EMGM 2001

European Monitoring Group on Meningococci
6th Meeting

Örebro Medical Centre Hospital, Sweden

Örebro
June 13 - 15

EMGM 2001

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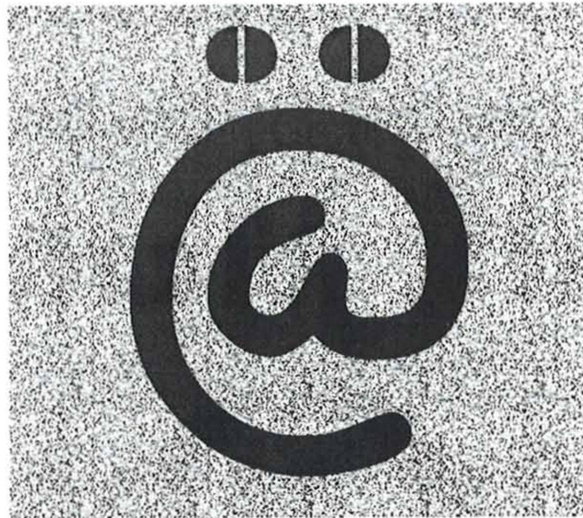
6th Meeting



The 6th meeting of European Monitoring Group on Meningococci (EMGM)

is organised by the Swedish Reference Laboratory for Pathogenic Neisseria, Department of Clinical Microbiology, Örebro Medical Centre Hospital.

Local Programme Committee: Per Olcén, Anders Bäckman, Hans Fredlund, Lennart Sjöberg.

International Advisory Committee: Keith Cartwright, Andrew Fox, Sigrid Heuberger, Georgina Tzanakaki.

Financial support:

SmithKline Beecham AB, major sponsor
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The meeting includes participation of European Centres of Surveillance on Meningococci, National Reference Laboratories and persons interested in the meningococci. Other participants are from WHO, United States of America (CDC), Israel, Australia.

It is a pleasure to invite you to attend this meeting in Örebro in the middle of Sweden during the beginning of the summer in Northern Europe. It is our wish that microbiologists, epidemiologists and specialists in infectious diseases from all parts of Europe will meet here in Örebro to share their experiences and that it will be lots of possibilities for fruitful discussions among all attendees on meningococci and meningococcal disease both during the different sessions but also outside the formal scientific programme.

For the Programme Committee

Per Olcén　　　　Hans Fredlund

Programme

Wednesday 13 June

08.50	Welcome: The local programme-committee
09.00	***Diagnosis and meningococcal characterisation*** Moderators: Dominique Caugant (Norway), Muhamed-Kheir Taha (France) • Introduction incl. background facts to the presentations by the moderators (10 min) • Diagnosis of meningococcal disease by PCR and non-culture strain characterisation. Malcolm Guiver and Andrew Fox (UK) (20 min) • MLST and associated web databases: Martin Maiden and Man-Suen Chan (UK) (20 min) • Genosubtyping and PFGE of *N. meningitidis*. Paula Mölling, Magnus Unemo (Sweden) (20 min) ***Stretching (5 min)*** • Current status of and future plans for monoclonal antibody reagents available from NIBSC. Janet Suker, Ian Feavers (UK) (25 min including discussion). • Report of the "QA working group": Andrew Fox (UK) (10 min)
10.50-11.20	Coffee break (coffees and lunches hosted by Örebro Medical Centre Hospital)
11.20-13.00	***Epidemiology I*** Moderators: Paula Kriz (Czech Rep.), Björn-Erik Kristiansen (Norway) • Introduction incl. background facts to the presentations by the moderators (10 min) • Epidemiological surveillance of McD in Europe 1998/1999. Norman Noah (UK) (20 min) • Report of the "Questionnaire on carriage studies in Europe": Paula Kriz (Czech Rep.), Del AlaÁldeen (UK) (20 min) ***Stretching (5 min)*** • "Prevention and control policies for meningococcal disease in Europe: a systematic review of the evidence". James Stuart

(UK), Susanne Samuelsson (Denmark), Sigrid Heuberger (Austria), Ingrid Ehrhard (Germany) et al. (45 min).

13.00-14.00 Lunch break and Poster viewing

14.00-15.30 ***Epidemiology II***

Moderators: Susanne Samuelsson (Denmark), Per Olcén (Sweden)

- Introduction incl. background facts to the presentations. The moderators (10 min)
- International outbreak of W-135 meningococcal disease linked to Hajj 2000.
  Anne Perrocheau (France) (30 min)
- EC-funded DG SANCO project: Surveillance of meningococcal disease in the European Union, 2000 - 2001. Sarah Handford (UK) (20 min)

Stretching (5 min)

- EU-MenNet: Impact of meningococcal epidemiology and population biology on public health in Europe – a consortium funded by the EU Quality of Life program 2001-2004. Matthias Frosch (Germany) (15 min)
- Discussion concerning pan-European MC surveillance (10 min)

15.30-16.00 Coffee break

16.00-17.15 ***Clinic and antibiotics***

Moderators: Colin Block (Israel), Hans Fredlund (Sweden)

- Introduction incl. background facts to the presentations. The moderators (10 min)
- The clinical picture of meningococcal disease: Alfred Halstensen (Norway) (15 min)
- Atypical manifestations of meningococcal disease: Hans Fredlund (10 min)

Stretching (5 min)

- Report of the "Antibiotic susceptibility testing working group" including Proposal for standardization. Julio Vazquez Moreno (Spain), Colin Block (Israel) (35 min)

Thursday 14 June

08.30-10.30 ***Immunology and vaccines I***
Moderators: Germie van den Dobbelsteen (Holland), David Salisbury (UK)

- Introduction incl. background facts to the presentations. The moderators (10 min)
- Immunology and the meningococcus. Elisabeth Wedege (Norway) (30 min)

Stretching (5 min)

- Vaccines against serogroup B meningococcal disease. Einar Rosenqvist (Norway) (25 min)
- The MCC vaccines. David Salisbury (UK) (30 min)

10.30-11.00 Coffee break

11.00-13.00 ***Immunology and vaccines II***
Moderators: Alfred Halstensen (Norway), Ray Borrow (UK)

- Introduction incl. background facts to the presentations. The moderators (10 min).
- Functional tests:
  # Serum bactericidal assays. Ray Borrow (UK) (20 min)
  # Opsonophagocytosis of meningococci. A. Halstensen (Norway) (20 min)

Stretching (10 min)

Measurement of T-cell responses against meningococcal antigens.
Lisbeth Meyer Næss (Norway) (25 min)

- Future meningococcal vaccines. Germie van den Dobbelsteen (Holland) (35 min)

13.00-14.00 Lunch break and Poster viewing

14.00-15.00 Time for members of the working groups and other constellations to meet

Friday 15 June

08.30-10.00 ***Genetics***

Moderators: Martin Maiden (UK), Anders Bäckman (Sweden)

- Introduction incl. background facts to the presentations. The moderators (10 min)
- The complete sequence of meningococci - what does it mean? Rino Rappuoli (Italy) (60 min)
- Discussion

10.00-10.30 Coffee break

10.30-13.00 ***Miscellaneous/Free papers***

Moderators: Sigrid Heuberger (Austria) and Arne Höiby (Norway)

- Introduction incl. background facts to the presentations. The moderators (10 min)
- Demonstration of search in the MLST web database. Martin Maiden and Man-Suen Chan (30 min)
- "Assessing the risk of laboratory aquired meningococcal disease". James Stuart, Stephen Gray, Ed Kaczmarski, (UK) (20 min)
- Discussion

13.00-14.00 Lunch break and Poster viewing

14.00-15.00 ***The take home lesson***

Mattias Frosch

15.00 ***"This is the End and where do we go from here?"***

The local programme-committee and the international advisory committee with

Coffee and Bye Good after nomination of country for the EMGM-2003.

Posters

Colin Block et al.
Meningococci isolated from cases of meningococcal disease in Israel – National laboratory data for the period 1983 – 2000.

Arijana Boras et al.
Meningococcal Disease in the Republic of Croatia in the Year 2000.

