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ORAL PRESENTATIONS O 001 – O 015

O 01

THE EPIDEMIOLOGY OF INVASIVE MENINGOCOCCAL DISEASE IN EUROPE, 2008 AND 2009

Ida Czumbel

The objective of the analysis was to describe the epidemiology and surveillance of invasive meningococcal disease (IMD) in Europe in 2008 and 2009.

Designated national experts from Member States (MS) reported data into The European Surveillance System, following EU-wide reporting standards. Out of the 29 countries, 28 submitted case base data, all have a comprehensive and passive reporting system. The application of case definitions differed between countries, with the majority applying 2008 EU case definition.

In 2008 and in 2009 a total number of 9615 cases of IMD were reported with an overall notification rate of 0.99/100,000 in 2008 and 0.92 in 2009/100,000. The highest notification rates were reported by Ireland (3.68/100,000 in 2008 and 3.37/100,000 in 2009) and the United Kingdom (2.29/100000 in 2008 and 2.02/100000 in 2009). The highest rates were notified in infants younger than 1year (18.3/100,000 in 2008 and 15.9/100,000 in 2009). Serogroup B formed the largest proportion of cases (71%) followed by serogroup C (13%). In countries with MenC vaccination (MCC) the incidences in 2009 were lower in age groups targeted by vaccination (<1year: 0.54/100000; 1-4 year: 0, 22/100000), compared with countries without MCC vaccination (<1year: 1.01/100000; 1-4 year: 0.45/100000).

The case fatality was 8.5% (422 deaths) in 2008 and 7.4% in 2009 (340 deaths). , Multilocus sequence showed that the bacterial population was highly diverse (11% data completeness) with 26.1% of isolates (n=256) belonging to CC ST-41 complex.

As the wide range of data completeness and various case definitions applied the results should be carefully interpreted.

O 02

INTRODUCTION TO THE SESSION "OBLIGATIONS FOR MC AND HI REFERENCE LABORATORIES", EMGM 2011

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Each European country's microbiological Reference laboratories have different capacities.
In order to look at the strengths and weaknesses, information from the questionnaire collected earlier this year will be interpreted.

In addition, reports will be given from two countries with different situations, one with a fairly small population (Finland) and one with a larger one (France). The ECDC perspective and requests have been explored and reported (Core functions of microbiology reference laboratories for communicable diseases. Technical report, June 2010)

As an introduction, the following are some qualities that should be found in a good reference laboratory:

1) Open for questions without delays
2) Providing help with problem samples without delays
3) Provide international and national ref. material (sera, strains, other things)
4) Providing officially accredited services?, including participation in relevant QA schemes
5) Actively teaching in the fields of the ref. lab.

6) Active research and development within the field with publications in international and national journals
7) Run some part of clinical routine service; such as a normal diagnostic lab. Thus making the ref. lab. an active part of basic services of the routine labs. Helping the ref. lab. to be an active part of ”the gang”
8) Having a good contact network
9) Participate and be active in the major international conferences within the ref. fields
10) Have member/s in national and international professional groups within the ref. field
11) Provide reports to the receiver including annual report for the country

O 03

DUTIES AND CHALLENGES OF A NATIONAL REFERENCE LABORATORY – HOW TO COPE IN A SMALL COUNTRY LIKE FINLAND

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Finland is a small country, not so in size but by population: 5,38 million people living in an area of about 338 000 square kilometers, i.e. roughly the size of Germany. Approximately one third of the population lives in the southernmost parts of the country around Helsinki, the capital.

For health services, the country is divided into five university and 20 regional hospital districts. Each of these is served by a hospital, which contains a clinical microbiology laboratory. In addition to these central public laboratories, few large private ones doing clinical microbiology also exist. All these laboratories are obliged by law to notify major microbiological findings into the National Infectious Diseases Register held at the National Institute for Health and Welfare, THL, and to send samples or isolates of some 25 microbial species to the reference laboratories at the THL.

In the Bacteriolgy Unit at the THL we have three of these reference laboratories. Our Anaerobic and Respiratory Bacteria Laboratory deals with anarobes like *Clostridium difficile*, and respiratory pathogens including *Legionella* sp., *Haemophilus* sp., meningococci and pneumococci. Our task is to confirm the identification of these bacterial species and to type the isolates for clinical, epidemiological and research purposes. We do our best to serve the clinical diagnostics, prevention of transmission in health care facilities, outbreak investigation, epidemiological surveillance, vaccine efficacy monitoring, and scientific research. In 2010, we analyzed 228 isolates of *C. difficile*, 1248 isolates of *Streptococcus pneumoniae*, of which a vast majority were invasive ones, 145

isolates of *Neisseria meningitidis*, 53 of *Haemophilus influenzae*, and 4 isolates of *Legionella* sp using conventional bacteriological methodologies along with many DNA based techniques.

Our personnel is funded partly by the institute, partly by external grants. The same people need to share their time and interest with many different tasks. During the last years, our laboratory has faced many administrative challenges arising from ongoing organizational changes and diminishing resources as well as new demands in relation to epidemiology or prevention of diseases caused by the bacteria we are dealing with. As examples, the emergence of the highly virulent strain of *C. difficile* PCR ribotype 027 in 2007, and the introduction of pneumococcal conjugate vaccine into the national childhood vaccination program in September 2010 have required prompt set up of new typing techniques. In addition, the constantly increasing ECDC surveillance and reporting requests have brought us other new challenges, which are important but nevertheless, laborious.

In order to cope with our reference laboratory duties, we welcome the support from the ECDC and IBD labnet. First, improved communication between the ECDC/IBD labnet and the corresponding epidemiologists and vaccinologists is needed, both within the member states and ECDC. For instance, their participation at the IBD labnet meetings might be beneficial. Second, every new task for the lab, epi and vaccine people needs to be considered carefully – can the information collected to TESSy really be analyzed and used for action at the national and the EU level? Finally, to ensure our competence also in the future, the essential work done in the national reference laboratories is of utmost importance to remember in the meetings of the National Microbiology Focal Points, the Advisory Forum and the Management Board of ECDC, at all levels of the decision making.

O 04

NATIONAL REFERENCE LABORATORIES – ECDC PERSPECTIVES

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Recognizing the need for close collaboration in microbiology, the European Centre for Disease Prevention and Control (ECDC) asked the EU Member States (MS) to appoint a National Microbiology Focal Point (NMFP) in each country. The first NMFP meeting was held in Stockholm in November 2007. The following issues were discussed at eight biannual meetings organized to date: strategy of collaboration between Public Health Microbiology (PHM) in MS and ECDC, selection and rating of the National Reference Laboratories (NRLs), EQA and accreditation of PHM laboratories, core function of the microbiology NRLs, training in PHM by ECDC (EUPHEM), coordination of PHM laboratories in crisis situations, biosafety, biosecurity, EU strain collections, and molecular typing. To rate NRLs in MS, two large surveys were conducted. The first one based on a 7-chapter questionnaire was organized by ECDC in 2008. The results are available in two ECDC Technical Reports („Core functions of microbiology reference laboratories for communicable diseases“ and „Fostering collaboration in public health microbiology in the European Union“), at http://www.ecdc.europa.eu/en/publications/technical_reports/Pages/index.aspx. The second survey „European System of Reference Laboratories for Pathogens for Humans - EURLOP“conducted by the Health Protection Agency is now under final evaluation. It is based on three questionnaires on

microbiology, finance and legal issues. EURLOP representatives visited most MS to discuss the NRLs and PHM issues on site. The EURLOP Options Workshop was held in March 2011. In conclusion, the microbiology NRLs play a crucial role in MS. ECDC promotes their quality and coordination to achieve safety from infectious diseases.

O 05

WHO NEEDS WHAT AND WHEN CONCERNING MENINGOCOCCAL CHARACTERISTICS? THE LOCAL MEDICAL OFFICER RESPONSIBLE FOR COMMUNICABLE DISEASES.

Hans Fredlund

The local medical officer requires correct and rapid diagnosis of meningococcal disease. It is advisable early in the course to have a discussion with the physician managing the patient to decide who should be offered antibiotic prophylaxis. Still it is not very urgent with an antibiogram since empiric treatment and prophylaxis rarely fail, but this situation can change rapidly. These primary laboratory investigations are the tasks for the local laboratory.

Sero/genogrouping is important when considering protective precautions such as vaccination for persons at increased risk for the disease. This analysis is the task for the local or reference laboratory depending on the laboratory-organisation in each country.

A more detailed characterization of meningococcal isolates is required to understand if cases are suspected to be linked are caused by “the same” meningococcus or “different” meningococcus as a random coincidence. These investigations are tasks for the reference laboratory. Requirements for a preliminary statement on the identity of isolates depend on the strength of epidemiological links. The assays used in individual situations depend partly on how fast data have to be available and on the experience of the individual laboratory.

It is not always easy to define the population at risk for intervention. The family in which the meningococcal case is diagnosed should be offered antibiotic prophylaxis and fairly often prophylaxis is given to the children at the same day-care. Today, we have vaccine against sero/genogroup A, C, Y, W-

135 on the market but a general vaccine against sero/genogroup B is not yet available. If vaccination is considered, a manageable population at risk must be identified before starting.

O 06

EPIDEMIOLOGY AND SURVEILLANCE OF MENINGOCOCCAL DISEASE IN GERMANY

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Invasive meningococcal disease (IMD) is notifiable in Germany. Most cases undergo detailed molecular typing permitting surveillance of circulating strains and identification of clusters by means of scan statistics. Routine vaccination against Serogroup (Sg) C IMD of 1-year old (y.o.) children was recommended in 2006, with individual catch-up recommended for older children. Vaccination coverage in 2009 in ≤18 y.o. was 50.5%, highest among 2-5 y.o. (78%)[1].

IMD incidence (>99% laboratory confirmed) in Germany decreased from 0.93 cases/100.000 inhabitants in 2001-2003 to 0.72 in 2004-2006, 0.56 in 2007-2009 and 0.47 in 2010. The peak in incidence usually seen in the first quarter was absent in 2010. IMD incidence was highest in <2 y.o. with a smaller peak in 15-19 y.o. Case fatality ranged from 6.9%-9.7% in 2001-2010, higher for SgC than SgB IMD (11.6% versus 7.9%, $p_X2=0.0007$).

Disproportionately greater decreases in SgC than SgB incidence were observed in 1-5, 6-14 and 15-19 y.o. since 2006, but not in <1 or >19 y.o.

Dominant *N. meningitidis* lineages were similar to those in other European countries. The most common SgB lineage 3 clone (B:P1.7-2,4:F1-5) continued to cause a disproportionately high number of cases in western North Rhine-Westphalia in 2009-2010. The most common SgC fine

type C:P1.5-2:F.3-3, clustered in parts of Bavaria in 2005, 2006 and 2009, decreased markedly in most regions in 2010.

IMD-incidence in Germany reached a record low in 2010, with MenC vaccination explaining only a small part of this decrease. There is no evidence for herd immunity in unvaccinated groups. The low incidence in early 2010 may have been related to the earlier than usual occurrence of the pandemic influenza wave in the fall of 2009.

References

1. GfKHealth. Representative Survey on Meningococcal C vaccination in Germany . GfK Health, 2010.

O 07

MONITORING THE BREADTH OF COVERAGE OF MENINGOCCOCAL VACCINES: AN OVERVIEW AND PROGRESS UPDATE ON PFIZER'S BIVALENT LIPIDATED RLP2086 VACCINE PROGRAM

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Background and aims: Pfizer is developing a bivalent factor H binding protein (fHBP/LP2086) vaccine to prevent *Neisseria meningitidis* serogroup B (MnB) disease. fHBP, an outer membrane lipoprotein, protects MnB from complement attack. >2,500 MnB isolates studied have the *fhbp* gene, and sequences segregate into two immunologically distinct subfamilies, A and B. Preclinical studies identified the importance of including one lipidated protein from each subfamily into the vaccine to achieve broad disease coverage. Vaccine coverage in the context of fHBP sequence diversity is an important consideration for licensure, and post-licensure surveillance with appropriate surveillance mechanisms.

Materials and methods: fHBP sequences, expression levels, patient age and epidemiological markers were analyzed for MnB invasive isolates. Breadth of vaccine coverage was evaluated by hSBA using bivalent, rLP2086 immune sera from phase 2 studies conducted in adolescents ages 11 – 18 and phase 1 studies conducted in young adults...

Results: The bivalent, rLP2086 vaccine candidate induced robust hSBA responses against diverse MnB strains. Clinical data from phase I/II will be reviewed in the context of epidemiological studies. Potential plans to monitor disease post-vaccine introduction also will be reviewed.

Conclusions: The bivalent, rLP2086 investigational vaccine confers broad protection against diverse MnB invasive strains. Efficacy will be determined and monitored using hSBA

responses (surrogate for protection) with invasive MnB strain expressing heterologous (compared to vaccine) fHBP sequences.

O 08

ESTIMATING THE POTENTIAL STRAIN COVERAGE IN EUROPE OF A MULTICOMPONENT VACCINE TARGETING SEROGROUP B MENINGOCOCCI

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Background: A multicomponent vaccine (4CMenB) is proposed for prevention of invasive serogroup B (MenB) meningococcal disease. Because MenB clinical isolates are diverse, it is necessary to assess the potential public health impact of 4CMenB. We defined a target population of MenB strains in Europe to estimate strain coverage by 4CMenB.

Methods: To evaluate strain coverage by 4CMenB we used the Meningococcal Antigen Typing System (MATS), which predicts the potential for bactericidal activity of sera from immunized 13-month-olds, based on quantity and crossreactivity with the vaccine-induced immune response of three antigens (factor H binding protein, Neisserial Heparin Binding Antigen, and Neisserial Adhesin A), and the genotype of a fourth antigen, PorA. As a recent and representative target strain population, we evaluated invasive MenB strains isolated mainly in a single epidemiologic year (July 2007-June 2008) by the national reference laboratories of England and

Wales, France, Germany, Norway, and Italy, a total of 1052 strains. Valid MATS results were obtained for 1011/1052 strains and were used to estimate coverage, and were linked to MLST and antigen sequence data.

Results/Conclusion: Using MATS, we estimated that 78% of the strains (95% confidence interval 66-91%) would be covered by 4CMenB. Half of the total strains (64% of the covered strains) potentially could be targeted by bactericidal antibodies against more than one antigen. Coverage estimates varied by country and ranged from 73% to 87%. 4CMenB has the potential to protect against a significant proportion of the MenB strains that have caused invasive disease recently in Europe.

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O 09

DEVELOPMENT OF A MENINGOCOCCAL ANTIGEN SURFACE EXPRESSION (MEASURE) ASSAY FOR THE PHENOTYPIC CHARACTERIZATION OF FHBP EXPRESSION BY NEISSERIA MENINGITIDIS

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Background and Aims: A bivalent factor H binding protein (fHBP/LP2086) vaccine is currently in Phase II clinical trials for prevention of invasive meningococcal disease. Previous studies determined that a bivalent fHBP vaccine elicits a broad serum bactericidal antibody response against diverse invasive MnB strains expressing fBHP variants heterologous to vaccine antigens. fHBP surface expression was the variable most related to the outcome of the serum bactericidal assay. To assess the phenotypic expression of fHBP, we have developed the Meningococcal Antigen Surface Expression (MeASurE) assay as a diagnostic to measure surface expression of fHBP variants on intact bacteria.

Material and Methods: Meningococcal strains were grown under the conditions used to assess serum bactericidal activity. fHBP surface expression was evaluated by flow cytometry using a broadly cross-reactive monoclonal antibody (MN86-994-11) that detects essentially all fHBP variants. The MeASurE assay was extensively tested to assess reproducibility. fHBP knockout strains were used as negative controls. Total fHBP expression was determined by quantitative Western immunoblotting performed utilizing polyclonal rabbit anti-bivalent rLP2086 sera as the primary

antibody and Cy5-labeled goat anti-rabbit IgG as the secondary antibody.

Results and Conclusions: Surface expression of fHBP determined by the MeASurE assay correlated with total fHBP expression as determined by western immunoblotting. The MeASurE assay is a specific, robust and precise method for determining the phenotypic expression of fHBPs utilizing a broadly cross reactive monoclonal antibody and flow cytometry. This assay can be used on globally collected strains to predict which MnB strains are susceptible to serum bactericidal activity.

O 10

MODELLING THE POTENTIAL IMPACT OF NEW 'MENB' VACCINES IN ENGLAND ALLOWING FOR HERD IMMUNITY

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Background and aims: New meningococcal vaccines able to protect against serogroup B disease are in advanced stages of development. It is not yet clear if these vaccines will also protect against carriage. This study uses a dynamic model to predict the potential impact and cost-effectiveness of introducing a new meningococcal vaccine in England, and explores different assumptions about the extent of herd immunity.

Methods: An age-structured transmission dynamic model of meningococcal carriage, disease and vaccination was developed. Epidemiological parameters and the costs of meningococcal disease and vaccination to the health service were estimated from contemporary data; future costs and benefits were discounted back to 2008. A range of routine and catch-up vaccination strategies were simulated.

Results and Conclusions: If the vaccine has a reasonable (60%) effect on carriage a strategy of routine infant immunisation (2,3,4+12 months) and a catch-up campaign (≤17 years) could reduce the annual number of cases by 71% after 10 years. However, this strategy is unlikely to be cost effective if the vaccine costs £40 per dose (£93,100 per QALY gained). Greater, sustained reductions in disease could be achieved in the longer term by routinely vaccinating teenagers if the vaccine protects against carriage. The results are

sensitive to assumptions around: the profile of the vaccine; carriage prevalence; population mixing patterns; and discount rates.

A universal meningococcal vaccine that can reduce carriage, in addition to disease, could have a substantial impact on the burden of disease in England and would be cost-effective if competitively priced.

O 11

MOLECULAR EPIDEMIOLOGY OF MENINGOCOCCAL MENINGITIS IN SUB-SAHARAN AFRICA, 2008-2010

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Background: Epidemics of meningitis are a major public health problem in countries of sub-Saharan Africa. Although effort has been put recently on capacity building, laboratory-based surveillance of meningococcal disease is still hampered by the lack of resources in the region. Using Trans-Isolate medium to grow and transport meningococci we have collected information regarding disease-causing strains in sub-Saharan Africa in the past two decades. We present here the molecular characterization of disease-causing isolates analyzed at the WHO Collaborating Centre in Oslo, in 2008-2010.

Materials and methods: A total of 443 cerebrospinal fluid samples or strains were received from 10 African countries and were analysed. Meningococcal isolates were serogrouped, tested for antibiotic susceptibility, sequence type (ST) and porA-fetA sequences. Cultures negative samples were analysed by PCR for capsule gene and porA sequencing.

Results and conclusions: Of samples, 169 gave growth to meningococci and 161 of the culture-negative samples were positive for meningococcal DNA in PCR. Overall serogroup A strains predominated (76% of the cases), followed by serogroup W135 (12%) and serogroup X (8%). The large epidemic in Nigeria in 2009 was caused by serogroup A of ST-7, as was the epidemic in Chad in 2010. In Burkina Faso a shift from serogroup A to serogroup X occurred during the epidemic season 2010.

Evaluation of the impact of the conjugate serogroup A vaccine, MenAfriVac, which was introduced in three countries of the region in December 2010 requires improved surveillance and detailed characterization of the circulating strains, in view of the naturally occurring epidemiological changes.

O 12

LABORATORY QUALITY CONTROL IN A MULTICENTRE MENINGOCOCCAL CARRIAGE STUDY IN BURKINA FASO

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Background: To investigate the potential herd immunity effect of MenAfriVac, a new conjugate vaccine against serogroup A Neisseria meningitidis (Nm), a large carriage study was initiated in three health districts in Burkina Faso before vaccine introduction. Carriage prevalence was assessed through cross-sectional studies every 3 months during the year 2009, yielding a total of 20 326 oropharyngeal samples. A major challenge was the harmonization of operational procedures and the documentation and control of correct results. We present the laboratory quality control system implemented.

Material and Methods: Laboratory analysis performed by the three Burkina Faso laboratories included macroscopic assessment of colony morphology, oxidase test, Gram stain, ONPG and GGT tests and slide agglutination serogrouping.

Internal quality control was performed by these laboratories on media, reagents, laboratory equipment and field conditions. At NIPH results were confirmed by serogrouping and molecular characterization of isolates identified as Nm in Burkina Faso. Additional external quality control was performed on 3% of samples where no colonies morphologically resembling Nm had been identified and on 10% of non-ONPG-GGT+ isolates.

Results and conclusions: Focusing on quality control helped us identify potential difficulties and keep good laboratory practices throughout the study. Using the results obtained at NIPH as “gold standard” the overall sensitivity was 89.0% and the specificity was 98.8%. The overall Nm carriage prevalence (3.98%) was probably slightly underestimated and the corrected prevalence was 4.48%. No serogroup A isolates were found in the quality control, thus the serogroup A carriage prevalence of 0.39% is reliable.

O 13

CLINICAL RESEARCH PROJECTS

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Systemic meningococcal disease (SMD) can be classified according to the dominant clinical symptoms on hospital admission (scoring systems, clinical definitions). Using such classifications have clarified important aspects of the underlying pathophysiology of SMD. The major determinant of the clinical symptoms is the degree of proliferation in the circulation and the cerebrospinal fluid. The infection is usually compartmentalized to one of areas. Comparatively low graded proliferation in the blood ($<10^3$ Nm-DNA copies/ml) leads to meningococcemia without shock which subsequently may proceed to distinct meningitis. Massive proliferation ($>10^6$ Nm-DNA copies/ml) in the circulation causes septic shock and multiple organ failure leading to antibiotic treatment or death before distinct meningitis has developed. As postulated >100 years ago endotoxin (LPS) is the major toxic principle triggering the innate immune system via CD14-TLR4-MD2. However, many clinical aspects related to Neisseria meningitidis infections remain unsolved.

