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Welcome address

On behalf of the organising committee it is with great pleasure that we welcome you to the 10th European Meningococci Disease Society (EMGM) meeting in Manchester, United Kingdom.

For the last 16 years EMGM has convened every two years to share best practice in epidemiological and laboratory methods and for the public health management of meningococcal disease. Recently it has also become a venue for the presentation and discussion of the latest vaccine developments, clinical studies, impact of different implementation strategies and much more. At this meeting we are widening the scope to more closely align with the activity of the ECDC Invasive Bacterial Diseases surveillance programme and for the first time will be including scientific presentations and discussions on *Haemophilus influenzae* and *Streptococcus pneumoniae*.

We hope that all participants will find the presentations stimulating for fruitful discussions and we wish everyone a pleasant stay in Manchester.

Dr Ed Kaczmarski and Dr Ray Borrow

EMGM/Health Protection Agency

History of the EMGM

1st EMGM, 1991, Graz, Austria

2nd EMGM, 1993, Bad Gleichenberg, Austria

3rd EMGM, 1995, Bad Gleichenberg, Austria

4th EMGM, 1997, Paris, France

5th EMGM, 1999, Crete, Greece

6th EMGM, 2001, Örebro, Sweden

7th EMGM, 2003, Lanzarote, Spain

8th EMGM, 2005, Dublin, Ireland

9th EMGM, 2007, Rome, Italy

10th EMGM, 2009, Manchester, United Kingdom

Committees

Local Organising Committee

Dlawer Ala'Aldeen, Nottingham, UK

Ray Borrow, Manchester, UK

Steve Gray, Manchester, UK

Ed Kaczmarski, Manchester, UK

Robert Read, Sheffield, UK

Caroline Trotter, Bristol, UK

Scientific Committee

Ray Borrow, UK

Dominique Caugant, Norway

Matthias Frosch, Germany

Steve Gray, UK

Ed Kaczmarski, UK

Sigrid Heuberger, Austria

Paula Kriz, Czech Republic

Martin Maiden, UK

Per Olcen, Sweden

Robert Read, UK

Muhamed Taha, France

Caroline Trotter, UK

Georgina Tzanakaki, Greece

James Stuart, UK

Arie van der Ende, The Netherlands

Julio Vázquez, Spain

Registration

The registration desk will be open on

Wednesday 17 June 2009	07:30 – 19:00 hrs
Thursday 18 June 2009	08:00 – 19:00 hrs
Friday 19 June 2009	08:00 – 18:30 hrs

Registration fee (on site)

EMGM members, from UK £250

EMGM members, not from UK €300

EMGM non members, from UK £290

EMGM non members, not from UK €350

The registration includes the following:

Admission to the meeting

Welcome reception

Programme and abstract book

Certificate of attendance

Three lunches

Coffee breaks

The registration desk is located in front of the Hallé Conference Suite on the second floor of the hotel.

General information

Refreshments during the coffee breaks will be served in the bar and adjacent room at the back of the Hallé Conference Suite on the second floor.

Lunch will be served in the Alto restaurant on the ground floor.

The Radisson Hotel has a wireless network which is available to all guests at the hotel.

Coats can be stored on the coat racks in the Elder Bar free of charge.

For those attending the Safety meeting at the Manchester Royal Infirmary on Wednesday 17 June, the coach (Hayton's coaches) will pick up at the main entrance to the hotel (Southmill Street) at 8.00am. Refreshments will be served that morning in the Hallé Conference Suite from 7.30am.

The entire programme for Thursday and Friday will be held in the Hallé Conference Suite (please see the programme for the location of the sessions for Wednesday).

A message board will be located by the registration desk.

Certificates for the conference will be available from the registration desk at the end of the conference or on your departure.

Please return conference badges to the registration desk at the end of the conference or on your departure.

A free bus service operates around Manchester City Centre, please see P.16 for more details and a map of the bus routes.

Useful telephone numbers:
Manchester Black Cabs – 0161 227 1888
Manchester taxis – 0161 297 0089

Introduction to the scientific programme and instructions for authors

Oral presentations

For oral presentations the prefix **O** has been used throughout the program.

Instructions for oral presentations

Please bring your presentation on a memory stick to the registration desk (Radisson Edwardian Hotel, located on the second floor) at the latest by the break before the start of your session. Technicians will assist you loading your presentation. The use of your own laptop is not allowed. Please make sure your presentation is suitable for IBM PC (no Macintosh presentations please). The conference programme is very compact, with little free time between sessions. Please respect the allotted time for presentations to ensure that your session stays on track. Please reserve five minutes time for discussion in your presentation.

Poster presentations

For poster presentations the prefix **P** has been used throughout the programme.

Instructions for poster presentations

The poster area is located in the rear of the Hallé Conference Suite which is on the second floor of the Radisson Edwardian Hotel. You will find the assigned poster numbers on the poster boards. The numbers on the poster boards correspond to the abstract numbers in the abstract book. Your poster can be attached to the poster panel using velcro microdots, which will be supplied by the conference organisers (at the registration desk).
Presenters must ensure their posters are displayed between 10.00 and 13.30 on Wednesday 17 June. Please arrange for a colleague to do this for you if you cannot do it yourself. Posters can be taken down between 15.30 and 18.00 on Friday 19 June. Please arrange for a colleague to do this for you if you cannot do it yourself. Posters not collected at the end of the conference cannot be returned.

SCIENTIFIC PROGRAMME

All sessions are taking place in the Hallé Conference Suite, second floor, apart from indicated.

Wednesday 17 June 2009

07:30 – 19:00 **Registration**

07:30 – 08:00 **Tea on arrival**

Workshops of EMGM Working groups

08:00 – 11:00 **Lab safety practical workshop** – how we do it here
Dr Ed Kaczmarski/Dr Steve Gray
Venue: Manchester Royal Infirmary
Pre-registration essential
(Prompt pick-up at 08:00 from the Southmill Street entrance of the conference hotel and transfer back to the conference hotel at 11:00)

11:00 – 11:30 **Tea break**

11:30 – 12:30 **Carriage studies** – open to all attendees
Dr Georgina Tzanakaki and Dr Caroline Trotter

12:30 – 13:30 **Lunch break**

13:45 – 14:45 **Public Health management issues**
Open to all attendees
Prof James Stuart and Dr Wiebke Hellenbrand

14:45 – 15:45 **Host-pathogen interactions and clinical implications**
Open to all attendees
Prof Robert Read

15:45 – 17:30 **Tea break & Poster viewing**

17:30 – 18:30 **Open Workshop: Vaccine development Forum**
Open to all attendees: Microbiology / Public health / Vaccine developers and producers – How do we best support each others' needs?
Dr Ray Borrow and Prof Ian Feavers

Parallel sessions- **ECDC IBD Labnet meeting**
Together with EMGM working groups and organised by Prof. Matthias Frosch
Closed session for IBDLabNet /EMGM full members
All to be held in the Dickens suite, fourth floor

13:30 – 13:45 **Opening and Introduction of IBD LabNet (ECDC)**
Prof Matthias Frosch

13:45 – 14:45 **Session 1: EQA**

Presentation of the results from the Meningo EQA
Dr Steve Gray

Presentation of the results from the *Haemophilus* EQA
Dr Mary Slack

Discussion: Definition of deficits and needs for training
All participants

14:45 – 15:45 **Session 2: Antibiotic resistance**

Harmonisation of antibiotic resistance testing for meningococci
Dr Muhamed Taha

Current state and future needs for harmonisation of protocols for antibiotic resistance testing in *Haemophilus*
Dr Mary Slack

Discussion
All participants

15:45 – 16:00 **Tea break**

16:00 – 17:25 **Session 3: Enhanced laboratory surveillance and strain collection**

Molecular typing – ECDC's point of view
Dr Lucia Pastore Celentano and Prof Matthias Frosch

Consensus of molecular typing for meningococci
Prof Martin Maiden

Integration of laboratory variables and molecular typing data in Tessy
Dr Lucia Pastore Celentano and Prof Martin Maiden

Towards the establishment of a European strain collection
Prof Dominique Caugant

Discussion
All participants

Thursday 18 June 2009

08:00 – 19:00 **Registration**

08:00 – 09:00 **Tea on arrival**

EMGM Scientific Sessions

09:00 – 09:10 **Welcome on behalf of the Health Protection Agency Meningococcal Reference Unit for England and Wales**
Dr Ed Kaczmarski

09:10 – 09:40 **Opening address**
Dr Dorian Kennedy

09:40 – 09:55 **ECDC**
Dr Lucia Pastore Celentano

09:55 – 10:10 **EMGM President**
Prof Matthias Frosch

10:10 – 10:40 **Tea break**

10:40 – 12:30 **VACCINES AND IMMUNISATION STRATEGIES I**
Moderators: Dr Ray Borrow and Dr Caroline Trotter

10:40 – 11:25 **O001. Issues surrounding conjugate vaccines for *N. meningitidis*, *S. pneumoniae* and *H. influenzae***
Prof. Elizabeth Miller

11:25 – 11:45 **O002. Meningococcal conjugate vs. meningococcal polysaccharide vaccine in Saudi Arabian adolescents previously vaccinated with multiple doses of meningococcal polysaccharide vaccine**
Dr Yagob Al-Mazrou

11:45 – 12:05 **O003. A new meningococcal vaccine to control meningitis in Africa. (On behalf of the Meningitis Vaccine Project (MVP) and partners)**
Dr Marie-Pierre Préziosi

12:05 – 12:25 **O004. Age-related immune responses following *Neisseria meningitidis* serogroup C conjugate vaccination in the Netherlands: A pre- and post-vaccination survey**
Dr Richarda de Voer

12:30 – 14:00 **Lunch break**

14:00 – 15:30 **EMGM General Assembly -** EMGM members only

15:30 – 16:30 **Tea break & Poster viewing**

16:30 – 18:00 **LABORATORY DIAGNOSIS & STRAIN CHARACTERISATION**
Moderators: Prof Martin Maiden and Prof Dominique Caugant

16:30 – 16:45 **Report back from Molecular typing and strain collection working groups**
Prof Martin Maiden and Dr Muhamed Taha
Prof Dominique Caugant and Dr Paula Kriz

16:45 – 17:00 **O005. European meningococcal epidemiology in real time (EMERT)**
Dr Arie van der Ende

17:00 – 17:15 **O006. VNTR-PCR in local epidemics: A useful tool for fine typing of *N. meningitidis***
Dr Konstantinos Kesanapoulos

17:15 – 17:30 **O007. Validation of the diagnostic value of position 310 of the serogroup W-135 and Y capsule polymerases for molecular serogroup discrimination**
Dr Heike Claus

17:30 – 17:45 **O008. Factor H binding protein diversity among *Neisseria meningitidis* isolates causing invasive disease in South Africa, 2005**
Dr Mignon du Plessis

17:45 – 18:00 **O009. Validation of a SODC-based real-time PCR assay for the detection of *N. meningitidis***
Dr Jennifer Dolan

18.00 – 18.30 **Poster viewing**

Friday 19 June 2008

08:00 – 18:30 **Registration**

07:30 – 08:30 **Tea on arrival**

EMGM Scientific Sessions

08:30 – 09:10 **PUBLIC HEALTH**
Moderators: Dr Ed Kaczmarski and Dr Sigrid Heuberger

08:30 – 08:50 **O010. Evidence for action, consensus or confusion: Public health management of sporadic cases of invasive meningococcal disease**
Dr Wiebke Hellenbrand

08:50 – 09:10 **O011. Public health management of close contacts of individuals with invasive *H. influenzae* serotype B (Hib) and pneumococcal disease in the era of routine childhood immunisation with conjugate vaccines**
Dr Shamez Ladhani

09:10 – 09:30 **ANTIBIOTIC RESISTANCE**
Working group report and announcement of further activities on antibiotic resistance in *N. meningitidis*, *S. pneumoniae* and *H. influenzae*
Dr Muhamed Taha and Dr Julio Vázquez

09:30 – 09:45 **CARRIAGE**
Report back from working group
Dr Georgina Tzanakaki

09:45 – 10:00 **VACCINE DEVELOPMENTS FORUM**
Report back from working group
Dr Ray Borrow

10:00 – 10:10 **SAFETY**
Report back from working group
Dr Steve Gray

10:10 – 10:20 **O012. Biological safety management of virulent meningococcal strains and GMOs in reference and research laboratories at the University of Würzburg, Germany**
Dr Ulrich Vogel

10:20 – 10:30 **O013. Meningos can't jump! Can they?**
Daniel Holme

10:30 – 10:35 Discussion

10:35 – 11:00 **Tea break**

11:00 – 12:00 **HOST INTERACTIONS**
Moderators: Prof Matthias Frosch and Prof Dlawer Ala'Aldeen

11:00 – 11:30 **O014. Host/microbial interactions and severity of meningococcal disease**
Prof Robert Read

11:30 – 11:45 **O015. Implications of the structural basis of recruitment of Factor H by *Neisseria meningitidis***
Dr Qian Zhang

11:45 – 12:00 **O016. Lipid A variants in *Neisseria meningitidis*: Effect on clinical course of meningococcal disease**
Dr Arie van der Ende

12:00 – 13:00 **Lunch break**

13:00 – 14:50 **EPIDEMIOLOGY**
Moderators: Dr Mary Ramsay and Dr Hans Fredlund

13:00 – 13:20 **Overview of meningococcal disease in Europe, a summary of the submitted European epidemiology posters**
Prof Dominique Caugant

13:20 – 13:35 **O017. The control of hyper-endemic serogroup B meningococcal infection in Normandy, France**
Dr Muhamed Taha

13:35 – 13:50 **O018. Distribution of B:P1.7-2,4:F1-5:ST-42:MLVA Type-19 in Germany and possible efficacy of MENZB™ against this notorious outbreak strain**
Dr Johannes Elias

13:50 – 14:10 **O019. The epidemiology of *Haemophilus influenzae* type b in the UK: lessons for Europe**
Dr Mary Ramsay

14:10 – 14:20 **O020. The epidemiology of invasive non-B *Haemophilus influenzae* in Europe**
Dr Mary Slack

14:20 – 14:40 **O021. Surveillance of invasive pneumococcal disease in 30 EU countries: Towards a European system?**
Dr Germaine Hanquet

14:40 – 14:55 **O022. First two years of experience with pneumococcal conjugate vaccine implementation in the Netherlands**
Dr Sabine de Greeff

14:55 – 15:20 **Tea break**

15:20 – 17:50 **VACCINES AND IMMUNISATION STRATEGIES II**
Prof Ian Feavers and Dr Arie van der Ende

15:20 – 15:55 **O023. Meningococcal vaccine developments**.
Prof Ian Feavers

15:55 – 16:15 **O024. Serum bactericidal antibody against an extended panel of meningococcal strains following immunisation with novel serogroup B meningococcal vaccines in infancy**
Dr Jamie Findlow

16:15 – 16:35 **O025. A phylogenic classification on meningococcal Factor H-binding proteins based on a modular architecture**
Dr Peter Beernink - Children's Hospital Oakland Research Institute

16:35 – 16:55 **O026. Development of a Factor H binding protein vaccine for broad protection against invasive *Neisseria meningitidis* serogroup B (MNB) disease**
Dr Annalisa Anderson

16:55 – 17:15 **O027. Variability of the antigens of the Novartis investigational MenB vaccine: A US vs. Europe comparison**
Dr Maurizio Comanducci

17:15 – 17:30 **Poster awards, conclusions and 2011 EMGM meeting announcement.**

Social programme

Wednesday 17 June 2009, 19:30 – 20:45

Welcome reception with light snacks and drinks at the Town Hall, St Peter's Square, Manchester, M60 2LA. The Town Hall is located approximately 5 minutes walk away from the conference hotel.

Thursday 18 June 2009, Evening Free

Suggestions for dining and experiencing Manchester include:

Dinning:
1. Rusholme (Indian) – Wilmslow Road, aka the Curry Mile (recommended: Sangam)
2. Chinatown – City Centre (recommended: Little Yan Sing or Red Chilli)
3. Italian restaurants – City centre (recommended: Giorgio's)
4. Noodle bars – city centre (recommended: Vina noodle bar)

Pubs/Bars:
1. Peveril of the Peak (in the centre of Great Bridgewater Street).
2. RainBar (halfway along Great Bridge Street).
3. Britain's Protection (junction of Great Bridge Street and Lower Mosely Street).
4. City Arms (on Kennedy Street, off Clarence Street near Albert Square).
5. Vine Inn (on Kennedy Street, off Clarence Street near Albert Square).
6. Mr Thomas' Chop House (on Cross Lane short way from King Street).
7. Circus Tavern (half way down Portland Street).
8. Sinclairs Oyster Bar (in Exchange Square behind Marks & Spencers).
9. The Old Wellington Inn (in Exchange Square behind Marks & Spencers).

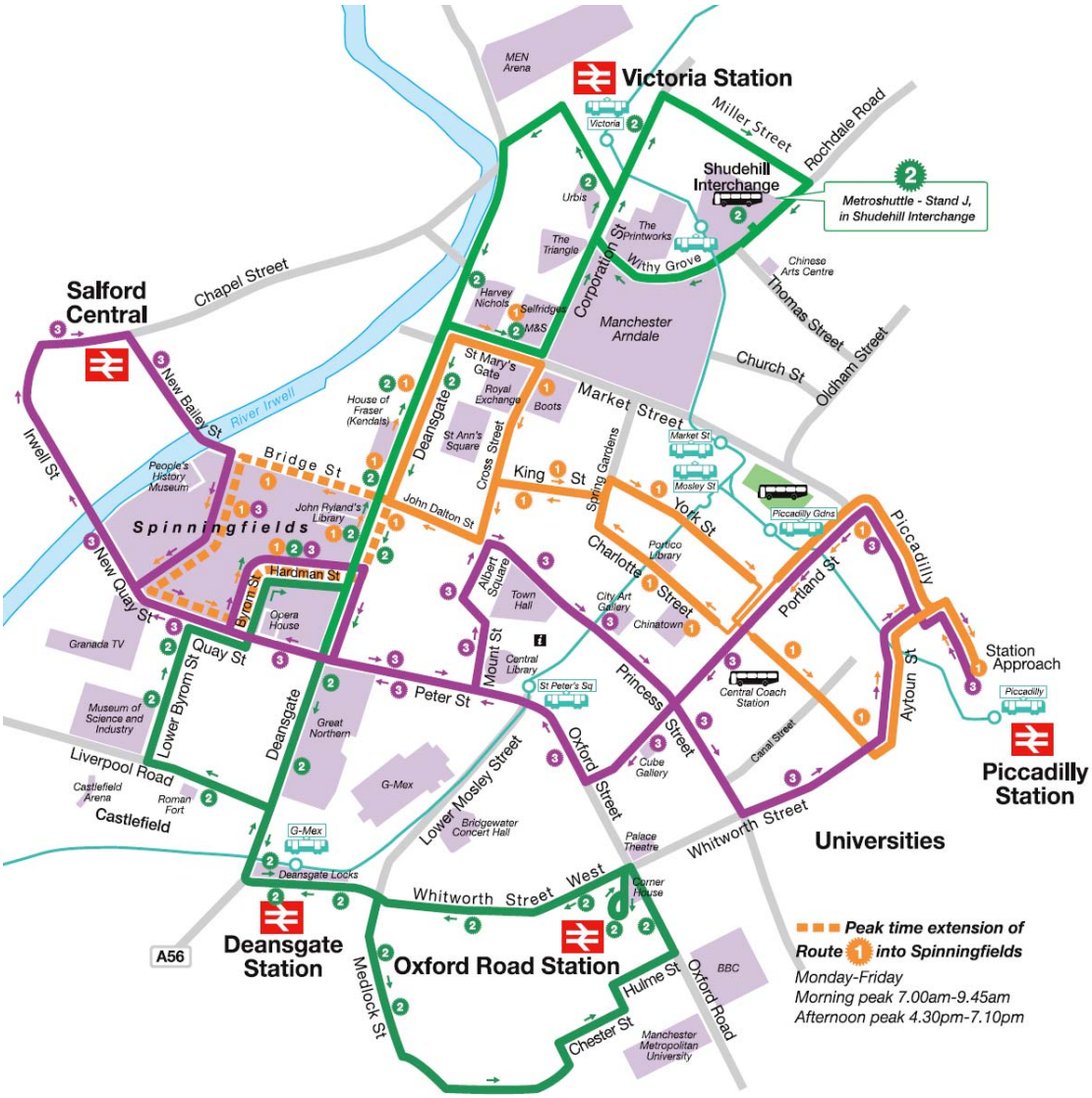
Friday 19 June 2009, 19:00

EMGM dinner at the Museum of Science and Technology (MOSI), Castlefield, Manchester, M3 4FP. The museum is located approximately a 15-20 minute walk away otherwise a short taxi journey.

Most people have booked to attend the dinner but if you have not, and would like to do so tickets may be purchased while registering for the conference at the registration desk (if still available). The cost of dinner is £40.

Please don't forget to bring your dinner voucher.

Manchester



This map outlines the route of the free metroshuttle buses. The Radisson Hotel is located on Peter Street.

Abstract
oral Presentations
O001 – O027

O001

ISSUES SURROUNDING CONJUGATE VACCINES FOR N.MENINGITIDIS, *S. PNEUMONIAE* AND *H. INFLUENZAE*

Elizabeth Miller

Centre for Infections, Health Protection Agency, UK

The advent of conjugate technology has revolutionised the ability to control invasive infections with bacteria that have a polysaccharide capsule. Conjugate polysaccharide vaccines have already made a major impact on the incidence of disease due to *Haemophilus influenzae* type b (Hib), meningoccoccal serogroup C and *Streptococcus pneumoniae,* both in vaccinated young children and their older contacts. The ability of meningococcal serogroup A conjugate vaccine to prevent disease in the epidemic belt of Africa will shortly be put to the test. The limitations of polysaccharide antigens have been long recognised, being in the main T cell independent antigens that simulate B cells without recruiting T cell help. Such antigens are poor immunogens in very young children and when generating antibodies in older age groups do not induce memory B cells. The discovery that presenting polysaccharide antigen covalently linked to a protein such as tetanus toxoid or CRM (a non-toxigenic natural variant of diphtheria toxin) overcame the limitations of plain polysaccharide antigens was revolutionary. Such conjugate vaccines were immunogenic in young infants, generated immune memory and reduced carriage thereby inducing herd immunity in the general population. However, expectations that induction of immune memory would necessarily translate to long term protection in the individual have not been realised and have prompted a re-evaluation of correlates of protection with greater emphasis on antibody persistence. In reality, the ability of Hib, meningococcal and pneumococcal conjugate vaccines to control disease in the individual and induce herd immunity in the population is a complex function determined by age-specific carriage rates, mixing patterns between age groups in the population, the degree and duration of protection of the vaccine against carriage, and in the case of pneumococcus, the competition between different serotypes for establishing carriage in the nasopharynx. Thus, proper understanding of the impact of a conjugate vaccination programme on the age-specific incidence of disease requires synthesis of knowledge from epidemiologists, microbiologists, immunologists and mathematical modellers. The generic similarities between conjugate vaccines and their important epidemiological and immunological differences will be presented and their success in controlling disease in different countries reviewed.

O002

MENINGOCOCCAL CONJUGATE VS MENINGOCOCCAL POLYSACCHARIDE VACCINE IN SAUDI ARABIAN ADOLESCENTS PREVIOUSLY VACCINATED WITH MULTIPLE DOSES OF MENINGOCOCCAL POLYSACCHARIDE VACCINE

Yagob Al-Mazrou[1], M. Khalil[1], C. Bravo[2], V. Bosch Castells[3], D. Johnson[2], H. Findlow[4], H. Chadha[4], R. Borrow[4]

[1]Ministry of Health, Riyadh, Saudi Arabia; [2]sanofi pasteur, Lyon, France; [3]sanofi pasteur, Marcy d; [4]Vaccine Evaluation Unit, Health Protection Agency, Manchester Royal Infirmary, UK

Background: Repeat dosing with meningococcal polysaccharide vaccine (MPSV) may induce immunological hyporesponsiveness.
Methods: Saudi Arabian adolescents who had previously received 1 dose of quadrivalent (A,C,Y,W-135) and ≥1 dose of bivalent (A,C) MSPV were randomised to receive 1 dose of either meningococcal (serogroups A,C,Y,W-135) polysaccharide diphtheria toxoid conjugate vaccine (Menactra®; sanofi pasteur, Swiftwater, PA, USA) or 1 dose of A,C,Y,W-135 MPSV (Mencevax®; GlaxoSmithKline, Rixensart, Belgium). A comparison group of meningococcal vaccine-naïve adolescents also received 1 dose of Menactra. Blood samples, collected before and 28 days post-vaccination, were assessed for serum bactericidal antibody activity using baby rabbit complement (rSBA).
Results: Of 446 participants, 144 MPSV-primed and 161 naïve participants received Menactra and 141 MPSV-primed received Mencevax. For each serogroup, the post-vaccination rSBA geometric mean titre (GMT) was significantly higher in the naïve Menactra recipients than in either MPSV-primed group. Among those primed with MPSV, the post-vaccination serogroup C rSBA GMT was significantly higher in the Menactra versus Mencevax group, when adjusting for pre-vaccination GMTs.

Post-vaccination rSBA GMTs (95% Confidence Interval) by Serogroup

Serogroup	Menactra MPSV-primed	Mencevax MPSV-primed	Menactra vaccine-naïve
A	4116 (3469, 4885)	3833 (3308, 4442)	6352 (5508, 7324)
C	288.3 (190.2, 437.0)	158.6 (99.7, 252.0)	993.4 (714.5, 1381.2)
Y	2770 (2197, 3493)	1936 (1426, 2630)	4205 (3458, 5112)
W-135	2436 (1715, 3459)	1456 (958.1, 2212)	6927 (5648, 8495)

Conclusions: These findings show that MPSV-induced hyporesponsiveness can occur with all vaccine serogroups and that the hyporesponsiveness can be at least partially overcome by using meningococcal conjugate vaccine when boosting.

O003

A NEW MENINGOCOCCAL VACCINE TO CONTROL MENINGITIS IN AFRICA. (ON BEHALF OF THE MENINGITIS VACCINE PROJECT (MVP) AND PARTNERS)

Marie-Pierre Préziosi

MVP, Initiative for Vaccine Research, World Health Organization, Geneva

Epidemic meningitis is one of the most feared diseases in Africa. Almost 50 years ago the African meningitis belt was first described as a vast sub-Saharan area extending from Senegal to Ethiopia with high endemic rates of meningococcal disease on which major epidemic waves occur periodically. Virtually all of the major epidemics are caused by group A *Neisseria meningitidis*. Over the last 30 years control activities using meningococcal polysaccharide (Ps) vaccines have been modestly successful in decreasing cases once epidemics have started but have had little success in halting these epidemics. Following international standards, the Meningitis Vaccine Project (MVP), a partnership between WHO and PATH has led the development of a new group A meningococcal conjugate vaccine priced at less than $US 0.50 per dose. This vaccine has been shown to be safe, highly and sustainably immunogenic with superiority to Ps vaccines in clinical trials in India and in African meningitis belt countries (Mali, Senegal, The Gambia) in subjects 1 to 29 years old. Introduction of this new conjugate vaccine at large scale through mass preventive immunization campaigns is highly likely to generate herd immunity and put an end to these epidemics. There is hope it will be licensed and ready for use before the end of 2009. The vaccine is eagerly anticipated; at a September 2008 meeting in Cameroon, health officials from the 25 countries that make up the African meningitis belt pledged to put in place funds for introduction of the vaccine as soon as it becomes available.