Mary Cafferkey et al.
Epidemiology of invasive meningococcal disease in the Republic of Ireland: A report on laboratory confirmed cases, July 1997 – June 2000.

Mary Cafferkey et al.
Early impact of introduction of meningococcal group C conjugate vaccines on the epidemiology of invasive meningococcal disease in the Republic of Ireland.

Françoise Carion.
Laboratory surveillance of meningococcal disease in Belgium 2000.

Stuart C. Clarke et al.
Automated methods for the laboratory confirmation of meningococcal disease.

Stuart C. Clarke et al.
Non-culture confirmation of meningococcal disease by *por*A PCR and DNA sequencing using automated methods.

G.F.S. Edwards et al.
Laboratory confirmation of Neisseria meningitidis: past, present and future.

Ingrid Ehrhard et al.
Epidemiology of meningococcal disease in Germany, 2000.

Ingrid Ehrhard et al.
Characterization of invasive *Neisseria meningitidis* serogroup C strains of Austria, Germany and Hungary with serological and molecular methods.

Ingrid Ehrhard et al.
First results of a longitudinal study of meningococcal carriership in teen-agers.

Hans Fredlund et al.
Invasive meningococcal disease in Sweden year 2000.

Simon Funnell et al.
Development of surface-labelling and opsonic assays for assessment of serogroup B meningococcal vaccines.

Sigrid Heuberger et al.
Epidemiology of Meningococcal Disease AUSTRIA 2000.

Edward Kaczmarski et al.
Epidemiology of meningococcal infection in England and Wales 1999-2001.

Jitka Kalmusova et al.
PCR diagnosis of invasive disease caused by *Neisseria meningitidis*, *Haemophilus influenzae* b and *Streptococcus pneumoniae* in the Czech Republic.

Jitka Kalmusova et al.
Multilocus Sequence Typing directly from clinical material.

Edita Karelová et al.
Analysis of the epidemiological situation in invasive meningococcal disease in the Slovak Republic in 2000.

Paula Kriz et al.
Invasive meningococcal disease in the Czech Republic in 2000.

Paula Kriz et al.
Multilocus Sequence Typing performed in the Czech Republic.

Ileana Levenet et al.
Meningococcal disease in Romania 1995 – 2000. Epidemiological and bacteriological studies.

Diana Martin et al.
New Zealand's Continuing Epidemic of Meningococcal Disease.

Mark Muscat.
The Epidemiology of Meningococcal Disease in Malta, 2000.

Martin Musilek et al.
Changing shape of clonal distribution of Czech *Neisseria meningitidis* isolates related to declining prevalence of the ET-37 complex strain in the early 2000's.

Alexander E. Platonov et al.
Meningococcal disease in Russia and in Moscow in 1999-2000.

Alexander E. Platonov et al.
Differential genodiagnostics of meningitis in Moscow.

Susanne Samuelsson et al.
Meningococcal disease in Denmark 2000.

Anna Skoczyńska et al.
Meningococcal meningitis in Poland in 2000.

Ingrid Smith et al.
FcγRIIa and FcγRIIIb allotypes were not associated with contraction or severity of systemic meningococcal disease in Norwegian teenagers and adults.

Elise Snitker Jensen et al.
A 20-year population based study of meningococcal disease in a Danish county with emphasis on meningococcal phenotypic and genotypic markers and case fatality rate.

Tonino Sofia et al.
Meningococcal meningitis in Italy in the year 1999-2000.

John Tapsall et al.
National Epidemiological Data - Australia 1 January to 31 December 2000.

Stefan Tyski et al.
Diversity of *Neisseria Meningitidis* type 22 strains isolated in Poland.

Ulrich Vogel et al.
The Bavarian meningococcal carriage study: genetic typing and cluster analysis.

Diagnosis of meningococcal Disease by PCR

Malcom Guiver, Andrew Fox
Molecular Biology, Public Health Laboratory Service, Manchester, UK

Non-culture confirmation of meningococcal disease by PCR was introduced into the Meningococcal Reference Laboratory (MRU) for England and Wales in 1995. The demand for this service has dramatically increased and currently approximately 17000 samples are processed annually. Non-culture diagnosis is now an important component in the repertoire of diagnostic tests for case confirmation, identifying outbreaks, and providing epidemiological data. Currently 42% of cases are confirmed by PCR alone and consequently PCR is now the essential diagnostic investigation in MCD.

Development of PCR assays have progressed from agarose-gel systems to PCR ELISA, and finally to automated TaqMan™ and LightCycler real-time PCR platforms. Using Applied Biosystems 7700, TaqMan™ assays targeting the capsule transfer gene (*ctrA)*, and the sialyltransferase gene, a screening assay and serogroup B and C specific assays have been developed. More recently serogroup W135 and Y assays have been developed in response to the increasing incidence of imported W135 disease. To complete the repertoire of serogroup specific assays a TaqMan™ serogroup A assay has also been developed targeting the *myn* gene operon.

Accurate monitoring of MCD throughout Europe will require standardisation of PCR assays with similar levels of sensitivity and specificity. While the ABI 7700 TaqMan™ provides a robust automated high-throughput system this technology is not available or necessarily suitable for use in other laboratories. To enable other laboratories who wish to use these primer sets we have evaluated most of these TaqMan™ assays using agarose detection, PCR ELISA and LightCycler formats, with comparable levels of sensitivity to the TaqMan™ system.

Sample type and extraction protocol has been shown to greatly affect PCR sensitivity in the detection of meningococcal DNA, however all described methods are labour intensive manual processes. We have recently been evaluating the Roche MagnaPure automated nucleic acid extractor which has been designed to complement the LightCycler system. This system provides walk-away automation and is capable of post extraction aliquoting of PCR master mix and inoculation of extracted samples. Initial results suggest a single protocol provides comparable sensitivity to existing manual methods for detection of meningococcal DNA from whole blood and CSF samples. This should minimise mistakes due to human error, and improve the accuracy and reproducibility of the assays.

TaqMan™ real-time PCR assays enable accurate quantification of DNA present in the clinical sample to be made. This information is used to assess the performance of each run using known standards. In addition a study has been carried out measuring meningococcal bacterial load from serial blood samples admitted with MCD to Alder Hey Childrens Hospital in Liverpool. Admission bacterial load is significantly higher in patients with severe disease (GMSPS > 8) and is independent of the duration of clinical symptoms or the decline in DNA load. This suggests the admission bacterial load is the result of variable multiplication rates of the meningococci probably due to differing immune responses in individual patients.

This information may therefore in future be used as a prognostic marker to aid selection of appropriate specific therapies in treating MCD.

Non-culture strain characterisation

Andrew Fox, Andrew Birtles, Suzanne Cooke, **Ed Kaczmarski**, **Steve Gray**, **Malcolm Guiver**
Molecular Biology, PHLS, Manchester, UK

In England and Wales increasing numbers of cases of meningococcal infection are being confirmed by PCR alone for which no isolate is available. Non-culture strain characterisation beyond serogroup identification is important to provide information for early case cluster and outbreak recognition.

The MRU has recently begun to carry out sequence typing for *por*A VR1 and VR2 variable regions and the seven housekeeping gene loci defined within the MultiLocus Sequence Typing (MLST) scheme for meningococci. We have also recently been working to adapt the PCR assays currently used to sequence type isolates to improve sensitivity to allow MLST of isolates direct from clinical specimens in the absence of a cultured isolate. The development of sensitive multilocus PCR assays has involved the adjustment of primer sequences and PCR product size including nested primer sets.

The development and application of both *porA* and MLST PCRs for non-culture strain characterisation will be described and preliminary data on the use of non-culture strain characterisation in different outbreak situations will be presented.

Non-culture strain characterisation using MLST will be of growing importance in the future surveillance for meningococcal disease.

MLST and associated web databases

M C J Maiden, K A Jolley and M S Chan
Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK

The MLST (multilocus sequence typing) scheme for *Neisseria* was the first to be established and the web-accessible data base remains the largest such resource in terms of numbers of submissions and user base. It has been collating data on isolates since early 1999 and is proving to be of value for both research and epidemiological surveillance. As the site is dependent on the submissions which it revives it does not represent a population sample of the meningococcus, although some subsets of the site do; however, as isolates with new or unusual sequence types are more likely to be submitted, the site provides a unique indication of the genetic diversity so far identified in the meningococcus.