Why does such a high percentage of the patients with systemic meningococcal disease (SMD) develop septic shock in Western countries? The persistent high case fatality rate (CFR) of 7-10% is primarily related to the development of septic shock. In 3 studies, comprising 862 patients studied prospectively, 33, 30, 30% developed shock. The CFRs were: *shock without meningitis*: 52, 29,16%, *shock with meningitis*: 12, 7, 11%, *meningitis without shock*: 0, 1, 1% and *meningococcemia without shock or meningitis*: 0, 0, 5% (Gedde-Dahl T. NIPH Ann 1983;6:155, Halstensen A. Scand J Infect Dis 1987;19:35, de Greeff SC. Eur J Clin Microbiol Infect Dis 2008;27:985). No other invasive human pathogens (E. coli, S. pneumoniae, S. aureus, S. pyogenes) cause septic shock as frequently. Why is not the immune system

recognizing intruding N. meningitidis as readily as the other pathogens?

Does serogroup A cause septic shock less frequently than serogroup B or C? From reported clinical experience fulminant meningococcal sepsis is uncommon in sub-Saharan Africa (Warrell D, Oxford University). Studies from Niger (Boisier P. Vaccine 2007;25S:A24) and Ethiopia (Norheim G. J Clin Microbiol 2006;44:861) support this observation. The reported CFRs were 5.5% and 4.2%, respectively. Is there a selection bias in these studies? Do the sickest patients reach the hospitals? During the serogroup A epidemic in Moscow in the 1970s and 1980s the clinical pictures, LPS levels in serum and CFR were similar to those observed in Western countries. Are cross reacting antibodies protecting sub-Saharan from septic shock and high CFR from serogroup A?

What protects a person from developing SMD the first week after acquisition of a new virulent N. meningitidis?

O 14

MENINGOCOCCAL FACTOR H BINDING PROTEIN POLYMORPHISM ASSOCIATED WITH CLINICAL COURSE OF MENINGITIS PATIENTS

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Factor H Binding protein (fHBP) is an important meningococcal virulence factor, enabling the meningococcus to evade the complement system, and is an important target for vaccination. Recently, the structure of fHBP in complex with factor H (fH) was published (Schneider *et al* Nature 2009;458:890). Two fHBP amino acids, E_{283} and E_{304}, were found to be important in the interaction with fH, involved in salt bridges to fH. Fifteen amino acids were identified to make hydrogen bonds to fH. *fHbp* of 254 isolates from adults with meningococcal meningitis was sequenced to study the effect of fHBP variants on meningococcal disease severity and outcome. E_{283} was conserved, while E_{304} was substituted by T_{304} in 40 of 254 fHBP sequences. The proportion of patients infected with meningococci with $fHBP_{T304}$ and developing septic shock during admission tended to be lower (2/40 [5%] *vs.* 28/214 [13%]; $P = 0.15$) resulting in a lower rate of unfavorable outcome (2/40 (5%) vs. 28/214 [13%]; $P = 0.15$). Interestingly, the charge of 2/15 amino acids (at position 184 and 306) forming hydrogen bonds was either basic or acidic. Patients infected with meningococci with $fHBP_{D184}$ (acidic residue) were more likely to develop septic shock during admission (11/42 [26%] *vs.* 19/212 [9%]; $P = 0.004$) resulting in a higher rate of unfavorable outcome (9/42 [21%] *vs.* 21/212

[10%]; *P* = 0.05). The 306 polymorphism was not associated with clinical course. In conclusion, we identified $fHBP_{D184}$ to be associated with sepsis and unfavorable outcome in patients with community-acquired meningitis.

O 15

GENOME TYPING AND ANNOTATION ON PUBMLST.ORG: INTRODUCTION OF THE BACTERIAL ISOLATE GENOME SEQUENCE DATABASE (BIGSDB) PLATFORM

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Background: The Neisseria databases hosted on PubMLST.org are now hosted using a new genomics platform, the Bacterial Isolate Genome Sequence Database (BIGSdb). In addition to the existing typing functionality of the site, BIGSdb facilitates a scalable and community-based approach to whole genome analysis.

Materials and methods: For each isolate provenance and phenotypic properties are linked to sequence data, which may range from a single gene fragment, through multilocus sequences and partial genome assemblies, to whole closed genomes. These sequence datasets can be queried with standard algorithms using curated reference datasets for given genetic loci. This enables the rapid discovery and characterisation of genetic variation and its association with known phenotypes. Loci can be grouped into schemes with unlimited numbers of members to reveal higher order structure, as in MLST, but schemes for metabolic processes, antibiotic resistance, and antigenicity are also possible. Fine-grained authentication of access permits multiple users to participate in community annotation by setting up or contributing to different schemes within the database.

Results and Conclusions: BIGSdb is currently running the reference and isolate databases for Neisseria sequences (http://pubmlst.org/neisseria/) where it hosts provenance and genotypic data for over 18000 isolates, including 29 complete genomes. It is also currently being used as an in-house tool to analyse the genomes of 200 N. meningitidis isolates, but there are no practical limits to the number of bacterial genomes, loci,

and schemes which can be accommodated. The platform is available as a resource for community annotation and investigation of phenotypic and genotypic variation.

POSTER PRESENTATION P 001 – P 014, Section: National epidemiology of invasive meningococcal disease

P 001

SURVEILLANCE OF INVASIVE MENINGOCOCCAL DISEASE IN POLAND IN 2010

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Background and aims: The aim of the study was to characterise epidemiological situation concerning invasive meningococcal disease (IMD) in Poland in 2010.

Material and methods: All invasive isolates of Neisseria meningitidis received by the National Reference Centre for Bacterial Meningitis (NRCBM) in 2010 were identified, serotyped and characterised by MLST, porA and fetA typing. A PCR technique was used for identification of the etiological agent directly from clinical materials in the case of a negative culture.

Results: In 2010, the NRCBM identified 227 of laboratory confirmed IMD cases (0.59/100.000), out of which for 220 a serogroup was defined. There were 151 invasive meningococcal isolates and 76 PCR-positive reactions with primers specific for Neisseria meningitidis. More than 51% of patients with IMD were under 5 years of age. Majority of IMD cases were caused by meningococci of serogroup B (n=114; 51.8%), followed by serogroup C (n=100; 45.4%), Y (n=5, 2.3%) and W135 (n=1; 0.5%). Decreased susceptibility to penicillin characterised 36.4% of isolates. All meningococci were susceptible to cefotaxime, chloramphenicol, rifampicin and ciprofloxacin. Amongst 107 meningococci analysed to date by MLST, 59 STs were found including 16 new STs. More than 74% of isolates belonged to 12 known clonal

complexes (cc). The most common were representatives of ST-103cc, ST-41/44cc, ST-18cc and ST-32/ET-5cc.

Conclusions: In 2010 the number of IMD cases decreased in Poland in comparison with previous years. Following a peak in 2002, the proportion of isolates of serogroup B and C have been stable.

P 002

AUSTRIA 2010, EPIDEMIOLOGY AND SURVEILLANCE OF MENINGOCOCCAL DISEASE

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Background: The National Reference Centre for Meningococci (NRCM) was founded by the Ministry of Health in 1981. The NRCM collects data about the isolates and issues an annual report.

Methods: The case definition is consistent with 2008/426/EG. The isolates referred to the NRCM are characterised by serogrouping, and by porA and fetA variable regions sequencing. The antibiotic resistance of all strains is determined with Epsilon-Test. The NRCM also offers the nonculture method PCR diagnosis to all hospitals free of charge.

Results: Eighty cases of meningococcal disease were laboratory confirmed in 2010. The incidence rate was 0.95/100 000. Ten deaths were registered, which results in a case-fatality ratio of 12.5% and a mortality rate of 0.12/100 000. The clinical presentation was 30% meningitis, 46% septicaemia only and 24% meningitis & septicaemia combined. The distribution of the serogroups in the 80 laboratory confirmed cases was serogroup B 58.75%, serogroup C 31.25%, serogroup Y 5.0% and serogroup W_{135} 5.0%.

The eighty strains show a wide diversity in the porA variable regions. In the serogroup B strains 19 different combinations were found. The most prevalent genosubtype was P1.7,16. The serogroup C strains were assigned to 6 different porA variable region combinations, here C:P1.5,2 was most frequent. Each of the Y and W_{135} isolates had a different genosubtype.

Fourteen different fetA variants were distributed across all serogroups According to the criteria of the Clinical and Laboratory Standards Institute (CLSI) 6 isolates were in vitro intermediate resistant to penicillin. All isolates were in vitro sensitive to ceftriaxon, rifampicin and ciprofloxacin.

Conclusions: The incidence in 2010 with 0.95/100,000 is lower than 2009 (1.25/100,000). The decrease in IMD, compared to 2009, is due to serogroup C disease. Serogroup C disease showed a reduction from 39.1% (2009) to 31.25%. An increase was seen in serogroup B disease (58.75% 2010, 52.9% 2009) and a slight increase in serogroup W_{135} (5% 2010, 2.3% 2009,).

P 003

REPORT ON HAEMOPHILUS AND MENINGOCOCCAL INVASIVE ISOLATES COLLECTED IN SERBIA IN TWO YEAR PERIOD (2009 - 2010)

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Background and aims: Serbian Haemophilus and Meningococcal Reference Laboratory (HMRL) was established in 2008, by the decree of Serbian Ministry of Health as part of the EU funded project "Strengthening the Services of Public Health Laboratories in Serbia". It is situated in Sombor, and it works as part of the Microbiology laboratory in the Institute of Public Health – Sombor. IPH Sombor is the Public Health Institution for West Backa District covering the region of about 200000 citizens.

Material and methods: Serbian HMRL provides basic Haemophilus and Meningococcal confirmation and characterization (phenotypic and genotypic) for isolates sent from laboratories throughout the country and works on creating national collection of characterized isolates.

Reference Laboratories from Hungary (National Center for Epidemiology) and Austria (National Reference Centre for Meningococci) offered major help in our work regarding molecular characterization of collected isolates.

Results and Conclusions: During the 2009 – 2010 period, Serbian HMRL collected ten N. meningitidis and two H. influenzae isolates that are characterized and prepared for long term preservation.

Keywords: Reference Laboratory, N. meningitidis, H. influenzae, isolate characterization.

P 004

THE EPIDEMIOLOGY AND SURVEILLANCE OF MENINGOCOCCAL DISEASE IN ENGLAND AND WALES

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Background and Aims: The HPA performs surveillance of invasive meningococcal disease for England and Wales to ascertain case numbers and characterise strains.

Materials and Methods: Clinicians notify suspected cases of meningococcal meningitis/septicaemia to the Office for National Statistics. Microbiology laboratories submit isolates for phenotypic characterisation and since, October 2007, by porA sequencing. MICs of penicillin, cefotaxime, rifampicin and ciprofloxacin are determined. Clinical samples are submitted for non-culture detection and serogroup confirmation by PCR.

Results and Conclusions: The increase in serogroup C cases from 1995-9 resulted in the introduction (November 1999) of serogroup C conjugate (MenC) vaccine into the UK population. Laboratory confirmed cases rose from 1,448 in 1995 to peak at 2,804 in 1999 falling to 917 in 2010. During 2010, 489 cases (53%) of invasive meningococcal disease were confirmed by PCR alone (comprising 16,607 samples representing 11,418 patients investigated by PCR). Serogroup B accounted for 88% of all confirmed cases (2010), 7% were serogroup Y (more than doubling from 28 cases in 2004 to 68

in 2010) whereas only 2% (20 cases) were confirmed as serogroup C and 3% serogroup W135. Phenotypic and genotypic shifts have been observed, specifically the relative contribution of clonal complexes ST-41/44, ST-269, ST-32, ST-213 and ST-11 to meningococcal epidemiology.

Surveillance has demonstrated a sustained reduction in serogroup C infections since 1999 to 2005 remaining at <20 cases per year, a direct consequence of the MenC programme and resultant herd immunity. A small but detectable increase in serogroup Y cases is now being closely monitored.

P 005

THE EPIDEMIOLOGY OF NEISSERIA MENINGITIDIS IN ITALY, 2009-2010

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Background: Incidence of Neisseria meningitidis invasive disease in Italy in the years 2000-2008 has been generally low ranging between 0.3 and 0.6/100,000. We here describe the national epidemiology of meningococcal invasive disease for the years 2009-2010.

Methods: Information on N. meningitidis invasive disease derives from the advanced surveillance since 1994. From 2007 individual notifications are reported on a website database by the Local Health Units

N. meningitidis isolates and clinical samples are sent to the National Reference laboratory, at ISS, for confirmation and typing. Analysis was performed by EPI-INFO version 3.5

Results: In 2009 and 2010* 188 and 136 cases of meningococcal invasive disease were reported, respectively, with an annual incidence rate of 0.3/100,000 and 0.2/100,000. The highest incidence rates were observed in children < 1 year of age (2.8 and 4.6/100,000 in 2009 and 2010), followed by the 1-4 year age group (1.6 and 1.0/100,000, respectively). In the 15-24 year age group incidence rates were 0.7/100,000, 0.2/100,000 respectively. Information on serogroup was available for 258**/324 (79,6%): 61% were due to serogroup B (158 cases) and 26 % to serogroup C (68 cases). Interestingly, compared to previous years a rise in proportion of cases due to other serogroups (A,Y,W135,X) was observed (12,4%).

Conclusion: Incidence of N.meningitidis invasive disease in Italy continues to be low although infants <1 year are still the most affected. Preliminary data show a decrease of number of

cases caused by serogroup C very likely due to MenC vaccination being recommended in almost all the Italian Regions.

* (preliminary data)

* notare che I tre CW non possono essere considerati tipizzati ne altro

P 006

INVASIVE MENINGOCOCCAL DISEASE IN SWEDEN 2010

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In the year 2010 a total of 68 cases of invasive meningococcal disease were identified in Sweden (9.4 million inhabitants) via the mandatory combined clinical and laboratory reporting systems. Incidence rate was 0.7, which is at a similar level as last years.

The diagnosis was confirmed by culture in 60 patients, by PCR in 7, and one case was diagnosed clinically. The invasive isolates were of group B (n=15), C (n=26), Y (n=23), and W-135 (n=3). Among the patients 37 were women and 31 men, aged from 10 months to 89 years. The case fatality rate was 10 % (n=7, 2-89 years of age).

Decreased laboratory susceptibility for penicillin G is a reality in 12% of the isolates if susceptibility is defined as ≤0.094 g/L and 40% if ≤0.064 g/L. Rifampicin resistance was seen in one isolate with MIC 0.38. Otherwise was the highest MIC of rifampicin 0.125 mg/L.

Further characterisation with genosubtype and antibiogram including sequencing of the penA gene show a split collection of endemic meningococci with one emerging clone Y:P1.5-2,10-1,36-2:F4-1:ST23(cc23). Due to the increase of group Y meningococci in Sweden during the last couple of years an investigation of the clonal pattern through genetic characterisation has been conducted on all group Y isolates collected in Sweden during 2000-2010*.

The annual report forms a basis for extended discussions around vaccine policy for meningococcal disease in Sweden.

* S. Thulin Hedberg, B. Törös, H. Fredlund, P. Olcén and P. Mölling. Genetic characterization of the emerging invasive Neisseria meningitidis serogroup Y isolates in Sweden/abstract EMGM 2011.

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P 007

EPIDEMIOLOGY AND SURVEILLANCE OF MENINGOCOCCAL DISEASE IN GREECE (2009-2010)

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A total of 143 cases of meningococcal disease were notified in Greece (85 and 58 cases for 2009 and 2010), corresponding to an incidence of 0.77 and 0.55 cases per 100 000 inhabitants for 2009 and 2010 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, 90.6% (77/85) and 91.4% (53/58) were confirmed for the two years (2009 and 2010).

For the period of two years, 39.1% of invasive cases originated from children aged <1-4 years, 11.2% from children aged 5-9 years, 5.6% and 14.7% from the age groups of 10-14 and 15-19 years respectively. In addition, 28% (40/143) were originated from adults (>20). The case fatality rate per year ranged from 5.9% (2009) to 6.9% (2010).

Serogroup B was responsible for 72.7% of the cases for both years (64.8% and 84.8% for 2009 and 2010), followed by serogroup C (2.8% (2009) and 2.2% (2010).

The highest incidence rate for serogroup B was noted in age groups of <1-4 and 5-9 years for both examined years.

For both years, the predominant phenotypes among the serogroup B isolates was B:4: NST while, predominant sequence type clonal complexes were: ST 269 cc followed by ST-162 cc and ST-41/44 cc.

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 for both examined years.

Among the 3 isolates belonging to serogroup C, only one was culture confirmed possessing the phenotype C:2a:P1.2,5, ST-11 while the rest 2 were confirmed only by PCR and there was no predominant porA gene combination. ST-32 cc was observed for the first time among the serogroup C isolates in Greece.

Reduced susceptibility to penicillin was found in both years; 15.4% (2/13) for 2009, while, the percentage was almost doubled for 2010 isolates (30.43%, 7/23). All were sensitive to chloraphenicol, rifampicin, cefaclor, ceftriaxone, ciprofloxacin and cefotaxime.

P 008

INVASIVE MENINGOCOCCAL DISEASE IN THE CZECH REPUBLIC IN 2010

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Background and aims: Nation-wide enhanced surveillance of invasive meningococcal disease (IMD) was implemented by the National Reference Laboratory for Meningococcal Infections (NRL) in 1993. Since then, valid and comparable data have been available. The aim of this study is to assess the epidemiological situation with the purpose of updating the vaccination strategy accordingly.

Material and methods: The case definition is consistent with the ECDC guidelines. Culture and PCR are used for confirmation of cases. Notification is compulsory and *Neisseria meningitidis* isolates from IMD cases are referred to the NRL to be characterized by serogrouping, *Por*A and *Fet*A sequencing (http://neisseria.org/nm/typing/), and multilocus sequence typing (MLST) (http://pubmlst.org/neisseria/).

Results and conclusions: In 2010 the IMD incidence decreased to 0.6/100 000 in 2010) and the case fatality rate was 8.9 %). The disease was caused mainly by serogroup B meningococci (58.2 %), followed by serogroups C (11.9 %) and Y (6 %). The following clonal complexes were most frequently associated with IMD: cc41/44 (20.7 %), cc18 (10.3 %), and cc32 (10.3 %), all related to serogroup B. Clonal complex cc11, related to serogroup C was found in 6.9 % isolates only.

The highest age-specific morbidity rates were observed in the lowest age groups, i.e. 0-11 months and 1-4 years (8.4/100 000 and 3.6/100 000, respectively), and were associated with high prevalence of serogroup B.

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P 009

EPIDEMIOLOGY OF INVASIVE MENINGOCOCCAL DISEASE IN FINLAND, 1995-2010

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Background and aims: Reporting of invasive meningococcal disease (IMD) is obligatory in Finland, and case-based data is available since 1995. We report here the trends in IMD in Finland in 1995-2010.

Material and methods: In Finland, reporting of IMD is based on notifications from clinical microbiology laboratories reporting all positive CSF/blood culture, antigen detection and/or PCR findings and clinicians reporting all laboratory-confirmed cases. All case isolates are requested to be sent to THL for confirmation and typing. In 1995-2010, most cases (>90%) were culture proven.

Results: Since a period in 1995 and 1996 with higher incidence caused by serogroup B and serogroup C strains, the incidence of IMD in Finland has fluctuated at low levels between 0.6 to 1.1 per 100 000 population (29-58 notified cases annually). Most cases (>70%) have been due to group B. Only 1-9 group C cases have occurred annually (incidence <0.2 per 100,000). In 2010, an increase in serogroup Y was detected. The phenotype (years 1995-2010) and the genotype (year 2010) distribution of the isolates will be presented.

Conclusions: The incidence of IMD in Finland has remained low during the past 14 years. Due to low incidence of group C disease, there are no plans to introduce serogroup C conjugate vaccine into the national vaccination program. Further studies are needed to investigate the reason behind the increase in serogroup Y disease in 2010. The introduction

of molecular typing methods in 2010 has significantly improved our capabilities for strain differentiation compared to previous years using only phenotyping methods.

P 010

INVASIVE MENINGOCOCCAL DISEASE: RECENT TRENDS IN BELGIUM.

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In 2010 the NRC for *Neisseria meningitidis* examined 130 samples of which 96 strains from invasive infections, were confirmed. The resulting incidence of the disease in 2010 was 0.9 cases per 100,000 inhabitants. Meningococcose hits especially children younger than 5 years (36.5%) and teenagers between 15 and 19 years (25%), with equal number of cases in men and women (sex ratio M/F = 1). These infections caused 8 deaths (CFR of 8.3%). Fifty percent of the cases were observed in Flanders, 32.3% in Wallonia and 17.7% of cases in Brussels. Serogroup B was determined in 79.2% of the samples and the serogroups C, Y, W135, X respectively in 10.4%, 4.2%, 4.2% and 1% of the samples. Most frequently observed in serogroup B were serotype P3.4 (33.3%) and subtypes P1.4 (33.3%) and P1.14 (13.5%). The main phenotypes were B:4:P1.4 (24%, ST-41/44) and B:NT:P1.14 (11.5%, ST-269). In serogroup C, serotype P2.2a (87.5%, ST-11) occurred most frequently. Eighty percent of the strains with serogroup C are from Wallonia, the remaining 20% from Brussels. The sensitivity of strains to benzylpenicillin dropped to 38.6 percent, 8 percent of the strains were resistant and 53.4% intermediate.

Over the last few years, the number of meningococcal infections in Belgium has slowly decreased and for serogroup C meningococci a complete shift was seen from north to south. The majority of strains were susceptible to antibiotics currently used for treatment and prophylaxis of meningococcal

disease but the prevalence of clinical isolates with reduced susceptibility to penicillin is alarmingly increasing.

P 011

LABORATORY AND EPIDEMIOLOGICAL SURVEILLANCE OF INVASIVE MENINGOCOCCAL DISEASE IN DENMARK 2009 AND 2010

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Background and Aims: The aim was to present data on invasive meningococcal disease (IMD).

Material and Methods: Data were obtained from both clinical and laboratory notifications, which are mandatory. All isolates submitted by the Departments of Clinical Microbiology to the Reference Laboratory were characterized by serogroup, serotype, serosubtype, and *por*A and *fet*A type. Data for 2010 are preliminary and may change.

Results and Conclusions: The incidence of IMD was 1.3 per 100,000 in both 2009 (72 cases) and in 2010 (72 cases). The case fatality rate was 3 % in 2009 (2 cases, one serogroup B and one serogroup C).

