in loose to mushy stools (57%). Furthermore, the majority of the positive watery samples was not sent for parasitological examination but only for bacterial culture.
We conclude that the widely used microscopy is a very specific but less sensitive method for the laboratory detection of *C. parvum* in feces. Both ELISA and Cryptostrip have good sensitivity and both positive and negative predictive values. Real time PCR is a very sensitive and specific method for the detection of *C. parvum*. The majority of positive *Cryptosporidium* samples were found in mushy stools from children younger than 10 years old. Examination of watery stools, sent only for bacteriological examination, for the presence of *C. parvum* yields additional positive samples which would otherwise not have been detected.

11.01

Microscopie in de parasitologische diagnostiek – kerntaak of specialistenwerk?

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Microscopisch onderzoek van patiëntenmateriaal op de aanwezigheid van pathogene organismen wordt algemeen beschouwd als de standaardmethode voor het aantonen van parasitaire infecties. Het is echter bekend dat deze diagnostische procedure verscheidene nadelen kent. Met name de noodzaak om microscopisch ontlastingonderzoek meerdere malen te herhalen voordat de uitslag 'geen parasieten' gesteld mag worden, legt een zware belasting op de routinediagnostiek. Dit speelt vooral in een Nederlandse setting, waar de prevalentie van intestinale parasieten relatief laag is. Ons laboratorium richt zich sterk op de ontwikkeling van alternatieve diagnostische procedures binnen de parasitologie en heeft een ruime ervaring opgebouwd in de ontwikkeling, evaluatie en implementatie van immunologische en moleculair technieken. Daarbij is de toepassing van de real-time-multiplex PCR een bijzonder succesvolle aanpak gebleken, waarbij op zeer gevoelige en specifieke wijze meerdere parasitaire infecties kunnen worden aangetoond en grote hoeveelheden monsters efficiënt kunnen worden verwerkt. De hierdoor veranderende positie van het microscopisch onderzoek binnen de routinediagnostiek en de mogelijke consequenties voor SKML rondzendingen zal tijdens deze presentatie worden toegelicht. Daarbij wordt een overzicht gegeven van de moleculaire diagnostische testen die momenteel op onze afdeling worden toegepast, en onze ervaringen binnen verschillende klinische populaties.

12.01

Structural analysis of a novel anionic polysaccharide in the oral pathogen *Porphyromonas gingivalis*

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The Arg-gingipains (RgpsA and B) of *Porphyromonas gingivalis* are a family of cysteine proteases and are important virulence factors of this oral pathogen. A monoclonal antibody, which recognizes an epitope on glycosylated monomeric RgpsA, cross-reacts wit a cell-surface polysaccharide of *P. gingivalis* W50 suggesting that the maturation pathway of the Arg-gingipains may be linked to the biosynthesis of a surface carbohydrate. This anionic polysaccharide is distinct from the lipopolysaccharide and the serotype capsule polysaccharide and appeared to be a phosphorylated branched mannan. This branched mannan represents a novel polysaccharide that is immunologically related to the post-translational additions of Arg-gingipains.

12.02

Oral biofilms: models for drug testing

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Introduction: The purpose of this study was to look into the interaction of *Streptococcus mutans*, a dental pathogen, with *Veillonella parvula*, a bacterium that utilizes the lactic acid produced by *S. mutans* in the dental plaque biofilm. Furthermore, we wanted to explore the possibility of proteomic and genomic analysis of this interaction. This information is used to explain differences in antimicrobial efficacy between single and multi-species biofilms.
Materials and methods: Single and dual species biofilms of *S. mutans* and *V. parvula* were grown on polystyrene in BHI supplemented with lactic acid. Growth, and survival after exposure to antimicrobials were assessed. Analysis of protein expression with 2D difference gel electrophoresis and analysis of *S. mutans* mRNA expression with 70-mer microarrays was evaluated.
Results: 48 h biofilms all had similar numbers of viable bacteria (appr. 10^9 CFU/cm^2). When grown in dual species biofilm, *S. mutans* and *V. parvula* were more resistant to chlorhexidine and *S. mutans* was more resistant to hydrogen peroxide. With the help of advanced statistical

methods we were able to calculate differences in protein expression between single species and dual species biofilms. Pilot experiments showed that a careful experimental design makes it possible to evaluate differences in gene expression between *S. mutans* grown in single species and in dual species biofilms.

Conclusions: 1) *S. mutans* and *V. parvula* grown in dual species have different resistance to antimicrobials than when grown in single species biofilms, 2) Analysis of protein and gene expression by dual species biofilms is possible.

12.03

Bacterial biota in the oropharynx

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The oral microbial flora plays important roles in prevention of disease, as a reservoir of pathogens and in maintenance of disease, depending on its composition. Different parts of the oral cavity form different niches for diverse microbial species. The oropharynx is a known reservoir for the causative agents of bacterial meningitis, but otherwise little is known about its biota. Our aim was to explore the bacterial biota in the oropharynx both quantitatively and qualitatively by culture independent methods. Total microbial DNA was isolated following suspension of microbial cells from throat swabs taken from two healthy volunteers. 16S rRNA gene libraries were constructed following amplification with generic 16S primers. Two different DNA isolation protocols were compared; one used in previous studies and one including achromopeptidase lysis. Five libraries yielded > 1600 sequences for phylogenetic analyses. Identical samples assessed by the different lysis protocols resulted in statistically significant differences between the resulting libraries: more *Firmicutes* sequences were retrieved with achromopeptidase. Prevalent phyla included *Fusobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. Large qualitative and quantitative differences between libraries of different individuals, or taken with large time intervals, were evident.

Two striking observations were made. One individual carried 88% (359/407) *Neisseria meningitidis* in her oropharynx. Clones from another individual contained sequences of the TM7 subdivision, a novel phylum of which no bacteria have been cultured to date.

Conclusions: 1) Currently used protocols for 16S library construction may result in underestimation of the number of Gram positive bacteria present. 2) The qualitative and quantitative composition of the bacterial biota of the oropharynx differs in time and between individuals. 3) DNA of bacteria from the TM7 phylum is present in the oropharynx. 4) The predominance of a single species (*N. meningitidis*) in an individual may have implications for transmission of this potential pathogen.

12.04

Response of *Streptococcus mutans* towards environmental stress

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Streptococcus mutans is an important pathogen in the initiation of dental caries. The acidogenic and aciduric nature of the organism is one of its important virulence determinants. We have shown that these determinants are even more distinctive when *S. mutans* is growing as a biofilm. It is known that several stress-responsive genes are involved in biofilm formation. Expression of these and several other genes in biofilms differs significantly from suspension-growth. However, the distribution of these stress-responsive gene-products in biofilms is unclear and the relation between physicochemical gradients and the (antimicrobial) resistance properties still needs to be explored.

To be able to do so we have constructed several promoter GFP fusions that allow us to study the expression of stress-responsive genes under various conditions.

In this study we aimed at determining the expression from the CovRS promoter. It has been shown that this two-component system plays an important role in the development of virulence factors in *Streptococci* (cov stands for control of virulence).

The CovRS promoter (which is being auto-regulated) from *S. mutans* UA159 was cloned into the pVA838 shuttle vector in front of the coding sequences for the fluorescent protein GFPmut2. The shuttle vector was transformed back into UA159 and the reporter strain was used to study expression from the promoter by determining fluorescence levels during growth under various conditions. Several independent experiments clearly indicate that CovRS is induced by oxidative stress including the presence of oxygen and hydrogen peroxide.

14.01

A genome-scale model of *Lactobacillus plantarum* WCFS1: useful for omics data integration and exploring metabolic capacities

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Systems biology took off because of the omics revolution, confronting biologists with the need of models for data integration, analysis, and - ultimately - understanding of the complexity of biological systems. Hence, if we want to make optimal use of functional genomics data, we need models of genome scale. We have built a genome-scale metabolic model of *Lactobacillus plantarum* WCFS1, an important industrial lactic acid bacterium, both for food and health applications. The complete model currently consists of 546 unblocked internal reactions and 434 corresponding metabolites, 97 exchange reactions, and 721 genes (23.5% of the genome). The model is based on bioinformatics, comparison with other genome-scale models, literature, and in-house generated experimental evidence for the presence of pathways. Interactive metabolic maps have been generated, enabling data projection onto these maps. Chemostat experiments were run to generate physiological data for model construction and validation. Fluxes and biomass composition were measured. From this data, maintenance and growth-associated ATP consumption rates were estimated. Using Flux Variability Analysis, we found a remarkable flexibility in ATP-producing and ATP-consuming pathways, including 28 futile cycles detected by genome-scale elementary flux mode analysis. Optimization of an objective function – referred to as flux balance analysis (FBA) – has been often used to predict flux distributions in metabolic networks, but it fails miserably in *L. plantarum*. Rather than predicting flux distributions, FBA does appear useful in *L. plantarum* for exploring potential contributions to metabolic objectives, such as ATP generation or biomass yield.

14.02

Culture-independent approaches to elucidate biodiversity and population dynamics in complex microbial ecosystems of food fermentations and the intestinal tract

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Introduction: In recent years, DNA-based culture-independent techniques have opened interesting perspectives to unravel the composition and population dynamics of microbial communities in various environments. Especially in highly complex microbial ecosystems such as fermented food products and the intestinal tract, there is ample evidence illustrating that the use of conventional culture methods alone is inadequate to assess the true diversity of predominant bacterial groups in food or faecal samples. Triggered by the universal availability of bacterial gene and genome sequences and by the development of new molecular tools, direct microbial analysis of minimally disturbed samples has become possible.

Methods: Sequence-dependent electrophoresis techniques such as Denaturing Gradient Gel Electrophoresis (DGGE) are one of the most commonly used approaches for microbial population profiling of fermented food and intestinal ecosystems. Through the use of universal and/or group-specific PCR primers targetting the 16S rDNA gene or single-copy housekeeping genes, the PCR-DGGE concept offers a wide range of possibilities to study the predominant members or a specific subpopulation in a given microbial community. On the other hand, it should be noted that PCR-DGGE – at its best performance – is a semi-quantitative technique. For the assessment of relative bacterial concentrations or the quantification of temporal shifts in complex microbial ecosystems, PCR-DGGE thus needs to be complemented with quantitative molecular tools such as Real-time PCR (RT-PCR).

Results: In the course of previous and ongoing research projects, the biodiversity and population dynamics of several traditional fermented foods have been studied with PCR-DGGE. The use of universal V_3-16S rDNA primers in PCR-DGGE in combination with digitized band position analysis and band sequencing allowed to assign predominant band fragments to specific taxa of the lactic acid bacteria (LAB) present in Belgian sourdoughs (mainly *Lactobacillus* and *Weissella* species), Flemish artisanal cheeses (mainly *Lactococcus, Lactobacillus* and *Pediococcus* species) and the South-African fermented sorghum product Ting (mainly *Lactobacillus* species). PCR-DGGE analysis also proved to be highly useful for temporal monitoring of semi-industrial or lab-scale fermentation processes and could give a reliable indication of the minimal fermentation time needed to develop a stable LAB community in each product. In another set of studies, the potential of PCR-DGGE to monitor the stability of predominant microbiota and specific bacterial subgroups in faecal samples was explored during placebo-controlled pro-, pre- and synbiotic administration trials in healthy human volunteers. Although the targetted populations remained fairly stable based on PCR-DGGE profiling with V_3-16S rDNA primers, one striking finding in these trials concerned the appearance or intensification of one specific DGGE band after intake of the prebiotic compound lactulose. Band sequence analysis showed that in 90% of

the subjects, this band could be assigned to *Bifidobacterium adolescentis*. Subsequent analysis with RT-PCR could link this single-band effect to a statistically significant increase ($P < 0.05$) in total bifidobacteria after lactulose intake.
Conclusions: In our hands, PCR-DGGE is a valuable addition to culture-dependent approaches to assess the biodiversity of LAB and other autochthonous bacteria of fermented food and intestinal environments. When combined with RT-PCR, it also provides a powerful means to monitor and quantify temporal shifts in complex microbial communities.

14.03

Metabolite target analysis and population dynamics of sourdough fermentation processes

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Introduction: Sourdough is a mixture of ground cereals and water that is spontaneously fermented by lactic acid bacteria and yeasts. Sourdough fermentations improve the properties of the dough, delay bread spoilage, and improve bread texture and flavour. The aim of this work was to study the population dynamics of spontaneously fermented sourdoughs and to perform an extensive metabolite target analysis to correlate the production of metabolites with the presence of certain sourdough species and to explain why strains disappear, adapt, or even become dominant during the sourdough fermentation process.
Methods: Six spontaneous laboratory sourdough fermentations (with wheat, rye, or spelt flour) were performed by means of daily back-slopping over a period of ten days. Culture-dependent microbial analysis was performed to monitor the population dynamics of the sourdoughs. Denaturing Gradient Gel Electrophoresis as molecular, culture-independent method was used to monitor the bacterial diversity and dynamics of the ecosystems. A combination of different chromatographic methods such as High Pressure Liquid Chromatography, High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection, Liquid Chromatography with Mass Spectrometry, and Gas Chromatography with Mass Spectrometry was used to quantify a range of important bacterial metabolites.
Results: A stable microbiota was established in all sourdoughs after four to five days. This stability was also noticed for the different sugar and amino acid metabolites quantified. Moreover, changes in DGGE profiles, i.e. appearance of bands, could be correlated to changes in metabolite profiles, i.e. appearance of certain metabolites (e.g. mannitol). Besides the more common metabolites, other compounds such as succinic acid were detected for the first time in a sourdough ecosystem.
Conclusion: A combination of both population dynamics and metabolite target analysis as performed in this study may contribute to the rational selection of starter cultures appropriate for controlled sourdough fermentations.

14.04

Role of thioredoxin reductase (trxB1) in oxidative stress response of *Lactobacillus plantarum* WCFS1

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Thiols are essential compounds that may act as antioxidants and it has been established in *Escherichia coli* that one of the two major thiol antioxidants, thioredoxin (TRX), is involved in a broad spectrum of cellular processes such as DNA synthesis, protein folding, stress response, and detoxification. We have focused on determining the impact of this redox-mediating thiol in the overall metabolism of the human isolate *Lactobacillus plantarum* WCFS1. This is a lactic acid bacterium encountered in many environmental niches, such as plant material and fermented foods, but is also a natural inhabitant of our intestine. TRX is activated through a NADPH dependent reaction catalyzed by thioredoxin reductase (TR). For this study we have created an extensive library of mutants with varying amount of active thiol and analyzed these strains for their growth kinetics, stress survival, and resistance towards oxidative stresses. We have observed that controlled overproduction of only one of the components (either TRX or TR) does not represent a burden to the strain. On the contrary, overproduction of the complete thioredoxin system (TRX and TR) was found to have a significant impact on the specific growth rate and biomass yield in batch cultures, especially under aerobic conditions. To establish the link between this overproduction and the physiological response, we determined the global transcriptional response of the *L. plantarum* WCFS1 strains grown in chemostat cultures and challenged by different oxidative stresses. We have determined through ANOVA statistical analysis that 45 genes are significantly affected due to the genomic modification. Most of these genes are involved in three major metabolic routes: purine metabolism, cysteine biosynthesis, and glycolysis. Interestingly, these routes were also found affected in the presence of oxidative stress together with other expected stress-affected genes. The results of this functional genomics study will be presented using interactive metabolic maps (Simpheny™) to highlight the role of the thioredoxin system in *L. plantarum* WCFS1.

15.01

Molecular typing of bacterial pathogens reveals a spectrum from clonal to panmictic population structures

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Introduction: Strain typing is an integral part of epidemiological investigations of bacterial infections. However, strain typing may also serve to study the composition of bacterial populations and the impact of human interference on these populations. Methods to distinguish bacterial strains have improved dramatically over the last decade, mainly due to the introduction of molecular typing technology.

Methods: Many studies have been performed to assess source and contacts in outbreaks of community acquired or nosocomial bacterial infections. Mostly these studies rely on rapid, easy to perform band based typing methods like pulsed-field gel electrophoresis and restriction fragment length polymorphism. However, more recent and reliable typing technology, such as multi-locus sequence typing (MLST), relies on DNA sequence information. This portable technique seems to be better suited to create (inter)national databases required for population studies. Furthermore, the availability of whole genome sequences over a large number of bacterial species has enabled the development of microarrays to perform comparative genome hybridizations.

Results: MLST has yielded a number of publicly available databases with typing results that have been used to study bacterial populations. Some of the best studied bacterial species are *Neisseria meningitidis* and *Streptococcus pneumoniae*. Analysis of the MLST databases showed that species like *Helicobacter pylori* are extremely diverse due to constant lateral transfer and genome reshuffeling. In other species like *N. meningitidis* where DNA is exchanged and recombined to a lesser extend MLST has proven to be extremely useful for population studies. However, some species like *Bordetella pertussis* hardly exchange DNA and have a nearly uniform population often referred to as clonal. For highly variable and clonal species MLST is unsuitable and other typing methods are required.

Conclusion: Molecular typing techniques, particularly MLST, have revealed that bacterial species may have population structures that vary from panmictic to clonal.

15.02

Phylogenomic Analysis of *Enterococcus faecium* (Efm) Using Mixed Whole Genome Microarray Technology Discerns a Globally Dispersed Hospital Clade

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Introduction: Efm, ubiquous colonizers of humans and animals, have emerged as nosocomial pathogens in the last decade. Comparative phylogenomic analysis (CPA) using a mixed whole genome microarray was used to elucidate the population structure of Efm, using 97 Efm isolates from different backgrounds (hospital outbreaks, hospitalized patients, healthy subjects and animals) typed by multilocus sequence typing (MLST).

Methods: A shotgun library constructed of DNA from 9 Efm strains and additional PCR products (virulence genes and resistance markers) were spotted. Mixtures of Cy5 labeled DNA of the 97 Efm isolates and Cy3 labeled DNA of the library strains were hybridized. 3474 inserts met quality criteria and were analyzed. 151 hospital clade associated inserts were amplified, sequenced and blasted in GenBank. Genomic mosaicism was visualized using split decomposition analysis (SDA) and a Bayesian-based algorithm on binary data was used to study phylogeny. Character evolution was studied with maximum likelihood-based models.

Results: 1183 inserts (34 %) were conserved among all Efm strains. CPA identified a clade, supported by Bayesian probabilities (p=1), containing all outbreak-associated strains and closely resembling the previously described MLST-based Clonal Complex-17. 447 inserts (13%) were associated with this clade. The predicted most prominent inserts of this clade encoded a mutator type transposase and a predicted metal-dependent hydrolase (98% sensitive, 100% specific). Clade-specific inserts (specificity > 78%, sensitivity 40-94%) included membrane proteins (n=8), hypothetical (n=26), resistance and (n=3), regulatory genes (n=7), mobile elements (n=35), phage (n=6) and plasmid genes (n=6). The genes were mainly located on 2 contigs of the unfinished Efm DO genome. In SDA presence and absence of genes on these contigs was inconsistent with a branched phylogeny, indicating a highly mosaic structure.

Conclusion: These findings demonstrate the evolution of a specific Efm subpopulation, associated with hospital outbreaks worldwide, and characterized by mobile elements, regulatory and potential virulence genes and a highly mosaic structure.

15.03

Non-typeable methicillin-resistant *Staphylococcus aureus* form a clonal cluster which seems to be related to pig farmers and pigs

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Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen which has emerged worldwide. Typing of MRSA is essential to detect outbreaks and to set up surveillance programs. Pulsed-field gel electrophoresis (PFGE) is considered to be the gold standard for MRSA strain typing. Recently, the number of MRSA isolates which could not be typed by PFGE using *Sma*I is increasing steadily in the Netherlands.
Methods: To characterize these PFGE non-typeable MRSA strains, different typing methods were used, including staphylococcal protein A (*spa*) gene typing, multi-locus sequence typing (MLST), Panton-Valentine leukocidin (PVL) PCR, accessory gene regulator (AGR) typing, and toxin gene profile. MRSA isolates, which were non-typeable by PFGE, from a pig farmer's family, 3 employees, and 10 pigs (from the pig farm consisting of 1500 pigs) were included in this study.
Results: All typing results showed a close relatedness between the non-typeable strains. The pig-related MRSA strains had identical typing results and were the same or could be grouped together with the typing results of all non-typeable MRSA strains. Pig-related MRSA were characterized by: PFGE non-typeable, *mecA* positive, *spa* type t108, sequence type 398, PVL negative, LukM negative, TSST negative, and AGR type 1.
Conclusion: All typing methods showed the clonal relatedness of the non-typeable MRSA strains, which seem to have diverged from a common ancestor. A strong link was found between these strains and pig farmers and/or pigs. The non-typeable MRSA strains seem capable of pig to human transmission (or vice versa) as well as human to human transmission. This could be a serious threat to public health and may hamper the successful Dutch search and destroy strategy aimed at containing the spread of MRSA in hospitals.

15.05

Spread of a persistent methicillin-resistant *Staphylococcus aureus* ST80 clone in the community of the northern part of the Netherlands

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Until 2002, community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) was virtually non-existent in the northern part of the Netherlands. In this study we describe the spread of a single persistent MRSA clone in the northern part of the Netherlands between 2002 and 2005. Outbreaks occurred predominantly in the community but also in hospital and nursing home environments.
MRSA strains were obtained from skin or wound infections of patients displaying staphylococcal disease syndromes (e.g. furuncles, abscesses) or routine culturing of patient contacts. PCR was used for determining genes of mec A, Panton-Valentine leukocidin (PVL), staphylococcal enterotoxines, and typing of the staphylococcal cassette chromosome mec element (SCCmec). Susceptibility testing was performed by disk diffusion (NCCLS) and MRSA strains were genotyped by pulsed-field-gel electrophoresis (PFGE).
Between 2002 and 2005 one persistent PVL positive MRSA clone, SCCmec type IVc, was found in 43 patients. Initially, a concentration of outbreaks was detected in the area of the city Groningen, which was followed by spreading of the clone to adjacent areas. MRSA was acquired in the community (74%), nursing homes (16%) and hospitals (9%). Patients of all ages harboured the MRSA clone (median age 48 years). All MRSA strains showed resistance to beta-lactam antibiotics and also to tetracycline and fusidic acid. Multilocus sequence typing (ST) characterized the clone as ST80, a prevalent CA-MRSA clone in Europe.
In conclusion, a single clone of PVL positive CA-MRSA with SCCmec type IVc, identical to the European ST80 clone, was found to be spreading in the community of the northern part of the resulting in the largest spread of CA-MRSA in the Netherlands.

17.01

Antibiotic resistance and fitness

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Antibiotic resistance is a global threat, which emerges through several different mechanisms including mutations in the genes that control the bacterial processes or structures that are their targets. It is often assumed that organisms pay a significant physiological price for the acquisition of a resistance determinant: most commonly, it is supposed that this 'price tag' includes a decrease in virulence. We have previously demonstrated, in *Mycobacterium tuberculosis*, that chromosomal mutations in the *rpo*B gene that produce rifampicin resistance have a limited repertoire and almost all are associated with a reduction in fitness, as measured by comparative growth velocity. The relative fitness of each of these mutations was significantly correlated with the frequency of clinical isolation in practice. This study has recently been confirmed by other workers in other bacterial species. The price-tag concept has a profound influence on biological thinking and public health approaches

to control resistance. It is argued that if the resistance pressure is removed, for example by stricter control of antibiotic use, then resistant organisms will be replaced by physiologically 'stronger' non-resistant strains. This simplistic approach has come under increasing challenge as examples demonstrating that strains adapt rapidly to the fitness deficit of resistance emerge. There is now considerable evidence that serial passage of any bacteria through any artificial biological system is associated with change (adaptation) that is mediated by compensatory mutation. Antibiotic resistance, whether caused by chromosomal mutation or acquisition of plasmid encoding resistance determinants, is also subject to this effect. Using a streptomycin resistant mutant *Escherichia coli* as a model system, Schrag *et al*. demonstrated that initial *rpsL* mutants had a 14-19% selective disadvantage per generation, as measured by the chain elongation rate. After serial passage in the absence of antibiotic selection, revertants to susceptibility did not appear, but the fitness deficit was eliminated. As there was no change in the sequence of the *rpsL* gene, it also suggests that the adaptation had arisen by mutation in other gene(s) that were not identified. If a susceptible genotype was then reinserted into the adapted resistant strain, this new strain showed a fitness deficit relative to the adapted resistant strain. In a further study, streptomycin resistant *Salmonella typhimurium* passaged *in vitro* and in mice were shown to have a different pattern of compensatory mutation. Those passaged *in vitro* contained extragenic suppressor mutants in the *rps*D or *rps*E gene; those passsaged through mice had a specific compensatory intragenic mutant. This suggests that bacterial adaptation may follow a different pathway *in vivo* to ameliorate the costs of resistance depending on its environment. In many instances the positions of these mutations are not within the antibiotic target genes making it difficult to identify them.

We have shown that, in different isolates of the same strain of *M. tuberculosis* isolated from patients involved in an outbreak, significant differences in fitness as measured by growth velocity are found. This suggests that the organisms adapt differently to different human hosts.

17.02

The tragedies in antimicrobial resistance

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The use of antimicrobials has caused a proliferation of resistant pathogens and, most worryingly, some pathogens resistant to multiple classes of drugs. Policies are now being implemented to reduce antimicrobial use with some encouraging successes. However, current policies may only partly solve the problem. In particular, they do not address the conundrum at the heart of antimicrobial resistance: the solution may ultimately require us to put society before the individual. This uncomfortable conclusion uses the logic of the well-known social dilemma 'the tragedy of the commons'.

It will be shown that rational, reputational and economic choices are the common drivers of widespread occurrence of antimicrobial resistance. In fact, four different tragedies are at the heart of this dilemma and it appears, that technical solutions defined as 'ones that requires a change only in the techniques of the natural sciences, demanding little or nothing in the way of change in human values or ideas of morality' will not be able to curb this trend.

17.03

Phenotypic and molecular tests for antimicrobial resistance detection, infection control measures and surveillance systems – predictions for the future

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Molecular tests for the detection and identification of medically relevant microorganisms have been developed in large numbers over the past decades: there is not a single known species of microorganism capable of infecting humans for which there is no nucleic acid amplification-based detection scheme available. This has strongly enhanced the efforts to make such tests amenable to application in the routine medical microbiology laboratory. Automation of nucleic acid isolation procedures has been attained and high-throughput extraction robots have become commercially available. In addition to classical PCR tests also quantitative molecular assays (e.g. using TaqMan hydrolysis probes) have been developed successfully. Consequently, the methodology to detect single base mutations and / or to define the presence of microbial genes or gene-specific fragments in any type of clinical specimen is available to the diagnostic community.

Detection and identification of bacterial strains with clinically relevant resistance spectra can be performed on a routine basis and the example of methicillin resistant *Staphylococcus aureus* (MRSA) will be highlighted. Detection of MRSA can be performed with different commercially available systems and two of these will be discussed. The main message here is that the suitability of such approaches does depend on the combination of test characteristics (sensitivity, specificity, positive and negative predictive values) and the local incidence of MRSA. A test to be used in a low endemicity area requires different qualities than one used for screening in cases of established high-level colonisation among patients. Next to straightforward single target PCR tests, current

literature is increasingly crowded with tests using nucleic acid-based arrays. Whether such tests are already useful to the "clinicus practicus" is currently doubtful but there are great promises in the technology. Arrays facilitate direct species identification in combination with antimicrobial susceptibility testing (at the genome level) and epidemiological typing. Next to straightforward microbial detection, (sub-species) identification of bacterial isolates is important as well. This can be used for tracking multi-resistant clones during their travels through clinical settings and the open population. Spectacular technological developments in this field have facilitated the detailed study of bacterial population genetics and dynamics. Two such methods, Amplified Fragment Length Polymorphism (AFLP) typing and multi-locus sequence typing (MLST) will be explained and results obtained during the analysis of strains of MRSA and MSSA will be discussed.
Finally, new and challenging methods are continuously emerging. This involves several biophysical methods, for instance. The combination of protein chemistry and mass spectrometry is new and combining mass spectrometry with DNA amplification is particularly appealing. Other methods, including Raman spectroscopy, putatively enable microbial species identification by direct analysis of microcolonies on solid agar media. In conclusion, molecular diagnostics for the detection of most if not all microbial species (and their phenotypes) has reached the clinical laboratory. Implementation of such tests, however, requires a thoughtful analysis of laboratory expertise, budget, clinical relevance, timeliness, throughput and many other factors. These need to be balanced prior to introduction of new technology, including the most recent biophysical approaches, into the clinical laboratory in order to warrant appropriate implementation and optimal diagnostic results.

17.04

Mutator bacteria and antibiotic-resistance

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Emerging antibiotic-resistant bacteria pose a serious threat to public health, for the infections they cause are much more difficult to treat. Drug resistance in bacteria are frequently caused by specific spontaneous mutations in their genome. Under non-selective conditions (i.e. when no antibiotics are taken) these mutations arise once in every 10^6-10^8 replications on average. Certain bacterial strains, called hypermutators or mutator strains, have an elevated mutation rate, thereby increasing the risk of acquiring drug resistance. It is thought that most drug resistance is acquired within the host, since most mutations impair the fitness of the bacteria, making transmission of (drug-resistant) hypermutators less likely. The general consensus on hypermutators, therefore, is that they form a risk for the patient rather than the population. However, the outcomes of various recent epidemiological and mathematical studies suggest that antibiotic-resistant bacteria in fact can and have caused epidemics. The increased mutation rate of hypermutators is thought to not only induce drug resistance at a higher rate, but also drive the evolution of resistant bacteria by the acquisition of compensatory mutations. These mutations restore the fitness of the bacteria and thereby fix the drug resistance mutations in the population. This phenomenon underlines the need for the development of new antibiotics, but even more so for methods to maintain the activity of new drugs, for instance by inhibiting the emergence of hypermutator bacteria. Knowledge of the genetic and molecular mechanisms underlying the mutator phenotype could facilitate inhibition of hypermutation and thus reduce drug resistance. The most important mechanisms, such as error-prone DNA repair, will be discussed. In addition, a review will be presented of methods that can possibly interfere with these mechanisms.

17.05

Determinants of inappropriate (IA) use of antibiotics identified in prevalence surveys

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Introduction: To improve appropriate use of antimicrobial therapy (AMT) in hospitals it is important to identify factors associated with IA use.
Methods: The study was performed in a 1350 bed teaching hospital. Six consecutive one-day prevalence surveys of inpatients were performed twice yearly, from 2001 to 2004. Demographic-, infection- and AMT-data were gathered. The appropriateness (AP) of AMT was assessed according to a standardised algorithm based on the local antibiotic prescription guidelines.
Results: A total of 4105 patients were included. 942 (22.9%) received AMT. In 60 (6.4%) patients the AP of AMT could not be judged. Of the remaining 882 patients, in 350 (39.7%) AMT was IA. The following factors were statistically significant associated with IA use: Quinolones, Co-amoxi-clavulanic acid, older age and patients on Urology. The following factors were statistically significant associated with AP use: cephalosporins, penicillins, meropenem, metronidazol, rifampicin, younger

age, infection at admission and patients on pediatrics. After multivariate analysis the use of quinolones was the only statistical significant factor associated with IA use. When patients on Orthopaedic surgery, Urology or Neurology were treated with quinolones more than 75% was IA. The results were consistent over time.

Conclusions: Prevalence surveys proved to be useful tools to determine the AP of AMT and to identify risk factors for IA use. The use of quinolones was an independent risk factor for IA use. Moreover, several areas in the hospital were identified with an extremely high rate of IA use. This provides targets for highly effective interventions to improve AMT. Subsequently, repeated prevalence surveys can be used to measure the effect of the interventions. In this way a quality circle for optimalisation of AMT can be achieved. This method is potentially an effective tool in the battle against the ever-increasing resistance rates.