O004

AGE-RELATED IMMUNE RESPONSES FOLLOWING NEISSERIA MENINGITIDIS SEROGROUP C CONJUGATE VACCINATION IN THE NETHERLANDS: A PRE- AND POST-VACCINATION SURVEY

Richarda de Voer

National Institute for Public Health and the Environment (RIVM)

Background/Aim: In 2002 a MenC conjugate (MenCC) vaccination was introduced at the age of 14 months and a mass catch-up campaign was performed targeting individuals aged between 1 and 18 years. We determined age-related immune responses before and after introduction of the MenCC vaccine.

Methods: In two population-based serum collections, established in pre- (1995/1996) and post-vaccination (2006/2007) periods, polysaccharide-specific IgG (n= 2303 and 6376), IgM (n=281 and 1097), IgG subclasses and avidity (654 post- sera) were determined by a multiplex immunoassay. In addition, in a subset of sera from both serum collections (n=735 and 1220) MenC-specific serum bactericidal antibody titers were determined.
Results: Overall SBA seroprevalence (≥8) was 22% [18.0-26.6%] and 45% [41.1-49.3%] in the pre- and post-vaccination period, respectively. SBA titers and PS-specific IgG show an age-specific trend, with the highest antibody persistence in the oldest vaccinated age-groups. SBA seroprevalence is not significantly different between the pre- and post-vaccination periods in unvaccinated adult groups, whereas the MenC PS-specific antibodies are. In all immunized age-groups higher levels of IgG1 compared to IgG2 were observed, while naturally derived immunity was mainly restricted to the IgG2 subclass.
Conclusions: MenCC vaccination induced higher IgG levels compared to natural exposure, but only older vaccinated age-groups seem to benefit from antibody persistence. Due to mass vaccination, MenC circulation probably decreased, resulting in lower IgG titers in the unvaccinated older age-groups, posing them at risk if MenC starts re-circulating. Induction of IgG1 in immunized age-groups may indicate activation of cellular mechanisms typical for a T-cell-dependent response.

O005

EUROPEAN MENINGOCOCCAL EPIDEMIOLOGY IN REAL TIME (EMERT)

Arie van der Ende[1], K. Jolley[2], M. Diggle[3], U. Vogel[4]

[1]Academic Medical Center, Amsterdam; [2]Department of Zoology, University of Oxford, UK; [3]Scottish Meningococcus and Pneumococcus Reference Laboratory (SMPRL); [4]NRZM, Institute for Hygiene and Microbiology, Würzburg, Germany

Meningococcal antigen sequence typing has harmonized typing strategies in Europe. Databases accessible at http://neisseria.org have been implemented, and these are fed and used by National Reference Laboratories (NRLs). The EMGM developed the EMERT database in order to share, between reference laboratories, real time information on antigen sequence types of invasive meningococci circulating in Europe. Participating NRLs are requested to submit details of all isolates causing disease, thus enabling data to be used for management of trans-national outbreaks and selection of a representative virtual strain collection.
The EMERT website (http://emgm.eu/emert/) has been operational since May 2007.
Currently, 12 NRLs have submitted data, resulting in over 3400 records. Of these, 76% were serogroup B, 13% serogroup C, 6% serogroup Y and 3% serogroup W-135. PorA finetyping is present for 70% of the cases with P1.7-2,4 (17.4%), P1.22,14 (9.3%), P1.22,9 (7.5%) and P1.5,2 (6.6%) being

predominant. FetA typing results are available for 37% of the cases, with F1-5 (10%) and F3-3 (6%) as dominant types. MLST is known for 26% of the cases; 30% belong to cc41/44, 14% to cc269, 13% to cc11 and 11% to cc32. In conclusion, this database will help to link European NRLs, thus providing for the first time a comprehensive overview on circulating strains in Europe in real-time and will help to identify new variants of antigens of interest.
The database does not contain clinical or detailed demographic data, and thus serves only as a tool for reference laboratories to complement non-overlapping databases under development by the ECDC.

O006

VNTR-PCR IN LOCAL EPIDEMICS: A USEFUL TOOL FOR FINE TYPING OF *N. MENINGITIDIS*?

Konstantinos Kesanopoulos, J. Kremastinou, G. Tzanakaki

National Meningitis Reference Laboratory, NSPH Athens Greece

Background: Three local outbreaks caused by serogroup B *Neisseria meningitidis* took place in the Athens area during the years 2003, 2004 and 2007 respectively.
Methods: In total 73 samples (isolates and biological fluids) were investigated by conventional and molecular techniques. The VNTR-PCR methodology was used either to meningococcal isolates or directly to the patients' clinical samples (blood and/or CSF). All outbreak samples were evaluated simultaneously by conventional (serogroup, serotype, serosubtype) and molecular techniques (MLST and porA typing). In addition, clinical samples and isolates form sporadic cases, with identical phenotypic and genotypic characteristics with the outbreak samples were included in the study.
Results: *Outbreak 1:* In total, 20 strains were investigated: 3 patient's and 7 carrier isolates, as well as 10 isolates from sporadic cases. Among those, 16 strains were phenotype B:4:P1.14, porA alleles 22, 14, 36 (VR 1, 2 & 3, respectively) belonging to the ST-162 while, 3 carrier strains were NG:NT:P1.6, ST-1136. *Outbreak 2.* In total, 10 isolates were investigated: 2 strains from the outbreak and 8 strains from sporadic cases all having identical phenotypes (B:15:P1.7) and genotypes (ST-32, porA alleles 7, 16, 35(VR-1, 2 & 3 respectively). *Outbreak 3*. A total of 13 samples obtained from 7 patients of the outbreak were compared with 30 samples from sporadic cases with identical phenotypic (B:4:P1.6,7) and genotypic characteristics (ST-269, porA 19-1, 15-11, 36 (VR-1, 2 & 3, respectively))
Discussion: In all above 3 outbreaks, the application of the VNTR- PCR, was a valuable tool for the identification of the clone caused the local epidemics among the strains from the sporadic cases with identical phenotypic and genotypic characteristics, by providing further information for the differentiation of meningococcal isolates that were identical by porA and MLST. Moreover, the application of the nested VNTR-PCR is sensitive and reliable and can be used for the plylogenetic correlation directly in clinical samples independently of strain isolation. The present methodology offers

rapid, simple and reproducible results with high discriminating power in less than 24 hours overcoming the high cost and time-consuming sequencing-based techniques.

O007

VALIDATION OF THE DIAGNOSTIC VALUE OF POSITION 310 OF THE SEROGROUP W-135 AND Y CAPSULE POLYMERASES FOR MOLECULAR SEROGROUP DISCRIMINATION

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The amino acid sequences of the capsule polymerases of serogroup W-135 and Y meningococci, i.e. $SiaD_{W-135}$ and $SiaD_Y$, are 98% identical. Recently, we identified amino acid 310 within the EX_7E motif of the enzyme's nucleotide recognition domain as the crucial position that determines the specificity towards galactose or glucose (Claus et al., 2009).
We analysed the sequence of the EX_7E motif of 206 meningococcal isolates which had been assigned to serogroup W-135 and Y, respectively, by slide agglutination. 62 of 64 W-135 isolates (96%) harboured the galactosyltransferase motif EX_2PX_4E and 140 of 140 serogroup Y isolates (100%) harboured the glucosyltransferase motif EX_2GX_4E. Four of 206 isolates (2%) harboured an EX_2SX_4E motif. Meningococcal strains with this motif have been suggested by Tsang et al., 2008, to express a polysaccharide capsule composed of both galactose and glucose. By agglutination with polyclonal antibodies from Remel, two of these isolates agglutinated with the W-135 antibody only, whereas the other two reacted with both the W-135 and the Y antibody. An ELISA with monoclonal antibodies unambiguously assigned the EX_2SX_4E motif to serogroup W-135.
In summary, for culture independent confirmation of serogroup W-135 and Y meningococcal disease, the EX_2PX_4E and EX_2GX_4E motifs provide a highly accurate discrimination. The EX_2SX_4E motif requires further biochemical and immunochemical characterisation; isolates with this motif should be reported as "either W135 or Y".

O008

FACTOR H BINDING PROTEIN DIVERSITY AMONG *NEISSERIA MENINGITIDIS* ISOLATES CAUSING INVASIVE DISEASE IN SOUTH AFRICA, 2005

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Introduction: Factor H binding protein (fHBP) is a meningococcal outer membrane protein that comprises two subfamilies and generates mainly subfamily-specific bactericidal responses. Vaccines containing fHBP are currently undergoing clinical trials. We report the diversity of fHBP among multiple serogroups.
Methods: All invasive serogroup B (n=58), and 80 serogroup A, C, Y and W135 (20 each) isolates from 2005 were characterised by MLST, PorA typing, FetA typing and *fHBP* sequence analysis. Isolates were collected through a national laboratory-based surveillance program.
Results: 543 cases of invasive meningococcal disease were reported; 414 had isolates available. Serogroup distribution was: A (24), B (58), C (21), Y (52), X (2) and W135 (257). Common genotypes among serogroups A, C, Y and W135 were: A:P1.5-2,10:F5-1:ST-1 (ccST-1/subgroupI/II) in 17/20 isolates; C:P1.7-1,1:F1-6:ccST-865 in 16/20 isolates; Y:P1.5-1,2-2:F5-8:ST-175 in 15/20 isolates; W135:P1.5,2:F1-1:ST-11(ccST-11/ET-37) in 19/20 isolates. fHBP variant B16 was predominant in serogroups A and Y, whereas A15 and B45 were common among serogroups C and W135, respectively. Serogroup B isolates were represented by 7 clonal complexes (42/58) and 16 unrelated isolates; and 20, 16 and 17 variants of PorA, FetA and (subfamily A and B) fHBP, respectively.
Conclusions: Serogroup A, C, Y and W135 isolates were relatively clonal whereas serogroup B was heterogeneous. Most fHBP variants in serogroups A, B and Y were similar to those described elsewhere in the world, while variants predominant among serogroups C and W135, although related to common variants, were rare elsewhere. This survey suggests that fHBP vaccines may have potential beyond serogroup B strains.

O009

VALIDATION OF A SODC-BASED REAL-TIME PCR ASSAY FOR THE DETECTION OF *N. MENINGITIDIS*

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Real-time PCR is a widely-used molecular method for *N. meningitidis* species identification. Current real-time PCR diagnostics for *N. meningitidis* target the capsule transport gene, *ctrA*. However, over 16% of meningococcal carriage isolates lack *ctrA*.
sodC is found in *N. meningitidis* but not in any other *Neisseria* species. To identify all *N. meningitidis*, regardless of capsule genotype or expression status, a *sodC*-based Taqman real-time PCR assay was developed and validated.
Cell lysates from 627 *N. meningitidis* and 154 non-*N. meningitidis* isolates collected during two meningococcal carriage studies were used to validate this assay. The sensitivity panel contained 389 nongroupable, 11 serogroup A, 61 B, 12 C, 110 Y, 11 W135, 10 X, 10 29e, and 13 Z isolates. The specificity panel included 93 *N. lactamica*, 5 *N. gonorrhoeae*, 6 *N.* spp., 20 *M. catarrhalis*, 18 *Haemophilus* spp. isolates, plus 12 other isolates from genera whose clinical presentation or laboratory identification might be confused with *N. meningitidis* . C_t values £35 were considered positive; C_ts in the range of 36-40 equivocal; C_t values >40 negative.
98.7% (619/627) of *N. meningitidis* isolates tested were *sodC* positive, with a range of C_t values from 13.2 to 29.9. The mean C_t value was 17.7 +/- 2.2.
Of 143 nongroupable *ctrA*- isolates, 97.9% (n=140) were detected by *sodC*. The assay was 98.1% specific, detecting only 3/154 non-*N. meningitidis*.
This *sodC* real-time PCR assay is a highly sensitive and specific method for detection of *N. meningitidis*, especially in carriage studies where meningococcal isolates may lack capsule genes.

O010

EVIDENCE VERSUS CONSENSUS OR CONFUSION: PUBLIC HEALTH MANAGEMENT OF SPORADIC CASES OF INVASIVE MENINGOCOCCAL DISEASE

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Public health management of invasive meningococcal disease (IMD) varies in Europe. We performed a systematic literature review to identify evidence-based measures for prevention of subsequent disease in contacts of sporadic IMD cases. Recommendations were based on quality of evidence and balance of benefits and harms, and classified as weak or strong according to GRADE methodology. We strongly recommended chemoprophylaxis for household contacts as meta-analysis of observational studies showed an ~85% reduction in IMD risk in treated versus untreated contacts. No direct evidence was available in other settings. However, meta-analysis of observational studies showed an elevated risk in IMD contacts in pre-school settings compared to the background incidence (pooled risk difference (RD): 58.2 cases/100,000 inhabitants (95% CI: 27.3-89.0). A weak recommendation was made to provide chemoprophylaxis to IMD contacts in pre-school but not other educational settings.We found weak evidence for persistent carriage of meningococci in the nasopharynx after inpatient therapy of IMD with non-eradicating antibiotics. As carriage in the case is likely to pose a continuing risk to close contacts, consensus was for a strong recommendation to provide chemoprophylaxis to such patients before hospital discharge. Moderate or high quality evidence exists that besides antibiotics most commonly used for chemoprophylaxis (rifampicin, ciprofloxacin, ceftriaxone), minocycline, azithromycin and cefixime also effectively eradicate meningococci. All but minocycline were strongly recommended for chemoprophylaxis. Surveillance of susceptibility of pathogenic meningococcal strains to these drugs is important. Evidence on the most appropriate dosage was lacking. Uncertainty remains regarding efficacy and safety of some preparations in infants, children and pregnant women. We found low quality evidence that exposure to saliva as might occur with sharing of drinks with an IMD case was not a risk factor for subsequent IMD. The same applied to sharing the same transport vehicle. Weak recommendations were made not to give chemoprophylaxis only based on such contact. This review permitted some clear evidence and consensus based recommendations, but areas of uncertainty with only weak recommendations remain. This may reasonably lead to different policies between countries. Yet if potential for confusion is high, e.g. in managing airplane contacts, consensus across Europe is desirable.

O011

PUBLIC HEALTH MANAGEMENT OF CLOSE CONTACTS OF INDIVIDUALS WITH INVASIVE *HAEMOPHILUS INFLUENZAE* SEROTYPE B (HIB) AND PNEUMOCOCCAL DISEASE IN THE ERA OF ROUTINE CHILDHOOD IMMUNISATION WITH CONJUGATE VACCINES

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Haemophilus influenzae serotype b (Hib) and *Streptococcus pneumoniae* can cause severe life threatening disease in healthy individuals, with the vast majority of cases occurring in young children and the elderly. The introduction of Hib and pneumococcal conjugate vaccines into routine childhood immunisation programmes has resulted in a dramatic reduction in invasive disease due to these organisms, particularly in the age-groups targeted for vaccination. Following a case of invasive Hib disease, household and pre-school contacts should receive Rifampicin prophylaxis for four days to eradicate pharyngeal carriage of Hib and reduce the risk of secondary Hib infection, particularly if the contact is a vulnerable individual (child <10 years or an immunosuppressed or asplenic individual of any age). If there is a vulnerable individual among the household contacts, all members of that household, including the index case, should receive chemoprophylaxis. Where more than one case occurs in a pre-school or primary school setting, chemoprophylaxis should be offered to all room contacts (including staff). Isolated, individual cases of invasive pneumococcal disease do not require public health intervention. Where two or more cases occur in a closed setting within a two-week period, close contacts should receive amoxicillin chemoprophylaxis for seven days. Pneumococcal vaccination for close contacts should be considered if the infection is due to a vaccine-preventable serotype or in cases where serotype information is not available. Additionally, all unimmunised and partially immunised children should complete their primary immunisations, including booster doses, as soon as possible.

O012

BIOLOGICAL SAFETY MANAGEMENT OF VIRULENT MENINGOCOCCAL STRAINS AND GMOS IN REFERENCE AND RESEARCH LABORATORIES AT THE UNIVERSITY OF WÜRZBURG, GERMANY

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Prevention of laboratory acquired meningococcal disease and of the release of GMOs from the laboratory are key components of quality control of meningococcal research and reference laboratories. Prevention is based on structural and organizational elements according to national and European regulations. At the Institute for Hygiene and Microbiology in Würzburg, regular

training of staff, MenACWY and MCC vaccination, safety audits concerning specific operational procedures, and retrospective analyses of events with potential biohazard constitute a backbone of safety management. We here report on the frequency and consequences of potential biohazardous events. For a staff size of approximately 25 including students, a total of 15 events were recorded since 2000 (on average 0.07 / person year). Each event was discussed and if necessary followed by changes in processes and infrastructure. Most events were related to spillage of tubes or ELISA plates in the safety cabinet and in rotary shakers, insufficient identification of cultures, or usage of spectrophotometers outside of the safety cabinet. To rule out colonisation of staff with GMOs, staff screening by voluntary retropharyngeal swabbing was conducted in 2005, 2007 and 2009. Results for 2005: 17 negative swabs, no carriers of GMO, 1 positive swab with a probably natural isolate cnl:P1.18,25:F5-2; 2007: 22 negative swabs, no carriers of GMO, 1 positive swab with a probably natural isolate B: P1.7,30-5:F1-5; 2009: 22 negative swabs, no carriers of GMOs, two carriers of probably natural isolates NG(serogenotype B):P1.5-1,2-58:F1-15 and cnl:P1.12-1,16:F5-1. Finally, prevention of laboratory acquired menB disease by vaccination remains to be an urgent desideratum.

O013

MENINGOS CAN'T JUMP! CAN THEY?

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Background: *Neisseria meningitidis* has been reported as a cause of laboratory-acquired infection. Safety is of paramount importance and processes are constantly reviewed to reduce risks to laboratory staff. Meningococci are transmissible via aerosols and hence not transmissible from solid agar cultures, however, these cultures are incubated in humid environments. As part of ongoing safety audits we investigated if during incubations, meningococci were capable of mobilising or "jumping" from their solid agar cultures in a form that could transmit infection.
Methods: *N. meningitidis* was sub-cultured onto Columbia horse blood agar (CHBA) plates and grown in a decontaminated, humidified incubator overnight at 37^{o}C +5% CO_2. The following morning, condensate which had collected in the incubator and in the CHBA plate lids was sampled. Condensate was used to inoculate fresh CHBA plates (to determine any viable meningococci) and was tested for the presence of meningococcal DNA by in-house PCR.
Results/Conclusions: No viable meningococci were recovered form any of the condensate samples taken from within the incubator or from the inside of CHBA plate lids. Positive PCR results were found for 2/16 condensate samples taken from CHBA plate lids. Condensate samples taken from the incubator were all PCR negative. The significance of meningococcal DNA being identified in a small number of condensate samples from CHBA plate lids remains undetermined, however, no viable meningococci were

recovered. These results suggest that condensate found within incubators and from solid media plate lids is unlikely to harbour viable meningococci capable if transmitting to laboratory staff.

O014

HOST/MICROBIAL INTERACTINS AND SEVERITY OF MENINGOCOCCAL DISEASE

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Physicians and paediatricians who treat meningococcal disease (md) observe a range of severity from relatively benign disease to severe physiological disruption leading to death. Study of factors associated with the severity of md is difficult and confounded by the sporadic nature of the disease and marked differences in access to health systems. Many studies have shown that the outcome of md is associated with the phenotype of the infecting organism. The odds of death from disease are highest for certain sequence types, for example ST-11/ET-37 complex and ST-32/ET-5 complex. The major virulence determinants of *Neisseria meningitidis* include the polysialic capsule, LPS immunotypes, sialylation, and outer membrane proteins including Opa and Opc. Whilst there is no consistent segregation of virulence determinants in clonal groups associated with the most severe md, organisms expressing serogroup C have been associated with fatal outcome and the highest bacterial loads in plama samples. A number of host factors have been identified as important in determining severity of md. Chief amongst these is age at presentation, with the worst prognosis being associated with adults. Twin studies show that death from infectious disease has a familial component and in the case of md the role of genes encoding components of the immune system, the inflammatory response and coagulation pathways has been studied. The difficulty faced by geneticists is that death is the most easily verifiable end-point, but the mortality rate is only 8% so very small cohorts have been assembled. Association studies have revealed a relationship between a number of genes and the likelihood of death in md. These include Fc receptors, polymorphisms of plasminogen activator inhibitor type 1, properdin deficiencies, and polymorphisms within interleukin 1 and the interleukin 1 receptor antagonist.

O015

IMPLICATIONS OF THE STRUCTURAL BASIS OF RECRUITMENT OF FACTOR H BY *NEISSERIA MENINGITIDIS*

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The meningococcus has several mechansims to avoid complement mediated lysis. The bacterium binds factor H, the human negative complement regulator, via a surface lipoprotein fHbp which is a leading vaccine candidate. Here we present the biochemical characteristics and structural basis of this interaction. fHbp is composed of two barrels, and it a novel structure. The high affinity binding of fH to fHbp is mediated by two salt bridges and multiple hydrostatic interactions between the partners. The implications for pathogenesis and vaccination will be discussed.

The meningococcus is an important cause of speticaemia and meningitis. To cause disease, the bacterium must avoid killing by the immune system, particularly complement mediated lysis. The bacterium has several mechanisms to evade the complement system. It can recruit factor H, the human negative complement regulator, via a surface lipoprotein fHbp which is a leading vaccine candidate. Here we present the biochemical characteristics and structural basis of this interaction.

fHbp has a novel structure and is composed of two distinct beta-barrels. The high affinity binding of fH to fHbp is mediated by two salt bridges and extensive hydrostatic interactions between the partners. These interactions occur over a large surface area that spans both barrels of fHbp. fH binds with high affinity to representative fHbps from each of the three families of this lipoproteins.

The location of the fH binding domains in relation to the conserved regions of fHbp and the immunogenic epitopes of the protein will be presented. The implications for pathogenesis and vaccination will be discussed.

O016

LIPID A VARIANTS IN *NEISSERIA MENINGITIDIS*: EFFECT ON CLINICAL COURSE OF MENINGOCOCCAL DISEASE

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LPS is sensed by the host through Toll-like receptor 4 (TLR4), resulting in activation of proinflammatory cytokine pathways. TLR4 recognises the lipid A moiety of LPS and the chemical composition of lipid A determines how well it is recognized by TLR4. *N. meningitidis* has been reported to produce hexa-acylated lipid A, optimally recognized by TLR4. Recently, we found that a significant proportion of meningococcal isolates from patients have penta-acylated lipid A, caused by mutations in *lpxL1*. Among 254 meningitis patients of a prospective nationwide study cohort, those infected with a meningococcal *lpxL1* mutant presented significantly less frequently with rash and had higher thrombocyte counts.Here, 414 patients with invasive meningococcal disease, 847 carriers and 10 patients with chronic meningococcemia were evaluated. The proportion of *lpxL1* mutants among meningococci from patients and from carriers was similar, 6.4% and 5.3%, respectively. The proportion of *lpxL1* mutants was higher among non-meningitis patients (11/121; 9.1%). Three (30%) patients with chronic meningococcemia were infected with an *lpxL1* mutant isolate. Among non-meningitis patients, only 1/11 infected with *lpxL1* mutant was admitted to the IC ward, while 44/104 (42%) with wt meningococcal infection were admitted ($P<0.05$). Non-meningitis patients infected with a *lpxL1* mutant were presented with less petechial rash (4/10 vs 81/104, $P<0.02$) and had lower lactate values ($P<0.05$).In conclusion, infection with *lpxL1* mutant meningococci results in less severe disease manifestation. Our results provide the first example of a specific mutation in *N. meningitidis* which can be correlated with the clinical course of meningococcal disease.

O017

THE CONTROL OF HYPER-ENDEMIC SEROGROUP B MENINGOCOCCAL INFECTION IN NORMANDY, FRANCE

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CNRM : Centre National de Référence des Méningocoques, Institut Pasteur, Paris, France InVS: Institut National de Veille Sanitaire, Saint Maurice, France, DGS: Direction Général de la Santé, AFSSAPS: Agence Française de Sécurité Sanitaire des Produits de Santé, DDASS: direction départementale des Affaires sanitaires et sociales and CHU Rouen: Centre Hospitalo-universitaire de Rouen, France

Background: Meningococcal disease due to isolates B:14:P1.7,16 of the ST-32 clonal complex started to increase in 2003 in Normandy, France.
Methods: An enhanced epidemiological and serological surveillance was implemented and a vaccination campaign has been launched mid-2006 for the under 20 year-old population.
Results/Discussion: The MenBvac® vaccine was used on the basis of similarity of the local strain to the vaccine strain and on the basis of cross protection of the vaccine against the Normandy strain.The vaccinations started in the most affected group represented by the children 1-5 year-old living in Dieppe area for which the incidence of B:14:P1.7,16 IMD cases reached 120/100,000 in 2006. Due to low number of vaccine doses, the vaccine scheme was 2 doses six weeks apart, a third dose was given after 7 months. SBA titres were assayed before, six weeks and 15 months after the third dose. The percentage of children with SBA titres ≥ 4 was 37% (95% CI: 31-43) before the third dose. This percentage increased significantly 6 weeks after the third dose to reach 88% (95%CI 83-92). However, it was only 56% (95% CI: 49-63) 15 months after. These data were used to valid a scheme with 2 doses and a booster in the other age groups for which vaccinations were carried out from December 2007 and June 2009 in Dieppe area. No case due to B:14:P1.7,16 strain occurred from August 2008 to date in this area suggesting an impact of the vaccination campaign on the epidemic strain.

O018

DISTRIBUTION OF B: P1.7-2,4:F1-5:ST-42:MLVA TYPE-19 IN GERMANY AND POSSIBLE EFFICACY OF MENZBTM AGAINST THIS NOTORIOUS OUTBREAK STRAIN

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Meningococci of the clonal complex ST-41/44 cause a major share of invasive meningococcal disease (IMD) in Europe. For years, Germany has been plagued by recalcitrant upsurges of IMD in an area around the city of Aachen with a population of 1.1m bordering the Netherlands, leading to incidences of up to 3.1/100,000 (2005). A previous collaboration between German and Dutch institutes showed that the responsible clone had the type profile B:P1.7-2,4:F1-5:ST-42:MLVA type-19, (Elias et al., in preparation). Interestingly, New Zealand had also experienced a country-wide epidemic caused, among other strains, by B:P1.7-2,4:F1-5:ST-42 starting in 1990, which lead to vaccination with MeNZBTM.
Screening of 215 serogroup B strains from Bavaria between 2002 to 2008 yielded 61 possible ST-41/44 strains, of which 59 were confirmed by MLST. Only one single ST-42 strain was found, which proved not to be MT-19. This finding suggests, that despite high regional incidences in Western Germany, spread of the outbreak clone to Bavaria has not taken place. A similar investigation of the distribution in other Federal States will be reported. Serum bactericidal antibodies (SBA) against an Aachen type strain DE9686/04 using sera of 20 individuals obtained before and after vaccination with MeNZB^{TM} indicate a rise of SBA-titres comparable to vaccine strain NZ98/254. All individuals had SBA-titres > 8 against DE9686/04 after vaccination. In conclusion, results indicate that the clone with the profile B: P1.7-2,4:F1-5:ST-42:MT-19 has not spread into control regions investigated, and that MeNZB^{TM} might be effective in controlling this clone.