From 64 cases (88%) in 2009 and 59 cases (81%) in 2010 an isolate was available. Among these isolates 57% were serogroup B (60% in 2009 and 54% in 2010), 37% group C (34% in 2009 and 41% in 2010), and 6% group Y, X or 29-E. Among 123 isolates grouped, serotyped and serosubtyped 28% were type C:2a:P1.2,5 (14 in 2009 and 21 in 2010), and 22% were B:15:P1.7,16 (13 in 2009 and 14 in 2010). A total of 113 isolates were also fully typed using *por*A and *fet*A partial sequencing. The most prevalent types were C:P1.5,2:F3-3

(32%; 15 isolates in 2009 and 21 in 2010), and B:P1.7,16:F3-3 (27%; 12 isolates in 2009 and 12 in 2010).

The numbers of cases of serogroup C were stable. The relative proportion of type C:P1.5,2:F3-3 increased from 68% in 2009 (15 of 22) to 88% in 2010 (21 of 24).

P 012

SURVEILLANCE OF INVASIVE MENINGOCOCCAL DISEASE IN SLOVENIA, 2000 – 2010

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Background and aims: Infections (invasive) caused by Neisseria meningitidis (Nm) represent an important public health problem worldwide. Beside very dangerous invasive disease they can cause local outbreaks, epidemics or even pandemics.The epidemiological surveillance and typing of bacteria is an important issue of Public Health authorities for tracking transmissions and recognition of disease clusters.

The aim of the study was to estimate the epidemiological situation in Slovenia to support immunisation program, if there some changes will be needed.

Material and Methods: 131 strains of Nm isolated from patients with invasive disease in Slovenia from 2000 to 2010 were evaluated. The isolates were typed phenotipically by slide agglutination and dot-blot method and genotipically with molecular typing method MLST (Multi Locus Sequence Typing, Oxford and RIVM). Antibiotic susceptibility was determined by E-test following the CLSI recommendations. Together corresponding epidemiological data were processed.

Results: The incidence rate varied from year to year and was the highest in children in the year 2009 (4,3/100.000) and coincide with the highest pneumococcal rate (24,8/100.000), the lowest was in the year 2000 (0,3/100.000). In adults the incidence rate was much lower, the highest was in the year 2008 (0,63/100.000). The most affected age groups were children from 0–1 year (43 strains) and from 15-19 years (28 strains). The most common was serogroup B (80 strains), followed by serogroup C (28 strains), which was increasing from the year 2003 to 2008. The collected isolates were very heterogenous. The most common ones were the ST types which did not belong to the main hypervirulent clonal complexes. Nevertheless we detected also the strains belonging to all the main hypervirulent complexes, which caused epidemics in Europe and Saudi Arabia. The most frequent were ST-41/44 complex/Lineage 3, ST-32 complex/ET5 (both of them mostly serogroup B) and ST11-complex/ET37 complex (mostly serogroup C).

Conclusions: The incidence rate is quite low and the epidemiologic situation is still endemic in Slovenia. The isolates are very heterogenous, but all the main hypervirulent clones are present, so surveillance is also essential for prevention and recognition of disease clusters.

The incidence of serogroup C is low and does not demand the introduction of conjugate meningococcal C vaccine for now.

P013

EPIDEMIOLOGY OF MENINGOCCOCAL DISEASE IN ESTONIA

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Background: Meningococcal infection is a notifiable disease in Estonia since 1965.

Aims: To determine the incidence and trends of meningococcal disease in Estonia.

Materials and Methods: The statistics of meningococcal disease incidence based on the Estonian Communicable Disease Registry. Existing countrywide, mandatory, passive surveillance system (clinical and laboratory) is using EU case definitions and ICD 10 code.

Results: Meningococcal infection has a low incidence in Estonia with the predominance of serogroup B. During the observation period 2001-2010 the proportion of serogroup B was 65%, serogroup C – 18,9%, serogroup A – 14,9% and other serogroups - 1%. A significant decline of the morbidity of meningococcal infection has been observed during the last 10 years. In 2010 the morbidity rate was 0,1/100 000 population. During the period 2001-2010 there were 11 deaths due to the invasive meningococcal disease, serogroup B was the main etiological agent. The fatality prevalence varied from 0 to 36,4% in the last decade, no fatal cases registered in 2002, 2003 and 2010. Clinical presentation: meningitis – 56,3%, meningococcal septicaemia - 38,8%, meningococcal encephalitis – 1% and other forms – 3,9%. Age distribution of meningococcal infection - the majority of cases (48,5%) were registered among 0-14 year old children, 22,5% among persons aged 50 years and over and 8,7% among children below 1 year old. 92% of cases (2001-2006) were laboratory

confirmed by culture, from 2007 all cases were laboratory confirmed either by culture or PCR. Currently vaccination against meningococcal infection is not included in the national schedule. It is recommended to travellers to endemic countries. The rate of imported cases has increased: 36,4% of cases in 2004 versus 8% in 2005, from 2006 all cases have been domestic origin.

Conclusions: Estonia is belonging to the group of European countries with a low meningococcal disease incidence rate varied between 0,1 and 1,0/100 000.

P014

INVASIVE MENINGOCOCCAL DISEASE IN FRANCE, 2009-2010

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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 2009 and 2010.

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. The completeness of mandatory notification system is > 90% since 2005.

Results: The incidence of the IMD has been decreasing in France since 2005 and was 1.1 (n=628) and 0.9 (n=518) per 100,000 in 2009 and 2010 respectively (after correction for under reporting). The global fatality rate of IMD was 10%. Among all the 1146 reported cases, 13 were reported in French overseas departments and 1034 (90%) were reported using culture and/or PCR.

Amongst the 1062 IMD cases with known serogroup (93%), 73% (n=777) belonged to B, 20% (n=208) to C, 2% (24) to W-135, 4% (44) to Y and 1% (9) to other serogroups (or were non groupable).

In France mainland, the distribution of the main B, C, W and Y serogroups has changed between 2009 and 2010 (p=0.05). The serogroup C decreased from 22% (n=125) to 17% (n=81) (p=0.03) whereas the serogroup Y increased from 3% (n=17) to 5.5% (n=26) (p=0.04).

In 2009, the predominant clonal complexes (CC) among 365 characterized isolates were ST-41/44 (29%), ST-11 (24%), ST-32 (16%) and ST-269 (8%). In 2010, these percentages were 22%, 15%, 18% and 10% respectively among the 342 characterized isolates. Other CCs also continued to increase such as ST-213, ST-461 and ST-23 that accounted together 13% and 15% in 2009 and 2010 respectively (<5% for the period 2006-2008). In 2009-2010, 25% of isolates showed reduced susceptibility to penicillin G, 2 isolates were resistant to ciprofloxacin but no resistance to rifampicin was detected.

The control of the prolonged outbreak in the Normandy continues using MenBvac® with a positive impact of the vaccination on the epidemiological situation.

Conclusions: IMD is predominated by several serogroup B isolates, the incidence has slightly decreased for serogroup C and increased for serogroup Y.

The decrease in IMD C incidence has been observed since 2003. With the introduction of the conjugate C in the French immunization schedule in April 2010 (one dose for toddlers between 1 and 2 years old and a 1-dose catch-up for the 2-24 years old), this trend should be confirmed.

P-??

INVASIVE CASES OF *H. INFLUENZAE* AND MENINGOCOCCI: MALTA, 2009/2010

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Heamophilus influenzae

For the year 2009, we isolated invasive *H. influenzae* from the blood of three patients, aged 23, 50 and 82 years.

For the year 2010, we isolated invasive *H. influenzae* from the blood of two patients, aged 35 and 73 years.

Both for 2009 and 2010, we did not isolate *H. influenzae* from CSF specimens.

Neisseria meningitides

For the year 2009, we isolated invasive *N. meningitides* from five patients as follows:

Patient no	Age	Isolated from	Serogroup
01	3	Blood	B
02	6	Blood	C
03	<1	Blood + CSF	B
04	52	Blood	C
05	2	Blood	C

For the year 2010, we isolated invasive *N. meningitides* as follows:

Patient no	Age	Isolated from	Serogroup
01	51	Blood	B
02	<1	Blood	B

POSTER PRESENTATION P 015 – P 025, Section: National epidemiology of invasive Haemophilus disease

P 015

THE EPIDEMIOLOGY OF HAEMOPHILUS INFLUENZAE IN ITALY, 2009-2010

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Background: In Italy, Haemophilus influenzae type b (Hib) vaccination was included in the national vaccination program in 1999. We describe the national epidemiology of invasive H. influenzae (Hi) disease, in 2009-2010.

Methods: Information on invasive Hi disease derives from the surveillance of Bacterial Invasive Disease on Neisseria meningitides, Hi and Streptococcus pneumoniae. Cases are reported on a website database including information on age, sex, Hib vaccination status, clinical diagnosis and outcome by the local health unit. Hi isolates are sent to the National Reference laboratory, at ISS, for species confirmation and PCR capsular serotyping.

Analysis was carried out by EPI-INFO version 3.5.

Results: In 2009 and 2010 (for 2010, data is preliminary) overall 108 cases of invasive Hi disease were reported, with an annual incidence rate of 0.09/100,000. The incidence by age group was 1.6/100.000, 0.2/100,000, <0.1/100,000, 0.36/100,0000 for < 1, 1-4, 5-64 and > 64 years, respectively.

Sixty-five Hi isolates (60.2%) were serotyped. Forty-five of these (69.2%) were nonencapsulated (ncHi), 10 strains (15.4%) were type b and 10 strains (15.4%) were encapsulated non-Hib. ncHi cases were more common among adults and the elderly (40% for both 5-64 and ≥ 65 years) but a substantial number of cases occurred in children ≤5 years (20%). Compared with encapsulated strains, ncHi was more frequently isolated in all age groups.

Conclusion: The impact of Hib vaccination resulted in an impressive reduction of invasive Hib infection incidence, in Italy. A marked change in the predominant serotype from Hib to ncHi has occurred.

P 016

IMPACT OF MASS VACCINATION AGAINST HIB ON INVASIVE H. INFLUENZAE POPULATION IN POLAND

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Background and aims: : To investigate the impact of mass vaccination against *H. influenzae* serotype b (Hib), which has started in 2007, on invasive *H. influenzae* population in Poland.

Material and methods: The study was performed on all *H. influenzae* isolates collected between 1997 and 2010 during the routine monitoring of bacterial invasive infections by the National Reference Centre in Poland. All strains were identified according to standard procedures. PCR reactions were run to confirm species identification, serotype determination, and to detect capsule-specific genes and *fts*I gene (BLNAR phenotype). MICs of antimicrobials were evaluated by E-test. β-lactamase production was detected by nitrocefin assay.

Results: During the study 397 invasive *H. influenzae* isolates were collected. Until 2007 most of them (73%) were recovered from children below 5 years. The majority of the strains were characterized as Hib (93%). *H. influenzae* serotype f (Hif) and non-typeable isolates (NTHI) were responsible for 1.2% and 6.5% of cases, respectively. From 2008 to 2010, most of the *H. influenzae* isolates were recovered from patients above 5 years (56%). NTHI were responsible for 57% of infections, followed by Hib (42%) and Hif (1%). Ampicillin resistance was associated with β-lactamase production (13%) and BLNAR phenotype (2%).

Conclusion: Three-years after introduction of Hib vaccine into Polish Calendar significant reduction of infections due to *H. influenzae* type b was observed. However, the surveillance data showed a shift in age of patients as well as in the number of infections caused by NTHI.

P 017

INVASIVE HAEMOPHILUS INFLUENZAE IN SLOVENIA, 1993 – 2010

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Background and aims: The objectives of our study were to characterise the invasive *Haemophilus influenzae* isolates from 1993 to 2010 in Slovenia, a country with routine *H. influenzae* serotype b (Hib) conjugate vaccination since the year 2000.

Material and Methods: All invasive isolates of *H. influenzae*, recovered from a normally sterile site, isolated in all Slovenian microbiological laboratories were collected at the National Institute of Public Health as a part of national surveillance system. The isolates were serotyped by slide agglutination and the serotype results since 2000 were confirmed with molecular capsule typing by PCR. Antibiotic susceptibility was determined by disc diffusion method and Etest following the recommendations of the CLSI. To study genetic relatedness PFGE using *SmaI* was performed.

Results: The incidence of invasive *H. influenzae* disease in children aged less than 5 years decreased rapidly after the introduction of the regular Hib vaccination; from 24.6/100.000 per year in the pre-vaccination era to 2.8 in the vaccination era. The overall proportion of serotype b decreased from 85.3% to 9.9% in the vaccine period and the proportion of NT increased from 12.0% to 83.0%. No case of Hib has been observed in the children aged less than five years since the year 2001. Production of beta-lactamase was the principal mechanism of ampicillin resistance, occurring in 8.5 % isolates in the period 2000 to 2008. The study of genetic relatedness by PFGE demonstrated that the isolates of serotype b and f were genetic homogeneous within the serotype.

Conclusions: The results of our national surveillance showed that the vaccine has been very efficient in preventing Hib invasive disease in Slovenia. Nevertheless we see a need for further monitoring of invasive *H. influenzae* infections at a national level.

P 018

AUSTRIA 2006-2010, EPIDEMIOLOGY AND SURVEILLANCE OF HAEMOPHILUS INFLUENZAE

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The National Reference Centre for *Haemophilus influenzae* (NRHI) was founded by the Ministry of Health in 2004. The NRCM collects data about the isolates and issues an annual report. Austria has vaccinated with *Haemophilus influenzae* serotype b (Hib) conjugate vaccine since 1991.

Methods: The case definition is consistent with 28/IV/2008. The isolates sent to the NRHI are characterised by serotyping, biotyping and the antibiotic resistance of all strains is determined. The NRHI also offers the nonculture method PCR diagnosis to all hospitals free of charge.

Results: In the time period 2006 – 2010, 51 isolates of invasive *H. influenzae* disease were sent to the National Reference Centre. Two cases were confirmed with PCR. The average incidence lies by 0.64/100 000 with the highest incidence of 6.5/100000 in the age group <1 year. The clinical presentations reported were 19 meningitis cases and 19 septicaemias. In 18 further cases with other clinical presentations *H. influenzae* was isolated from blood. One death was reported with the diagnosis meningitis and three with septicaemia. The serotype distribution of the meningitis cases was 2 *H. influenzae* type b, 3 type f and 14 noncapsulated strains. One of the meningitis cases was <1 year, and six were in the age group 1 - 4 years. The 19 septicaemia cases were caused by two *H. influenzae* type b,

one type f and 16 noncapsulated strains. According to the criteria of the CLSI, one isolate was intermediate resistant to ampicillin. No isolates were resistant to cefotaxim, cefuroxim, tetracycline, ciprofloxacin and rifampicin.

Conclusions: The number of *H.influenzae* isolates sent to the NRHI is low. Invasive disease with *H. influenzae* is still associated primarily with serotype b. Through the good vaccination coverage, invasive disease with Hib has become very rare. From the other serotypes only three serotype f strains were sent to the NRHI in the last 5 years. The proportion of non-capsulated isolates is however increasing. We hope with the increasing awareness of H. influenzae invasive disease caused by non-b serotypes and non-capsulated strains a better surveillance and epidemiology for H. influenzae can be achieved in the future.

P 019

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

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Background: In the Czech Republic, the surveillance of invasive disease caused by *Haemophilus influenzae* b (Hib) was started in 1999. Since 2009, it has been extended to monitoring non-b *Haemophilus influenzae* invasive disease. The routine Hib vaccination was launched in July 2001. The vaccine schedule consists of four doses.

Material and methods: The case definition is consistent with the ECDC guidelines. The surveillance has also included the investigation of Hib vaccine failure since 2002. Serotypes were verified using PCR and biotyping was carried out in all strains.

Results: In 1999-2010, invasive Hib disease presented mostly as meningitis, followed by epiglottitis. 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 cases of Hib invasive disease. The morbidity rate decreased by 100 % in children aged 0 to 14 years after nine years of routine Hib vaccination. Invasive Hib disease is uncommon in older age groups. Hib vaccine failure has been very rare (14 true cases, 5 possible cases and 1 apparent case).

Conclusions: The surveillance results indicate a rapid decrease in Hib invasive disease in the target age group following the introduction of routine Hib vaccination in infants in the Czech Republic in July 2001.

P 020

ENHANCED SURVEILLANCE OF INVASIVE INFECTIONS BY HAEMOPHILUS INFLUENZAE IN BADEN-WUERTTEMBERG

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The epidemiology of disease caused by *H. influenzae* has changed considerably within the last decade. After implementation of the polysaccharide-conjugate vaccine against *H. influenzae*, serotype b, incidence of invasive disease has decreased to 0.1/100,000 in 2000 in Germany. Nevertheless, since 2001 incidence rates have risen again to 0.18/100,000 in 2008 (data from the statutory notification system). This rise is mainly due to non-typeable *H. influenzae* (NTHi) strains, which do not express a polysaccharide capsule, among older adults. Reported incidence rates were highest in the state of Baden-Wuerttemberg (BW) with 0.34 cases per 100,000 inhabitants in 2008. This prompted collaboration between KLHi and the state authority in BW (LGA) with the aim of documenting disease manifestations, and performing strain typing. In 2009, 23 strains were received by KLHi from BW, while 31 cases were reported to LGA. Most strains processed were NTHi (19 of 23, 83%). Serotypes b (3 of 23, 13%), and f (1 of 23, 4%) were additionally found. The mean and median age of patients was 54 and 69 years, respectively. The majority of isolates were cultured from blood (87%) and the remaining isolates were grown from central spinal fluid. Multi-Locus-Sequence-Typing (MLST) revealed a highly diverse bacterial population, suggesting that invasive cases represent sporadic cases with no evidence for epidemiological links. In addition to above data, analysis of strains from 2010 is underway and results will be included. In

summary, invasive disease by *H. influenzae* in BW mostly affects the elderly, and is caused by highly diverse NTHi.

P 021

BACTERIAL MENINGITIS DUE TO H. INFLUENZAE IN GREECE: AN 8 YEAR EPIDEMIOLOGICAL DATA (2003-2010).

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A total of 52 cases due to *H. influenzae* were notified for an 8 year period. Of those, 35 cases were due to *H. influenzae* type b (Hib) and 17 were identified as *H. influenzae* non type b. The average incidence was 0.04 per 100 000.

All cases were confirmed by either PCR assays (42 cases; 80.8%) while, only 10 cases (19.2%) 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 (16.92/100 000; 18/35) followed by the age group of 1-4 years (1.34/100 000, 7/35). There was only one case of Hib at the ages 5-9 years; while 7 cases were identified in adults (>20 years). Positive samples of *H. influenzae* non-type b were found mainly in older ages (>20 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 at ages less than 1 year old, especially at the ages of 3-6 months.

P 022

EPIDEMIOLOGY OF INVASIVE HAEMOPHILUS INFLUENZAE DISEASE IN FINLAND, 1995-2010

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Background and aims: Surveillance of invasive *Haemophilus influenzae* (Hi) disease is needed to monitor the effectiveness of the Hi type b (Hib) vaccination program and to detect possible serotype replacement. We report here the trends in invasive Hi disease in Finland in 1995-2010.

Materials and methods: Since 1995, the National Infectious Disease Registry (NIDR) at the National Institute for Health and Welfare (THL) in Finland has collected invasive Hi isolates for serotyping as a part of Hi surveillance program. All isolates were serotyped by latex agglutination and counterimmunoelectrophoresis. A conventional PCR targeting the capsular locus genes was used to confirm the serotype and to differentiate the capsulate and non-capsulate isolates.

Results: Altogether 525 invasive Hi isolates were sent to NIDR in 1995-2010. The number of isolates varied from 12 in 1995 to 33 in 2010. Most isolates were non-capsulated (N=387; 74 %). Among the capsulated Hi isolates (N=138; 26 %), type b was the most common (N=72; 52 %), followed by f (N=55; 40 %), e (N=9) and a (N=2). The number of invasive Hib cases varied between 1 to 8 per year, and the overall incidence between 0,02/100 000 to 0,15/100 000. No upward trend was observed. The serotyping results obtained by conventional and PCR methods were fully concordant.

Conclusions: Most invasive Hi isolates were non-capsulated. Since the introduction of the routine childhood Hib conjugate

vaccination in 1986, the number and proportion of Hib cases has been low. Our data do not suggest serotype replacement.

P 023

LABORATORY AND EPIDEMIOLOGICAL SURVEILLANCE OF HAEMOPHILUS INFLUENZAE IN DENMARK 2009-2010

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Background and Aims: To present data on invasive *Haemophilus influenzae* (HI) isolates. Vaccination against HI serotype b (HIB) is part of the Danish childhood immunization program since June 1993.

Material and Methods: Isolates from blood or cerebrospinal fluid (CSF) were submitted from Departments of Clinical Microbiology (DCMs). The National Reference laboratory performed biotyping and serotyping using antisera.

Results and Conclusions: In 2009 and 2010 we received 29 and 43 isolates, respectively, corresponding to incidence rates of 0.53 and 0.78 per 100,000. Six percent of the isolates were from CSFs, 8% were from children below 5 years and 43% from patients above 65 years of age.

Eleven percent were serotype b, 8% serotype f, 7% serotype e, and 74% non-capsular, and 44% were biotype II, 26% biotype I, 20% biotype III, and 8% biotype IV or V. The prevalent types were; non-capsular, biotype II (42%) or non-capsular, biotype III (21%). Non-capsular, biotype II isolates increased from 28% in 2009 to 51% in 2010.

Seven among eight HIB-cases were adults between 26 and 61 years, and one was a two-dose-vaccinated 10-month-old-child, who survived meningitis without sequelae.

More isolates were received in 2010 compared to 2009, possibly because it became mandatory to submit invasive isolates by October 2007. We received an increasing number of isolates from 2006 to 2010 from an increasing number among the 15 DCMs. A high proportion of our isolates were non-capsular according to conventional serotyping. In 2010 we therefore introduced use of multiplex-PCR.

P 024

NATIONAL EPIDEMIOLOGY OF INVASIVE HAEMOPHILUS DISEASE

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The Scottish Haemophilus, Legionella, Meningococcus and Pneumococcus Reference Laboratory (SHLMPRL) has phenotypically (capsular serotype) and genotypically (Multilocus Sequence Typing (MLST)) characterised all invasive *H.influenzae* isolated in Scotland since 2002. By facilitating enhanced surveillance of this organism we are able to monitor changes in the epidemiology of *H.influenzae* disease. Here we present data obtained between 2003 and 2010.