18.02

The presence of the protective HLA-B27 allele results in increased responsiveness of HIV-1 specific CTL restricted by HLA-A2

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Introduction: HLA-B57 is associated with relatively slow progression to AIDS. Previous work revealed that CTL responses specific for a HIV-1 Gag derived epitope presented by HLA-B57 were relatively high compared to Gag derived epitopes presented by HLA-A2 or HLA-B8. Because HLA-B27 is also associated with a delay in AIDS progression, we aimed to extend this work, now also including HLA-B27 restricted epitopes.

Methods: We measured HIV-1 specific CTL responses in 6 HIV-1 infected individuals expressing HLA-A2 and 8 individuals expressing both HLA-A2 and HLA-B27 using the IFN-γ ELIspot assay. Responses to in total 50 (30 HLA-A2 and 20 HLA-B27 restricted) peptides derived from the entire HIV-1 genome were studied. These peptides were a combination of known (published in the Los Alamos database) and predicted epitopes (based on MHC-peptide binding profiles, proteasomal cleavage and TAP transport efficiency).

Results: In individuals expressing both HLA-A2 and HLA-B27, we found no differences in either the breadth or magnitude of CTL responses specific for HLA-B27 restricted epitopes compared to HLA-A2 restricted epitopes. Interestingly, we found that individuals expressing both HLA-A2 and HLA-B27 responded to significantly more HLA-A2 restricted peptides compared to individuals without HLA-B27 (p=0.023, Mann-Whitney). The magnitude of responses towards HLA-A2 restricted peptides was also significantly higher in individuals expressing both HLA-A2 and HLA-B27 (p=0.015, Mann-Whitney). Viral load was significantly lower in individuals expressing both HLA molecules compared to individuals without HLA-B27 (p=0.031, Mann-Whitney) and correlated with CTL responsiveness (p=0.013, Spearman's correlation test).

Conclusion: In conclusion, individuals expressing both HLA-A2 and HLA-B27 have a lower viral load and respond significantly better to HIV-1 derived peptides restricted by HLA-A2 compared to individuals that don't have this 'protective' HLA molecule. These data suggest that proper suppression of viral load can preserve CTL function.

18.03

HIV-1 variants with multiple protease mutations can persist because loss of single resistance mutations reduces replicative capacity and blocks evolution to wild type

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Objective: The development of HIV-1 protease inhibitor resistance comes at the cost of a reduction in viral replicative capacity (RC). In individual cases it has been shown that full compensation of this reduced RC is possible through the acquisition of compensatory mutations. However, in most cases protease inhibitor resistant viruses are not able to fully compensate their reduced RC. Cumulative data indicate that protease inhibitor resistant viruses can persist *in vivo* in absence of protease inhibitors (e.g after transmission), despite their often-reduced RC. In this study the mechanism behind the *in vivo* persistence of protease inhibitor resistant viruses in absence of protease inhibitors was investigated.

Methods: Longitudinal genotypic analyses were performed on sequential samples drawn from two HIV-1 infected patients, who interrupted their protease therapy for four years while maintaining their nucleoside/non nucleoside RT therapy (partial treatment interruption: PTI). RC was determined using recombinant viruses containing protease and C-terminus of Gag derived from sequential time points. Subsequently, the effect of changing individual protease mutations back to wild type on RC was determined.

Results: All primary mutations persisted in absence of protease inhibitors for four years, despite the fact that the RC was severely reduced. Changing individual protease mutations (M46I, I54V or V82A) to wild type reduced RC even further.

Conclusions: We propose the following mechanism for the *in vivo* persistence of variants with multiple protease mutations in the absence of protease inhibitors. Since the HIV-1 population is generally small, reversion is most likely to occur through sequential single changes. However, any intermediate with a single change that is generated, can only become dominant if its RC is higher than its predecessor. In cases where changing of individual amino acids leads to a reduction in RC, reversion to wild type is blocked and the virus with multiple mutations will persist.

18.04

A novel and rare amino acid substitution E40F in HIV-1 reverse transcriptase (RT) increases zidovudine (AZT) resistance and decreases replication capacity

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Introduction: After almost twenty years of HIV treatment with the reverse transcriptase inhibitor zidovudine (AZT) the complete pattern of resistance against AZT is still not completely clear. Classical AZT-resistance-associated amino acid substitutions have been mapped at positions 41, 67, 70, 210, 215 and 219 in RT. We have identified several patient-derived viruses containing the E40F change in the background of the classical AZT-mutations. In this study, we have investigated the effects of this change on resistance and replication capacity (RC) and its frequency in the treated population.
Methods: Patient-derived HIV RT variants harboring the E40F change in the background of classical AZT-mutations were investigated. We have cloned the N-terminal part of the RT gene (amino acid 25 through 314) in a reference HIV strain and the E40F change was reverted to wild type by site-directed mutagenesis. The impact on RC and drug susceptibility for AZT and d4T were analyzed.
Results: The patient-derived virus clones contained the E40F change in the background of the classical AZT-mutations M41L, L210W, T215Y +/- D67N. These viral clones demonstrated high-level resistance against both AZT and d4T but had a reduced RC compared to wild type. Changing the E40F substitution back to wild type resulted in a five-fold decrease in resistance for AZT and a slight increase in RC.
Analysis of a large database revealed the presence of this change in 0.45% of the treated population, while it was not present in the naïve population.
Conclusions: In this study we found a novel amino acid substitution in HIV-1 RT that contributes to AZT resistance. Selection of the E40F change results in a five-fold increase in resistance to AZT at the price of a reduction in RC. Further research is warranted to determine why this amino acid change is relatively seldom observed in treated patients.

18.05

Estimating the costs and benefits of CTL escape mutations in SIV/HIV infection

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Mutations that allow SIV/HIV to avoid the CTL response are well documented. Recently, there have been a few attempts of estimating the costs of CTL escape mutations in terms of the reduction in viral fitness and the killing rate at which the CTL response specific to
one viral epitope clears virus-infected cells. Using a mathematical model we show that estimation of both parameters depends critically on the underlying changes in the replication rate of the virus, and the changes in the killing rate over time (which in previous studies
were assumed to be constant). We provide a theoretical basis for estimation of these parameters using it *in vivo* data. In particular, we show that 1) by assuming unlimited virus growth one can obtain a minimal estimate of the fitness cost of the escape mutation, and 2) by assuming no virus growth during the escape, one can obtain a minimal estimate of the average killing rate. We also discuss the conditions under which better estimates of the average killing rate can be obtained.

19.01

What is the role of γ-butyrolactones in *Streptomyces coelicolor* A3(2)?

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Streptomyces, the Gram-positive filamentous bacteria, produces almost 70% of antibiotics. However, the regulation system of antibiotic production is unclear. Among the numerous factors influencing the onset of antibiotic production in *streptomyces*, a family of small diffusible compounds, γ-butyrolactones, which act as extracellular signalling molecules and also described as 'microbial hormones' is present. The most well characterised γ-butyrolactone is the A-factor found in *Streptomyces griseus* which is required for both streptomycin production and aerial mycelium formation (Ohnishi *et al*, 1999).
In *Streptomyces coelicolor* A3(2), the model streptomycete, we have previously reported the identification of a γ-butyrolactone, SCB1 isolated from transition and stationary phase culture supernatants which stimulate the production of actinorhodin (Act) and undecylprodigiosin

(Red).[1] We have also cloned *scbA*, which is involved in SCB1 synthesis and found it to be located divergently from *scbR* which encodes a γ-butyrolactone receptor. Transcription analysis indicates that ScbR represses its own expression while regulating that of *scbA*. *scbA* and *scbR* mutants produced no γ-butyrolactones, yet *scbA* mutant overproduced Act and Red, while *scbR* mutant showed delayed production of Red.[2] These phenotypes together with the transcription results suggest that ScbR represses one or several genes directly in the γ-butyrolactone cascade leading into antibiotic production. To determine these regulons, transcriptome analysis was conducted using the *scbR* mutant. Several genes were identified whose expression was repressed by ScbR which included potential biosynthesis genes encoding for a type I PKS cluster. One gene in this cluster (*kasO*, a SARP family of regulatory genes) was identified to be directly regulated by ScbR.[3]
However several questions still remain. Are there more targets of ScbR? How are the butyrolactone synthesized? How does *kasO* regulate antibiotic production? Do the ScbR homologues compete with binding sites? Is the sole role for butyrolactones to regulate antibiotic production?

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19.02

A novel nutrient sensory system that controls central metabolism, morphogenesis and antibiotic production in streptomycetes

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Soil-dwelling bacteria are indispensable for the recycling of the most abundant polysaccharides (cellulose, xylan, chitin) on earth, and as such fulfill a crucial position in the hunt for renewable energy sources. Members of the prokaryotic genus *Streptomyces* produce a wide range of industrial enzymes and over 60% of all known antibiotics. A leading theme in microbiology is, how do these organisms sense the nutritional state of the environment, and what controls the signal for the switch to morphological differentiation and antibiotic production? Correct timing of the decision when to sporulate is a precarious one, and crucial for survival. While it becomes increasingly clear which genes coordinate the developmental programme, little is known about how these organisms make the principal decision that precedes this, namely how do they sense the nutritional state and translate this information into a signal for morphological differentiation? We recently discovered the GntR-family regulator DasR as a regulatory master switch for carbon and nitrogen utilization in *Streptomyces coelicolor*. An integrated approach combining *in silico* analysis, proteomics and individual target analysis unveiled that the DasR regulon is one of the largest ever identified in bacteria, including regulons for central metabolism, polysaccharide and cell wall degradation, and secondary metabolism. Also, we could directly link DasR with early developmental control, and thus paint a picture of a novel type of carbon regulator that senses the nutritional state of the habitat, maintaining soil-bound vegetative growth until changing circumstances necessitate the switch to sporulation. A model integrating our data will be presented, including a proposed key effector molecule.

19.03

Engineering the steroid catabolic pathway of *Rhodococcus*: inactivation of multiple gene homologues

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Steroids constitute a large class of organic compounds with different bioactive properties and biological functions. Steroids have a major pharmaceutical value for mankind, for example as anti-inflammatory, diuretic, anabolic, contraceptive, anti-androgen, or antibiotic drug. Process routes that are currently in use for the industrial synthesis of steroids (precursors) involve multiple chemical steps, sometimes combined with a few microbial (enzyme) steps. Cleaner biocatalytic routes, starting from abundantly available and cheap precursors such as cholesterol and β-sitosterol, are highly desirable. Setting up routes for steroid biotransformation requires gene technology as well as knowledge of the steroid catabolic pathway on a molecular level to be able to genetically engineer microbial catalysts that will selectively degrade the sterol side-chain without degrading the steroid polycyclic ring. In order to prevent polycyclic steroid ring degradation, the biocatalyst should be devoid of the key enzymatic activities involved in steroid ring opening, i.e. 3-ketosteroid Δ^1-dehydrogenase (KSTD) and 3-ketosteroid-9α-hydroxylase (KSH) activities. A firm knowledge on the molecular level of these two critically important activities thus is needed to generate molecularly defined mutants with blocked steroid polycyclic ring opening.
Rhodococcus species have been widely acknowledged as micro-organisms able to rapidly degrade sterols, producing the steroid pathway intermediates 4-androstene-3,17-dione (AD), 9α-hydroxy-4-androstene-3,17-dione (9OHAD) and

1,4-androstadiene-3,17-dione (ADD) utilizable as precursors in drug synthesis. Molecular toolboxes for *Rhodococcus* have increased substantially over the past several years. We have developed a method for unmarked gene deletion for *Rhodococcus* species, which allowed us to sequentially inactivate multiple genes in one *Rhodococcus* strain. The method can be applied to several *Rhodococcus* species: *R. erythropolis, R. rhodochrous, R. opacus, Rhodococcus* sp. RHA1. Mutants generated by unmarked gene deletion can be used for multiple rounds of gene inactivation by the same system, allowing metabolic engineering of complex pathways.
3-Ketosteroid 9α-hydroxylase (KSH) catalyzes the C9(α) monohydroxylation of the steroid poly-cyclic ring structure. Analysis of deduced amino acid sequences of the *kshA* and *kshB* genes of *R. erythropolis* SQ1, encoding KSH activity, showed that this enzyme is an iron-sulfur containing, two-component class IA monooxygenase with KshA as the terminal oxygenase component and KshB as the oxygenase ferredoxin reductase component. *Rhodococcus* strains were shown to contain several genes homologous to *kshA* in their genomes. Using degenerate PCR primers based on conserved amino acid sequences in KshA, we have been able to identify and clone a total of 8 homologues of the *kshA* gene from two *Rhodococcus* strains. The results of multiple gene inactivations of these homologues will be discussed.

19.04

Members of the SALP family play a role in peptidoglycan assembly and degradation of sporulation-specific cell division

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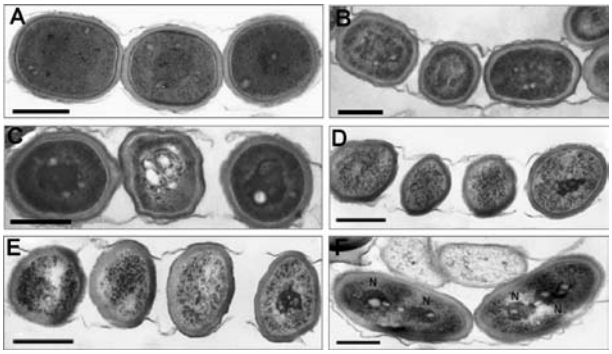
Growth on solid media starts with the germination of a single spore that develops into a complex vegetative mycelium of branching hyphae that are divided into connected multinucleoid compartments by vegetative septa or cross-walls. Environmental signals result in the development of initially aseptate aerial hyphae. Developmental cell division results in the simultaneous production of up to a hundred spore septa in close harmony with chromosome segregation, resulting in chains of hydrophobic, uninucleoid spores. The molecular mechanisms underlying this process, which requires unparalleled complex coordination of cell wall synthesis, DNA segregation, and autolysis, has long been a mystery. The family of SsgA-like proteins (SALPs) is apparently unique to sporulating filamentous bacteria. Four of the SALPs are present in all so far sequenced streptomycetes, suggesting an important role in *Streptomyces* life-cycle. Previously we have shown that *ssgA* and *ssgB* are essential for correct sporulation in *Streptomyces coelicolor*. Knock-out mutants of *ssgC-D-E-F-G* were created in *S. coelicolor* and the effect of the mutations on development and cell division were examined using electron microscopy and confocal fluorescence microscopy. These experiments revealed that *ssgC-G* are involved in septum localization, spore wall synthesis and autolytic spore separation. Confocal fluorescence microscopy was also used to study the localization of the SALPs, using fusions with Green Fluorescent Protein (GFP). Here, we discuss the role of the SALPs, a novel family of proteins, involved in the control of specific aspects of the sporulation process, from initiation of septal peptidoglycan synthesis to the separation of spores. Our observations tell us that the SALPs might function by binding to other proteins, such as enzymes responsible for the synthesis and autolysis of peptidoglycan. Currently, we are looking for interaction partners for the SALPs using genomic approaches.



Transmission electron micrographs of hyphae and spores of *S. coelicolor* and its SALP mutants.
The parental strain M145 produced regular mature spore chains (A) while the spore chains of ssgC (B) and ssgD (C) mutant were highly heteromorphous. Deletion of the late sporulation gene ssgE resulted in almost completely normal mature spores (D). A large proportion of the mature spore chains of ssgF mutant had spores rotated by 90° (E). Around 50% of all spores produced by ssgG mutant were twice, three or even four times the length of normal spores (F). Bar=0.5 μm.

20.01

A CTX-M Extended-Spectrum β-Lactamase in *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*

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Introduction: During a prevalence study on Extended-Spectrum β-Lactamases (ESBLs) in Amsterdam, a *Pseudomonas aeruginosa* strain was isolated from the sputum of a 21 year old male cystic fibrosis outpatient and a *Stenotrophomonas maltophilia* strain was isolated from the sputum of a hospitalized male neonate at the department of neonatology at AMC. Both strains were cefotaxime

resistant and produced ESBL. This remarkable observation was further analyzed.

Methods: The species determination was performed using the VITEK-2 and confirmed by PCR and sequence analysis of the 16S rRNA gene. ESBL production was detected with a combination of the double disk and the combined disk test (DCDT), which includes disks of ceftazidime, cefotaxime, cefpodoxime and cefepime placed around a disk containing amoxicillin plus clavulanate. ESBL genes, SHV, TEM and CTX-M were investigated with PCR and sequence analysis.

Results: The DCDT confirmed the production of ESBL. The Molecular analysis confirmed the species, as *P. aeruginosa* and *S. maltophilia* and showed that both strains contain CTX-M-1 genes.

Conclusion: This is the first description of CTX-M ESBLs in *P. aeruginosa* and *S. maltophilia*. The CTX-M ESBLs provide these pathogens with an additional powerful resistance mechanism, which may have serious clinical implications as *P. aeruginosa* and *S. malthophilia* may become hidden reservoirs for such ESBLs and the adequate therapeutic options become limited.

20.02

Clostridium difficile PCR ribotype 027 toxinotype III in The Netherlands

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June 2005, the more virulent *Clostridium difficile* PCR ribotype 027, toxinotype III was first detected in The Netherlands. In response, the Dutch Centre for Infectious Disease Control, in collaboration with the first known affected hospital and various experts, has drawn up *C. difficile* 027 guidelines for infection control and treatment. The Leiden University Medical Centre serves as a reference centre for diagnostics and typing of *C. difficile*. Laboratories are encouraged to send in samples for typing in case of an outbreak of *C. difficile* associated diarrhoea (CDAD) or clinically suspect cases. Organisation-based surveillance was set up: institutions with CDAD outbreaks are contacted monthly to inquire after incidence, testing strategies, antibiotics use and control measures.

Measures taken in 027-affected hospitals include: treatment of CDAD with vancomycin instead of metronidazole, frequent and thorough cleaning and disinfection, isolation of all patients with diarrhoea until tested negative for *C. difficile* toxin and restriction of certain antibiotics, including fluorochinolones.

Until December 16th, 2005, 344 samples from 29 institutions have been sent in for typing, resulting in 93 type 027 positives from 12 institutions. Epidemic spread of type 027 has been detected in 8 hospitals and one nursing home. Isolated cases of type 027 were detected in retrospective studies in 3 hospitals without further spread. In one region with 3 hospitals, the CDAD incidence appeared to have risen already in the past few years.

In the hospitals with epidemic spread of type 027, a wide range in the monthly incidence of CDAD was observed, from 50 to 114 per 10,000 admissions during the outbreaks. The pre-epidemic incidence varied from 3 to 38 (*figure 1*). By the end of 2005 the incidence has decreased in several institutions. The outbreaks are difficult to control: most hospitals continue to have new cases for a long time. Fortunately, once a *C. difficile* 027 outbreak in a hospital is recognised, spread to other hospitals has not been observed.

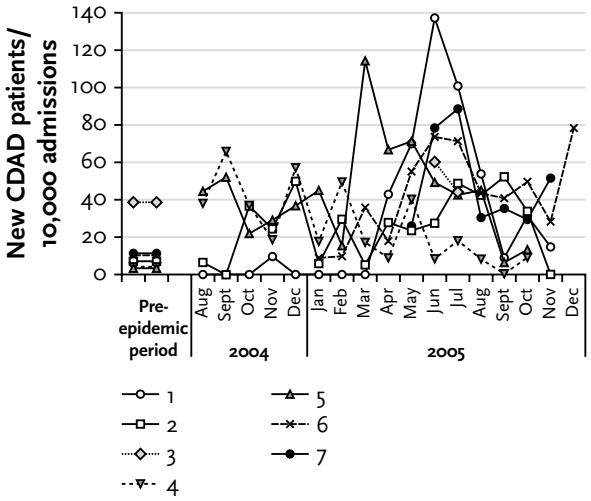

Figure 1. Incidence data of 7 hospitals

20.03

Increase in patients with impetigo caused by a *Staphylococcus aureus* clone intermediate resistant to fusidic acid

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Introduction: In 2002 an increase in impetigo was observed in the Netherlands. Most *Staphylococus aureus* strains (75%) belonged to cluster 105 (Infectieziekten Bulletin 2002;13:11), suggesting clonal relationship. In general practice fusidic acid is often used in the treatment of impetigo.

In 2003, we started in our laboratory, serving mainly general practioners, to determine incidence of impetigo, to study susceptibility patterns and analyzing clonal relationship of S.aureus strains isolated from patients with impetigo. Here we present the results for 2003-2004.

Methods: After isolation and identification of *S. aureus*, strains were tested for routine susceptibility testing (Vitek-2). Disk diffusion was used for susceptibility to mupirocin. Strains were investigated by pulsed-field gel electrophoresis (PFG) for clonal relationship.

Results: In 2003, 51 strains of *S. aureus* were isolated from patients with impetigo. 81% of these strains belonged to cluster 105 and 90% of these strains were intermediate resistant (IR) against fusidic acid. In 2004, 59 *S. aureus* impetigo strains were isolated; 77% belonged to cluster 105 and 92% were IR against fusidic acid. All strains were susceptible to mupirocin.

Conclusion: Clonal spread of a fusidic acid resistant *S. aureus* strain has occurred in impetigo patients in the Netherlands. Therefore the empiric use of fusidic acid in the treatment of impetigo needs reevaluation.

20.04

Investigation of an outbreak of *Salmonella typhimurium* DT104 in the September–November 2005

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Background: *Salmonella typhimurium* definitive phage type (DT) 104 has emerged as an important pathogen in the last two decades. The Dutch national *Salmonella* surveillance relies on isolates from 16 public health laboratories. During September to November 2005, 169 isolates of *S. typhimurium* DT104 were received, which is 10-fold more than expected. This large outbreak prompted an investigation to identify the source of infection in order to enable preventive measures.

Methods: A subset of outbreak isolates was typed by pulsed-field gel electrophoresis (PFGE) and multiple-locus variable-number tandem-repeats analysis (MLVA), the latter performed at the Norwegian Institute for Public Health. In a case-control study, cases (n=109) and population controls (n=411) matched by age and geographical region, were invited to complete a self-administered questionnaire.

Results: Salmonellosis is not notifiable in The Netherlands and various permissions have to be obtained before a patient can be contacted. This caused a two-month delay before questionnaires could be distributed. The cases were dispersed throughout the Netherlands and males and females were equally represented. The age distribution of cases was shifted towards the ages 6-20 years compared to historical data. The molecular typing corroborated the clonality of the isolates and suggested a link to a recent outbreak of *S. typhimurium* DT104 in Denmark, associated with imported beef. The incriminated shipment was traced in the Netherlands by the Food and Consumer Product Safety Authority (VWA) after being distributed through various EU member states. Sampling of the beef identified *S. typhimurium* DT104 of the same molecular type as the outbreak isolates. Cases were more likely than controls to have eaten a particular raw beef product.

Conclusions: Our preliminary results are consistent with this *S. typhimurium* DT104 outbreak being caused by contaminated beef. This investigation underlines the importance of European collaboration, traceability of consumer products and a need for timely intervention into distribution chains.

20.05

Strong increase in integron prevalence in intestinal flora of young children due to cotrimoxazole use

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Background: Although resistance to sulfamethoxazole-trimethroprim (COT) is common among Enterobacteriaceae, COT remains an alternative in the treatment of several infectious diseases, including chronic suppurative otitis media (CSOM). Resistance to sulfamethoxazole (SUL) may be encoded by the *sul1* gene present on integrons. Integrons are strongly associated with multidrug resistance.

Objective: The aim of this study was to determine the influence of long-term COT treatment on the SUL resistance rates and integron prevalence in Enterobacteriaceae in the intestinal flora of young children.

Materials and methods: Faecal samples of 2 groups were studied: 1) 101 children with CSOM recruited for a controlled trial on the effectiveness of prolonged treatment with COT. These were divided into a) 6 wks COT (COT I), b) 12 wks of COT (COT II), and c) placebo group (PLAC). Faecal samples were cultured on day 0 (T0), 6 wks (T1) and 12 (T2) wks, and 2) healthy children (SCHOOL; n=61),visiting day-care centres in Bilthoven. Integron specific PCR primers (*Int1*) were used.

Results: Enterobacteriaceae were cultured from resp 51, 51 pts and 53 children in resp COT, PLAC and SCHOOL. Among these SUL resistance rates at T0, T1 and T2 were 51-52% in the PLAC and 53% in SCHOOL. For both COT I and II the SUL resistance rate increased from 58% at T0 to 95% at T1. For COT I the resistance rate decreased to

58% at T2 while for COT II this rate remained very high (93%). *Int1* genes were positive in 24% of the SUL resistant pt isolates at T0, as well as in the SCHOOL isolates. Interestingly, the relative prevalence of integrons among SUL resistant isolates increased during COT treatment to 48% at T1 to 78% at T2 for the COT II.
Conclusion: Around 12% of Dutch young children carried integrons in the intestinal flora. Treatment with COT caused an increase in the prevalence of integrons and associated multidrug resistance in the intestinal flora. The prevalence rate was positively correlated with the duration of treatment. This finding is another warning that antibiotic treatment should be prescribed with great care since the intestinal flora is an important reservoir for infections.

20.06

Update of *Echinococcus multilocularis* in the Netherlands: evidence of increasing presence in the southern border area in the Netherlands

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Between January 2002 and March 2003, a base line prevalence study was carried out to get a better insight in possible spread of *Echinococcus multilocularis*, the cause of alveolar echinococcosis, since its first recognition in 1996. Foxes in Limburg were investigated for *E. multilocularis* by microscopical examination of the jejunum and PCR method of colon contents. In addition, the same foxes were examined for ecological factors, such as sex, age, condition and stomach contents, to get a better insight into the interaction between the parasite and the fox population dynamics. Of the 196 foxes examined, 25 were positive by either microscopy or by PCR. Most positive foxes were identified during the winter period of January and February 2003. Worm burdens were significantly higher in this study compared to the previous study. In addition, spatial analysis using GPS. A mathematical model describing the parasite population dynamics both in time and in space was fitted to the worm burdens of 229 foxes sampled between 1996 and 2003 in the NL. We found a strong indication that the parasite's reproduction number R0 is greater than 1 and that the parasite is spreading to a wider region in Limburg. Based on the R0 derived from the mathematical model of the parasite's transmission, we explore the effect of public health measures aimed to control the infection. This is the first report of increased infection pressure of *E. multilocularis* in north-western Europe. Prevention and possible public health measures will be discussed.

22.01

Comparative genome analysis in the study of host-microbe interactions

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The study of genome sequence information can provide insight into mechanisms playing a role in the interaction of bacteria with their hosts.
A variety of extracellular proteins of bacteria have been demonstrated to have proteins and/or carbohydrates binding properties, thereby mediating their adherence to host cells. In depth in silico genome mining and comparative genomics can help to firstly identify relevant proteins and their domains and, secondly elucidate the mechanisms underlying these interactions.
As an illustration of such methods, the bioinformatics analysis of a domain involved in the adherence of bacteria to intestinal mucus will be presented. Based on the sequence of a Lactobacillus reuteri protein that was shown to be involved in the adherence to intestinal mucus, we have characterized the domain thought to be responsible for this interaction and identified proteins in other bacteria containing similar domains.

23.01

Detection of hepatitis C virus RNA by transcription-mediated amplification in PCR negative samples during antiviral treatment

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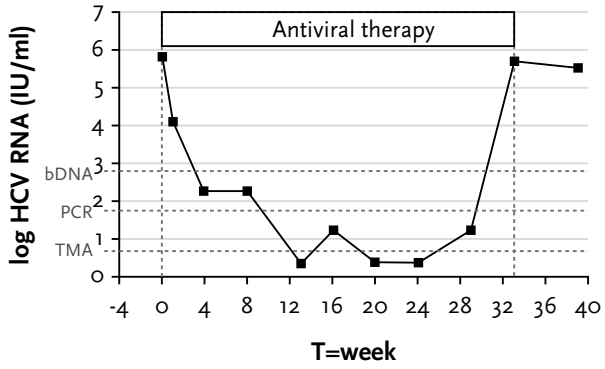
Qualitative assays for the detection of hepatitis C virus (HCV) RNA become more sensitive. The prevalence and significance of low level (i.e. PCR negative) viremia during antiviral therapy are unknown. The aim of our study was to evaluate whether low level viremia occurs during treatment with amantadine hydrochloride, ribavirin and 6 weeks of interferon-alfa2b induction followed by weekly pegylated interferon alfa-2b for a total of 24 or 48 weeks.
HCV-RNA was assessed in plasma at various timepoints before, during and after treatment by: quantitative bDNA (VERSANT® HCV 3.0 assay, Bayer Diagnostics, Berkeley, CA, lower limit of detection (LOD) 615 IU/ml), qualitative RT-PCR (AMPLICOR® HCV Test, v2.0, Roche Diagnostic Systems, Branchburg, NJ, LOD 50 IU/ml), and TMA (Transcription-Mediated Amplification, VERSANT® HCV qualitative assay, Bayer Diagnostics, LOD 5 IU/ml).

115 patients were enrolled. Analysis (ongoing) of a subgroup of 23 initial responders who later broke through or relapsed, revealed 8 patients with either (transient) reappearance (blips), or persistence of HCV at very low levels (PCR negative, 5-10 HCV-RNA IU/ml) during treatment. We observed 2 new patterns in HCV viremia: (1) Persisting low level viremia eventually resulting in relapse; (2) Disappearance and transient reappearance of viremia eventually resulting in breakthrough or relapse (*figure*). Both patterns are characterised by viremia around the LOD of TMA. Review of the literature reveals that low level viremia (5-10 IU/ml) at end-of-treatment results in relapse in most patients. In our patientgroup, reappearance of low levels of HCV-RNA in plasma during treatment was invariably followed by either breakthrough or relapse, and therefore a sign of impending treatment failure.



Figure

23.02

Epstein-Barr virus as a possible pathogen in interstitial lung abnormalities

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Introduction: Interstitial lung abnormalities seen on thoracic images may be due to a wide range of disorders. Extensive medical work-up including a bronchoalveolar lavage (BAL) not always results in a diagnosis. In addition to the standard tests, Epstein-Barr virus (EBV) viral load was determined in BAL fluid and serum in this population, to elucidate a possible role of EBV in the pathogenesis of interstitial lung abnormalities.

Methods: In this prospective study, 33 consecutive patients with unexplained interstitial lung abnormalities on thoracic imaging, who underwent a BAL, were included. In most cases matching serum was obtained. BAL fluids and sera were analysed for the presence of EBV DNA using real-time EBV PCR. In addition all BAL fluids were analyzed by real-time PCR for cytomegalovirus (CMV), human herpes virus (HHV) 6 and respiratory viruses. EBV and CMV serology was carried out on all serum specimens.

Results: Seventeen (52%) out of 33 BAL specimens contained EBV DNA (median 3043 genome equivalents (geq)/mL, range 183 – 4,000,000 geq/mL). From 24 of 33 patients serum was available. In 6 (46%) out of 13 patients with an EBV positive BAL fluid, EBV DNA was present in serum (median 427 geq/mL, range 70 – 2,098 geq/mL), indicating systemic reactivation. EBV DNA was detectable in none of the sera (0/11) of patients with an EBV negative BAL fluid (p=0.012). EBV serology of all patients with EBV positive BAL fluids was consistent with past infection.

CMV DNA was detectable in 5 out of 30 (17%) BAL specimens (median 1725 geq/mL, range 163-2,800,435 geq/mL). One HHV 6 and five rhinoviruses, including two mixed infections with a coronavirus and a para-influenzavirus, were detected in both the EBV positive and negative group.

Conclusions: 1) EBV is detectable in BAL fluid in a remarkable number of cases of unexplained interstitial lung abnormalities, whereas CMV and respiratory viruses seem to play a much less important role. 2) Systemic reactivation of EBV infection was only seen in patients with EBV PCR positive BAL fluids, suggesting a pulmonary origin of reactivation.

23.03

Fatal cases of influenza-associated encephalopathy in the Netherlands

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Introduction: Fatal influenza-associated encephalopathy (IAE) is extremely rare except among Japanese children with 50-100 deaths each year during 1995-2001.