O019 and O020

THE EPIDEMIOLOGY OF *HAEMOPHILUS INFLUENZAE* TYPE B IN THE UK: LESSONS FOR EUROPE

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THE EPIDEMIOLOGY OF INVASIVE NON-TYPE B *HAEMOPHILUS INFLUENZAE* IN EUROPE

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The introduction of Hib conjugate vaccine into national immunisation schedules in the 1990s resulted in a dramatic reduction of over 90% in the incidence of invasive Hib infections. The possibility of disease replacement by other serotypes or non-capsulated strains has been raised.
From 1999 to 2006 the European Union Invasive Bacterial Infections Surveillance Network (EU-IBIS) monitored the impact of Hib conjugate vaccine on invasive *Haemophilus influenzae* (Hi) disease in EU countries; this analysis includes data from 13 countries that routinely serotyped Hi isolates across all age groups. 7,211 Hi invasive infections were reported from 2000 - 2006.Of these 2,005(27.8%) were caused by Hib, 552 (7.7%) were due to other capsulated Hi and 3,172(44.0%) were caused by non-capsulated Hi (ncHi).Serotype was not reported for 1436 (19.9%) isolates. The average annual incidence of non-b Hi infections (0.28/100,000) was almost twice that of Hib disease (0.15/100,000). There was no increasing trend in non-b infections. Both Hib and ncHi infections showed similar age distributions with the highest incidence in children <1 y followed by a decline into adulthood and an increase in those aged >65y. The median age of Hib disease was 4.5 y (range 1 day-98y) with 17.2% cases occurring in the first year of life, 30.2% of cases in the first two years and 51.4% in the first 5 years of life. Meningitis was the most common presentation (29%) followed by bacteraemia (24%). In contrast the median age of presentation of ncHi infections was 58.2 y (range 1 day-103y). Bacteraemia was the most common clinical manifestation (36%).ncHi strains were responsible for 33% of all cases of meningitis, where the serotype was known. The median age of ncHi meningitis was 19.7 y compared with 1.6 y for Hib meningitis. The mortality rate for ncHi [366/3172 (11.5%)] was almost three-fold greater than for Hib [88/2005(4.4%)] and in children <6m the mortality of ncHi was six-fold greater than for Hib (20.2% vs 4.1%). Concerns regarding replacement disease by non-b Hi are unfounded. Long-term surveillance of all Hi infections in all age groups is essential to assess the long term impact of Hib vaccination, potential changes in circulating strains and monitor changes in the epidemiology of invasive Hi disease.

O021

SURVEILLANCE OF INVASIVE PNEUMOCOCCAL DISEASE IN 30 EU COUNTRIES: TOWARDS A EUROPEAN SYSTEM?

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Background: Surveillance systems for invasive pneumococcal disease (IPD) differ across European countries, hampering the comparison of incidence rates and serotype distribution across countries. However, Europe-wide surveillance is essential to monitor the impact of conjugate vaccines and detect serotype changes. This study, done for the ECDC by the Belgian Institute of Public Health, describes and compares IPD surveillance in Europe. Its goal is to provide key elements for European IPD surveillance.
Methods: *S. pneumoniae* experts were nominated in 31 European countries and requested to fill in questionnaires covering the epidemiological surveillance, the surveillance conducted at the National Reference Laboratory (NRL) and the vaccination policies. Data were analysed and interpreted with the support of a steering committee.
Results: Response rate was 97% for each surveillance questionnaire. IPD cases are reported by laboratories and clinicians in 79% and 66% countries respectively, mostly based in hospitals. Case definitions differ but all countries report meningitis. The surveillance sensitivity has been calculated recently in 36% countries. All countries have a NRL or equivalent, 83% perform serotyping and 59% molecular typing. 67% countries report a recent or future reinforcement of IPD surveillance in general.
Conclusions: Our survey confirms the heterogeneity of surveillance systems but also shows strengths on which a European-wide surveillance system could be based. Two major aspects are the wide availability of serotyping and the surveillance of pneumococcal meningitis in all countries. More efforts are required for estimating surveillance sensitivity to correct for under-ascertainment at national level. Differences in medical practices need further investigation.

O022

FIRST TWO YEARS OF EXPERIENCE WITH PNEUMOCOCCAL CONJUGATE VACCINE IMPLEMENTATION IN THE NETHERLANDS

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Background: The 7-valent pneumococcal conjugate vaccine (PCV-7) wasimplemented in the Dutch national immunization protocol (NIP) in June 2006.This study aims to assess the impact of PCV-7 vaccination on the incidence and clinical manifestation of invasive pneumococcal disease (IPD) in the Netherlands from June 2004- June 2008.
Methods: Isolates received by the Netherlands Reference Laboratory for BacterialMeningitis were serotyped. Isolates from meningitis cases (all ages) had nationwide coverage and other IPD (all ages) covered 9 sentinel laboratories, representing 25% of the Dutch population. Clinical manifestation and outcome of IPD patients was derived from hospital records.
Results: Among children eligible for vaccination, the number of IPD cases caused by vaccine type pneumococci was reduced by ± 98%. A reduction of 80% was observed for meningitis in children <2 years of age. However, in this age-group the number of cases of IPD due non-vaccine types increased. Serotypes that had increased in post-vaccination period were 1, 7F, 19A, 33F. None of the 19A strains were penicillin resistant. In other age groups no significant effect of vaccination was observed.
Conclusion: Two years after the introduction of PCV-7 a reduction of vaccine-type IPD was observed in vaccinated cohorts. Increase of cases of IPD due to pneumococci with non-vaccine types among children eligible to vaccination partly abolished the effect of vaccination. Until now no herd-immunity effect has been observed.

O023

MENINGOCOCCAL VACCINE DEVELOPMENTS

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It has been almost a decade since the introduction of the group C polysaccharide conjugate vaccine in the United Kingdom and its subsequent licensure throughout Europe. In that time no new vaccines have been licensed that offer the prospect of protection against virulent meningococci

expressing other capsular polysaccharides. The successful use of MenC conjugates in a number of European countries has, in particular, highlighted the lack of suitable vaccines to prevent disease caused by group B meningococci. However, there are now a number of meningococcal vaccine formulations at an advanced stage of clinical development that have the potential to provide more comprehensive protection against virulent meningococci. This presentation will review the composition of some of these vaccine candidates and their progress towards licensure.
Given the low incidence of endemic meningococcal disease, it is likely that most, if not all, of these new vaccines will be licensed without direct evidence of protection in controlled phase 3 trials. Instead, licensure will rely on evidence of immunological correlates of protection. In the case of meningococcal vaccines the principal immunological correlate is serum bactericidal antibody. The measurement of bacterial killing mediated by antibodies and complement is complex and presents particular difficulties for the regulatory processes involved in the licensing of new vaccine formulations.

O024

SERUM BACTERICIDAL ANTIBODY AGAINST AN EXTENDED PANEL OF MENINGOCOCCAL STRAINS FOLLOWING IMMUNISATION WITH NOVEL SEROGROUP B MENINGOCOCCAL VACCINES IN INFANCY

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Background: Following early infant (2, 4, 6 and 12 month) or late infant (6, 8 and 12 month) courses of a investigational serogroup B meningococcal (MenB) vaccine (rMenB+OMV), we previously reported that ≥93% of infants had human complement serum bactericidal antibody (hSBA) titres ≥1:4 against 3 meningococcal strains expressing vaccine antigens (44/76-SL, NZ98/254 and 5/99). We have now further investigated immunogenicity against a further four MenB strains.
Methods: hSBA titres were determined, following early or late infant courses of rMenB (containing factor H binding protein (fHBP) sub-variant 1.1, NadA and GNA2132) or rMenB+OMV (rMenB plus outer membrane vesicles from strain NZ98/254, expressing PorA P1.7-2,4) against: M00-242922 (ST:41, fHBP 1.4, NadA-ve, PorA:P1.7-2,4); M01-240101 (ST:1049, fHBP 1.11, NadA-ve, PorA:1.19-1,15-11); M01-240355 (ST:213, fHBP 3.4, NadA low expression, PorA:P1.22,14) and M01-240364 (ST:11, fHBP 3.4, NadA+ve, PorA:P1.5,2).

Results: Following an early infant course of rMenB+OMV, 80%, 57%, 11% and 78% of subjects had hSBA titres ≥1:4 against M00-242922 (PorA homologous), M01-240101 (related fHBP), M01-240355 (fHBP, PorA and NadA heterologous) and M01-240364 (NadA homologous), respectively. Following a late infant course of rMenB+OMV, 100%, 70%, 17% and 90% of subjects had hSBA titres ≥1:4 against M00-242922, M01-240101, M01-240355 and M01-240364, respectively. For these four strains, rMenB alone was only immunogenic for M01-240364 (hSBA ≥1:4 in 70% and 88% of early and late infant subjects, respectively).
Conclusion: These results confirm that a course of rMenB+OMV in early or late infancy induces SBA against strains expressing vaccine antigens, further demonstrating the potential for improved vaccine prevention of MenB disease.

O025

A PHYLOGENIC CLASSIFICATION OF MENINGOCOCCAL FACTOR H-BINDING PROTEINS BASED ON A MODULAR ARCHITECTURE

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Meningococcal factor H-binding protein (fHbp) is a promising vaccine antigen that promotes binding of human factor H to the surface of the bacteria, which in turn down-regulates the alternative complement pathway. fHbp has been classified into three antigenic variant groups by one team of investigators, and into two sub-families, A and B, by another team. We performed a phylogenetic analysis of 61 unique fHbp amino acid sequences that showed the presence of five variable spans, each separated by blocks of two to five invariant residues. For each variable span, there were two progenitor sequences designated as type I or II. Amino acid identity of the respective segments within a progenitor type ranged from 80 to 100%, while that of segments between progenitor types ranged from 69 to 78% for a segment in the N-terminal portion of the molecule, to 32% to 45% in a segment encompassing resides 98-158. Each of the respective segments of the prototype fHbp variant 1 (strain MC58) and variant 3 (strain M1239) were phylogenetically distinct. However, the segments of other fHbps in the variant 2 or 3 groups represented natural chimeras of spans from types I and II. There were a total of two parental and four distinct chimeric types of fHbp sequences among 57 strains. The remaining four strains each had fHbps with different junctional positions at other invariant blocks of sequences. Thus two progenitor sequences appeared to have given rise to all of the fHbp variants through recombination of genetic modules.

O026

DEVELOPMENT OF A FACTOR H BINDING PROTEIN VACCINE FOR BROAD PROTECTION AGAINST INVASIVE *NEISSERIA MENIGITIDIS* SEROGROUP B (MNB) DISEASE.

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Background and Aims: Invasive meningococcal serogroup B disease (IMDB) though rare is devastating. There is no licensed broadly protective vaccine available. Wyeth is conducting clinical trials with a rLP2086 vaccine composed of two MnB human factor H binding proteins (fHBP). fHBP is a recognized virulence factor associated with immune evasion. Extensive prevalence and sequence analyses of fHBP have been conducted on MnB clinical isolates. All isolates have a *fHBP* gene and the >190 unique sequence variants identified to date segregate into 2 distinct subfamilies (A and B) in all countries surveyed. The purpose of this study was to evaluate the ability of a pool of human fHBP immune sera from human clinical trials of the rLP2086 vaccine to broadly kill IMDB isolates.
Methods: Serum bactericidal antibody (SBA) assays for ~100 IMDB isolates, representative of the diversity of fHBP expression and sequences, were developed and used to assess a pool of human immune sera from clinical trials of the fHBP vaccine.
Results:Human immune sera generated by a bivalent fHBP vaccine were able to kill a high proportion of IMDB isolates in SBA assays. Susceptibility to killing was independent of fHBP variant sequence, PorA and MLST type.
Conclusions: A vaccine that includes fHBP from each subfamily (A and B) elicits antibodies that are broadly bactericidal against epidemiologically diverse IMDB isolates. Since fHBP can be detected by flow cytometry on >98% of IMDB isolates, broad protection from an fHBP based vaccine containing members of both subfamilies can be expected.

O027

VARIABILITY OF THE ANTIGENS OF THE NOVARTIS INVESTIGATIONAL MENB VACCINE: A US VS EUROPE COMPARISON.

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Background: *Neisseria meningitidis* is a very diverse pathogen. Different local clonal expansions could cause big differencies of the molecular epidemiology in different countries. Active molecular surveys are needed for understanding the rate and degree of possible changes. The degree of diversity may also be responsible for the different impact of chemotherapeutics and vaccines in different countries.
Methods: We have compared the meningococcal genetic sequence variability between two panels representative of US and Europe strains.The genetic variability was assessed by Multi Locus Sequence Typing (MLST) diversity, and by the variability of the three recombinant proteins included in the Novartis MenB vaccine. The US strain panel was composed of isolates collected during ABCs (Active Bacterial Core surveillance) between 2000 and 2008, whereas the European strain panel was composed by 143 strains isolated from 13 different European countries over a 40 yrs time period.
Results/Conclusions: In terms of MLST, the most striking differences between US and Europe are the predominance of cc32, with respect to cc41/44, the absence of cc8 strains and the importance of cc162 in the US. Interestingly, in the US the sub-complex ST-44 is more frequently associated with disease than in Europe, where it is mostly associated with carriage. Each clonal complex is associated with the same vaccine antigen variants in both regions. Only the proportion of different variants within each clonal complex changes. Conversely, the antigenic repertoires are not significantly different between US and Europe, indicating similar clonal expansions. The most important exceptions are reported.

Abstract

Poster Presentations

P001-P087

P001 – P013 VACCINES AND IMMUNISATION STRATEGIES I

P014 – P020 LABORATORY DIAGNOSIS AND STRAIN CHARACTERISATION

P021 – P025 ANTIBIOTIC RESISTANCE

P026 – P077 EPIDEMIOLOGY

P078 – P088 VACCINES AND IMMUNISATION STRATEGIES II

P001

GROUP C MENINGOCOCCI IN ITALY IN THE ERA OF CONJUGATED MENC VACCINATION

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To investigate possible changes in the pattern of invasive meningococcal disease (IMD) in Italy after the introduction of conjugate menC vaccine in 2005 and to provide information for developing timely and appropriate public health interventions, analysis of microbiological features of isolates and clinical information of cases has been carried out. In 2005 and 2008, IMD showed an incidence of 0.5 and 0.2 x 100.000 inhabitants, respectively. While the incidence due to serogroup B remained quite stable, IMD incidence due to serogroup C has decreased since 2005. In particular, the fall was markedly significant among infants with an incidence of 0.5 per 100,000 inhabitants in 2007, vs. 1.69 in 2004. A smaller decrease was observed among adolescents and young adults. Clinical manifestations and outcome of infections underlined more severe disease associated with C:2a isolates, with an increase in septicaemia from 28% in 2005 to 70% in 2007. In the same period, fatal cases due to C:2a meningococci increased, from 7% to 55%. The majority of C:2b (82%) showed decreased susceptibility to penicillin. In Italy, immunization with the conjugate vaccine is recommended by the Ministry of Health but has not been evenly implemented since the regions may apply different strategies on the basis of local health priorities. Therefore, this setting may provide insight on possible effects of a partial vaccination coverage of the whole population at risk not only on the incidence of the disease but also on the spreading of specific meningococcal types. Data from ongoing surveillance of IMD will be important to evaluate effects of this kind of vaccination policy and to monitor the disease burden, predominantly caused by serogroup B meningococci.

P002

IMPACT OF GROUP C MENINGOCOCCAL VACCINE - PORTUGAL

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In Portugal an increasing tendency in the incidence of meningococcal disease was observed since 1998. By that time there was no available laboratory data about serogroups, the notification was clinical based. The conjugate vaccine against group C *Neisseria meningitidis* was licensed in Portugal in 2001. In 2002 an enhanced surveillance system for meningococcal disease was

implemented, which included a laboratory surveillance system. The information gathered by that system supported the decision to include the group C meningococcal conjugate vaccine in the National Programme of Immunisation. The high coverage of MenC even before the vaccination with the group C meningococcal conjugate vaccine started to be part of the National Programme of Immunisation (January 2006), can be explained by the acceptability of the vaccine by parents and clinicians. A significant impact on the incidence of the disease was observed in the period 2003-2005, particularly in the under five age group. The estimated overall annual incidence of group C meningococcal disease was $2.39/10^5$ in 2002 and decreased to 0.77 in 2003 and to 0.21 in 2004 and 2005. In January 2006, the schedule adopted by National Programme of Immunisation was a 3-dose schedule (3, 5 and 15 months of age) and a two years catch-up campaign was initiated. An information campaign took place in health centres, schools, other settings and in the media. Data from the evaluation showed vaccine coverage higher in the youngest cohorts, varying by cohort between 88 % and 95 %. Vaccine coverage in the adolescents varied between 74% and 89 %. The impact of the vaccine can be expressed by the comparison of the annual estimated incidence of group C meningococcal disease in 2002 (2.39) with those observed in 2006 (0.19), in 2007 (< 0.06) and 2008. Since 2003 the incidence by age group shows a greater impact in the under five, and in the under one year an important impact was observed since 2006. The success of the vaccination campaign against C-meningitis in Portugal is related with the performance of the National Immunisation Programme. The sustainability of the National Programme of Immunisation and of the Integrated Surveillance System of Meningococcal Disease are crucial in order to maintain the control of the group C disease, as well as to attain the control of future epidemics of meningococcal disease caused by other serogroups.

P003

SEROLOGICAL ANTIBODY KINETICS AFTER PRIMARY IMMUNIZATION WITH MENINGOCOCCAL SEROGROUP C CONJUGATE VACCINE OR SECONDARY IMMUNIZATION WITH EITHER CONJUGATE OR POLYSACCHARIDE VACCINE IN ADULTS

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Introduction/Aim: A single MenC conjugate (MenCC) vaccination was introduced at the age of 14 months in 2002. In addition, in a mass catch-up campaign all persons between the age of 1 and 18 years received one dose of MenCC vaccine. Here we investigate the characteristics and development of antibody (iso)types after secondary immunization with MenCC or plain polysaccharide and the possible role of certain antibody responses in maintaining immunity after vaccination.
Methods: Volunteers, age 18-55 years, were immunized with MenCC or received a secondary immunization with MenCC or plain MenC PS. Blood

samples were obtained before and seven time-points after immunisation. IgG, IgA, IgM, IgG1, IgG2 and avidity were assessed by a multiplex immunoassay. Functional antibodies were determined by a serum bactericidal assay.
Results: High levels of antibodies were still present 5 years after primary MenCC immunization. Secondary immunisation resulted in increased IgG and SBA titers after 5 to 7 days. In primed individuals, IgM was still present, and this only increased following a secondary immunization with plain PS. In addition, immunization with PS induced a higher IgG2 response compared to MenCC immunization.
Discussion: Secondary immune responses are quiet slow. The composition of the Ig (iso)type distribution is different between MenCC and plain PS and might be of influence on functional titers. Although this study indicates that immunological memory was previously induced by a single MenCC vaccination, it highlights the importance to sustain protective antibody levels against a rapid invasive organism such as *N. meningitidis*.

P004

IMMUNOGENICITY AND SAFETY IN UK LABORATORY WORKERS OF A COMBINATION *HAEMOPHILUS INFLUENZAE* TYPE B AND MENINGOCOCCAL CONJUGATE VACCINE (MENITORIX).

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Background: Despite laboratory staff within the Vaccine Evaluation Unit having potential occupational exposure to *Haemophilus influenzae* type b (Hib), occupational vaccination is not currently offered. This issue is complicated by the unavailability of a monovalent Hib vaccine. We therefore undertook a trial of the conjugated Hib/meningococcal serogroup C (MenC) vaccine Menitorix (GSK, Belgium) in laboratory workers.
Methods: Staff considered to be at potential occupational exposure to Hib were enrolled into a study of a single dose of Menitorix. Serum samples were taken pre- and 4 weeks post-vaccination and tested for functional antibody in the standardised MenC serum bactericidal antibody (SBA) assay utilising baby rabbit complement. Anti-Hib and MenC specific IgG concentrations were determined using a bead based assay.
Results: Overall the vaccine was well tolerated. Pre-vaccination, 24/30 (80 %) of subjects had protective SBA titres $\geq$ 8 which increased to 29/30 (97%) post-vaccination. Anti-MenC IgG geometric mean concentrations (GMC) increased from 4.3 (95 % CI: 2.1-8.8) pre-vaccination to 6.6 (95% CI: 3.3-13.1) post-vaccination. All subjects had received 1 or more prior doses of meningococcal polysaccharide vaccine. Anti-Hib IgG GMC rose significantly from 0.2 (95% CI: 0.1-0.6) pre-vaccination to 50.7 (95% CI: 24.3-105.8) post-vaccination. Proportions of subjects with protective anti-Hib concentrations $\geq$

0.15 mg/mL rose from 19/30 (63%) pre-vaccination to 30/30 (100%) post-vaccination.
Conclusion: Menitorix was safe and immunogenic and may be used to provide protection in laboratory workers which may be at potential occupational exposure to Hib.

P005

THE AUSTRALIAN MENINGOCOCCAL C VACCINATION PROGRAM: 5 YEARS ONWARDS

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In May 2003, a national, publicly-funded meningococcal C vaccination program for Australian children aged 12 months commenced and has been successful in reducing serogroup C meningococcal disease in all age groups, even those not targeted by the catch-up program.
In May 2003, 3 serogroup C meningococcal conjugate vaccines (MenCCV) were funded for inclusion on the Australian National Immunisation Program (NIP) as a single dose to all children aged 12 months. A nationally-funded catch-up program was also undertaken in a phased approach with priority given to the 15/19 years age group. Children aged >5 years were targeted via a school-based program, presenting a number of logistical challenges for some States and Territories where NIP vaccines had generally been administered via a GP.
Young adults not in the school system proved difficult to target, resulting in poor coverage estimated to be ~20%. MenCCV coverage in the target age group, 12 months, increased each year since the program introduction. Vaccine coverage at 24 months for one dose of MenCCV was approximately 88% in 2006 and 93% in 2008.
Prior to the MenC vaccination program, notified cases (both clinical and laboratory confirmed) had begun to decline in 2001 from ~700 to ~550 in 2003. In 2008 ~280 cases were notified. Laboratory confirmed serogroup C meningococcal cases declined from 162 cases in 2002 to 14 in 2007. There have been 7 vaccine failures reported, 5 of which were in the 2-4 years age group. Herd-effects are evident across all age-groups, including adults not targeted by the program and older adolescents where coverage was low. Serogroup B meningococci now account for ~85% of all laboratory confirmed notifications in Australia.
In 2005 substantial legislative changes were introduced in Australia which altered the funding process for vaccines. The quadrivalent conjugate meningococcal vaccines and serogroup B meningococcal vaccines, which are not currently registered for use in Australia, will now be required to meet a number of stringent criteria in order to be funded via the NIP.

P006

INFLUENCE OF PRIOR VACCINATION WITH FRACTIONAL DOSES OF A TETRAVALENT MENINGOCOCCAL POLYSACCHARIDE VACCINE ON SERUM BACTERICIDAL ANTIBODY RESPONSES AFTER REVACCINATION

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Background: Immunological hyporesponsiveness, measured as impaired antibody responses after repeated immunisations with polysaccharide vaccines (PS), have been clearly observed for meningococcal C-PS, whereas for the A-PS results have been conflicting. Studies on hyporesponsiveness to group W135 and Y polysaccharide are scarce. The mechanism for hyporesponsiveness is poorly understood, but antigen dose could possibly play a role. Here, we investigated in an African population whether immunisation with fractional doses of an A/C/Y/W135 PS vaccine might result in hyporesponsiveness after revaccination with a full dose of the PS vaccine.
Methods: One year after vaccination with full dose (50µg) or fractional doses (1/5 and 1/10) of a licensed A/C/Y/W135 PS vaccine (Menomune®, Sanofi Aventis) in a clinical trial in Uganda, 120 individuals (2-19 years) receiving full, 1/5 or 1/10 dose (N=40 per group) were all revaccinated with a full dose (50 µg). Sera were taken before and 4 weeks after each immunisation and analysed by measuring serum bactericidal activity (SBA) against all 4 serogroups using rabbit complement.
Results/Conclusion: After prior immunisation with a full dose, significant hyporesponsiveness was observed for serogroups A, C and W135, but not for serogroup Y. For serogroup C, W135 and Y hyporesponsiveness was not observed when using fractional doses as primary immunisation. For the A polysaccharide, some degree of hyporesponsiveness was observed for the 1/5 dose, but not for the 1/10 dose.

P007

AVIDITY OF IGG ANTIBODIES AGAINST SEROGROUP A MENINGOCOCCAL POLYSACCHARIDE AFTER VACCINATION WITH REDUCED DOSES OF A/C/Y/W135 VACCINE IN UGANDA

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Background/Aim: Currently, there might be shortage of vaccines in case of large meningitis epidemics in sub-Saharan Africa. We have shown earlier that lower doses of polysaccharide induce similar levels of bactericidal antibodies as a full dose. We investigated here the IgG antibodies avidity elicited by reduced doses in comparison to a full dose of polysaccharide vaccine and the effect of revaccination with a full dose. We also analyzed the IgG responses in those who had received reduced doses.
Methods: A clinical trial in 2-19 years old was performed in Uganda, using a tetravalent meningococcal polysaccharide vaccine (Menomune®). Full dose, 1/5 dose and 1/10 dose were compared. One year later, a randomized subset of 115 individuals received a second, full dose of 50µg. Sera were drawn before, and 1 and 12 months following both vaccinations. A chaotropic ELISA method was used to detect high avidity IgG antibodies against serogroup A meningococcal polysaccharide. Antibody avidity was calculated as percentage IgG still bound after adding ammonium thiocyanate.
Results: No significant differences in IgG avidity were seen between the three doses 1 month after first vaccination (geometric mean avidity: 5µg dose: 41,2; 10µg dose:43,1; 50µg dose: 38,0), but avidity significantly increased for reduced doses from 1 to 12 months following the 1st dose. Following the second vaccination with a full dose, no significant differences were seen between the groups. One month after vaccination, IgG responses were dose-dependent.
Conclusion: Overall, our results demonstrated that fractional doses induce antibodies of, at least, the same avidity as a full dose.

P008

RESPONSE TO QUADRIVALENT MENINGOCOCCAL CONJUGATE VACCINE IN SAUDI ARABIAN CHILDREN WHO PREVIOUSLY RECEIVED 2 DOSES OF QUADRIVALENT MENINGOCOCCAL POLYSACCHARIDE VACCINE BEFORE 2 YEARS OF AGE

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Background: Very young Saudi Arabian children have demonstrated relatively poor immune responses to quadrivalent (A,C,Y,W135) meningococcal polysaccharide vaccine (MPSV4). This study explored how these children later responded to quadrivalent meningococcal conjugate vaccine (MCV4).
Methods: In a multicentre study, Saudi Arabian children, 5-8 years of age and previously vaccinated with 2 doses of MPSV4 when < 2 years of age, received a single dose of MCV4 (Menactra®; sanofi pasteur, Swiftwater, PA, USA) as did an age-matched comparison group of meningococcal vaccine-naïve children. Blood samples, collected before and 28 days post-vaccination, were assessed for serum bactericidal antibody activity using baby rabbit complement (rSBA).
Results: Across all serogroups, there were no statistically significant differences in either the pre-or in the post-vaccination geometric mean titres (GMTs) for the booster versus comparison groups, with one exception (see table).
Pre- and Post-Vaccination rSBA GMTs (1/dilution) by Serogroup

	Booster Group n = 140		Comparison Group n = 81	
Serogroup	**pre**	**post**	**pre**	**post**
A	168.9	6992	196.4	8263
C	2.5	167.2*	4.1	512.0*
Y	10.2	2120	12.4	2558
W-135	3.0	2388	2.7	2763

*P-value < 0.001, booster vs comparison group
The proportion of participants with post-vaccination serogroup C titres ≥1:8 was 86.4% in the booster group and 95.1% (*P* value = 0.07) in the comparison group. For serogroups A, Y, and W135, all participants had post-vaccination titres ≥1:8.
Conclusions: This MCV4 induced robust immune responses, both in participants who previously received MPSV4 and in those who were meningococcal-vaccine naïve.