On average 58 case, per annum, of invasive *H.influenzae* infection were reported to Health Protection Scotland (HPS) between 2003 and 2010 (2003 n=57, 2004 n=64, 2005 n=51, 2006 n=50, 2007 n=58, 2008 n=68, 2009 n=63 and 2010 n=52).

Isolates associated with 391 episodes of invasive *H.influenzae* disease were referred to the SHLMPRL between 2003 and 2010 (ranging from 37 (2007) to 56 (2004) per year). Over 80% (373/439) of isolates were recovered from blood. *H.influenzae* was also isolated from CSF, abscesses, joint fluid, eye, pleural fluid, peritoneal fluid, HVS and placenta swabs.

In 2003 53% (29/55) of *H.influenzae* were capsular serotype b, 3.6% serotype f, 1.8% serotype d and the remaining 42% were capsule non-typable (NT). By 2010, over 70% of referred isolates were described as NT, with serotype's b and f accounting for 13% of strains each and type e 2%.

On the basis of Multilocus sequence typing (MLST) data, the genetic diversity of *H.influenzae* isolates causing invasive disease in Scotland has increased, year on year, from an

Index of Diversity (D) of 0.687 in 2002 to 0.988 in 2010. The greatest level of genetic diversity is observed among the phenotypically NT strains.

P 025

THE EPIDEMIOLOGY OF INVASIVE HAEMOPHILUS INFLUENZAE DISEASE IN ENGLAND AND WALES

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Background and Aims: Routine immunisation against serotype b (Hib) from 1992 in the UK resulted in a dramatic reduction in invasive Hib disease. Resurgence in cases in 1999, due to declining vaccine effectiveness, has been controlled by an additional dose of Hib vaccine for children aged 6 months to 4years in 2003 and the introduction in 2006 of a routine booster dose at 12m. This study reports epidemiological data for 2010.

Methods: The HPA conducts national surveillance of invasive H. influenzae disease in England and Wales through a combination of laboratory and clinical reporting schemes, and provides a national service for serotyping invasive H. influenzae isolates.

Results: In 2010, there were only 30 cases of invasive Hib disease, accounting for 5.5% (30/541) of all invasive H. influenzae cases. Fewer cases were recorded in infants aged <1 year (4 cases) and toddlers aged 1-4 years (2 cases) in 2010 than any other year since Hib vaccine was introduced and no deaths from Hib disease have been reported in these age groups since 2007. Non-capsulated H. influenzae accounted for 55.5% (300 /541) of all H. influenzae infections,

with most cases (243/300, 81.0%) occurring in adults. Invasive infections caused by other capsulated serotypes were also more common than Hib (66/541, 12.2%) occurring mainly in adults (54/66, 81.8%).

Conclusions: The number of Hib cases in England and Wales reached the lowest recorded levels in 2010. The majority of invasive H. influenzae infections are now caused by non-capsulated H. influenzae and occur mainly in adults.

POSTER PRESENTATION P 026 – P 034, Section: Epidemiology of invasive MC/Hi disease

P 026

REPORTED CASES OF MENINGOCOCCAL MENINGITIS AND VACCINATION AGAINST INVASIVE MENINGOCOCCAL DISEASE FROM 2008 TO 2010 IN THE CELJE REGION IN SLOVENIA

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Background and aims: Institute of Public Health Celje monitors the regional epidemiological situation and provides different vaccinations. The regional doctors are obliged to report the suspicion and a case of invasive meningococcal disease to the regional epidemiologist in 6 hours. Close contacts of the patient with confirmed meningococcal disease are prescribed chemoprophylaxis and suggested appropriate vaccination by epidemiologist.

Material and methods: Analysis of existent epidemiological data on reported cases of meningococcal meningitis and data on vaccination against invasive meningococcal diseases.

Results and conclusions: From 2008 to 2010, the Institute of Public Health Celje had dealt with close contacts of eight reported patients with meningococcal meningitis, most in 2008 (5 reports). In three cases the cause was *Neiserria meningitidis* serogroup C, in two serogroup B, in one serogroup Z' and in one serogroup Y. One case was not typed.

Chemoprophylaxis was prescribed to 103 close contacts, 40 close contacts were vaccinated with quadrivalent

polysaccharide or monovalent conjugate vaccine. In 2010, we reported on the identification of the first serogroup Z' meningococcal meningitis in Slovenia, caused to a 19-year-old student.

From 2008 to 2010 we also vaccinated against the invasive meningococcal disease members of Slovenian army, patients without spleen, passengers and persons after the bone marrow transplantation. For these groups we used quadrivalent polysaccharide meningococcal vaccine. We protected 756 professional soldiers (missions to Kosovo, Afghanistan, Lebanon, and Bosnia and Herzegovina) and 178 people, most of whom were passengers (113) and people without spleen (44).

P 027

POPULATION SNAPSHOT OF INVASIVE SEROGROUP B MENINGOCOCCI IN SOUTH AFRICA, 2005-2008

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Background: In South Africa, serogroup B meningococcal (MenB) disease is sporadic. Historically ST-32/ET-5 was predominant in the Western Cape.

Aim: To characterise MenB strains causing invasive meningococcal disease (IMD) in South Africa from 2005 to 2008.

Methods: Isolates, collected through a national laboratory-based surveillance program for IMD, were characterised by multilocus sequence typing (MLST).

Results: 2234 cases were reported; 1601 (72%) had a serogroup assigned. MenB was the second most common serogroup (17%, 276/1601) and increased from 14% (58/414) in 2005 to 23% (81/360) in 2008 ($p<0.05$). For 71% (174/244) of MenB strains, 82 sequence types (ST), including 17 new ST's, were associated with one of 15 clonal complexes (cc).

An additional 44 ST's (including 27 new ST's), not associated with any cc, were identified among the remaining 70 strains. ST was not available for 7 strains, although a cc could be assigned. ST-32/ET-5 and ST-41/44 accounted for 22% (54/251) and 25% (62/251), respectively. In Gauteng, ST-32/ET-5 increased from 8% (2/24) in 2005 to 38% (9/24) in 2008 (p=0.04). ST-32/ET-5 comprised 15 ST's with ST-33 (n=25 strains) as the dominant strain. ST-7212 (n=14) emerged as a subgroup founder of ST-33 in 2007. ST-41/44 comprised two groups of 13 and 11 ST's each, of which ST-154 (n=12) and ST-6590 (n=11) were most prevalent.

Conclusion: MenB disease was characterised by heterogeneous strains and several new ST's. ST-32/ET-5 and ST-41/44 continue to predominate in South Africa. The expansion of ST-7212 appears to be driving the increase in ST-32/ET-5 in Gauteng.

P 028

EPIDEMIOLOGY OF INVASIVE MENINGOCOCCAL DISEASE IN RUSSIAN FEDERATION

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Background and aims: The incidence of invasive meningococcal disease (IMD) in Russia during the last 4 years has decreased and in 2009 it was 1.26 per 100,000 population. Reported annual incidence in different regions of the country usually ranges from 1 to 2 per 100,000. However, in some regions IMD incidence could be as high as 3-4 cases per 100,000.

Materials and Methods: Serogroup distribution of meningococci isolated from blood and cerebrospinal fluid in children and adults was investigated for 2009.

Results and conclusions: In total, 1231 cases of IMD were registered in Russia in 2009. Annual incidence of IMD in Russia for 2006-2009 was: 1,7 (2006), 1,56 (2007), 1,45 (2008), and 1,26 (2009). IMD incidence in most regions was within the range from 0 to 2 per 100,000 (73 regions out of 83 regions). Only two regions of Russia were within the range from 3 to >4 per 100,000 (Astrakhan region and Far East Jewish Autonomous region). In 2009 the highest IMD incidence was observed in children <1 y.o. – 16.9 per 100,000. Incidence in children <5 y.o. was 8.8 per 100,000. IMD incidence in adolescents 15-19 years of age was 0.95 per 100,000. Most IMD cases were caused by meningococci of serogroup A (29.3%), B (28.9%), and C (22.9%), whereas some cases were caused by meningococci of other

serogroups (W-135 – 4 cases, Y – 2 cases, and X – 2 cases). Overall IMD case fatality rate in 2009 was 11.6%.

P 029

MOLECULAR CHARACTERISATION OF NEISSERIA MENINGITIDIS SEROGROUP B ISOLATES CAUSING INVASIVE DISEASE IN SOUTH AFRICA, 2002-2006

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Introduction: From August 1999 through July 2002 ST-32/ET-5 and ST-42/44/lineage 3 were predominant clones of serogroup B meningococci (MenB) in Western Cape, South Africa.

Aim: To genotype MenB in South Africa from 2002 through 2006.

Methods: Invasive isolates, submitted to a national, laboratory-based surveillance system, were characterised by pulsed-field gel electrophoresis (PFGE). Clusters were defined as ≥5 isolates sharing ≥80% similarity on dendrogram. Multilocus sequence typing was performed on randomly selected isolates.

Results: 2144 invasive cases were reported. Gauteng (1234/2144,58%) and Western Cape (354/2144,17%) recorded the highest number of cases among the 9 provinces. 76% (1627/2144) of cases had viable isolates and 307 (19%) were MenB. MenB prevalence decreased from 24% (47/192)

in 2002 to 14% (64/474) in 2006 (p<0.001). PFGE results were available for 302/307 (98%) isolates, dividing 68% (206/302) into 13 clusters. The largest cluster, B1, accounted for 25% (76/302). Selected isolates (6/76,11%) belonged to clonal complex (cc) ST-32/ET-5. ST33 (n=5) and new ST6589 (n=1) were identified. B1 isolates decreased over time, from 43% (20/47) in 2002 to 13% (8/62) in 2006 (p<0.05) and were common to Western Cape (58/76,76%). Cluster B2 comprised 31/302 (10%) isolates. Selected isolates (10/29,31%) belonged to cc ST-41/44/lineage 3. B2 isolates showed no significant change over time and were prevalent in Gauteng (18/31,58%). ST154 (n=2), ST41 (n=1) and new ST6698 (n=1) were identified.

Conclusion: MenB isolates were mostly diverse but could be divided into clonal groups, with no single dominant clone. Strains of the global cc ST-32/ET-5 and ST-41/44/lineage 3 are still circulating.

P 030

INVASIVE MENINGOCOCCAL DISEASE AND THE DOMINATING PORA GENOTYPE, AUSTRIA, 1995-2010

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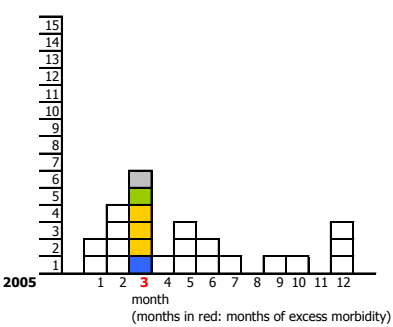
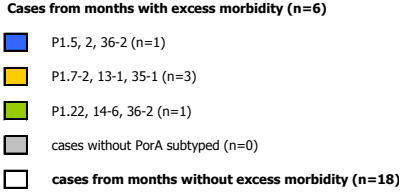
Background: The National Meningococci Reference Centre receiving the majority of isolates from local laboratories across Austria has operated the meningococci surveillance database since 1995. Aims of our surveillance data analysis was to describe the IMD morbidity from 1995-2010 by demographics, lethality and trends, and to identify dominating meningococci sg C strains by *porA* gene typing.

Methods: A total of 1320 cases of laboratory confirmed IMD remained for descriptive analyses. A periodogram function was applied on a time-series of monthly IMD sg C incidence data of 1995-2010. By use of a cyclic regression model the expected monthly incidence values were estimated; its upper 95% confidence limit (upper 95% CL) was used as alert threshold for excess morbidity. A high excess morbidity was defined as monthly incidence exceeding the upper 95% CL of the averaged monthly excess incidence values. The occurrence of high excess morbidity was analyzed by identifying the *porA* genotypes.

Results: The annual incidence rate was 1.02/100,000pyr, dominated by IMD sg B. IMD sg B morbidity decreased by an annual average percentage change (AAPC) of 0.93% and IMD sg C increased by an AAPC of 1.84%. The annual lethality total was 8.33%. The < 1-years old were most affected (15.92/100,000pyr). The IMD morbidity showed a 12-month periodicity. Among cases of months with high IMD sg C excess morbidity observed in 2001, 2002, 2008 and 2009 cases infected with genotype P1.5, 2, 36-2 were dominant (25/45 cases; 56%).

Discussion: As observed in other countries without mass vaccination against sg C meningococci, Austria experienced an IMD sg C morbidity increase from 1995-2010. Months exceeding considerably the expected IMD sg C morbidity were sporadically observed. In these months the *porA* genotype P1.5, 2, 36-2 was dominant.





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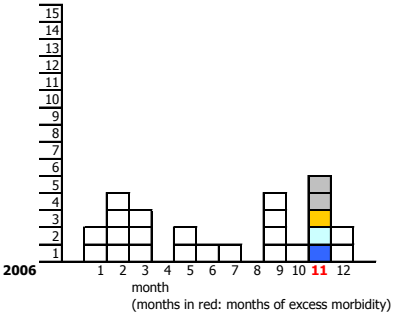

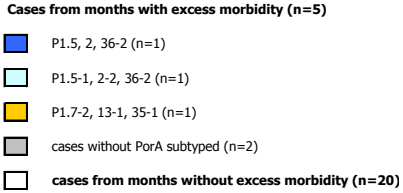

P 031

FIRST GENETIC CHARACTERIZATION OF NEISSERIA MENINGITIDIS ISOLATES IN THE REPUBLIC OF BELARUS

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Background and Aim: In 2010, 133 cases of meningococcal meningitis were notified in Belarus (1.39/100,000 inhabitants). The aim was to genetically characterize *N. meningitidis* from different regions of Belarus in 2006-2010.

Material and Methods: Twenty-two *N. meningitidis* isolates, from patients and contacts, were species-confirmed using biochemistry, serology, and a real-time *ctrA* PCR, and subsequently subjected to genogrouping, genosubtyping (*porA* sequencing), FetA typing, and MLST. Furthermore, *penA*, *rpoB*, and *gyrA* were sequenced for detection of mutations causing a decreased susceptibility/resistance to penicillins, rifampicin, and ciprofloxacin, respectively.

Results and Conclusions: The isolates were assigned genogroup B (n=16; 73%), C (n=5; 23%), and one isolate was not possible to genogroup. Sixteen different MLST STs (12 not previously reported), 12 genosubtypes (one new) and 13 FetA types (two new) were identified. Five isolates (23%) contained a *penA* mosaic allele causing a decreased penicillin susceptibility, however, no resistance mutations causing a decreased susceptibility/resistance to rifampicin or ciprofloxacin were identified.

Several meningococcal strains that have not been previously described internationally are circulating in Belarus. Enhanced information of the molecular epidemiology and genetic determination of decreased susceptibility and/or resistance to antimicrobials used for treatment and chemoprophylaxis of meningococcal infection in Belarus is crucial.

P 032

THE CHANGING EPIDEMIOLOGY OF INVASIVE MENINGOCOCCAL DISEASE IN CANADA, 1995 TO 2009

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Background: National goals for the reduction of invasive meningococcal disease (IMD) were developed after meningococcal C conjugate programs were implemented in Canada between 2002 and 2005.

Aim: To describe the epidemiology of IMD in Canada pre- and post-vaccine introduction and ascertain our attainment of national goals.

Methods: Provinces/territories report epidemiologic data on all IMD cases. Isolates are sent to the National Microbiology Laboratory for confirmation of serogroup and further studies. Epidemiologic and laboratory data are linked retrospectively.

Results: There has been a significant decrease in IMD incidence in Canada from 1.04 cases per 100,000 in 1995 to 0.61 in 2009. A 90% reduction occurred in serogroup C incidence but no significant change in other serogroups. In the post-vaccine period (2005 to 2009), serogroup B accounted for the majority of cases (59%), followed by C (17%), Y (16%), and W-135 (6%). In the pre-vaccine period (1995 to 2001), C accounted for 41% of cases. The most common phenotypes post-vaccine are B:17:P1.19, B:4:P1.4, and C:2a:P1.2,5. Serogroup B continues to affect the young, with a median age of 16 years, versus 38 years for C and W-135, and 46.5 years

for Y. The age group with the highest average incidence remains those less than one year olds (7.51 cases per 100,000), followed by one to four year olds (1.88) and 15 to 19 year olds (1.21).

Conclusions: Canada has achieved its goals of a sustained reduction in IMD serogroup C incidence. This experience will be reviewed for consideration of new vaccine programs.

P 033

MENINGOCOCCAL MENINGITIS AND INVASIVE HAEMOPHILUS INFLUENZAE TYPE B DISEASE IN TAIWAN, 1999-2010

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Background and aims: Both meningococcal meningitis and invasive *Haemophilus influenzae* type b (Hib) disease are listed as notifiable diseases by the Centers for Disease Control in Taiwan, the former in the second-category and the latter in the third-category. The epidemiological situations for these diseases are analyzed.

Material and methods: The isolated bacteria or less often the clinical specimens from the suspected cases are delivered to our laboratory for confirmation or identification, followed by bacterial serotyping.

Results and Conclusions: From 1999 to 2010, there were 249 confirmed cases of meningococcal meningitis and 291 confirmed cases of invasive Hib disease. For both diseases, the incidence rate is about 0.1 per 100,000, with the highest rate occurring in children less than one year old. Since 2005, more than 75% of the *Neisseria meningitidis* isolates obtained from the cases of meningococcal meningitis are serogroup B. The Hib vaccine was introduced as a client-paid vaccine in Taiwan in 1993. Since 2008, more than 70% of *H. influenzae* obtained from the notified cases of invasive Hib disease were nontypable. In conclusion, for the evaluation of vaccine policy and disease prevention, it is important to continue the surveillance for both diseases.

P 034

2010 - VIEW AT INVASIVE BACTERIAL DISEASES AT UNIVERSITY HOSPITAL FOR INFECTIOUS DISEASES "DR FRAN MIHALJEVIĆ" ZAGREB, CROATIA

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Introduction: Early recognition and appropriate management of invasive bacterial disease (IBD) is important task for clinician. Diagnostic tool used routinely at our hospital was conventional bacteriologic method – cultivation followed by antibiotic susceptibility testing. From 2005 real time PCR for five common microbiological causes of IBD was also introduced: *N.meningitidis, H.influenzae, S.pneumoniae, S.aureus and CoNS* and *L.monocytogenes.*

Materials and methods: Data of IBD caused with *N.meningitidis, H.influenza, S. pneumoniae* and *L.monocytogenes* were included.Two methods for the detection are used, cultivation with antibiotic susceptibility testing and *in house* real-time PCR detection based on specific primers.

Results: In our hospital during 2010 fifty eight patients **(58**) with IBD were hospitalized, 26(45 %) presented as sepsis, 22 (38 %) as meningitis and 10 (17 %) as sepsis and meningitis. There were 29 children and 29 adults. *S.pneumoniae* caused 27/58 (47%) IBD and *N. meningitidis* 29/58 (50%). In adults main etiological agents were *S.pneumoniae* 18/27 (67%) and in children *N.meningitidis* 20/29 (71%). Sepsis was dominated presentation of IBD in children 17/25 (68%) and it was mainly caused by *N.meningitidis* 12/14(86%). Group B *N.meningitidis* predominated, also 1 isolate was *N.meningitidis* group W135 and 1 *N.meningitidis* group C. Only 14% (4/29) cases were confirmed by cultivation, 65% by PCR only and rest by both methods. Six isolates were recorded as penicillin intermediate. Meningitis was predominant clinical presentation in adults

16/20 (80%) and it was mainly caused by *S.pneumoniae* 11/13 (85%). *L.monocytogenes* caused meningitis in two adults. *H.influenzae* was not detected.

Conclusion: The predominance of S.pneumoniae as the main cause of IBD in adults and N.meningitidis as the main cause in children was recorded. S.pneumoniae caused mainly meningitis in adults. N.meningitidis causes mainly sepsis in children. As N.meningitidis group B is predominant isolate genotyping procedure should be in routine work leading us towards possible usage of future vaccine.

POSTER PRESENTATION P 035 – P040,

Section: Emergence of serogroup Y

P 035

INVESTIGATIONS INTO AN INCREASE IN REPORTED MENINGOCOCCAL SEROGROUP Y DISEASE IN ENGLAND AND WALES IN 2007-9

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[2]Health Protection Agency, Manchester, U.K.

Background and Aims: We investigated the clinical, epidemiological and microbiological characteristics of invasive meningococcal serogroup Y (MenY) disease in England and Wales following a recent increase in cases reported to the HPA Meningococcal Reference Unit (MRU).

Material and Methods: For all MenY cases diagnosed in 2009, clinical details, risk factors and outcome were obtained. In addition, all MenY isolates submitted to the MRU between 2007-2009 were characterised by multilocus sequence typing and *porA, feta and fhbp* genotyping.

Results and Conclusions: In 2009, there were 66 MenY cases, with 45%, 39% and 11% occurring among those aged 15-65, >65 and in children, respectively. Underlying medical conditions were present in 38% of cases and most prevalent among >65 years-olds (65%). Meningitis was the most common clinical presentation (35%), followed by pneumonia (29%) and septicaemia (26%). Overall case-fatality was 18% and, after adjusting for age, the presence of underlying conditions (OR 24.6; 95% CI, 2.3-266; P=0.008) and clinical presentation with pneumonia (OR 9.8; 95% CI, 1.3-75.4; P=0.029) were independently associated with death. Among 114 isolates from 2007-2009, over 90% belonged to clonal complexes ST23 (63%), ST174 (24%), ST16 (12%) and ST22

(9%). Three FetA types, namely 4-1 (49%), 3-7 (22%) and 5-8 (17%), were present in 88% of strains, while *porA* were mainly P1.5-1,10-1 (23%), P1.5-1,10-4 (20%) and P1.5-1,2-2 (16%). Interestingly, 95% of *fhbp* variants were variant 2.

Although MenY disease remains uncommon, it is associated with significant morbidity and mortality particularly among the elderly and those with underlying medical conditions.