Methods: Two fatal cases of IAE, occurring in 2004 and 2005, were analyzed for clinical details and by pathological and molecular analysis on post-mortem tissue in one case. A 5-year hospital database search (2000–2005) and a MEDLINE literature search (1955-2005) were performed for

documented fatal cases in the hospital and the Netherlands respectively.

Results: Two unrelated native Dutch patients, a 17-year-old male adolescent and a 9-year-old girl, were admitted to the hospital in shock and coma, with signs of profuse bleeding from the gastrointestinal or respiratory tract. The patients had a short prodromal illness consisting of flu-like symptoms, vomiting and increasing drowsiness. Influenza A virus H3N2 was cultured from the respiratory tract of both patients. Only the 9-year-old girl had respiratory symptoms on physical examination and an infiltrate on the chest radiograph. Neuroimaging was normal in both patients. Both patients died within 24 hours after admission following rapidly progressive multi-organ failure, disseminated intravascular coagulation and shock. Post-mortem analysis of the lungs of the 17-year-old male revealed extensive hemorrhages, bronchitis, early diffuse alveolar damage and bronchial epithelial cells positive for influenza A virus by immunohistochemistry. Influenza RNA was detected in multiple organs including the brain, but not in blood specimens. No additional fatal cases were uncovered in the hospital from 2000-2005. To our knowledge, no fatal cases of IAE are documented in the Netherlands after the 1957 Asian influenza pandemic, when 68 cases were reported.

Conclusions: Fatal IAE is described in previously healthy unvaccinated Dutch children and warrants monitoring of severe influenza. Influenza RNA was detected in multiple organs and the brain of one patient, suggesting a causal relationship between viral dissemination and severe neurological and systemic disease.

23.04

Herpes zoster caused by wild-type varicella zoster virus in a vaccinated patient with immunosuppression

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Varicella zoster virus (VZV) in immunocompromised patients is a serious infection, which can lead to significant morbidity. It has been shown that vaccination with live-attenuated VZV (Oka-strain) results in a high seroconversion rate, and provides protection against generalized varicella and local re-activation (herpes zoster) in seronegative patients with chronic kidney disease. We present an immunosuppressed patient who developed herpes zoster despite adequate vaccination.

The patient was 3 years old and had developed end-stage renal failure. In anticipation of future renal transplantation with subsequent immunosuppressive medication, he was vaccinated for VZV in June 2004. Seroconversion was confirmed in August 2004. Renal transplantation followed in August 2005, and immunosuppressive medication was subsequently started. Early November 2005, there was an epidemic outbreak of children with varicella in the day care institution that the patient was attending. On November 10th, he developed pain in his left knee and lower leg, without fever. Two days later, the ankle was swollen, and there was an eruption of vesicles at the ventral side of his lower leg. Examination showed multiple vesicles in the distribution of dermatome L5. VZV was cultured from the vesicles. Treatment was started with acyclovir and the vesicles disappeared completely over the course of 8 days. The VZV-strain from the patient was compared to vaccine-type and several wild-type strains by using PCR-based RFLP of the PSL-region.[1] Results showed that the skin lesions were caused by wild-type VZV, and not by the vaccine-strain. This proves that the patient had been infected with wild-type VZV despite adequate vaccination.

In conclusion, latent infection with vaccine-type VZV does not prevent additional infection with wild-type strains. We speculate that boostering by exogenous VZV in immunocompromised patients leads to a primary infection with localized (i.e. zoster-like) lesions, instead of generalized varicella.

Reference

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23.05

Identification of a fourth human parechovirus serotype

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Introduction: Since the recent discovery of a novel human parechovirus (HPeV) serotype in Japan, three different serotypes are recognized within the genus of HPeVs (formerly echovirus 22 and 23). While genotyping HPeV isolates from our laboratory, we identified a deviant HPeV isolate (K255176-02) from a stool specimen from a neonate with high fever. Complete genome analysis and serotyping was carried out to characterize this isolate further.

Method: For serotyping, neutralisation assays were carried out with antisera directed against the known serotypes

HPeV1, HPeV2 and HPeV3. The complete genome sequence was determined by combinations of consensus primers to generate partially overlapping PCR fragments, which were subsequently sequenced. Extensive phylogenetic analysis was carried out using the Jukes and Cantor substitution model and Neighbor-Joining trees were constructed.
Results: K255176-02 could not be neutralized by antisera directed against HPeV1, HPeV2 and HPeV3. Phylogenetic analysis of the complete genome showed that K255176-02 was most related to the HPeV2 prototype CT86-6760. However, the genetic distance is considerable (0.313) and comparable with the distances to other HPeV prototypes.
Conclusion: A fourth HPeV serotype was identified from a child with a mild febrile illness.

24.01

Zorg van de toekomst en ICT van de toekomst

G. Freriks
Convenor CEN/TC251 WG1, TNO Quality of Life

In deze presentatie komt een aantal ontwikkelingen rond het Elektronisch patiëntendossier (EPD) aan bod:
- Ontwikkelingen in de ICT
- Ontwikkelingen in de zorg
- Europese en Nationale EPD-norm: ICT van de toekomst voor zorg van de toekomst
- Ondersteunende ICT-Infrastructuur, xISsen, Eénheid van Taal
- De komende taak voor de wetenschappelijke verenigingen
- Wat moet de zorgverlener er allemaal mee?

P001

Characterization and expression sites of newly identified chicken collectins

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Collectins are members of the family of vertebrate C-type lectins. They have been found almost exclusively in mammals, with the exception of chicken MBL. Because of their important role in innate immunity, we sought to identify other collectins in chicken. Using the amino acid sequences of known collectins, the EST database was searched and related to the chicken genome. Three chicken collectins were found and designated chicken Collectin 1 (cCL-1), chicken Collectin 2 (cCL-2), and chicken Collectin 3 (cCL-3), which resemble the mammalian proteins Collectin Liver 1, Collectin 11 and Collectin Placenta 1, respectively. Additionally, a lectin was found which resembled Surfactant Protein A, but lacked the collagen domain. Therefore, it was named chicken Lung Lectin (cLL). Tissue distribution analysis showed cCL-1, cCL-2 and cCL-3 are expressed in a wide range of tissues throughout the digestive, the reproductive and the lymphatic system. Similar to SP-A, cLL is mainly localized in lung tissue.
Phylogenetic analysis indicates that cCL-1, cCL-2 and cCL-3 represent new subgroups within the collectin family. The newly found collectins may have an important function in avian host defence. Elucidation of the role of these pattern-recognition molecules could lead to strategies that thwart infectious diseases in poultry, which could also be beneficial for public health.

P002

Recombinant production of the antimicrobial peptide thrombocidine-1 and derivatives in *Pichia pastoris*

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Thrombocidins (TCs) are microbicidal peptides from human blood platelets contributing to innate immunity. TC-1 and TC-2 are derivatives of CXC-chemokines NAP-2 and CTAP-III, respectively, differing from these chemokines by 2 amino acid C-terminal truncations. TC-1 is the most potent thrombocidin, with broad-range microbicidal activity at low micromolar concentrations. The C-terminal truncation is imperative for microbicidal activity. We hypothesize that the C-terminal truncation of NAP-2 results in a conformational change required for the microbicidal activity of TC-1, and aim to study this by nuclear magnetic resonance (NMR). As a first step a *Pichia pastoris* recombinant protein production system was tested for production of sufficient amounts of NAP-2 and TC-1 for NMR analysis.
In earlier studies with 15-mer synthetic peptides covering the entire TC sequence, peptides with highest microbicidal activity were identified in a region near the N-terminus of TC-1. The cysteines in the CXC-motive were imperative for the microbicidal activity of these peptides. To study the structural requirements of the N-terminus in the complete TC-1 protein, N-terminally truncated TC-1 variants, lacking the first or both cysteine residues of the CXC-motive, were also produced using *P. pastoris*.
Recombinant NAP-2 and TC-1 were produced highly efficiently by *P. pastoris*, yielding concentrations of about 100 mg/l in culture supernatant. However, production of the TC variants lacking the first or both cysteine residues only yielded about 10 mg/l and 0.25 mg/l in culture supernatant, respectively. All recombinant proteins were subsequently purified using cation-exchange chromatography. Currently, the recombinant NAP-2 and TC variants are being analyzed by mass spectrometry, and their antimicrobial activity is investigated.

P003

***Capnocytophaga canimorsus* infection in a rabbit**

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A 2 year old healthy pet rabbit was bitten in the head by a dog. The rabbit was treated with a trimethoprim/sulfonamide combination, but its condition deteriorated and an abscess developed. A sample was taken from the abscess and a pure culture of slow growing bacteria was found on sheep blood agar under anaerobic conditions after 48 h incubation at 37°C. Gram-staining of the colonies showed that it were slender fusiform gram-negatieve rods. Upon subculturing on chocolate agar the strain grew well under microaerophilic conditions. Rapid ANA identified the organism as *Capnocytophaga* spp. Real time PCR with

Capnocytophaga canimorsus specific primers and nucleotide sequencing of the PCR product showed that the isolate was actually *C. canimorsus*. The rabbit was treated with doxycycline per os and amoxicillin and clavulanic acid soaked tampons were inserted in the abscess cavities and the rabbit recovered.

Many cases of *C. canimorsus* infections following dog bites have been reported in human medicine.[1,2] To our knowledge, this is the first report of a *C. canimorsus* infection in a species other than man.

References

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P004

Intravenous therapy with colistin for ventilator associated pneumonia with multidrug-resistant *Pseudomonas aeruginosa*

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Clinical case: The patient concerns a 57-year old man who was admitted to the surgical intensive care unit (ICU) of our hospital after resection of oesophageal carcinoma and gastric tube reconstruction. Post-operatively the patient developed a acute respiratory distress syndrome and a ventilator-associated pneumonia (VAP): *Pseudomonas aeruginosa* and *Klebsiella oxytoca* were isolated as causative pathogens of the VAP and treatment was initiated with antibiotics and corticosteroids.

Under antibiotic treatment the patient remained colonized with multiple strains of *P. aeruginosa*; at attempts to stop the antibiotics, the pulmonary function deteriorated and the antibiotic treatment had to be restarted. Under this treatment the *P. aeruginosa* strains became increasingly resistant, untill, ten weeks after admittance to the ICU, the patient became infected with a strain resistant to all convential antibiotics with activity against pseudomonads. *Figure 1* depicts the antibiotic treatment the patient received and the susceptibility pattern of the isolated pseudomanas strains.

Decided was on a regimen with intravenous colistin methanesulphonate, in addition to oral azithromycin and aerosolised colistin and tobramycin. Under this regimen, the pulmonary function improved and four weeks later the patient could be detubated. Six months after his operation the patient was discharged to a revalidation center.

Discussion: The use of the antibiotic colistin was abandoned in the nineteen-seventies, due to reports of its nephrotoxicity and neurotoxicity. In recent studies however (see table 1), the drug seems to have far less toxic side-effects than previously thought, and its use may therefore be an option in the treatment of infections with multidrug-resistant, Gram-negative bacteria.

Table 1. Nefrotoxic and neurotoxic side effects of intravenous colistin therapy

	NUMBER OF PATIENTS	NEFRO-TOXICITY	NEURO-TOXICITY	MOR-TALITY
Price et al. Br Med J 1970	14	100%	unclear	57%
Koch-Weser et al. Ann Int Med 1970	288	20%	7%	56%
Conway et al. Thorax 1997	53	unclear	96%	2%
Levin et al. Clin Infect Dis 1999	59	27%	0%	37%
Markou et al. Crit Care 2003	24	14%	unclear	42%
Linden et al. Clin Infect Dis 2003	23	n.v.t.	4%	61%
Li et al. J. Antimicrob Chemother 2003	12	0%	8%	0%
Garnacho-Montero et al. Clin Infect Dis 2003	21	24%	0%	62%
Reina et al. Intensive Care Med 2005	55	0%	unclear	29%
Michalopouloset et al. Clin Microbiol Infect 2005	43	19%	0%	28%
Falagas et al. BMC Infect Dis 2005	17	unclear	5%	41%
Berlana et al. Am J Health Syst Pharm 2005	12	0%	none reported	18%
Petrosillo et al. Clin Microbiol Infect 2005	14	7%	none reported	50%

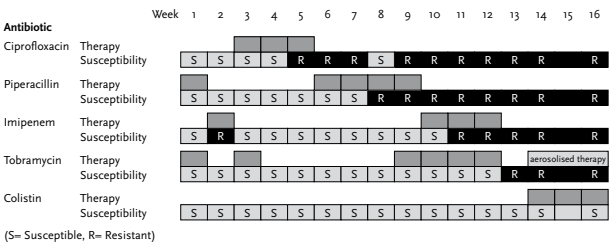

Figure 1.

P005

Lymphogranuloma venereum proctocolitis: two-year follow-up as Crohn's disease

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Introduction: Lymphogranuloma venereum (LGV) proctocolitis is an emerging sexually transmitted disease among men who have sex with men (MSM).
Methods: A patient with proctocolitis suggestive of Crohn's disease is described, and additional pathological and molecular studies were performed on rectal tissue.
Results: A 33-year-old man, known to have sex with men, was referred to the hospital with a 2-year history of slowly progressive complaints of constipation, rectal mucopurulent discharge, bleeding and pain. Crohn's disease was previously diagnosed and considered therapy-resistant following unsuccessful treatment with sulfasalazine, corticosteroids, azathioprine and infliximab respectively. Stool frequency was once a week and followed by fever after each passage of stool. Symptoms had reduced remarkably during recent minocycline treatment for acne vulgaris. Laboratory findings showed anemia and thrombocytopenia. Testing for HIV infection, previously refused by the patient, was positive (CD4 count 69 x 10^6/L; HIV-RNA 229.000 copies/ml). A rectal biopsy showed both mucosal and submucosal ulceration with predominantly lymphocyte and plasma cell infiltration in the lamina propia. *Chlamydia trachomatis* serovar L2 was detected by a LGV-specific real-time PCR and genotyping using restriction fragment length polymorphism analysis on rectal biopsies obtained during 2 years. A diagnosis of LGV proctocolitis was made and Crohn's disease was rejected. Concomitant infections (*Treponema pallidum, Neisseria gonorrhoeae,* herpes simplex virus type 2, *Mycobacterium* sp., *Clostridium difficile, Entamoeba histolytica*, and other less common pathogens) were excluded by microscopy, culture or PCR. Immunosuppressive therapy was discontinued and azithromycin was administered for 4 weeks with good clinical response.
Conclusion: A prolonged course of LGV proctocolitis is described in a patient with HIV and additional immunosuppressive therapy. LGV can resemble Crohn's disease both clinically and histologically, and should be excluded in MSM with proctocolitis.

P006

Sero-epidemiology of *Campylobacter* in The Netherlands

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Introduction: The annual number of episodes of gastroenteritis due to *Campylobacter* in The Netherlands is estimated to be 100.000. This number is based on extrapolation of culture results from population based studies. The number of culture confirmed cases of *Campylobacter* infection peaks in the first five years of life. A sero-epidemiological study of *Campylobacter* will clarify the relation between age and presence of former infection with *Campylobacter*.
Methods: Using a validated ELISA system, IgG antibodies to *Campylobacter* are measured in a randomly selected sample (n=500) of the PIENTER serum collection.
Results: Seroprevalence of *Campylobacter* IgG antibodies increases with age, starting with approximately 25% in the first year to > 95% at the age of 20. After seroconversion, antibody levels further increase with age, suggestive of repeated re-infection. Modeling of data points to one infectious episode every 6-7 years, yielding 2-3 million infections/year in The Netherlands.
Conclusion: Sero-epidemiological data demonstrate repeated infections with *Campylobacter* throughout youth. From young adulthood, > 95% of the Dutch population has serological evidence for a *Campylobacter* infection in the past.

P007

Enhanced surveillance of *Listeria monocytogenes* in the Netherlands preliminary results of the first half year

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In January 2005, an enhanced surveillance of *Listeria monocytogenes* has started in The Netherlands. All laboratories were requested to report positive cases to the public health services and submit *Listeria* isolates of patients with meningitis or septicaemia to the Netherlands Reference Laboratory for Bacterial Meningitis (RBM). The RBM

sends these strains to the National Institute for Public Health and the Environment (RIVM) for serotyping and pulsed-field gel electrophoresis (PFGE). The laboratories send *Listeria* isolates of cases with other clinical manifestations directly to the RIVM. The public health services collect background information from patients, using a standardised questionnaire.

Thirty-five patients with symptom onset between 1 January and 1 July were reported, including 2 pregnancy-related cases. Seven patients (20%) died, as well as the newborn child of one pregnant woman. Serotypes 1/2a (63%) and 4b (31%) were predominating. Four clusters were identified, including one cluster of isolates of 15 patients with serotype 1/2a and a genotype that was only found since 2004. A clear source for this cluster was not found. A questionnaire was returned for 28 patients (80%). Of these, 75% had predisposing conditions for listeriosis. Septicemia was the most common diagnosis (37%), followed by meningitis (22%), gastroenteritis (22%) and pneumonia (15%). High percentages of patients reported eating sausage (75%), cooked or smoked ham (64%), smoked salmon (54%), Brie (36%), raw vegetables or salad (93%), or eating in a restaurant (57%).

In the first half of 2005, the number of cases in the enhanced surveillance was higher than expected (incidence of 4.3 per million inhabitants per year), which is in accordance with the increasing number of reported cases since 2003. The surveillance also seems to lead to improved submission of *Listeria* isolates by the laboratories, as a result of which also less severe *Listeria* infections are notified. When the surveillance includes more patients, it should become clear if risk factors and symptoms are serotype/genotype-specific, as is indicated by these first data.

P008

Optimizing use of ciprofloxacin in a large teaching hospital: a prospective intervention study

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Objective: Antimicrobial resistance to ciprofloxacin, a valuable second-line antibiotic, is increasing. To limit this increase the inappropriate use of ciprofloxacin should be discouraged. The objective of this study was to reduce the number of inappropriate prescriptions and improve the quality of ciprofloxacin prescriptions by way of educational intervention.

Methods: Five units (197 beds) of the departments of Internal Medicine, Gastro-Enterology, Surgery, Urology and Pulmonary Diseases, selected because of a high rate of ciprofloxacin prescription, participated in a prospective intervention study. The study comprised three periods of three months: 2 observation periods (phase 1 and 3) and an intervention period (phase 2). During the two observation periods all ciprofloxacin prescriptions were registered and the quality of each ciprofloxacin prescription was evaluated in a standardized manner by two experts in infectious diseases independently. During the intervention period physicians prescribing ciprofloxacin were interviewed by a medical microbiologist, and educational presentations were given to physicians of the participating units.

Results: During phase one 491 prescriptions/1000 admissions of ciprofloxacin were prescribed, declining to 184 prescriptions/1000 admissions in phase three, a reduction of 62.5%. The greatest reduction was observed in units of the Departments of Surgery and Urology (83.9% and 75.6% respectively), mainly due to a reduction of erratic prophylactic use. Unjustified prescriptions (no use of antibiotics indicated) decreased with 25.9%. Inappropriate prescriptions (wrong choice of antibiotic or duration of prescription) declined from 69.5% to 57.7%, mainly due to the decrease of ciprofloxacin courses of too long duration. Definitely appropriate prescriptions increased with 33.5%.

Conclusion: Intervention by direct consultation of a medical microbiologist and educational presentations led to markedly reduced use of ciprofloxacin and improvement of the quality of ciprofloxacin prescription in the three months following the intervention.

P009

Regional, seasonal differences and secular trends in incidence and antibiotic resistance of *Campylobacter* in a nationwide surveillance study in The Netherlands: an overview 2000-2004

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Objectives: *Campylobacter* is the most frequent cause of bacterial gastroenteritis worldwide. We describe epidemiological features of culture-proven *Campylobacter* infections in The Netherlands over the years 2000-2004.

Methods: Data from two ongoing projects for surveillance of infectious diseases in The Netherlands were used, covering 3 and 8 million inhabitants, respectively. Incidence and resistance rates were analysed over time, by region, by level of urbanization, for seasonal variation and for recent travel history.

Results: The incidence of culture-proven *Campylobacter* infections showed an incidental decrease in the year 2003, in time related to an avian flu outbreak in poultry. The incidence of *Campylobacter* infections was highest in the southern part of The Netherlands; 55.7 per 100.000 in the south versus an average of 39.1 per 100.000 in the other parts of The Netherlands. The incidence was much lower in the rural than the urban areas. High stable rates of resistance were observed for fluoroquinolones (35%). Resistance to erythromycine was low but increasing over the years. Highest resistance rates to erythromycine were found in the south of The Netherlands. Resistance rates increased with increasing urbanization level. Analysis of fluoroquinolone and macrolide resistance in *Campylobacter* isolates demonstrated an inverse seasonal pattern with higher incidence and lower resistance rates in summer and lower incidence and higher resistance rates in winter. Resistance to predominantly fluoroquinolones was considerably higher in travel- related infections (54%), as compared to endemic ones (33%).
Conclusion: We found regional differences in incidence and resistance rates, both being highest in the south of The Netherlands. Furthermore we found an inverse relationship between resistance rates to fluoroquinolones and macrolides and the incidence of campylobacteriosis. The high resistance rates to fluoroquinolones warrants reconsideration of its use as drug of first choice in the empiric treatment of presumed *Campylobacter* infections.

P010

Nosocomial rota- and adenovirus gastroenteritis; a retrospective study over 5 years in 2 hospitals in The Netherlands

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Introduction: Rota- and adenoviruses are highly contagious pathogens causing infectious gastroenteritis in children. In order to determine the nosocomial infection rate, a retrospective study over 5 years was performed in one neonatal and two pediatric wards from two hospitals in the east of the Netherlands.
Methods: Over a 5 years period (1999-2003), all rota- and adenovirus latex-agglutination tests were selected. Diarrhea at admission or within two days thereafter was assumed to be community acquired. If the positive test was requested > 2 days after hospital admission, the reason for admission was traced. The possible source was searched for in case of nosocomial infections. A patient with a positive test in the same ward ≤ 6 days (rotavirus) or ≤ 10 days (adenovirus), was assumed to be the index patient.
Results: From the 943 requests from the pediatric wards, 250 tests were rotavirus positive (26,5%). Of these 250, 38 (15,2%) were acquired in the hospital. According to our definitions, 31 (81,6%) of those, 38 were nosocomial infections an index patient in the same ward was found. 955 requests for adenovirus were tested, from which 60 tests (6%) were positive. 16 of those 60 (26,5%) were found to be acquired in the hospital. From those 16, 6 (37,5%) were transmitted by an index patient in the same ward. In the neonatal ward, no rota- or adenovirus infections were found.
Conclusion: This is the first report on the epidemiology of nosoconial rota-and adenovirus infections in the Netherlands. From the positive tests, 15,2% represented rotavirus infection acquired in the hospital, of which 81,6% was transmitted by an index patient. For adenovirus, the percentages were 26,5% and 37,5% respectively. These results may be useful for both data comparisons in other hospitals and as a starting point for adjustment of hygiene measurement.
In conclusion, existing prevention measures could not prevent nosocomial transmission of rota- and adenovirus.

P011

Methicillin-Resistant *Staphylococcus aureus* in the Dutch community

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Introduction: The emergence of community acquired methicillin-resistant *Staphylococcus aureus* (MRSA) infections in the community, especially strains possessing the Panton-Valentine leucocidin (PVL) genes, is a concern. We studied the incidence of MRSA among specimens send to a laboratory for general practitioners.
Methods: Methicillin resistance was determined by Vitek2,oxacillin disk diffusion and E test. Mec A presence by MRSA screen test. Positive results were confirmed by a reference laboratory(RIVM), which also performed genotyping and PVL PCR.
Results: From 2002-2006, 20 patients with a MRSA infection were seen. A dramatic increase, not attributable to an outbreak, was seen in 2005 with 12 patients. All strains, except 1, were community-acquired. 8 strains (6 isolated in 2005) were associated with furunculosis and all were PVL+.
Conclusion: Isolation of MRSA, especially PVL+, is rapidly increasing in this laboratory serving general practitioners in the center of the country.

P012

Molecular genetic analysis of *Cryptosporidium* found in fecal samples from human patients in the Netherlands

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Cryptosporidium is an intestinal protozoan parasite causing diarrhea in animals and man. Molecular genetic typing is the most effective method to distinguish the different (sub)species. In humans, mainly two species are found: *Cryptosporidium hominis*, also called *Cryptosporidium parvum* genotype H or type 1, and *C. parvum* (genotype C or type 2). *C. parvum* is also found in animals. To study the epidemiology and the genetic diversity of *Cryptosporidium* in the Dutch population, we genotyped a cohort of 97 human *Cryptoporidium* positive patients with diarrhea. Stool isolates came from different regions in the Netherlands and were genotyped by DNA sequence analysis for five loci on the *Cryptosporidium* genome: 18S rRNA gene (18S), the *Cryptosporidium* outer wall protein (COWP), the heat shock protein 70 (HSP70), and the two microsatellite markers ML1 and ML2. Of the 97 samples, 91 gave interpretable sequence data. Our results showed that, except for one C. felis isolate, all isolates were identified as *C. parvum* (22%), *C. hominis* (70%) or both (7%), the latter depending on the marker considered. HSP70, ML1, COWP and 18S sequencing was most successful. ML2 sequencing was most difficult due to long GA-repeats. For COWP we found one *C. parvum* and one *C. hominis* genotype, making it the most conserved marker. HSP70 showed one *C. hominis* and two *C. parvum* genotypes. ML1 and ML2 showed the highest variability for *C. parvum*. For 18S we found only one *C. parvum* and two *C. hominis* genotypes. HSP70 was the only marker that identified the C. felis isolate. The total number of *C. hominis* cases showed a peak in the period September – November, coinciding with the period that most cases of cryptosporidiosis were reported. Gender difference did not correlate with genotypes and there were only slight differences between regions. The majority (80%) of the cases originated from children between 0 -7 years, mainly with (>70%) *C. hominis*. The remainder was from patients > 29 years and showed a tendency for more *C. parvum*.
Concluding, *C. hominis*, for which we find two subspecies, is the most important genotype in the Dutch human population.

P013

Occupational risk of cytomegalovirus infections in female day-care personnel in the Netherlands

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Cytomegalovirus (CMV) is an ubiquitous prevalent infection with a usual subclinical course in the immunocompetent host. Nevertheless, primary or recurrent CMV infection acquired during pregnancy may affect the foetus resulting in developmental disabilities. High risk women should be identified to offer preventive or therapeutic strategies. Children in day care centres harbour a high risk of acquiring a CMV infection and can thus potentially transmit the virus to pregnant day care personnel. The aim of the study was to evaluate the occupational risk of CMV infection in female day care personnel.
As such, we determined the CMV IgG seroprevalence and its co-variates in 319 female day-care personnel recruited from 56 regional day-care facilities belonging to 14 different municipalities in the Netherlands who were for 95% of Dutch origin. The participating response was 95%. The seroprevalence was 12.5% in the age group £ 19 years (n=8), and showed a sharp increase to 50% (40/80) in the age group 20-24 years. In women above 35 years a seroprevalence of maximum 65% was reached (n=113). Comparing day care personnel (20-24 years of age) without own children to an equal female control group recruited from the general population, 31% (16/52) was found positive for CMV IgG antibodies in the latter group (Chisquare=4,78, p=0,03). Having own children was not related to CMV-seroprevalence in day care personnel, whereas seniority significantly influenced seropositivity (OR adjusted for age 1,1; p=0,03). The overall occupational risk of CMV infection in female day-care personnel was increased (OR adjusted for age 2,47; p < 0,001). In conclusion, these data suggest the need to provide preventive measures for female day-care personnel.

P015

***Mycobacteria* attacked with a Troian trick**

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Introduction: Using a site-specific and site-avoidance drug delivery tool (carrier), the objective of this study is to improve and shorten the drug treatment of tuberculosis, using existing antimycobacterial agents. The addition of this carrier to the conventional therapy is called the TaRgeting Obtaining Improved ANtimycobacterial-therapy or short: Troian-therapy.
Methods: Different antimycobacterial agents were evaluated *in vitro*, on their bacteriostatic versus bactericidal activity against *Mycobacterium avium* and *Mycobacterium tuberculosis*, in relation to the time of exposure and the growth phase of the mycobacteria. In C57Bl/6 mice, a disseminated *M. avium* infection was established, to determine the therapeutic efficacy of the Troian-therapy in comparison to the conventional treatment.
Results: The *in vitro* study revealed the rapid and high killing capacity of Amikacin. In the experimental *M. avium* infection, application of Amikacin in the carrier effected in a rapid decrease of the mycobacterial load in the infected organs and complete elimination after 12 weeks. This in contrast to the conventional treatment without the Troian-therapy, which resulted in a substantially decreased but non-eliminated load after 24 weeks.
Conclusion: The addition of the Troian-therapy, allows a reduction of the treatment duration in a *M. avium* infection in mice, to 12 weeks, without a risk for relapse. These results open new ways in the treatment of tuberculosis and thus will value of this new drug delivery-based treatment be studied in a mouse model of pulmonary tuberculosis.

P016

Evaluation of three automated enzyme immunoassays for the detection of HIV combined antibody and antigen

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Introduction: Fourth generation (4th gen) HIV assays that detect p24 and HIV-1/2 antibodies (Ab) simultaneously, reduce the diagnostic window in recent HIV infection as compared to 3rd gen HIV-1/2 Ab only assays. Despite the increased diagnostic sensitivity, there has been concern about the specificity of 4th gen HIV assays. The present study focussed on the specificity and precision of the recently introduced HIV Ag/Ab Combo for the Abbott Architect i 2000 SR processor (HIV Ar) compared to the HIV Ag/Ab Combo for the Abbott Axsym Plus processor (HIV Ax) and the HIV Duo Ultra Ag/Ab test for the bioMérieux Mini Vidas (HIV bM).
Methods: The specificity of the assays was assessed in 553 routine plasma samples of pregnant women (group 1), 20 serum samples with positive IgM for CMV, EBV, hepatitis A virus, Toxoplasma gondii and rheumatoid factor (group 2), 99 plasma samples from solid organ transplant donors (group 4) and 35 serum samples from routine HIV testing (group 5). One HIV-1 seroconversion panel (n=6) was tested for sensitivity (group 6). To determine the ability of the HIV Ar and HIV bM to detect HIV-1 group M and group O p24/Ab and HIV-2 Ab, 33 HIV group M, 5 group O and 10 HIV-2 HIV Ax positive serum samples were analyzed (group 7). Finally, the intra- and interrun coefficients of variation (CV) were determined with negative, low positive and high positive samples.
Results: No reactive samples were found for all 3 assays in groups 1-4. One sample of group 5 was false reactive in the HIV Ar and HIV Ax (specificity for HIV Ar and HIV Ax > 99% and for HIV bM 100%). In group 6 complete concordance was found for all assays. Both HIV Ar and HIV bM were reactive in all samples of group 7. Overall intrarun CV's for HIV Ar, HIV Ax and HIV bM were 7.8, 18.2 and 5.0%. Overall interrun CV's for HIV Ar, HIV Ax and HIV bM were 20.1, 7.5 and 2.6%.
Conclusion: We conclude that the HIV Ar is highly specific as compared to the established HIV Ax and HIV bM assays. The precision of the HIV Ar was comparable to the HIV Ax, with better intrarun CV for the HIV Ar and better interrun CV for the HIV Ax, The HIV bM showed the highest precision of the assays tested.