P009

LEVELS OF ANTIBODIES TO PNEUMOCOCCI AND MENINGOCOCCI IN BATCHES OF INTRAVENOUS IMMUNOGLOBULIN MANUFACTURED FROM PLASMA POOLS SOURCED FROM EUROPE OR THE UNITED STATES.

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Background/Aim: Patients with primary antibody deficiencies are treated with intravenous immunoglobulin (IVIG) prepared from pooled immunoglobulin which reflects the microbial flora to which the donor community has been exposed. We hypothesised that different batches of IVIG manufactured from plasma pools sourced from the United States and Europe would vary in the levels and distribution of anti-pneumococcal and anti-menigococcal antibody subtypes.
Methods: A total of 45 batches of lyophilised IVIG (22 from US and 23 from Europe) were reconstituted to 6% protein concentration according to the recommendation of the manufacturer and analysed using a standard in house (CSL Behring) nephelometric assay to determine subclass and total IgG concentrations. Specific IgG concentrations were determined using a 13 bead multiplex assay against nine *S. pneumoniae* serotypes (1, 4, 5, 6B, 9V, 14, 18C, 19F, 23F) and four *N. meningitidis* serogroups (A, C, Y and W135). Functional antibody against *N. meningitidis* was determined using a standardised serum bactericidal antibody (SBA) assay against 11 strains (serogroups A, B, C, W-135, and Y). All assays were calibrated for use with serum, not IVIG.
Results/Conclusions: Individual batches of IVIG sourced in the US and Europe were observed to vary in total and subclass IgG and specific anti-pneumococcal and anti-meningococcal IgG concentrations. Substantial concentrations of pneumococcal serotype specific IgG were measured in all IVIG batches with significant differences between Europe and USA sourced IVIGs for some serotypes (1, 4, 5). Serogroup B SBA titres varied by target strain and region of origin.

P010

MENACWY-CRM, A QUADRIVALENT MENINGOCOCCAL CONJUGATE VACCINE SUITABLE FROM INFANCY THROUGH ADULTHOOD

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Background: Meningococcal disease remains a global public health concern affecting all ages, with a varying epidemiology including dynamic changes in serogroup distribution. The best current prophylaxis is vaccination with quadrivalent vaccine, but limited availability of conjugate vaccine, and unsuitability of current vaccines in all age groups remain problematic. The Novartis Vaccines' investigational MenACWY-CRM quadrivalent conjugate is intended to be used in all age groups from 2 months to 65 years of age.
Methods: Phase II and III studies were performed in infants (2-12 months), toddlers (1-2 years), children (2-10 years), adolescents, (11-18 years) and adults (19-65 years) to determine safety and immunogenicity of MenACWY-CRM with age-appropriate licensed quadrivalent vaccines as comparators (monovalent men C conjugate in infants). Safety was assessed as solicited and unsolicited adverse events throughout the studies; immunogenicity was assessed by measuring serum bactericidal activity using human complement (hSBA).
Results: MenACWY-CRM is well tolerated in all age groups, with comparable reactogenicity to the age-appropriate licensed comparators. Similarly, immune responses, whether assessed as seroreponse, seroprotection rates (hSBA ≥ 1:8) or SBA titers, were non-inferior to current vaccines, and in many cases were statistically superior for one or more serogroups in all age ranges.
Conclusions: The cumulative data from a series of clinical studies show that the investigational vaccine, MenACWY-CRM, is suitable for use in all ages from 2 months to 65 years with safety and tolerability equivalent to currently available vaccines. Furthermore, immune responses were non-inferior, and frequently statistically superior, to the current options for meningococcal vaccination.

P011

NATURALLY-ACQUIRED IMMUNITY AGAINST *NEISSERIA MENINGITIDIS* SEROGROUP X

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Objectives: To get insight into the naturally acquired immunity against *Neisseria meningitidis* serogroup X by a case-control study set up one year after the 2006 epidemic.
The long-term aim is to contribute to the understanding of protection and the development of a vaccine.
Methods: Thirty one triplets were selected in July-August 2007: 31 patients having recovered from a meningitis due to *N. meningitidis* X in 2006; 31 "exposed" controls (same village than the cases) and 31 "non-exposed" controls recruited in an area where *N. meningitidis* X was not supposed to have circulated but in an area concerned by a *N. meningitidis* A epidemic in April 2006.Pharyngeal swab was obtained from each study subject and plated onto selective medium plate for carriage study. Blood samples were also collected for serum bactericidal activity (SBA) and ELISA for IgG antibodies to serogroup X capsular polysaccharide.
Results:

- Only 9 *N. meningitidis* strains, all originating from the non-exposed controls, were isolated from pharyngeal swabs. Agglutination tests revealed 2 *N. meningitidis* A and 7 poly-agglutinable *N. meningitidis*.
- Genogrouping by PCR and immuno-chromatographic tests identified 7 *N. meningitidis* A, 1 *N. meningitidis* X and 1 *N. meningitidis* W135.
- SBA analyses and ELISA tests showed that 78.1% of the patients who recovered from their meningitis had a protective bactericidal titre and 84.4% a protective IgG antibody titre.
  Among the exposed controls, 51% were protected according to SBA results and 71% according to ELISA test results.

Only 9.4% among the non-exposed presented a protective bactericidal activity and 25% had a protective IgG antibody titre.
Conclusions: As expected, protective SBA and IgG antibodies titres against *N. meningitidis* X were detected in patients who recovered from meningitis due to *N. meningitidis* X and in their close contacts. However, significantly lower titers were observed in non-exposed persons in spite of a higher rate of meningococcal carriage suggesting that the exposure to capsulated X isolates is crucial in mounting response against serogroup X isolates.

P012

MENINGOCOCCAL GROUP C AND W135 IMMUNOLOGICAL HYPORESPONSIVENESS IN AFRICAN TODDLERS

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A phase II clinical study was conducted in African toddlers enrolled at 12-23 months of age to receive either a new meningococcal A conjugate (PsA-TT), a meningococcal ACWY polysaccharide or Hib-TT vaccine. Ten months following primary vaccination, the 3 study groups were further randomised to receive either a dose of PsA-TT, 1/5th PsACWY or Hib-TT vaccine. Immunogenicity was assessed by serum bactericidal antibody (SBA) assay using rabbit complement for groups A, C and W135. Group A results have been reported previously. PsA-TT demonstrated superior immunogenicity *vs.* the Men A component of the PsACWY vaccine. The group C and W135 SBA titres in the three arms who received 1/5th dose of PsACWY vaccine were compared. Blood samples were taken prior to, 7 and 28 days post vaccination with 1/5th PsACWY. The GMTs are shown in the table below. For those primed with a full dose of PsACWY a 1.78 and 0.9 fold increase in group C GMT was seen from pre- to days 7 and 28 post-vaccination with 1/5th PsACWY. For those primed with PsA-TT, the fold increases were 45.8 and 2.5 from pre- to days 7 and 28 post-vaccination with 1/5th PsACWY. For those primed with Hib-TT, the fold increases were 53.25 and 3.5 from pre- to days 7 and 28 post-vaccination with 1/5th PsACWY. For W135 these fold increases were 9.32 and 1.57 in those primed with a full dose of PsACWY; 528.5 and 8.8 for those primed with PsA-TT and 270.75 and 4.6 for those primed with Hib-TT at day 7 and 28 post-vaccination with 1/5th PsACWY. The significant increases in SBA GMT at day 7 then decline to day 28 requires further investigation into antibody isotypes. Hyporesponsiveness to both C and W135 was evident at day 7 though less clear at day 28.

Primary Vaccine	**PsA-TT**	**Hib-TT 1/5th**	**PsACWY**
MenC			
Pre	2(n/a)	2.3(1.9-2.8)	2(2-2.1)
7 days post	91.6(45.6-183.9)	4.1(2.7-6.1)	106.5(53.5-212.0)
28 days post	5(3.2-7.8)	3.2(2.3-4.6)	7(4.1-11.9)
Men W135			
Pre	2.2(1.8-2.7)	2.8(2.1-3.9)	2.4(1.8-3.2)
7 days post	1162.8(595.7-2269.5)	26.1(11.7-57.9)	649.8(293.7-1437.2)
28 days post	19.3(9.2-40.6)	4.4(2.7-7.3)	11.1(5.6-21.7)

P013

MEASUREMENT OF FUNCTIONAL ANTI-MENINGOCOCCAL SEROGROUP A ACTIVITY: EFFECT OF IMMUNOTYPES L10 (STRAIN 3125) AND L11 (F8238) ON SERUM BACTERICIDAL NATURAL IMMUNITY

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Background: Functional anti-meningococcal serogroup A (MenA) activity is measured by serum bactericidal assay using rabbit complement (rSBA) according to the CDC protocol. Although the recommended MenA strain is of L11 immunotype (strain F8238), most MenA invasive strains express L10 immunotype.
Methods: Sera from random subsets of infants, children and adolescents in 5 clinical trials conducted with MenA-conjugate-containing vaccines were tested for SBA using strain F8238 (L11) and re-tested with strain 3125 (L10).
Results: The L10 immunotype showed a higher level of differentiation between protection and susceptibility in vaccinated and unvaccinated subjects: 1) The percentage of unvaccinated subjects who developed natural immunity over time was substantially lower when the L10 immunotype assay was used compared to the L11 immunotype. 2) In subjects vaccinated with a conjugate MenA vaccine, the percentage with a post-vaccination response (4 weeks) or with persisting (up to 1.5 year) rSBA-MenA ≥1:8 was generally similar using either immunotype.
Conclusions: L10 and L11 immunotypes provide discordant results in terms of detection of natural immunity with rSBA. The MenA L10 3125 SBA target strain may therefore avoid measurement of falsely high levels of natural immunity observed with the F8238 MenA L11 strain. Consequently, assays using the L10 immunotype may help to better reflect protection/susceptibility of immunised and non immunised subjects.

P014

CHARACTERISATION OF FHBP, GNA2132, SEQUENCE TYPE, PORA AND THE GENOMIC PRESENCE OF IS1301 IN ENGLISH AND WELSH GROUP B MENINGOCOCCAL ST-269 CLONAL COMPLEX ISOLATES, SUGGESTS THE EXISTENCE OF 2 BROADLY DISTINCT AND WELL-DEFINED LINEAGES.

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Objectives: During an ongoing international effort to assess the potential coverage of the Novartis Vaccines MenB vaccine, English and Welsh ST-269 clonal complex (269cpx) isolates were genetically characterised with respect to 4 of the vaccines major components - fHBP, GNA2132, NadA and PorA. To expose underlying antigenic trends, data were profiled against Sequence Type and the genomic presence of a further marker, insertion sequence IS1301.
Methods: We investigated a randomised selection of 269cpx isolates, received by the Health Protection Agency Meningococcal Reference Unit (MRU) in 2000, 2001, 2005, 2006, 2007 and 2008 (n=21, 22, 32, 41, 25 and 27, respectively). Presence and genetic diversity of gna2132, fHbp and nadA was determined using PCR and sequence analysis, and the genomic presence of IS1301 was determined using internally directed primers.
Results: nadA was present in 4 of the isolates. All isolates harboured fHbp and gna2132. The major variants, fHbp 1.11, gna2132 5, PorA P1.19-1,15-11 (and most minor subtypes), centred around ST269. fHbp 1.9-3 and 2.4, gna2132 12 and PorA P1.22-9 centred around ST275. IS1301 was present in 100% and 1% of isolates centred around ST269 and ST275, respectively.
Conclusion: 269cpx consists of 2 broadly distinct and well-defined lineages transcending house keeping genes and surface antigens alike, and including the presence of a specific mobile genetic element.

P015

SEROTYPING OF PNEUMOCOCCAL MENINGITIS CASES IN THE MENINGITIS BELT USING SEQUENTIAL MULTIPLEX PCR DIRECTLY ON CEREBROSPINAL FLUID

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Introduction: Data on serotype distribution in pneumococcal disease in sub-Saharan Africa are rare, mostly due to difficulties with culture and technical requirements for serotyping.
Methods: Based on pneumococcal meningitis isolates collected in Burkina Faso and Togo between 2002 and 2008, we reformulated a sequential multiplex PCR (SM-PCR) algorithm (*Pai et al. 2006)*. The resulting method was used to identify pneumococcal serotypes from isolates, or directly from cerebrospinal fluid (CSF). After evaluation in Germany, the technique was transferred to Burkina Faso.
Results: In all 77 cases serotyped by Quellung, SM-PCR serotyping on the isolate was successful, while on CSF from eight cases, no serotype was defined. SM-PCR serotypes correlated with those found by Quellung, except for five serotypes that were not included in the SM-PCR algorithm and therefore were non-typable. SM-PCR could not distinguish between serotypes 38/25F or serotypes 6A/6B. Using pneumococcal meningitis cases from surveillance in Burkina Faso and Togo during 2007-08, an isolate was tested by both Quellung and SM-PCR for 55 cases, and a CSF sample by SM-PCR for 133 cases. In each of these groups, the number (proportion) of cases successfully serotyped was 54 (98%), 51 (93%) and 123 (93%), respectively; serotype 1 accounted for 38%, 38% and 53% of cases, respectively.
Discussion: The SM-PCR technique can be adapted to the pneumococcal epidemiology in sub-Saharan Africa and used directly on CSF samples. It could be useful for serotype surveillance around vaccine introduction in the meningitis belt and substantially increase the number of cases with serotype information.

P016

COMPETITIVE INHIBITION FLOW ANALYSIS ASSAY FOR THE NON-CULTURE DETECTION OF PNEUMOCOCCAL SEROTYPE CAPSULAR POLYSACCHARIDE.

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Diagnostic procedures for the identification of a pneumococcal serotype require a positive isolate. There are non-culture diagnostic protocols available, but these only confirm the presence of pneumococci and do not identify the serotype. The increased use of pneumococcal polysaccharide and conjugate vaccines, especially in high risk groups and the likely increase in the number of serotypes included in future versions of the conjugate vaccines has necessitated the need for improved enhanced surveillance (and diagnosis) in order to assess their impact on public health. Recently, a multiplex bead-based assay was described using serotype specific monoclonal antibodies to identify serotypes which was demonstrated to have good specificity in identifying previously characterised pneumococcal isolates. We have developed, a competitive inhibition assay for the identification of pneumococcal serotypes from clinical samples (blood, CSF or urine) without the restriction of requiring monoclonal antibodies and has the potential for expansion of the repertoire of serotypes currently included in the assay. Pneumococcal capsular polysaccharides were conjugated to microspheres and mixed with the unknown sample and pneumococcal antisera (89-SF). The presence of a pneumococcal serotype-specific polysaccharide is identified by an inhibition of fluorescence in comparison to a control (microspheres plus antisera only). 23 serotypes were included in the assay and has shown good specificity following the addition of homologous polysaccharide. Analysis of pneumolysin PCR positive CSF samples with the 23-plex assay gave 89% samples positively identified. Of the negative samples, 88% had low bacterial loads with a PCR cycle of 45. A multiplex, rapid non-culture assay for pneumococcal serotype identification has been developed which will be useful in assessing the impact of pneumococcal vaccines.

P017

STRENGTHENING MICROBIOLOGICAL SURVEILLANCE AND PCR DIAGNOSIS OF BACTERIAL MENINGITIS IN SIX AFRICAN COUNTRIES

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Introduction: *N. meningitidis* serogroup A causes major epidemics within the African meningitis belt. An additional concern is related to the recent change in the epidemiological pattern of meningococcal outbreaks due to the presence of serogroups W135 and X, as well as *S. pneumoniae* serotype 1, as epidemic strains. Strengthening the capacity for epidemiological and microbiological surveillance is therefore a prerequisite for effective prevention and control of meningococcal outbreaks in Africa. Moreover, most of the CSF arrives either refrigerated or frozen at the typing laboratories due to the long distances to remote healthcare centers, hampering the culture. Implementation of the PCR assay for diagnosis and surveillance of bacterial meningitis in six countries among the most stricken countries was therefore an important milestone for the meningitis national control programmes.
Results: The polymerase chain reaction (PCR) method for the diagnosis of acute bacterial meningitis and serogroup prediction of *N. meningitidis* has been transferred from the Pasteur Institute (Paris, France) into six laboratories from six sub-Saharan countries. However, the number of processed samples/declared cases considerably varies from a country to another depending on the integration of the laboratories into the national surveillance frameworks. The starting Workshop in 2007 has gathered 19 scientists (biologists and epidemiologists). Since then, 10 biologists and 17 technicians have been trained during the last two years. In addition, the staff is provided with financial support to participate in international conferences and training and on return are encouraged to conduct similar training courses for the benefit of other staff of the laboratories. This has helped considerably in strengthening the technical skills of most of the scientists/technicians. Each laboratory was upgraded to international standards and acquired adequate equipment (notably 11 thermocyclers) for PCR applications. An EQA scheme has been recently conducted and results are soon expected.
Conclusion: The six laboratories continued their focus to enhance capacity building in the strengthening of human resources and infrastructure/equipment. Training to develop existing skills and expertise but also standardization has been the mainstays of the laboratories.

P018

DEVELOPMENT AND CLINICAL VALIDATION OF A LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY FOR THE RAPID DETECTION OF PATHOGENIC *NEISSERIA MENINGITIDIS*

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The novel DNA amplification technique Loop mediated isothermal amplification (LAMP) facilitates the rapid and highly specific amplification of DNA targets under isothermal conditions. Advantages include high amplification efficiency, reaction simplicity and ability to discern positive reactions visually negating need for expensive or specialised equipment. Described here is the development and clinical validation of a rapid, highly sensitive and specific LAMP assay for the detection of *N. meningitidis ctrA* gene. The assay was 100% specific for 8 different capsular *N. meningitidis* strains tested showing no cross reactivity with other Neisseria spp. (n=6) or with 47 different bacterial and fungal targets. The assay had a detection limit of 28 ctrA gene copies per reaction. Comparison with an existing 'Gold Standard' Real Time PCR *ctrA* assay for detection of *N. meningitidis* in a range of clinical specimens (n=373) demonstrated that *ctrA* LAMP assay had a sensitivity of 97% and specificity of 98.8%. Crucially *ctrA* LAMP assay was simple to perform with positives confirmed in less than 50 minutes. Due to its simplicity, specificity and low cost *ctrA* LAMP has the potential to be adapted as a rapid Point of Care Test (POCT).

P019

VNTR ANALYSIS OF CLUSTERS OF INVASIVE MENINGOCOCCAL DISEASE IN AUSTRIA

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Introduction: Clusters of meningococcal infection are rare in Austria. The disease occurs as sporadic cases with an annual incidence around 1 per 100.000 inhabitants. The documented clusters comprise 2 to 3 cases in defined geographical settings. Until now we sent the isolates and/or non-culture samples to other reference laboratories for comparison, which means a delay of 24 to 48 hours. After evaluating the different methods we implemented the variable number of tandem repeats (VNTR) assay.
Method: DNA was extracted by the use of the the MagNA Pure Compact Nucleic Acid Isolation Kit (Roche Applied Science, Germany) on the MagNA Pure Compact Instrument (Roche Applied Science, Germany) according to the manufacturer instructions. Samples were tested by the VNTR-PCR assay described previously (Yazdankhah et al. 2005; Kesanopoulos et al. 2008).

Capillary electrophoresis was carried out on the 3130 Genetic Analyzer (Applied Biosystems). The validation of the assay was performed with positive non-culture clinical samples and the corresponding isolates.
Results: Comparison of clinical material (CFS or blood samples) to the corresponding isolates showed the same VNTR profile. Different ST-complexes gave different VNTR patterns. Analysis of confirmed clusters revealed epi-linked cases.
Discussion: Numerous genotyping techniques have been employed to characterize *N. meningitidis*. Among these, multilocus sequence typing (MLST) has become the gold standard and is currently the most widely used approach for studying genetic variations of *N. meningitidis.* VNTR analysis could be a rapid alternative for smaller labs for cluster investigation within 6-8 hours.

P020

THE DISTRIBUTION AND BIOLOGICAL CONSEQUENCES OF EXPRESSION OF CLASS II PILIN IN *NEISSERIA MENINGITIDIS*.

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The type IV pilus (tfp) of *Neisseria meningitidis* is an important virulence factor as it is important for processes such as adhesion and DNA uptake. The pilus fibre is composed primarily of subunits encoded by the *pilE* gene (pilin). Based on the cross-reaction with different antibodies, two types of pili (class I and II) have been described. We have compared the protein sequence of class I and class II pilin and found that the latter lacks part of an exposed loop and we propose that this could have significant consequences on pilus properties and function. In addition, analysis of meningococcal genome sequences reveals important differences in genetic organisation of the *pilE* locus in class I and II strains. Therefore, we have begun a project to examine the frequency and distribution of class II pilin among isolates of *N. meningitidis* and to investigate the biological consequences of expressing class II pilin. We have analysed a total of 85 isolates by PCR including strains from different serogroups and clonal complexes. Preliminary data indicates that the class II pilin gene is found in 74% (64/85) of these isolates and almost all (62/63) of these strains belong to the ST11 complex. Furthermore, we have sequenced *pilE* genes from these isolates and found that the class II pilin protein sequence is highly conserved; this is in striking contrast to class I pilin which shows significant sequence variation. The immune responses and phenotypes associated with class II pilin expression are currently under investigation.

P021

FLUOROQUINOLONE RESISTANCE IN *NEISSERIA MENINGITIDIS* ISOLATES IN THE PEDIATRIC HOSPITAL OF TUNIS

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The aim of our study is to determine the point mutations in the quinolone resistance determining regions (QRDRs) in *parC* and *gyrA* genes among invasive meningococcal strains resistant to fluoroquinolone (FQ). From 1998 to 2008, a total of 79 invasive meningococcal strains were collected and studied. Antigenic formulae were determined using conventional methods. Quinolone resistance were detected using the disk of nalidixic acid. MICs of all antibiotics tested were determined using the E-test. Strains with MICs of ciprofloxacin value ≥0.004mg/l and one susceptible meningococci strain (MIC<0.002mg/l) were characterised by analysis for mutations within the QRDRs in the *gyrA* and *parC* genes. Those genes were amplified by PCR and sequenced using probe NG-GYRA-A, NG-GYRA-B and parC-Forward, parC-Reverse. The MIC determination of FQ revealed 7 strains with ciprofloxacin MICs =0.004mg/l and 1 with ciprofloxacin MIC=0.064mg/l. All strains with an MIC value of 0.004mg/l were with decreased susceptibility to penicillin and belonged to serogroups A, B and C. The strain with MIC_{CIP} 0.064mg/l was a respiratory strain isolated from a 19-year-old male. It was susceptible to penicillin and its antigenic formula was C:2a :P1-2,5. No mutations were noted for the QRDR sequencing of *parC* gene for all strains tested. The QRDRs sequencing of *gyrA* gene have shown that only the strain with MIC_{CIP} of 0.064mg/l contained a mutation which result in an Asp95-to-Asn change. This is a recognised alteration in the QRDR of *N. gonorrhoeae gyrA* gene and was reported then for meningococcal strains isolated in France and Australia. We report in this study the first case of resistance to fluoroquinolone (ciprofloxacin) by *gyrA* mutation in *N. meningitidis* strains isolated in Tunisia.

P022

COMPARATIVE ANTIBACTERIAL ACTIVITY OF 11 ANTIBIOTICS AGAINST *STREPTOCOCCUS PNEUMONIAE* STRAINS, ISOLATED IN ROMANIA BETWEEN 1997-2008

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Objective: the aim of the study was to analyse data obtained in the last 12 years in Romania on a number of *S.pneumoniae* strains of different capsular types, displaying significant antibiotic resistance.
Methods: 1964 strains of *S. pneumoniae*, coming from blood, CSF, pleural fluid, tracheal aspirate (TA) or sputum and others (middle ear fluid and sinus), isolated between 1997-2008, were analysed at Respiratory Bacterial Infections Lab., from Cantacuzino Institute. The strains were serotyped and tested for susceptibility to the following antibiotics: penicillin (Pc), erythromycin (Em), cephalothin (Kf), cefuroxim (Cxm), cefotaxim (Ctx), trimethoprim/sulfamethoxazole (Sxt), chloramphenicol (Cm),tetracycline (Te), ofloxacin (Ofx), amoxicillin (Amx), vancomycin (Va), by standard dilution MIC testing.
Results: breakpoints were used as proposed by CLSI 2008. Strains isolated from TA or sputum, pleural fluid, blood and CSF showed lower levels of antibiotic resistance (38.8 % Pc, 20.6 % Kf, 7.2 Cxm, 4.9 % Ctx, 2.7% Amx, 24 % Em, 2.4 % Ofx, 68 % Sxt) against strains coming from middle ear fluid and sinus which revealed high levels of resistance (70 % Pc, 26.5 % Kf, 10 % Cxm, 5.9 % Ctx, 3.4 % Amx, 58.4 % Em, 3.8 % Ofx, 73 % Sxt). No resistant strain to vancomycin was found.The pneumococcal strains isolated from others belonged only to a few serotypes: 23, 14, 19 and 6, closely correlated with the antibiotic-resistance. The most frequently serotypes encountered in TA or sputum, pleural fluid, blood and CSF were: 8, 7, 1, 3, 19, 14, 23, 6.
Conclusions: during the study period the most efficient antibiotics were: Ctx, Amx and Ofx. There is an urgent need in Romania for surveillance, prevention and control of antibiotic resistant pneumococci and to enhance the use of an efficient pneumococcal vaccine.

P023

TARGET GENE (RPOB) SEQUENCING TO DEFINE THE MENINGOCOCCAL BREAKPOINT(S) FOR RIFAMPICIN

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Reference laboratories for meningococci from: [1]France; [2]Belgium; [3]Czech Republic; [4]Germany; [5]Greece; [6]Italy; [7]Poland; [8]Spain; [9]Sweden; [10]University of Oxford, UK

Background: Rifampicin is one of the antibiotics of choice for chemoprophylaxis of meningococcal disease. Few resistant isolates have been reported. The identification of these isolates is hindered by the absence of a reliable and harmonised breakpoint. Moreover, different breakpoints are employed by different laboratories. The EUCAST has recently suggested MIC of ≤ 0.250 µg/mL for susceptible isolates while resistant isolates show MIC > 0.250 µg/mL. However, different values are used by the CLSI. We aimed to explore breakpoints on the basis of molecular characterization of the *rpoB* gene.
Methods: A 660 bp DNA fragment of the *rpoB* gene was sequenced from a collection of meningococcal isolates from several countries. Sequences differing by at least one nucleotide defined a unique allele. The MICs of rifampicin were determined using E test®. Geometric means of MIC were calculated for isolates displaying the same allele.
Results/Discussion: Rif-R isolates which were defined on the basis of MIC >1µg/mL possessed mutations in the *rpoB* gene resulting in substitutions at the codon 552 and less frequently at nearby codons, (542, 548, 557 and 560) which were absent in non-resistant isolates (≤ 1mg/L). The Rif-R isolates belonged to diverse types (serogroups:PorB:PorA:FetA) and to different clonal complexes. Isolates with MIC between >0.250 µg/mL and ≤ 1 µg/mL showed similar alleles to those with MIC<0.250 µg/ml. Our data suggest that strains with rifampicin MIC above 1.0 µg/mL, but not below this threshold, consistently show genetic alterations. This finding needs to be integrated into pharmacological concepts of breakpoint definition.