As of May 2001, the database contained information derived from 1878 isolates, mainly *Neisseria meningitidis*. Isolates from 48 different countries were present and more than 1200 unique sequence types had been identified. The seven MLST loci employed by the scheme had between 80 and 155 alleles. Just over half of the isolates were sampled from asymptomatic carriers and mostly these did do not belong to recognised hyperinvasive lineages.

Curation of the data base ensures the accuracy of the data which it contains and the data base is regularly examined to identify and annotate the relationships among the isolate data.

The MLST site has been highly successful in its current form, but further developments are planned to enhance the value of its content. These include the development of associated databases containing information on other genes, especially those encoding antigens, and ways of linking the data electronically with epidemiological surveillance.

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Genosubtyping and pulsed-field gel electrophoresis of *Neisseria meningitidis*; Two useful molecular techniques for epidemiological investigations

Mölling P, Unemo M, Issa M, Olcén P, Fredlund H. National Ref. Lab. for Pathogenic Neisseria, Dept. of Clin. Microbiol. & Immunol., Örebro Medical Centre Hospital, Sweden.

Introduction

N. meningitidis (Mc) strains are traditionally characterized with phenotypical markers e.g. capsular polysaccharides (serogrouping), outer membrane proteins (serotyping and –subtyping), bacterial enzymes, lipopolysaccharides and antibiotic susceptibility. The serological characterization has some inherent limitations concerning typeability and discriminatory ability. Some powerful typing methods are based on DNA characterization for investigations of clonal relationships. Sequencing the variable regions of the *porA* gene, which encode the outer membrane protein PorA, and deducing the amino acid sequences gives the genosubtype. As complement to the genosubtyping, that examines one single genetic locus, pulsed-field gel electrophoresis (PFGE) after restriction endonuclease cleavage potentially index the whole genome.

Aim

To illustrate the usefulness and performance characteristics of the two methods exemplified with the Swedish/Sudanese collection of McW-135 isolates as well as McC isolates from a suspected cluster of meningococcal disease.

Materials and Methods

McW-135 with the Hajj 2000 genosubtype P1.5,2,36b isolated in Sweden during 1979-2000 (invasive and carrier isolates, n=17) and in Sudan late in the year 2000 (isolates from carriers, n=5), a McW-135 Hajj-2000 reference strain (M7034) and four McC isolates from a presumed Swedish cluster were included. All isolates were phenotypically characterized (co-agglutination[1], whole-cell ELISA[2] and antibiograms) and genetically characterized by genosubtyping[3] and PFGE[4] using *Spe*I and *Nhe*I.

Results

None of the Swedish or Sudanese McW-135 isolates was serosubtypeable using the co-agglutination. By the ELISA 91% (20/22) were serosubtypeable as P1.2 with available monoclonal antibodies. The PFGE detected a considerable genetic diversity (11 different PFGE fingerprints using *Spe*I and 10 using *Nhe*I)

among the isolates. Five of the Swedish isolates as well as all the strains from Sudan were indistinguishable from the Hajj-2000 reference strain using genosubtyping and PFGE.

Concerning the suspected McC cluster the co-agglutination subtype reagents could neither confirm nor falsify the cluster situation; the ELISA however identified two pairs of isolates with serosubtypes P1.2 and P1.7,16 respectively. The genetical characterization also identified the pairs of isolates with genosubtypes P1.5,2,36b and P1.7,16new,35 respectively. Also the PFGE identified the same indistinguishable pairs of isolates. These results and the evident epidemiological connections confirmed the existence of two different clusters.

Discussion

The two illustrated epidemiological situations show that the routinely used phenotypical characterization is not enough. Reliable molecular methods like genosubtyping and PFGE are needed for understanding the epidemiology of meningococcal disease. These methods have an excellent typeability, reproducibility and also a high discriminatory ability (especially PFGE). A moderate ease of interpretation and performance are also advantages but the expensive equipment, time-consuming procedures and lack of phenotypical information are some disadvantages. PFGE standardization in the parameters of the procedure is desirable for an optimal intra- and interlaboratory reproducibility of the fingerprints, especially the choice of restriction enzyme(s), agarose, and controls as well as the electrophoretic conditions.

References

1. Olcén P, Danielsson D, Kjellander J. The use of protein A-containing staphylococci sensitized with anti-meningococcal antibodies for grouping *Neisseria meningitidis* and demonstration of meningococcal antigen in cerebrospinal fluid. Acta Path Microbiol Scand B 1975;83:387-396.

2. Abdillahi H, Poolman J. Whole-cell ELISA for typing *Neisseria meningitidis* with monoclonal antibodies. FEMS Microbiol Lett 1987;48:367-371.

3. Mölling P, Unemo M, Bäckman A, Olcén P. Genosubtyping by sequencing group A, B and C meningococci; a tool for epidemiological studies of epidemics, clusters and sporadic cases. APMIS 2000; 108:509-516.

4. Mölling P, Bäckman A, Olcén P, Fredlund H. Comparison of serogroup W-135 meningococci isolated in Sweden during a 22-year period and those associated with a recent Hajj pilgrimage. J Clin Microbiol 2001;39(7):in press.

Current status of and future plans for monoclonal antibody reagents available from NIBSC

Janet Suker and Ian Feavers
Division of Bacteriology, National Institute for Biological Standards and Control (NIBSC), Blanche Lane, South Mimms, Potters Bar, Herts, EN6 3QG, UK.

Historical Background

Traditionally, serological typing of meningococcal antigens recognised by specific monoclonal antibodies (mAbs) provided epidemiological information on meningococcal outbreak strains. The mAbs have been used to define: serogroup (capsular polysaccharide), serotype (class 2 or 3 outer membrane protein, PorB), serosubtype (class 1 outer membrane protein, PorA) and immunotype (lipooligosaccharide) and thus have also been used in the development of vaccines based on these components. In 1993, NIBSC agreed to act as a safe-depository for hybridoma cells expressing antibody to meningococcal antigens and to distribute a panel of the most commonly used mAbs. Collaborators worldwide have donated 63 different hybridoma lines from which 24 mAbs were produced in large amounts as ascitic fluids and characterised. This set of 24 mAbs are listed in the NIBSC reagent catalogue (see http://www.nibsc.ac.uk/catalog/index.html) and have been available for general distribution since 1993. One set of the mAbs was provided without charge to each European Meningococcal Reference Laboratory that requested it. Subsequent supplies were subject to the usual NIBSC handling charges (these do not reflect the cost of actually producing the reagents). A similar set of mAbs have also been available from the RIVM (P.O. Box 457, 3720 AL Bilthoven, the Netherlands). The remaining mAbs have been provided on an *ad hoc* basis.

Current situation

As reported at the last meeting of EMGM in Crete 1999, there are several difficulties in continuing the supply of the mAb reagents. First, and most importantly, the panel of mAbs are becoming less representative of strains circulating in meningococcal populations. Horizontal genetic exchange between *por*A and *por*B genes gives rise to antigenic variants that are not recognised by the current reagent panel and so are recorded as not-typeable or only partially typed. For example, a study of the nucleotide sequences of *por*A genes in meningococcal strains isolated in England and Wales during a 20 year period illustrated the rapidity with which the predominant PorA antigen changed with time (1). A greater understanding of the genetic basis for antigenic variation has allowed the development of alternative, PCR-based methods for strain

characterisation. However, not all laboratories are able to adopt this technology within the timescale required to phase-out use of mAbs for typing purposes.

The current set of reagents are already running out and alternative strategies are being investigated to replenish stocks. There is now a ban on ascites production in the UK so the reagents would have to be provided in the form of concentrated cell culture supernatants, the stability of which needs to be tested for each individual mAb. As this potentially requires considerable resources, clarification of the future demand for these reagents is a prerequisite for the development of a replacement strategy. We would therefore welcome discussion at EMGM 2001 on the following points:

- Is there still a requirement for mAbs as serological typing reagents? (If so, for how long?)
- How representative are the current mAbs of strains now circulating in Europe/worldwide?
- If the current mAbs are not representative, but demand for serological reagents still exists, is there a need for isolation of new mAbs? (If so, who will do this?)
- Is there a demand for particular mAbs as research reagents and would this be better met by supplying the mAbs on request rather than holding stocks which have finite shelf-life?