P017

Nonfermenters and yeasts under the influence: ethanol disinfection of saline dispenser causes misidentification in Vitek 2 GN and YST cards

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Introduction: Vitek 2 is an automated system for the identification of bacterial pathogens and yeasts. Recently the new GN and YST cards were introduced, which enable identification of more taxa of Gram-negative bacilli and yeasts respectively than before. We found that in the new cards *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were constantly misidentified and that yeasts gave poor results overall. Attention was focused on the bottletop dispenser used to fill test tubes with saline solution for suspension preparation. According to a local protocol the dispenser was cleaned with ethanol every night and reconnected to the saline bottle in use the next day. We tested the bottle in use for ethanol and found a concentration of 0.4 g/litre. We conducted experiments to determine the effect of ethanol on identification results.
Methods: We inoculated GN cards with four strains of *P. aeruginosa* and three strains of *A. baumannii*, and YST cards with three strains of *Candida glabrata*, one *Saccharomyces cerevisiae* and one *Candida parapsilosis*, each strain from suspensions with 0, 0.1, 0.2, 0.3 and 0.4 g/litre ethanol.

Results: Three strains of *P. aeruginosa* were identified correctly with 0-0.1 g/liter ethanol, but identified as *Burkholderia cepacia* with ≥ 0.2 g/litre ethanol; one strain was identified correctly with and without ethanol. *A. baumannii* was identified correctly without ethanol only, with ethanol results were *Klebsiella oxytoca* or 'Unidentified'. Without ethanol all yeast strains were identified correctly except one *C. glabrata* (*Candida lipolytica*). With ethanol all yeast strains were 'Unidentified' except *C. parapsilosis*, which was identified correctly with 0.1-0.2 g/litre ethanol, but 'Unidentified' with ≥ 0.3 g/litre ethanol.

Conclusions: Residual ethanol on the filling tube of the dispenser interfered with the reactions or the viability of the tested bacilli and yeasts in the cards. The problem was solved after we started using disposable materials for saline dispensing and stopped ethanol disinfection.

P018

Real-time detection of ciprofloxacin resistance in *N. gonorrhoeae* in clinical samples

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In both STI populations and patients of general practioners PCR is suitable for diagnosing gonorrhea caused by infection with *Neisseria gonorrhoeae* (NG). Positive samples need to be confirmed targetting the NG *opa* genes. In the past decade the proportion of NG strains with resistance to ciprofloxacin has risen dramatically in STI patients.[1] By sequencing 170 cultured NG strains we confirmed other studies that the GyrA gene AA95Asp to 95Gly/Ala/Asn mutations are associated with Intermediate (I) or Resistant (R) ciprofloxacin phenotype.

We evaluated a paper [2] describing FRET real time mutation analysis targetting the GyrA gene overlapping AA95 performed directly on clinical samples. A prospective panel of 79 STI patients was tested on available PCR screening samples from different locations (urethra, proctum, cervix). Of these, 41 were negative in both the real-time *opa* confirmation PCR and the AA95 FRET mutation assay, whereas 20/21 were also culture negative, proving a high specificity. The other 38 NG culture positive samples showed some discrepancies in antibiotic resistance patterns derived by culture versus real-time FRET assay however. One sample was negative in both confirmation and mutation real time assays but was ciprofloxacinR (MIC ≥ 1). All other culture positive samples were positive in the confirmation PCR. Of two culture positive samples with cipro I (0.06 < MIC).

Reference

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P019

Clinical evaluation of five commercial EBV ELISA assays

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Background: In our laboratory for serology and virology an immune fluorescence assay (IFA) is performed for the determination of EBV anti-VCA IgM and EBV anti-VCA IgG. If needed an anti-EBNA IgG assay is performed. We are planning to switch to an EBV ELISA assay measuring these three parameters in one run. We therefore evaluated five commercial EBV ELISA assays on 72 clinical samples.

Methods: 72 serum samples were selected: 20 sent to us for serological screening, and 52 sent to us for EBV serological testing in order to exclude or prove EBV infection. Based on IFA and clinical presentation 21 sera (14 pt.) were from patients with acute EBV infection, three sera (2 pt.) from patients with acute CMV infection and in 28 sera (28 pt.) EBV infection was less likely. The commercial assays for VCA IgG, VCA IgM, EBNA IgG included were: BIOTEST, PANBIO, TRINITY, SERION ELISA, and VIRONOSTICA. All assays were performed according to the manufacturer's instructions. From the three test results of each ELISA an overall interpretation per sample was made. At least 3/5 interpretations had to be identical in each sample.

Results: In 37/72 samples (51%) all test parameters had an identical result and in 60/72 samples (83%) the interpretation of the result of three parameters were identical. One sample probably was of bad quality, because all assays revealed a different conclusion. In 11 samples 14 essential differences were observed: EBNA IgG positive in seronegative sample (4x), VCA IgG positive in seronegative sample (2x), VCA IgG and EBNA IgG positive in seronegative sample (1x), seronegative in primo infection (5x), EBNA IgG negative in EBNA IgG positive sample (2x). All ELISA assays had one or more wrong interpretations (range 1/72 (1,4%)-5/72 (6,9%).

Conclusion: Evaluation of five commercial EBV ELISA assays in 72 clinical serum samples revealed a poor agreement if all parameters were considered (51% agreement). An identical interpretation of the results was observed in 83% of the samples. This study shows that none of the tested assays has a 100% positive or negative predictive value for past or present EBV infection.

P020

Evaluation of VIRCELL enzyme immunoassay and indirect immunofluorescent assay for the detection of antibodies against *Legionella pneumophila*

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Introduction: Bacteria of the family Legionellaceae are ubiquitous in both natural and man-made aqueous environments and inhalation or aspiration of contaminated water can cause Legionnaires' disease (LD), a severe pneumonia. The aim of our study was to evaluate commercial ELISA and IFA assays (VIRCELL, S.L., Santa Fe, Granada, Spain) for the detection of antibodies against *Legionella pneumophila*.
Methods: We evaluated the ability of VIRCELL *L. pneumophila* serogroup 1 immunoglobulin G (IgG) and IgM indirect immunofluorescent assay, *L. pneumophila* serogroup 1 IgM and IgG enzyme-linked immunosorbent assay (ELISA) and *L. pneumophila* serogroup 1-6 IgM plus IgG combined ELISA to diagnose LD in a well described sample of patients with and without LD. Also, we determined the agreement, sensitivity and specificity of the different VIRCELL assays in comparison to a validated ELISA assay (SERION classic ELISA).
Results: We included 129 serum samples of 65 patients with proven LD and 50 serum samples of 29 patients with respiratory tract infections other than *Legionella*. Clinical sensitivity and specificity were respectively 74.6% and 96.6% for IFA IgM, 65.1% and 88.0% for IFA IgG, 92.3% and 100% for ELISA IgM, 43.3% and 96.6% for ELISA IgG and 90.8% and 100% for IgM plus IgG combined ELISA. Compared to SERION classic ELISA, agreement, sensitivity and specificity were respectively 80.0%, 83.1% and 78.4% for IFA IgM, 75.2%, 66.0% and 79.5% for IFA IgG, 89.5%, 82.0% and 97.6% for ELISA IgM, 81.9%, 88.9% and 78.0% for ELISA IgG and 93.5%, 90.0% and 96.6% for IgM plus IgG combined ELISA.
Conclusion: The value of a positive diagnostic result obtained by VIRCELL IFA IgM, VIRCELL IFA IgG and VIRCELL IgG ELISA might not be acceptable for a diagnostic assay. Both the high specificity and sensitivity of VIRCELL ELISA IgM and IgM plus IgG combined ELISA, and the high correlation with SERION classic ELISA, indicates that they are useful in the diagnosis of LD.

P021

Evaluation of two new immunochromatographic assays (Rapid U *Legionella* antigen test and SD Bioline *Legionella* antigen test) for the detection of *Legionella pneumophila* serogroup 1 antigen in urine

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Introduction: Since antigen detection in urine has proved to be a sensitive and rapid method for detecting *Legionella pneumophila* serogroup 1, this technique has become one of the most used tools for the diagnosis of Legionnaires' disease (LD). The aim of our study was to evaluate the Rapid U *Legionella* antigen test (Diamondial, Sees, France) and SD Bioline *Legionella* urinary antigen test (Standard Diagnostics, inc., Kyonggi-do, Korea) for the detection of *L. pneumophila* serogroup 1 in urine.
Methods: We evaluated the ability of the new antigen tests to diagnose LD in a well described sample of patients with and without LD using frozen urine samples. We compared the sensitivity and specificity of the assays to the Binax Now urinary antigen test (Binax, Portland, Maine). All tests were used as specified by the manufacturer.
Results: We included 98 urine samples (98 patients) from patients with proven LD and 89 urine samples (89 patients) of patients with respiratory tract infections other than *Legionella*, mainly community-acquired pneumonia due to *Streptococcus pneumoniae*. Sensitivity and specificity were respectively 71.4% (70/98) and 96.6% (3/89) for the Rapid U *Legionella* antigen test, 27.6% (27/98) and 98.9% (1/89) for SD Bioline *Legionella* urinary antigen test and 92.9% (91/98) and 100% (80/89) for the Binax Now urinary antigen test. The sensitivity of the rapid U test increased to 80.6%(79/98) (p=0.18) if tests were reexamined after 45 min. The differences in specificity between the three tests were not statistically significant. The Binax test showed sensitivity levels significantly higher than that of the Rapid U and SD Bioline test ($P < 0.001$).
Conclusion: The Binax Now urinary antigen test is superior for the diagnosis of infection caused by *L. pneumophila* serogroup 1 compared to the Rapid U *Legionella* antigen test and the SD Bioline *Legionella* urinary antigen test. The Rapid U *Legionella* antigen test showed a reasonable degree of sensitivity, increasing after a prolonged incubation time. We therefore recommend to use an incubation time of 60 min instead of 15 min for the Rapid U *Legionella* antigen test.

P022

Detection of *Bartonella henselae* DNA in serum samples from patients with cat scratch disease

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Introduction: *Bartonella henselae* is the causative agent of cat scratch disease (CSD), which usually presents as a self-limiting lymphadenopathy in children and young adults. Clinically, CSD cannot be distinguished from lymphadenopathy caused by other microbial pathogens or malignancy, and invasive sample taking is often needed to confirm a suspected case of CSD. In this study, the diagnostic performance of *B. henselae*-specific PCR on serum samples was evaluated.
Methods: 65 patients with a clinical presentation of CSD based on retrospective analysis of clinical data and laboratory evidence for infection with *B. henselae* were included. Laboratory evidence included one or more of the following criteria: (i) a positive PCR result on pus aspirates or biopsy specimens from lymph nodes using a 16 S rRNA assay and/or (ii) a single positive titer in IgM antibodies against *B. henselae* in sera using an Indirect Fluorescent Assay (IFA). Cases included were 65 patients (38 males and 27 females) between 4 and 64 years old (mean age 26.0 years). 21 patients were PCR positive and 61 patients were seropositive. A control group consisted of 50 patients whose serum was sent for *B. henselae* testing and tested negative for *B. henselae* antibodies. Controls included were 50 patients (16 males and 34 females) between 2 and 78 years old (mean age 34.6 years).
Results: We included 65 serum samples (65 patients) with proven CSD and 50 control serum samples (50 patients). The control samples all tested negative in PCR. Of the 65 patients with proven CSD, 4 sera (6.2%) tested positive in PCR.
Conclusion: Detection of *B. henselae* DNA in serum is not a useful tool in addition to existing tests for the diagnosis of CSD. Studies suggest that *Bartonella* spp. can be detected more efficiently from whole blood. Further (prospective) studies are required to determine the exact sensitivity and specificity of *Bartonella*-specific PCR in whole blood samples.

P023

Rapid immunographic assays for the detection of *Clostridium difficile* toxins; comparison with the fibroblast cytotoxicity assay

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Introduction: *Clostridium difficile* is the most common cause of antibiotic-associated diarrhea and frequently causes hospital-acquired diarrhea. Rapid diagnosis is important to prevent progression of the disease to pseudomembranous colitis and nosocomial transmission. The enteropathogenicity of *C. difficile* is due to the production of enterotoxin A and cytoxin B. The cytotoxicity assay is considered the golden standard because of high sensitivity and specificity. However, the turn-around time is more than 48 hours. The objective of this study is to compare two rapid immunochromatographic assays, the ImmunoCard Toxins A&B (ICTAB, Meridian) and the X/PECT Toxin A/B (Remel), with the cytotoxicity assay (CTA).
Methods: This study consisted of two parts. In the first study, 695 unselected stool samples were tested with the ICTAB assay and with the CTA . In the second study, 33 selected stool samples were also tested by the X/PECT assay. Selection was based on the results obtained with the ICTAB assay and the CTA. The CTA was performed on human embryonic lung fibroblasts. All cytotoxic samples were retested in a neutralisation assay.
Results: From the 695 selected stool samples, 40 were positive and 633 were negative in both assays. In addition 16 samples were CTA positive/ICTAB negative and 6 were CTA negative/ ICTAB positive. The sensitivity and specificity of the ICTAB assay were 71% and 99%, respectively.
In the second study, of 11 concordant CTA/ICTAB positive samples one was negative in the X/PECT assay. Three concordant CTA/ICTAB negative samples were all negative in the X/PECT assay. From 16 CTA positive/ICTAB negative samples 7 were positive and 9 negative with the X/PECT assay. Three CTA negative/ICTAB positive samples were all negative with the X/PECT assay.
An additional experiment showed that the recently discovered *C. difficile* ribotype 027 can easily be detected by the X/PECT assay.
Conclusions: The clinical sensitivity of the ICTAB assay is much lower than the CTA. Preliminary data suggest that the X/PECT assay is more sensitive than the ICTAB assay. The X/PECT assay is easier to perform and interpretation of test results is better.

P024

SSI medium: can it replace a combination of selective media for the identification of enteropathogens?

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Introduction: Statens Serum Institut medium (SSI) is being used for the detection and identification of enteropathogens in Denmark. *Salmonella* spp., *Shigella* spp., *Yersina enterocolitica, Escherichia coli* and phenylalanine deaminase positive bacteria can grow on it. It is a selective medium like MacConkey medium. The identification is

based on four different properties: H_2S production, phenylalanine deaminase reaction, indol reaction and specific colony morphology.
Our aim was to evaluate whether the SSI medium could replace our combination of selective media used for fecal culture.
Methods: From 2/5/2005 to 4/8/2005 all feces received on Monday, Tuesday, Wednesday and Thursday were streaked on SSI, XLD, DCL and SS. Definite identification was performed according to our laboratory procedures.
Results: Results of our comparison are represented in table 1. We cultured 436 fecal samples. The performance of SSI was deemed 'good' if separate colonies were present. It was considered as 'bad/difficult' if it was overgrown with non enteric pathogens.
Conclusion: Enteric pathogens do grow on this medium, but so can other bacteria. In nearly 25% of all cultures, the overgrowth of other bacteria impairs the sensitivity to detect enteric pathogens. In addition suspected colony morphology requiring further biochemical testing, was less present on SSI (nearly 6%). Using SSI we would miss 1.4% positive stool cultures in comparison to our current method. We therefore conclude that the SSI medium can not replace our combination of selective media for fecal culture.

Table 1. Results

	SSI	STANDARD PROCEDURES
Positive test results	10 (2.3%)	16 (3.7%)
Suspected colony morphology	100 (22.9%)	124 (28.4%)
	Good	Bad/difficult
Performance	331 (75.9%)	105 (24.1%)

P025

Detection of Methicilline-Resistant *Staphylococcus aureus* using a TaqMan PCR assay

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Introduction: A reliable and rapid detection of methicillin resistant *Staphylococcus aureus* (MRSA) is important to minimise the stress caused to patients, and decrease costs, labour and transmission associated with MRSA.
Huletsky, et al. have described a PCR method based on Staphylococcal Chromosomal Cassette: SCCmec sequences specific for MRSA. This method has been made commercially available with ready-to-use kits. Kits however, are expensive, have limited tenability, and processing of clinical material according to the kit is rather labour intensive.
The objective of our study is to investigate whether an 'in house' protocol for MRSA detection can also reliably detect MRSA.
Methods: We have adjusted the method described by Huletsky et al. by choosing TaqMan probes instead of molecular beacons, and have optimized the test for default Taqman settings. The lower detection limit was 1 femtogram of DNA. In a separate PCR reaction, inhibition of PCR was measured by addition of Phocine Herpes Virus. 134 samples from patients known for MRSA carriership were analysed. One sample set included at least a nasal swab, a throat swab, and an anal swab, and if appropriate swabs from wounds, hands, or medical devices were taken. PCR was performed on separate swabs compared to culture. Swabs to be used for PCR were suspended in 300 µl Tris-EDTA buffer and samples purified using MagNAPure total nucleic acid kit. A second PCR was performed on azthreonam culture media (100 µl) after overnight incubation of swabs used for culturing.
Results: Of 134 samples, 33 were positive in PCR and 25 in culture. The negative predictive value of PCR was 98%. The positive predictive value of PCR was 72% and 96% on respectively swabs and culture media. Among PCR positive/culture negative patients 78% were receiving treatment during sampling, or had received antibiotics 1 week before.
Conclusion: Despite the limited number of samples analysed, PCR results are promising with regard to MRSA negative screening. Future comparison with the commercial kit will reveal whether 'in house' protocols can be used for screening of MRSA.

P026

Erythromycin susceptibility of *Campylobacter jejuni* and *Campylobacter coli*; accuracy of susceptibility tests and detection of a new resistance mutation

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Introduction: Macrolides are considered as agents of first choice for treatment of *Campylobacter jejuni* and *Campylobacter coli* infections. The aim of this study was to determine the accuracy of erythromycin resistance of *Campylobacter* spp. by phenotypical assays, and to identify the molecular basis for the resistant strains.
Materials and methods: Forty-eight erythromycin resistant *C. jejuni* (n=36) and *C. coli* (n=12) strains were selected from a Dutch gastroenteritis case-control study. The

participating laboratories performed susceptibility testing according to their routine procedures.
At the LUMC, susceptibility for erythromycin was determined using E-test and disk diffusion. Classification of strains as susceptible, intermediate susceptible or resistant (R) was performed using CRG criteria (Committee for Guidelines on Susceptibility testing, R: MIC > 2 mg/l or zone < 23mm) and CLSI criteria (Clinical and Laboratory Standards Institute, R: MIC ≥ 8 mg/l or zone ≤ 18mm). Detection of mutations in the 23S rDNA codon was performed on 30 strains by sequence analysis of the nucleotides 2058 and 2059 (*E. coli* equivalents).
Results: Results of the E-test showed that 6 (17%) and 5 (14%) of the 36 *C. jejuni* isolates were erythromycin resistant according to CRG and CLSI criteria, respectively. Of 12 *C. coli* isolates, 11 (92%) and 8 (67%) were considered as resistant using these criteria. Using disk diffusion, resistance rates for *C. jejuni* and *C. coli* were 25% and 67% according to both criteria. All isolates with MIC values < 256 mg/l (n=17, range 0.25-12 mg/l) had the AA-genotype. All 8 *C. coli* and 3 of 5 *C. jejuni* isolates with MIC values of > 256 mg/l had the well-known A2059G mutation. Two resistant *C. jejuni* strains showed an A2058T mutation that has not been described in *Campylobacter* previously.
Conclusions: 1) Results of erythromycin susceptibility tests of *C. jejuni* and *C. coli* revealed important differences between the laboratories and emphasize the need for standardization of methods and breakpoints. 2) A new mutation (A2058T) encoding erythromycin resistance was found in 2 resistant *C. jejuni* strains.

P027

The occurrence of the AdeABC efflux system in a genotypically diverse population of *Acinetobacter baumannii*

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Introduction: The AdeABC efflux system with three structural (*adeA, adeB, adeC*) and two regulatory genes (*adeR, adeS*) has been associated with multidrug resistance in a number of *Acinetobacter baumannii* strains. Upregulation of this system has been ascribed to mutation in *adeR* or *adeS*. The aim of this study was to assess the occurrence of the AdeABC efflux system and its association with multidrug resistance in a well-documented set of *A. baumannii* strains.
Methods: A genotypically and epidemiologically heterogeneous set of 120 *A. baumannii* strains was investigated. The strains were allocated to six multidrug resistant (MDR) clonal groups (n=71) or to unique genotypes (n=49) by AFLP analysis. PCR detection of the *adeA, adeB, adeR* and *adeS* gene was performed. Susceptibility to 11 antibiotics was tested by disk diffusion. In addition, MICs to netilmicin, a phenotypical marker for upregulation of the AdeABC system, were determined.
Results: Ninety-nine strains (83%) including all but one strains of EU clone I-III were positive for all four genes; 11 strains were negative. Ten strains were positive for one to three genes. Eight strains negative for all four genes were fully susceptible (FS). Strains positive for all genes were MDR (n=75) or FS (n=23). Strains with netilmicin MIC ≥ 8 mg/l (n=56) but without known netilmicin resistance genes (*aacA4, aacC2*) were MDR and positive for all four genes. Forty-nine strains with a netilmicin MIC ≤ 1 mg/l included 33 fully susceptible strains. The latter were genotypically diverse, both in efflux gene content and by AFLP analysis.
Conclusion: AdeABC is common in MDR and FS *A. baumannii*, but may be absent in some strains. Strains with AdeABC genes present but not expressed, as derived from susceptibility to netilmicin, are not uncommon and are usually susceptible to many other antibiotics. We postulate that multidrug resistance in *A. baumannii* is generally associated with the presence of upregulated AdeABC.
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P028

Comparison of Five Phenotypic Methods to Detect Extended-Spectrum β-Lactamases (ESBL)

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Background: Acquired resistance to third-generation cephalosporins among *Enterobacteriaceae* is rising. Extended-Spectrum β-Lactamases (ESBLs) and plasmid encoded AmpC β-lactamases are generally the cause. Phenotypic detection of ESBL is based on the reduction of the MIC against cephalosporins by clavulanic acid, but previously we noted a difference between the disk diffusion test and Etest ESBL in the ability to detect ESBL (Florijn A, et al. Eur J Clin Microbiol Infect Dis 2002;21:241). This is partly caused by the large number of families of β-lactamases, which may have a large number of different members. But sometimes the presence of multiple different β-lactamases in a single bacterial cell may iterfere with detection. Therefore we compared five phenotypic tests for the detection of ESBL.
Methods: A total of 145 Enterobacteriaceae (79 *Escherichia coli*, 27 *Klebsiella oxytoca*, 30 *Klebsiella pneumoniae*, and 1 *Klebsiella ozaenae*) suspected for ESBL carriage (MIC

for ceftriaxone, ceftazidime, aztreonam ≥ 2 μg/ml) from The Netherlands and Europe were tested with Etest ESBL (both the ceftazidime and ceftriaxone strips; EE), Etest cefepime (EC), Phoenix (Becton Dickinson; P), the Oxoid test Combination test (O), and the double disk test with ceftriaxone, ceftazidime, and.aztreonam (DD).

Results: A total of 36 different combinations of results were obtained for the five assays. For only 51% of the isolates the results were concordant (all tests positive or all tests negative). Pair wise comparisons of the methods yielded the following results: EE-P: 67%; EC-P: 75%; O-P: 72%; DD-P: 70%; EC-EE: 73%; O-EE: 69%; DD-EE: 68%; O-EC: 79%; DD-EC:79%; DD-O: 81%.

Conclusion: The concordance between any two methods is poor (67-81%). Genotypic identification of the ESBLs and AmpC β-lactamases is required to establish which phenotypic method yields the best prediction for the presence of an ESBL.

P029

Emergence of multidrug-resistant Gram-negative bacteria during selective decontamination of the digestive tract on an Intensive Care Unit

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Introduction: During treatment with selective decontamination of the digestive tract (SDD), four different strains of multidrug-resistant (MDR) Gram-negative bacteria (three *Escherichia coli* strains and one *Klebsiella pneumoniae*) were isolated from four patients which were determined as non-carriers for such MDR strains before their admission to the Intensive Care Unit (ICU) in the Academic Medical Center (AMC) in Amsterdam. These isolates were extended spectrum beta-lactamase (ESBL) positive. We investigated whether this was due to interspecies transfer of resistance genes.

Methods: The MDR strains were typed by amplified fragment length polymorphism analysis (AFLP). The plasmids from these strains were characterized by restriction fragment length polymorphism and the resistance genes (SHV, TEM and CTX-M) were characterized by PCR and sequence analysis.

Results: AFLP analysis confirmed that the three MDR *E. coli* isolates represented three different strains. The *E. coli* and *K. pneumoniae* strains were resistant for tobramycin, gentamicin, ciprofloxacin, cefotaxime, ceftazidime, cefepime, cefpodoxime and intermediately sensitive to polymyxin E. The strains harboured the same plasmid with identical extended-spectrum β-lactamase (ESBL) genes.

Conclusion: We identified the transfer of a resistance plasmid, which carried identical ESBL genes among different Gram-negative bacterial strains during SDD treatment in the ICU. The use of the third generation cephalosporins in SDD may be associated with emergence of ESBL producing strains. Therefore, for accurate evaluation of the SDD application in ICUs, the screening for ESBLs should be included.

P030

Phenotypic and genotypic characterization of antimicrobial resistance among Dutch *Salmonella* isolates

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In the present study, 237 epidemiologically unrelated Dutch *Salmonella* isolates originating from food producing animals and human cases of salmonellosis were tested for their susceptibility to 15 antimicrobial agents. Resistance to 14 of these antimicrobials, including the third-generation cephalosporins was detected. Resistance to sulphonamides, ampicillin, tetracycline, streptomycin, trimethoprim and nalidixic acid was common (28.7, 20.7, 17.3, 16.5, 13.5, and 11.8% of the isolates, respectively). Resistance to three or more antimicrobials was observed in 57 isolates. These isolates were investigated for the prevalence of class 1 integrons, the gene cassettes present in these integrons and the presence of *Salmonella* Genomic Island 1 (SGI1). Thirty-six (15.2%) isolates carried class 1 integrons. Ten distinct integron profiles were found based on the size of the integron and restriction fragment length polymorphism analysis. Integrons were detected for the first time in serovars Indiana and Senftenberg. Multidrug-resistance was strongly associated with the presence of class 1 integrons in which the *aadA2*, *aadA1*, bla_{PSE-1}, *dfrA1*, *dfrV*, *dfrA14* or *sat* genes were present as determined by nucleotide sequencing. The presence of gene cassettes or combinations of gene cassettes not found before in integrons in *Salmonella* was also observed. *Salmonella* Genomic Island 1 and its variants (SGI-B, -C and -F) were found in 14 isolates belonging to either serovar Typhimurium, Derby or Albany. Similar phenotypes, integron profiles and SGI1 structures were found regardless of the source of the isolate (human or animal). These data indicate that antimicrobial resistance genes including integrons and SGI1 can be exchanged between different *Salmonella* serovars and reservoirs.

P031

Prevalence surveys are reliable tools to determine the appropriateness of antimicrobial therapy

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Introduction: Prudent use of antibiotics is mandatory to control antibiotic resistance. The objective of this study was to determine if prevalence surveys are reliable tools to determine the appropriateness (AP) of antimicrobial therapy (AMT).

Methods: The study was performed in a 1350 bed teaching hospital. Six consecutive one-day prevalence surveys of inpatients were performed twice yearly, from 2001 to 2004. Demographic-, infection- and AMT-data were gathered. The AP of AMT was assessed according to a standardised algorithm based on the local antibiotic prescription guidelines.

Results: On average 684 patients were included in each survey (total 4105). 16.7% of the patients had an infection on admission to the hospital and 8.7% of the patients had at least one nosocomial infection on the day of the survey. 942 (22.9%) of the patients received AMT. In 350 (37.2%) of the patients on AMT, AMT was inappropriate (IA). IA-AMT consisted of: AMT unjustified: 123 (35%), IA choice: 140 (40%) and IA dosage: 87 (25%). 0.6% of all patients did not receive AMT although this was indicated. The use of AMT did not differ significantly between the six surveys (figure). The prevalence of AMT (23/100 patients) to the annual data from the pharmacy department (24 PDD/100 patient days).

Conclusions: Prevalence surveys proved to be reliable tools to measure the use of AMT. As it relates AMT to an individual patient, it can also determine the AP of AMT, collect determinants for IA use and provide an estimate of the proportion of patients that did not receive AMT while this was indicated. This study shows that in a setting with an extremely low use of AMT (the Netherlands is among the countries with the lowest use in Europe and this hospital is among the lowest in the country) there are few patients who inadvertently do not receive AMT. On the other hand a substantial part of the patients with AMT are treated IA. Using prevalence surveys these patients are easily identified, offering opportunities for targeted interventions. Furthermore, this method can be used for benchmarking AMT in hospitals.

P032

Did the fungal genome arise after a major genome transfer event?

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The phylogenetic position of the fission yeast *Schizosaccharomyces pombe* in the fungal tree of life is still controversial. Three alternative phylogenetic positions have been proposed in the literature namely 1) a position basal to the Hemiascomycetes and Euascomycetes, 2) a position as a sistergroup to the Euascomycetes with the Hemiascomycetes as a basal branch or 3) as a sister group to the Hemiascomycetes with Euascomycetes as a basal branch. Here we compared 91 clusters of orthologous proteins containing a single orthologue, that are shared by 19 fungal genomes. The major part of these 91 orthologues supports a phylogenetic position of *S. pombe* basal as a basal lineage among the Ascomycetes, thus supporting the second hypothesis. Interestingly, part of the orthologous proteins supported a fourth, not yet described hypothesis, in which *S. pombe* is basal to both Basidiomycetes and Ascomycetes. This suggests that the nuclear genome of fungi has a heterogeneous composition that may be explained by a major genome transfer event in its early history. If this hypothesis is true, it may have great consequences of our insights in the origin of the fungi as a kingdom.

P033

Functional Analysis of PrmC of *Neisseria meningitidis*

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The universally conserved methyltransferase PrmC plays a key role in the accuracy of termination of translation in *Escherichia coli, Chlamydia trachomatis* and *Saccharomyces cerevisiae* by the methylation of protein release factors. In addition, PrmC seems to be essential for virulence in *Yersinia pseudotuberculosis*. The function of PrmC in *Neisseria meningitidis* is unknown. We investigated the genetic organization and functionality of *prmC* of *N. meningitidis*. In *N. meningitidis* Z2491 one ORF (NMA0369) of 822 bp was found, encoding a 30 kDa putative PrmC (42% identity with *E. coli* PrmC). NMA0368 (462 bp) upstream of NMA0369 is annotated as a putative membrane protein (PMP). The stop codon of *pmp* is located 5 nucleotides downstream of the start codon of *prmC*. Interestingly, the corresponding genome region of strain MC58 contains

one large ORF (NMB2065) of 1272 bp, encoding a putative protein of 46 kDa. Here, deletion of one nucleotide near the junction of *pmp* and *prmC* created a frame shift, resulting in loss of the stop codon of *pmp*, thus creating an in-frame fusion between the *pmp* and *prmC*. Homologues of PMP in other bacterial species, or any other translational fusions between *prmC* and other genes were not found. NMA0369 as well as NMB2065 could trans-complement the growth defect of the *prmC* knockout *E. coli* strain SC5, indicating functionality. Genome analyses and RT-PCR data indicated that in Z2491 and MC58 *prmC* and *pmp/prmC* is third and second ORF of a polycistronic operon, respectively. This operon contains 4 (MC58) or 5 ORFs (Z2491) of which the function of the gene products is largely unknown. Immunoblotting of the cytosolic fractions of MC58 and Z2491 using anti-PrmC demonstrated that both strains express similar sized immuno-reactive proteins of approx. 30 kDa, suggesting that in MC58 only the part of the transcript encoding PrmC is being translated into functional PrmC. Conclusion: In *N. meningitidis* MC58, PrmC is encoded by an unusual large ORF. This ORF is part of a polycistronic operon. Translation into functional PrmC in MC58 is most likely from an internal ribosomal binding site.

P034

Too few, too late: submission patterns of bronchoalveolar lavage fluid samples obtained under the suspicion of the diagnosis of ventilator associated pneumonia.