P024

DELETION EVENT IN *NEISSERIA MENINGITIDIS* MTRR GENE: ANTIMICROBIAL RESISTANCE AND EPIDEMIOLOGICAL FEATURE

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The mtrR gene sequence was checked in 585 meningococcal strains representing different Clonal Complexes and serogroups to analyze the presence of a particular 154 bp deletion.
Two serogroup Y invasive meningococcal strains showing the same deletion of 154 bp in mtrR has been described. The deletion affect the mtrR gene from the mtrRCDE gene complex, which encodes an energy-dependent efflux pump system, and it has been suggested as responsible for the reduced susceptibility to ciprofloxacin (cip). Although the mtrCDE genes in gonococci constitute a single transcriptional unit that is negatively regulated by mtrR gene, it has been suggested that the MtrCDE efflux system in meningococci may not be subject to the MtrR regulatory scheme.
To define the association between this event and resistance to ciprofloxacin and other drugs we checked the mtrR sequence in a collection of 8 serogroup Y cipS strains belonging to the same ST (ST-1624) to which the 2 serogroup Y cipR strains previously mentioned belonged. Both, cipS and cipR isolated, showed the same deletion in mtrR ruling out the association between the deletion and the decrease susceptibility to cip. No alterations in the susceptibility to other antimicrobial drugs (penicillin G, rifampicin, erythromycin and ceftriaxone) associated with this deletion were found.
Owing to the fact that all strains showing the mtrR deletion were characterized as ST-1624 serogroup Y we decided to check if this event if a specific characteristic for this particular ST or if it is something present in other STs: We analyzed 243 serogroup Y strains belonging to more than 40 different STs most of them included in ST-167 CC, ST-23 CC and ST-5770. All 51 ST-1624 (ST-167 CC) showed the 154 bp deletion, that was absent in the rest of the serogroup Y isolates. Only two different deletions were found among serogroup Y ST-23 CC: one of them is a 152 bp deletion affecting to the Correia element presented in 5 isolates, and the other one of 114 bp only found in 1 strain. Most of the meningococci belonging to the ST-23 CC did not presented any deletion. In addition 110 group B, 95 group C, 95 group W135, 8 group A, 23 group 29E and 11 group X strains were also checked for the presence of the 154 bp deletion. None of them presented this event. However 1 serogroup B ST-41/44 CC and 1 serogroup A ST-35 CC presented the same 152 bp deletion described above among group Y strains. In conclusion the 154 bp deletion is specific for the ST-1624 being not present in other STs from the same CC or in other CCs and serogroups. Other deletions at the mtrR level appear sporadically among different serogroups and CCs.

P025

WHICH ANTIBIOTIC REGIMES SHOULD BE ADVISED FOR CHEMOPROPHYLAXIS: A REVIEW OF EVIDENCE

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Introduction: Most European countries recommend antibiotic prophylaxis for close contacts of meningococcal cases, but the recommended type and dosage of antibiotics vary across countries. We investigated which antibiotic regimes are most effective for prophylaxis in adults, children and pregnant women to assist decision making across European countries.
Methods: We reviewed the literature on effectiveness of antibiotic regimes in eradicating carriage. Because the risk of disease is highest within the week following exposure, effectiveness was defined as eradication at ≥7 days of follow up. Recommendations were developed based on GRADE methodology.
Results: We found high level evidence for eradication of carriage with rifampicin, ciprofloxacin and mino-cycline and moderate evidence for ceftriaxone, azithromycin and cefixime (≥1 RCT, ≥93% eradica-tion). The same antibiotics were also effective in children and pregnant women (moderate evidence). Rifampicin resistance develops rapidly after prophylaxis, in up to 27% of initial carriers. Though ciprofloxacin is not recommended in young children, we found numerous controlled studies showing no association between ciprofloxacin therapy and joint damage in this group, and similar frequency of adverse events to that found with other antibiotics. Minocycline presents a high rate of vestibular side effects.
Conclusions: We made a strong recommendation that rifampicin, ciprofloxacin, ceftriaxone, azithromycin and ce-fixime could be used for chemoprophylaxis. Ciprofloxacin, azithromycin and ceftriaxone are preferred as they can be used in single dose, have similar effectiveness, can be given to adults and children, and were not shown to induce resistance Surveillance of susceptibility of pathogenic strains to antibiotics used for prophylaxis is essential to inform decisions on the appropriate agent.

P026

NEISSERIA MENINGITIDIS: A REVIEW OF NASOPHARYNGEAL CARRIAGE IN THE EUROPEAN UNION

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Background and Aims: New serogroup ACWY meningococcal conjugate vaccines are available or under development. A better understanding of *Neisseria meningitidis* carriage will contribute to the assessment of potential vaccine impacts in Europe.
Methods: A literature search traced existing information on *N. meningitidis* carriage (prior to MenC conjugate vaccination) in the 27 European Union (EU) countries.
Results: Most studies assessed special populations at high risk of invasive meningococcal disease. Only a few studies evaluated carriage in the general population. In countries with available data, overall carriage prevalence differed within and between countries, varying across age-groups and serogroup distribution, and over time. Carriage prevalence increased during childhood and was highest between 15-24 years. No clear difference in serogroup distribution across age-groups was apparent. Serogroup B was the dominant serogroupable carriage strain in Spain, Norway, Czech Republic, most UK studies, and The Netherlands. In Germany, serogroups B, Y and 29E were roughly equally distributed. Serogroup C carriage prevalence varied greatly between and across countries. Serial surveys 16 years apart in the Czech Republic showed increased serogroup C carriage and disease over time. Serogroup A was isolated in carriage studies in Greece. Non-groupable isolates were commonly encountered. Overall and serogroup-specific carriage prevalence tended to be similar in communities with and without local invasive disease outbreaks. During outbreaks, carriage of the outbreak strain was infrequently detected, suggesting high virulence but low transmissibility of the outbreak strain.
Discussion: Data describing meningococcal carriage epidemiology across the 27-EU are incomplete. Longitudinal carriage studies in the general population will increase understanding of relationships between disease, carriage, and will allow modelling of the impact of conjugate vaccines on disease transmission.

P027

THE EPIDEMIOLOGY AND SURVEILLANCE OF MENINGOCOCCAL DISEASE IN ENGLAND AND WALES.

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Background: The HPA performs surveillance of invasive meningococcal disease for England and Wales to ascertain case numbers and characterise organisms.
Methods: Clinicians notify suspected cases of meningococcal meningitis/septicaemia. Microbiology laboratories submit isolates and samples for meningococcal DNA detection by PCR. Isolates are characterised by serogroup, serotype and serosubtype. MICs of penicillin, cefotaxime, rifampicin and ciprofloxacin are determined. Non-culture confirmation utilises PCR assays.
Results: Laboratory confirmed cases rose from 1,448 in 1995 to peak at 2,804 in 1999 falling to 1,233 in 2008. The increase in serogroup C cases from 1995-9 resulted in the introduction (commencing November 1999) of serogroup C conjugate (MenC) vaccine into the UK population.
In 2008, 54% of cases were confirmed by PCR alone. The proportion of disease attributed to serogroups B, C, Y or W135 altered markedly following MenC introduction. In 2008, 90% of all confirmed cases were serogroup B, 3% were serogroup Y and serogroups C and W135 comprised just 2% each. Transient increases in W135 infections in 2000-1 were associated with Hajj pilgrims and their contacts in the UK. Phenotypic and genotypic shifts have been observed, specifically the relative proportions of MLST clonal complexes ST-41/44, ST-269, ST-32, ST-213 and ST-11 to meningococcal epidemiology.
Conclusions: Surveillance as described has demonstrated a sustained reduction in serogroup C infections since 1999. Observations of MenC vaccine failures more than a year after infant immunisation some children resulted in the addition of a booster dose to the routine childhood schedule in 2006.

P028

EPIDEMIOLOGY AND SURVEILLANCE OF MENINGOCOCCAL DISEASE IN GREECE (2007-2008)

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A total of 180 cases of meningococcal disease were notified in Greece (106 and 74 cases for 2007 and 2008), corresponding to an incidence of 0.95 and 0.67 cases per 100 000 inhabitants for 2007 and 2008 respectively Clinical notification is compulsory for Greece. Samples (biological fluid samples (CSF, blood) as well as cultures) from nearly 90% of the cases, were sent for either confirmation by PCR or further identification by conventional and molecular methods. Clinical records were reconciled with laboratory records on national scale. From the notified cases, 87.8% (93/106) and 92% (68/74) were confirmed for the two years (2007 and 2008). For the period of two years, 36.1% of invasive cases originated from children aged <1-4 years, 11.7% from children aged 5-9 years, 7.8% and 11.1% from the age groups of 10-14 and 15-19 years respectively. In addition, 30.6% were originated from adults (>20). The case fatality rate per year ranged from 4.7% (2007) to 9.45% (2008). Serogroup B was responsible for 80% of the cases for both years (76.8% and 84.4% for 2007 and 2008), followed by serogroup C (8.1% (2007) and 7.8% (2008). Serogroup cases have been increased during the past 2 years compared with the previous years (3.8%, 2005 and 6.5% 2006). The highest incidence rate for serogroup B was noted in age groups of <1-4 and 5-9 years for both examined years.The predominant phenotypes among the serogroup B isolates was B:4:P1.14 (2007) and B:4:P1.6 (2008). The predominant sequence types were, ST 269 complex (2007) and ST 32 complex (2008). Analysis of the variable regions (VR) sequences of the porA gene, revealed that the combinations of 19-1, 15-11, 36 for the VR-1, VR-2 and VR-3 respectively, predominated during the year 2007, while the combination of 18-1, 3 and 38 was predominant among the 2008 samples. Among the 12 isolates belonging to serogroup C, in both years their predominant phenotypic characteristics were C:2a:P1.2,5, ST-11 with VR combinations of 5-1, 10, 36-2. Reduced susceptibility to penicillin was found in both years8.3% of the strains (5/36) showed reduced susceptibility to penicillin. for 2007, while, the percentage was higher for the 2008 isolates (11.5%, 3/26). One strain isolated in 2007 was resistant to rifampicin; all were sensitive to chloraphenicol, rifampicin, cefaclor, ceftriaxone, ciprofloxacin and cefotaxime.

P029

INTRODUCTION OF THE MENINGOCOCCAL A CONJUGATE VACCINE IN THE AFRICAN BELT: CHALLENGES FOR SURVEILLANCE

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Background: The epidemiology of meningococcus meningitis in the African belt is characterized by a state of hyperendemicity and massive outbreaks during the dry season. Approximately 500 million people in the 25 countries of the belt are at risk, resulting in a human toll of over 250,000 cases and 25,000 deaths over the past decade.
Discussion: The current enhanced surveillance strategy implemented in African meningitis belt countries is based on early outbreak detection, identification of the causative organism, and reactive immunization campaign. The enhanced surveillance monitors the weekly meningitis attack rates at the district level, to identify the causative organism on a fraction of the first cases, and, in case of meningococcus, to launch a reactive immunization campaign once a predefined WHO recommended threshold is reached, with a vaccine that covers the circulating serogroup. To be efficient, vaccination needs be implemented as soon as the epidemic threshold is reached. The new meningococcal A conjugate vaccine will allow to move from reactive to preventive strategy and will overcome some of the limitations of the current strategy. The current surveillance strategy will not be able to estimate the impact of the vaccine on the infection risk and carriage prevalence. This will require strengthening epidemiological and microbiological surveillance in order to obtain case-based data, to conduct carriage studies before and after vaccine introduction, and implement surveillance of adverse events following immunization.
Conclusion: The introduction of the Men A conjugate vaccine poses major challenges in terms of surveillance. WHO will contribute to carriage studies and support countries to implement case-based surveillance.

P030

ESTIMATING THE AGE-SPECIFIC PREVALENCE OF MENINGOCOCCAL CARRIAGE: A SYSTEMATIC REVIEW

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Aims: To estimate the age-specific prevalence of meningococcal carriage, quantify the variation in prevalence and explore reasons for heterogeneity.
Methods: We conducted a systematic review of meningococcal carriage studies published between 1970-2007. Papers in any language reporting on ≥100 individuals were included; studies in settings not comparable to the UK were excluded. References of relevant papers were hand searched. We used mixed-effects logistic regression using splines to model carriage prevalence as a function of age, as this relationship is known to be non-linear.
Results: Results presented here are based on 66 papers reporting European studies that satisfied the inclusion criteria. 12 papers included longitudinal studies of carriage, which were analysed separately. After controlling for country and study as random effects, background disease level (endemic/increased incidence), setting (civilian/hospital/military/mixed) and decade of the study were independently associated with carriage prevalence by age (likelihood-ratio test $p<0.005$). Carriage prevalence predictions for UK civilians in an endemic setting between 2000-2007, for individuals aged 2, 10, 17, 40 and 60 were 4%, 6%, 15%, 8% and 5% respectively (likelihood-ratio test $p<0.05$).
Conclusion: Whilst the population prevalence of meningococcal carriage is often quoted as 10% this review shows carriage is highly variable by age. Estimates of carriage by age also vary with disease incidence, setting, and over time. In the absence of a recent large-scale carriage study across all age groups, this review allows the production of robust age-specific carriage prevalence estimates, which will be used to parameterise transmission dynamic models of meningococcal carriage, disease and vaccination.

P031

NEISSERIA MENINGITIDIS SEROGROUP W135: AN EMERGING PATHOGEN. A SYSTEMATIC REVIEW

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Invasive Meningococcal Disease (IMD) fulfils all requirements of an emerging pathogen. Recognized as an outbreak-causing disease for the first time in 1805, IMD has since then been identified as the cause of sporadic cases and epidemics throughout the world. The authors made a systematic search of Medline, LILACS, EMBASE, BIOSIS and Web of Science (ISI) with search

words: *Neisseria meningitidis* W135, *Neisseria meningitidis 135*, no language or date restriction.The purpose of this review is to summarize the current epidemiology of *N.meningitidis serogroup* W135 and discuss its current and possible future trends.First described in the USA in 1968, invasive disease cases were described 10 years later. However, until the Hajj-associated outbreaks in 2000 and 2001, little attention was given to this then apparently rare serogroup. A virulent strain of *N.meningitidis* serogroup W135, (W) ET-37 / ST-11, is currently circulating widely. This strain has caused outbreaks in the African "meningitis belt", in Saudi Arabia, in Europe and South America for almost 10 years. It seems to have become endemic in Turkey, the African Meningitis Belt, South Africa, Argentina and Brazil. At the present, there is clear indication of continuing dissemination and the there is a potential risk of a W135-pandemic spread.The capacity of *N. meningitidis* to change its capsule and to exchange genes within and outside the species makes it almost impossible to predict future trends, making it a constant emerging pathogen. This calls for intensified epidemiologic surveillance and pro-active control policies.

P032

CHARACTERIZATION AND ATB SUSCEPTIBILITY OF NEISSERIA MENINGITIDIS STRAINS ISOLATED IN THE AFRICAN MENINGITIS BELT IN 2007 AND 2008.

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Objectives: the WHO Collaborating Centre in Marseilles surveys meningococcal meningitis in the African meningitis belt.
Methods: group, type, subtype, Sequence Type were determined after culture or directly on CSF. MICs were measured by Etest.
Results: in 2007-2008 in Burkina Faso, 123 isolates were group A ST-2859, 3 ST-6968. In Niger, 28 isolates were A ST-7 and 8 A ST-2859. In Togo 19 isolates were A ST-2859, 13 W135 ST-2881, 11 X ST-181. In Benin 2 isolates were W135 ST-2881, one Y ST-767, one X ST-181. In 2007 in Chad, one isolate W135 was ST-11, one W135, ST-2881. In 2008 in Cameroon, 9 isolates were W135 ST-2881, one W135 ST-11. Five isolates were less susceptible to penicillin (0.125≤MIC≤1), all were susceptible to ceftriaxone and chloramphenicol.
Conclusion: the 2007 and 2008 epidemics in Burkina Faso were due to group A meningococci belonging to ST-2859 (cc5). In Niger ST-7 (cc5) continues its expansion. In 2007 ST-2859 emerged for the first time in Niger

and in Togo. The group W135 was predominant in the North Cameroon. Most of isolates were ST-2881 and gave only sporadic cases, that were different from ST-11 that was responsible for the 2000 outbreak in Burkina Faso. Serogroup X was present in several countries (Benin, Niger, Togo, Burkina Faso). The treatments of meningococcal meningitis by chloramphenicol or ceftriaxone that are recommended by WHO are relevant. It is hoped that the new monovalent A-conjugate vaccine will be soon available to diminish the burden of the disease in the region.

P033

SEROPREVALENCE OF SERUM BACTERICIDAL ANTIBODIES TO *NEISSERIA MENINGITIDIS* SEROGROUP A IN BURKINA FASO

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Aim: To describe the age-specific seroprevalence of serum bactericidal antibodies to *Neisseria meningitidis* serogroup A in Burkina Faso during the meningitis season of 2008 (non-epidemic year).
Methods: In February and March 2008, a representative sample of residents of urban Bobo-Dioulasso, Burkina Faso aged 0–59 years participated in a meningococcal seroprevalence (N=1008) and carriage (N=500) study. Serum bactericidal antibody (SBA) titres to strain F8238 (A:4:P1.20,9) were determined using standard methods (rabbit complement).
Results: The geometric mean titres (GMT) and prevalence of titres ≥8 varied substantially with age. In infants, only 4/107 (4%) had SBA titres >4, but 60% of 1-4 year olds had SBA titres ≥8. Both the GMTs and proportion with SBA titres ≥8 increased in each subsequent age group, peaking in 20-24 year olds (GMT = 488µg/ml, 90% with SBA titre ≥8) before declining in older adults. Provisional results indicate that overall meningococcal carriage prevalence was low (<2%), and no serogroup A carriers were detected.
Discussion: Culture and PCR-based surveillance in Bobo-Dioulasso in 2008 showed that the annual meningococcal meningitis incidence rate was highest at around 30 per 100.000 in infants <6 months and 15 -19 year-olds, falling below 10 per 100,000 in adults >30 years [see abstract by Yaro et al.] The classic Goldschneider curves cannot be replicated in this population, with both high SBA titres and high (hyper-)endemic disease incidence being reported in individuals aged 5-29 years. The absence of detectable serogroup A carriage suggests that exposure to other organisms may induce serum bactericidal activity.

P034

EPIDEMIOLOGY AND SURVEILLANCE OF INVASIVE MENINGOCOCCAL DISEASE (IMD) IN IRELAND, 2008

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The incidence rate of IMD notifications continues to decline from the peak in 1999 (14.8/100,000). In 2008, 169 cases of IMD were notified (4.0/100,000), a decrease from 2007 when 179 cases (4.8/100,000) were notified. Since the introduction of the MenC vaccine in 2000 the incidence of serogroup C disease has declined from 3.9/100,000 (1999) to 0.07/100,000 (2008), a 98% reduction. During the same period meningococcal B disease has declined from 8.1/100,000 to 3.3/100,000 (2008), a 59% reduction. In 2008, 82% of IMD cases were due to serogroup B, 60% of which were in children under 5 years of age. Three IMD cases caused by serogroup C were notified (1.8% of all IMD), all in adults. Other serogroups accounted for 3.6% of cases. No organism was detected in the remaining cases. The overall pattern of IMD by age group is relatively unchanged since 1999; disease incidence rates have declined in all age groups under 25 years by 68.5% since 1999. In 2008, 93% of IMD notifications were laboratory confirmed: 47% were confirmed by PCR alone; 39% by both PCR and culture; 8% by culture alone; 3% by microscopy; and 2% by serology. In 2008, there were seven IMD related deaths in Ireland (case fatality ratio of 4.1%). Five deaths were due to serogroup B and there was one each from serogroups C and W135. The last reported serogroup C death In Ireland occurred in 2004.

P035

CLONAL LINEAGES EVOLUTION OF SEROGROUP B INVASIVE MENINGOCOCAL STRAINS IN SPAIN (2001-2007)

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The Spanish Reference Laboratory for Meningococci receive around 70% of the confirmed cases of meningococcal disease all over the country. All the strains are characterised at phenotypical level by serogroup, serotype and serosubtype determination. The clonal complexes (CC) of a representative proportion (15%) of the strains were defined by MLST in both, B and C serogroups. A clonal substitution has been previously observed in Spain among serogroup C isolates: strains belonging to the ST-11 CC were predominant before 1993 changing to the ST-8 CC over 1996-1999 and finally ST-11 CC strains displaced the ST-8 CC isolates since 2000. These strains

displacements were associated with epidemic waves of serogroup C meningococcal disease in Spain. With the aim to know the CCs evolution of serogroup B in Spain we analyzed 417 invasive strains isolates over 2001-2007 period (average of 58 strains per year). All strains were genosubtyped by definition of VR1 and VR2 PorA sequence and they were assigned to CCs by MLST.
Althought 67 VR1/VR2 PorA combination were found, 24% of the strains were characterizated as 19,15. Other representative combinations were 22,9 (10.3%), 7-2.4 (9.8%) and 5-1.10-8 (8.4%). The percentages were homogeneous over the analysed period. Three hundred and foty three strains were distributed among 17 CCs while 74 isolates (17,7%) showed STs with non CC assigned. St-32 CC (26.6%), ST-41/44 CC (12.7%), ST-269 CC (9.8%) and ST-11 CC (7.9%) were the most frequently found. There were not relevance changes in the strains distribution among the most frequent CCs over the study period. The ST-35 CC, usual in 2001 and 2002, became rare during the next years; by contrast the ST-11 CC showed an opposite feature. The results obtained from the study show an homogeneous distribution of a relatively small number of CCs among invasive serogroup B meningococci in Spain.

P036

EPIDEMIOLOGICAL AND LABORATORY SURVEILLANCE OF INVASIVE MENINGOCOCCAL DIS-EASE IN DENMARK 2007 AND 2008

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Objectives: To present data on invasive meningococcal disease (IMD) in Denmark 2007-2008.
Methods: Data are obtained from mandatory clinical and laboratory notifications. All Departments of Clinical Microbiology send isolates to the Reference Laboratory where grouping, sero- and serosubtyping, antibiotic resistance determination, and sequence based typing on *por*A, *por*B and *fet*A are performed.
Results
In 2007, 78 cases (1.4 pr 100,000 population) and in 2008 66 cases (1.2 pr 100,000) of IMD were reported. The highest incidence occurred in infants and teenagers. Diagnostic methods were: 119 (83%) confirmed by culture, 19 (13%) by other methods, and 6 (4%) only clinically. Manifestations were classified as meningitis (41 cases), sepsis (44 cases), both (56 cases) or arthritis (3 cases). Seven persons acquired infection abroad, and 12 died (case fatality rate 8.3%).
The most common serogroups were B (78 cases), and C (40 cases). Of all isolates grouped, sero- and serosubtyped the most prevalent types were B:15:P1.7,16 (19 isolates), B:1:P1.14 (10 isolates) and C:2a:P1.2,5 (10 isolates). By using *por*A and *fet*A sequence typing the prevalent types were B:P1.7,16:F3-3 (20 isolates) and C:P1.5,2:F3-3 (10 isolates).

Discussion: During the past 20 years the incidence of IMD has declined from around 5 per 100,000 per year. This trend continued in 2007-08. The sequence based typing tool has improved the laboratory surveillance and outbreak investigation possibilities. The dominant serogroups were B and C. With a low incidence of group C, there is no universal immunisation. The reasons for the declining incidence are unknown.

P037

EPIDEMIOLOGICAL SITUATION OF INVASIVE MENINGOCOCCAL DISEASE AND VACCINATION STRATEGY IN THE CZECH REPUBLIC

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Background: Nation-wide enhanced surveillance of invasive meningococcal disease was implemented by the National Reference Laboratory for Meningococcal Infections (NRL) in 1993.
Methods: The case definition is consistent with the ECDC guidelines. Notification is compulsory and *Neisseria meningitidis* isolates from IMD cases are referred to the NRL to be characterised by serogrouping, PorA and FetA sequencing (http://neisseria.org/nm/typing/) and multilocus sequence typing (MLST) (http://pubmlst.org/neisseria/).
Results: Despite the stable trend in IMD incidence (0.8/100 000) since 2005, the case fatality rate was high (11.8 %) in 2007. The disease was caused mainly by serogroup B meningococci (67.4 %) in 2007, followed by serogroups C (20.9 %) and Y (9.3 %). The following clonal complexes were most frequently associated with IMD: cc11, cc18, cc41/44 and cc32 (18,6 %, 13,9 %, 9,3 % and 9,3 %, respectively). The highest age-specific morbidity rates were observed in the lowest age groups, i.e. 0-11 months and 1-4 years (11.4/100 000 and 4.5/100 000, respectively), and were associated with high prevalence of serogroup B. The case fatality rate was the highest in infants under 1 year of age (38.5 %). The incidence of IMD caused by serogroup C is currently low and there is no indication for mass vaccination with MenC conjugate vaccine. MenB vaccine is needed for infants, but the sero/subtype coverage by the currently developed porin-based vaccines is low for Czech meningococcal isolates.
Conclusion: Other than porin-based vaccine effective against *N. meningitidis* B needs to be developed.

P038

MOLECULAR METHODS IN EPIDEMIOLOGY OF INVASIVE MENINGOCOCCAL DISEASE

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Background: The National Reference Laboratory (NRL) for meningococcal infections has been conducting surveillance of invasive meningococcal disease (IMD) in the Czech Republic (CR) since 1993. High morbidity and mortality in the mid 1990s was caused by *N. meningitidis* C hypervirulent clone cc11. Serogroup B is currently prevalent.
Methods: All isolates from IMD cases in CR referred to NRL in 2005-2008 were characterized by multilocus sequence typing (MLST) and porA gene and fetA gene sequencing. Non-culture PCR detection of *N. meningitidis*, *H. influenzae* and *S. pneumoniae* directly from clinical specimens enabled diagnosis in culture-negative cases and deaths.
Results: Molecular methods allow investigation of epidemiological relationships. For example, in October 2005, *N. meningitidis* B was detected by PCR in a culture-negative IMD patient, a 16-month-old male twin. His father was a carrier of *N. meningitidis* B, phenotype B:1:P1.14, genotype ST-5128, cc213. Eleven months later, the female twin developed IMD and the causative strain was identical in phenotype and genotype to the paternal carrier strain. In 2006, the male twin tested positive for carriage of the same strain (B:1:P1.14, ST-5128, cc213). In 2005-2007, no secondary case of IMD was detected by the analysis of epidemiological data and isolate genotypes. Majority of case/close contact isolates had identical genotypes in contrast to case/non-close contact isolates that differed in genotype.
Conclusion: Molecular methods are essential in IMD surveillance. No secondary case of IMD has been detected in the Czech Republic. The measures taken in IMD foci are efficacious and need to be targeted to close contacts.

P039

THE CASE FATALITY RATE OF MENINGOCOCCAL DISEASE IN ENGLAND AND WALES; 1998-2008.