A draft strategy document will be prepared for discussion at EMGM 2001 and the strategy for supply will be finalised on the basis of these discussions.

Reference

J.E. Russell, I.M. Feavers, A.J. Fox, S. Gray, M.C.J. Maiden.
Persistence of complexes of disease-causing *Neisseria meningitidis* isolated in England and Wales over twenty years 1975-1995.
Abstracts of the Twelfth International Pathogenic Neisseria Conference 2000.

Epidemiological Surveillance of McD in Europe 1998/99

Norman Noah* and Brian Henderson.
PHLS Communicable Disease Surveillance Centre and
*London School of Hygiene and Tropical Medicine

In Europe 30 countries and one region provided statistics for the period July 1998 to June 1999, which was supplemented with data from USA, Australia and New Zealand, and China. 16 of these countries which have been reporting regularly for many years, constitute a core data set, and we present some of the information using the core data only. Some countries still send in aggregated data.

During this period, 7317 laboratory confirmed cases were reported, giving an incidence rate of 1.7 (2.1 for core group). Of these England and Wales reported 38 % (2783) of the cases, which was by far the highest from any country in Europe with a similar sized population. The highest incidence rates tended to be found in islands - Malta (6.9), Ireland (7.9) and Scotland/England and Wales (6.0/5.5).

Of those countries with more than about 20 cases, the septicaemia rate (*ie* septicaemia alone), was between 15 and 29%. In Greece 70% (89), Republic of Ireland 57% (242) and in N. Ireland 70% (62) of cases were reported as septicaemia, whereas in Poland there were only 3 of 39 cases (8%). There seemed to be no obvious correlation between group or type and septicaemia rate.

Reporting case fatality rates is difficult because of missing information, but the overall increase in CFR with age has been consistent. In this period, the lowest case-fatalities were in children aged 5-14.

The proportions of groups B and C 63% : 31% were similar to previous years, but group C accounted for 50% or more in Czech and Slovak Republics (consistently for several years), Scotland and Iceland (7/14 cases). More than 80% of group B infections were noted in Germany, Denmark, Netherlands, Norway and Poland. Group Y cases accounted for 20% of cases in Israel and 10% in Finland. This group accounts for 1/4-1/3 of cases in USA.

The most common serotypes were B;4, B:15, and C:2a and C:2b. The age pattern of C:2b showed a predilection for those aged 1-4y. 37% of all C:2b infections were in this age group, compared with 25% for B:4 and B:15, and 22% for C:2a. More than 20% of B:15 and C:2a infections were in older teenagers.

Compared with the previous year, resistance to penicillin increased from 0.3% to 0.8%, to rifampicin more than a 100-fold from 0.002% to 0.2%, and sulphonamide from 40.6% to 48.3%. Penicillin and rifampicin resistances were reported on very small numbers of cases.

Summary

The surveillance continues to improve, and we are grateful to our contributors for this. The main problem remains one of **timeliness**. That some countries still only send their statistics in aggregated form is only one reason for this, and we need to discuss other reasons at the conference. Another problem is that of **representativeness. Completeness,** though important if we are to make any sense of incidence, is not such a serious problem, but we need more countries to give fair estimates of what - and how much of the whole - their data represent. The **typing** system makes it difficult to interpret the data epidemiologically, but improvements in characterising the organism bode well for the future. In surveillance, we need to plan well in advance however if there are going to be major changes in the typing system.

On the more positive side, in spite of the disparate sources of data, we can be fairly confident that the surveillance has succeeded in providing us with a broad picture of what is happening with McD in Europe, and being able to compare it with some other parts of the world. Nevertheless, there is certainly room for improvement and we hope that, by the end of the conference, the size of this room will be smaller.

Report of the "Questionnaire on carriage studies in Europe"

Kriz Paula[1], Ala'Aldeen Dlawer[2]
[1]National Reference Laboratory for Meningococcal Infections, Center of Epidemiology and Microbiology, NIPH, Prague, Czech Republic
[2]Molecular Bacteriology and Immunology Group, Division of Microbiology, Nottingham University Hospital, Nottingham, United Kingdom

Introduction

In the previous EMGM meeting (Crete 1999), a session was devoted to carriage studies. It became obvious that different studies carried out by different centres lacked common design and protocols, and were not easily comparable. We agreed to gather information, assess current practice and offer useful suggestions to participants at EMGM 2001.

Materials and Methods

A questionnaire was designed to address different variables, and sent to 87 participants. 25 of these responded, 13 of whom were engaged in carriage studies. However, only 12 returned completed questionnaires that could be analysed. Non-responders were: hospital clinicians, laboratory researchers, WHO and EC colleagues outside Europe: USA & Australia, colleagues performing studies in Africa.

Results

The final analysis showed that carriage studies vary in their overall design and type of epidemiological data and clinical material collected. They also vary in the swabbing techniques used, qualification (training) of the swabbers, time delay before plating swabs and the choice of culture media used. Phenotypic characterisation of strains were very similar in most studies, however, they varied in their choice of molecular characterisation and antibiotic susceptibility testing. Most centres were willing to make their data available and to collaborate with other centres.

Conclusion

It would be useful to discuss at EMGM 2001, issues relating to these variables. Reaching firm consensus might be difficult but, if achieved, would be of great value.

Prevention and control policies for meningococcal disease in Europe: A systematic review of the evidence

Purcell B, Hahne S, **Samuelsson S**, Camaroni I, **Heuberger S, Ehrhard I, Stuart J.**

Background

The severity of invasive meningococcal disease and its tendency to cluster within households, schools and other communities mean that high public health priority is given to strategies to reduce case fatality and the risk of further cases. However a lack of evidence to underpin policy has resulted in a marked variation in approach between countries in Europe.

Objective

To review the evidence for the effectiveness of control measures for meningococcal disease and to make recommendations to improve harmonisation of policy across Europe

Methods

From surveys of national control policies in Europe, control measures were selected for review where there was greatest variation between countries. These were identified as (1) Preventing further cases of meningococcal disease through chemoprophylaxis +/- vaccination (a) to the index case, household contacts and in child care settings following a single case, and (b) to students/children in colleges, schools, and pre-school settings following two or more linked cases;
(2) Reducing case fatality rate by giving antibiotics before admission to hospital.

The review included experimental and non-experimental studies with a minimum of ten cases per study, defined outcomes, and comparisons between intervention and non-intervention groups. There was no restriction on date of publication or language of publication. Relevant studies were identified by searching electronic data bases (Medline,Embase, CAB Health), by contacting the WHO and the European meningococcal disease surveillance network and by examining bibliographies of review articles .

Results

The database search identified 3527 possible articles. After an initial screening process, studies were identified in each subject area and two reviewers independently checked the selected papers for inclusion in the review. Data were extracted and quality assessed on standardised forms. Results and conclusions will be presented at the EMGM meeting.

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International outbreak of W135 meningococcal disease linked to Hajj 2000

Perrocheau A[1], Meffre C[2], Hahné S[3], Aguilera JF[4], and the members of the European investigation team of the W135 meningococcal outbreak.
1 InVS, St Maurice, France. 2 RIVM, EPIET, the Netherlands. 3 JF Aguilera, PHLS, EPIET, CDSC, London, UK. 4 PHLS, EPIET, CDSC, Cardiff, Wales

Introduction

Late March 2000, national reference laboratories for Meningococci in the UK and in France detected an increase in serogroup W135 isolates. Most of them were isolated from pilgrims recently returned from the Hajj, the annual Muslim pilgrimage to Mecca which took place from the 15th to the 18th of March (week 11), or from their household contacts. At the same time W135 Meningococcal Disease (MD) cases linked to the Hajj were reported from Saudi-Arabia, other European countries, the USA, and Asia. National surveillance centres for infectious diseases in Europe conducted a combined outbreak investigation. In France, the 8th of April, national health authorities recommended preventive chemoprophylaxis with rifampicin to all pilgrims and their household contacts to prevent further cases and to limit the spread of the epidemic strain in the population. We describe the outbreak characteristics and present results of the control measures assessment in France.