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Introduction: At our hospital, ventilator associated pneumonia (VAP) is diagnosed by microbiological and cytological analysis of bronchoalveolar lavage (BAL) fluid. Opening hours of the in-house microbiological laboratory are between 8.00 a.m. to 5.00 p.m. During off-hours a laboratory technician is on call for urgent samples including BAL fluid. The total laboratory work-up of the BAL fluid takes two hours. The present study aimed to analyse the day and hour submission patterns of BAL fluid samples.
Material and Methods: During a 58-month period (January 2000 till October 2005), day and hour of submission of all consecutive BAL fluid samples obtained from patients suspected of VAP were recorded. The diagnosis of VAP was made if quantitative cultures reached ≥ 10e4 colony forming units/ml and/or if ≥ 2% infected cells were enumerated on May-Grunwald Giemsa stained cytocentifuged preparations.
Results: A total of 433 BAL fluid samples was included. On week days, a total of 69.8 ± 5.8 samples for each day were submitted, compared to 38 and 46 samples on Saturday and Sunday. For nearly half (199, 46.0%) of the samples, the on-duty lab technician was required: 99 (23.0%) samples arrived within one hour before closing, and an additional 100 (23.0 %) were submitted after closing. VAP was diagnosed in 168 (38.8%) samples, 92 (54.8%) of these diagnoses were made after closing hours. VAP was diagnosed in 76/220 (34.5%) of samples submitted during opening hours, and tended to be diagnosed proportionally more after closing hours during week days (54/129 41.8%) and during weekends (38/84 samples, 45.2%, p = 0.09).
Conclusions: 1) The high number of BAL fluids processed after laboratory opening hours is of concern because of the suboptimal working conditions (fatigue, lack of supervision). 2) Technician time spent to these samples put a strain on the laboratory in terms of costs and absence of the technicians because of legal recuperation. 3) The low number of BAL fluids submitted during the weekends combined to the higher proportion of VAP in these samples points to poor compliance with in-hospital quidelines for the diagnosis of VAP and could suggest that we missed a number of (unconfirmed) episodes of VAP.

P035

Campylobacter jejuni strains harboring the LOS class A or B biosynthesis gene cluster expressing GM1 are hyper-invasive into Caco-2 cells

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Campylobacter jejuni is the most frequent cause of bacterial gastroenteritis worldwide. Lipo-oligosaccharide (LOS) class A and B biosynthesis gene clusters (containing genes required for expression of the ganglioside mimic GM1) of *C. jejuni* are crucial for the induction of anti-ganglioside antibodies that are involved in the pathogenesis of Guillain-Barré syndrome (GBS). Whether there is a correlation between GBS-inducing potential, LOS, and invasion of *C. jejuni* into the intestinal epithelium is still unclear.
We studied 7 GBS- and 13 enteritis-associated *C. jejuni* strains isolated from Dutch patients and 6 Penner reference strains for invasion into Caco-2 cells using the gentamicin exclusion assay. As LOS A and B classes are marked by the unique presence of the genes cstII and orf11, we generated mutants for these genes in 3 LOS class A GBS-associated strains. The role of GM1-mimicking LOS in invasion was studied by blocking experiments using dead, formalin fixed, GM1-deficient mutant and wild type strains.
C. jejuni with LOS class A or B, expressing the ganglioside mimic GM1, invaded significantly better than *C. jejuni* with

LOS class C, D or E ($p<0.005$), irrespective of the clinical diagnosis of GBS. Invasion was significantly reduced in all three strains upon deletion of either cstII or orf11. The invasion of two GBS associated wild type strains could be inhibited by pre-incubation of Caco-2 cells with the killed wt and isogenic orf11 mutants (both GM1+), but not by killed cstII (GM1-) mutants. In contrast, the invasion of a third strain could be blocked by killed wt and both orf11 (GM1+) and cstII (GM1-) mutants.
Our data show that the *C. jejuni* genes cstII and orf11 play an important role in invasion of caco-2 cells, and indicate the co-existence of GM1-dependent and GM1-independent invasion mechanisms.

P036

A physiological approach to studying sporulation and *B. subtilis* general stress response; Induction levels under nutrient limitation versus true starvation

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Initiation of sporulation was studied under physiologically well-defined conditions in continuous cultures at a range of specific growth rates. Expression of the early sporulation gene *spoIIA* under varying limitations was monitored using fluorescence microscopy and flow cytometry. It was shown that under carbon, nitrogen, and phosphate limitations at any given dilution rate a percentage of the cell population is induced to sporulate. The number of spores formed increased with a decrease in dilution rate (although the extent of sporulation for a given limitation varied). Fluorescence microscopy confirmed these results as the level of expression of *spoIIA*-gfp also increased with a decrease in the dilution rate showing that sporulation is initiated at a higher frequency at slow growth rates. Further induction of sporulation could not be achieved on complete starvation of the cells (switching off the chemostat pump) in any of the limitations tested. Cell density had no effect on sporulation in the chemostat. Flow cytometry results showed that sporulation was heterogenous under all steady-state conditions tested. On reaching stationary growth phase, *Bacillus subtilis* cells may choose from a number of distinct stress response pathways including next to sporulation induction of the general stress response and competence development. This highly ubiquitous pathway for survival was chosen for further study. We measured the response using both a Lac Z and ctc-gfp reporter and monitored their expression at varying dilution rates in a carbon limited chemostat. A very small induction could be seen at a low growth rate ($D=0.1\ h^{-1}$). On nutrient starvation a transient increase in the expression of sigma B could be seen after 2 and 3 hours. These results indicate that the sporulation and general stress response pathways are not intimately intertwined under carbon limited conditions. Current studies aim at assessing at the level of cell populations the relation between these two stress adaptation responses.

P037

How effective is the disinfection of endoscopes?

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Introduction: Several endoscopy related outbreaks have been reported. Transmission tends to be incidentally identified when associated with an unusual species or a microorganism (m.o.) with uncommon resistance profile. Spread of common m.o.'s may remain unrecognized. Therefore, we designed a microbiological surveillance system with standard sampling intervals. The system includes the endoscopes and, if indicated, the automatic Washer Disinfectors (WD's).
Methods:
• Surveillance protocol: 216 anterograde and 216 retrograde samples of therapeutic gastroscopes and duodenoscopes used for Endoscopic Retrograde Cholangiopancreaticography and decision scheme;
• Electron Microscopy (EM) on the exchanged sheaths of channels and the elevator wire of frequently contaminated endoscopes.
Results: With retrograde sampling (modification from [1]), 31% of all surveillance cultures were positive (*table 1*).

Table 1. Results of surveillance sampling of herapeutic duodenoscopes and therapeutic gastroscopes September 2002 – January 2005

SAMPLES	ANTEROGRADE	RETROGRADE*
Total	216	216
Positive for		
- Any microorganism	17 (7.9%)	67 (31%)
- Yeast**	6 (2.8%)	54 (25%)

*Retrograde frequently more than one m.o. were found in one sample.

**Nine of 15 (60%) sampled endoscopes were found at least once contaminated with *Candida* spp. other than *C. albicans*. Retrograde 77% and anterograde 100% of all further identified non-albicans *Candida* spp. were *C. parapsilosis*.

We found an increase of *Candida* spp., in particular *Candida parapsilosis*. These yeasts were also isolated from the WD's. However, directly after the once daily auto-disinfection process in the morning no yeasts were found, but after regular cycles during the working day *Candida* cells appeared in the WD's. The range of CFU/ml was between 1-10 to 3000 for endoscopes and 0.02 to 0.06 for the WD's. Biofilmforming in endoscopes was found by Electron Microscopy.

Conclusions: 1) According to reference 1, retrograde sampling is much more sensitive than anterograde sampling; 2) Without retrograde sampling the structural problem with *C. parapsilosis* would not have been detected; 3) Biofilmformig in endoscopes is not prevented by the disinfection procedure; 4) We were able to show the likelihood of cross-contamination between endoscopes and the WD's; 5) Transmission of microorganisms in an endoscopy center is despite a well-controlled disinfection process [2] likely to occur and may pose a risk for patients.

References:

1. Srinivasan A, Wolfenden LL, Song X, et al. An outbreak of Pseudomonas aeruginosa infections associated with flexible bronchoscopes. N Engl J Med 2003;16;348(3):221-7.
2. Guideline of the Dutch Infection Prevention Working Party (WIP) for cleaning and disinfection of endoscopes. 2004 (http://www.wip.nl).

P038

Capnocytophaga canimorsus in patients and dogs

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Introduction: *Capnocytophaga canimorsus* is reported to occur in normal oral flora from dogs and can cause severe invasive infections in humans. In 2004-2005, we saw two cases of severe sepsis and one case of prolonged keratitis by *C. canimorsus* in the LUMC. The aim of the present study was to assess the incidence of infections with *C. canimorsus* and a related, but less virulent pathogen, *Capnocytophaga cynodegmi*, in the Netherlands. In addition, we wanted to know the incidence of these bacteria in normal dogs.

Materials and methods: An enquiry was sent to all laboratories for Medical Microbiology in the Netherlands asking for the number of *C. canimorsus* and *C. cynodegmi* isolates cultured in 2003-2005 and for clinical information on the patients from which they had been cultured. From dogs visiting a veterinary practice oral swabs were taken for culture on blood agar plates containing gentamicin. In addition, a PCR targeting the rpoB gene was developed for identification of cultured strains and for direct detection of Capnocytophaga-DNA from canine specimens.

Results: So far 12 out of 50 laboratories replied and reported 12 cases of infections with *C. canimorsus*. If we assume that these laboratories take care of diagnostics of 24% of the Dutch population, the yearly incidence of *C. canimorsus* infections would be 1 per 10^6 persons. This is twice the numer as reported in Denmark (Pers et al. CID 1996;3:71). No infections with *C. cynodegmi* were reported. So far we cultured 4 strains from 9 dogs, of which only one was *C. canimorsus* and 3 were *C. cynodegmi*.

Conclusions: The incidence of *C. canimorsus* infections in the Netherlands is as least as high as in Denmark. *C. canimorsus* does not seem to be a universally present commensal bacterium in dogs. Data from more laboratories and direct PCR analysis of more canine samples, which will be performed in the near future, are necessary to support these preliminary conclusions.

P039

Internal quality assurance of antibody detection with ELISA assays on an automated system (DSX)

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Introduction: Elisa assays against different Infectious Disease Markers should have an internal quality control that can check both interassay – and intraassay variation. We are using a pooled serum that is used as a 'patient' serum when we are running our tests.

Methods:

- We have 3 Dynex-DSX instruments* that are used to perform over 75000 Infectious Disease ELISA tests on 21 different parameters. The ELISA assays that are being used in our laboratory are *Bordetella* IgA and IgG, *Chlamydophila* IgA and IgG, *Chlamydia trachomatis* IgA and IgG, CMV IgM and IgG, *Coxiella burnetti* phase II IgM and IgG, EBNA IgG, HCV IgG, HIV Ag-Ab, HSV IgM and IgG, *Legionella* IgM and IgG, Mycoplasma IgM and IgG, VCA IgM and IgG and VZV IgM and IgG.
- After we test the 'pooled' serum we import the results into QC Today**. In this poster, we present to you the results of the last 9 months of VCA IgG and IgM for the DSX2 (*table 1*).
- On the Dynex-DSX we have the possibility to use the Levey-Jennings option to validate the results of the Infectious Disease ELISA's. In this poster we present as an example the results for the OD of the standard of the VCA IgG – and IgM assay over a period of the last 12 months.

Conclusion: We will continue to use the 'pooled' serum samples for the internal control of our routine elisa testing

to monitor the quality of our routine testing; because of the ease of use the Levey-Jennings option on the DSX will be taken into consideration to facilitate the use of looking at the Quality Controls.

* The DYNEX DSX ELISA Automate is distributed in The Netherlands by Clindia Benelux BV, Leusden.

** QC Today is a program from Instrumentation Laboratories.

P040

The incidence of sepsis in a large Dutch university hospital

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Introduction: In 1992 the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine (SCCM) published definitions of sepsis and SIRS (Systemic Inflammatory Response Syndrome). These were confirmed during the latest consensus conference in 2001. Sepsis is now defined as infection with two or more SIRS criteria. In this study the incidence, etiology and outcome of sepsis is evaluated in adult hospitalized patients in a Dutch University Hospital.

Methods: For a period of one year all adult patients admitted at the UMCU from whom blood cultures were drawn were analysed. Medical information was obtained by reviewing the medical records, discharge letters and the culture results. The (possible) sepsis episodes in these patients were counted. Outcome was measured as survival after 28 days.

Results: A total of 2025 suspected sepsis episodes were identified in 1676 patients. From these patients 1022 were classified as sepsis episodes, 541 as possible sepsis and 436 as none sepsis. The incidence of sepsis was 22.6 per 1000 patients admitted, the incidence of sepsis syndrome was 21.7 per 1000, and the incidence of septic shock was 5.0 per 1000. The predominant infection site was the respiratory tract (35.4%). Of all septic patients, 34.8% had positive blood cultures. From these 344 positive bloodcultures, 57.6% were caused by gram positive micro-organisms. *Staphylococcus aureus* was recovered in 75 sepsis episodes (21.8%), coagulase-negative *Staphylococci* in 45 (13.1%), *Escherichia coli* in 58 (16.9%) and *Klebsiella pneumoniae* in 20 (5.8%). The mortality for patients with sepsis was 5.5% (22/399), for patients with sepsis syndrome 16.4% (63/383) and for patients with septic shock 39.3% (35/89).

Conclusion: 1) There is a slight increase in the incidence of sepsis at the UMCU in the past decade. However, the mortality shows a small decrease. 2) Sepsis diagnosis can be very hard and difficult to distinguish from other causes of the systemic inflammatory response syndrome. 3) Recognizing sepsis in an early stage may reduce mortality.

P041

Decrease of external ventricular or lumbar drain related infections by a multidisciplinary approach

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Introduction: Extraventricular (EVD) and lumbar drains (LD) are important temporary measures for patients requiring continuous cerebrospinal fluid (CSF) drainage. Reported incidences of drain related meningitis have varied from 2.4 to 15%. In the UMCU, microbiologically confirmed CSF infections in patients with EVD/LD increased from 28% in 2001 to 39% in 2003. The aim of this prospective study was to reduce the incidence of drain-related meningitis to less than 10% in 2005. Patients: All patients who received EVD or LD from Jan-April 2004 (period I), Aug-Dec 2004 (period II), and Jan-May 2005 (period III).

Interventions: A multidisciplinary team (neurosurgery, clinical microbiology, and hospital hygiene) designed a strategy based on 4 pillars: 1) implementation of drain management protocols for medical and nursing staff based on the "no-touch" concept, optimal hygiene and strict criteria for placement and removal of drains, 2) implementation of an algorithm on diagnostic and therapeutic management of patients suspect of drain-related meningitis, 3) implementation of a new protocol on pre-operative prophylaxis, 4) introduction of a closed drain system.

Results: Results are depicted in the table. The decrease of the infection rate in period III versus I was more pronounced for ELD than for EVD. The RR to acquire an infection per 100 days at risk decreased for the ELD with 0.2 (95%-CI: 0.03-1.7) and for the EVD with 0.7 (95%-CI: 0.2-2.2). From period I to III a sharp shift was observed in the kind of micro-organisms isolated from the CSF; in period III the typical nosocomial pathogens had disappeared.

Conclusions: The incidence of drain-related meningitis has decreased from 39% in 2003 to less than 10% in 2005. Awareness of the problem and the interventions performed in 2004 were probably important causative factors in this reduction. Adequate prophylaxis remains a point of concern and new strategy seems needed for improvement.

PERIOD	BASELINE[1]	I	II	III
Interventions				
1) - Awareness	-	+	+	+
- Protocol development	-	+	+	+
- Implementation of protocol	-	-	+	+
- Enforcement of protocol	-	-	-	+
- Insertion LD in special room	-	-	+	+
2) Diagnostic and therapeutic management	-	-	+	+
3) Protocol on surgical prophylaxis	-	-	+	+
4) Introduction of closed drain-system	-	-	-	- / +
Number of patients with EVD or LD	1152	67	64	533
Patients with drain-related infections (%)	45 (39.1)[2]	12 (17.9)	9 (14.1)	5 (9.4)
Drain-related infections per drain-episode (%)	-	12 (14.6)	9 (13.4)	5 (7.6)
EVD infections per 100 days at risk	-	1.8	2.1	0.9
LD infections per 100 days at risk	-	4.5	1.3	1.8
Multiple drain infection episodes	-	4	0	0
Total number of nosocomial infections	-	46	28	18
% patients with nosocomial infections	-	40.3	28.1	28.3
Adequate antibiotic prophylaxis % EVD	-	37	38	35
No prophylaxis EVD %	-	25	19	28

[1]baseline period January 2002-June 2003; [2]only EVD; [3]incomplete data.

P042

National surveillance to the incidence of *Clostridium difficile*-associated diarrhea in the Netherlands

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Objectives: The recent outbreaks of *Clostridium difficile*-associated diarrhea (CDAD) due to the new emerging PCR-ribotype 027, toxinotype III strains has renewed the interest of CDAD as an important nosocomial infection. To determine the incidence of CDAD in the we conducted a prospective surveillance study in 14 hospitals in the Netherlands.

Methods: From May 1st to July 1st of 2005, 14 participating hospitals registered all patients diagnosed with CDAD. A standardized questionnaire was devised to obtain patient information. Faecal samples or isolated strains were sent to the Reference Laboratory at the LUMC for culture and further characterization by PCR-ribotyping, toxinotyping, and the presence of genes for toxin A (tcdA) and toxin B (tcdB).

Results: In total, 101 patients with CDAD were reported. The overall incidence (median) of CDAD was 17 for 10.000 patient admissions and varied from 1 to 75. Of 101 patients with CDAD, 41 % was community acquired. The median age of 54 patients with nosocomial acquired CDAD was 59 years. Of 54 patients with CDAD, 7 (13.9%) died during the study period. At least 41 different PCR-ribotypes could be recognized among 91 strains. Type 027 was identified in 9 patients, all from 1 hospital. Toxinotyping revealed the presence of at least 7 different types. Of 91 strains, 87% were TcdA+/TcdB+, 10% TcdA-/TcdB- and 3% TcdA-/TcdB+.

Conclusions: The incidence of CDAD in The Netherlands is lower than reported in USA and Canada, but varied considerably per hospital. The new emerging type 027 was found in 9 patients from 1 hospital with a high incidence of CDAD (39 per 10,000 admissions).

P043

Outbreak of three related cases of psittacosis detected by real-time PCR

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Introduction: Psittacosis is a zoonosis caused by an infection with the intracellular microorganism *Chlamydophila psittaci*. Clinical manifestations include high fever, severe headache and cough, and range from mild to life threatening disease. The obligate intracellular habitat makes culture difficult and time consuming. Moreover, isolates of *C. psittaci* are highly infectious and should be handled under bio-safety level 3 conditions. Molecular techniques like real-time PCR are therefore ideal alternatives for the detection of *C. psittaci* to aid the diagnosis of psittacosis.

Methods: We developed an internally controlled real-time PCR that targeted the ribosomal intergenic spacer of *C. psittaci*. The PCR assay was validated on a set of clinical samples from serologically confirmed patients with psittacosis and controls.

Results: Using this PCR system we detected an outbreak of psittacosis among members of a veterinary unit. One of these members was admitted to the ICU, because of multi organ failure.

Conclusions: With a new developed real-time PCR for *C. psittaci*, we were able to identify three patients with psittacosis related to a common source. The symptoms of the patients varied from mild to severe. This real-time PCR for *C. psittaci* enables the diagnosis of psittacosis in prior clinical undetected cases.

P044

Comparison of a molecular screening method with traditional culture for the detection of *Salmonella* spp. and *Campylobacter jejuni* in feces

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Background: *Salmonella* spp. and *Campylobacter jejuni* are the major causes of bacterial gastro-enteritis in the Netherlands. Conventional diagnosis is based on detection of both species in feces by traditional culture, which can take several days. We developed a sensitive molecular screening method for the detection of both species which decreases the turn-around time significantly. This study describes the comparison of this real-time PCR based screening method with routine culture for the detection of *Salmonella* spp. and *C. jejuni* in feces.

Methods: A total number of 1742 stool samples were received at our laboratory. Routine culture was performed on fecal samples and consisted of enrichment, selective culture and phenotypic identification. The molecular method consisted of a semi-automatic DNA extraction in combination with real-time PCR assays for *Salmonella* spp. and *C. jejuni*. PCR positive samples as well as samples which demonstrated PCR inhibition were cultured afterwards, consisting of the identical procedure as described for routine culture. Also, data regarding costs made for personnel and consumables were collected for both methods.

Results: A total number of 1724 samples were included for validation of *Salmonella* spp. The detection of *Salmonella* spp. improved by 18% with molecular screening; sensitivity was 100% and specificity 99%. For *C. jejuni* 1680 samples were included and detection improved by 23%; sensitivity was 98% and specificity 97%. PCR inhibition was observed in less than 1.6% of all samples. Average costs were € 10,94 per sample for routine culture and € 18,00 per sample for molecular screening.

Conclusion: 1) The molecular screening method has a great potential for rapid detection of *Salmonella* spp. and *C. jejuni* in feces. 2) The detection of *Salmonella* spp. and *C. jejuni* will improve with molecular screening. 3) Average costs per sample for the molecular screening method are 65% higher then routine culture. However, further automation of the extraction en detection procedures will reduce the costs of the molecular screening.

P045

Construction and validation of an internal control for detection of *P. gingivalis* in clinical samples

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Background: Real-Time PCR has been described as a convenient detection and quantification assay for several periodontal pathogens. None of the PCR amplifications has been described with an internal PCR inhibition control. This control recognizes false negative RT-PCR results and improves the reliability of quantitative RT-PCR for diagnosis. In this study a *Porphyromonas gingivalis* 16s rRNA RT-PCR was used for construction of a non-competitive internal positive control. The construct was developed by insertion of a non-bacterial sequence within a specific *P. gingivalis* probe recognition site. Subsequently, this modified *P. gingivalis* construct is integrated into the genome of *E. coli* by site directed mutagenesis. Spiking of subgingival plaque samples, before DNA extraction, with low amounts of the modified *Escherichia coli* provides insight into inhibitory effects in clinical oral specimens.

Methods: Two probe sequences were constructed by overlap extension technique. After amplification the resulting product was cloned in a cloning vector and subsequently recombined in the genome of *E. coli*.

Results: The application of the IPC was validated in an assay for the detection of *P. gingivalis* in subgingival plaque samples from periodontitis patients. The presence of inhibitors of the amplification reaction was confirmed after adding known amount of *P. gingivals* after the DNA isolation.

Conclusion: The designed IPC has proven to be an effective tool for monitoring inhibition of RT-PCR and confirms the correct interpretation for quantitative diagnostic results. This methodology could be used as a quality control in molecular diagnosis of periodontal diseases.

P046

Value of real time PCR for the detection of *Mycoplasma pneumoniae*, *Legionella pneumophila*, and *Chlamydophila pneumoniae* in diagnostics for community acquired pneumonia

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Introduction: Molecular diagnostic techniques are promising tools for the rapid etiological diagnosis of respiratory tract infections. We investigated the value PCR for the detection of *Mycoplasma pneumoniae, Legionella pneumophila* and *Chlamydophila pneumoniae* as causative agents in community acquired pneumonia (CAP).

Methods: Sputum samples, throat swabs and paired blood samples were collected from adult patients with acute CAP seen by pulmonologists in Friesland. Results of PCR on respiratory samples were compared to results of paired serology and, for L. pneumophila also with results from urine-antigen-detection (Leg-Ag).

For molecular detection of *Legionella* and *M. pneumoniae,* DNA extracts were prepared from sputum or throat swabs using a MagNA Pure Compact (Roche). For *C. pneumoniae,* the manual Boom extraction was performed. Real time PCR was performed using a LightCycler-I (Roche) and reactions were optimalised for separate detection of *L. pneumophila* plus *L. non-pneumophila, M. pneumoniae* or *Chlamydophila.*

Results: 83 patients were included in the study. Sputum samples were available from 35.

M. pneumonia was detected six times in PCR and in the same patients serology was positive. Three of these patients were PCR positive in sputum, but negative in throat swab.

L. pneumophila was detected by PCR in four patients. Twice in sputum only, and twice in both sputum and throat. Three of these PCR positive patients had a positive Leg-Ag. The fourth was positive in serology. In serology five positives were found, three of these were PCR negative in throat swabs, no sputa were available.

For *C. pneumonia* no PCR positives were found, whereas serology detected seven infections.

Conclusions: For *C. pneumoniae,* PCR on respiratory material is not a sensitive tool.

For *M. pneumoniae* and *L. pneumophila* results from PCR in respiratory material correlate well with other diagnostic results and because PCR can be performed in acute phase samples molecular testing will be a useful diagnostic tool in CAP. For *M. pneumoniae* and *L. pneumophila* PCR is less sensitive from throat swabs than from sputum.

P047

Construction and validation of an internal control for quantitative Real Time amplification of *Chlamydia trachomatis*

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Chlamydia trachomatis (CT) is a major cause of sexual transmitted infections. A large number of commercial and in house nucleic acid amplification tests have been described for the detection of CT. Among all described amplification procedures for CT there is no internal control used for monitoring the complete process. In this study we developed an internal control (IC) detecting inhibition of the amplification reaction, and performance of nucleic acid extraction simultaneous with the CT amplification procedure.

The cryptic plasmid found in CT was used as a amplification template for CT detection. For construction of the IC we used a chromosomal chlamydial sequence in which we cloned an artificial probe sequence. This IC was subsequently flanked with two CT specific primers. This amplification product was cloned in a suicide vector and transformed to a Dh5a *Escherichia coli* strain. After recombination with the chromosome of this *E. coli* strain this strain is validated as an internal control for the CT detection procedure. For validation three CT patient panels were used. 1) multiple bacterial strains and different CT serovars for the determination of specificity, 2) 100 CT positive or negative females for validation of the assay with DNA extraction by HPPTP Kit (Roche) and 3) 20 CT Cobas positive and 60 CT Cobas negative swaps to validate the assay with the commercial Cobas CT DNA Preparation Kit (CDPK, Roche). As expected after validation it was demonstrated that less IC amount was needed for the HPPTP isolation (200 cfu) as compared to the CDPK sample preparation (2000 cfu) since only the HPPTP generates pure DNA.

Based on the variation detected after testing the negative samples the inhibition limit was calculated at Ct 40.1 for the HPPTP protocol and Ct 38.2 for the CDPK. Our new assay detected all CT serovars and showed no cross reactions with other bacteria. Addition of the IC did not influence the sensitivity of 0.01 IFU.

The assay described shows that the use of the internal control is a rapid, sensitive, easy to use and cost effective monitoring for detection of CT in clinical samples.

P048

Nucleic acid detection in urine samples as control for treatment of a *Chlamydia trachomatis* infection

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Introduction: *Chlamydia trachomatis* is the most common sexually transmitted bacterial pathogen worldwide. Because most infected individuals are asymptomatic, the actual number of reported cases represents only a fraction of the infected population. If left untreated, this silent infection can evolve in women into pelvic inflammatory disease or lead to infertility. Since the infection is easily and effectively treatable with antibiotics (e.g., azitromycin or doxycycline), detection and treatment of infected individuals is an important part of chlamydia control programs. The purpose of the present study was to evaluate whether a polymerase chain reaction (PCR) assay for *C. trachomatis* in urine samples is a useful tool to study the response to treatment of a chlamydia infection.

Methods: One hundred ten urine samples, one specimen per patient (35 male urine samples and 75 female urine samples), collected between one and nine weeks after treatment of a *C. trachomatis* infection, were tested with the COBAS amplicor assay.

Results: This study on monitoring of *C. trachomatis* infection showed that DNA detection in urine samples in the observation period after treatment were negative (94%). Only three of the 110 samples were positive in the period 0-2 weeks after treatment and were probably taken too soon after treatment. Four samples taken between two and four weeks after treatment were also positive, probably due to reinfection with *C. trachomatis*.

Conclusion: In conclusion, our study on the monitoring of *C. trachomatis* infection showed that DNA detection in urine samples two weeks after treatment were negative, indicating successful treatment. One option for the few cases in which the sample remains positive two weeks after treatment would be to determine the presence of DNA again four weeks after treatment to exclude treatment failure or a reinfection with *C. trachomatis*.

P049

Comparison of different genes for the identification of atypical Mycobacteriae by DNA sequencing

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Introduction: Identification of atypical mycobacteria supports proper treatment of infected patients. Sequencing of different gene fragments for the identification of mycobacterial species is a widely used method. However, several genomic targets have been described for this purpose and none has been determined superior.

Methods: Six genetic targets (16S, ITS, *sodA, secA1, hsp65* and *rpoB*) were sequenced. In 2004, the six most encountered mycobacterial species in the Netherlands were *Mycobacterium tuberculosis, Mycobacterium avium, Mycobacterium kansasii, Mycobacterium gordonae, Mycobacterium chelonae/abscessus,* and *Mycobacterium malmoense*. In total, 18 isolates of these species, identified by the RIVM, were used to compare the utility of the different genomic targets for identification. The usefulness of the targets for identification of mycobacteria was determined by the quality of sequences, the inter- and intra- species variation and availability of sequences in public databases.

Results: Sequence analysis of the 16S, ITS and *secA1* regions showed good quality sequences for all 18 strains. For the other targets sequencing failed for one strain or more. With sequenced products of all 6 targets that could be analysed, the correct species (to complex level) could be determined, and *secA1*, *sodA* and *rpoB* could be used for differentiation to the sub-species level. Availability of sequences in the public databases is best for the 16S gene and there is some information on the *rpoB*, the *hsp65* gene and ITS.

Conclusion: A more complete panel of strains from different species will be subjected to DNA sequence analysis to determine whether one of the less-frequently used targets are superior to the currently most frequently used ones (16S, ITS and *hsp65*).

P050

Reliable performance of molecular microbiological methods for diagnosis of infectious diseases

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Nucleic acid amplification based methods are currently used in clinical microbiology laboratories for the detection of pathogens in clinical samples, both qualitatively and quantitatively, as well as for the identification of cultured micro organisms.

In order to produce reliable results for the diagnosis of infectious diseases, guidelines are proposed describing the professional qualifications required from the head of the laboratory and the technicians, the requirements for laboratory construction and adequate working instructions including continuous education.

In a laboratory manual the workflow and the procedures for the individual tests is described with special attention for prevention of contamination. Instructions for sampling of specimens, transportation and storage, and criteria for acceptable clinical specimens are included.

Separate rooms with one-directional workflow, proper equipment and restricted access are a prerequisite. No clinical samples nor purified nucleic acids should be allowed in the amplification mixture preparation room. In the pre-amplification room vials containing viable organisms should be opened only in a laminar flow cabinet. Rules on the quality control of equipment and their maintenance should be specified. There should be an accurate inventory of the necessary reagents.

Commercial tests with appropriate controls should be performed strictly as instructed by the manufacturer. For in-house developed tests, the sensitivity and specificity should be thoroughly investigated with purified nucleic acids and with micro-organisms in the same matrix background as the expected in clinical samples. Specific or general- use internal controls should always be included as well as a sufficient number of negative samples to detect contamination.

The laboratory should participate in external quality control programs. Exchange of reagents and/ or samples between laboratories is encouraged. All activities and results should be accurately registered. These guidelines should help laboratories to meet the requirements for reliable performance of molecular diagnostics in infectious diseases to be evaluated in internal and external quality control audits.

P051

Nucleic acid extraction from micro organisms in clinical samples, comparison of the manual Boom method with extractions using the MiniMAG (Biomerieux)

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The sensitivity of nucleic acid amplification tests for detection of low numbers of micro-organisms is dependent on the efficiency of nucleic acid (NA) extraction from the clinical samples. With increasing numbers of samples, automation is needed in order to save time and to avoid cross contamination between samples. In our search for an efficient, robust and fast method we compared the manual silica based Boom method with the MiniMAG method provided by Biomerieux. The MiniMAG is not automated and uses the same reagents as the NA extraction robot EasyMAG, recently launched by Biomerieux on the Dutch market.