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Aims: To look at the case fatality rate (CFR) for confirmed meningococcal disease by serogroup, age and method of diagnosis over a 10 year period.
Methods: Isolates from cases of meningococcal disease in England and Wales are sent by microbiology laboratories to the Health Protection Agency (HPA) Meningococcal Reference Unit (MRU) where they are classified into serogroup, serotype and serosubtype. Samples are also submitted for meningococcal DNA detection by PCR. Data from the Office of National Statistics have been used to generate information on deaths from meningococcal disease and additional deaths have been identified by linking to HPA MRU laboratory confirmed reports. These data have been used to generate case fatality rates by sergroup, age and method of diagnosis between 1998 and 2008. These were further analysed by cycle threshold (CT) counts for PCR confirmed cases.
Results: Case fatality trends indicated that between 1998 and 2008 the CFR was declining in cases confirmed by PCR only. This suggests that PCR sensitivity could be increasing, resulting in the diagnosis of milder cases. Analysis by CT count shows that CFR is higher in cases with lower counts. This suggests that presence of lower DNA levels is associated with a lowered risk of death. This is consistent for Groups B and C but the relationship is less clear for adults than for children.
Discussion: The findings are compatible with milder disease being detected by the increased sensitivity PCR or with inclusion of “false positive” results that were not in fact meningococcal sepsis.

P040

AUSTRIA 2008, EPIDEMIOLOGY AND SURVEILLANCE OF MENINGOCOCCAL DISEASE

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Aims: The National Reference Centre for Meningococci (NRCM) was founded by the Ministry of Health 1981. All invasive meningococcal disease (IMD) isolates are sent to the NRCM. The NRCM collects the data and reports to the Ministry of Health.
Methods: The serogrouping is performed with sero-agglutination using polyclonal sera. The serotype is determined with whole cell elisa using monoclonal antibodies. The serosubtype is identified by DNA sequencing of the *porA* variable regions. The antibiotic resistance is determined with E-test on Müller Hinton with 5% sheep blood.
Results: 95 cases of meningococcal disease were reported 2008. The incidence rate was 1.14/100000. 10 deaths were registered, which results in case-fatality ratio of 10.6% and mortality rate of 0.12/100 000. The clinical presentation was 41.5% meningitis, 30.53% septicaemia only, 28.42% meningitis & septicaemia combined. In 84 cases the case definition was laboratory confirmed. 11 cases were diagnosed only clinically. The distribution of the serogroups in the 84 laboratory confirmed cases was serogroup B 69.05%, serogroup C 28.57%, serogroup Y 1.19% and serogroup W_{135} 1.19%. According to the criteria of the Clinical and Laboratory Standards Institute (CLSI) 5 isolates were intermediate resistant to penicillin. No isolates were resistant to penicillin, rifampicin, ciprofloxacin and ceftriaxon.
Conclusions: The incidence 2008 with 1.14/100000 is the first slow rise after two years of incidence rates <1.0/ 100.000. The increase in IMD, compared to 2007, is due solely to serogroup B disease. Serogroup B disease showed an overall escalation from 56.5% (2007: 37; 2008: 58).

P041

A HIGH PREVALENCE OF SEROGROUP Y MENINGOCOCCAL ISOLATES IN STUDENTS FROM THE UNIVERSITY OF NOTTINGHAM

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Longitudinal carriage of meningococci was surveyed in students from the University of Nottingham between November 2008 and May 2009. A total of 192 volunteers were recruited from six separate halls of residence, of which 90% were first year university entrants. A phayrngeal swab was taken and immediately inoculated onto selective media prior to performing a PCR-based detection protocol on suspected meningococcal isolates. Meningococcal

carriage was indicated by PCR-positive reactions for the meningococcal crgA, ctrA and porA genes. Serogrouping and PorA typing were then performed on confirmed meningococcal isolates. A carriage rate of 48% was detected at the first time point with clearance rates being less than 5% at 4 and 12 weeks. Among the 89 meningococcal isolates 11, 1 and 2 were positive for the type B, C and W-135 capsular serotypes, respectively, whilst 39 isolates were positive for serogroup Y. Analysis for unusual variants of ctrA indicated that 21 and 4 isolates were of the 29E and Z serogroups, respectively. Two separate clusters of serogroup Y isolates with identical PorA-types were present in two halls of residence indicative of rapid, intra-hall transmission. PorA- and FetA-typing demonstrated that some of these isolates exhibited a high level of similarity with a concomitant fatal case of serogroup Y meningococcal disease in Nottingham.

P042

ASSOCIATION OF MENINGOCOCCAL FINE TYPES WITH CLINICAL MANIFESTATION AND PENICILLIN SENSITIVITY IN GERMANY, 2002-2008

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We investigated molecular genetic fine types of *N. meningitidis* in relation to clinical manifestation and penicillin sensitivity in patients with invasive meningococcal disease (IMD). The German National Reference Centre for Meningococci (NRCM) determined fine type (Serogroup:PorA-variable region (VR) 1, PorA-VR2:FetA-VR) and penicillin in IMD patient isolates from 2002-2008. Results were matched to statutorily notified cases. Fine types were analysed according to age, over time and region, clinical picture, case-fatality (CF) and penicillin susceptibility (Chi-square test, multivariate logistic regression). Analysis of 2895 matched NRCM-cases revealed 625 fine types. The most common were B:P1.7-2,4:F1-5 (11.6%), C:P1.5,2:F3-3 (10.3%), B:P1.7,16:F3-3 (6.9%). Compared to all other fine types, B:P1.7,16:F3-3 and B:P1.7-2,16:F3-3 caused a disproportionately higher proportion of disease in adolescents ($pc^2<0.00001$, $pc^2=0.007$), while for C:P1.5-1,10-8:F3-6 ($pc^2<0.00001$), and Y:P1.5-2,10-1:F4-1 ($pc^2<0.00001$) this was the case in younger (20-49 years) and older (≥50 years) adults, respectively. The highest diversity of fine types was observed in infants with 1-39 cases/finetype (460 cases/233 fine types) and the lowest diversity among adolescents aged 15-19 years (1-79 cases/fine type, 616 cases/149 fine types). Only a few fine types deviated from the overall temporal distribution, e.g. B:P1.7-2,4:F1-5 increased disproportionately in 2004-2005 causing a marked increase in IMD incidence in western North Rhine-Westphalia.Fine types B:P1.7-2,4:F1-5, C:P1.5,2:F3-3, B:P1.19-15:F5-1, C:P1.5-1,10-8:F3-6, C:P1.5-1,10-8:F4-1 and C:P1.5-2,F3-6 were significantly associated with higher CF in multivariate logistic regression analysis. The latter 3 fine types belonged almost exclusively to the ET-15 clone, and were also significantly associated with a septic clinical

course. Intermediate penicillin susceptibility (MIC≥0.125) was not associated with age, sex, CF or clinical manifestation but was significantly (p<0,001) associated with 2 fine types B:P1.19-15:F5-1 (23,5%) and C:P1.5,2:F5-8 (94,1%) that accounted for 40/147 (27.2%) intermediately susceptible isolates. More severe IMD, high CF and intermediate penicillin susceptibility are associated with particular fine types. Corroboration of the latter finding is planned through investigation of associations between disease outcome, fine type and intermediate *penA*-alleles.

P043

THE MENINGOCOCCAL CARRIAGE CONSORTIUM: STUDIES OF MENINGOCOCCAL CARRIAGE AND THE IMPACT OF SEROGROUP A CONJUGATE VACCINATION ACROSS THE AFRICAN MENINGITIS BELT.

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Meningococcal disease remains a major problem in countries of the African meningitis belt, but it is expected that an affordable serogroup A conjugate vaccine being developed by the Meningitis Vaccine Project will be rolled-out across this region, beginning in Burkina Faso in 2009/10. This vaccine should provide high levels of direct protection to immunised individuals but, as for serogroup C conjugate vaccines, a greater public health impact will be achieved if carriage and transmission of the infection are also prevented.
A Meningococcal Carriage Consortium, combining the expertise of 8 centres in developed countries and 9 African partners, has been created to conduct a series of studies to establish the pattern of carriage across the meningitis belt before vaccine introduction and subsequently to determine the impact of the vaccine on carriage. Seroprevalence studies of serogroup A-specific antibodies will also be performed.
Pilot studies will take place in mid-2009 to obtain preliminary information on carriage and antibody prevalence in each of the study populations, to test field and laboratory methods and to compare two swabbing techniques. Following the pilot study, cross-sectional carriage and seroprevalence surveys will be undertaken in 2,000 subjects in each country, once during the dry season and once during the rainy season. Detailed longitudinal studies will be performed in individuals identified as serogroup A carriers to examine rates of acquisition and loss and describe patterns of transmission within households. To investigate the impact of vaccination on carriage, pre- and post- vaccination carriage surveys will be undertaken in 3 countries.

P044

MAJOR SEQUENCE TYPES AND CLONAL COMPLEXES AMONGST NON-INVASIVE MENINGOCOCCAL ISOLATES IN SCOTLAND 1974 - 2004

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Aims: The characterisation of non-invasive *Neisseria meningitidis* isolates and examination of the sequence types and associated MLST complexes.
Methods: Non-invasive meningococcal isolates were identified from the SMPRL isolate collection. These isolates were characterised by Multi-locus sequence typing (MLST) and *porA* variable region (VR) sequencing. Non-groupable isolates were also characterised by genogrouping PCR tests specific for genes encoding different capsular polysaccharides.
Results: Isolates were assigned to at least twenty-eight different MLST complexes; most commonly this included the ST-41-44 complex/Lineage 3, ST-8, ST-35, ST-22, ST-53, ST-269, ST-213, ST-23/Cluster A3, ST-32/ET-5 and ST-254 complexes. A significant number of isolates could not be assigned to a known MLST complex. Many new STs were assigned to the ST-35 complex. Temporal changes were evident within the data set; for example the emergence of the ST-213 complex in the mid 1990's. Around one third of isolates were non-groupable and genogrouping PCR testing revealed a significant proportion of these isolates possessed the capsule null locus (*cnl*). A new *cnl* allele sequence was identified in isolates of the ST-53 complex. In some instances particular capsular polysaccharides and *porA* VR sequences appear to be associated with certain lineages, whereas in other lineages this is not the case.
Conclusions: MLST has been used extensively for a number of years by the SMPRL for the characterisation of invasive meningococcal disease isolates in Scotland. This project has characterised non-invasive meningococci and the data generated will be an important resource for a more detailed examination of meningococcal lineages over a 30-year period.

P045

EPIDEMIOLOGY OF THE SEROGROUP B *NEISSERIA MENINGITIDIS* (MNB) FACTOR H BINDING PROTEIN IN STRAINS SAMPLED FROM SPAIN AND GERMANY IN THE YEARS 2001-2006

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Background: Recombinant LP2086, also known as factor H binding protein (fHBP), is a promising vaccine candidate for prevention of MnB disease. The protein exists in two genetically distinct subfamilies, designated A and B. An earlier study investigated the genetic diversity of fHBP in a large collection of MnB isolates (n=1,263) from the USA, UK, France, Norway, and the Czech Republic. The current study extends the observations of this earlier work by examining the genetic and phenotypic diversity of isolates from Spain and Germany.
Methods: The fHBP expression levels and genetic diversity were determined from a collection of 539 isolates, representing every eighth isolate from Spain and Germany during the years 2001-2006.
Results/Conclusions: All meningococcal isolates contained the *fhbp* gene and >98% expressed antibody-accessible fHBP on their cell surface at levels ≥4X background using in vitro growth conditions. Though the proportion of strains in subfamily A or B was variable depending upon the country surveyed, the diversity of fHBP sequences observed fell within the previously described subfamilies, and the surface expression profiles were similar to the reference collection. fHBP is a target for a universal MnB vaccine (rLP2086) and the importance of providing coverage against MnB strains from both fHBP subfamilies should be noted, as the percentage of subfamily A expressing strains was observed to be ~40% in Spain and 21% in Germany. The epidemiological observations reported here support the continued development of a fHBP vaccine containing both A and B subfamily variants for a universal MnB vaccine.

P046

MENINGOCOCCAL DISEASE IN EUROPE

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Background: A comprehensive survey of meningococcal disease isolates from the years 2000-2002 from 18 participating countries of the EUMenNet project linked with EU-IBIS epidemiological information. Strain type information (serogroup, PorA, FetA and Sequence Type (ST)) was available for 2456 isolates.
Results: A total of 1130 unique strain types were present. Of these, a total of 15 (1.3%) strain types were observed 20 times or more, accounting for 794 (32.3%) of these isolates. Of the remaining strain types, 896 (36.5%) were observed only once. Together with isolates belonging to the same clonal complex, the most prevalent strain types accounted for 43% of isolates. Age data were available for 3226 isolates. Significant age effects were seen for a number of complexes, and those unassigned to any complex. The ST-41/44 complex was over-represented in the <1 year and under-represented in the 65+ year age ranges. The ST-11 complex was under-represented in the under five year olds and over-represented in the 15-44 year age range. Increased disease was associated with ST-32 complex in the 5-14 year age group and with ST-8 complex in the 1-4 year age range. The ST-23 complex was significantly associated with the 15-19 years old age group and the over 65s. Unassigned STs were over-represented in children <1 year old and under-represented in the 15-19 year olds. The ST-8 complex was encountered comparatively more frequently in females than expected (56.4%, $p<0.005$). Conversely, the ST-213 complex was more associated with males (71.4%, $p<0.007$). Similarly, STs unassigned to any complex were also more associated with males: (59.0%, $p<0.05$). These differences were, however, no longer significant when the Bonferroni correction was applied.
Summary: This large and comprehensive dataset of combined epidemiological and molecular typing information will give a unique insight into meningococcal disease across Europe. An important use of the data will be the examination of the potential impact of different types of meningococcal vaccines. This will assist in the development of future vaccine strategies.

P047

VARIABILITY OF THREE ANTIGENS INCLUDED IN THE NOVARTIS INVESTIGATIONAL MENB VACCINE: AN OVER TIME ANALYSIS

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Background: Broad-range non-capsular meningococcal vaccines are still not available. Recently, a novel Vaccine against Meningococcus B, has been developed and is being tested in clinical trials. Studies on sequence variability of the vaccine components are important to evaluate their conservation, and potential strain coverage. We have assessed antigenic variation of NadA, fHbp and GNA2132 in a panel of meningococcal strains collected in the Netherlands over a period of 40 years.
Methods: 135 isolates, belonging to serogroups B and C, were randomly selected at the Netherlands Reference Laboratory for Bacterial Meningitis, and characterized by MLST. Approximately 30 strains were included from years 1960, 1970, 1980, 1990 and 2000, each. Genes encoding for fHbp, GNA2132 and NadA were sequenced.
Results/Conclusions: 27% of the isolates belong to clonal complex (cc)41/44, 16% to cc8, 10% to cc11. The remaining 47% included 16 different clonal complexes. cc41/44 was predominant in 1960 (30%), 1990 (30%) and 2000 (50%). In 1970, cc8 was predominant (53%), whereas cc11 was 22%. The three protein variants appeared to be always associated with clonal complexes. Furthermore, the repertoire of the three antigens remained the same over time. However, the relative prevalence of variants for each antigen varied: in the case of cc41/44 and cc11, the fHbp variants more represented in 1960 were different from those predominant in 1990 and 2000.Strains associated with unique antigenic repertoires were found only once in the present, and in other, strain panels, suggesting that these are less fit and excluded by selection.

P048

INVASIVE BACTERIAL DISEASES DURING 2008 IN UNIVERSITY HOSPITAL FOR INFECTIOUS DISEASES "DR FRAN MIHALJEVIC" ZAGREB, CROATIA - SHORT OVERVIEW

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Introduction: Invasive bacterial disease (IBD) undoubtedly is of grate importance for the clinicians and at the same time it is grate diagnostic challenge for the microbiologists. The International Circumpolar Surveillance (ICE) network registers Invasive bacterial disease (IBD) caused by *Neisseria meningitidis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Listeria monocytogenes*, group A streptococcus and group B streptococcus. Therefore we analysed the distribution of four microbiological causes of IBD, *N. meningitidis*, *H .influenzae*, *S. pneumoniae*, and L.monocytogene, during 2008 in our hospital.
Methods: The patients with IBD caused by chosen bacteria treated in the University Hospital for Infectious Diseases Zagreb during 2008 were included. Laboratory information's for all isolates from blood cultures and cerebrospinal fluids were recorded and correlated.
Results: A total of 96 patients with IBD caused by chosen bacteria were hospitalized during 2008 and 52 were children (54 %). At the same time 101 isolates were detected. *S. pneumoniae* was predominant (63/96), in adults (35/63) and children (28/63). It was followed by *N. meningitidis* 31/96. IBD in 23/31 children was caused by N.meningitidis and in 8/31 adults. *H. influenzae* was cause of one IBD episode in child (sepsis), while L.monocytogenes caused IBD (meningitis) in one adult. Sepsis was recorded in 74/97 patients and meningitis in rest of patients. In adults sepsis and meningitis were caused mainly with *S. pneumoniae* (26/31 and 9/13). Meningitis in children was mainly caused by *N. meningitidis* (6/9), but sepsis was caused by *S. pneumoniae* (25/43 as well as *N. meningitidis* (17/43).
Conclusion: The predominance of S.pneumoniae as main cause of IBD in our hospital was confirmed. It was dominant cause of sepsis as well as of meningitis in adults. *N. meningitidis* is still main cause of meningitis of children. In Croatia vaccination against *H .influenzae* type B was introduced in 2002 and therefore only one case of meningitis during 2008 was recorded.

P049

VARIABILITY OF THE ANTIGENS OF THE NOVARTIS INVESTIGATIONAL MENB VACCINE IN A CARRIER/DISEASE STRAIN PANEL

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Background: The definition of the differences between pathogenic and carrier meningococcal strains is of great interest for molecular epidemiology. In general, a higher genetic and antigenic diversity is associated with carriage than with disease. We have analysed and compared the gene variability of three components of a MenB vaccine in all isolates obtained from cases of disease and from a concomitant sample of carriage over a one year time period and from the same European country.
Methods: The sequence variability of the vaccine components NadA, fHbp and GNA2132 was evaluated in 270 strains isolated in 1993 in the Czech Republic. Full gene sequences were assessed for *gna2132* and *fHbp*, whereas the *nadA* gene was amplified in all strains and the sequence determined in a few cases only. The *gna2132* and *fHbp* sequences, and the *nadA* presence-absence genotypes, were compared between carrier and pathogenic isolates belonging to the same clonal complexes, and with isolates from other pathogenic panels.
Results/Conclusions: Both fHbp and GNA2132 displayed a higher degree of diversity in the carrier isolates than in the disease associated isolates. NadA was only present in a subset of isolates. Interesting differences in the antigenic repertoires between carriage and pathogenic strains were found in almost all clonal complexes. In particular, cc41/44 and cc11 displayed antigenic repertoires, which appeared to be associated with carriage only. The differences will be confirmed by similar studies performed in other countries, and at other times. Web-accessible databases for GNA2132 and NadA have been set up at http://neisseria.org/nm/typing/ to complement the existing fHbp database.

P050

TRENDS IN MENINGOCOCCAL DISEASE BEFORE AND DURING IMPLEMENTATION OF MENINGOCOCCAL CONJUGATE VACCINE, UNITED STATES, 1998-2007

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Background: This report describes the epidemiologic features of meningococcal disease in the United States from January 1998 through December 2007, before and during implementation of adolescent quadrivalent meningococcal conjugate vaccine (MCV4).
Methods: Data were collected from active surveillance for disease caused by N. meningitidis conducted through the Active Bacterial Core Surveillance (ABCs) sites during 1998-2007. Isolates from cases were serogrouped at the ABCs site and confirmed at CDC. Incidence rates were calculated using U.S census data for the ABCs sites, and estimates of the number of cases in the 50 U.S. states were calculated standardizing for race and age group.
Results: In the years 1998-2007, the estimated U.S. average annual incidence of meningococcal disease was 0.53 cases per 100,000 population (0.51-0.55, 95% credible interval [CI]); an estimated 12.1% of these cases were fatal. The annual incidence decreased 64.5%, from 0.93 in 1998 to 0.33 in 2007. Infants aged <1 year have the highest incidence of meningococcal disease (3.03 cases per 100,000 population). Differences in the incidence of meningococcal disease by race decreased during 1998-2007. No significant decrease in serogroup C or Y meningococcal disease was seen among 10-19 year-olds in 2006-2007, after introduction of MCV4.
Conclusions: Prior to introduction of MCV4, the incidence of meningococcal disease in the United States decreased three-fold. However, meningococcal disease still causes a substantial burden of disease among all age groups. In the first two years after introduction, no impact from MCV4 could be observed.

P051

THE EPIDEMIOLOGY OF *NEISSERIA MENINGITIDIS* IN ITALY, 2007-2008

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Background: In Italy, the average annual incidence of invasive meningococcal disease (IMD) in 2007-2008 was 0.3 cases/100,000 inhabitants. We describe the national epidemiology of meningococcal invasive disease, in years 2007-2008.
Methods: Information on *N. meningitidis* disease derives from two sources of data*, i.e:*1) statutory notification of meningitis due to *N. meningitidis*, since 1991. Within this system, clinicians report each confirmed case to the Local Health Unit (LHU), that transmits data to the Regional and National Authorities. 2) surveillance of Bacterial Meningitis, since 1994. From 2007 this system was extended to all invasive bacterial diseases. All *N. meningitidis* isolates are sent to the National Reference laboratory, at ISS, for confirmation, serotyping, molecular typing and antibiotic susceptibility.
Results: A total of 185 and 179 cases of IMD were reported in 2007 and 2008 respectively, with an annual incidence rate of 0.3/100,000. The highest incidence rates were observed in children < 1 year of age (3.2 and 4.1/100.000 in 2007 and 2008), followed by the 1-4 year age group (1.8 and 1.0/100.000, respectively) and 5-9 year age-group (0.5 and 0.6/100,000, respectively).
Conclusion: Preliminary analysis shows that annual incidence of IMD in Italy was stable in the period 2007-2008. The MenC vaccination coverage by Region should be properly monitored, in order to assess the impact of vaccination on *N.meningitidis* invasive disease.

P052

SIMULATION OF MENINGOCOCCAL DISEASE SCENARIOS IN PORTUGAL

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Many factors are involved in the dynamics of *Neisseria meningitidis* colonization and infection. Some of these factors are related to virulence and transmissibility characteristics of bacterial strains; other factors are related to host susceptibility and are influenced by environmental conditions and human social behavior. Those factors act as a system.The aim of study is to evaluate how the interplay of all these factors, or variables, influences the

incidence and mortality of meningococcal disease in Portugal. We use the System Dynamics methodology for this purpose. In particular, we build a dynamic model to simulate what combinations of variables replicate the patterns of incidence observed in previous years.The construction of a system dynamic model starts with the identification of the variables and their behavior over time. Behaviors result from inter-relationships that we describe in causal diagrams. The final model for simulation builds on this causal diagram by using suitable computer software (Vensim or other). In the end, the statistical comparison of data generated by the model with the observed data provides the empirical validation of the model.Meningococcal strains have been characterized since October 2002, in order to know the serogroup, type, subtype and sequence type. Meningococcal disease variables considered to create a simulator include age group incidences, vaccination rates (meningococcal serogroup C), variability of genotypes, flu incidence, and mortality rates. These have been studied over five years.

P053

SARIMA MODEL USE IN FORECASTING EARLY MENINGITIS OUTBREAK INTENSITY

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Background: Bacterial meningitis remains a major public health problem in the African belt meningitis with recurrent and large outbreaks still observed with a high lethality.
Objective: To forecast weekly cases in 2009 using data of a microbiological surveillance performed between 2003 and 2008.
Methods: A case of bacterial meningitis is defined by any subject suspected of meningitis, which is microbiologically confirmed using a previously described procedure. Cerebral spinal fluids are collected through a nationwide surveillance system. A model of the Box-Jenkins family, SARIMA, was chosen to forecast the weekly cases for the year 2009. Parameters were selected with the function auto.arima of R software and AIC was used to choose the most appropriate model. Pacf allowed checking absence of autocorrelation. Forecast predictions were compared to the observed one.
Results: Highest incidences were observed in 2006 and 2008 with more than 1200 cases and more than three daily cases in average versus one for the remaining years.
Forecast predictions for 2009 show an earlier onset of the outbreak at the 13th week (peak at week 17) than observed. However the number of cases in the 2009 season is presently between three and five times more than expected by the model and occurred earlier (4th week).
Conclusion: The model was able to detect the early beginning of the 2009 outbreak but failed to forecast the unusual huge increase in the number of

cases. This demonstrates that outbreak predictions should take into account additional factors, especially climate, to improve more realistic forecasting.

P054

BACTERIAL MENINGITIS DUE TO *S. PNEUMONIAE* IN GREECE: A 6 YEAR EPIDEMIOLOGICAL STUDY (2003-2008).

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Introduction: Notification of bacterial meningitis is compulsory in Greece. Samples (CSF, blood, cultures) from nearly 90% of bacterial meningitis cases, are sent for identification by conventional and molecular methods.
Results: A total of 369 cases of meningitis due to *S. pneumoniae* were notified for a 6 year period, with the average incidence of 0.55 per 100 000. Of those, 354 (95.3%) cases were confirmed either by culture (129; 36.4%) or by PCR (225; 63.5%). For their identification, 3 multiplex PCR assays were performed; one for genus specific and 2 for the identification of 9 main serotypes (1, 3, 4, 6B, 14, 18C, 19A, 19F, 23F). Almost 50% of the cases were related to the age groups of <1-4 (26.4%) (group A) and >60 years of age (23.6%) (group E). The most prevalent serotypes were 19F (24.7%), 6B (20.8%) and 3 (14.3%). Analysis by age showed that there is an equal distribution of those at the age groups of A and E. In contrast, serotype 19A was found mainly at the age group of <1-4 years (7.8%).
Conclusion: Although the pneumonococcal conjugated 7-valent vaccine is included in the National Vaccination Program since 2005, no decrease in the cases was observed. In addition, serotype 3, which is not included in the 7valent vaccine, has been identified the last three years.

P055

BACTERIAL MENINGITIS DUE TO *H. INFLUENZAE* IN GREECE: A 6 YEAR EPIDEMIOLOGICAL STUDY (2003-2008)

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A total of 35 cases due to *H. influenzae* were notified for a 6 year period. Of those, 26 cases were due to *H. influenzae* type b (Hib) and 9 were identified as *H. influenzae* non type b. The average incidence was 0.05 per 100 000. All cases were confirmed by either PCR assays (27 cases; 77%) while, only 6 (23%) were confirmed by culture. For their identification, 2 multiplex PCR assays were carried out. The *bex* and *hel* genes were used for Hib and *H.*

influenzae non-type b respectively. The highest incidence of Hib was observed in the children aged less than 1 year (10.34/100 000; 11/26) followed by the age group of 1-4 years (1.45/100 000, 6/26). There was no isolation of Hib at the ages 5-9 years; while 9 cases were identified in adults (>20 years). Positive samples of *H. influenzae* non-type b were found mainly in older ages (>40 years).
Hib vaccination in Greece is compulsory since 1995. This resulted in impressive reduction in disease. Nevertheless, there are still few cases reported each year especially at ages less than 1 year old, especially at the ages of 3-6 months.