Methods

A confirmed case was defined as a person with W135 *Neisseria meningitidis* (Nm) isolated from a normally sterile site with antigenic formula 2a:P1.2,P1.5 or belonging to the ET-37 clonal complex. A probable case was defined as a pilgrim or a person who had been in contact with a pilgrim, with either identification of MD due to Nm serogroup W135 (PCR, detection of specific antigens) or with a clinical diagnosis of MD without laboratory confirmation. Cases admitted from 18th March to 31st July 2000, i.e. week 12 to 30 were included. A standardised questionnaire was sent to all European countries participating in the European Monitoring Group for Meningococci (EMGM). As they accounted for most of the cases, results were presented by country for France and the UK, and they were combined for other countries.

To evaluate the impact of the French preventive measures, we compared the change in incidence after/before the intervention in France and in the UK (1) for pilgrims and their household contacts, and (2) for cases with pilgrim contact outside the household and cases with no known contact. A odds ratio France / UK greater than one indicated no impact of the measures.

Results

From week 12 to week 30, 87 cases (79 confirmed and 8 probable) of W135 MD were ascertained from 8 European countries. The outbreak peaked at week 14 (18 cases) and 86% of the cases occurred by week 19. Among pilgrims (n=12), the peak of incidence appeared at week 13, among household contacts (n=31) at week 14, among non-household contacts (n=19) at week 16 and among cases with unknown or no contact with a pilgrim (n=25) at week 19. The sex ratio male to female was 0.7. The proportion of patients older than 20 years old decreased from 41% during the first four weeks of the outbreak to 18% after that period (p=0.02). Clinical symptoms were reported for 67 cases : meningitis (29 cases), septicaemia (23) or both (15). They were associated with arthritis in six cases, osteomyelitis in one and pneumonia in one. Purpura fulminans was reported for 12 (18%) cases. The overall CFR was 16%, not significantly higher than the CFR observed in France in 1999, around 10%.

The ratio of ratios of France / UK regarding changes before / after the measures among non-household contacts and no-known contacts was lower than one, suggesting that the control measures may have reduced the number of cases in this group. The ratio of ratios concerning pilgrim cases and household contacts higher than one suggested an absence of impact. None of these results were significant.

Discussion

Following the occurrence of outbreaks of MD serogroup A associated with Hajj that occurred in 1987 and 1992 the Saudi health authorities have implemented mandatory vaccination against AC Nm for all pilgrims entering Saudi Arabia. The described outbreak was caused by Nm serogroup W135 that accounted for less than 4% of cases notified to the EMGM between 1995 and 1999 in Europe. The outbreak strain belonged to the ET-37 clonal complex that has caused hyperendemic disease activity and outbreaks in the recent past in Europe. Although the CFR associated with the outbreak appeared high initially due to the oldest age of the first cases, mostly pilgrims or household contact of pilgrims, the overall CFR did not differ from the CFR observed in France or in the UK. Most of the cases occurred during the first weeks after the introduction of the strain in the countries by the returning pilgrims. Then the number of incident cases decreased rapidly, indicating that the transmission of the outbreak strain among the general population reflect what is usually observed for NM.
Following this outbreak, several countries such as the UK, France, the Netherlands recommended vaccination with A-C-Y-W135 polysaccharide vaccine to pilgrims travelling to Hajj in 2001. At the end of March 2001, 2 cases of W135 linked to Hajj 2001 had been reported in France and 11 cases in the UK. Reasons for these differences are still unclear.

EC-funded DG SANCO project : Surveillance of meningococcal disease in the European Union, 2000-2001

Sarah Handford, Andrew Fox, Mary Ramsay, Brian Henderson, Norman Noah

European Commission Decision No. 2119/98/EC for setting up a network for the epidemiological surveillance and control of communicable diseases in the European Community stated as a priority 'bacterial meningitis'. Invasive disease due to *Neisseria meningitidis* comes within this priority.

The surveillance network for meningococcal disease was established amongst the European Union member states, and a number of other countries, in year 2000, with the following aims:

- To improve the epidemiological information on invasive meningococcal disease within the European Union;
- To improve the laboratory capacity to accurately characterise the isolates of *N. meningitidis* using standardised methods.

As meningococcal disease is relatively uncommon, this project allows pooling of such data to increase the power of any epidemiological analysis. European wide analysis should be able to detect changes in serogroup and serotype distribution, which is important in both disease management and the formulation of vaccination strategies.

The minimum dataset data has been collected from participant countries for 1999 and 2000 and the descriptive epidemiology analysed. The minimum dataset includes age, sex, date of onset, method of confirmation, site of identification, serogrouping, typing and sero-subtyping. Age-specific incidence rates, seasonality, temporal trends and the phenotypic variation between disease causing meningococcal isolates will be compared between countries.

Provisional data for 1999 shows that of the 7671 reported cases of invasive meningococcal disease in participating countries, 5899 were serogrouped, 2833 were serotyped and 3078 were sero-subtyped. For 2000, the provisional figures show that of the current reported total of 4639 cases, 3895 were serogrouped, 1738 serotyped and 2205 sero-subtyped.

These epidemiological analyses, the ability to identify changes in the distribution of serogroup, serotype and subtype distribution, and the importance of these in the formulation of vaccination strategies, are reliant on participant countries providing disaggregated case data.

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EU-MenNet: Impact of meningococcal epidemiology and population biology on public health in Europe - a consortium funded by the EU Quality of Life program 2001-2004

Matthias Frosch
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Objectives

1. Creation of a pan-European infrastructure for the investigation and surveillance of meningococcal disease. Existing infrastructure and expertise will be extended and linked by electronic information systems.
2. Generation and dissemination of Europe-wide resources and knowledge and harmonisation of methodologies for meningococcal research and surveillance.
3. Analysis of meningococcal population structure and dynamics by exploiting of the outputs of 1 and 2. Specifically, to define disease–associated and antibiotic resistant meningococci and to measure and understand their spread in Europe.
4. Accurate ongoing assessment of the meningococcal disease burden in Europe and prediction of future trends.
5. Integration of the insights obtained into improve public health interventions and sharing and dissemination of best practice in meningococcal disease surveillance and management.

The project is divided into three tasks:

Task 1. Will create the infrastructure required for population studies of meningococcal disease. Three European resource centres will be created: a European Meningococcal Epidemiology Centre (EMEC); a European Meningococcal MLST Centre (EMMC); and a European Meningococcal Strain Collection (EMSC). Linked websites at the EMEC will be created to provide integrated epidemiological and isolate characterisation data. Improved methods for non-culture diagnosis and isolate characterisation will be developed.

Task 2. Will exploit the infrastructure and techniques generated by Task 1, by means of hypothesis-driven research programmes involving the characterisation and comparison of large defined meningococcal isolate collections. These studies will identify and define:

- the meningococcal lineages that are responsible for disease;
- those meningococcal lineage which are rarely or never associated with disease;

- the meningococcal lineages that are responsible for the most severe manefestations of meningococcal disease;
- the potential impact of population-scale vaccination on meningococcal evolution;
- the extent and nature of antibiotic resistance in meningococci, and the association of antibiotic resistance determinants with particular lineages.

These data will be used to construct predictive models of meningococcal population structure and spread.

Task 3. Will co-ordinate the activities of Tasks 1 and 2 and will ensure that the outputs of these tasks are appropriately exploited for the benefit of public health in Europe. This will be achieved by the establishment of a unified management structure and the promotion of information exchange by the organisation of meetings, the co-ordination of fellowship exchanges among members of the network, the publication of results in international peer reviewed journals, and the promotion of best practice for public health and clinical management of meningococcal disease throughout Europe.

The clinical pictures of meningococcal disease

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Aims
A major aim in meningococcal disease (MCD) is to reduce the high case fatality rate (CFR). Early diagnosis and treatment can save more patients. Therefore, adequate and simple information of the early clinical signs of the disease, especially the "dangerous" signs, should be given to the public and to health personnel.