In order to test the MiniMAG, sputum and pus samples were spiked with different numbers of *Mycobacterium bovis* BCG or *Bartonella henselae*. We choose these combinations of sample background and species because for these samples only the manual Boom method gives sufficient sensitivity and other methods such as Qiagen columns or MagNA Pure Compact (Roche) do not respond to our demands. All extractions were performed in duplicate with the manual Boom or the MiniMAG followed by a species specific quantitative real time PCR. Before extraction with the MiniMAG an 'off board' lysis was performed, using a home made lysis buffer with proteïnase K. In total 112 different samples were tested and the Ct values derived after MiniMAG extraction were comparable with the Ct values with the manual Boom. Sometimes, extraction with the manual Boom resulted in a slightly better extraction efficiency (Ct 1-2). For adequate extraction of DNA from different clinical samples an "off board" pretreatment with an SDS-proteïnase K containing lysis buffer is necessary. For detection of micro-organisms with a low load in viscous or purulent samples the extraction efficiency of the MiniMAG is sufficient and this procedure is promising for application at the EasyMAG extraction robot.

P052

Detection of enterovirus and parechovirus in clinical samples

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In our laboratory, detection of enteroviruses (EV) in CSF samples is performed by a two-step real time PCR. The

sensitivity and speed of this PCR is better than culture and we have stopped culturing CFS samples. Fecal samples are cultured in order to perform typing of positives. The EV-PCR is very specific and does not detect rhinoviruses and parechovirus (PEV), described formerly as enterovirus type 22 and 23. Recently a third PEV is described, PEV-3. PEV types 1, 2 and 3 cause similar clinical symptoms in infants as enteroviruses and detection of PEV is indicated in the same samples as detection of EV. Like EV, PEV can be isolated in viral culture, however no monoclonal antibodies are available to identify PEV in a positive culture.

We have set up a real time PCR for PEV 1-3, based on primers and probes described by Corless, et al. J Med Virol 2002;67:555-62. The sequences were slightly changed in order to combine the PEV PCR in the same LightCycler run with the EV PCR.

Two methods of RNA extraction were compared, QiAamp Viral RNA MiniKit (Qiagen) versus MiniMAG (Biomerieux). Sensiscript (reversed transcriptase, Qiagen) was used to prepare cDNA and real time PCR was performed in three capillaries one for EV, one PEV and one for phocid distemper virus (PDV) which was used as internal control to monitor the extraction efficiency and inhibition of the reaction. Each target was detected with its own set of primers and a specific 5'-nuclease probe (Taqman) in channel F1 of a LightCycler I (Roche).

Isolation of viral RNA with the MiniMAG system was more efficient than Qiagen extraction. The PCRs were specific and no cross reaction was detected between the targets EV and PEV. PEV-3 was detected in a recent quality control panel (SKML 2005). The sensitivity of the PCR assays in comparison to culture (TCID50) for various types of EV and PEV with will be presented.

P053

Application of minimal sequence quality values prevents misidentification of bla_{SHV} type in single bacterial isolates carrying different SHV extended spectrum beta-lactamase genes

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Nucleotide sequencing is the standard molecular method for determination of the type of beta-lactamase gene present in an isolate. During a study on ESBL epidemiology, SHV genes of *Escherichia coli, Enterobacter cloacae* and *Pseudomonas aeruginosa* were amplified by PCR and the amplicons were sequenced. The sequence assembly of individual sequence traces of bla_{SHV} genes of the three different isolates, using default parameters, yielded an SHV-2 gene for the *E. coli* and *E. cloacae* isolates and an SHV-5 gene for the *P. aeruginosa* isolate. After visual inspection of the sequence traces, using minimal sequence quality values, there were several low quality positions in the bla_{SHV} genes of the three strains. At three different positions in the sequence traces double signals were visible. A or T at position 92, A or G at position 402 and A or G at position 703. Different nucleotides at position 92 and 403 lead to amino acid substitutions and this will lead to different SHV types. The four possible combinations correspond to SHV types SHV-2, SHV-2a, SHV-5 or SHV-12, which differ in spectrum and activity. A possible explanation for the double signals could be that two or more bla_{SHV} alleles were amplified for each isolate. To asses the number and type of bla_{SHV} alleles in the PCR amplicons, these were ligated into cloning vector PCR2.1. Ligation products were transformed to E.coli DH5 alpha. Insert size was confirmed by colony PCR. Eight different clones for each of the PCR amplicons of the three different isolates were sequenced, and the signals at nucleotide positions 92, 402 and 703 were compared. Only two of the four possible combinations were present: $A_{92}G_{402}A_{703}$ which corresponds to SHV-2 and $T_{92}A_{402}G_{703}$ which corresponds to SHV-12.

Conclusion: We conclude that 1) two different bla_{SHV} genes were present in the isolates of the three different species: SHV-2 and SHV-12; 2) Genotypic detection using default parameters may lead to misidentification of the number and sort of SHV genes; 3) Using minimal sequence values will prevent misidentification of bla_{SHV} genes.

P054

Development and validation of real-time pcr assays for, and preliminary results of a multi-center study to assess the prevalence and epidemiology of Shiga-toxin producing *Escherichia coli* in the Netherlands

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Introduction: A nationwide screening program for shiga-toxin producing *Escherichia coli* (STEC) will take place in the Netherlands from November 2005 to November 2006

(Abstract NVMM 2006, Van Zwet, et al). Prior to the start of this program, real time (RT) PCR assays were developed and validated.

Methods: Assays targeting the *stx*$_1$ and *stx*$_2$ genes were developed for both LightCycler (LC) and TaqMan (TM). Stools were processed by a miniMAG stool protocol. The phocine herpes virus-1 was used as an internal control (IC). Both assays were validated with a panel of well characterized *E. coli* (n=31) and non-*E. coli* strains (n=38). Intra-, inter-assay variation and analytical sensitivity were assessed by dilution series (n=8), spiked in 2 fecal matrices, analyzed in 5-fold on the same day and once daily on 4 subsequent days. Starting November 2005, 8 laboratories will weekly screen 10 stools each for the presence of STEC by RT-PCR. Isolation and typing of STEC from the positive stools will be performed at the RIVM.

Results: Both assays proved specific for *stx*$_1$ and *stx*$_2$ genes and no cross-reaction was observed. The TM assay was capable of detecting approximately 10^4 CFU/g of stool (100% hit rate) for semi-solid and liquid stools. Lower hit rates were observed at approximately 10^3 CFU/g (22% and 67%, respectively). The LC assay proved to be 1 log less sensitive (100% hit rate) compared to the TM assay for semi-solid stools. Furthermore, the LC assay did not detect approximately 10^3 CFU/g. Coefficients of variation (CV) were < 5% for both TM and LC assays. During the first 2 months of the study, 705 stools were screened, resulting in 7 RT-PCR positives, of which 2 were also positive for *E. coli* O157 by routine culture. From 2 RT-PCR positives, STEC have been isolated and typed, resulting in O157 and O8 serotypes, whereas the remaining RT-PCR positive stools await further testing.

Conclusion: Both TM and LC assays proved to be very reproducible, although their sensitivities were not equal. Although based on limited data, our results might suggest that RT-PCR screening for STEC is more sensitive compared to the current culture-based approach that only targets *E. coli* O157.

P055

Presence of *Legionella pneumophila* DNA in serum samples during Legionnaires' disease in relation to C-reactive protein levels

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It has been shown that *Legionella pneumophila* DNA can be detected in serum from patients with Legionnaires' disease. The reported sensitivity of *L. pneumophila* PCR on serum samples ranges from 30 to 80%. We further explored this observation studying the kinetics of *L. pneumophila* DNA in serum samples in relation to C-reactive protein (CRP) and urea levels. Fifteen hospitalized patients with confirmed Legionnaires' disease were studied on day of admission up to 10 days thereafter. Diagnosis was made by *Legionella* urinary antigen test in 12/15 patients and seroconversion in 3/15 patients. A macrophage infectivity potentiator (MIP) real-time PCR was performed on 38 serum samples, including 23 follow-up serum samples obtained from 12/15 patients. A serum sample obtained on day of admission was available from 11/15 patients.

Serum samples obtained on day of admission were MIP PCR positive in 7/11 (64%) patients and MIP PCR negative in 4/11 (36%) patients. In 3 patients with a MIP PCR negative serum sample on day of admission, one or more follow-up serum samples were positive. Overall, *L. pneumophila* DNA was detected in serum samples from 13/15 (87%) patients. Despite appropriate antibiotic treatment, *L. pneumophila* DNA remained detectable up to day 10 following admission in one patient. The lowest Ct value by far (26,65) was detected in the one patient that died on day 5 following admission.

CRP level in the 7 patients with a MIP PCR positive serum sample on day of admission was 499 ± 144 mg/l (median ± SD). In this group, admission urea level was > 7 mmol/l (CURB-65 criteria) in 3/7 (43%) patients. In the 4 patients with a MIP PCR negative serum sample on day of admission, CRP level was 244 ± 97 mg/l. Admission urea level in this group was > 7 mmol/l in 1/4 (25%) patients.

Thus, the presence of *L. pneumophila* DNA in serum is a common phenomenon in hospitalized patients with Legionnaires' disease although in some cases not yet present on day of admission. The presence of *L. pneumophila* DNA in serum on day of admission seems to correlate with a strong acute phase response as reflected in high CRP levels.

P056

Rapid detection of human parechovirus in CSF of young children by real-time PCR

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Introduction: Human parechoviruses (HPeVs) have been associated with mild respiratory and gastrointestinal symptoms in predominantly young children, but more severe conditions such as transient paralysis, neonatal sepsis en meningitis have also been reported. Therefore,

a rapid detection of HPeVs in clinical samples from young children is essential in viral diagnostics.

Materials and methods: We have developed a 5'UTR real time taqman PCR assay specific for HPeVs using an enteroviral internal control. Serial dilutions of HPeV wild type RNA were tested to determine the dynamic range and lower limit of detection of the assay. The sensitivity and specificity of the assay were tested by using high positive and low positive cell cultures of three previously described HPeV serotypes, the new HPeV serotype we found (HPeV4), enterovirus serotypes (EVs), rhinoviruses and hepatitis A (HAV). To establish diagnostic relevance 482 cerebral spine fluid (CSF) samples from children <5 years negative for enterovirus were tested.

Results: The HPeV taqman assay has an analytical sensitivity of 100 copies in PCR. All 4 HPeV serotypes could be detected while EVs, rhinoviruses and HAV remained negative. Testing of the 482 clinical samples in our assay demonstrated HPeV infection in 16 children (3.3%) with severe conditions such as sepsis and meningitis.

Conclusions: HPeV infections in young children may lead to serious conditions as neonatal sepsis and meningitis. Therefore, HPeV should be included in viral diagnostics of neonatal sepsis and meningitis in young children. We have developed a rapid 5'UTR real time taqman PCR assay specific for HPeV that will be introduced in our diagnostic setting.

P057

Novel restriction/methylation system leading to uninterpretable PFGE results in methicilline resistant *Staphylococcus aureus*

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Introduction: Many micro-organisms protect themselves against negative influences of foreign DNA by using restriction endonucleases that cut the DNA at a specific recognition sequence. In order to protect their own DNA from being digested, this is usually modified by methylation of specific adenine or cytosine residues at the corresponding recognition sites.

During an investigation of pig farming as a possible source of MRSA in the Netherlands

with Pulsed-field gel electrophoresis some strains were not digested using the standard restriction enzyme SmaI. We investigated if this could be the result of DNA methylation.

Methods: *Staphylococcus aureus* isolates were recovered from pigs and pigs farmers. An unrelated control ATCC strains was used in all experiments. All strains were analysed by PFGE using methylation sensitive (SmaI) and methylation insensitive (XmaI) restriction enzymes. DNA sequence analysis was performed with sodium bisulphite treated and untreated DNA to look for methylated cytosine's.

Results: The DNA of the pig-farming related strains was clearly protected from digestion by SmaI but not by XmaI while the DNA of the control strain yielded identical banding patterns with both enzymes. Furthermore, a number of C residues were identified that were protected from conversion by sodium bisulphite in the tested strains but not in the control strain.

Conclusions: Taken together the restriction enzyme analyses and the bisulphite sequencing results convincingly show that the DNA from these pig-farming related strains was indeed methylated. The putative methylation recognition sequence for the DNA methyltransferase in the methylated strains is CC*NGG. For the genus Staphylococcus as a whole, no such enzyme has yet been reported.

P058

IS *6110* Primer Mediated AFLP for strain differentiation within the *Mycobacterium tuberculosis* complex

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IS *6110* Restriction Fragment Length Polymorphism (RFLP) typing is considered the golden standard in genetic typing of strains of the *Mycobacterium tuberculosis* complex, but the procedure is laborious and requires much chromosomal DNA. An alternative method could be Amplified Fragment Length Polymorphism (AFLP) typing, which is rapid, easy to perform, requiring low amounts of DNA and it has the possibility for database construction. Standard AFLP is a highly discriminating typing technique for many micro-organisms, but it does not differentiate between species and strains within the *M. tuberculosis* complex. Therefore this method was modified as follows: Chromosomal DNA is digested with two restriction enzymes, followed by ligation of the corresponding adaptors, according to the standard AFLP procedure. Subsequent amplification of the restriction fragments was performed with one standard adaptor primer and one labelled IS *6110* specific primer. Addition of the IS *6110* specific primer will amplify the region adjacent to the insertion element until the first restriction site. By labelling the IS primer a IS *6110* specific pattern is created. Number and lengths of fragments were determined on a ABI Prism® 3100 genetic analyser with Genescan software. Based on in silico genome AFLP analysis a high discrimination was predicted between different species and strains within the *M. tuberculosis* complex. We tested

strain H37Rv of *M. tuberculosis* and strain Copenhagen of *Mycobacterium bovis*/BCG and compared the experimental AFLP pattern with the in silico pattern, which we found to be highly identical. To confirm its discriminatory power we tested 7 additional strains of the *M. tuberculosis* complex: *Mycobacterium microti*, *Mycobacterium africanum* and 5 strains of *M. tuberculosis* with different genotypes. The similarity coefficient between the AFLP patterns of each of these strains was less than 70%, which is appropriate for reliable differentiation.
IS *6110* Primer Mediated AFLP might well be an attractive alternative for discriminating species, genotypes and strains within the *M. tuberculosis* complex and thereby being a powerful tool in molecular epidemiology.

P059

Genotyping with amplified fragment length polymorphism of *Mycobacterium haemophilum* from different continents

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Introduction: The application of specific culturing and molecular detection methods has identified *Mycobacterium haemophilum* as an increasingly common pathogen in the Netherlands. The species was previously rarely diagnosed, but has now been encountered as the involved pathogen in several diseases like skin inflammation, lymphadenitis and arthritis. In 2003-2004 a sudden increase of patients with cervicofacial lymphadenitis caused by *M. haemophilum* was observed in the Amsterdam region. As a part of an epidemiological study, the genetic diversity of these strains was investigated and compared to unrelated strains originated from different parts of the world.
Methods: 130 clinical *M. haemophilum* isolates were collected: 30 European strains (of which 20 from the Amsterdam region) and 100 strains from different continents (among which 43 Australian strains and 40 USA strains). Amplified Fragment Length Polymorphism (AFLP) methodology was optimised for the genotyping of *M. haemophilum*. DNA was extracted using the MoBio® UltraClean Microbial DNA kit. An enzyme combination of *Eco*RI and *Mse*I with selective priming was used to obtain a high discriminatory power. AFLP Patterns were compared using Dice calculation. Clinical, geographical and time-frame differences between the strains have been analysed.
Results: The AFLP method enabled inter- and intra-species differentiation of *M. haemophilum*. No differences were observed in the AFLP patterns of the 20 Amsterdam strains while genetic diversity was present in 10 other European *M. haemophilum* strains.
In general, strains belonging to a certain continent showed clustering of AFLP patterns. The 43 Australian strains represented 2 separate clusters, encompassing 21 and 14 strains. Among the 40 strains from USA, 36 were from the New York area. Within these 36 strains, AFLP discriminated 5 types, including 1 large cluster of 23 strains.
Conclusion: *M. haemophilum* is a highly conserved mycobacterial species, but AFLP shows sufficient diversity for epidemiological studies. Clustering of *M. haemophilum* was clearly present in the region around Amsterdam.

P060

Molecular detection and genotyping of *Pneumocystis jirovecii* in a cluster of renal transplant patients

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Introduction: We tested the applicability of real-time PCR for diagnosing *Pneumocystis jiiroveci* pneumonia (PcP) and sequence analysis of the internal transcribed spacer (ITS) for genotyping, on a cluster of renal transplant patients infected with PcP at the Leiden University Hospital medical Center (LUMC). The cluster was compared with a control group of other PcP infected patients.
Methods: In the period from the first of January until the first of October 2005, 20 patients with possible PcP were included. Thirteen patients from the Leiden region with PcP diagnosed in 2003 and 2004, served as controls. The diagnosis of PcP was established by direct microscopy or real-time PCR (targeting the dihydropteroate synthase (DHPS) gene) on bronchial alveolar lavage (BAL) fluid. Clinical and demographical data of the patients were collected. Genotyping of the patient strains was performed by sequence analysis of the internal transcribed spacer 1 (ITS1) and ITS2 regions.
Results: Of 20 patients, 16 were diagnosed with PcP; 10 with microscopy and real-time PCR, 6 with only PCR. All patients had clinical symptoms compatible with PcP, and none died. Sequence analysis of the ITS1 and ITS2 gene regions was successful in 13 of 16 PcP positive patients and yielded the combined type 'Ne' in 9/13 samples and type 'Bi' in 1 sample. In 3 samples only the ITS2 genotypes could be determined (type 'e' twice and 'g' once). Preliminary typing results of the control group showed that the 'Ne' type was uncommon in the region.
Conclusion: 1) Real-time PCR increased the diagnostic yield of PcP in renal transplant patients with 60%. 2) In contrast

with the control group, the Ne type was predominantly present in the cluster of renal transplant patients.

P061

Extended spectrum beta-lactamases among *E. coli* isolates obtained in the Twente-Achterhoek region

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Introduction: In recent years, 84 *Escherichia coli* isolates obtained in our region, were found to carry the ESBL-phenotype as demonstrated by the disk-approximation test (DAT). We characterized the ESBL's of these isolates by PCR and sequence analysis and retested the ESBL-phenotype with the VITEK II compact (BioMerieux).
Methods: PCR amplification was used for the detection of the most frequently present beta-lactame- (bla-)gene families: bla-TEM, bla-SHV, bla-CTX-M and bla-OXA. When positive a second PCR followed by sequence analysis was applied for the characterization of the nucleotide sequences of the bla-genes. The ESBL-phenotype, as demonstrated by the DAT, was also tested with the vitek. Clonal relatedness of isolates was determined by amplified fragment length polymorphism (AFLP).
Results: The PCR results showed that 78 *E. coli* isolates harbored one or more members of the bla-gene families, 25 harbored bla-TEM, 7 harbored bla-TEM and bla-SHV, 23 harbored bla-TEM and bla-CTX-M, 7 harbored bla-SHV, 1 harbored bla-SHV and bla-CTX-M and 15 harbored bla-CTX-M. No PCR reactivity was observed in 6 isolates. No bla-OXA genes were detected. Sequence analysis of the bla-TEM genes showed that only few ESBL-genes were present: 1 isolate harbored a TEM-12, 1 isolate harbored a TEM-20 and 3 isolates harbored a TEM-52. Also in the bla-SHV genes only 1 isolate harbored a SHV-12. Based on these molecular findings a total of 28 isolates harbored no ESBL-genes while 50 isolates did. Analysis by the VITEK showed that only 2 of the ESBL-gene negative isolates were phenotypic ESBL's. On the other hand all but 2 isolates of the ESBL-gene positive isolates were confirmed by the VITEK. There was no clonal relatedness between isolates harboring the same ESBL-genes.
Conclusion: Based on the molecular characterization of the ESBL's we conclude that the VITEK II provides a better method for the phenotypic confirmation of possible ESBL's than the DAT. The lack of clonal relatedness of the isolates demonstrates a polyclonal origin of the ESBL-genes in our region despite a predominance of the CTM-X gene.

P062

Emerging High Pathogenicity Island associated with an *Enterobacter cloacae* outbreak

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Introduction: *Yersinia* spp. or *Escherichia coli* containing the High Pathogenicity Island (HPI) are more virulent than strains without the HPI. The HPI (36-72 kb) consists mainly of genes encoding for iron uptake, including *irp1*, *irp2*, and *intB*, encoding HMWP1, HMWP2, and integrase B, resp. Also *Enterobacter cloacae* (EC) may contain the HPI.
Aim of the study was to investigate the prevalence, expression, and mobility of the HPI in EC.
Methods: The presence of the HPI was examined in 4 groups by PCR amplification of a fragment of *irp2*. I: 153 EC (83 *E. cloacae* clone I (OEC), 70 unique genotypes) isolated in the UMCU during an outbreak (2001-2003); II: 35 isolates from other, non EC, gram-negative species from 23 patients harbouring the OEC; III: 89 non-repeat clinical EC from different European centers; IV: 146 non-repeat EC blood isolates from the UMCU (1989-2004). HMWP1 and HMWP2 expression was detected by SDS-PAGE in HPI-positive strains. HMWP2 was confirmed by Edman degradation. The *intB* genes of *irp2*-positive isolates were sequenced to examine the possibility of horizontal transfer of HPI.
Results: Prevalence of HPI; Group I: 96% (80/83) of the OEC harbored *irp2*, whereas 4% (3/70) of the unique EC harboured *irp2*. In Group II: 29% (10/35) of the isolates were *irp2* positive, 9 *E. coli* and 1 *Enterobacter aerogenes*. In group III 2% (2/89) were positive (1 blood and 1 wound). Of group IV 3% (4/146) isolates were positive. HMWP1 and HMWP2 were expressed in the 2/2 OEC, 2/3 of the unique EC and 1/1 *E. aerogenes* tested. The sequences of the *IntB* genes of three genotypic different EC and an *E. aerogenes* were identical to each other, indicating transfer of the HPI.
Conclusions: 1) Nearly all OEC harboured the HPI, while almost no other *E. cloacae* contained the HPI, indicating a possible association between the HPI and increased epidemicity of strain. 2) At least two HPI encoded proteins were expressed under iron limited conditions, indicating that the HPI was functional. 3) Data indicated that HPI is mobile between different Enterobacteriaceae. Possible transfer of the HPI occurred also during the *E. cloacae* outbreak.

P063

Comparison of REA and AFLP typing of *Porphyromonas gingivalis* strains isolated from spouses

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Introduction: *Porphyromonas gingivalis* is an obligate anaerobic bacterium that is strongly associated with rampant periodontitis. Previous studies have indicated the presence of transmission of these bacteria between subjects. To control infection in closed populations, such as families, it might be usefull to treat the patient as well as other colonised persons within these families. For those studies, it proved to be necessary to perform reliable typing. Typing of *P. gingivalis* is done by a lot of different methods.

Aim: We performed a REA based typing method to study whether spouses are colonised by similar strains of *P. gingivalis*. Recently, an AFLP was developed for similar studies. The aim of this study was to compare REA results with AFLP.

Methods: We investigated a group of strains isolated from 6 patients and their spouse both with periodontitis. The *P. gingivalis* strains were isolated from different sites of the mouth and typed with the REA method and with AFLP using MSEI and PSTI restriction enzymes.

Results: Results suggested transmission between spouses in four of the six couples. In two couples no transmission was found by the REA methode. By using the AFLP method, similar results were found.

Conclusion: It is suggested that both REA and AFLP can be used for typing studies on *P. gingivalis*. The advantages of AFLP typing is that the method is more reliable and more robust. Furthermore automated pattern comparison is possible by using a database of many strains, enabeling comparing strains over time.

P064

Dynamics in the population structure of *Mycobacterium tuberculosis* in The Netherlands 1993-2004

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Background: The incidence of tuberculosis (TB) has been relatively stable in The Netherlands over recent years. This is related to a decreasing incidence among elderly native residents and an increasing incidence among younger immigrants. We hypothesised that this changing epidemiological profile among patients could be reflected in the population structure of circulating *Mycobacterium tuberculosis* strains.

Methods: Between 1 January 1993 and 31 December 2004, all culture-positive TB strains diagnosed in The Netherlands were genotyped at the RIVM by IS*6110* RFLP. Using a computer algorithm, the RFLP patterns of the strains were grouped into clades with 60% similarity. To assess changes over time in the population structure of circulating TB strains, we compared the distribution of clades among patients upto the age of 30 and among patients aged 70 and above in the periods 1993-1995 and 2002-2004.

Results: In total 3104 genotyped isolates were available for analysis; 37% of them could be grouped into 5 major clades, 28% into 24 smaller clades, 15% did not belong to a clade, and 11% were low copy samples and could therefore not be further analysed. Adjusted for time period, sex and area, the 70+ population had a lower risk of being infected with a Beijng clade strain (OR 0.5, 95%CI 0.3-0.8). Adjusting for age, sex and area, there were no differences in risk of infection with any specific clade between the two time periods. However, stratified by age, the risk of infection with a Beijing clade among the 70+ population adjusted for sex and area was higher in the more recent time period (OR 3.3; 95%CI 1.4-7.8).

Discussion: While the overall risk of infection with a Beijing strain decreases with age, the chances of being infected with a Beijing strain have increased among the 70+ population in the period 2002-2004 compared with the period 1993-1995, adjusted for sex and geographical area of diagnosis. A more complete and detailed analysis of all genotyped isolates is being prepared to assess contributing factors to changes in the population structure of *M. tuberculosis*.

P065

Use of Raman spectroscopy for rapid subspecies identification of vancomycin resistant enterococci

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Vancomycin resistant enterococci (VRE) can cause problems in a hospital, especially in outbreak situations. The transfer of vancomycin resistance to other organisms[1], such as MRSA, renders the latter practically untreatable and is a logical concern for clinicians. Using standard techniques, identification of enterococci can take up to 3 days; genotyping (e.g., PFGE) for epidemiological analysis requires an additional 2 days.

Raman spectroscopy is a nondestructive, optical technique that provides high-resolution optical fingerprints of any kind of tissue. In microbiology, Raman spectroscopy is gaining interest due to developments in equipment, data analysis and the resulting ease-of-use. The usefulness of Raman spectroscopy for species identification[2] and, the potential for subspecies identification[3] has been shown.

We have used a collection of 19 *Enterococcus faecium* isolates[4] from 8 different PFGE types to evaluate the typing capabilities of Raman spectroscopy. After one overnight culture, 5 sample suspensions were prepared per isolate; in the dried suspensions, 100 spectra of 1 second were recorded; this way, the set was measured on the same day the overnight cultures were available. The experiment was repeated on a second day to evaluate reproducibility.

Hierarchical clustering of the Raman spectra showed to be comparable to PFGE clustering; 10 identical isolates from PFGE type 'I' were also identical according to Raman. All 3 isolates from PFGE type 'IV' could be discriminated from each other by Raman. Of the 6 isolates of independent PFGE types, 4 were fully discriminated; the remaining 2 clustered with isolates from PFGE type 'IV'.

On the second day, these independent isolates also clustered with type IV isolates. If these differences in classification are caused by the choice of the 86% similarity cut-off level for the PFGE types or by the fundamental differences in the methods (genotype vs phenotype) will be discussed in the presentation.

Conclusion: We conclude that Raman spectroscopy has the potential for the rapid typing of VRE, with minimal sample handling and fast turnaround times.

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P066

High-resolution molecular fingerprinting of *Aspergillus fumigatus* from a suspected outbreak in the Intensive Care Unit

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Introduction: In October 2005, a possible outbreak of *Aspergillus fumigatus* among intensive care unit (ICU) patients was investigated using a high resolution molecular fingerprinting assay.

Methods: A total of 28 *A. fumigatus* isolates (mainly from respiratory samples) from 4 different patients and 4 environmental isolates were studied. All isolates were analyzed using the STRAf (Short Tandem Repeats of Aspergillus fumigatus) assay. This is an exact typing method for A. fumigatus, which yield a genotype of up to 9 different markers that can easily be compared to each other.

Results: All but one of the intrapatient isolates were of the same genotype, all interpatient isolates belonged to different genotypes. The isolates collected from the environment were all unique; two samples proved to be a mixture of two or more strains. Two out of 4 patients had probable aspergillosis and were treated with systemic antifungal (itraconazole and caspofungin) at autopsy invasive aspergillosis was diagnosed.

Conclusion: Since all environmental isolates were of a different genotype, they most likely do not fully represent the actual variation of strains present in the ICU which may be much larger. To have an accurate reflection of the environmental strains, multiple isolates will have to be of the same genotype. Therefore, it was of no surprise that we didn't uncover any relation (if present) between the patient isolates and the few environmental isolates collected in the same period. Molecular epidemiology was of little refuse in this outbreak. We conclude that we were dealing with nosocomial aspergillosis during construction work, but to be able to prove such relationships, continuous monitoring of the endogenous population will be mandatory.

P067

The development of a new typing method for *Clostridium difficile*: Multi-Locus Variable Number of Tandem Repeat Analysis (MLVA) and its application for the epidemiology of the virulent PCR-ribotype 027 strain

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Introduction: To study the epidemiology of *Clostridium difficile,* a typing method with a higher discriminatory power, typeability and reproducibility than currently available methods is required. Multi-Locus Variable Number of Tandem Repeat Analysis (MLVA) is a new candidate technique, that has already been tested successfully on a number of bacterial and fungal species. Using the whole genomic sequence, we developed MLVA for *C. difficile* and compared the method to the standardized PCR-ribotyping. Additionally, MLVA was tested on a collection

of the new emerging hypervirulent PCR-ribotype 027 strains.

Methods: Short tandem repeat loci (3-9bp) were identified using Tandem Repeat Finder v3.21 on the genome of *C. difficile* strain 630. Amplification was performed using a single PCR-protocol. PCR-fragments were analysed using multi-coloured capillary electrophoresis on an ABI3100, with a ROX500-marker as internal marker. The number of repeats per fragment was subsequently determined.

The discriminatory power was tested on 23 reference strains representing 11 serogroups and 12 toxinotypes. The ability to subtype specific PCR-ribotypes was investigated with 7 subtypes of PCR-ribotype 001 (rep-PCR types 1-7), 6 TcdA-/TcdB+ strains of PCR-ribotype 017, and 11 strains belonging to PCR-ribotype 027. Of these 11 type 027 strains, 9 were isolated from 3 outbreaks and 2 from endemic cases.

Results: A total of 7 regions with short tandem repeats were identified. MLVA discriminated all 23 reference strains and the 7 known subtypes of PCR-ribotype 001 (rep-PCR 1-7). Two MLVA-types were recognized among 6 TcdA-/TcdB+ strains; the differences were present in only one repeat-region. Of 11 PCR-ribotype 027 strains, 9 outbreak-related strains were identical to each other. Interestingly, two endemic type 027 strains differed from the other strains in 3 of 7 regions.

Conclusion: 1) MLVA is a highly discriminatory genotyping method for *C. difficile* and is capable to subtype various PCR-ribotypes. 2) MLVA is also an important new tool to study the epidemiology of the emerging PCR-ribotype 027 strains.

P068

Optimisation of screening for methicillin-resistant *Staphylococcus aureus* by using the chromogenic medium MRSA ID

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Introduction: In Dutch hospitals, a 'search and destroy' policy to prevent the spread of methicillin-resistant *Staphylococcus aureus* (MRSA) has so far kept the incidence under 1%. To shorten patient isolation periods and control costs, screening methods need to be rapid, highly sensitive, specific and inexpensive. We prospectively analysed whether using the new chromogenic medium MRSA ID (bioMérieux, Marcy l'Etoile, France) instead of the currently used CHROMagar Staph aureus (CHROMagar, Paris, France) improved our MRSA screening protocol with respect to turnaround time and workload.