P056

EPIDEMIOLOGY OF MENINGOCOCCAL DISEASE IN RUSSIA

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Introduction: The present study describes: the notification incidence of invasive meningococcal disease (IMD); the age distribution of invasive meningococcal infection; the serogroup distribution of meningococci in different regions of Russia; the mortality of invasive meningococcal infection.
Aims: The epidemiology and serogroup prevalence of meningococcal infections have been studied in 47 regions of Russia.
Methods: For the period 2007 the serogroup prevalence of meningococci isolated from specimens of blood or cerebrospinal fluid from children and adults was investigation. In total 601 of serogrouped cases were registered. Individual data about all cases of IMD were collected and analysed.
Results: The notification annual mean of incidence in all Russia were: 2005 – 1,92; 2006 – 1,7; 2007 – 1,56 and the average for this period was 1,72 per 100000 population. Between regions the incidence ranged widely from 0 to 6, although most regions were within the range from 0 to 3 (69 regions out of all 84 regions). Over half the cases were in children under 5 years (55,2%), 13,2% of patients were in adults 15-24 years, 14,8% - were in adults 25 – 64 years and 2,57% – were in elder over 65 years. Most cases IMD in Russia were caused serogroup A (36,1%), B (24,1%), C (18,5%). There was some variation between regions of Russia. In North-Western regions nearly 46,4% of grouped cases in the period 2007 were attributed to serogroup B, 17,8% - to serogroup C and 3,6% - to serogroup A. In Central regions of Russia nearly 56,1% of grouped cases were attributed to serogroup A , 20% - to serogroup B and 17,2% - to serogroup C. In Southern regions of Russia predominated of serogroup A – 52,5%, percent of serogroup C was 19,2% and B – 12,1%. In Siberia and Eastern regions of Russia out of all grouped cases predominated serogroup B (38,5% and 50% respectively) and C (11,5% and 8,3% respectively). The average mean of mortality (overall case fatality rate – CFR) of IMD was 12,1%.
Conclusion: The meningococcal disease has a variation in rates of incidence and serogroup distribution around the different part of Russia. In

Central and Southern regions of Russia serogroup A largely predominated. By contrast, in North-Western and Eastern regions of Russia out of all serogrouped cases IMD predominated meningococci of serogroup B.

P057

EPIDEMIOLOGY OF MENINGOCOCCAL DISEASE IN FINLAND IN 1995-2008

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Introduction: Reporting of invasive meningococcal disease (IMD) is obligatory in Finland, and case-based data is available since 1995.
Aims: We report the trends in IMD in Finland in 1995-2008.
Methods: In Finland, the surveillance of IMD is based on statutory notifications from clinicians and microbial laboratories to the National Infectious Disease Register at the National Institute for Health and Welfare (THL; formerly National Public Health Institute, KTL) and the phenotypical characterization of the corresponding isolates submitted to the reference laboratory. In 1995-2008, 87-100% of the IMD case isolates were sent annually to the reference laboratory.
Results: Since a period with higher incidence in 1995 and 1996, caused by both serogroup B and serogroup C strains, the incidence of IMD in Finland has fluctuated at low levels between 0.5 to 1.1 per 100 000 population (29-58 notified cases per year). The majority of cases were due to serogroup B (73%), followed by serogroup C (16.4%). Since 1996, the number of serogroup C cases has remained low, with 1-9 cases occurring per year (incidence 0.02-0.17 per 100,000). In 2004-2008, the most common serogroup B phenotype was B:4:P1.4, and 27-54% of all isolates were non-serosubtypable.
Conclusions: The incidence of IMD in Finland has remained low during the past 14 years. The majority of cases have been due to serogroup B. There are currently no plans to introduce serogroup C conjugate vaccine into the national vaccination program. The high proportion of non-serosubtypable isolates emphasizes the need of *porA* sequence typing for epidemiological surveillance.

P058

HAEMOPHILUS INFLUENZAE STRAINS ISOLATED FROM PATIENTS WITH MENINGITIS AND SEPSIS BEFORE AND AFTER THE INTRODUCTION OF HIB VACCINE INTO POLISH UNIVERSAL VACCINATION CALENDAR

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Objective: To investigate the impact of mass vaccination against *H. influenzae* serotype b (Hib), which has started in 2007, on *H. influenzae* population responsible for meningitis and sepsis in Poland.
Methods: The study was performed on all *H. influenzae* isolates collected between 1997 and 2008 during the routine monitoring of bacterial invasive infections by the National Reference Center for Bacterial Meningitis in Poland. All strains were identified according to standard procedures. PCR reactions were run to confirm species identification and serotype determination, and to detect capsule-specific genes. MICs of antimicrobials were evaluated by E-test. β-lactamase production was detected by nitrocefin assay.
Results: 350 *H. influenzae* strains isolated from CSF and blood were collected. Until the year 2007 most of them (83%) were recovered from children below the age of five. The majority of the strains were characterized as Hib (93%). *H. influenzae* serotype f (Hif) and non-capsulated isolates (NCHI) were responsible for 1% and 6% of cases, respectively.In 2008, most of the *H. influenzae* strains were recovered from patients above 5 years of age (67%). NCHI were responsible for 50% of infections, following by Hib (47%) and Hif (3%).Only 12% of all 350 isolates were resistant to ampicillin and this was strictly correlated with β-lactamase production.
Conclusion: Two-years after introduction of Hib vaccine into Polish Calendar significant reduction of infections due to *H. influenzae* type b was observed. However, the enhanced surveillance showed a shift in age patients as well as in number of infections caused by NCHI.

P059

CLUSTER OF MENINGOCOCCAL DISEASE CAUSED BY ST-11 MENINGOCOCCI IN THE NORTH-WEST OF POLAND

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Aim: To investigate a cluster of meningococcal infections in Goleniow county in the North-West of Poland.

Methods: Isolates of *Neisseria meningitidis* received by the National Reference Centre for Bacterial Meningitis were identified by standard methods. They were serotyped and characterized by RFLP-PFGE, multilocus sequence typing (MLST), *porA* and *fetA* typing.
Results: From 10.03.2009 until 25.03.2009 five cases of invasive meningococcal disease (IMD) emerged in the small Goleniow county (33000 habitants), giving overall incidence rate of 15.2/100000. Three cases were diagnosed as sepsis with meningitis, one as sepsis and one as meningitis. Except the meningitis case all patients developed petechial rash. The IMD affected persons aged from 7 until 25 years. Serogroup C of *N. meningitidis* was responsible for all cases. The MLST analysis of three isolates classified them to the ST-11 clonal complex. No epidemiologial links were established amongst the patients. Since the oldest patient belonged to the risk group of IMD and there are logistic problems concerning vaccinations of young adults, the decission to vaccinate Goleniow population from 6 until 19 years old (with incidence 70.2/100000) was taken. The further analysis is in progress.
Conclusions: The aforementioned cluster of cases in Goleniow county is the first one caused by ST-11cc in the wider community in Poland, since several outbreaks in 2006-2007 caused by isolates of this clone, involved patients with established epidemiological links.

P060

SURVEILLANCE OF INVASIVE MENINGOCOCCAL DISEASE IN POLAND IN 2008

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Aim: To characterise epidemiological situation concerning invasive meningococcal disease (IMD) in Poland in 2008.
Methods: In 2008 the enhanced surveillance of invasive bacterial infections including IMD was established by building a net comprising 130 Polish microbiological labs. All invasive isolates of *Neisseria meningitidis* received by the National Reference Centre for Bacterial Meningitis (NRCBM) were identified and characterised by standard methods. A PCR technique was used for identification of the etiological agent directly from clinical materials in the case of a negative culture.
Results: In 2008, the NRCBM identified 335 of laboratory confirmed IMD cases, out of which for 277 a serogroup of aetiologic agent was defined. There were 226 invasive meningococcal isolates and 109 PCR-positive reactions with primers specific for meningococcal species. Majority of IMD cases were caused by meningococci of serogroup B (n=143; 51.6%), followed by serogroup C (n=122; 44.0%), W135 (n=7; 2.5%) and Y (n=3, 1.1%). Subsequent isolates subtyping revealed that the most predominant phenotypes among serogroup B meningococci were B:15:P1.(7),16; B:22:P1.14 and B:NT:NST. Among serogroup C meningococci, the most

common were phenotypes C:NT:P1.3,6 and C:2a:P1.5. Ten percent of isolates showed decreased susceptibility to penicillin. In 2008 the first meningococcus resistant to rifampicin was isolated in Poland. All meningococci were susceptible to cefotaxime, chloramphenicol and ciprofloxacin.
Conclusion: Enhanced surveillance along with wider implementation of non-culture techniques led to laboratory confirmation of 90% of all notified IMD cases in Poland in 2008.

P061

SURVEILLANCE OF INVASIVE DISEASE CAUSED BY *HAEMOPHILUS INFLUENZAE* IN THE CZECH REPUBLIC

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Background: We present the results of a ten-year surveillance in the Czech Republic (1999-2008); it evaluates the efficacy of routine *Haemophilus influenzae* type b (Hib) vaccination that started in July 2001. The vaccine schedule consists of four doses.
Methods: The case definition is consistent with the ECDC guidelines. The surveillance also included the investigation of Hib vaccine failure since 2002 and non-b *Haemophilus influenzae* invasive disease since 2007. Serotypes were verified using PCR, biotyping was carried out in all strains.
Results: In the years 1999-2008 invasive Hib disease presented mostly as meningitis, followed by epiglottitis. Case fatality rate due to an invasive Hib disease was 2.8 % in the years 1999-2008. Among Hib strains isolated in invasive disease biotype I prevailed. Following the introduction of routine Hib vaccination in the Czech Republic there was an overall drop in morbidity due to Hib invasive disease. Seven years after the introduction of routine Hib vaccination the morbidity dropped by 100 % in children aged 0 to 1 year. In higher age groups there also was noted a visible decrease in the number of invasive Hib disease. Neither was there an increase in ‘non-b’ haemophilus invasive disease. Hib vaccine failure has been very rare (14 true, 5 possible and 1 apparent vaccine failure).
Conclusions: The results of surveillance indicate a rapid decrease in Hib invasive disease incidence in the target age group following the introduction of routine Hib vaccination in infants in the Czech Republic in July 2001.

P062

EPIDEMIOLOGY OF *HAEMOPHILUS INFLUENZAE* MENINGITIS IN TUNISIAN CHILDREN (2000-2008)

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We conducted a retrospective survey of *H. influenzae* (Hi) meningitis in children before (2000-2002), during (2003-2005) and after (2006-2008) the use of Hib conjugate vaccine in Tunisia. We analyzed all laboratory confirmed *H. influenzae* meningitis between January 2000 and December 2008 in children's hospital of Tunis. Data on demographics and vaccination status were collected. All cerebrospinal fluid (CSF) specimens were studied by conventional methods. Since 2006, we introduced PCR to amplify the *bexA* gene of Hi directly from CSF. The isolates serotype was determined by slide agglutination. Antibiotic susceptibility was determined by disk diffusion method. Beta-lactamase production was analyzed using cefinase test, and the type was determined by PCR using bla_{TEM} and bla_{ROB} primers. Sixty seven cases of Hi meningitis were enrolled, 2 of them were diagnosed only by PCR. Most cases (91%) occurred in children under 3 years of age. Before the introduction of Hib vaccine, 39 cases were isolated, during the period of use; only 9 cases (4 in 2003, 4 in 2004 and 1 in 2005) were identified. After stopping Hib vaccine, 19 cases were notified. The b serotype was the predominant (63 cases), while 3 cases were uncapsulated and 1 case belongs to serotype f. Resistance to ampicillin was almost 30%, all with beta-lactamase production of TEM type. This study confirms previous data in the literature by demonstrating that immunisation by Hi conjugate vaccine has decreased the incidence of Hi meningitis. In fact, after stopping this vaccination, we notified a considerable increase in such disease.

P063

EPIDEMIOLOGY AND SURVEILLANCE OF MENINGOCOCCAL DISEASE IN GERMANY

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Invasive meningococcal disease (IMD) is statutorily notifiable in Germany. Laboratories send isolates or samples to NRZM for confirmation, typing (Serogroup:PorA:FetA) and antibiotic sensitivity testing, permitting identification of clusters by means of scan statistics, international comparison of data, and surveillance of circulating antigenic variants. Vaccination against Serogroup (Sg) C disease was recommended for 1-year old children in July 2006.The incidence of notified IMD (>99% laboratory confirmed) in Germany decreased from 0.9 cases/100.000 inhabitants from 2001-2003 to 0.7 from

2004-2006, and 0.5 from 2007-2008. From 2001-2008, the incidence of Sg C IMD ranged between 0.1 (2008) and 0.3 (2003) cases/100,000 inhabitants and of Sg B IMD between 0.4 (2007) and 0.6 (2001). Since 2006, incidence of Sg C declined disproportionately more (33.5%) than Sg B (13.4%), particularly in 1 to 3 year olds targeted for vaccination (63.5% versus 12.1%). IMD incidence was highest in <2 year olds with a smaller peak in 15-19 year olds. From 2001-2008, case fatality (CF) ranged from 6.9%-9.7% with no clear time trend and was consistently higher for Sg C than Sg B IMD (8.1%, versus 11.7%, $p_X2=0.002$). IMD incidence ranged from 0.3 to 1.0/100.000 in the 16 states in 2008, and was highest in the northeast. Vaccination coverage of 2 year old children was estimated at ~50% in 2007 based on billing data from one state.Dominant lineages of *N. meningitidis* were similar to those seen in other European countries. The most common Sg B lineage 3 clone (B:P1.7-2,4:F1-5) continues to cause a disproportionately high number of cases in western North Rhine-Westphalia, with further spread to other states since 2006. The most common Sg C fine type C:P1.5-2:F.3-3 was found to be clustered in parts of Bavaria in 2005, 2006 and again in the first quarter of 2009. Identified spatiotemperal IMD clusters were small in size (2-10 cases). From 2005-2008, only 1.7% of cases were notified as epidemiologically linked; however, 11% of cases referred to the national reference laboratory were found to be spatiotemporally clustered in sensitive prospective scans.IMD-incidence in Germany has decreased further since 2006, with MenC vaccination explaining only a small part of this decrease. Surveillance is comprehensive, with a high proportion of cases undergoing detailed molecular typing enabling rational responses to changes in IMD epidemiology and evaluation of vaccination recommendations.

P064

INVASIVE DISEASE BY *HAEMOPHILUS INFLUENZAE* IN GERMANY: BACK IN NEW GUISE AND ON THE RISE

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Invasive disease caused by *Haemophilus influenzae* is rare in Germany. In 2008, 152 cases have been notified according to the Infection Protection Act, with the federal state Baden-Wuerttemberg reporting the highest number of cases (37). For 2009, the trend seems to continue, with 51 cases reported in the first quarter of 2009 as opposed to 32 cases reported in the same period 2008. A sizeable proportion of invasive isolates was sampled from elderly patients, shifting the median age of patients with invasive strains to 56. Furthermore, two discrete age-groups could be discerned among patients with invasive disease, which differed in their proportion of strains isolated from blood (45% in the younger vs. 84% in the older age group, p=0.01, Fisher´s Exact Test). In contrast, the proportion of acapsulate strains did not differ significantly (63% vs. 83%, p=0.10, Fisher´s Exact Test), highlighting the age-independent dominance of non-typeable strains. The absence of the capsule

was confirmed by missing amplification of *bexA*, a gene involved in capsule polysaccharide export. The most common serotype among invasive strains was f (8 of 57, 14%), followed by a and b (2 each out of 57, 4%). One serotype b isolate originated from an individual previously vaccinated with Hib conjugate vaccine. The considerable proportion of older adults afflicted by invasive disease and the dominance of acapsulate strains mark changes in the epidemiological profile of *H. influenzae*, once infamous for causing a large share of bacterial meningitides in infants. Similar developments have also been described in other developed countries including the USA and Canada. As a consequence, the public health authority of Baden-Wuerttemberg and the consulting laboratory for H. influenzae have agreed to prospectively follow up all cases in 2009 to a) assess regional incidence, b) timely record disease manifestation, and c) assure that full typing, including multi-locus-sequence-typing (MLST) is done on all invasive strains.

P065

INVASIVE MENINGOCOCCAL DISEASE IN ESTONIA

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Meningococcal infection is a notifiable disease in Estonia since 1965. Data on meningococcal disease in Estonia are based on the mandatory notification (clinical and laboratory). Estonia belonged to the group of European countries with a low meningococcal disease incidence rate varied between 0,4 and 1,0/100 000. Most of cases are sporadic. In Estonia the data on meningococcal disease is based on the mandatory notification. The passive surveillance system consist of mandatory reporting, using ICD-10 coding of cases, to the county division of the health protection service by clinicians and microbiologists. The county division reports to the National Health Protection Inspectorate (HPI). Meningococcal infection has a low incidence in Estonia with the predominance of serogroup B. A significant decline of the morbidity of meningococcal infection has been observed during the last 10 years. In 2008 the morbidity rate was 0,5/100 000 inhabitants. The case fatality rate was about 12% in the last decade. Age distribution of meningococcal infection has changed since 1992: in 1977-1992 the majority of cases (62,2%) were registrated among 0-4 year old children. From the year 1993 the number of persons aged 30 years and over has increased and the number of 0-4 year old children has decreased (38%). 84% of cases (1995-2008) were laboratory confirmed by culture. No antibiotic resistant strains were found. Currently vaccination against meningococcal infection is not included in the national schedule. It is recommended to travellers to endemic countries. Imported cases has increased: 36,4% of cases in 2004 versus 8% in 2005.

P066

INVASIVE MENINGOCOCCAL DISEASE IN FRANCE, 2007-2008

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Background: For more than 20 years in France, incidence rates of invasive Meningococcal disease (IMD) have been varying between 1 and 2 cases per 100,000 inhabitants. We describe the epidemiology of IMD in France in 2007 and 2008.
Methods: In France, epidemiological follow-up of IMD is based on mandatory notification of cases to the French institute for public health surveillance and microbiological characterization of invasive strains at the National Reference Centre for meningococci.
Results: In 2007 and 2008, 721 and 688 IMD cases were notified respectively, corresponding to incidence rates, corrected for under-reporting, equal to 1.3 and 1.2 per 100,000. Compiled 2007-2008 analysis shows that 65 % of the cases were aged 20 years or less. The highest mean incidence rates (per 100,000) were observed in the <1 year old (13.1), the 1-4 years old (4.9) and in the 14-19 years old (2.5). Amongst the IMD cases with known serogroup, 67 % belonged to B, 24 % to C, 3 % to W135 and 4 % to Y. Case fatality ratio was 12 % (18% for serogroup C and 10% for serogroup B). To control a prolonged outbreak in the Normandy region due to the B:14:P1.7,16 strain, ST-32 clonal complex, a vaccination campaign with MenBvacâ started in June 2006.Incidence of C IMD increased in two districts leading to vaccination campaigns with a Men C conjugate vaccine. A new clone C:2a:P1.1,7, ST-11 clonal complex, emerging since 2005, was involved in 1 community and 3 school setting clusters in 2007 and 2008.
Conclusions: IMD-incidence is stable with a predominance of serogroup B disease. The C IMD outbreaks/clusters observed in the past few years led the Health authorities to re-assess the relevance of Men-C routine vaccination through a cost-effectiveness analysis.

P067

EPIDEMIOLOGY OF INVASIVE MENINGOCOCCAL DISEASE IN BELGIUM, 2001-2008

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Introduction: In December 2001, because of the increase in incidence of serogroup C meningococcal disease (clonal complex ST-11), health authorities decided to implement vaccination campaigns at regional level. In Flanders, where a steep rise of serogroup C disease was reported, the campaign covered the 1-18 years cohort while in Wallonia, it targeted the children aged 1-5 years. Since 2002, vaccination against serogroup C meningococcal infection was included in the national calendar, with one dose of conjugate vaccine at the age of 12-15 months.
Methods: Data were collected by the National Reference Centre (NRC) which characterises meningococcal isolates by serological (serogouping, serotyping) and molecular (multilocus sequence typing) methods.
Results: Between 2001 and 2008, the annual incidence rate of invasive meningococcal disease dropped from 3.7/100,000 to 1/100,000 inhabitants.. During this period the number of serogroup C isolates decreased from 179 to 13 and the number of serogroup B isolates from 172 to 92. The emergence of new phenotypes as B:NT:P1.14 and B:21:P1.14 was observed in 2007 while the proportion of the dominant phenotype B:4:P1.4 (complex ST-41/44) among serogroup B meningococci decreased from 44% in 2006 to 27% in 2007 and 2008. Most strains B:NT:P1.14 belonged to complex ST-269 and were isolated in West Flanders. Strains B:21:P1.14 belonged to complex ST-213.
Conclusion: Since 2002, the incidence of meningococcal disease in Belgium is declining. The vaccination against serogroup C infections contributed significantly to this decline. Moreover, since 2004, a natural decrease in incidence of serogroup B infections has been observed.

P068

INVASIVE MENINGOCOCCAL DISEASE IN SWEDEN 2008

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In the year 2008 a total of 49 cases of invasive meningococcal disease were identified in Sweden (9 million inhabitants) via the mandatory combined clinical and laboratory reporting systems (incidence rate 0.54). The diagnosis

was confirmed by culture in 40 patients, and by PCR in 8.
Mortality came to 7 cases (14%), 12 – 96 years of age.Serogroup B was found in 17 cases, C in 18, Y in 10, and W-135 in one. Further characterization with serotype, genosubtype and antibiogram including sequencing of the *penA* gene show a split collection of endemic meningococci without dominating clones.Decreased laboratory sensitivity for penicillin G is a reality in 5% of the isolates if sensitivity is defined as ≤0.096 g/L and 29% if ≤0.064 g/L.The annual report forms a basis for extended discussions around vaccine policy for meningococcal disease in Sweden.

P069

STATUS PRAESENS OF INVASIVE MANINGOCOCCAL DISEASE IN CROATIA - FIRST STEP TOWARDS FUTURE VACCINATION

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Introduction: The incidence of invasive meningococcal disease (IMD) in Croatia is not very high and the meningococcal disease is mainly sporadic, commonly caused by *Neisseria meningitidis* serogroup B. Still the prevention of the IMD with the broad ranged, efficient, well tolerated vaccine against *N. meningitidis* group B is desirable. Invasive disease caused by *N. meningitidis* group B is not caused by single clone, but number of hypervirulent lineages. At the moment there are a lot of vaccine candidates. Croatian invasive isolates were PorA genotyped as a part of European mapping.
Methods: All the patients treated in the University Hospital for Infectious Diseases during 2008 were included. The incidence and prevalence of serogroup were recorded as well as age distribution, methods of detection and genogrouping. PorA genotyping was done for the 34 strains collected during 2007 thanks to S.Grey and E.Kaczmarski, Manchester, UK.
Results: A total of 28 patients with IMD were hospitalized and 21 were children (75 %). The recorded incidence from our data was 0,6 cases per 100 000 inhabitants. All these case were sporadic. Serogroup B was predominant group 24/28 (85,7 %) and was recorded for 19/24 children (79,1 %). The equal proportion of children and adults were infected with N.meningitidis group C. In 12 patients (43 %) N.meningitidis was detected only by PCR. The genotype diversity was seen and twenty geno-subtypes were recorded. The most prevalent geno-subtype was P1.7-2,4,37 (30 %), followed by geno-subtype P1.5-1,10-4,36-2 (25 %). Five geno-subtypes were recorded each two times (10 %) and rest of them only once (5 %).
Conclusions: The *N.meningitidis* serogroup B is highly predominant in Croatia. Introduction of MenB vaccine could be foreseen in the future because IMD is still challenging public health attention. The data of our study represents good resource of information about PorA distribution among N.meningitidis group B invasive isolates in Croatia and could be valuable for

considering multivalent PorA based vaccine or new recombinant vaccine. Summerized content of abstract: The data of PorA characterisation of Croatian invasive isolates could be starting resource for selection of future vaccine candidate at least in Europe.

P070

LESSONS FROM MENINGOCOCCAL CARRIAGE STUDIES IN WEST AFRICA

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Objectives: Conjugate vaccines have the ability to reduce carriage and thus, transmission of the target bacteria in the population. To be able to estimate the impact that meningococcal conjugate vaccines will have in Africa, it is necessary to better understand the pattern of meningococcal carriage in countries of the African meningitis belt.
Methods: About 900 individuals between 2 and 29 years old (300 in Mali, 300 in The Gambia, and 300 in Senegal) were enrolled as part of a clinical trial with MenAfriVac. The volunteers were sampled four times, just before vaccination and at 1, 6, and 12 months after vaccination. Pharyngeal samples were plated directly on selective medium. Growth from the plate was collected and sent frozen to Norway where meningococci were identified by standard procedures. The strains were serogrouped using slide agglutination and further analysed by molecular methods.
Results: Overall meningococcal carriage rates were low (about 10%) and little seasonal variation was observed. The majority of the isolates (202 of 327) were non-serogroupable. Among strains with expressed capsular polysaccharide, serogroup W135 predominated in Mali, while serogroup Y predominated in The Gambia and Senegal. Serogroup A was identified in only 3 individual carriers from Mali, in only one sample each.
Conclusions: Low meningococcal carriage rate and geographic differences in serogroup distribution of the meningococcal isolates are the main findings of our study. In the absence of epidemic, carriage of serogroup A might be much less than 1%. These preliminary results are important to consider when planning larger meningococcal carriage studies in Africa to assess the impact of mass vaccination campaigns with meningococcal conjugate vaccines.

P071

LABORATORY SURVEILLANCE OF INVASIVE MENINGOCOCCAL DISEASES IN ITALY: 2007-2008

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In Italy, data on invasive diseases due to *Neisseria meningitidis* derive from two sources of data: statutory notification since 1991 and since 1994 National Surveillance of Bacterial Meningitis, now Surveillance of Bacterial Invasive Diseases, coordinated by the Istituto Superiore di Sanità. Of 308 cases of meningococcal disease, 255 were culture confirmed by hospital laboratories and 176 strains (69 %) were sent to our Institute for characterization. 55.7% were serogroup B, 36.4% C, 3.3 % W135 and 3.3% Y. Six culture-negative samples were analyzed by Realtime PCR confirming the presence of *N. meningitidis* serogroup B (2 samples) and serogroup C (4 samples). Among serogroup B the most frequent sero/subtypes were: 15:P1.16, 15:P1.4, 1:P1.6, 4:P1.13, nt:nst, nt:P1.4 and among serogroup C the 2aP1.5, 2a P1.5,2, 2bP1.5, 2bP1.5,2. All meningococci were fully susceptible to ceftriaxone, ciprofloxacin and rifampicin; whereas 34.5 % of B and 26.7% of C strains showed decreased susceptibility to penicillin. InMLST all C:2a strains belonged to ST-11 clonal complex and all C:2b to ST-8/A4. Two clusters of C:2a/ST-11 were reported in the North of Italy with a high rate of septicaemia and fatal outcome. Serogroup B strains belong to ST-41/44 and ST-32.Public health surveillance of invasive meningococcal disease and characterization of the isolates will continue to monitor the circulation of the two main serogroups since the introduction of the menC conjugate vaccine in Italy started to reduce circulation of group C leaving the incidence of serogroup B stable. Incidence of other serogroups is unchanged and remains low.

P072

EPIDEMIOLOGY OF INVASIVE PNEUMOCOCCAL DISEASE IN FINLAND IN 1995-2008

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Introduction: Surveillance for invasive pneumococcal disease (IPD) is important for the introduction of pneumococcal conjugate vaccine and the assessment of its effectiveness. National Infectious Disease Registry (NIDR) at the National Institute for Health and Welfare (THL; formerly National Public Health Institute, KTL) has since 1995 collected the *S. pneumoniae* isolates from cases of IPD for serotyping as part of NIDR.