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P 036

SEROPREVALENCE OF SERUM BACTERICIDAL ANTIBODIES AGAINST GROUP W135 AND Y MENINGOCOCCI IN ENGLAND IN 2009

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Background and aims: Serological surveillance has been used in the United Kingdom to inform vaccine policy for several infections, including group C meningococci. Quadrivalent meningococcal conjugate vaccines, containing groups A, C, W135 and Y, are now available. The aim of this study is to establish a baseline for immunity for groups W135 and Y in England.

Methods: Serum samples collected in 2009 from individuals across all ages (N=1191) were obtained from the Health Protection Agency Seroepidemiology Unit, which collects residual sera from participating laboratories across the country. Serum bactericidal activity (SBA) against serogroup Y (strain M03 241125) and W135 (strain M01 240070) was determined using a standardized complement-mediated SBA assay, with complement derived from baby rabbits. The age-specific geometric mean titres (GMTs) and percentage of individuals with SBA ≥8 (the putative protective level) were calculated, together with 95% confidence intervals.

Results and conclusions: Antibody prevalence varied according to age and serogroup. In general, titres were low in younger children with 7% and 13% of children under 5 years

achieving titres ≥8 against group W135 and Y respectively. GMTs peaked in 20-24year olds for group W135 (GMT= 7.1 95% CI 4.7, 10.9) and in 30-44 year olds for group Y (GMT=8.6, 95% CI 5.9, 12.7). Unlike seroprevalence against group B meningococci, there was not an obvious peak in titres in teenagers.

Natural immunity against group W135 and Y meningococci in England is low.

P 037

CHARACTERISTICS OF INVASIVE MENINGOCOCCAL SEROGROUP Y ISOLATES IN FINLAND, 1995-2010

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Background and aims: In Finland, invasive meningococcal diseases (IMD) caused by serogroup Y has been rare with an average of 4 (range 1-8) culture confirmed cases notified annually in 1995-2009. Here, we report an increase of IMD caused by serogroup Y in 2010 and, present the phenotypic and genotypic characteristics of the invasive meningococcal serogroup Y isolates in Finland in 1995-2010.

Material and methods: In Finland, the surveillance of invasive meningococcal disease (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). All case isolates are requested to be sent to meningococcal reference laboratory at THL for confirmation and typing. For the present study, all invasive meningococcal serogroup Y isolates were serotyped by whole-cell enzyme-linked immunosorbent assay and genotyped by sequencing the variable regions of the *porA* (VR1 and VR2) and *fetA* genes.

Results and Conclusions: In total, 67 culture-confirmed IMD cases caused by serogroup Y were detected in 1995-2010. In 2010, both the number and the proportion of cases caused by serogroup Y increased significantly from an average of 4 cases (8%) in 1995-2009 to 13 cases (38%) in 2010. 62% (8/13) of the cases occurred in people over the age of 20.Thus far, no epidemic link between the cases has been recognized. The phenotype and genotype distribution, and the possible

clonality of the serogroup Y isolates in 1995-2010 will be presented.

P 038

GENETIC CHARACTERIZATION OF THE EMERGING INVASIVE NEISSERIA MENINGITIDIS SEROGROUP Y ISOLATES IN SWEDEN

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Background and aims: In Sweden, serogroup B and C have until 2008 been responsible for the majority of invasive meningococcal disease. Recently, an increase of serogroup Y has been noted in Sweden (from 2% in 2000 to 39% 2010), as in a few other countries. The aim of the present study was to investigate the clonal pattern of the emerging serogroup Y in Sweden during the last decade, through genetic characterization.

Material and methods: Invasive serogroup Y meningococcal isolates in Sweden from 2000 to 2010 (n=85) were genetically characterized by sequencing the *fetA, fHbp, penA, porA* and *porB* genes and by multilocus sequence typing (MLST). Each sequence was aligned and assigned an allele number using the *Neisseria* Sequence Typing Home Page (http://pubmlst.org/neisseria/).

Results and Conclusions: The characterization data showed a recent increase of one specific clone with identical allele combinations, which is responsible for the observed increase of meningococcal serogroup Y (n=28). It was sulfadiazine resistant, has genosubtype P1.5-2,10-1,36-2, ST 23, cc23, *porB* allele 3-36, *fetA* allele F4-1, *fHbp* allele 25 and *penA* allele 22. This clone seems to be introduced in the beginning of the last decade with one case in 2002 and 2004 each, followed by six cases during 2006-2007, eight cases during

2008-2009 and peaking with 12 cases in 2010. An unusal increase of invasive disease in adolescents caused by this clone was also shown, but no increased mortality rate was observed.

Further research with additional discriminating methods, such as variable number tandem repeat (VNTR) analysis, could provide more information about the clone responsible for the recent emergence of serogroup Y.

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P 039

INVASIVE MENINGOCOCCAL DISEASE CAUSED BY SEROGROUP Y IN THE CZECH REPUBLIC

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Background and aims: The most frequent serogroup causing invasive meningococcal disease (IMD) in the Czech Republic is serogroup B, followed by serogroup C. The involvement of serogroup Y in IMD cases has increased in last years, causing the highest serogroup-specific case fatality rate. The aim of this study is to investigate which clonal complex is responsible for this highest case fatality rate.

Material and methods: Isolates of *Neisseria meningitidis* Y from 29 IMD patients and 50 healthy carriers recovered in 1970 – 2010 and characterized by serogrouping, *Por*A and *Fet*A sequencing (http://neisseria.org/nm/typing/), and multilocus sequence typing (MLST) (http://pubmlst.org/neisseria/) were compared.

Results and conclusions: Among 29 IMD isolates, 11 sequence types (STs) were identified, belonging to three clonal complexes: cc23 (16 isolates), cc92 (2 isolates), and cc167 (2 isolates), while 9 isolates were not assigned to the clonal complex. The most frequent fine type was *N. meningitidis* Y,P1.5-2,10-1,36-2:F4-1:CC23, followed by *N. meningitidis* Y,P1.5-1,2-2,36-2:F5-8:CC23. Among 50 carrier isolates, 35 STs were identified, belonging to 10 clonal complexes, while 15 isolates were not assigned to the clonal complex. The meningococcal carrier population is more heterogeneous than that from IMD. Hypervirulent clonal complex cc23 was rare among the isolates from carriers (in 2 isolates only). The data suggest that the high case fatality rate of IMD caused by serogroup Y is due to clonal complex cc23.

The work was in part supported by IGA MZ CR grant NT11424-4/10

P 040

MENINGOCOCCAL DISEASE IN NORWAY, 2009-2010: EMERGENCE OF SEROGROUP Y

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The incidence of meningococcal disease in Norway has been at a stable low level since 2002. Only 44 and 39 cases were notified in 2009 and 2010, respectively; the incidence in 2010 was 0.8 cases per 100,000. In these two years 20% of the cases were under 5 years of age and 9 patients (11%) were reported to have died as a result of the disease. Of the 83 cases, 35 were caused by serogroup B, 18 by serogroup C, 3 by serogroup W135 and 24 by serogroup Y. The serogroup was not determined for the remaining cases.

Methods: A total of 74 strains (89% of the 83 cases) of *Neisseria meningitidis* were sent to the National Reference Laboratory for further characterization. These strains were serogrouped and serotyped with monoclonal antibodies and analysed by multilocus sequence typing, *porA* and *fetA* sequencing as described at http://pubmlst.org/neisseria/.

Results: Of the strains received, 24 from were isolated cerebrospinal fluid, 49 from blood, and 1 from fluid in the lung. Only 32 (43.2%) strains were serogroup B and 15 (20.3%) were serogroup C. A marked increase in serogroup Y disease occurred in this period (in total 23 cases, 31.1%); in 2010 the number of serogroup Y cases was equivalent to the number of serogroup B cases. A total of 40 sequence types (STs) were identified. The ST-23 complex predominated with 31.1% of the strains (all serogroup Y), followed by the ST-41/44 complex (21.6%) and the ST-11 complex (20.3%). Sequencing of the *porA* and *fetA* genes revealed a total of 27 *porA* types and 16 *fetA* types.

Conclusions: While the incidence of meningococcal disease in Norway is keeping at a very low level, a marked increase in serogroup Y disease occurred in the past two years. Cases were geographically spread, but many occurred in young adults. This new epidemiological situation warrants special awareness and use of a tetravalent conjugate vaccine must be considered.

POSTER PRESENTATION P 041 – P071,

Section: Vaccines and serology

P 041

TARGET COVERAGE OF 4CMENB IN GERMAN SEROGROUP B MENINGOCOCCI OF THE EPIDEMIOLOGICAL YEAR 2007/2008

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Background and aims: The multi-component vaccine 4CMenB has been developed to target the antigenically variable population of serogroup B meningococci. Aim of the study was to estimate the coverage of 4CMenB against serogroup B meningococci in Germany.

Material and methods: Antigen sequence typing of *nadA* (Neisserial adhesion A), *fHbp* (factor H binding protein), *nhba* (Neisserial heparin binding antigen), variable region 2 of porin A and measurement of the relative antigen potency of three vaccine antigens (NadA, fHbp, and NHBA) by the Meningococcal Antigen Typing System (MATS, Donnelly *et al*. PNAS 2010) were performed on 223 German serogroup B strains isolated in the epidemiological year 2007/2008.

Results and conclusions: Sequence typing revealed the presence of fHbp-1 subvariants in 74.7%, *nadA* in 23.8%, and PorA P1.4 in 21.1% of the isolates. The proportion of strains harbouring at least one of the target genes was 77.1% (95% confidence interval 71.6-82.6%). Relative potencies above the positive bactericidal threshold were observed for fHbp in 68.7%, for NadA in 1.4%, and for NHBA in 63.1% of the isolates. The coverage of 4CMenB according to MATS analysis and PorA P1.4 was 81.1% (95% confidence interval 71.0-93.1%).

P 042

VACCINE ANTIGEN DIVERSITY IN 106 DIVERSE MENINGOCOCCAL ISOLATES

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Materials and Methods: The Illumina genome sequencing platform was used to generate whole-genome data from a collection of 106 diverse *Neisseria meningitidis* isolates. These genome data were deposited in a BIGS database (Jolley *et al*, 2010). An XMFA file with aligned and concatenated nucleotide sequences of the *tbpA*, *tbpB*, *lbpA*, *lbpB*, *porA*, *porB*, *fetA*, *fHbp* and *IgA1* genes was created at the 106 project BIGSdb. These sequences were aligned, concatenated and used for various phylogenetic analyses using ClonalFrame, Neighbour-Joining (NJ) and Maximum Likelihood (ML) algorithms.

Results: Phylogenies revealed a level of structuring of antigens. Clusters were evident that corresponded to clonal groupings. This is consistent with previous work showing clonal complex-antigen association. Both the sequencing and database technologies allowed for the easy comparison of the antigen gene repertoires amongst the diverse dataset.

Conclusions: Despite high levels of diversity in antigen genes, structuring is evident. In spite of much immune pressure, antigen repertoires may persist over wide temporal and geographical spans. This has important implications for vaccines based on such proteins in terms of their coverage both over time and geography. The advent of whole-genome sequencing technology will greatly add to vaccine development as it will be possible, using appropriately-sampled datasets, to easily identify combinations of many

antigens that persist and that may give the best possible coverage.

P 043

THE EPIDEMIOLOGY OF INVASIVE MENINGOCOCCAL DISEASE IN ENGLAND AND WALES: IMPLICATIONS FOR POTENTIAL INFANT IMMUNISATION SCHEDULES

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Background: Several meningococcal quadrivalent conjugate vaccines have recently been licensed, while licensure for serogroup B (MenB) vaccines is imminent. This study describes the epidemiology of invasive meningococcal disease, with a focus on infants (aged <1 year) in order to inform potential scheduling of such vaccines.

Methods: The Health Protection Agency's Meningococcal Reference Unit routinely receives clinical samples for PCR investigation and isolates from cases throughout England and Wales for confirmation and typing. Data for four epidemiological years (2006/07-2009/10) were analysed.

Results: The overall average annual incidence of invasive meningococcal disease was 2.0/100,000 population with a case fatality of 5.3% (234/4435). The highest average annual

incidence was among <1 year-olds (41/100,000) who accounted for 25% (1111/4435) of all cases. In this age group, MenB was responsible for 94% (1042/1111) of infections, with case fatality of 4.9% (51/1042). Cases increased from birth to 5 months of age, then declined gradually. There were an average of 76 MenB cases per year in infants aged ≤4 months, 142 among ≤6 month-olds and 261 among <1 year-olds (8%, 15% and 27% of all MenB cases, respectively).

Conclusions: Infants have the highest incidence of invasive meningococcal disease, with MenB accounting for almost all cases in this age group. Because of the high burden of disease in early infancy, immunisation schedules for MenB should start as early as possible to have the greatest impact. There is currently little evidence to support the introduction of a quadrivalent meningococcal conjugate vaccine into the UK infant schedule.

P 044

DEVELOPMENT OF A MENINGOCOCCAL DISEASE VACCINE ENRICHED IN HEAT SHOCK PROTEINS

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Background: Heat shock proteins (Hsps) are molecular chaperones that bind polypeptides, prevent aggregation and support protein folding. Hsps can also induce innate immune responses, activate dendritic cells and deliver peptides leading to antigen presentation and development of adaptive immunity to a broad range of antigens. We have developed a reproducible process for preparation of a Hsp-enriched vaccine for meningococcal disease and are investigating immune responses in mice.

Methods: The Hsp vaccine is prepared from Neisseria that have been subjected to a heat shock. Bacteria are broken by homogenisation and a Hsp-enriched fraction is prepared from the clarified supernatant using anion exchange chromatography. Sera from mice immunised with the Hsp-enriched vaccine have been assessed for antibody-mediated complement component C3 and C5b-9 deposition, opsonophagocytosis (OPA) and serum bactericidal activity (SBA) against a panel of meningococcal strains.

Results and conclusions: Optimisation of the vaccine preparation process has significantly increased the Hsp content and the process has been shown to be reproducible from a 5L to a 60L fermentation scale. Cross strain antibody-mediated complement C3 and C5b-9 deposition responses were seen with the Hsp-enriched vaccine, with greater responses seen with the optimised vaccine with greater Hsp

content. Strong cross strain OPA responses were also observed against all strains in the meningococcal panel with responses greater than or equal to the homologous strain OMV serum control for 4/6 strains. SBA responses against a panel of strains will be presented. This approach shows promise for a low cost multi-antigen vaccine to prevent meningococcal disease.

P 045

FACTOR H BINDING PROTEIN (FHBP) VARIABILITY WITHIN THE ST-41 AND ST-44 SUB-COMPLEXES OF CC41/44

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Background: *Neisseria meningitidis* clonal complex ST-41/44 contains two main sub-complexes: the ST-41 sub-complex is generally associated with invasive disease (in the MLST database the 73% of strains of this sub-complex are from disease), whereas ST-44 shows a relevant proportion of strains associated with carriage (51% of strains of this sub-complex are from carriers). Little is known about the relative genetic diversity of the factor-H binding protein (*fHbp*) in these two sub-complexes.

Method: We analysed the genetic variability of *fHbp* of strains belonging to the ST-44 sub-complex, and compared it with the diversity of strains belonging to the ST-41 sub-complex. The full *fHbp* gene sequence of 255 strains belonging to the cc41/44 were determined.

Results: Most of the isolates obtained from carriers belonged to the ST-44 sub-complex, and represented the 53% of this sub-complex isolates. Conversely, all of the strains belonging to the ST-41 sub-complex originated from disease. We

observed different levels of gene variability among carriage and pathogenic strains and among ST-41 and ST-44 sub-complexes. A significant correlation was observed between *fHbp* variant 1 and ST-41, as well as between ST-44 and variants 2 and 3.

P 046

A PHASE I/II OPEN-LABEL SAFETYAND IMMUNOGENICITY STUDY OF A MENINGOCOCCAL B BIVALENT RLP2086 VACCINE IN HEALTHY ADULTS

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Background and aims: *Neisseria meningitidis* serogroup B (MnB) is a leading cause of meningococcal disease in the industrialized world, but a broadly protective vaccine is not commercially available. A bivalent factor H-binding protein (rLP2086) vaccine has shown immunogenicity in early phase, dose-ranging studies. Here, safety and immunogenicity for the 120µg rLP2086 dose was assessed in healthy adults.

Methods: Participants (N=60; ≥18 to ≤40) were vaccinated with rLP2086 at 0, 1, and 6 months. Local and systemic reactions were recorded using electronic diaries for 7 days following vaccination. Unsolicited adverse events (AEs) were reported. Immune responses were evaluated by rLP2086-

specific immunoglobulin G titres and serum bactericidal assay using human compliment (hSBA).

Results: For MnB strains expressing LP2086 variants homologous or nonhomologous to the vaccine antigens, 94% had hSBA titres ≥1:4 after the third vaccination. In a subgroup of young adults (≥18 to ≤25), >80% had hSBA titres ≥1:4 against diverse MnB invasive strains. After each vaccination, local reactions, such as pain at injection site (91.2%–92.7%), induration (21.1%–27.3%) and erythema (10.0%–14.5%) were generally mild or moderate. There were 3 cases of severe induration and 4 cases of severe erythema. Fatigue and headache were the most common systemic events. Upper respiratory infection, the most common AE, occurred in 31.7%. One severe AE, respiratory track infection commencing on day 7, was considered related to vaccination. Two recipients reported 3 serious AEs unrelated to the vaccine.

Conclusions: The 120µg rLP2086 dose was well-tolerated and immunogenic in adults, supporting continued vaccine development.

P 047

ANTIGEN DIVERSITY OF THE 4CMENB VACCINE COMPONENTS IN SEROGROUP B MENINGOCOCCAL PATIENT ISOLATES FROM FIVE EUROPEAN COUNTRIES

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Background: To evaluate presence and degree of conservation of the main antigens of 4CMenB vaccine in the epidemiological background of some European countries, we analysed molecular diversity of the vaccine components in patient isolates. Molecular data were used with Meningococcal Antigen Typing System (MATS) results, for an estimation of 4CMenB coverage

Methods: A total of 1052 invasive MenB strains, isolated in five European countries mainly in a single epidemiologic year were analysed by MultiLocus Sequence Typing (MLST) and tested for PorA genotypes. Presence and diversity of the vaccine components NadA, fHbp and NHBA were assessed.

Results: The clonal complex (cc) 41/44 was prevalent in 4 of the 5 countries considered. In those countries, cc32 was the second most represented cluster. The exception to such trend was UK, where cc269 was predominant, and exceeded cc41/44 by 1.5%. The association between vaccine antigen variability and clonal complexes was confirmed. As a consequence, subvariants associated with cc41/44 (fHbp-

1.14, 1.4 and NHBA-2) were predominant. fHbp-1.1, that is associated with cc32 only, accounted for more than 20% in France, and for 19, 12 and 9 % in Germany, Norway and Italy, respectively. The occurrence of NadA reflected those of the NadA (+) clonal complexes, namely cc32, 8 and 213.

Conclusions: The distribution and the vaccine antigen repertoire of each clonal complex differed among European countries. For compiling coverage estimates, genetic data are to be linked with expression and bactericidal data. This is accomplished by MATS, as seen in poster..., where all coverage estimates are extrapolated.

P 048

NORWEGIAN ISOLATES FOR EVALUATION OF A PROTEIN BASED VACCINE DIRECTED AGAINST SEROGROUP B MENINGOCOCCAL DISEASE; AIMING FOR BROAD COVERAGE

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The study of vaccine component sequence variability and distribution among isolates from different epidemiological situations is a key step for evaluation of protein-based vaccines intended for broad coverage. The distribution and sequence variation of the genes coding for five recombinant antigens (NadA; NHBA; fHbp; GNA1030; GNA2091) included in a multi-component meningococcal serogroup B (4CMenB) vaccine, was assessed. The variability was traced in isolates from well characterized Norwegian epidemiological situations; one endemic period with low incidence of meningococcal disease and another from a serogroup B outbreak. In spite of high diversity of the genes coding for the vaccine antigens under consideration, the sequence match for 4CMenB vaccine holds good promise regarding potential coverage. Best match was found among the MenB isolates (72-95% in the endemic period). However, the vaccine is likely to be protective against a high number of strains belonging to other serogroups as well (56-95%). Antigen expression and accessibility on the bacterial surface was evaluated by FACS analysis. However, this gives only a rough indication of the amount of antigen expressed by each strain. MATS analysis will be needed for a more appropriate estimate of the real vaccine coverage and protective potential in various clinical situations.

P 049

POTENTIAL COVERAGE OF ENGLISH AND WELSH GROUP B ISOLATES BY AN INVESTIGATIONAL MENINGOCOCCAL GROUP B VACCINE

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Background and Aims: An investigational multicomponent meningococcal group B vaccine (4CMenB) has proven safe and immunogenic in Phase I-III trials. The vaccine contains three recombinant proteins; factor H binding protein (fHBP), Neisserial Heparin Binding Antigen (NHBA) and Neisserial adhesin A (NadA) formulated with Outer Membrane Vesicles containing the immunodominant PorA protein. Protection afforded by 4CMenB will depend upon antigen expression and cross-reactivity of induced antibody to antigen variants. To address this issue, a meningococcal antigen typing system (MATS) was developed to predict whether 4CMenB covers individual isolates. As group B currently accounts for approximately 90% of meningococcal disease in England and Wales, we investigated the potential coverage of 4CMenB using MATS.

Material and Methods: All 535 group B case isolates received at the Health Protection Agency Meningococcal Reference Unit from the epidemiological year 2007/2008 were characterised genetically for PorA and by MATS for recombinant antigens.

Results and Conclusions: We found that 63.3, 54.7 and 0.6% of isolates had positive MATS phenotype for fHBP, NHBA and NadA, respectively. Additionally, 20.3% harboured

the homologous PorA. Coverage (determined as ≥1 antigen with positive phenotype/homologous PorA genotype) was estimated at 72.9%. Among covered strains, 69% were positive for more than one antigen. We conclude that 4CMenB has the potential to protect against a significant proportion of MenB disease in England and Wales.

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P 050

POTENTIAL COVERAGE OF ENGLISH AND WELSH NON-GROUP B ISOLATES BY AN INVESTIGATIONAL MENINGOCOCCAL GROUP B VACCINE

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Background and aims: An investigational multicomponent meningococcal group B vaccine (4CMenB) has proven safe and immunogenic in Phase I-III trials. The vaccine contains three recombinant proteins; factor H binding protein (fHBP), neisserial heparin binding antigen (NHBA) and neisserial adhesin A (NadA) formulated with outer membrane vesicles, containing the immunodominant PorA protein. As these vaccine proteins are sub-capsular, protection afforded by the vaccine may not be restricted to group B meningococci. We therefore, investigated the potential coverage of 4CMenB against non-group B isolates from England and Wales via genetic characterisation of vaccine proteins.