Methods: Swabs submitted for MRSA screening were inoculated into a mannitol broth with 2.5% NaCl. After 18-24 hrs broths were subcultured onto MRSA ID (MID) and CHROMagar Staph aureus (CSA), which were incubated at 35°C. MID was read after 24 and 48 hrs, CSA after 48 hrs because in our experience 24 hrs yielded insufficient growth and colony pigmentation. Green colonies on MID and mauve colonies on CSA were tested for DNase production and screened for methicillin resistance on Columbia sheep blood agar containing 6 mg/litre oxacillin and with a methicillin strip. Suspected MRSA was confirmed by Vitek 2 and by PCR for *mec*A and *sa442* genes.

Results: In total 449 cultures from 190 individuals were analysed. After 24 hrs green colonies were found on MID in 43 cultures, of which 29 were positive for MRSA. After 48 hrs, another 35 cultures on MID were suspect but MRSA negative. Of the 29 MRSA positives, 26 were found on CSA. Sensitivities and specificities of the media were 100% and 97% for MID after 24 hrs, 100% and 88% for MID after 48 hrs, and 90% and 55% for CSA after 48 hrs respectively.

Conclusions: In this study, MID was found to be a 100% sensitive, easy to interpret medium with the highest specificity after 24 hrs. On CSA, three MRSA isolates were not found because of overgrowing colonies of methicillin-susceptible *S. aureus* and other flora. Using MID shortens the turnaround time of our MRSA screening by 24 hrs and its high specificity limits confirmative testing considerably, thus resulting in lower costs.

P069

Optimisation of MRSA screening protocol by determining salt tolerance of *Staphylococcus aureus*

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Introduction: According to the Dutch 'search and destroy' policy to control the transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals, patients and staff in risk categories are routinely screened for carriership of MRSA. To increase the sensitivity of culture methods, a mannitol salt enrichment broth is commonly used, which contains 7.5% sodium chloride as a selective agent. Because there is evidence that this amount of NaCl inhibits the growth of MRSA, we determined the salt tolerance of a collection of methicillin-resistant and methicillin-susceptible *S. aureus* (MSSA) strains.

Methods: We prepared mannitol salt broths with concentrations of NaCl ranging from 1.5 to 12.5% with intervals of 1% and determined the maximal non-inhibiting concentration (MaNIC) and the minimal inhibiting concentration (MIC) of NaCl for 41 MRSA and 48 MSSA isolates after 24 hrs and 48 hrs incubation.

Results: After 24 hrs the MaNIC values for MRSA ranged from 2.5 to 4.5% NaCl. Eleven (27%) MRSA strains of different phagetypes had a MaNIC of 2.5%. After 48 hrs MRSA MaNIC values ranged from 2.5 to 6.5%. The MIC_{50} and MIC_{90} of NaCl for MRSA after 24 hrs were 5.5% and 6.5% respectively (range 3.5-9.5%), after 48 hrs both were 6.5% (range 4.5-12.5%). For MSSA the MaNIC and MIC values of NaCl were similar.
Conclusions: The NaCl MIC_{90} after 24 hrs was 6.5% for both MRSA and MSSA, which is lower than the often-used concentration of NaCl of 7.5%. The MaNIC values after 24 hrs indicated that for 27% of MRSA growth was inhibited by > 2.5% NaCl. For optimal sensitivity, an enrichment broth for MRSA screening should therefore not contain more than 2.5% NaCl. This, however, will render it inadequately selective. Our results preclude the use of NaCl for selectivity purposes. Antimicrobial agents should be used instead.

P070

Molecular analysis of broth enriched screening samples: a sensitive, high throughput method to exclude the presence of methicillin-resistant *Staphylococcus aureus*

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Introduction: In the the spread of methicillin-resistant *Staphylococcus aureus* (MRSA) is successfully controlled by the policy of 'search and destroy'. In our hospital, suspected patients are kept in strict cohort isolation which is only lifted after MRSA colonization has been excluded. Typically, screening consists of overnight pre-enrichment of clinical samples in oxacillin broth and subsequent culturing on blood agar plates for 48 h. The return of negative results (i.e. no MRSA colonization or infection) can take up 4 to 5 days after a patient entered isolation. Clearly, strict isolation of MRSA suspected patients is costly and reduction in isolation period is desirable. We, therefore designed a molecular MRSA screening tool with a turn-around time of 24 hours. This molecular tool is especially designed to exclude the presence of MRSA. Confirmation of MRSA colonization or infection is done by classical culture.
Methods: An internally controlled multiplex *S. aureus*/mecA TaqMan real time PCR was designed and tested. DNA was extracted from pooled clinical oxacillin pre-enriched samples (nose, throat and perineum of one patient) on the Magna Pure LC system using a modified DNA isolation program. Molecular analysis was done in parallel with classical culture.
Results: In total, 1187 clinical samples (394 patients and personnel) from 2 outbreaks and 14 weeks of ICU MRSA screening were analysed. The MRSA screening assay has a specificity and sensitivity of 95,8% and 100%, respectively. The predictive negative value was 100%. Using this assay in an outbreak setting, the number of total patient-isolation-days was reduced from 66 to 37 days.
Conclusion: We developed a rapid and high throughput MRSA assay that gave a reduction of a 44% in isolation days in an outbreak setting.

P071

Evaluation of a new chromogenic agar (MRSA-select) for detection of methicillin-resistant *Staphylococcus aureus* with clinical samples in The Netherlands

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A novel chromogenic medium for the detection of MRSA, MRSA-select (Bio-Rad, Hercules, California), was evaluated with clinical samples in a public health laboratory in The Netherlands. In total, 3000 samples were tested in the period January to March 2005, including 972 nose, 972 throat, 968 perineum and 88 wound and urine samples. Presumptive MRSA colonies appear pink/mauve on the MRSA-select medium. We evaluated the colonies that grew on the MRSA select medium and we compared the performance of MRSA select medium with the routine screening procedure.
We found that all confirmed MRSA strains appeared as pink/mauve colonies, while none of the uncolored colonies were MRSA strains. Specificity of pink/mauve colonies decreased from 98.1% after 20-24 hrs incubation to 93.1% after 40-48 hrs incubation.
In total 70 MRSA strains were isolated, 55 of which were detected by the MRSA-select medium and 54 were detected by the routine screening procedure. Sensitivity of the MRSA-select medium was 78.5% and specificity was 99.5%, compared to sensitivity of 76.9% and specificity of 99.5% of the routine screening procedure. In total, 18 patients were MRSA positive, 4 of which were detected by the MRSA-select medium only and 1 of which was detected by routine screening only. Sensitivity on patient level was 94.4% and 77.8% for the select medium and the routine screening procedure, respectively, while specificities were 99.7% and 99.0%. We conclude that MRSA-select medium is as useful as our screening procedure for MRSA detection.

P072

Evidence for the effectiveness of 'search and destroy' of methicillin-resistant *Staphylococcus aureus* (MRSA) in The Netherlands

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Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen causing nosocomial infections. Prevalence of MRSA varies widely among countries. In the Netherlands, MRSA prevalence in clinical isolates is below 1%, due to a national 'Search and destroy' policy. The efficacy of this policy in endemic situations is debated. In this study we describe a large MRSA outbreak with a highly transmissible strain in a Dutch hospital, and the measures that were taken to control the epidemic.
Methods: MRSA was isolated using conventional culturing, including a selective broth. MRSA-isolates were typed using Pulsed Field Gel Electrophoresis. Measures used to control the epidemic included: screening of contacts (patients and hospital staff), screening at re-admission and in some departments at regular intervals and at discharge. In addition, strict isolation of colonized or infected patients, decolonisation of patient and hospital staff MRSA carriers using topical agents. Also an electronic patient signalling, and personnel culture-information system was developed.
Results: The epidemic started around November 2001. As the involved strain had a low oxacillin MIC, it was not immediately recognized as MRSA. In January, when gradually more contacts were screened, it appeared that MRSA had spread to many departments, and that members of staff were also colonized. During Nov 2001- Dec 2004 722 new isolates were detected among 559 patients and 129 staff members respectively; some persons were infected more than once. Of these isolates, 87% belonged to four epidemic clones, the remainder being sporadic types. Since May-June 2004, when a last upsurge of one of these clones occurred, only sporadic cases have been detected.
Conclusion: This study shows that with application of a strict 'Search and destroy' policy even endemic MRSA can be controlled.

P073

Staphylococcus epidermidis O-47 constitutively expressing Green Fluorescent Protein (GFP) to study staphylococcal survival in RAW264.7 Cells

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Background: *Staphylococcus epidermidis*, responsible for the majority of biomaterial associated infections, survives inside macrophages around biomaterials implanted in mice. To investigate this survival *in vitro* by FACS analysis, we aimed to construct an *S. epidermidis* strain constitutively expressing green fluorescent protein (GFP).
Methods: Plasmid WVW189GFP carrying a constitutive *Staphylococcus aureus* promoter in front of the GFP gene was used to transform *S. epidermidis* strain O-47, *S. aureus* RN4220 and *Escherichia coli* DH5α. *S. epidermidis* transformation was optimized by pre-treatment with achromopeptidase (ACP). Transformants were analysed for GFP expression by FACS analysis, for plasmid stability, and for effect of GFP presence on growth rate. Phagocytosis assays were performed using the RAW246.7 monocyte/macrophage cell line. RAW cells were incubated with bacteria and after 45 minutes extracellular bacteria were removed. At several time points RAW cells were lysed and the intracellular bacteria were analysed by FACS. Propidium iodide (PI) was used to discriminate between dead and viable bacteria after phagocytosis.
Results: Transformants of all 3 strains had a high level of GFP expression. *S. aureus* GFP and *S .epidermidis* GFP remained highly GFP-positive upon repeated culture without antibiotics. GFP had no adverse effect on growth in several bacterial and cell culture media. After phagocytosis the transformants could be well distinguished from the cell debris. The PI staining at 22h after phagocytosis showed PI negativity for 45-75% of both *S. epidermidis* GFP and *S. aureus* GFP.
Conclusions: An *S. epidermidis* strain stably and constitutively expressing GFP was constructed. GFP had no effects on growth in the tested media and was effective for FACS analysis. The number of PI positive particles, considered to represent dead bacteria, of both *S. epidermidis* and *S. aureus* after 22h of phagocytosis was remarkably low. This indicates a high level of survival of the staphylocci after phagocytosis. Alternatively, PI may have a low efficiency to stain dead *Staphylococci*. This is presently being investigated.

P074

Antibodies increase adherence of *Staphylococcus epidermidis* to biomaterials in murine experimental biomaterial-associated infection

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Biomaterial associated infections (BAI), mostly caused by *Staphylococcus epidermidis* are a significant problem

in modern medicine. In a mouse exp. BAI model, we have observed that *S. epi* persists longer in peri-implant tissue than on the implanted biomaterial (BM) itself. We therefore aimed to study the clearance of *S. epi* from the BM and from the surrounding tissue by (i) antibodies against surface protein antigens of *S. epi*, and (ii) a regimen of rifampicin/vancomycin (rif/vanco).

C57Bl/6 mice were immunized with a *S.epi* cell wall protein prep. to obtain monoclonal antibodies (mAbs). mAbs against LTA were obtained from QED Biosciences, UK. For experimental BAI, 2 Polyvinyl-pyrrolidone-coated silicon elastomer (SEpvp) BM segments were implanted subcutaneously in C57Bl/6 mice. Mice (9/group) were challenged with 107 cfu of the clinical isolate *S. epi* AMC5 30 min after a single subcutaneous injection of mAb or saline (repeated after 3 d in selected cases), and sacrificed after 8 d. BM and peri-BM tissue were processed and cultured on blood agar plates and in Brewer-Tween liquid medium.

To assess efficacy of the model, mice challenged with 107 cfu received daily i.p. injections of rif/vanco or saline, and were sacrificed after 1 or 8 days.

The major antigen recognized by sera of the immunized mice was Accumulation Associated Protein (AAP). a-AAP and a-LTA mAbs were used for passive immunization of C57Bl/6 mice. Neither of the two mAbs showed any protective effect. In contrast, bacterial adherence to the BM *in vivo* was sign. increased in the group treated with 80ug a-LTA. a-AAP also increased bacterial adherence, but this effect was not sign. Antibiotic treatment sterilized the BM, but not the peri-implant tissue.

Abs against *S. epi* LTA or AAP did not protect mice against BAI. a-LTA even increased bacterial adherence to the BM. Therefore, this study indicates that Abs against *S. epi* at the concentrations tested may contribute to rather than prevent BAI. Even after rif/vanco treatment, *S. epi* persisted in peri-implant tissue. Therefore, these data suggest that biofilm formation on the BM surface is not necessarily the only cause of persistent BAI.

P075

Coagulase-negative *Staphylococci* colonize peri-catheter tissue in human patients

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Introduction: Since an increasing number of patients will receive a biomedical device, the incidence of biomaterial-associated infections will rise. Skin bacteria such as *Staphylococcus epidermidis* and other coagulase-negative staphylococci are frequently associated with these infections.

Aim: To assess the presence of bacteria around intravenous and intra-arterial lines in critically ill patients.

Method: Under maximal sterility precautions intravenous and intra-arterial lines and surrounding tissues were excised from deceased intensive care unit (ICU)-patients. Appropriate swabs and blood samples, and bacterial culture plates placed next to the excision area and left open during the sampling procedure, were cultured as controls. The excised parts of the catheters were cut in segments of 1cm length and were rolled on blood agar plates according to a roll-plate method. After this, the segments were rinsed and sonicated. The sonicate was quantitatively cultured and the segments were placed in liquid broth. The tissue samples were homogenized and quantitatively cultured.

Results: Thirty-five lines from 18 patients were collected. The plates left open during the excision procedure yielded no or only very few bacterial colonies. Eight out of 35 (23%) peri-implant tissues tested were highly culture positive (> 100 colonies/biopsy). Catheter segments were found to be culture negative or only slightly culture positive. The bacteria cultured from different sites of the catheter and the surrounding tissue were compared by molecular typing methods. In two patients, the *S. epidermidis* colonizing the tissue were identical to the bacteria retrieved from the catheter.

Conclusion: The tissues surrounding intravenous or intra-arterial lines of critically ill patients form a niche for *Staphylococci* causing foreign body infections. This may have important consequences for the choice of antibiotic regime or frequency of catheter-changing, and is a novel element in the pathogenesis of foreign body infections.

P076

Clinical and molecular epidemiological characteristics of coagulase-negative staphylococcal bloodstream infections in intensive care neonates

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Introduction: Coagulase-negative *Staphylococci* (CoNS) are a major cause of late-onset sepsis in neonatal intensive care units (NICU). This study aimed to quantify neonatal CoNS sepsis, to determine clinical characteristics of CoNS sepsis patients, to assess the molecular epidemiology and biofilm forming properties of isolated strains, and to assess antibiotic susceptibility of clonal strains compared to incidentally occurring strains.

Methods: Retrospective study of late-onset CoNS bloodstream infection including infants admitted at a NICU in The Netherlands in 2003. Clinical and bacteriological data, were collected. CoNS isolates from bloodstream infection were retrieved and genotyped by restriction fragment end labeling (RFEL). Biofilm production was determined by quantitative assay.
Results: Included in the analysis were 339 patients. Ninety-four (28%) of total and 78 (51%) of very low birth weight infants developed late-onset sepsis; 64 of these 94 (68%) were due to CoNS. Seventy-six CoNS strains were isolated. Small for gestational age (SGA) infants had a 2.7 higher risk of developing CoNS sepsis. Dominant clones were absent. Biofilm producing strains were more frequently icaA positive than non-biofilm formers (58% vs 31%; p=0.022). Nearly all strains were resistant to one or more antibiotics. The mecA gene was present in 87.5% of the strains. Multiresistance occurred in 50% of all strains and in 40% of the clustered strains. Multiresistance occurred more often in biofilm forming strains than in non-biofilm formers (67% vs. 36%; p=.009).
Conclusions: 1) CoNS sepsis occurred frequently; 2) SGA is a risk factor for CoNS sepsis; 3) there were no dominant clones identified; 4) biofilm forming strains were significantly more frequently multiresistant, unlike clonal strains.

P077

EUREGIO MRSA-net Twente/Münsterland: Fighting (Community Onset-) methicillin-resistant *Staphylococcus aureus* internationally under different cultural perspectives

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Introduction: Goal is to establish a cross-border Dutch-German network (www.mrsa-net.org) providing a user-friendly knowledge centre for hospitals, public health authorities, GPs, nursing homes and laboratories which aids in the reduction of methicillin-resistant *Staphylococcus aureus*-rates (MRSA) and limit the cross-border transmission of MRSA. Guidelines and their implementation play a significant role in reaching these aims. Cross-border MRSA guidelines will be redesigned according to international standards and socio-cultural differences between the nations.
Methods: Based on quality standards for safety and healthcare documentation used in high risk chemical organizations, a framework for a systematic content analysis of current national Dutch and German MRSA-guidelines was developed. All guidelines were analysed on the basis of this framework.
Results: The MRSA-guidelines showed five dominating MRSA-perspectives: rule-, expert-, risk-, demand- and community-driven. German guidelines are mainly dominated by the rule- and expert-driven perspectives (guidelines are literally derived from law and follow the infection transmission route), in contrast to the Dutch which focus on the demand of the user and the community (addressed to public health and acceptability of guidelines by users).
Conclusion: 1) The fact that there are different guideline-perspectives results in an enormous, confusing set of guidelines. The management and use of guidelines becomes uncontrollable and leads to an illusory organisation where healthcare workers don't act in accordance with the guidelines and start applying their own insights. This might lead to cost-increasing and contrasting situations. 2) To implement guidelines successfully in a cross-border situation, a cultural and technical synchronisation alongside an integrated approach of the different perspectives of guidelines is necessary, inline with the current disease management models. Further research about the redesign and the evaluation of those guidelines in practice, will help achieving this.

P078

Serum- and animal tissue-free medium for transport and growth of *Helicobacter pylori*

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Introduction: The fastidious and microaerophilic nature of *Helicobacter pylori* makes exchange of *H. pylori* isolates between laboratories difficult. An additional problem is that all media that are commonly used for growth and shipment of live *H. pylori* strains contain serum and animal tissue-derived materials. As such materials potentially carry the risk of spreading infectious diseases (like Bovine Spongiform Encephalopathy), and hence the use of these transport media may be subject to regulations aiming to prevent the spread of such diseases. We therefore aimed to develop a growth and transport medium for *H. pylori* that would not contain serum- or animal tissue-derived components associated with the transfer of infectious agents.
Methods: Serum- and animal tissue free medium (SATFM) was based on serum-supplemented Brucella medium (BBN). Beef extract and peptic digest of animal tissue were replaced by papain digest soja pepton, bovine serum was

replaced by b-cyclodextrins, and pancreatic digest of casein was replaced by acid-hydrolysed casein.
Results: SATFM supported growth to similar levels as obtained with BBN. In addition, the protein profile of *H. pylori* reference strain 26695 was not significantly altered after growth in SATFM when compared to BBN. Finally, SATFM with 0.5% agar supported transport and storage of *H. pylori* strains, as 4/4 reference strains and 11/11 clinical isolates survived for up to 3 days at room temperature. Some strains (2/15) also survived for up to 7 days at room temperature in SATFM, although better survival rates at day 7 were obtained with Wang transport medium (10/15), which was used as reference transport medium.
Conclusions: SATFM can be used both as transport and growth medium for *H. pylori*. The formulation of SATFM may allow future certification enabling international transport of *H. pylori* and other bacterial pathogens.

P079

Cross-feeding between *Bifidobacterium longum* BB536 and acetate-converting, butyrate-producing colon bacteria during growth on oligofructose

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Introduction: Cross-feeding between colon bacteria is thought to be the link between the bifidogenic and the butyrogenic effect caused by the addition of oligofructose to the diet. Acetate and lactate produced by bifidobacteria may form the substrates for butyrate-producing colon bacteria that are reported to account for 2-3 % of the human gut microbiota. In this study, cross-feeding between *Bifidobacterium longum* BB536 and acetate-converting colon bacteria was studied through *in vitro* mono- and coculture fermentations.
Methods: Fermentations were carried out in anaerobic conditions with fructose, acetate and oligofructose as substrates. Monocultures of *B. longum* BB536, *Anaerostipes caccae* DSM 14662, and *Roseburia intestinalis* DSM 14610 were studied, as well as cocultures between *B. longum* BB536 and both butyrate-producing strains. Bacterial growth was followed up by measurement of optical density at 600 nm. Metabolite formation was studied using HPLC, GC/MS, LC/MS, and GC. Results: Growth of *B. longum* BB536 on fructose or oligofructose led to the production of acetic acid and, in lesser amounts, formic acid, lactic acid, ethanol, and succinic acid. *A. caccae* DSM 14662 produced only butyric acid and gasses when growing on fructose; no growth on oligofructose was observed. In a coculture with *B. longum* BB536, production of butyric acid by *A. caccae* DSM 14662 was detected, attributed to growth of the latter strain on the acetic acid, lactic acid, and fructose formed during the degradation of oligofructose by *B. longum* BB536. *R. intestinalis* DSM 14610 grew on both fructose and oligofructose, but showed an absolute requirement for acetate. Only butyric acid and gasses were formed. A coculture of *R. intestinalis* DSM 14610 with *B. longum* BB536 on oligofructose showed initial growth and acetate production by the *Bifidobacterium* strain, followed by oligofructose degradation and acetate conversion by the *Roseburia*, leading to the production of butyrate.
Conclusion: Two distinct types of cross-feeding between *B. longum* BB536 and acetate-converting colon bacteria were observed, both leading to the production of butyrate out of oligofructose.

P080

Effect of food processing conditions on degradation of phytate and release of iron and zinc in pearl millet

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As part of the European CEREFER (CEReal FERmentations) project, the effect of processing of pearl millet (*Pennisetum glaucum*, variety IKMP-5) was evaluated (4), to reduce the phytic acid concentration and hence, to increase the availability of iron and zinc from millet based diets. Pearl millet is a valuable staple food in the semi-arid areas of Africa, it is a major source of energy, proteins and minerals. Next to its high nutritional value, pearl millet contains some antinutritional factors (ANF) that can limit protein and starch digestibility and lower mineral availability. One major ANF is phytic acid (IP6). IP6 diminishes the availability of phosphorous to both humans and animals, and has an adverse effect on the utilisation of Ca, Zn, Fe and Mg. Millet grains were subjected to dehulling, fermentation, enzyme incubation, soaking, germination and cooking either in tap water or in kanwa (local potash) solution. *In vitro* availability was measured as solubility under physiological conditions using an *in vitro* digestion method (2). The final supernatant/digest was analysed for soluble iron and zinc by ICP-OES (Inductively Coupled Plasma - Optical Emission Spectrometer) (3). IP6 content was determined by HPLC (1).
Cooking in tap water improved in-vitro digestibility from 17% to 60%. Germination had the highest impact on improving iron bioavailability, up to 50%; followed by soaking and fermentation (35% and 27% respectively). Available zinc was also enhanced by these operations, but to a lower extent. Finally, IP6 was considerably reduced by fermentation, (microbial phytase on pre-fermented samples: 100% reduction). In a second phase, a more

detailed experimental design focussed on the single and combined effects of soaking, natural and pure culture lactic acid fermentation, phytase treatment and cooking. In particular soaking, combined with cooking appears to be very efficient in removing IP6. On the other hand, cooking sometimes leads to reduced mineral availability, presumably as a result of the formation of chemical complexes.

P081

Activity of herb volatile oils and their constituents against *Escherichia coli* O157:H7

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Introduction: The aim was to select volatile oils that are effective against *Escherichia coli* O157:H7, to identify the antibacterial components and possible interactions between them and to determine the mode of action.

Methods: 1) Minimum inhibitory concentrations (MIC) of oregano and thyme volatile oils (0.0078-2% v/v) were determined by means of a microdilution method using alamarBlue™ indicator of bacterial growth against *E. coli* O157:H7 strain RR98089. Experiments were repeated with the addition of 0.25% w/v soy lecithin or 0.05% w/v agar as stabilisers. 2) Percentage composition of the volatile oils was determined by HPLC-UV/LC-MS-MS. MIC assays for the major components were carried out and the fractional inhibitory concentration (FIC) index was calculated to detect possible interactions between the components. 3) SDS-PAGE with lysed whole cell samples was carried out to identify changes in protein synthesis under the influence of sub-MIC concentrations of individual volatile oil components.

Results: 1) MICs were as follows: oregano oil 625 µl/ml, thyme oil 625 µl/ml. Agar reduced the MICs to 78 µl/ml and lecithin increased the MICs to 1250 µl/ml. 2) The major components of oregano oils are thymol, carvacrol, cymene and terpinene (28%, 15%, 6% and 4% v/v respectively). MICs were as follows: carvacrol 1.2 mM, thymol 1.2 mM; cymene and terpinene were not antibacterial and did not influence the activity of the other components. Carvacrol and thymol were additive (FIC index = 1.1). 3) Preliminary results indicate that heat shock proteins were produced when cells were grown in the presence of carvacrol.

Conclusions: 1) Oregano and thyme volatile oils are effective against *E. coli* O157:H7. Agar as stabiliser improves the activity and lecithin reduces it. 2) Carvacrol and thymol are the major antibacterial components and their effects are additive. 3) Heat shock proteins are produced when cells are exposed to carvacrol.

P082

WATCH: Worldwide Analysis of Resistance Transmission over Time of Chronically and Acute HIV-1 infected persons

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Background: Patients infected with drug resistant HIV-1 experience a less efficient viral response to antiviral therapy. Worldwide many studies have been performed to investigate the magnitude of the problem of primary resistant HIV. These studies indicate that the prevalence of primary resistance varies from < 5% to > 25%. This variation can be attributed to differences in patient populations and behaviour, treatment guidelines, transmissibility and fitness of the resistant strains. Part of the variation is also attributed to the use of different algorithms to interpret transmitted resistance profiles.

The WATCH study aims to collect the raw data of these studies and to analyse all these data together in a standardised manner, in order to be able to make a good comparison of resistance figures.

Objectives: To collect HIV-1 PR and protease sequences from all over the world and analyse them together in a standardised manner.

Methods: We did systematic searches in Pubmed and in abstract books of international conferences (search terms: HIV primary resistance, transmitted resistance, HIV antiretroviral naive) in order to identify the researchers that have published on the subject. We approached these researches and asked them to collaborate with the study by contributing their (already published) data. The data we asked them to contribute were the RT and protease sequences and some clinical and demographic data.

Results: A total of 60 researchers agreed to collaborate with the WATCH study. Over 5500 sequences have been collected from 40 countries from all over the world.

Conclusion: This is the largest collection of HIV-1 RT and protease sequences of untreated patients in the world. It contributes to a better understanding of the transmission of resistant HIV-1.

P083

Novel HIV Gag based protease drug resistance mechanism caused by an increased processing of the NC/p1 cleavage site

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Background: HIV resistance mechanisms described so far involve mutations in the target gene of the drug. We

demonstrated that *in vitro* selection (IVS) experiments, using the protease inhibitor (PI) R0033-4649, resulted in the selection of viruses displaying 5-8 fold resistance to all clinically approved PI, in the absence of mutations in protease, proving a novel mechanism. Part of this resistance is caused by changes in the 3'-region of the viral gag gene, which contains the ribosomal frameshift signal as well as two protease cleavage sites separating the nucleocapsid (NC) and p6 domains. Two sets of experiments were performed to investigate whether an increase in efficiency of frameshifting and thereby in HIV protease levels and/or increased protease cleavage is responsible for the observed resistance.

Methods: In the first set of experiments the effect of the observed nucleotide changes on ribosomal frameshifting was determined. Since these nucleotide changes also confer amino acid changes in the NC/p1 protease cleavage site a second set of experiments investigating viral cleavage was performed. Therefore, wildtype and mutant proviral plasmids were transfected in 293T cells and viral antigens in cell lysates and concentrated virus preparations were analysed by immunoblotting. All experiments were performed in the absence and increased presence of R0033-4649.

Results: It was demonstrated that the observed nucleotide changes did not alter the frameshift efficiency. Immunoblot analysis using NC antibodies showed more efficient cleavage of the NC-p1-p6 fragment both in the absence as in the presence of R0033-4649.

Conclusion: We have identified that increased NC/p1 processing is the underlying mechanism explaining Gag related HIV protease drug resistance in the absence of protease mutations. The improved processing at this site may allow the virus to tolerate an inhibitor-induced reduction in protease activity and may thus be a general principle in resistance to protease inhibitors.

P084

Comparative disease progression observed in newly diagnosed patients infected with drug resistant and susceptible HIV-1: no signs for increased virulence

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Introduction: Recently rapid progression of disease due to transmission of a potential highly virulent, drug-resistant HIV-variant was reported in a patient from New York (USA). To rule out the potential spread of highly virulent drug-resistant viruses in Europe we compared disease progression in prospectively followed newly diagnosed patients infected with drug-resistant or sensitive viruses.

Methods: From a large prospective multi-centered cohort of 1415 patients diagnosed in 2003, we identified individuals with primary resistant viruses (R) and compared disease progression to a random selection of patients diagnosed with sensitive viruses (S). Resistance testing was performed using genotypic analysis. IAS-USA was used to identify primary resistance mutations. As endpoints of disease progress were used CD4 decline < 200 cells/mm^3, initiation of therapy, or occurrence of an AIDS defining event. Time to progression was compared using Cox proportional hazards analysis.

Results: Disease progression was compared between 78 patients infected with R-virus and 77 individuals infected with S-virus. Median follow-up was 16 months in both groups. Baseline CD4 and HIV-RNA in R and S were respectively 359 and 365 cells/m3 (p=0.9) and 4.8 and 4.7 log10 copies/ml (p=0.4). Multi-class resistance was identified in ten patients. At time of diagnosis, the endpoint was reached in 24 patients in R (31%) and 20 individuals in S (26%), OR=1.3 (95% confidence interval 0.6-2.6, p=0.5). During follow-up, 18 of 41 in R (44%) and 26 of 47 in S (55%) reached one of the endpoints during follow-up, hazard ratio = 0.7 (0.3-1.1, p=0.1).

Conclusions: In this systematic approach patients recently diagnosed with resistant viruses experienced a similar disease progression as patients infected with drug-sensitive viruses. Currently, there are no indications that multi-drug HIV variants with increased virulence are circulating in Europe. Further follow up is needed to determine whether clinical response to therapy once initiated may affect disease outcome.

P085

N-linked carbohydrate mediated interactions of porcine lung collectins and influenza A virus

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Introduction: The innate immune response against influenza A viruses (IAV) plays a significant role in containment of IAV in the airways. Important components of this first line defense barrier are a subgroup of mammalian collagenous Ca2+-dependent lectins known as the 'collectins'. Earlier studies have shown that surfactant

protein A (SP-A) and D (SP-D), collectins which are strongly expressed in lungs of mammalian species, are potent inhibitors of IAV infectivity and contribute to pulmonary antiviral defense.
Having receptors for both human and avian viruses, pigs can serve as important intermediate hosts for transmission of avian IAV strains to humans, and for the generation of reassortant strains. Studies on the interactions between porcine SP-A and SP-D with IAV, will contribute to a better understanding of the role of the innate immune response during influenza A virus infections in pigs.
Methods: Production of transiently expressed recombinant porcine SP-A and SP-D by HEK 293 cell secretion, modification of N-linked glycans by site-directed mutagenesis (deletion mutants, insertion of Asn-glycosylation motif sequences) and cotransfections of HEK cells with 2,3- and 2,6 sialyltransferases, IAV induced hemagglutination inhibition assays.
Results: Interaction studies of IAV with natural porcine SP-A and SP-D revealed that their more potent IAV neutralization activity results from interactions mediated by the Asn-linked sugars present in the lectin domains of both proteins. Furthermore it was shown that the distinct interactions of pSP-A and pSP-D with IAV depend on the terminal sialic acid residues present on this carbohydrate as well as their type of linkage to the penultimate galactose residues of the N-linked sugars.
Conclusions: Collectin glycosylation plays a crucial role in IAV recognition and neutralization. Therefore we recently started the production of recombinant pSP-A and pSP-D, as well as various mutants that differ in oligosaccharide profile. Screening of these recombinant proteins using infectivity neutralization assays allows us to determine the functional implications of glycan modifications for the antiviral efficacy of collectins in more detail.