Aims: We report the trends in IPD, and clonality of the isolates in Finland in 1995-2008.
Methods: Notifiable IPD was defined as a clinical condition with *S. pneumoniae* isolated from normally sterile site (blood, CSF). The isolates were serotyped by latex agglutination, counterimmunoelectrophoresis and, when needed, by quellung reaction. PCR methods and MLST were used on selected sets of isolates.
Results: During 1995-2008, the overall IPD incidence grew from 9/100 000 in 1995 to 17/100 000 in 2008. The clinical microbiological laboratories sent 8794 invasive pneumococcal isolates to NIDR. The number of invasive isolates serotyped increased from 351 in 1995 to 925 in 2008, and from 2007 to 2008 alone, by 15 %. In 1995-2001 (N= 3473), the ten most common serotypes were 4 (13%), 14 (12%), 7F (9%), 3 (8%), 23F (7%), 6B (7%), 9V (5%), 19F (5%), 19A (4%) and 22F (4%) and in 2002-2008 (N= 5337), 14 (17%), 4 (11%), 23F(8%), 6B (7%), 3 (7%), 9V (6%), 7F (6%), 19F (5%), 18C (4%) and 19A (4%), respectively. Since 1995, the proportion of serotypes 14 and 9V has increased, and 4 and 7F decreased, while the proportion of others has remained rather constant. Between 1995-2001 and 2002-2008 the 7-valent conjugate vaccine would have covered 49 % and 58 % of the cases by serotype, respectively.
Conclusions: The incidence of IPD has increased in Finland in the past 14 years. The theoretical serotype coverage of the 7-valent conjugate vaccine has also increased during this time although fluctuation in the rank order of the prevalent pneumococcal serotypes was observed.

P073

EPIDEMIOLOGY OF INVASIVE *HAEMOPHILUS INFLUENZAE* (HI) DISEASE IN FINLAND, 1995-2008

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Introduction: Surveillance for invasive *Haemophilus influenzae* type b (Hib) is important for the monitoring of the effectiveness of the Hib vaccination in the national vaccination program. National Infectious Disease Registry (NIDR) at the National Institute for Health and Welfare (THL; formerly National Public Health Institute, KTL) has since 1995 collected invasive *H. influenzae* (Hi) isolates from cases of invasive disease for serotyping as part of NIDR.
Aims: We report the trends in invasive Hi disease in Finland in 1995-2008.
Methods: Notifiable invasive Hi disease was defined as a clinical condition with *H. influenzae* isolated from normally sterile site (blood, CSF). The isolates were serotyped by latex agglutination and counterimmunoelectrophoresis. Presence or absence of capsule and serotypes of all invasive Hi isolates were confirmed by commonly used PCR methods based on the capsular genes.

Results: During the years 1995-2008, the absolute number of invasive Hib cases varied between 1 to 8 per year, and the overall incidence fluctuated between 0,02/100 000 and 0,15/100 000. No upward trend was observed. The clinical microbiological laboratories sent 446 invasive isolates to NIDR. The number of Hi isolates varied from 12 in 1995 to 46 in 2008, with an average of 32. Most of the isolates were noncapsulated (N=331; 74 %). Among the capsulated Hi isolates (N=115; 26 %), type b was the most common (N=62; 54 %) during the period. The second most common serotype was f (N=45; 39 %), followed by e (N=6) and a (N=2). Serotypes c and d were not found. Conventional and PCR serotyping results were fully concordant.
Conclusions: Most invasive Hi isolates were noncapsulated. Except for the first year of surveillance, the number and proportion of Hib cases were low, and the data do not suggest serotype replacement.

P074

LABORATORY SURVEILLANCE OF INVASIVE *HAEMOPHILUS INFLUENZAE* ISOLATES IN DENMARK 2007 AND 2008

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Objectives: To present data on invasive Haemophilus influenzae isolates in Den-mark 2007 and 2008. H. influenzae type b vaccine is part of the Danish child vac-cination program. From October 2007 it became mandatory to send invasive sero-type b isolates to the reference.
Methods: Isolates from blood or cerebrospinal fluids were received from Danish clinical microbiology laboratories and examined for presence of capsule (serol-ogy) and biotyped (phenotypic characterisation).
Results: In 2007 and 2008, 13 and 31 isolates, respectively, were received. This corresponds to an incidence rate of 0.23 and 0.57 pr 100,000 population. Fourteen percent of the isolates were from meningitis cases. Fifty two percent of the iso-lates were from males, 18% from children below 5 years of age and 43% were from persons above 65 years of age. Among all isolates 25% (n = 11) were of capsular serotype f, 20%(9) of serotype b, 7%(3) of serotype e, and 48%(21) were non-capsular. Most isolates were also biotyped, 57%(25) were biotype I, 23%(10) biotype II, 11%(5) biotype III and 5%(2) biotype IV. The most common combination among all isolates was capsu-lar serotype f and biotype I (25%(11)); 23%(10) were non-capsular, biotype II and 16% were capsular serotype b and biotype I.
Discussion: A high number of invasive H. influenzae isolates were received in 2008 compared to 2007. We assume this is because it became mandatory from October 2007. Most isolates were from persons above 65 years of age. The most common type among all isolates was capsular serotype f and biotype I.

P075

EPIDEMIOLOGY OF BACTERIAL MENINGITIS IN CHILDREN-BOSNIAN ASPECT

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Introduction: Bacterial meningitis is a medical emergency and has a high mortality rate if untreated in children. Clinical manifestations are vague and non specific in newborn and infants under one two of age.
Methods: Clinical manifestations in younger children should be ruled out. The most important test in identifying or ruling out meningitis is analysis of the cerebrospinal fluid through lumbar puncture.
Results: Approximately one fourth of infants with septicemia develop meningitis. The incidence of neonatal meningitis varies greatly among different institutions and geographic areas with approxiamate rates of 2 to 10 cases per 10,000 live births. The incidence rate of residual abnormalities in postmeningitic children is about 15%.
Discussion: The prognosis in individual infants and newborns patients with meningitis predicted on many factors including age of kid, duration and type disease before effective antibiotic therapy is instituted, type of causative agents, intesity of he host's inflammatory response and time needed to sterilise CSF culture.
Conclusion: Bacterial meningitis is a potentially fatal acute infectious disease. Possible interventions to reduce brain injury associated with infection might include earlier diagnosis and improved therapies, including efforts to stabilize blood pressure and maintain adequate oxygenation and pharmacologic interventions.

P076

MENINGOCOCCAL MENINGITIS SURVEILLANCE IN BURKINA FASO, 2006-2009

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Objective: To describe epidemiological and microbiological characteristics of meningococcal meningitis in Burkina Faso during 2006-2009.

Methods: A programme of bacterial meningitis surveillance was conducted in western Burkina Faso, adding multiplex PCR to bacteriology. This included an exhaustive surveillance study in the urban and rural area around Bobo-Dioulasso, epidemic investigations using a mobile laboratory and regional surveillance with PCR in four sanitary regions.
Results: About 900 suspected meningitis cases were evaluated during 2006-2008, of which one third were confirmed for meningococcus. Outside localised epidemics, the annual incidence rate of confirmed meningococcal meningitis (average 17/100.000) peaked at 30/100.000 in children aged <6 months and 5-19 years. During a localised epidemic in 2006, the cumulative incidence of confirmed cases (suspected cases) was 0.3% (3.5%) and peaked at 0.6% in children aged 1-19 years (10% in children aged 1-4 years). Most cases were due to serogroup A (NmA), with sporadic identification of NmW135, NmX and nongroupable Nm. Sequence types of NmA isolates were almost exclusively ST-2859 (clonal complex 5), but three cases of ST-6968 (single-locus variant of ST-2859) were found. During 14 outbreak investigations in 2007-2008, NmA could be confirmed as the causal agent and A/C vaccination campaigns were conducted in nine of the according districts. These results will be updated with data of the 2009 season.
Conclusion: The results show the value of a combination of various designs and techniques for surveillance of this complex disease. This experience will be useful for monitoring the upcoming introduction of serogroup A conjugate vaccine.

P077

INVASIVE DISEASES CAUSED BY *NEISSERIA MENINGITIDIS* AND *HAEMOPHILUS INFLUENZAE* IN SLOVENIA

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Aims: The epidemiologic situation of invasive diseases in Slovenia has been constantly monitored via laboratory and epidemiological surveillance (compulsory notification). The aim of the study was to investigate the epidemiological pattern of invasive diseases in order to formulate optimal vaccination strategy.
Methods: Via laboratory surveillance, from the year 1993 to 2008, these two bacteria and *Streptococcus pneumoniae* were nationally collected. All isolates were typed and antibiotic resistance was determinated. We collected 183 meningococcal and 293 haemophilus invasive isolates.
Results: The incidence in the year 2008 of invasive diseases showed the highest proportion in *S. pneumoniae* strains (19,6/100.000 in children and 7,9/100.000 in adults), followed by *N. meningitidis* (3,2/100.000 in children and 0,63/100.000 in adults), and *H. influenzae* (1,8/100.000 in children and 0,4/100.000 in adults), that coincide with introduction of universal compulsory *H.influenzae* b vaccination in the year 1999. The incidence before the

vaccine introduction was 197.9/100.000 (children 0 – 1 year of age) whereas in the period after was 38.2/100.000. From the year 2002 we have no longer noticed *H. influenzae* serotype b in children.
The collected meningococcal isolates were very heterogeneous. The most common was serogroup B (131 strains), followed by serogroup C (30 strains) which is increasing from the year 2003. The most affected age group were children from 0–1 year (60 strains) and children from 2-4 years (24 strains) with another peak in children from 15-19 years (31 strains). The epidemiologic situation is still endemic, nevertheless, all the main hypervirulent meningococcal clones are present.
Conclusions: The introduction of universal compulsory *H. influenzae* b vaccination showed drastical decline of invasive *H. influenzae* disease in children under 5 years of age. The rising incidence of invasive serogroup C menigococcal strains demands constant monitoring regarding the introduction of conjugate meningococcal C vaccine.

P078

IMMUNOLOGICAL EVALUATION AND SEQUENCE CONSTANCY OF TWO NEW MENINGOCOCCAL VACCINE CANDIDATE PROTEINS

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Neisseria meningitidis serogroup B is the major cause of invasive meningococcal disease in many countries. Recent advances in genomic technology and the availability of genome sequences provide the opportunity to examine protective antigens, which might form the basis of a universal vaccine against this pathogen. In our laboratory, two novel meningococcal antigens, NMA0939 and NMB0938, were identified by mining meningococcal genomic sequence databases and we evaluated these proteins as potential vaccine candidates. By combining PCR and nucleotide sequencing, we investigated the conservation of the deduced amino acid sequence of these proteins in different meningococcal strains, including strains isolated from healthy carriers. After immunisation with the recombinant variant of the proteins NMA0939 and NMB0938, the immune response was evaluated by whole cell ELISA and Fluorescence-activated cell sorter (FACS)-related assays. Functional activity of antibodies was evaluated by serum bactericidal activity and infant rat protection assays. The genes were present in 100% of the strains evaluated. Animals developed cross-reactive IgG antibodies in their sera, as determined by ELISA and Western blotting using whole cells of homologous and heterologous strains. FACS analysis showed binding of mouse polyclonal sera to live *N. meningitidis* from the CU385 strain, suggesting that these proteins are exposed on the surface of the cells. Besides, the immunization induced a functional response characterized by bactericidal antibodies and protective activity against meningococcal

bacteremia in the infant rat model. Taking into account these findings, NMA0939 and NMB0938 are promising vaccine candidates to be included in a future vaccine against meningococcal disease.

P079

DEVELOPMENT AND VALIDATION OF A MULTIPLEX ASSAY TO EVALUATE RESPONSES TO MENINGOCOCCAL ANTIGENS FOLLOWING IMMUNISATION WITH GROUP B MENINGOCOCCAL VACCINES.

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To achieve broad protection against diverse meningococcal isolates, many developmental vaccines consist of a combination of proteins antigens found on the cell surface, or in the outer membrane vesicle (OMV). A multiplex assay, based on X-map technology, was developed to evaluate antibody responses to meningococcal protein antigens in vaccinees and the general population. Eight PorA variants, including a VR1 and VR2 deleted variant, were cloned and expressed as His-tagged proteins in *E.coli* cells. The purified, refolded proteins were used to coat sets of Ni-NTA (nitrilotriacetic acid) conjugated microspheres, each bearing a unique fluorescent label. Antibody responses were measured using Liquichip analyser (Qiagen). Within the analyzer, lasers excite the internal dyes that identify each microsphere particle, as well as the reporter dye attached to the anti-immunoglobulin conjugate bound during the assay, allowing the amount of antibody bound by each individual microsphere set to be determined. Assay specificity was evaluated using mouse monoclonal antibodies and adsorbance assays using unbound proteins to block antibody responses. Inter- and intra-assay variation was assessed using pooled post immunisation human serum. These validation experiments showed that this approach provided specific and reproducible data. This assay is to be extended to include other antigens, such as PorB, FetA and factor H Binding Protein (fHBP) variants, and will be used to measure exposure to these antigens in 15-19 year olds. Antibody responses in vaccine trial sera will also be evaluated using this method.

P080

DEVELOPMENT OF A HIGH THROUGHPUT MENINGOCOCCAL BACTERICIDAL ASSAY

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Complement-mediated bactericidal activity has long been regarded the serological correlate of protective immunity against *Neisseria meningitidis*. This was affirmed in 2005 at a WHO sponsored meningococcal serology standardization workshop. The method currently employed by most laboratories involves determining surviving bacterial colony counts as a readout which is labor-intensive, time-consuming, and not amendable to rapid data analysis from clinical trials. Consequently there is an acute need to develop a sensitive, high throughput bactericidal assay to enable a rapid assessment of the effectiveness of vaccine candidates. To this end, we are developing an assay based on a fluorescent readout. This approach may shorten assay time and support the development of a higher throughput system. In a pilot study, 8 post immunization serum samples from human subjects vaccinated with MenB recombinant protein +/- OMV vaccines were assayed using the standard agar based counting method and fluorescence as a readout. Normal human serum lacking intrinsic bactericidal activity was used as an exogenous complement source. The geomean titers obtained against strain 5/99 were 191 by the standard method, and 173 when fluorescence is measured. Of six preimmune samples tested, five had titers <4 in both assays, and one sample had titers of <4 and 11 with the standard and fluorescence methods, respectively. These results suggest that use of fluorescence as an alternative readout is a promising approach for the development of a high throughput bactericidal assay.

P081

IMMUNOGENICITY OF A RECOMBINANT MENINGOCOCCAL VACCINE WITH AND WITHOUT OUTER MEMBRANE VESICLES, AGAINST A GROUP B STRAIN REPRESENTATIVE OF THE NORMANDY OUTBREAK.

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Background: The Normandy region of France is experiencing an ongoing outbreak of *Neisseria meningitidis* group B disease, caused by a ST32, B:14:P1.7,16 strain. An investigational recombinant meningococcal vaccine

(rMenB) containing the NadA protein and fusion proteins of 2132-1030 and 2091-factor H binding protein (fHBP) has been developed. The vaccine has been trialled with and without outer membrane vesicles (OMV) from the New Zealand outbreak strain NZ98/254. We therefore investigated the immunogenicity of these vaccines against a strain representative of the Normandy outbreak.
Methods: The fHBP of 4 outbreak strains was examined both genetically and for phenotypic expression, with one strain (LNP 20404) chosen for analysis in the standardised serum bactericidal antibody (SBA) assay with human complement (hSBA). Serum samples from healthy infants, vaccinated during a phase II study with either rMenB or rMenB+OMV in a 2, 4 and 6 month schedule were utilised.
Results/Discussion: The outbreak strains were all shown to harbour a genetically identical fHBP expressed in similar quantities, and strain LNP 20404 was demonstrated to be representative. The fHBP variant expressed by the strains was identical to that contained within the vaccine. The proportion of subjects attaining $\geq$ 4 fold rises in SBA from pre- to post-3rd dose were 5/8 (63%) and 12/12 (100%) and the proportion of subjects with SBA titres $\geq$ 4 post-3rd dose were 4/8 (50%) and 11/12 (92%) for those receiving rMenB and rMenB+OMV, respectively.
Conclusions: The rMenB+OMV vaccine was demonstrated to elicit protective hSBA against the current Normandy outbreak strain.

P082

SEROPREVALENCE OF ANTIBODIES AGAINST FHBP AND NADA

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The antibody (IgG) levels directed against fHbp and NadA, two meningococcal vaccine antigen candidates, have been examined in sera from 1036 persons in order to investigate the extent of natural immunisation against these antigens in different agegroups of a Swedish population. As a comparison the seroprevalences of anti-*Haemophilus influenzae* type b (anti-Hib) IgG were measured. The results show that anti-fHbp and anti-NadA both had rather low levels in the two youngest agegroups, below 10 years of age. Anti-fHbp then had a 9-fold increase in the agegroups up to 29 yoa, followed by a slow decrease with age. Anti-NadA showed a much lower and even increase up to 49 years. The anti-Hib levels were as expected high (and protective) for the agegroups vaccinated (start 1992/93). Older groups, not routinely vaccinated, had slowly falling but protective levels by age. Our results indicate that an immune response with anti-fHbp IgG is mainly taking place during the ages 10 to 29 years, a period that include the ages with high incidences of meningococcal group B and C disease and other droplet/saliva born infections like primary EBV infection (mononucleosis). Theoretically this immune response can be accomplished by meningococci, other Neisserial

species or any other cross-reacting microorganisms. The site(s) for this is purely speculative but could be the upper respiratory tract/throat and the gastrointestinal canal.

P083

COMPARISON OF COMPLEMENT SOURCES IN THE MENINGOCOCCAL SEROGROUP B SERUM BACTERICIDAL ANTIBODY ASSAY

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Introduction: The surrogate of protection against *Neisseria meningitidis* serogroup B (MenB) is the serum bactericidal antibody (SBA) assay. Pioneering studies utilised an exogenous human complement source, but problems in procuring human complement of sufficient quality and quantity have resulted in rabbit complement being used in serogroup A, C, Y and W135 assays. However, it is not feasible to use rabbit complement in the MenB SBA assay due to elevated titres caused by low avidity anti-MenB capsular antibody in test sera. Complement from other small species has also been unsuccessfully investigated. We therefore investigated the use of Bovine and Porcine complement in the MenB SBA assay.
Methods: Pre- and post-vaccination sera (n=150) from an adult study of the New Zealand OMV vaccine MeNZB^{TM} were assayed in the SBA assay against NZ 98/254 (B:4:P1.7-2,4) with human complement, three lots of bovine complement (b1, b2, b3), porcine complement (p) and rabbit complement.
Results: Preliminary correlation coefficients of 0.78, 0.72, 0.77, 0.55 and 0.47 were achieved between human complement and b1, b2, b3, porcine and rabbit complement, respectively. Compared to human complement results, mean reductions in SBA titre step of 1.0, 2.0, 1.4 and 3.5 were achieved with b1, b2, b3 and porcine complement, respectively. Conversely, rabbit complement resulted in a mean 3.3 increase in SBA titre step, as compared to human complement results.
Conclusions: Bovine and porcine complement gave stronger correlations between SBA titres gained with human complement than that achieved between rabbit and human complement. Further investigations of these complement sources are warranted.

P084

MEASURING ANTIGEN SPECIFIC BACTERICIDAL RESPONSES TO A MULTICOMPONENT VACCINE AGAINST SEROGROUP B MENINGOCOCCUS

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A multicomponent recombinant protein vaccine against meningococcus B combined with OMV was developed and tested in the ongoing clinical trials. Serum bactericidal antibodies detected with human complement are widely accepted as a surrogate marker of resistance to meningococcal meningitis and this vaccine elicits a bactericidal response with broad crossreactivity in both laboratory animals and human. The aim of the present work is to assess vaccine effectiveness testing the immune response to each major antigenic components: fHBP, NadA, GNA 2132 and PorA P1.4 in the OMV.To measure the bactericidal antibodies specifically directed against these antigens we screened for MenB strains matching the vaccine for individual components included in it, then we performed competitive bactericidal assays on serum from human vaccinees, using the soluble recombinant vaccine antigens, to determine whether killing of a particular strain in the SBA was inhibited by a specific antigen or combination of antigens. We identified a panel of MenB strains that when used as targets in the SBA each demonstrate that one of the major components is able to evoke a protective bactericidal response independently, and that recognition of any one of the components is sufficient to provide a bactericidal response. We found that most adult human subjects made bactericidal antibodies against each of the major components. The results of a typing assay that detects the presence of the vaccine components on different MenB strains, can be linked to killing of the strains in the SBA, and used for demonstration of the effectiveness of the vaccine.

P085

CORRELATION OF HIGH THROUGHPUT FLOW CYTOMETRY OPSONOPHAGOCYTOSIS AND ANTIBODY-MEDIATED MEMBRANE ATTACK COMPLEX ASSAYS WITH KILLING OPSONOPHAGOCYTOSIS AND BACTERICIDAL ANTIBODY ASSAYS

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Serum bactericidal activity has been established as a correlate of protection for polysaccharide-based meningococcal vaccines. However, for protein-

based vaccines the correlates of protection are less clear. We have developed high throughput opsonophagocytosis and antibody-mediated complement deposition assays, although correlation with protection is currently undetermined. This study has aimed to correlate the responses from these qualified high throughput assays with more labour-intensive functional assays to ascertain their usefulness in clinical trial sera assessment.
A high-throughput flow cytometry-based opsonophagocytosis assay (OPA) performed using BCECF-labelled fixed *N. meningitidis*, IgG-depleted human plasma and a DMF-differentiated HL60 granulocytic cell line was compared with an opsonic killing assay adapted from that reported by Plested and Granoff (Clin Vacc Immunol 2008). Also, a high throughput antibody-mediated complement deposition assay using fixed meningococci, IgG-depleted human plasma, and fluorescent anti C3c and C5-C9 antibodies was correlated with a standard serum bactericidal assay. The assays were performed using a panel of human vaccinee sera and Pearson correlation coefficients determined. Good correlations (95% confidence) between deposition of C5b-9 and bactericidal titres were determined and the high throughput opsonic assay correlated well (95% confidence) with the opsonic killing assay. We have also demonstrated strong correlation with opsonic responses and C3c deposition. These results show that these high-throughput assays performed on azide-fixed targets are useful in a large scale clinical serology testing.

P086

GENERATING CONSISTENT LOTS OF HUMAN SERUM COMPLEMENT FOR USE IN *NEISSERIA MENINGITIDIS* SEROGROUP B (MNB) VACCINE CLINICAL TRIALS

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Background: Forty to seventy percent of invasive meningococcal disease (IMD) in the US and Europe is caused by MnB The bivalent *Neisseria meningitidis* serogroup B (MnB) vaccine currently under development by Wyeth (rLP2086) contains two factor H binding proteins (fHBPs) covering all fHBP sequence variants. The serum bactericidal assay (SBA), an accepted surrogate of protection, will be used to assess vaccine efficacy since cases of IMD are rare. The SBA measures complement-mediated bacterial killing in the presence of immune serum. As there are no standardized MnB SBAs, assays in support of vaccine licensure must be carefully controlled and validated. The human complement is a key component of the SBA that can affect titers and, consequently, the assignment of responder status in meningococcal vaccine trials. A method has been developed to generate consistent complement lots for use in MnB fHBP vaccine trials.
Methods: Human immune sera from rLP2086 vaccinees were used in an SBA to screen at least thirty individual human serum complements. A statistical algorithm was used to identify complements yielding “average” titers. Selected individual complements yielding “average” titers were pooled.

The pooled complement lots were tested for performance using a proficiency panel of sera from human vaccinees.
Results: Pooled complement lots were generated and their performance in the SBA compared. The 90% CI of the mean bias were contained completely within 0.67 – 1.5.
Conclusion: Pooling of statistically-identified individual human serum complements yields lots that perform consistently and reproducibly facilitating reliable within- and between-clinical study comparisons.

P087

VARIABILITY OF GENES ENCODING FIVE VACCINE CANDIDATES AMONG NORWEGIAN MENINGOCOCCAL SEROGROUP B STRAINS FROM AN EPIDEMIC (1985-90) AND A LOW-ENDEMIC PERIOD (2005-06)

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Introduction: A key step in evaluating protein-based vaccines providing broad coverage against serogroup B meningococcus is the study of amino acid sequence variability of the vaccine candidates in various strains in order to estimate the degree of vaccine coverage.
Methods: Two strain panels were selected from a Norwegian collection. One panel (27 strains) was from an epidemic period (1985-90) and 66 strains from a low-endemic situation (2005-06). We have sequenced the genes of five recombinant antigens (NadA; GNA1030; GNA2091; fHbp; GNA2132) included in a MenB vaccine currently in phase III studies.
Results: The strains from the epidemic period were all ST-32, had fHbp protein variant 1 and GNA2132 variant 2. All but two strains harboured NadA-1. The strains from the low-endemic period were heterogeneous on the basis of both MLST [14 different clonal complexes, with the ST-32/ET-5 (24%) and ST-41/44/lin3 (30%) predominating] and genetic variation in the vaccine antigens. All main fHbp variants were found with a prevalence of the variant 1 (54%); two variants of GNA2132 were predominant: 2.1 (17%) and 1.2 (21%); and of the 28% NadA positive strains from the endemic period, NadA-1 was still prevalent.
Conclusion: Evaluation of the current and upcoming protein-based MenB vaccines requires extensive access to representative strain collections from various parts of the world and different epidemiological situations. While the Norwegian strains causing disease during the epidemic period were also homogeneous regarding the vaccine antigen assortment, the strains from the endemic period showed the presence of a more complex antigen repertoire.

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CONTROL OF NEW ZEALAND'S EPIDEMIC OF MENINGOCOCCAL DISEASE USING MENZB™ VACCINE

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Introduction: From 1991 New Zealand experienced an increasing number of meningococcal disease cases caused by a strain, B:4:P1.7-2,4, not previously identified in New Zealand. The highest rate of disease (17.4/100,000) occurred in 2001 when 75.7% (370/489) of cases were caused by this strain. A vaccine, MeNZB was developed by Chiron (Novartis) specifically for the purposes of epidemic control.
Methods: In 2001, a contract was signed with Chiron (Novartis) Vaccines to design a strain-specific vaccine for epidemic control. Following age-group trials demonstrating safety and immunogenicity, MeNZB was delivered to individuals aged <20 years from July 2004 through 2006.
Results: Based on the immunisation register close to 1.02 million individuals received three or more doses. Vaccine coverage for children aged <5 years was 84.8%, for 5-17 year-olds 86.8%, and for 18-19 yr olds 54%. The number of confirmed epidemic strain cases in all ages fell from184 in 2004 to 44 in 2008 ($p<0.001$). For those under 20 years of age epidemic strain numbers decreased from 129 in 2004 to 31 in 2008 giving a rate of 1.1/100,000, in contrast to the rate of 9.9/100,000 in 2001 ($p<0.001$).
Conclusions: The impact of MeNZB on epidemic strain case numbers is evident with greater decreases occurring in 2004 -2005 (39%) than the period 2001-2004. In 2008, case numbers totalled 123, but only 43.6% (44/101) for which a type was determined, were the epidemic strain. Although the epidemic strain continues to dominate disease rates, particularly in infants, it is considered unlikely that the epidemic would have undergone such rapid decline without the assistance of MeNZB vaccine.

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