Material and methods: From the epidemiological year 2007/2008 all non-group B case isolates received at the Health Protection Agency Meningococcal Reference Unit were genetically characterised with respect to multilocus sequence type, fHBP, NHBA, NadA and PorA. The 74 meningococcal strains comprised of groups; A (n=1), 29E (n=2), C (n=16), NG (n=2), W135 (n=24), Y (n=28) and Z (n=1) isolate.

Results and conclusions: Preliminary results demonstrated that while no isolates harboured the homologous PorA P1.4, all isolates possessed alleles for NHBA. For fHBP, 27% of

isolates harboured the variant 1 alleles (corresponding to the vaccine variant) and for NadA, 18% of isolates harboured nadA alleles. Based upon this genotypic data and potential cross-reactivity of induced antibody, 4CMenB has the potential to protect against a significant proportion of non-group B disease in England and Wales.

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P 051

TEMPORAL DYNAMIC OF SERUM-BACTERICIDAL ACTIVITY AFTER IMMUNIZATION WITH ACWY POLYSACCHARIDE VACCINE IN ADULTS

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Until recently, immunization with polysaccharide vaccine against Meningococcal serogroups A, C, W135, and Y has been recommended in most European Countries for individuals working with Neisseria meningitidis. We have performed a cross-sectional study with 20 individuals between 18 and 50 years, who were vaccinated with the same quadrivalent polysaccharide vaccine, to assess the temporal decline of serum-bactericidal activity and to estimate the duration of protection from invasive disease. Timepoint of immunization was retrospectively obtained from immunization cards. Analysis of serum-bactericidal titres and duration (in months) after vaccination revealed a significant negative log-linear correlation for all serogroups. The minimal duration of protection was estimated by reading the intersection of the lower 95% confidence band of the linear model with a horizontal line representing a protective titre of 1:8. Minimal protective duration was 35, 30, 0, and 25 months for serogroups A, C, W135, and Y, respectively. Mean protective duration, however, exceeded 100 months for all serogroups. In conclusion, our study might point to less effective immunization against serogroup W135 by a polysaccharide-only vaccine. Results, however, need to be confirmed by quantification of antibody levels using non-functional assays.

P 052

INTER-LABORATORY STANDARDIZATION OF THE MATS ELISA: A REPRODUCIBLE TYPING METHOD FOR *N. MENINGITIDIS* SEROGROUP B VACCINE STRAIN COVERAGE PREDICTION.

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Background: The Meningococcal Antigen Typing System (MATS) combines phenotypic information and PorA-typing to assess whether a MenB strain may be covered by 4CMenB. A sandwich ELISA (MATS ELISA) measures the fHbp, NadA, and NHBA content of MenB isolates relative to a reference

strain (*relative potency*). The relative potency is sensitive to variations in both the immunologic recognition and expression level of each antigen. In preliminary studies, MATS accurately predicted killing in the serum bactericidal assay using human complement.

Methods: We conducted an inter-laboratory standardization study at seven laboratories to assess the robustness of relative potencies for fHbp, NadA and NHBA and establish qualification parameters for laboratories performing the test. Each laboratory performed MATS on a set of 17 reference strains, each assayed multiple times. The reproducibility of relative potencies among laboratories and against consensus values derived from the present data was evaluated using a mixed-model analysis of variance (ANOVA).

Conclusions: We found very good agreement among the seven laboratories participating in this study, with Pearson correlation coefficients (r), coefficients of accuracy (C_a), and concordance correlation coefficients (r_c) exceeding 99% in the large majority of comparisons. Summary measures of reproducibility (between-laboratory variance) expressed as CV's were 7.85% (fHbp), 16.51% (NadA), and 12.60% (NHBA), and the aggregate measure of variation over all strains, adjusted for laboratory, expressed as CV's were 19.8% (fHbp), 28.8% (NHBA) and 38.3% (NadA).The MATS is a standardized typing system that can be used by local laboratories to obtain reproducible estimates of vaccine strain coverage in different geographical settings.

P 053

SEQUENCE TYPING AND EXPRESSION ANALYSIS OF 4CMENB TARGETS IN CAPSULE NULL LOCUS MENINGOCOCCI

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Background and aims: The multi-component vaccine against serogroup B meningococci (4CMenB) comprises three major recombinant antigens including Neisserial adhesion A (NadA), factor H binding protein (fHbp), and Neisserial heparin binding antigen (NHBA) as well as outer-membrane vesicles, which especially induce antibodies against P1.4 of porin A. The purpose of this study was to estimate the effect of 4CMenB on capsule null locus (cnl) meningococci.

Material and methods: Forty-one strains of cnl meningococci from three countries (Burkina Faso, Czech Republic, and Germany) belonging to nine sequence types (ST) and four clonal complexes were antigen sequence typed and analysed for antigen expression by genetic typing and the recently published Meningococcal Antigen Typing System (MATS, Donnelly *et al*. 2010).

Results and conclusions: Strains of the ST-198 complex harboured an fHbp variant, which is included in the vaccine, and expressed the antigen above the positive bactericidal threshold (PBT) as defined for serogroup B strains. Expression of NHBA above the PBT was seen in strains of the ST-198 complex and ST-845. Only one strain harboured the *nadA* gene and P1.4 of porin A was not present among all analysed cnl strains. The data suggest that bactericidal

antibodies will be effective only on a subset of cnl meningococci. A biological effect on the ST-198 complex and on ST-845 will depend on antibody levels at the mucosal surface and their capacity to block transmission, for which data are currently not available.

P 054

IMMUNE STATUS OF POPULATION 5 YEARS AFTER INTRODUCTION OF NEISSERIA MENINGITIDIS||SEROGROUP C CONJUGATE VACCINATION IN THE NETHERLANDS

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Background and aims: In 2002 a MenC vaccination was introduced as a single administration at 14 months in the Netherlands accompanied by a mass catch-up campaign targeting individuals between 1 and 18 years. We aimed to determine the immune status of the population before and after introduction.

Material and Methods: Two cross-sectional population-based studies in the Netherlands in pre- and post-vaccination periods allowed the measurement of polysaccharide-specific IgG(subclasses), IgM and avidity by a multiplex immunoassay. In addition, in a subset of sera MenC-specific serum bactericidal antibody titers were determined.

Results and conclusions: PS-specific IgG and SBA titers showed an age-specific trend, with the highest antibody persistence in the oldest vaccinated age-groups 5 years after vaccination. After the single vaccination PS-specific IgG levels dropped rapidly in the younger age cohorts. In all immunized age-groups higher levels of IgG1 compared to IgG2 were observed, while naturally derived immunity was mainly restricted to IgG2 subclass. An age-related increase in IgM levels was also observed. Noteworthy, the increase in IgG2 correlated with a reduced IgG-avidity with age. The MenC vaccine response appeared to be a mixture of both T-cell dependent and T-cell independent characteristics.

Due to mass vaccination, MenC circulation probably decreased drastically, resulting in lower IgG titers in the unvaccinated older age-groups, posing them at risk if MenC starts re-circulating. To maintain the present herd immunity and to offer better protection in the younger age cohorts a study to define the proper age for a booster vaccination will be conducted in 2011.

P 055

NATIVE EXPOSURE OF VACCINE CANDIDATE PROTEIN FHBP IN CLINICAL ISOLATES OF NEISSERIA MENINGITIDIS.

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Background and aims. The factor H binding protein (fHbp) in *Neisseria meningitidis* (Nm) is currently evaluated in clinical trails as a vaccine candidate for a meningococcal serogroup B vaccine. The aim of the present study was to investigate the native expression of the antigen and sequence variation of the gene in clinical isolates to explore fHbp´s potential as a vaccine.

Material and methods. The material consists of different Nm isolates collected in Sweden: invasive isolates from 2001-2002 (n=95), invasive isolates from patients with a fatal outcome collected in 1995-2004 (n=62), and carrier isolates from 1995-2004 (n=62). The fHbp was DNA sequenced and allele numbers were designed using the Neisseria Sequence Typing Home Page (http://pubmlst.org/neisseria/). Protein expression was examined under in vivo like conditions using Fluorescence-activated cell sorting (FACS) with a monoclonal antibody (502 anti fHbp, variant 1 from MC58) and a polyclonal antibody (mouse serum anti fHbp variant 1.1 from MC58).

Results and Conclusions. The sequence analysis showed that allele 1 was the most common type in both the isolates

from patients with a fatal outcome and in the invasive isolates (42% and 29% respectively). In comparison to the carrier isolates this allele was only identified in 2%. The fHbp expression was identified in all except seven isolates. These negative samples are currently being investigated further by Western blot analysis. In 18% of the isolates the monoclonal antibody gave a very weak response, and the comparable number for the polyclonal antibody was 31%.

fHbp is widely expressed in clinical isolates and is therefore a potentially vaccine candidate against serogroup B Nm.

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P 056

INSERTION SEQUENCES IN THE PROMOTER REGION OF FHBP

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Background and aims: Insertion sequences (IS) are mobile elements involved in genomic microevolution. They can induce reversible encapsulation, and/or inactivation of virulence genes encoding outer membrane proteins. The *Neisseria meningitidis* genome contains a relatively large amount of insertion sequences. In this study we analysed the presence and spreading of mobile elements in the promoter region of factor H binding protein (fHbp) of *Neisseria meningitidis*. fHbp is included in the multicomponent protein-based vaccine (4CMenB) being evaluated in Phase III clinical trials.

Material and methods: fHbp gene, including the promoter region, was amplified and sequenced in 327 invasive strains isolated in Europe and USA, in both pathogenic and carrier strains from the Czech Republic. All fHbp sequences are present in the MLST database accessible at http://pubmlst.org/neisseria/fHbp/.

Results and Conclusions: The insertion of a 181 bp element, named 'ATR' (A+T Rich), was present in the promoter region of fHbp-1.4, -1.5 and -2.24 sub-variants. All isolates carrying fHbp-1.4 and fHbp-1.5 have 'ATR', whereas only some isolates harbouring fHbp-2.24 have this insertion.

All strains carrying 'ATR' in the promoter of 2.24 were pathogenic. fHbp-1.4 is usually harboured by cc41/44 pathogenic strains. fHbp-1.5, which shares 99% identity with fHbp-1.4, was found in serogroup A strains.

The insertion sequence IS*1655* was also detected in the promoter region of fHbp in a subset of invasive strains harbouring fHbp 2.19, while the IS*1106* element was found in carrier strains harbouring sub-variant 2.333.

The influence of mobile elements on fHbp trascription/expression is under investigation through the analysis of natural and recombinant *N. meningitidis* strains.

P 057

VARIATION OF THE *NEISSERIA* HEPARIN BINDING ANTIGEN OF MENINGOCOCCUS

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Background: *Neisseria* Heparin Binding Antigen (NHBA), a meningococcal surface protein, is a novel vaccine antigen that induces protective immunity in humans. NHBA is structured into a substantial number of alleles that have some association with clonal complexes and Sequence Types (STs), and also with several sub-variants of factor H binding protein (fHbp), another novel vaccine antigen.

Methods: NHBA was characterized in 1530 pathogenic isolates collected after 1960 and before 2009 by several European Reference Laboratories and the US CDC. Strains were characterized by MLST (http://pubmlst.org/neisseria/). Molecular variability of NHBA was studied to assess the evolutionary mechanisms of selection and recombination.

Results: The *nhba* gene was present in all strains: 93 amino acid sequences were identified. A nomenclature scheme was introduced. NHBA peptide 2 (22%), 21 (15%), 20 (11%), 17 (10%), 18 (5%), 3 (4%), 5 (4%) and 29 (4%) were the most frequently observed in this panel. Associations between NHBA amino acid sequences and clonal complexes were consistent with observations made in other strain panels (1). Moreover, we assessed associations of several NHBA peptides with STs,

in particular with the two central genotypes of cc41/44, ST-41 and ST-44, respectively, and with the ST-269 and ST-275 of cc269. Associations between several NHBA peptides and fHbp sub-variants were also noticed. Analysis of recombination and selection on the allele sequences demonstrated that parts of the *nhba* gene are subject to selection for non-synonymous mutations, in agreement with the presence of immune selective pressure.

1 Bambini et al, Vaccine 2009

P 058

IN VITRO LEVELS OF NADA EXPRESSION MAY UNDERESTIMATE THE POTENTIAL EFFECTIVENESS OF IMMUNE RESPONSES AGAINST *NADA* IN VIVO.

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Background and aims: NadA is a component of 4CMenB, a recombinant MenB vaccine being evaluated in Phase III clinical trials. We used a novel meningococcal antigen typing system (MATS) that correlates with killing in the serum bactericidal assay using human complement (hSBA) to define the quantity of the NadA expression that allows strains to be killed in hSBA. Under the *in vitro* conditions of growth of the bacteria for hSBA and for typing, NadA antigen is repressed by a regulatory protein NadR/NMB1843.

Methods and Results: NadA expression is induced by incubation of the bacteria with Human saliva (HS) or exposure to the small molecule inducer 4HPA that is present in HS by relieving NadR repression. A strain panel covering a range of NadA levels was selected and when repression is relieved through knocking out of the NadR regulator, *nadR*$^-$ strains all expressed comparably high levels of the NadA antigen. Sera from clinical trial subjects of different age groups immunized with the rMenB were able to kill all of the *nadR*$^-$ strains confirming that NadA is a powerful immunogen.

Conclusions: In this study, all strains with the *nadA* gene (*nadA*+) had the ability to express high levels of NadA antigen when its repression was relieved. In vivo, we expect NadR

repression to be alleviated due to niche-specific signals, resulting in high levels of NadA expression from any *nadA*+ strain and therefore efficient killing by the anti-NadA antibodies.

P 059

UPDATE OF THE VACCINATION STRATEGY IN THE CZECH REPUBLIC IN 2010

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Background and aims: The vaccination strategy against meningococcal disease is regularly updated in the Czech Republic according to the epidemiological situation and currently available meningococcal vaccines. The incidence of invasive meningococcal disease (IMD) in the Czech Republic is low and meningococcal vaccination is voluntary. A new tetravalent conjugate vaccine A,C,Y,W135 was launched in the Czech Republic in October 2010.

Material and methods: The National Reference Laboratory for Meningococcal infections analysed the surveillance data and prepared a new recommendation for vaccination against meningococcal disease. This recommendation was passed to the Ministry of Health and to the National Immunisation Committee.

Results and conclusions: The incidence of IMD caused by serogroup C is currently low and there is no indication for mass vaccination with MenC conjugate vaccine. The involvement of serogroup Y in IMD cases has increased in last years, causing the highest serogroup-specific case fatality rate. The recent expert recommendations for booster vaccination with conjugate meningococcal vaccines were considered.

A new recommendation was issued by the National Immunisation Committee in November 2010:

- Vaccination of infants aged 2-6 years with MenC conjugate vaccine.
- Revaccination (primovaccination) of adolescents aged 11-14 years with tetravalent conjugate vaccine A,C,Y,W235.
- The 7-10-year revaccination interval should be shortened when epidemiologically or clinically indicated.
- MenB vaccine is needed for infants, but the sero/subtype coverage by the porin-based vaccines is low for Czech meningococcal isolates. A vaccine other than porin-based, effective against *N. meningitidis* B is needed.

P 060

AN OUTER MEMBRANE VESICLE VACCINE AGAINST MENINGOCOCCAL DISEASE FOR AFRICA

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Background and aims. Serogroups A and W_{135} meningococci are the main causes of meningococcal disease epidemics in sub-Saharan Africa. The outbreaks are clonal and express the same surface proteins PorA and PorB over time. In this situation an outer membrane vesicle (OMV) vaccines may be effective. NIPH and Finlay Institute intend to bring forward an A+W OMV vaccine candidate to clinical phase trial in Cuba in 2011.

Materials and methods. The vaccine is produced from representative epidemic serogroup A and W_{135} strains from Africa. Assays to assess identity, purity and potency of the vaccine were tailored to the A+W OMV vaccine. Several pilot batches of OMVs from each serogroup were tested in these assays. Preliminary toxicological evaluation was performed in rabbits and rats. The OMV vaccine was compared with commercially available conjugate and plain polysaccharide vaccines for immunogenicity in mice. IgG antibody responses was measured by ELISA, and functional activities were detected by bactericidal (SBA) and opsono-phagocytic (OPA) assays.

Results: A process to produce the OMV vaccine at 100L fermentor scale has been successfully established at Finlay

Institute. Quality assurance testing indicated that the vaccine passed the standard acceptance criteria for pyrogenicity and endotoxin content. The functional antibody titres (SBA and OPA) against the A and W_{135} strains were significantly higher in mice immunised with the OMV vaccine than with those immunised with the conjugate or plain polysaccharides vaccines.

Conclusions: These results indicate that the vaccine is suitable for testing in humans, and that it has the potential to become an effective vaccine for prevention of meningococcal disease in Africa.

P 061

DETERMINATION OF SERUM BACTERICIDAL TITERS AGAINST GROUP B MENINGOCOCCUS BY FLOW CYTOMETRY USING FLUORESCENT VIABILITY DYES.

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Background and aims: Measurement of serum bactericidal activity (SBA) is a long-accepted correlate of protection against meningococcal disease recognized by regulatory agencies around the world including the FDA and EMEA. The conventional agar-based assay used to assess bactericidal antibody responses to meningococcal vaccines is both labor intensive and time consuming. Alternative SBA assays readout systems are being developed to increase sample through-put over the conventional assay without compromising the accuracy and reproducibility of results.

Methods and Results: We report here a flow cytometric based assay using the fluorescent viability stains Syto9 and propidium iodide to distinguish live from dead cells respectively based on their differential membrane permeability properties. Complement mediated serum killing was measured in both MenB and E. coli.

Results obtained using the flow cytometer showed good agreement to the standard agar plating method. Flow cytometric SBA results were typically within 2-fold of titers obtained by cfus determination on agar plates.

Conclusions: Measurement of bactericidal activity by flow cytometry is a promising alternative to the conventional agar-

based assay. Data are obtained within hours following complement mediated bacterial killing.

P 062

IMPACT OF COVERAGE WITH QUADRIVALENT MENINGOCOCCAL CONJUGATE VACCINE (MENACWY) ON DISEASE INCIDENCE, UNITED STATES, 2005-2009

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Background: MenACWY was recommended by the Advisory Committee on Immunization Practices for adolescents in the United States in 2005. National vaccination coverage was 53.6% in 2009 with a range of 19.3% to 75.7% among the states.

Methods: The National Immunization Survey - Teen measures vaccination coverage among 13-17 year-olds. We divided the 50 U.S. states and the District of Columbia into quartiles based on the mean vaccination coverage in 2008-2009 (Q1: 17.1%-34.6%, Q2: 35.4%-43.2%, Q3: 44.2%-54.0%, Q4: 56.7%-69.2%). We used all cases of meningococcal disease (all case statuses and serogroups) reported to the National Notifiable Diseases Surveillance System (NNDSS) to determine disease incidence by quartile group during 2005-2007, and 2008-2009. We compared changes in disease incidence among 13-17 year-olds, 11-19 year-olds, children <5 years, and adults ≥25 years.

Results: The incidence of all cause meningococcal disease reported to NNDSS was 0.47 per 100,000 population in 2005-2007, and 0.31 per 100,000 population in 2008-2009. The table below shows the percent change in meningococcal disease incidence between 2005-2007 and 2008-2009 by quartiles:

MenACWY Coverage	13-17 year-olds	11-19 year-olds	children <5 years	adults ≥25 years
	% change	% change	% change	% change
Q1	+5	+4	-27	+10
Q2	-2	-13	-18	+6
Q3	-32	-24	-11	+13
Q4	-63	-46	-31	-23

Conclusions: There have been greater declines in meningococcal disease (all serogroups) among adolescents living in states with higher vaccination coverage. Increasing overall MenACWY vaccination coverage will likely have further impact on adolescent disease.

P 063

AN IMPROVED METHODOLOGY TO ENHANCE THE THROUGHPUT OF A MENINGOCOCCAL ANTIGEN TYPING SYSTEM (MATS)

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Background: Meningococcal Antigen Typing System (MATS), a sandwich ELISA, measures the level of protein (fHBP, NadA, and NHBA) surface expression on isolates of *Neisseria meninigitidis* serogroup B (MenB). A detergent extract (lysate) containing these proteins and used to competitively bind antibodies in the assay, is a time-consuming step and limits throughput. Here we describe a procedure to improve assay throughput.

Materials and methods: MATS lysates for MenB strains (n=6) were compared for their MATS immunoreactivity (OD_{490}) and relative potency using 1) a standard protocol (freshly prepared, non-frozen lysate) and, 2) a modified protocol (lysates frozen with dry ice-ethanol). Lysates were tested with all antigens three times over 30 days.

Results and conclusions: Frozen lysates were stable (at least 30 days) with minimal loss (<10%) in immunoreactivity. Strong correlation (r^2>0.9; P≥1.0) in MATS immunoreactivity was seen between fresh and frozen lysate especially in strains with high immunoreactivity. Two strains with extremely low immunoreactivity and relative potency exhibited modest correlation (r^2=0.69 and 0.77) supporting the substitution of frozen lysates for freshly prepared, non-frozen lysates. Replacing fresh lysate with frozen lysate for the reference

strain alone will reduce the lysate and assay preparation time by 33% and thus greatly improve throughput.

P 064

BEAD BASED MULTIPLEX MENINGOCOCCAL ANTIGEN TYPING SYSTEM (BMATS)

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Background: The Meningococcal Antigen Typing System (MATS) is an ELISA based immune-phenotyping technique used for *Neisseria meningitidis* B (MenB) strains. Novartis MenB vaccine, 4CMenB, has 4 major components of which 3 are detected in a bacterial lysate by ELISA. To test a single strain, 3 individual MATS ELISAs have to be performed. We have developed a multiplexed bead based meningococcal antigen typing system, BMATS, where three or more antigens are measured simultaneously.