P086

Fur mediates iron-responsive repression of urease expression in *Helicobacter hepaticus*

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Introduction: The murine pathogen *Helicobacter hepaticus* colonizes the enteric and hepatobiliary tract of rodents and causes inflammatory bowel lesions, hepatitis, gall-stones and hepatic malignancies. Urease is an environmentally regulated key-virulence factor for gastric *Helicobacter* species, but little is known on its role or its regulation in enterohepatic *Helicobacter* species like *H. hepaticus*. Here it is reported that urease expression and activity of *H. hepaticus* is iron-repressed, and this regulation is mediated by the trancriptional regulator Fur.
Methods: *H. hepaticus* strain ATCC51449 and its isogenic fur mutant were grown both under low-iron and high-iron conditions. Gene expression was monitored by Northern hybridization, and protein expression was monitored by SDS-PAGE and Western blotting. Urease activity was measured via a colorimetric reaction representing production of ammonia.
Results: Iron-restriction of growth medium resulted in a three-fold increase in urease activity in wild-type *H. hepaticus* strain ATCC 51449. Using Western blotting and Northern hybridization it was demonstrated that iron-responsive regulation of urease expression was mediated at the transcriptional level. Insertional inactivation of the fur gene abolished the effect of iron-restriction, indicating that Fur is responsible for iron-responsive regulation of urease expression and activity. A direct role of Fur in urease regulation was confirmed using gel-shift and DNase footprint assays, which revealed that Fur displays metal-dependent binding to a Furbox-like sequence in the promoter region of the urease gene.
Conclusions: *H. hepaticus* regulates its urease expression and activity in response to iron-availability via Fur, and this represents a novel type of urease regulation in bacteria, as well as a novel function for Fur in regulation of virulence determinants. Since iron-restriction is often used by pathogenic bacteria as a signal for entering the host, this suggests that this form of regulation may be required for the chronic colonization of the murine hepatobiliary tract by *H. hepaticus*.

P087

Campylobacter jejuni binds to Sialic acid-binding immunoglobulin-like lectins (Siglecs) expressed on monocytes and macrophages

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Introduction: *Campylobacter jejuni* is the predominant cause of enteric infection in industrialized countries. *C. jejuni* can induce post-infectious immune-mediated polyneuropathy (Guillain-Barré Syndrome, GBS) through molecular mimicry between the bacterial lipooligosaccharide (LOS) and gangliosides expressed on nerve cells. However, not all *C. jejuni* strains that express ganglioside-mimics induce GBS. *C. jejuni* is one of the few bacteria that, like host cells, express sialic acid (Sia). Sia, which is a key component of gangliosides, is a specific ligand for sialic acid-binding immunoglobulin-like lectins (Siglecs) that provide inhibitory signals to cells of the immune system. Bacterial and host factors that determine the outcome of *C.*

jejuni infection and confer a risk of post-infectious GBS are still largely unknown.

Methods: In ELISA, we studied binding of 13 *C. jejuni* strains (8 Penner reference strains and 5 GBS-associated strains, expressing different ganglioside mimics and combinations thereof) to several mouse- and human Siglecs with distinct Sia-specificities.

Results: *C. jejuni* with sialylated gangliosides were bound specifically by several members of the Siglec family that are expressed on monocytes and macrophages. The binding pattern of *C. jejuni* strains to distinct Siglecs reflected the expression of ganglioside mimics in their LOS. Siglec-binding of *C. jejuni* could be inhibited by monoclonal antibodies that block the Siglec Sia-binding site. In addition, knockout mutagenesis of *CstII*, a *C. jejuni* gene essential for ganglioside expression, abolished the binding of *C. jejuni* to its specific Siglec.

Conclusions: This is the first study that addressed lectin-mediated interaction between *C. jejuni* and the host at the monocyte/macrophage interface. Our result suggest that ganglioside expression by *C. jejuni* influences binding and uptake by phagocytes and may affect host intracellular signalling. These data may provide insight in the innate immune response to *C. jejuni* and may open up the way to future identification of risk factors and pathogenic processes involved in *C. jejuni*-associated post-infectious complications.

P088

CD14 gene polymorphism in relation to caries

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In multibacterial diseases, such as caries and periodontitis, presentation of antigens to the immunesystem is essential for a sufficient hostdefence. Bacterial components are recognized by CD14 and toll-like receptors 2 and 4 resulting in a NF-κB-based inflammatory response by activating the production of cytokines. It is suggested that an increase in CD14 molecules may result in higher inflammation, for example after a deep carious lesion. We hypothesise that individuals, who produce more *CD14*, due to a genetic variation in the *CD14*-260 promotor region, will react with more abcesses or fistulae after a carious attack than controls. The *CD14*-260T genotype was found in 45% of the population. From the individuals with numers of decayed, missed or filled teeth > 7 and the presence of abcesses or fistulae, 78% carried the *CD14* -260T –allele, while in the no-abscess group 41% carried the T-allele (Fisher exact test p=0.02, OR 3.8, 95% CI 1.1-11.9).

From these results we conclude that carriers of a *CD14*-260T-allele are more susceptible for inflammation after a carious lesion.

P089

Contribution of glutamine synthetase GlnA and its transcriptional repressor GlnR to pneumococcal virulence

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Central nitrogen metabolism is of utmost importance in bacterial survival. In the pneumococcus, several systems involved in amino acid metabolism, such as peptide uptake systems, have been shown to contribute to virulence. Signature tagged mutagenesis studies have shown that in pneumococcus, *glnA*, the gene encoding Glutamine Synthetase (GlnA) and *glnQ*, a glutamine ABC transport system, are involved in virulence. In *Streptococcus pneumoniae*, GlnR controls, together with GlnA, the expression of the *glnRA* and *glnPQ* operons.

To assess the relevance of this regulatory system *in vivo*, we used D39 wild type, Δ*glnA* and Δ*glnR* in a murine model of colonization and infection. We observed that the *glnA*-mutant had a reduced ability to colonize the murine nasopharynx ($p < 0.03$). The *glnR*-mutant, however, did not show any reduction in colonization. *In vitro* adherence to human nasopharyngeal cells of these mutants correlated with these observations, since reduced adherence to Detroit 562 cells was only observed for Δ*glnA*. Furthermore, upon intravenous infection, mutants for both *glnR* and *glnA* showed reduced levels of bacteremia (p=0.0182 and p=0.0002, respectively), and mice infected with these mutants showed increased survival times (p=0.0260 and p=0.0043, respectively).

We hypothesize that the maintenance of a glutamine pool in the bacterium contributes to full virulence. Furthermore, GlnR-mediated regulation also plays a role in virulence, in particular during sepsis, albeit to a lower extent. Individual targets of GlnR are currently assessed for their contribution to virulence.

P090

Regulation of gene expression in *Streptococcus pneumoniae* by Two-Component System 09 is strain-dependent

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Recent murine studies have demonstrated that TCS09 of *Streptococcus pneumoniae* is virulence-associated, moreover, is strain-specific. In the present study, we used a murine model of infection to assess the virulence of a TIGR4 *rr09*-mutant, and found that TIGR4Δ*rr09* was attenuated after intranasal infection and mice infected with *rr09*-mutant had significantly longer survival times than the wild-type infected mice. Further, we investigated the transcriptional changes in pneumococcal mutants lacking the response regulator of TCS09 (Δ*rr09*) by microarray analysis of two strains, D39 and TIGR4. The transcriptional pattern of D39Δ*rr09* and TIGR4Δ*rr09* displayed clear differences as compared to their parental wild type strains. Moreover, TCS09 appeared to (directly or indirectly) regulate different genes in D39 and a TIGR4. In D39Δ*rr09*, genes involved in competence (e.g. *comAB*) were upregulated, while several genes involved in sugar uptake (e.g. PTS systems) were downregulated. In TIGR4Δ*rr09* fewer genes were found to be regulated by RR09, most prominently genes located on the rlrA pathogenicity islet. Furthermore, we found that the genes encoding a β-galactosidase, a putative mannose-specific PTS, a putative sugar isomerase and putative aldose epimerase were strongly downregulated in D39, and not regulated in TIGR4. Real-time PCR confirmed these findings and this was extended to strain 0100993 in which these genes were not regulated by RR09.
In conclusion, our results indicate strain-specific regulation of pneumococcal genes by TCS09.

P091

Filamentous bacteriophages and small colony variants in *Pseudomonas aeruginosa*

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The capacity of *Pseudomonas aeruginosa* to form biofilms is an important requirement for colonization of human tissues. Bacteria in biofilms often develop into phenotypic variants that show highly different gene expression as compared to planktonic cells (1). It has been described that the filamentous bacteriophage pf4 is highly upregulated in biofilms, and that the emerge of small colony variants (SCVs) in biofilms is related to the plaque-forming pf4 phage in PAO1 (2). Those SCVs containing pf4 at their surface show enhanced attachment and microcolony formation. We therefore hypothesized that the presence of SCVs and pf4 in isolates might be of clinical importance.
Biofilm related *P. aeruginosa* strains were collected from clinical material and screened for the presence of pf1 and pf4 genes by PCR. Moreover, we also screened for genes of pf5 (PA14), which we identified to be highly homologous to phage pf4. In addition, the ability to form SCVs was determined by a static growth assay. These analyses showed that in several isolates phage genes were found, but that these putative bacteriophages were not identical to pf1, pf4 or pf5. Furthermore, the presence of phage genes could not be correlated to the ability to form SCVs.
P. aeruginosa strain PA14 forms SCVs very efficiently (~70%) upon static culturing, in contrast to PAO1. The role of phage pf5 in small colony formation was studied in more detail. We detected and characterized the replicative form of phage pf5. However, using induction experiments, immunoblot analysis and transcriptional profiling of the major coat protein we did not find evidence for the production phage proteins and virions. Moreover, mutants with transposon insertions in phage genes showed decreased production of the replicative form, but formed SCVs as efficiently as wildtype cells. Taken all data together, we concluded that small colony formation in PA14 is not caused by phage pf5.

P092

Translationally controlled tumour protein from *Madurella mycetomatis*, a marker for tumorous mycetoma progression

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About forty years ago antibodies against the fungus *Madurella mycetomatis* were first demonstrated in eumycetoma patients. To date nothing is known about the individual immuno-reactive antigens present in this fungus. We here identify its first immuno-dominant antigen, a protein homologous to the translationally controlled tumour protein (TCTP), a well-conserved

histamine release factor in a range of eukaryotes. Many functions have been recorded for TCTP.
The gene for this antigen was demonstrated to be present in two variants in *M. mycetomatis*, with 13% amino acid difference between the two proteins encoded. Differences were also recorded for the two TCTP signature sequences. TCTP variant I had a deviated TCTP2 signature sequence and TCTP variant II had a deviated TCTP1 signature sequence. The two variants were similarly represented in our collection with variant I found in 53% of our isolates and variant II in 47%. The two proteins shared epitopes, since variant II of the protein could be recognised by antibodies raised against variant I. *In vivo*, TCTP was found to be expressed at the fungal cell surface in developing stages of the eumycetoma-characteristic black grain but not in completely developed grains.
IgG and IgM immune responses against the whole TCTP protein and selected *M. mycetomatis*-specific peptides were determined. The antibody levels correlated with lesion size and disease duration. Overall, the patients with the largest lesions had the highest antibody titre, the titre lowering with decreasing size of the lesion. After 6-15 years of disease duration the antibody titres were the highest. Unfortunately some cross-reactivity, was observed between the mycetoma patients and the Sudanese healthy control population. Cross-reactivity appeared to be less when peptides were used which were developed on *M. mycetomatis* specific domains in the TCTP sequence.
TCTP is the first well-characterised immuno-dominant antigen and although TCTP may not be the best diagnostic tool, the ELISAs presented here could be useful in seroprevalence studies. In addition, studies into the influence of TCTP on mycetoma development or its use as a vaccine in the prevention of infection are urgently warranted.

P093

Genetic variability of *Tannerella forsythensis* in periodontitis patients and healthy subjects

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Background: The periodontopathic pathogen *Tannerella forsythensis* is strongly associated with aggressive forms of periodontitis. The prevalence in periodontal healthy subjects is however high and reaches levels of almost 48% in The Netherlands. Previous studies to elucidate whether the numbers in subgingival plaque or intrinsic characteristics of this pathogen accounts for disease development, failed to find differences in pathogenic genotypes. We aimed to study the genetic variation of isolates of *T. forsythensis* from healthy subjects and periodontitis patients using amplified fragment length polymorphism analysis (AFLP).
Methods: An AFLP technique was developed to observe whole-genome variation of *T. forsythensis*. A combination of restriction enzymes to observe sufficient variation was chosen and validated on isolates from twenty-seven individual non-linked subjects with periodontitis. In addition, intra- and interexperimental variation was determined. Clonality of *T. forsythensis* within one subject was determined from isolation of thirty strains from one subject using two isolates per sample.
Results: Based on restriction enzymes *MseI* and *PstI*, 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 all *T. forsythensis* isolates from twenty-seven periodontitis patients using one isolate per patient were different. The same was found for a group of fourteen periodontal healthy subjects using up to four isolates per subject. The genetic variation between strains from periodontitis patients was equal to the variation between healthy subjects what suggest that there were no clusters observed that assume virulent clones within the species *T. forsythensis* using AFLP.
Conclusion: Based on our results it is shown that each subject carries a *T. forsythensis* with a unique genotype. None of the subjects carries more than one *T. forsythensis* gentotype and there were no typical patterns that discriminate strains from healthy subjects from periodontitis patients.

P095

Interaction of *Streptococcus mutans* with *Veillonella parvula* grown in dual species biofilm

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Introduction: The purpose of this study was to look into the interaction of *Streptococcus mutans*, a dental pathogen, with *Veillonella parvula*, a bacterium that utilizes the lactic acid produced by *S. mutans* in the dental plaque biofilm. Furthermore, we wanted to explore the possibility of proteomic and genomic analysis of this interaction.
Materials and methods: Single and dual species biofilms of *S. mutans* and *V. parvula* were grown on polystyrene in BHI supplemented with lactic acid. Growth, and survival after exposure to antimicrobials were assessed. Analysis of protein expression with 2D difference gel electrophoresis

and analysis of *S. mutans* mRNA expression with 70-mer microarrays was evaluated.

Results: 48 h biofilms all had similar numbers of viable bacteria (appr. 109 CFU/cm^2). When grown in dual species biofilm, *S. mutans* and *V. parvula* were more resistant to chlorhexidine and *S. mutans* was more resistant to hydrogen peroxide. With the help of advanced statistical methods we were able to calculate differences in protein expression between single species and dual species biofilms. Pilot experiments showed that a careful experimental design makes it possible to evaluate differences in gene expression between *S. mutans* grown in single species and in dual species biofilms.

Conclusions: 1) *S. mutans* and *V. parvula* grown in dual species have different resistance to antimicrobials than when grown in single species biofilms. 2) Analysis of protein and gene expression by dual species biofilms is possible.

P097

Evaluation of the variation in carriage rate of *N. meningitidis*

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Neisseria meningitidis causes life-threatening meningitis and septicaemia. Evaluation of the effect of vaccination on carriage and of the extent of carriage in outbreak situations is of importance. Reported carriage rates assessed by culture of throat swab specimens vary from 5% to 30%, which is partly explained by age dependence of carriage rate. We hypothesized that the site of swabbing is also of importance in the assessment of meningococcal carriage. Therefore, we evaluated carriage by culture of swab specimens taken from two closely spaced sites in the throat.

Medical students (n=314) were asked to participate; 6 used antibiotics three weeks prior investigation and were excluded; 11 did not give consent. Of each student a swab was taken form the oropharynx, and from the anterior faucial pillar, respectively. All swabs were taken by the same experienced investigator. Swabs were plated on blood agar and subsequently on VCN agar, selective for meningococci. Of 294 students, 94 (32%) carried meningococci in their throat. Meningococci were found in the culture of both swabs of 10 subjects, while of 83 subjects only the oropharyngeal swab yielded meningococci. In addition, the blood agar plate culture of the swab of 13 subjects yielded exclusively meningococci, while that of another 15 subjects showed partial inhibition of the throat flora. In contrast, growth of meningococci was partly inhibited on blood agar cultures of the swab of 4 subjects, most probably by the microbial throat flora.

In conclusion, meningococci colonize a very specific site in the human throat. Therefore, to assess carriage, throat swabs have to be taken carefully and precisely from the oropharynx. At this site, in some individuals, meningococci may outgrow their competitors from the microbial throat flora. Phenotyping and genotyping of such strains may give insight in possible specific determinants of this property. Moreover, inhibition of meningococcal growth by microbial throat flora is of interest for development of novel therapeutics for prophylaxis.

P098

Polymorphisms in the *CD14* gene and peri-implant infection

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CD14 is a pattern-recognition receptor for bacterial components. It binds and transfers bacterial ligands to Toll-like receptors resulting in NF-κB activation, release of inflammatory cytokines and up-regulation of co-stimulatory molecules, providing signals to direct adaptive immune response. The genes involved in the innate immunity and regulation of the subsequent pro-inflammatory cytokine release may play an important role in the pathogenesis of dental implant infections (peri-implantitis). We have recently shown that the *CD14*-260T/T genotype contributes to periodontitis susceptibility. We aimed to determine the *CD14*-260 gene polymorphisms in peri-implantitis patients. Further, a relationship between genotypes, *Porphyromonas gingivalis* (Pg) and *Actinobacillus actinomycetemcomitans* (Aa) infection and smoking was investigated.

The study included 118 North Caucasian individuals. 72 patients (mean age 69 years, 76% smokers) demonstrating peri-implantitis at ≥ 1 implants as evidenced by bone loss > 3 threads on Brånemark implants, bleeding and/or pus on probing and 46 controls (mean age 65 years, 35.5% smokers) with clinical healthy mucosa and no bone loss were recruited for the study. The implants had been in function at least two years. Mouthwash samples were collected and genotyped for *CD14*-260 polymorphisms using PCR-technique. The presence of the two major periodontal pathogens, Pg and Aa, was assessed by standard culture techniques.

The genotype and allele frequencies for *CD14*-260 were comparable in patients and controls. The *CD14*-260T/T genotype was found in 19.4% of patients and 21.7% of

controls. Pg was detected in 13.3% and 4.4% in patients and controls, respectively, and Aa in 16.7% and 2.2% in patients and controls, respectively.
We conclude that the *CD14*–260 polymorphism does not contribute to the susceptibility to peri-implantitis.

P099

Diagnosis of *Giardia lamblia* with microscopy, striptest, ELISA and real time PCR

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Giardia lamblia is the most frequently diagnosed pathogenic intestinal parasite in the Netherlands. We compared four different diagnostic methods for the detection of *G. lamblia* in feces in both acute and chronic diarrhea.
Microscopic examination on stained samples collected with Triple Feces Test, ELISA (Novitec and Novatec Giardia lamblia ELISA), Giardia-strip (Coris Bioconcept), and real time PCR for the detection of *G. lamblia* were compared.
From July to September 2005, 515 feces were included. 154 watery specimens from acute diarrhea were sent for bacteriological examination and 361 triple feces test (TFT)-samples, representing a more chronic form of diarrhea, were sent to the parasitology department.
Using real time PCR as the golden standard, the positive predictive values of microscopy, ELISA and *Giardia*-strip were 100%, 99% and 50%, respectively. The sensitivity of microscopic detection was 71% while for *Giardia*-strip this was only 5%. Novitec ELISA was more sensitive (67%) than Novatec ELISA (51%). Specificity of all methods was never lower than 97%.
Microscopy with Triple Feces Test is a very specific method for the laboratory detection of *G. lamblia* in feces with good sensitivity. Both ELISA's also have good sensitivity and can be acceptable alternatives for microscopy. *Giardia*-strip can not be used as an alternative, because of very low sensitivity. Real time PCR is a very sensitive and specific method for the detection of *G. lamblia*.

P100

Pruritus and itchy papulovesicular skin eruptions caused by Dermanyssus gallinae

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Case report: A 48-year old healthy female patient developed a diffuse, pruritic erythematous maculopapular rash on her trunk, arms and legs. Her general practitioner felt that the most likely diagnosis was scabies and treated her with lindane and lidocaine/levomenthol. She returned the following day. The patient mentioned hundreds of 'bugs' infesting her bedroom. After visiting the internet, she suspected that she might be infested by chicken mite (Dermanyssus gallinae). A bug was examined in the Laboratory for Medical Microbiology and identified as Dermanyssus gallinae. The chicken mite is a mite usually parasitizing birds like sparrows, starlings and pigeons. Pigeons are 'pest birds' that have created numerous health problems, mainly in metropolitan areas, but reports of pigeon mite infestation and dermatitis are uncommon. In this case the mites originated from a pigeon nest situated just under the roof next to the ceiling of the bedroom. Since the pigeon had died, the mites had migrated into the bedroom in search of alternative hosts. Chicken mites cannot survive on humans in the long term, but its bite can cause urticarial and itchy papulovesicular skin eruptions. After removing the old nest and cleaning the bedroom the symptoms disappeared.
Conclusion: Epizoonosis belongs in the differential diagnosis of itching and an infestation with chicken mites should be considered.

P101

Serodiagnosis of *Strongyloides stercoralis* infection for routine clinical practice

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Imported strongyloidiasis can result in life-threatening disease in case of immunosuppression. Therefore, fast and reliable screening of patients at risk is mandatory. In this study, a home-made ELISA (AMC-ELISA) and dipstick-assay (Dipstick) for the detection of anti *Strongyloides stercoralis* antibodies in serum were developed and evaluated together with two commercially available ELISAs (IVD-ELISA, IVD Research, Inc. and Bordier-ELISA, Bordier Affinity Products SA) for their use in serodiagnosis of imported strongyloidiasis. Both commercially available ELISAs have not been evaluated before. The sensitivity of the assays was evaluated using sera from 90 patients with parasitologically proven intestinal strongyloidiasis, and from 9 patients with clinical larva currens. The sensitivities of the AMC-ELISA, Dipstick, IVD-ELISA and Bordier-ELISA were 93, 91, 89 and 83% for intestinal strongyloidiasis, respectively. In all tests 8/9 sera from

patients with larva currens were positive. The specificity was assessed using a large serum bank of 220 sera from patients with various parasitic (with the exception of filariasis, because of partial cross-reactivity; data not shown in this abstract), bacterial, viral and fungal infectious diseases, sera containing autoimmune antibodies and sera from healthy blood donors. The specificities of AMC-ELISA, Dipstick, IVD-ELISA and Bordier-ELISA were 95.0, 97.7, 97.2 and 97.2%, respectively. Our data suggest that all four assays are highly sensitive tests for the diagnosis of both intestinal strongyloidiasis and larva currens.

P102

Effectiveness of protocols for preventing occupational exposure to blood and body fluids (BBF) in Dutch hospitals

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Introduction: Healthcare associated occupational accidents have a great economic impact due to loss of manpower from occupational injuries and loss of reputation when infected staff members contaminate patients. Modification of protocols to make them more effective, vaccination programmes and newer technology reduce risks, but may not influence the risk behaviour of HCWs. HCWs may not realise that they too are responsible for a safe environment, for themselves, their coworkers and patients. Compliance has been well documented in literature, but little is known about the HCWs' actual ability to prevent or deal with infection problems.

Methods: Compliance of HCWs (nurses, physicians, laboratory technicians, cleaners) with protocols to prevent exposure to BBF was studied by means of a questionnaire (n=70) to assess HCWs' knowledge and attitude towards and compliance with the safety precautions stipulated in the protocols. A practical test (n=42) with scenarios to examine performance of the protocols in practice and to detect any problems the different HCWs faced while using them. In-depth interviews (n=48) with infection experts, management and quality control staff to investigate the development and implementation of protocols and see how far they are in line with the organisational infection policy and the needs of different HCWs.

Results: HCWs encountered problems with comprehension, acceptability and applicability of the protocols, especially for post-exposure precautions. The protocols are not tailored to the differences in knowledge, risk perception and practical needs of different professionals, they are governed more by legal considerations than applicability. Most HCWs experienced a lack of organisational support to aid compliance.

Conclusion: 1) The perceived risk of being exposed to serious blood-borne pathogens appears to be an important determinant of compliance with universal precautions. 2) Compliance appears not to depend on lack of time or resources but on the degree it impedes provision of care and on uncertainties about responsibilities and obligations.

P103

A defect in natural transformation contributes to clonality in *Campylobacter jejuni*

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Campylobacter jejuni is the most common cause of bacterial gastro-enteritis in humans. Genotypic analysis by multi locus sequence typing and other genotyping methods identified the *C. jejuni* population as genetically diverse with a few clonal lineages.

The aim of this study was to identify the molecular mechanisms involved in generation of diversity and preservation of clonality in the *C. jejuni* population. As most of the *C. jejuni* strains are naturally competent and the majority of genetic diversity is caused by horizontal gene transfer, we investigated the transformability of a selected group of *C. jejuni* strains.

With amplified fragment length polymorphism (AFLP) the genetic relationship of 27 *C. jejuni* strains of Penner serotypes O:1, O:2, O:19, O:41 and O:55 was determined. Electro- and natural transformation assays were used to investigate their transformability. For most of the strains belonging to serotypes O:1 and O:2 AFLP analysis yielded unique patterns, indicating that genetic diversity exists within these lineages (non-clonal). Very homologous AFLP profiles were found within serotypes O:19, O:41 and O:55. This suggests that in these lineages genetic diversity was minimal (clonal). Natural transformation experiments with homologous plasmid DNA showed that many of the non-clonal strains could be transformed, whereas the majority of the clonal strains could not be transformed. The results suggest that a defect in natural transformation contributes to the preservation of clonal lineages in the *C. jejuni* population.

Preliminary results from radio-active DNA uptake assays suggest that inefficient DNA-uptake contributes to inefficient transformation, and, in some cases, to non-transformability. The results also suggest that in some strains other defects must play a role as the basic level of DNA uptake is similar to that in some transformable strains. In the near future micro-array analysis with DNA and/or RNA isolated from the selected group of *C. jejuni* strains will be performed. These experiments are necessary to investigate differences at DNA and/or expression level between natural transformable and non-natural transformable strains.

P104

Investigation of the applicability of antimicrobial peptides against *Helicobacter pylori*

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Symptomatic *Helicobacter pylori* infections demand triple therapy to radicate the bacterium. Antimicrobial peptides provide a rapid expanding source of new bactericidal agents that are indispensable for filling-in the gap in potent therapeutics inflicted by increased resistance of bacteria to conventional antibiotics. Antimicrobial peptides generally have a net positive charge and an amphipatic character, endowing interaction with the membrane of the micro-organism. The membrane is destabilized and essential molecules can leak out. The unique conditions of the niche of *H. pylori* as present in the stomach, e.g., low pH, proteolytic activity, and a mucous environment, hamper the bactericidal activity of defence systems.

The purpose of this study was to investigate whether antimicrobial peptides are efficacious *in vitro* against *H. pylori*, considering its niche.

H. pylori was screened against previously developed antimicrobial peptides, that effectively kill a large series of bacterial pathogens, including antibiotic-resistant variants. One particular compound, the human cathelicidin peptide LL-37, emerged as potential candidate. Further study on LL-37 revealed the following: The all-D isomer possessed a higher bactericidal activity indicating proteolytic cleavage of LL-37 by *H. pylori*. Sub-lethal doses of LL-37 increased the sensitivity toward the antibiotic amoxicillin in a clinical amoxicillin-resistant strain of *H. pylori*. LL-37 interfered in the pH-dependent adherence of *H. pylori* to the salivary mucin MUC5b and in the pH-undependable binding to MUC7.

The killing activity of LL-37, synergic with antibiotics, and the interference in *H. pylori*-to-mucin adhesion justify further investigation.

P105

Prevalence of methicillin-resistant *Staphylococcus aureus* in Dutch veterinarians and veterinary students

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Introduction: In the Netherlands the prevalence of MRSA in clinical isolates of *Staphylococcus aureus* is with 1.0%, one of the lowest in Europe. This low prevalence is best explained by the national 'search and destroy' policy, that asks for admission screening and isolation of all patients that are at risk of MRSA carriership. So far, the at-risk patients mainly consisted of patients who have been admitted to and/or treated in foreign hospitals. In 2004, three patients in our hospital were found to be colonized with MRSA associated with exposure to pigs. We tried to determine whether repeated exposure to animals, especially livestock, poses a risk of acquiring MRSA.

Methods: 80 Dutch veterinary students and 99 veterinarians were screened for MRSA carriership. Cultures were taken from both anterior nares and throat. All were asked to fill in a questionnaire about the type of animal contacts and possible exposure to known MRSA risk factors.

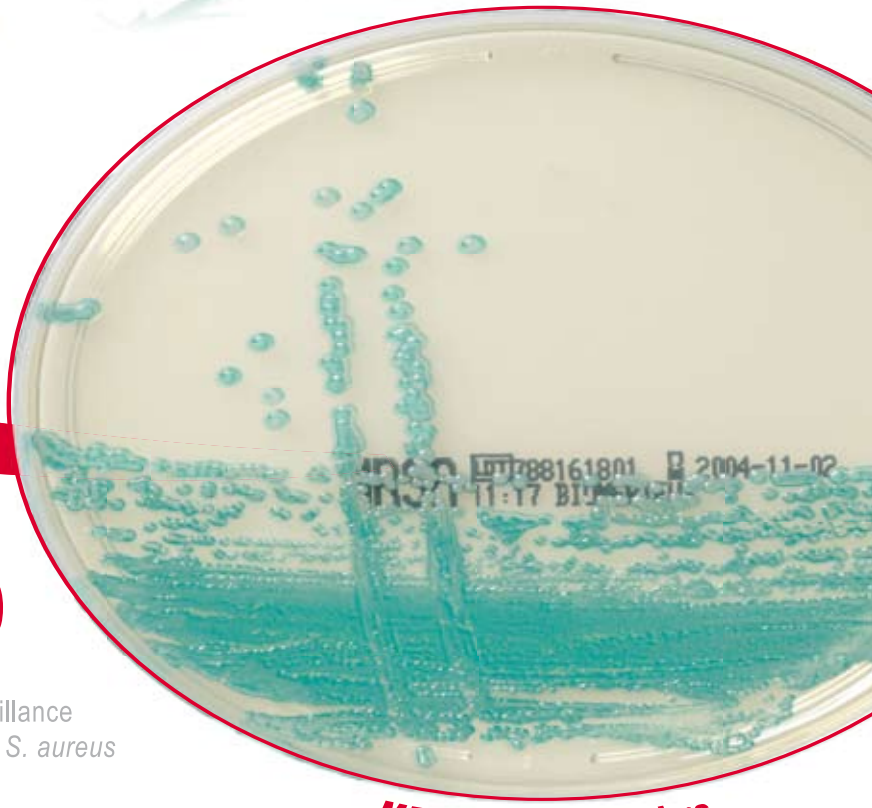
Results: Two students and five veterinarians were found to be MRSA positive. They had no known risk factors for MRSA carriership.

Conclusions: With a prevalence of 3.7% this is similar to that found in patients transferred from foreign hospitals and about a 120 times higher than the one found among patients at hospital admissions. With regard to the Dutch 'search and destroy' policy, Dutch veterinarians that come in contact with the health-care system may therefore qualify as 'risk-patients' warranting screening and/or isolation at admission to hospitals.

Advertentie

ISOLATE MRSA COLONIES & START PATIENT ISOLATION

MULTIRESISTANT BACTERIA



MRSA ID →18-24 hrs

MRSA ID

Chromogenic medium for the rapid & reliable Surveillance
Culture of Methicillin-Resistant *S. aureus*

BIOMÉRIEUX

from diagnosis,
the seeds of better health