Material & methods
In order to give such information we have, from 1976, systematically recorded a number of clinical and laboratory factors of all MCD patients admitted to Haukeland University Hospital in Bergen.

Results
More than 500 patients, 3 months to 93 years old, have been studied.
Altogether 44 of them died (CFR=8.7%). The annual CFR ranged from 0-22%.
The dominating clinical pictures were meningitis, septicaemia or the combination of meningitis and septicaemia. Additional information about skin rash and blood pressure were mandatory. Based on these clinical manifestations on admission to the hospital we have classified the patients into 4 clinical disease categories (CFR in %):

I. Meningitis [≥100 cells/μl of CSF or back rigidity] and no hypotension (< 1%)
II. Septicaemia with hypotension and/or ecchymoses, but no signs of meningitis (>50 %)
III. Septicaemia with hypotension and/or ecchymoses and signs of meningitis (10 %)
IV. Septicaemia with or without meningitis, with no hypotension or ecchymoses (< 5%)

In addition:
Petechiae were present in 80% of the patients, and in all but one fatal case.
The most severe MCD developed rapidly, and all fatal cases had been ill for ≤24 hours.
A majority of the fatal cases were admitted during the morning hours (nightly delay).
Low levels of platelets (<100 x 10^9/L) and leukocytes (<5 x 10^9/L) were associated with increased probability for fatal outcome.

Discussion

Symptoms and signs of MCD vary. Meningitis and/or septicaemia are most common.

A simple diagnostic and classification system of MCD have been useful to decide on the extent and urgency of therapeutic measures required, even prior to hospitalisation. The data driven system is based on admission recordings of: Duration of illness, meningitis, septicaemia or both, shock and skin bleedings. Briefly, rapid progression to septic shock without meningitis is most severe, and meningitis only has good prognosis.

Note also unexplained fever accompanied by reduced general condition, strong shivering, persistent vomiting, seizure, and signs of meningitis (headache, vomiting, irritability, photofobia, reduced consciousness, nuchal or back rigidity).

Low annual CFR could be associated with media information of the early clinical signs of MC septicaemia. Accordingly, the public and health personnel should repeatedly be informed about the clinical picture of this manifestation of the disease.

Conclusions

1. Simplified and regularly repeated information to the public about the early signs of MCD, especially MC septicaemia, is necessary to reduce CFR.
2. Core information: During the first 24 hours of unexplained fever, especially in children and teenagers, look for skin rash or skin bleedings at regular intervals (2-4 hours), even during the night.
3. Colour prints of petechiae and the "glass test" in newspapers are most efficient.

Atypical manifestations of meningococcal disease

Hans Fredlund MD PhD
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The spectrum of meningococcal disease

The spectrum of meningococcal disease (MCd) is wide, ranging from mild local infections to meningitis and fulminant, fatal septicemia. It is not known why some previously healthy persons develop a benign MCd, and others a fulminant septicemia culminating in death within hours. A combination of host factors, environmental factors, co-infection, and organism characteristics seem, however, to be important. Man is the only natural host for MC and carriage peaks at 15-20 years of age. Signs and/or symptoms from various organs may be due to local non-invasive infection or be a part of a general invasive disease.

Symptoms from the ***upper respiratory tract*** are often found as prodromal symptoms in patients developing invasive MCd but have also been reported without any other symtom of MCd. Primary MC ***conjungtivitis*** is seen as a non-invasive form and an invasive form which is followed by systemic MCd after a variable interval of time. The number of cases with MC as the etiology of ***lower respiratory tract*** infections may be underestimated, due to a suboptimal sampling for culturing of MC. Patients with MC pneumonia often suffer from a relatively mild disease with few complications.

Urogenital infections have been reported among women as well as men and are probably spread by oro-genital sexual behaviour. The liability to confusion with gonococcal infections was early reported.

Sometimes ***abdominal symptoms*** are described initially in invasive MCd, such as pain, vomiting and diarrhoea, which has misled patients, their families and physicians to adopt a diagnosis of acute abdominal disease.

Benign/chronic meningococcemia was also early described and is characterized by spikes of fever, skin manifestations and arthralgia-arthritis in a relatively healthy-appearing individual. The most typical finding is the skin eruptions, a maculopapular exanthema with infiltration, and sometimes a haemorrhagic character.

MC associated arthritis can be either bacterial or reactive. Isolated acute, bacterial arthritis is unusal. It is more often reported in children than adults. Reactive arthritis can have an early onset with polyarthritis or a postinfectious onset often involving only a few joints.

Peri-myocarditids is often present in fulminant cases of MCd. ***Endocarditis*** is nowadays an unusual complication but was extensively documented in the preantibiotic era.

These manifestations and a lot of different other clinical pictures of MCd have also been published as case reports but have not recently been reviewed in the literature.

Proposal for standardization of media and methods to be used on the determination of the susceptibility level of resistance to antibiotics in *Neisseria meningitidis*

Luisa Arreaza and **Julio A. Vázquez** (Spanish Reference Laboratory for Meningococci – National Institute of Health Carlos III), representing 14 different Reference Laboratories (listed bellow).

Introduction

During the last EMGM meeting in Greece 1999, Colin Block from Israel presented data about a questionnaire which had been send to different laboratories in Europe. The main conclusion in that study was the heterogeneity on the methods and media used in each laboratory, making it difficult to compare data between not only different countries but also between different laboratories. For this reason it was presented a proposal around an study to try to standardise method and media to be used on the MIC determination in meningococcal strains. According with the preferences of some laboratories it was decided to include penicillin G, rifampicin, ciprofloxacin, ofloxacin, ceftriaxone and cefotaxime.

Material and Methods

A total of 14 laboratories were interested to participate in the study:

Anna Skoczynska	Sera & Vaccines Central Res Lab – Poland
Colin Block	Hadassah University Hospital - Israel
Georgina Tzanakaki	National School of Public Health – Greece
Ingrid Ehrhard	National Reference Center for Meningococci - Germany
Julio A Vázquez	National Institut of Health Carlos III - Spain
L Spanjaard	Academic Medical Center – The Netherlands
Muhamed K Taha	Institut Pasteur - France
Paola Mastrantonio	Instituto Superiore di Sanitá – Italy
Paula Krizova	Nat. Refer. Lab. for meningococcal infections – Czech Republic
Per Olcen	Orebro Medical Center Hospital - Sweden
Pierre Nicolas	Unite meningocoque, WHO collaborating Centre - France
Sigrid Heuberger	Bundesstaatiche Bakt-serolog - Austria
Steeve Gray	Withington Hospital – United Kingdom
Steen Hoffmann	Statens Serum Institute - Denmark

The Spanish Reference laboratory for meningococci (SRLM) prepared 14 freeze dried collections of 18 meningococcal strains with different levels of susceptibility particularly for penicillin and rifampicin. The SRLM sent to all the laboratories a collection with 18 strains identify as EMGM1, EMGM2......until EMGM18.

All the participant laboratories also received:

- A 500 grs bottle of Muller Hinton medium belonging all of them to the same Comercial Company and to the same lot.
- The 6 different antimicrobial agents to use (same lot and power)
- A protocol for the agar dilution method using Muller Hinton agar, MH agar supplemented with sheep blood and MH agar supplemented with chocolated sheep blood

In order to decide objectively the strains with moderate susceptibility to penicillin, the SRLM sequenced the *penA* gene in all of them. The group of the Institut Pasteur did the same analysis with the same conclusions: 12 meningococci Pen^{ms} were included on the study.

So, each laboratory was to analyse the collection of 18 different meningococcal strains with the three previous media by agar dilution method and also by Etest. There were some laboratories that decided to include also their own media or method on the study. All the data were sent to the SRLM to be analyzed.

For the analysis, the first critical question was to know the best medium in each method with each antibiotic, and the selection was done by analysing the agreement between laboratories in each method-medium and antibiotic.

Once the best medium was decided, the second critical issue was to choose the "correct" MIC for the strains in each antibiotic: the mode/result was choose in each case.