Materials and methods: A multiplex IgG capture assay was developed to quantify anti-4CMenB IgG. BMATS detects the homologous competition between the surface expressed/exposed antigens in the bacterial lysate and antigen conjugated fluorescent beads for antigen specific antibody (IgG) in the capture assay. Using xMAP technology, fluorescent beads are screened for captured antigen specific IgG and expressed as median fluorescence index (MFI). A strain with high density and/or crossreactivity of antigen results in specific reduction in MFI. This inverse relationship establishes the MenB antigen phenotype. Eighteen MenB strains were tested by both methods.

Results and conclusions: Assay sensitivity, specificity, reproducibility and robustness are presented. BMATS successfully detected antigens on all MenB strains tested. Reference strains for fHbp and NHBA yielded 100% reduction in MFI, and NadA yielded 70% reduction. BMATS was in

agreement with MATS ELISA for all 18 strains tested. We plan to validate BMATS and develop data reduction software to calculate antigen specific relative potency for each isolate.

P 065

IMMUNOGENICITY AND SAFETY OF A QUADRIVALENT MENINGOCOCCAL POLYSACCHARIDE DIPHTHERIA TOXOID CONJUGATE VACCINE IN INFANTS AND TODDLERS

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Background and Aims: The highest incidence of meningococcal disease is in children <2 years of age. A phase II study evaluated immunogenicity and tolerability of 2 doses (≥3 months apart) of sanofi pasteur's Menactra (MCV4) in healthy children ≥9 months of age. Safety was further examined in a later phase III study.

Materials and Methods: Participants in the phase II study received 2 doses of MCV4: at ages 9 and 12 months, 9 and 15 months, or 12 and 15 months. A control group of 3–5 year olds received 1 dose of licensed quadrivalent polysaccharide vaccine (MPSV4). Sera, obtained at baseline and 28 days after Dose 2, were assessed by serum bactericidal assay (SBA) with human complement. In the phase III safety study, participants received MCV4 at ages 9 and 12 months. Safety was followed until 6 months postvaccination in both studies.

Results: The 2-dose MCV4 schedules elicited seroprotective titers ≥1:8 in 85%–89%, 100%, 94%–96%, and 92%–96% of participants for serogroups A, C, Y, and W-135, respectively, all higher than those observed in the control group. Reactogenicity after MCV4 Dose 2 was comparable to that observed after Dose 1.

Conclusions: Robust increases in SBA titers against all 4 serogroups after MCV4 Dose 2 exceeded those seen in the group receiving MPSV4. Two MCV4 doses were well tolerated by infants and toddlers.

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P 066

VALIDATION OF SERUM BACTERICIDAL ASSAY USING HUMAN COMPLEMENT (SBA-HC)

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Background and Aims: The serum bactericidal assay (SBA) has been the gold standard *in vitro* assessment to measure complement-mediated antibodies that kill *Neisseria meningitidis* (Nm) bacteria. The SBA has become an accepted surrogate for the immune correlate of protection for vaccines targeting Nm. Human complement (HC) was the initial source of assay complement, but baby rabbit complement (BRC) has also been accepted as an alternative source. Both SBA-HC and SBA-BRC assays have been used in licensing Menactra® [MCV4, Sanofi Pasteur]. This presentation describes the validation of SBA-HC to assess the functional immune response for conjugate polysaccharide vaccines against Nm serogroups A, C, Y, and W-135. Methods to identify and qualify HC are also described.

Materials and Methods: The assay was developed and performed under GCLP, and validated following International Conference on Harmonization guidelines. Assay validation parameters included precision, accuracy, dilutability, specificity, lower limit of quantitation (LLOQ) and robustness.

Results and Conclusions: The assay validation results will be presented. The data analyses demonstrated that the SBA-HC met pre-defined acceptance criteria for validation and could measure anti-polysaccharide bactericidal activity to Nm serogroups A, C, Y, and W-135 in human serum.

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P 067

EVALUATION OF THE IMMUNOLOGICAL PROPERTIES OF THE NEISSERIAL HEPARIN BINDING ANTIGEN (NHBA)

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Background: Neisserial Heparin Binding Antigen (NHBA) is a surface-exposed lipoprotein of *Neisseria meningitidis* which binds heparin-like molecules. NHBA is an antigen of the multicomponent 4CMenB vaccine able to induce bactericidal antibodies in laboratory animals and humans. The aim of this study is to investigate the potential cross protection of NHBA-induced bactericidal antibodies against a panel of *N. meningitidis* strains.

Methods: We used various approaches to investigate the level of cross protection mediated by human anti-NHBA antibodies. In order to characterize only the immunological properties of NHBA we selected *N. meningitidis* strains mismatched for the other vaccine antigens (fHbp, NadA and PorA1.4). These strains have been tested in a Serum Bactericidal Assay using human complement (hSBA) and human sera from different age groups vaccinated with the 4CMenB vaccine. To further prove that the immune response was directed against NHBA, we performed a competitive hSBA using the NHBA recombinant antigen and also generated NHBA deletion mutants in different genetic backgrounds.

Results and Conclusions: The hSBA analysis showed that human sera raised against the 4CMenB vaccine are able to kill natural *N. meningitids* strains harboring different NHBA amino acidic sequences. We also demonstrated that the addition of

recombinant NHBA antigen or the deletion of *nhba* gene abolished or significantly decreases bactericidal titers.

These results demonstrate that NHBA is an important vaccine antigen able to induce cross-protective bactericidal antibodies against genetically different strains in different age groups vaccinated with the 4CMenB vaccine.

P 068

PREVALENCE OF SERUM BACTERICIDAL ANTIBODY TO SEROGROUP C *NEISSERIA MENINGITIDIS* IN ENGLAND, A DECADE AFTER VACCINE INTRODUCTION

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Background and aims: Meningococcal serogroup C conjugate vaccination started in the UK in 1999, with three doses for infants and single-dose "catch-up" up from age 1 to 18 years. Disease incidence and carriage rapidly fell, but antibody levels waned quickly in infants. In 2006, a revised schedule of 2 priming doses plus booster at 1 year was introduced. This study assessed age-specific protection using 2009 samples, compared with similarly-designed historical pre-vaccination and early post-vaccination data.

Method: Serum bactericidal antibody (SBA) was measured in anonymously banked serum samples collected in 2009 (n=1174), taking SBA titres ≥8 as protective.

Results and conclusions: Antibody titres were higher than before vaccine introduction, with 35% (95%CI 33-38) having titres ≥8. Protective levels were moderate (32%; 24-40) in children on the current schedule. In cohorts that received only infant doses without booster, levels declined markedly after 6

years, suggesting poor antibody persistence. In cohorts eligible for catch-up vaccination, higher levels resulted from school-age and adolescent vaccination, peaking in those vaccinated at 14 years (70%; 50-90).

Among non-immunised adults, the marked decline observed in the early post-vaccine period (presumed due to vaccine-interrupted circulation of the organism) has been reversed, possibly indicating some restored circulation.

Together, these observations underscore a likely need for adolescent boosters to maintain antibody persistence and prevent potential increased circulation.

P 069

HIGH FREQUENCY OF MNB DISEASE IN INFANTS CAUSED BY STRAINS CARRYING SUBFAMILY A FACTOR H BINDING PROTEIN INDICATES THE NEED FOR A BIVALENT FHBP VACCINE

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Background: *Neisseria meningitidis* serogroup B (MnB) disease afflicts all age groups, with peaks of disease observed in infants and young children (<5 yr) and adolescents/young adults (11-25 yr). Adolescents are an important age group for vaccination efforts. As with infants, vaccination provides direct protection. Adolescents also exhibit the highest rate of carriage; thus an efficacious vaccine may also lead to herd immunity and disease reduction in other age groups. Factor H binding proteins (fHBPs) are promising vaccine candidates that exist as two immunologically distinct subfamilies (A and B).

Results: We examined the distribution of fHBP sequences and multilocus sequence types (STs) among 1814 systematically selected MnB isolates from 6 European countries and the US[1,2] from 2000-2006; 392 isolates were from infants and 440 from adolescents. There was no consistent link between STs and age. Analysis of the distribution of fHBP subfamily with respect to patient age indicated that infants have a significantly higher proportion of subfamily A disease than adolescents (47.4% vs. 18.4%,

p<0.0001). Minor country to country differences were observed. This analysis was not weighted to account for epidemic strains, the impact of which will be presented.

Conclusions: The high frequency of disease in infants caused by subfamily A carrying strains underscores the value of including a subfamily A component within a fHBP based vaccine.

[1] Murphy et al J Infect Dis. 2009 Aug 1;200(3):379-89

[2] Zlotnick et al #P045 p.81 EMGM 2009.

P 070

PREVALENCE OF FACTOR H BINDING PROTEIN (FHBP) VARIANTS IN *N. MENINGITIDIS* CARRIAGE AND DISEASE ISOLATES IN THE UK

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Background and aims: fHBP is undergoing clinical trials as a vaccine for *N. meningitidis* serogroup B invasive disease. fHBP variants fall into one of two subfamilies, A or B, with a limited number of variants accounting for the majority of disease strains. In contrast, little is known concerning the diversity of fHBP variants in carriage isolates. It is important to monitor changes in the fHBP variants circulating in both carriers and disease patients over time to determine the potential coverage of fHBP-containing vaccines. Here we investigate changes in the prevalence of fHBP variants in carriage and MenB disease isolates in the UK between 1999 and 2009.

Materials and Methods: fHBP, MLST and serogroup were determined for *Neisseria meningitidis* strains isolated in three separate carriage studies undertaken in Nottingham, UK, in 1997, 1999-2001 and 2008-09. The same typing data was also determined for UK MenB disease strains isolated in 2001 and 2006.

Results and Conclusions: In invasive MenB isolates, the prevalent fHBP variants were B16, B44, B09, A22, B03 and A05 and their distribution remained constant between 2001 and 2006. In contrast, different variants were predominant in meningogcoccal carriage strains compared to invasive MnB strains. Significant differences were also apparent between 1999-2001 and 2008-09 meningococcal carriage isolates that

are not associated with disease. In the older carriage isolates, A19, A22 and B09 variants predominated; whereas in those from recent years, B09, A15, A26 and A07 predominated.

P 071

RETROSPECTIVE ANALYSIS OF FHBP, NADA AND NHBA CODING GENE PREVALENCE WITHIN THE 162 CLONAL COMPLEX STRAINS ISOLATED IN GREECE

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Objectives: The aim of this study was to elucidate the presence as well as the variation of *fHbp*, *nhba* and *nadA* genes which are included in 4CMenB vaccine in epidemiologically relevant Greek serogroup B meningococcal isolates belonging to 162 clonal complex.

Methods: A total of 70 serogroup B- 162 cc strains isolated in Greece, during the period 1999-2009 were investigated. These were obtained from 58 IMD cases as well as 12 carriers. In addition to MLST, the isolates were characterised by serogroup, serotype and porA genosubtype. Presence and genetic diversity of fHbp, NHBA and NadA was determined using PCR and sequence analysis.

Results: All isolates harboured *fHbp* and *nhba* genes. In contrast, no presence of *nadA* gene was observed. According to fHbp sequence analysis, the examined strains were distributed to variants 1 (46%), 2 (45%) and 3 (9%). The most frequent fHbp peptide was the 21 (38%) and for the NHBA was the peptide 20 (93%). The predominant combination was fHbp 2.21, NHBA 20 and PorA P1.22,14 (18.5%).

Conclusion: Among the 162cc strains examined, the presence of *fHbp* and *nhba* genes is universal, while no *nadA* gene was present. The fHbp sequence analysis reveals a high variability in contrast to NHBA. Further investigation on

variability of the vaccine components is necessary for evaluation of the potential strain coverage

POSTER PRESENTATION P 072 – P 086,

Section: Diagnosis and typing

P 072

ASSESSMENT OF FINETYPE-SPECIFIC CLUSTERS OF INVASIVE AND CARRIED MENINGOCOCCAL STRAINS WITH MULTIPLE LOCUS VNTR ANALYSIS (MLVA)

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Using genomes from carrier (α14, α153, α275, α710) and invasive (NMCC053442) isolates we adapted the Multiple Locus Variable-Number-Of-Tandem-Repeats Analysis (MLVA) scheme by Schouls et al. (JCM, 2006) to a multiplex approach requiring 2 amplification rounds per strain. Spatiotemporal clusters were retrospectively detected with the program SaTScan (version 5.1.3) in a dataset comprising fully finetyped (i.e. PorA and FetA typed) strains of 3417 non-duplicate cases of invasive disease between 2002 and 2009. Maximum cluster duration was 30 days, whereas maximum cluster area was defined by a population of 5.8 million people. Strains with high evidence of clustering (p=0.001) were matched at a 1:2 ratio to randomly selected controls of the same finetype. Strains were subsequently typed with MLVA; variation within case-control groups was measured using the Gower distance between MLVA profiles. Similarly, 7 and 6 finetype-specific clusters from carriers and invasive disease (latter caused exclusively by ET-15), respectively, were analyzed by MLVA. Interestingly, mean variation within spatiotemporal clusters and controls did not differ significantly (0.15 vs. 0.16, p=0.69). Nevertheless, distances within carrier and invasive clusters by ET-15 were significantly reduced (0.05 and 0.01, respectively). Less variation within carrier compared to spatiotemporal case-control groups may be due to the smaller number of transmissions. However, conservation of MLVA profiles within ET-15 may be due to the less recombinant nature of this type. To further clarify the picture, MLST will be done for all carrier

and case-control isolates; also, a collection of strains obtained from cases and close contacts will be included.

P 073

GENETIC ANALYSIS OF THE *NEISSERIA MENINGITIDIS* CAPSULE LOCUS

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Background and Aims: Thirteen serogroups have been described: A, B, C, D, 29E, H, I, K, L, W-135, X, Y and Z. With the exception of D, capsular polysaccharides have been characterised chemically and structurally with genes involved in capsule synthesis sequenced in serogroups 29E, L, W-135, X, Y and Z. This study explored the genetic relatedness of the meningococcal capsular locus among all thirteen serogroups and has compared these to capsule genes belonging to other bacterial species.

Materials and Methods: The capsule operon was sequenced in a representative *N. meningitidis* isolate from each serogroup D, 29e, H, I, K, L, W-135 (2 isolates), Y (2 isolates), X and Z.

Results and Conclusions: All of the serogroups examined had similar organisation of the capsule. Serogroup H contained genes involved in serogroup Z capsule synthesis and these shared significant sequence identity with *cps* genes belonging to *Actinobacillus pleuropneumoniae*. Serogroups I and K had identical capsule loci sharing sequence identity with genes involved in capsule synthesis in *Mannheimia haemolytica*. Phylogenetic analyses revealed common ancestry of some of the capsule genes among all of the bacterial species analysed.

P 074

AN UPDATE OF STANDARD REAGENTS AVAILABLE FROM THE NIBSC

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A number of meningococcal typing and ELISA reagents are available from the NIBSC. These include monoclonal antibodies specific for meningococcal surface structures, meningococcal polysaccharides, methylated human serum albumin (mHSA) and CDC1992 human reference serum.

Monoclonal Antibodies (mAbs): NIBSC holds a panel of hybridoma cell lines which express mAbs specific for different meningococcal surface antigens including outer membrane proteins (OMPs), polysaccharides and lipo-oligosaccharides (LOS). Between 2001 and 2004 the original panel of standards made from ascities fluid were replaced with cell culture supernatant. The mAbs were produced to characterise disease isolates and are primarily directed against the capsule (serogroup), PorB (serotype) and PorA (serosubtype). Overtime the panel have become less representative of circulating strains and PCR-based technologies are now the methods of choice for strain characterisation. However, mAbs are still used by typing laboratories and increasingly for vaccine characterisation and as reagents for research and biological assays.

ELISA reagents: Polysaccharides are used as coating antigens in ELISAs for screening serum from patients following vaccination with polysaccharide based vaccines. In addition, mHSA (used to bind the PS to the surface of the ELISA plate) and anti-meningococcal serogroup A/C/W-135/Y CDC1992 reference serum are available.

New Reagents: Recently an anti-factor H binding protein (JAR4) has been added to the panel of mAbs and anti-FetA mAbs are in production.

Under the auspices of WHO, NIBSC have prepared a meningococcal serogroup C polysaccharide International Standard which will be available in the fourth quarter of 2011.

P 075

NEISSERIA MENINGITIDIS NUCLEIC ACID AMPLIFICATION TESTING (NAT) STANDARD 09/320 FOR USE IN QUANTITATIVE PCR DIAGNOSTIC ASSAYS

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Background: Real-time PCR has become the standard diagnostic technology for numerous pathogens including the meningococcus. The lack of a standardised positive control has resulted in variability in the detection thresholds of these assays. NIBSC is developing a range of 'run control' working reagents for use in clinical infection laboratories. The reagents are designed to be similar to clinical samples and should be extracted and amplified in parallel to assess the performance of the entire assay. The reagents are designed to be a low positive to ensure the sensitivity of the assay with a real time PCR threshold or Ct of approximately 30.

Results and Conclusions: The *N. meningitidis* control consists of a frozen dilute heat killed suspension of strain H44/76 grown in Columbia agar. A collaborative study involving seven laboratories was undertaken each participant assessed three working reagents (WR) at different dilutions (WR1, 2 and 3). Different levels of intra-laboratory variation were observed but in general the variation was low. Fifty percent of the datasets produced 2SD values of less than 0.5 cycles, whilst 25% of the datasets had 2SD values of 1 cycle or above. The overall 2SD values across the laboratories were 3.08 (WR1), 2.26 (WR2) and 1.93 (WR3) cycles, equating to 1 $\log_{10}$ difference or less in a 100% efficient PCR reaction. The overall laboratory mean C_t values were 29.92, 33.47 and 36.64 for WR1, WR2 and WR3 respectively. Based upon this data WR1 was selected to produce the Nucleic acid

Amplification Testing (NAT) standard 09/320 available from the NIBSC.

P 076

ADDED VALUE OF PCR TESTING FOR DIAGNOSIS OF MENINGOCOCCAL MENINGITIS IN ENGLAND AND WALES

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Background and Aims: The Health Protection Agency Meningococcal Reference Unit (MRU) provides a free PCR service in England and Wales. This study assessed the added value of PCR testing for confirmation of meningococcal disease in relation to laboratory culture confirmation.

Material and Methods: The proportion of patients with a positive PCR test at the MRU in 2009-10 was assessed according to patient demographics, month of test, specimen type and number of PCR tests performed. Results were compared with laboratory culture results.

Results: There were 27,087 specimens from 23,527 patients. A total of 1,601 patients (6.8%) tested PCR positive of whom 1,182 (73.8%) were culture-negative. The proportion PCR-positive was highest amongst 17-20 year olds (152/720, 21.1%) and lowest in infants <3 months (35/2,073, 1.7%). Positivity rate was highest in January (255/2015, 12.7%), and lowest in August (57/1501, 3.8%). Among patients with a single sample submitted, the proportion positive was 6.9% (16/2,313) for CSF, 5.4% (810/15,147) for EDTA blood and 3.7% (116/3,112) for other samples. Multiple samples were submitted for 2,955 patients (12.6%). Results were discordant for seven patients (0.2%), including two patients initially testing

negative, and then positive. A total of 503 patients were confirmed by culture only, of whom 38 were PCR-negative and 465 were not tested by PCR.

Conclusion: Out of 2104 patients tested positive for meningococcal disease in 2009 and 2010, 1,182 (56%) patients would not have been confirmed without PCR testing. PCR testing could be made more cost-effective by testing only one sample per patient.

P 077

SEQUENCING OF *CAP* REGION II OF *HAEMOPHILUS INFLUENZAE* SEROTYPES C AND D

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Background and aims: The sequences of the capsule synthesis (*cap*) regions II of *H. influenzae* serotypes a, b, f and recently e have been published previously, whereas they have remained unknown for serotypes c and d (Hic and Hid). We here report the serotype specific capsule regions of Hic and Hid and discuss the phylogenetic development of bacterial capsules based on similarity searches.

Material and methods: The sequences of the *cap* region II of Hic ATCC 9007 and Hid ATCC 9008 were identified by PCR and primer walking. Assembly was controlled by sequential overlapping PCRs derived from the obtained contig.

Results and Conclusions: The sequences of Hic and Hid revealed four (*ccs1-4*) and five (*dcs1-5*) open reading frames, respectively. Ccs1, a capsular polysaccharide phosphotransferase as identified by sequence comparison, and Ccs2, a member of glycosyltransferase family 2, may be involved in the capsule synthesis of Hic, where a phosphate linkage connects O-acetylated β-D-GlcNAc to α-D-Gal-1. Ccs4 showed similarities to the capsule O-acetyltransferase of *Neisseria meningitidis* serogroup W-135 and was found to be subject to phase variation. In Hid, the putative functions of Dcs1 (UDP-N-acetyl-D-glucosamine 2-epimerase) and Dcs2 (UDP-N-acetyl-D-mannosaminuronic acid dehydrogenase) suggest a role in the conversion of β-D-GlcNAc to β-D-ManANAc, which both consitute the repetitive disccharide unit of the capsule polysaccharide. The similarity of genes and gene organization found in Hic and Hid to capsulation genes

and operons in *Actinobacillus spp.* as well as to more distant genera including *N. meningitidis* suggest horizontal gene transfer during capsule evolution across the bacterial classes.

P 078

MENINGOCOCCI OF ST-11 CLONAL COMPLEX IN POLAND

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Aim: The purpose of the study was to characterize *Neisseria meningitidis* isolates of clonal complex (cc) ST-11 responsible for invasive meningococcal disease (IMD) in Poland.

Material and methods: All invasive meningococcal isolates collected since 1997 until 2010 were identified, serotyped and characterized by MLST, *por*A and *fet*A typing. Minimal inhibitory concentrations were assessed by the Etests.

Results: Hundred and twenty one cases of IMD caused by isolates of ST-11cc, including 23 fatal cases were analyzed. Until 2006 sporadic cases were registered only, but afterwards at least 7 outbreaks in various parts of the country were observed. Teenagers and young adults were mostly affected. The majority of isolates belonged to serogroup C (81.8%), 14.1% to W-135 and 4.1% to B. All were susceptible to cefotaxime, chloramphenicol, ciprofloxacin and rifampicin, while 13.4% were nonsusceptible to penicillin. Among isolates of ST-11cc the most frequent were ST-11 (80.2%) and ST-247 (11.6%). Until 2003 the most common porA variant was 5/2 whereas in later years, 5-1/10-1. In 2009, again most frequent was 5/2. Sequencing of fetA indicated that until 2009 all ST-11 isolates of serogroup C, but one, had F3-6 variant. Interestingly, isolates causing last outbreak in 2009 represented variant F3-3.