The third question was to know the agreement in each laboratory and also be able to see the strains with a result with a deviation equal or higher than 2 dilutions with respect to the "consensus MIC" in all the laboratories.

Finally, it should be very interesting to analyze the efficacy of each laboratory to detect the Pen^{ms} isolates included in the study.

Results and Discussion

All the laboratories will be identified with a number, all the information being confidential. During the meeting in Örebro all the data will be presented in this anonymous way. Later on, a summary will be send to each laboratory identifying only the results belonging to the laboratory comparing with the other, identified with a number.

Each laboratory generated around 18x6x6 MIC values (648) so we should manage around 9000 MIC values.

There were 12 laboratories sending the results in time. The data from the remaining two laboratories will be included in the final analysis.

The data linked to the "local media" will be analysed in the future.

Up to now, we have only analysed the data from penicillin, rifampicin, cefotaxime and ciprofloxacin. We hope to be finished in time for the meeting in Örebro.

The first important result was that we included one strain (the EMGM3) that was showing very variable results, even in each laboratory. The strain is now being analysed in our laboratory but finally we decided not to consider it in our analysis. So, 10 isolates with moderate susceptibility to penicillin will be finally analysed.
The agreement in the different medium with each antibiotic was:

	penicil	rifamp	ciprofloxac	Cefotaxime
AD method				
MH	73.7	77	86.1	83.9
MH+blood	88.7	83.4	87.7	89.2
MH+chocolate	88.7	90.9	90.9	87.7
Etest				
MH	76.9	72.6	79.1	83.8
MH+blood	83.3	79.6	97.3	98.2
MH+chocolate	81.3	73.6	91.9	96.1

There were some laboratories having problems with Muller Hinton agar without supplement, and perhaps this was the reason for a low agreement compare with the other two media. Generally speaking, MH supplemented with blood or with chocolated blood offered similar results. Both media also gave similar results detecting Pen^{ms} strains. However, because it is an easier medium to prepare, we hope to recommend Muller Hinton supplemented with blood.

We will show the MICs "consensus " for all the strains.

In relation with the Pen^{ms} meningococci, by using the agar dilution method, 4 laboratories were able to detect all the 11 isolates, 6 were inefficients to detect one of these strains, and one group did not detect two Pen^{ms} meningococci. The same analysis on the Etest method showed that 5 laboratories detected all the "intermediate" isolates, 4 missed one, 2 groups missed 2 and there was one laboratory which was unable to detect any of them. It might be interesting to develop a PCR based method or some other asay to confirm those strains with 0.06 μg/ml.

Only one group classified one susceptible strain as Pen^{ms}.

The most interesting data about the agreement in each laboratory were generated with penicillin and rifampicin because all the strains presented a very homogeneous profile of susceptibility to the other antibiotics:

	AD PENICILLIN	Etest PENICILLIN	AD RIFAMPICIN	Etest RIFAMPICIN
LAB nº 1	94.1%	100	88.2	100
LAB nº 2	58.8%	82.3	76.5	68.7
LAB nº 3	100%	100	82.3	68.7
LAB nº 4	100%	70.6	76.5	75
LAB nº 5	88.2%	100	88.2	----
LAB nº 6	94.1%	94.1	100	50
LAB nº 7	94.1%	94.1	76.5	75
LAB nº 8	100%	100	94.1	93.3
LAB nº 9	100%	100	58.8	80
LAB nº 10	94.1	100	64.7	50
LAB nº 11	82.3	100	64.7	87.5
LAB nº 12		17.6		93.7

The results were in general much better for penicillin than for rifampicin.

Finally, during the meeting in Örebro we should discuss some aspects about the final analysis:

- Is there consensus about how to decide the "consensus MIC"?
- Is one dilution difference enough to analyse the agreement or should we permit two dilutions of difference?
- Results particularly different from those defined as "consensus MICs", should they or should they not be taken out in order to improve the final agreement?

Immunology and the meningococcus

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This review will focus on natural immunity and immune responses following meningococcal disease. *Neisseria meningitidis* is carried in the nasopharynx by about 10% of the adult population. This figure contrasts with the much lower incidence of meningococcal disease, so meningococcal disease may be considered as an opportunistic infection involving different elements of host susceptibility and bacterial virulence. Asymptomatic meningococcal carriage can be regarded as an immunising process that initiates and broadens protective antibody activity. Natural immunity is also developed by colonisation of *N. lactamica,* and the intestinal flora may enhance the immunity through cross-reacting antigens. Meningococcal carriage rate is lower in small children, and from the pioneering work by Goldschneider *et al.* an inverse relationship between serum bactericidal activity and age-related incidence of disease was demonstrated. Antibodies are most important in protection against disease through their binding to the bacterium and complement activation leading to bactericidal killing and phagocytosis. Which of these mechanisms that is the most important for protection is not fully elucidated, but studies suggest that their relative importance may depend on the serogroup of the infecting meningococcus. Bactericidal antibodies are induced by the serogroup A, C, Y and W135 capsules, but the group B capsule is poorly immunogenic, and the acquired immunity to group B meningococci is directed to various outer membrane proteins and LPS. Complement is central in preventing disease as demonstrated by studies of individuals with deficiencies of either terminal complement factors or factors in the alternative pathway. For the third way of complement activation, namely that through the mannose-binding protein, divergent findings have been presented for the association between genetic variants of this lectin and susceptibility to meningococcal disease. Cellular immune responses have been studied after infection and vaccination and several T-cell epitopes, present in conserved regions of outer membrane proteins, identified.

In some but not all respects, infants and smaller children, the age groups most affected with meningococcal disease, respond differently to meningococcal antigens compared to children above 10 years of age. The low levels of bactericidal antibodies in sera from infants were not reflected in the levels of IgG1 and IgG3 subclasses which were similar in infants and older children. This age-independent level of complement-fixing antibodies also contrasted with the cytokine profiles being skewed to Th1 in the youngest and to Th2 in the older children. No difference was observed in

the proliferative responses of peripheral blood mononuclear cells from smaller and older children. Further studies on the cytokine milieu will be needed to elucidate the lack of bactericidal responses in small children.

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Vaccines against serogroup B meningococcal disease

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During the last three decades serogroup B *Neisseria meningitidis* has emerged as the cause of epidemics in several European countries, the Americas, and South Africa. Whereas polysaccharide vaccines of serogroup A, C, Y and W135 offer good protection against meningococcal disease in adults and in children over 2 years, the pure serogroup B polysaccharide (B-ps) is poorly immunogenic. Therefore, alternative vaccine approaches, based on non-capsular surface antigens or chemically modified B-ps, have been suggested.

One approach has been a chemical **modification (N-propionylation) of the B-ps capsule** itself. The NPr-B-ps induced bactericidal antibodies in monkeys, which did not cross-react with human tissues, and is a potential vaccine candidate. Another approach is **detoxified lipopolysaccharide** (LPS) based vaccines. About 80-90% of invasive group B and C meningococcal strains have the L3,7,9 LPS immunotype. LPS is highly immunogenic in man and induce bactericidal antibodies. However, use of LPS-based vaccines has met with limited success. In contrast, the **PorA protein** has been shown in several studies to induce bactericidal antibodies and is therefore a critical protein to include in any group B protein vaccine. The PorA protein is exposed on the surface of the outer membrane and exists in the membrane as a trimer, and some data suggest that its natural configuration is as a heterotrimer with the PorB porin protein. In addition, several other outer membrane proteins, inducing cross-reactive antibodies among a variety of meningococcal strains, have been identified as potential vaccine candidates. Among these are the **Opc (5C) invasin**, the **iron regulated proteins (IRP), transferrin** and **lactoferrin binding proteins** (Tbps and Lbps), **PilQ**, and the **neisserial surface protein A** (NspA). Inclusion of these proteins in a vaccine may be important due to the antigenic diversity within PorA, and because these other proteins can potentially extend vaccine coverage and protection.