Conclusions: Since 2006 occurrence of outbreaks may be partially explained by the observed antigenic changes in the PorA and FetA proteins, since it has been suggested that even

very small alterations in antigenic characteristics may result in an increase in the number of IMD cases.

P 079

MOLECULAR TYPING OVERVIEW OF CZECH SEROGROUP B MENINGOCOCCI

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Background and aims: Serogroup B strains represent a major cause of meningococcal disease in the Czech Republic in the third millennium. A similar status applies across the continent and MenB vaccine is under pharmaceutical development. Sequence typing of serogroup B isolates from invasive disease identifies clonal shape of strains in the local host population supposed to be protected by vaccination.

Material and methods: Three hundred and forty serogroup B invasive isolates from 2001-2011, i.e. all isolates referred to the NRL by clinical laboratories in the period indicated, were typed by MLST and *porA*, *fetA* and *penA* sequencing.

Results and conclusions: Sixteen clonal complexes were detected, involving 85% of the isolate collection. ST-41/44 complex was the most frequent (22% in the whole, 9% related to ST-41 ancestor), ST-32 complex represented 20% of the collection, ST-18 and ST-269 complex 10% each and ST-213 and ST-35 complex 4% each. The clonal composition did not undergo a significant shift along the period. Overall allelic features of the *penA* gene indicated good antibiotic sensitivity of strains. P1.4 specificity related to porin A vaccine

component was detected in 2% of serogroup B isolates only. In spite of an efficient GNA antigenic vaccine component coverage reported for major hypervirulent complexes as ST-269 or ST-32, genetic and antigenic analyses of overall feasibility of the vaccine are necessitated because of the substantial clonal split in the Czech serogroup B meningococcal population.

The work was supported in part by grant No. NT11424-4/10 from the Internal Grant Agency of the Ministry of Health of the Czech Republic

P 080

MULTILOCUS SEQUENCE TYPING OF *HAEMOPHILUS INFLUENZAE* IN THE CZECH REPUBLIC

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Background: We present the results of multilocus sequence typing (MLST) of *Haemophilus influenzae* strains isolated from invasive and non-invasive cases in the Czech Republic in 2001–2009.

Material and methods: A total of 207 *H. influenzae* strains, of which 153 were *H. influenzae* type b (Hib), 2 *H. influenzae* type e (Hie), 2 *H. influenzae* type f (Hif) and 50 non-capsulated *H. influenzae* (Hinc), were tested by the MLST method.

Results: MLST classified 73 % of 153 Hib strains into sequence type (ST) ST-6 and 15 % of them into related ST-83. Other sequence types were uncommon (ST-92, ST-95, ST-108, ST-190, and ST-326). Five new STs were found (ST-663 to ST-667). Of 12 strains isolated from clinical specimens of Hib vaccine failure cases, nine were of ST-6, two of ST-83 and one of ST-190. The Hie isolates were of ST-18 and the Hif isolates belonged to ST-124. A Hinc strain isolated from the cerebrospinal fluid that might be a Hib strain that had lost the capsule was of ST-6. Characterization of other 49 Hinc strains revealed 37 different STs. Nine new STs were identified (ST-668 to ST-676).

Conclusions: The population of the Czech Hib isolates is highly homogeneous and all 12 STs detected are members of the same clonal complex cc6, with ST-6 being the central

genotype. Clonal complex cc6 was found in all vaccine failure cases. The Hinc population is highly heterogeneous.

P 081

GENETIC DIVERSITY OF INVASIVE STRAINS OF *NEISSERIA MENINGITIDIS* SEROGROUP B IN ITALY

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Objectives: To assess the characteristics of serogroup B strains circulating in Italy serological and molecular analyses have been carried out.

Methods: Serogrouping and antibiotic susceptibility test against penicillin, ciprofloxacin, ceftriaxone and rifampin were determined for 742 meningococci received at the National Reference Laboratory of the Istituto Superiore di Sanità in the period 2005-2010. MultiLocus Sequence Typing (MLST), *por*A VRs and FetA typing were performed on a subsample of more recent serogroup B strains (N=336).

Results: Since 2005 an increase of serogroup B strains (more than 50%) has been observed paralleled by a significant decrease of serogroup C strains up to 24% in 2010. 32% of serogroup B strains showed a decreased susceptibility to penicillin. Analysis of MLST showed heterogeneity among the isolates analysed: 13 clonal complexes. However, 76% belonged to four CCs: ST-41/44 (55.4%), ST-32 (12.5%), ST-269 (4.8%), ST-461 (3.6%). A limited number of strains (11%) was not assignable to a known CC. The PorA VR1, VR2 and FetA variants more frequently detected were : 7-2, 4 and F1-5 for the ST41/44; 19, 15 and F3-3 for the ST-32; 19-1, 15-11 and F1-7 for the ST-269; 19-2, 13 and F5-5 for the ST-461.

Conclusion: Serogroup B meningococci have been predominant in Italy during the last five years. Moreover, they are characterized by few CCs, *por*AVR and FetA variants. The

molecular characterization of serogroup B meningococci is essential for both epidemiological and vaccine development purposes.

P 082

MODIFICATIONS TO A PUBLISHED *CTRA* PCR ASSAY FOR THE IMPROVED NON-CULTURE CONFIRMATION OF MENINGOCOCCAL DISEASE IN ENGLAND AND WALES.

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Background and Aims: The HPA MRU has used a realtime Taqman™ *ctrA* PCR to detect capsulated *Neisseria meningitidis* in clinical samples (non-culture confirmation) since 1997. In 2003 it was observed that a small number of culture proven cases were matched with clinical samples negative with the screening *ctrA* assay (Corless *et al*., 2001). It is with interest we note recent publications from other groups reporting the same phenomenon (Cavrini *et al*., 2010 and Jaton *et al*., 2010).

Methods and Results: Nucleotide sequencing was performed on a 142bp region spanning the *ctrA* real-time PCR assay primer binding regions on 7 cultured isolates that were *ctrA* assay negative. The isolates were B:NT:P1.9 (4) B:NT:P1.5,2 (2) and B:NT:P1.7 (1) but all MLST CC ST-269. The nucleotide sequence data identified 4 nucleotide substitutions in the reverse primer sequence (A702G, T705G,

A708C and A717G) and a single substitution in the probe binding region (G606A) based on the full length *ctrA* gene sequence (AF520903). A modification of the published assay (Corless *et al*., 2001) to incorporate an additional reverse primer (5'-TTGCCGCGGATTGGCCACCA-3') has been made to enable detection of this variant strain.

Results and Conclusions: Since March 2003 approximately 80,000 samples have been screened with the modified *ctrA* assay and there have been no obvious culture proven only cases where appropriately matched clinical samples have been *ctrA* negative. This is an illustration of the potential for the emergence of variants that may present problems in PCR based assays and highlights the need for continued surveillance of isolates.

P 083

CHANGES IN THE CLONAL LINEAGES OF SEROGROUP B INVASIVE MENINGOCOCCAL POPULATION||IN SPAIN (2008-2010)

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The Spanish Reference Laboratory receives around 70% of the confirmed cases of meningococcal disease (MD) all over the country. All the strains are genosubtyped by definition of VR1 and VR2 PorA sequence. The clonal complex (CC) of a representative proportion (15%) of the strains is defined by MLST.

Preceding clonal substitutions among serogroup C isolates has been associated with epidemic waves of serogroup C MD in Spain. A previous study to know the CCs evolution of serogroup B in Spain, including more than 400 meningococcal isolates from patients over 2001-2007 period, showed that the most prevalent characterization was 19, 15 ST-32 CC, with a homogeneous distribution over the analyzed period, with a steadily increase of ST-11 CC associated with VR1 5-1, VR2 10-8 PorA combination.

With the aim to follow the evolution of the CCs among serogroup B strains in Spain, a new study including 254 strains isolated from patients over 2008-2010 period is now presented. ST-11 CC associated with VR1 5-1, VR2 10-8 still appear in a significant frequency. ST-32 CC mainly associated with VR1 19, VR2 15 has been predominating since 2001. However, after 2007 an important decrease can be observed. By contrast, ST-269 mainly associated with VR1 22, VR2 9 became the most frequent in 2009 (18.5%), with a similar trend observed for ST-213 CC associated with VR1 22, VR2 14 (15%). Strains showing a VR1 7-2, VR2 4 combination (potentially covered by B vaccines under development) only represented less than 10% of the isolates in Spain.

P 084

IMPLEMENTATION OF A PCR-SCHEME FOR NON-CULTURE SCREENING AND CAPSULAR TYPING OF *HAEMOPHILUS INFLUENZAE*

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The National Reference Centre for *Haemophilus influenzae* (NRHI) was founded by the Ministry of Health in 2004. Austria has vaccinated with *Haemophilus influenzae* serotype b (Hib) conjugate vaccine since 1991. After the implementation of the ECDC case definition 28/IV/2008, the *H. influenzae* non-culture diagnosis with PCR was extended to include all *H.influenzae* serotypes and non-capsulated strains.

Methods: The agarose-gel based PCR-scheme from Falla *et.al.* (1) was adapted for use with a real time PCR. Instead of their *bex*A specific primers we use *H. influenzae* P6 outer membrane specific primers for the detection of all *H. influenzae*, capsulated and non-capsulated. The PCR product is confirmed with FAM-Tamra labelled *Taq*Man probes.

Results: In the time period 2007 – 2010, 592 samples from invasive disease (blood and CSF) and 194 samples from non-invasive disease were screened for *H. influenzae*, capsulated and non-capsulated. Sixteen samples were positive. They were further analyzed with the *H. influenzae* a-f specific primers and the result was: 2 x Hib, 2 x Hif and 12 x non-capsulated. In this time period also 103 conventionally typed strains were confirmed by this PCR scheme with 100% accordance.

Conclusions: Through the good vaccination coverage, invasive disease with Hib has become very rare. Allthough it

still is the most prevalent serotype causing invasive disease in Austria the other serotypes and non-capsulated strains are becoming more prominent. The above described PCR-scheme enables the screening for capsulated and non-capsulated *H. influenzae* in non-culture samples and serotyping of a-f and non-capsulated strains.

1. Falla TJ, Crook DWM, Brophy LN, Maskell D, Kroll JS, Moxon ER. PCR for capsular typing of *Haemophilus influenzae*. Journal of Clinical Microbiology, Oct. 1994; 2382-2386

P 085

ASSESSMENT OF REAL-TIME PCR ASSAYS FOR CLASSIFICATION AND GENOGROUPING OF *NEISSERIA MENINGITIDIS*

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Background and Aims: To monitor meningococcal carriage, sensitive and reliable diagnostics to characterize colonizing strains are required. We have developed real-time PCR based assays to be used in an integrated strategy to identify and genogroup *Neisseria meningitidis* across the spectrum of clinically relevant strains.

Material and Methods: Extensive sequence alignments were conducted to design consensus primer and probe sets for *Neisseria* speciation (16S rRNA, *crgA*, *porA* and *ctrA* genes) and for capsule genogrouping (A, B, C, W135, X, Y, 29E and Z). Real-time PCR assays were optimized and specificity evaluated with a panel of bacteria (*Neisseria* and other relevant bacteria), including Men C and capsule null *Neisseria* strains. Capsule genogrouping real-time PCR assays were evaluated with a broad collection of serologically typed *N. meningitidis* strains.

Results and Conclusions: Only amplicons derived from *porA* and *ctrA* showed sufficient specificity, accuracy and robustness to identify *N. meningitidis* from other *Neisseria* or pathogenic bacteria. The incidence of false negatives (due to MenC porA- strains) was found to be negligible and the number of ctrA+ unencapsulated *N. meningitidis* strains was low. The combined speciation and capsule genogrouping assays were found to be specific, accurate and precise in identifying and classifying *N. meningitidis*. They were also in

agreement with results reported previously. We expect to use this set of assays for the epidemiological study of geographically diverse populations of healthy subjects.

P 086

ANALYSIS OF N. MENINGITIDIS SEROGROUP B PORA GENOTYPES AND EXPRESSION IN THE REPUBLIC OF IRELAND

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Background: The PorA protein is a major immunogenic component on the outer surface of the *N. meningitides* cell wall. With the construction of several PorA containing SgB OMP based vaccines, some of which are specific to circulating epidemic clones, knowledge of PorA expression and PorA allelic diversity are of significant epidemiological value.

Results: A total of 874 isolates and 1214 clinical samples, sent to the Irish Meningococcal Reference Laboratory (I.M.M.R.L.) between the years 1997 – 2009, were sequenced and the VR1 and VR2 variable regions were determined, yielding 52 unique genotypes and 5 novel variable regions. The most common genotype was B:p1.7,p1.4. To assess expression in the Irish *N. meningitidis* population, isolates received between 1997 & 2009 were probed with a panel of monoclonal antisera.

POSTER PRESENTATION P 087 – P 090,

Section: Antimicrobial resistance

P 087

THE MTRCDE OPERON: AN UNSTABLE REGION IN NEISSERIA MENINGITIDES.

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The *mtr* gene complex in meningococci encodes an efflux pump theoretically responsible for export of antibacterial hydrophobic agents. In gonococci, the *mtrCDE* operon is negatively regulated by the adjacent *mtrR* gene. However, in meningococci, the promoter region of the operon harbours an insertion sequence known as a Correia Element (CE), and a binding site for the integration host factor (IHF) at the centre of the CE. The expression of the operon in meningococci has been suggested to be subject to transcriptional regulation by the IHF.

Nevertheless, a recent study shows absence of the CE at that location in some strains. The present study attempts to expand the analysis to a broad panel of meningococci isolated from 1992 to 2010 and belonging to a large spectrum of

STs/CCs: 266 strains from France, 150 from Germany, 100 from Italy, 221 from Poland, 630 from Spain and 203 from Sweden. The strategy was based on the amplification of a 1033 bp fragment corresponding to the first 27 bp of the *mtrC* gene, the *mtrR*/*mtrCDE* intergenic region containing the Correia element, and the first 800 bp of the *mtrR* gene. A significant number of strains (108) showed different deletions, some of them affecting to the CE. Moreover, 7 strains showed different DNA insertions affecting to the intergenic region. No changes in antibiotic susceptibility were associated with these findings.

In conclusion, multiple variations are present in this region. Further investigations are required in order to elucidate the role of the *mtr* efflux system in meningococci.

P 088

TARGET GENE SEQUENCING TO DEFINE THE MENINGOCOCCAL BREAKPOINTS FOR CIPROFLOXACIN

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Background: Ciprofloxacin (Cip) is one of the antibiotics of choice for chemoprophylaxis of meningococcal disease. Few resistant isolates have been reported worldwide. The identification of these isolates is important, however, hindered by the absence of harmonised breakpoints. In the present study, we aimed to identify such breakpoints on the basis of sequencing of the *gyrA, parC* and *parE* resistance genes, and MICs of ciprofloxacin.

Methods: Isolates with MICs of ≥0.032 µg/ml of ciprofloxacin (n=23 isolates) cultured between 1995 to 2010 in 4 European countries were examined. DNA fragments of 847 bp, 822 bp and 444 bp of the *gyrA, parC* and *parE* genes, respectively, were amplified and sequenced. MICs of ciprofloxacin were determined using Etest.

Results and Discussion: Sequence data and MICs of ciprofloxacin were used to define "wild-type" alleles of the three genes and to define the breakpoint for susceptibility to ciprofloxacin. Isolates (n=17) with MIC of ciprofloxacin of ≥0.06 µg/ml have mutations in *gyrA* (most frequently at the codon 91). No mutations were detected in the QRDR of *parC* or *parE*. Isolates with MIC < 0.06 µg/ml (n=6) have no modifications in the QRDR of the three examined genes. These data support defining MIC of <0.06 mg/L as a breakpoint for meningococcal susceptibility to ciprofloxacin.

Molecular surveillance should continue including more strains and possible exceptions to these findings.

P 089

RESISTANCE TO ANTIBIOTICS STUDY OF HAEMOPHILUS STRAINS ISOLATED IN 2010 AT THE NATIONAL REFERENCE CENTRE FOR HAEMOPHILUS

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Background: *Haemophilus influenzae* causes diseases in many organ systems, but usually attacks the respiratory system.

Objective: studying and monitoring antibacterial resistance of Haemophilus strains, isolated in 2010, in the Reference Centre for Haemophilus.

Materials si methods: 33 *Haemophilus* strains isolated / received in 2010. These strains were isolated from patients aged between 1.6 - 60 years, with various diagnoses (acute meningitis, pneumonia, bronchiolitis, pharyngitis etc.). Identification was based on phenotypic characteristics and antibiotic susceptibility testing was performed by diffusion method according to CLSI standards 2010.

Results: The bacterial strains belonged to *H. influenzae* (n = 18) and *H. parainfluenzae* (n=15), species isolated from the cerebrospinal fluid, sputum, ear and conjunctival secretions, throat and nasal exudates. *H. influenzae* serotyping revealed the predominance of b type in invasive infections. 11 strains of *H. influenzae* were beta-lactamase positive and showed resistance to ampicillin. All strains were susceptible to amoxicillin/clavulanic acid, azithromycin and clarithromycin. One strain of *H. influenzae* showed low level resistance to ceftriaxone, one strain was resistant to chloramphenicol and other one to ciprofloxacin. 77.8% and 38.9% of *H. influenzae*

strains were resistant to tetracycline and sulfametoxazole/trimethoprim, respectivelly.

Conclusions: to know the real incidence of invasive Hib disease requires a sustained and continuous monitoring at the national level and use of molecular methods for diagnosis and study of antibiotic resistance.

P 090

THE IN VITRO SUSCEPTIBILITY TO ANTIBIOTICS OF NEISSERIA MENINGITIDIS STRAINS||ISOLATED IN THE LAST YEARS IN ROMANIA

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Background: meningococcal disease, caused by *N. meningitidis*, is most common in young children, with a secondary peak of disease incidence in teenagers In Europe. Disease is caused most frequently by serogroups B and C. In the last 10 years, average of the annual incidence of meningococcal meningitis in Romania was 1.1%.ooo

Aim: to study the antibiotic resistance and serogroups of meningococci isolated in the last years in Romania

Materials and Methods: 98 meningococcal strains isolated from CSF or blood, were sent (according to Romanian Ministry of Health no.860/2004) by District Public Health Authorities or hospital laboratories, at the National Reference Laboratory for Meningococci (2006 – 2010), to be confirmed and for determination of serogroups and antibiotic susceptibily (to penicillin, ceftriaxone, rifampin and ciprofloxacin).

Results: interpretation was done according to European Monitoring Group for Meningococci and revealed 20.5 % intermediate resistance to penicillin. No resistant strain to cefotaxime was found. Serogrouping showed the following aspects: 71.9 % group B, 18.8 % group C, 5.1 % group W135, 3.2 % Group A and 1% group X.

Conclusions: serogroups B and C were predominant in this period in Romania. The increase in resistance to Penicillin of *Neisseria meningitidis*, demands the use of molecular biology techniques that could show the existent relationship between changes in penA genes and the decreased susceptibility. There is an urgent need for a stronger partnership between clinical medicine and public health and for new vaccines in today's society.

POSTER PRESENTATION P 091 – P 092, Section: Clinical aspects of meningococcal disease

P 091

COUNTING THE COST OF MENINGITIS: A SEVERE CASE OF MENINGOCOCCAL MENINGITIS AND SEPTICAEMIA

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Background and aims: Cost benefit analysis is used to aid decisions on vaccination policy, and has become an increasingly important factor in reaching such decisions; however accurate cost of illness data is essential for this to be reliable.

This work aims to provide detailed information about the costs to society of treating severe long-term sequelae of meningococcal disease over the lifetime of a survivor, particularly the costs of specialist rehabilitation for people disabled by their illness.

Material and methods: Information on the management of long-term sequelae was gathered from interviews with individuals and with parents of children affected. Healthcare and educational professionals involved in rehabilitation, needs assessment, and providing support were also interviewed. A reference document was validated by all of our professional consultees, including health and educational professionals, economists and academics. Costs were derived from published National Health Service databases and other government sources and discounted at 3.5%.

Results and conclusions: It was found that a case of meningococcal meningitis with a severe outcome could cost the state approximately £1,600,000 (medical £326,171, educational £172,721 and social £1,101,336) over a lifespan of 50 years. A severe case of meningococcal septicaemia was estimated to cost £1,390,000 (medical £478,032, educational £153,832 and social £758,287) over a lifespan of 70 years.

At present there is little information in the UK about the direct and indirect lifetime costs associated with long-term sequelae of meningococcal disease. Our contact with families affected and professionals involved in rehabilitation enable us to contribute new information in this area.

P 092

AFTERCARE AND SUPPORT FOR CHILDREN AFTER MENINGITIS AND SEPTICAEMIA: THE PARENTS' VIEW

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Background and Aims: There is a considerable knowledge base regarding the incidence of meningitis and septicaemia, disease aetiology, and risk of associated sequelae. However, there is little published research on the needs and provision of aftercare for survivors. This study aimed (1) to gain an account of the aftercare and support services parents felt were required following their child's acute episode of meningitis or septicaemia, (2) to assess parents' satisfaction with provision and access to aftercare services, (3) to qualitatively explore the factors affecting parents' opinions of whether their child's needs for aftercare have been met.

Materials and Methods: Participants were recruited from Meningitis Research Foundation's member database and social media sites. Eligible participants were parents of children who survived meningitis or septicaemia between January 2000 and May 2010, living in the UK or Ireland. We used a mixed methods design. In stage one, participants completed a multiple choice questionnaire, either online or by post. In stage two, twenty participants were invited to take part in a follow-up interview, based on their answer to the survey question, 'overall, to what extent does / did the aftercare and support received meet your child's needs?'

Results and Conclusions: The deadline for questionnaire submission is mid-March and preliminary results will be available in May. We have so far received completed questionnaires from 340 participants and are currently

conducting follow-up interviews. We anticipate that the study will provide important information on the burden and costs of sequelae in meningitis/ septicaemia survivors and their families.

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