The most promising approach to date has been the use of outer membrane vesicle (OMV) vaccines which contain several of these antigens. Such group B meningococcal OMV vaccines have been extensively evaluated in clinical trials in several countries and have involved several million adults, older children and infants. The vaccines have shown a good safety (reactogenicity) profile and induce functional antibodies as measured by induction of both bactericidal and opsonic antibodies, as well as immunological memory. Most importantly, they have demonstrated a significant efficacy against group B disease in controlled clinical trials in Norway, Cuba and Brazil. However, waning immunogenicity and strain restricted protection in infants, has been observed with a two-dose

schedule. More recently, studies with a three-dose immunization schedule showed increased and more long-lasting bactericidal responses and higher level of cross-reactive bactericidal antibodies.

There are approximately 20 different serosubtypes, based upon antigenic differences in the two major variable regions in PorA. Although this represents a broad antigenic diversity, hyperendemic levels and epidemics caused by group B meningococci tend to be clonal and of long duration. Thus, OMVs from one or two PorA serosubtypes may provide broad coverage for a given geographic region and point in time. The fact that the important hyperendemic and epidemic group B strains change over time, but slowly, means that the manufacturing process for preparation of group B outer membrane protein vaccines should be sufficiently robust to apply equally to various group B outbreak strains.

Serum bactericidal assays

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The role of circulating antibody and complement in protection from meningococcal disease was clearly demonstrated in the 1900s.

Serum bactericidal antibody (SBA) activity has been shown to highly correlate with immunity to meningococcal disease and an inverse correlation has observed between the age-related incidence of disease and the age-specific prevalence of complement-dependent SBA activity. Induction of complement-dependent bactericidal antibodies after vaccination with meningococcal polysaccharide or protein conjugate vaccines is regarded as acceptable evidence of the potential efficacy of these vaccines.

In 1976, the WHO Expert Committee on Biological Standardisation recommended a SBA to satisfy the requirements for production and release of meningococcal polysaccharide vaccine. These SBA requirements have also been used to support vaccine licensure. A four-fold or greater rise in SBA titre is currently used to estimate the potential efficacy of meningococcal vaccines during field trials, as well as to determine seroconversion after immunisation with the currently licensed polysaccharide vaccine or protein-polysaccharide conjugate vaccines. The complement source used in the SBA appears to be of critical importance.

Unlike the reported serogroup B SBAs that use human complement, the WHO recommended procedure for serogroups A and C uses baby rabbit serum as a complement source. Intuitively, a normal human complement source would be desirable; however, in practice, large volumes of a suitable human source are not available or difficult to obtain. When SBA titres have been compared after using baby rabbit and human serum as a complement source, significant differences in antibody titres have been observed; human complement tends to give lower titres. The original assays that associated bactericidal antibody with protection used normal human serum that lacked bactericidal activity to strains used in the study as a complement source with test sera from individuals after natural infection.

Vaccine licensure, however, was supported using SBA titres obtained using baby rabbit complement with sera before and after vaccination. A recent multi-laboratory study standardised the serogroup C SBA and compared it to the recommended WHO procedure. The modified assay will facilitate inter-laboratory comparisons of the functional antibody produced in response to current or developing serogroup C meningococcal vaccines. The SBA can be used to measure large numbers of sera in an immunogenicity study. Semi-

automation can be achieved by the use of colony counters and dilutors. In fact, more sera can be assayed by SBA than by ELISA in the same amount of time.

In conclusion, for the serogroup C SBA, some questions still need to be answered and some research may need to be completed but we can now more confidently substitute these laboratory surrogates for clinical efficacy trials. As for serogroup C, the SBA has become the primary serologic assay used to assess protective immunity stimulated by serogroup B meningococcal vaccine components a number of SBA assays have been described which differ greatly.

Like serogroup C SBAs, the choice of complement is also unclear for serogroup B SBAs. Human post-vaccination antibody to meningococcal serogroup B polysaccharide has been shown to be strongly bactericidal with rabbit complement, but to have little or no bactericidal activity in conjunction with human complement. The specificity of human antibodies that are bactericidal for serogroup B meningococci have been investigated in a number of studies, often with conflicting results. Some discrepancies may be attributable to the use of different complement sources and to differences in the assays used.

Opsonophagocytosis of meningococci

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Aims

To present methods for measurement of opsonophagocytosis of meningococci (MC) and discuss the role of opsonophagocytosis in human host defence against MC.

Unspecific complement and specific antibodies can opsonize MC and stimulate phagocytic internalisation and killing of the bacteria. Such opsonophagocytosis can be measured *in vitro* by different methods, e.g. by microscopy, by leukocyte uptake of radiolabelled MC, by intracellular killing of MC using pour-plate technique and by chemiluminescence production from phagocytosing leukocytes. Presently, we use flowcytometry to quantitate the number of internalised bacteria and the intracellular "oxidative burst" of the phagocytes. Each of the 4 key factors – antibodies, complement, MC and leukocytes – can be studied by keeping the 3 others constant.

We have focused on the level and quality of antimeningococcal opsonizing antibodies during and after MC disease, and before and after vaccination. During MC disease opsonic antibodies peak about 2 weeks after onset of the disease, level off and decrease, but remains high for several years. After vaccination with the Norwegian serogroup B MC vaccine, most of the volunteers responded with increased opsonic antibodies and a booster response. However, the antibody response had shorter duration than after clinical disease. Remarkably, high cross-reacting opsonic activities can be recorded after systemic infection with different serogroups and serotypes/subtypes, while less cross reactions were found after the vaccination.

To study more antigen-specific opsonophagocytic responses, we have developed a multiparameter flowcytometric assay: MC were replaced by fluorescent polystyrene beads coated with a number of outer membrane structures, such as outer membrane vesicles, PorA, PorB, and transferrin binding complexes. Results from these studies suggest that the employed MC components have different potential as inducers of human opsonic antibodies. The method may be of great value for the selection of MC antigens for future MC vaccines.

The major advantages of the opsonophagocytic flowcytometer assay are the possibility to quantify functional species-specific and antigen-specific opsonic antibodies, and to measure large numbers of samples with small volumes. The technology offers rapid multiparameter measurements of single cells, allowing several phagocyte functions to be measured simultaneously. The assay are applicable to both experimental and clinical work. However, the phagocytosis

and oxidative processes are very complex and *in vitro* studies of these processes are indeed a great challenge. Sufficient knowledge about phagocytosis and flowcytometry are necessary to reach the aim of the experiments and to draw adequate conclusions. Technical complexity, and biological variation of donor leukocytes and viable MC (if used), are the most actual limitations of the assay.

Since phagocytosis is of major importance in host defence against encapsulated bacteria, and since encapsulated MC are phagocytized, studies of serum opsonins during MC disease with different MC and MC antigens as well as following vaccination may provide valuable information about the complex human immunity to MC infection.

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Measurement of T-cell responses against meningococcal antigens

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Introduction

Although protective immunity against meningococci relies on antibody-mediated effector functions (serum bactericidal activity and opsonophagocytosis), T-cells play an important role in the regulation of the immune response, including stimulation of B-cells for antibody production. T-cells are also necessary for the establishment of immunological memory, and to promote activation of phagocytic cells, thereby facilitating the uptake and destruction of meningococci.

Methods

Meningococcal disease and vaccination lead to activation and proliferation of antigen-specific CD4+ T-cells in peripheral blood. By using a thymidine incorporation assay, performed in microtiter plates, human CD4+ T-cell responses to meningococcal antigens can be measured *in vitro*. This assay can be combined with measurement of cytokines by ELISA of supernatants from antigen-stimulated T-cells or by flow cytometry at the intracellular level.

Peripheral blood mononuclear cells (PBMC) are isolated from fresh whole blood drawn from vaccinees or convalescents from meningococcal disease. PBMC are cultivated *in vitro* in the presence of meningococcal antigens (purified protein antigens, outer-membrane preparations or whole inactivated bacteria). Recognition of meningococcal antigens presented by APC will stimulate antigen-specific CD4+ T-cells to proliferate. T-cell proliferation is measured by pulsing the cultures at day 6 for 18 hours with [^{3}H] thymidine, which is incorporated into the DNA of dividing cells. The amount of radioactivity present in DNA harvested from the cell cultures is detected in a scintillation counter and represents a measurement of antigen-specific CD4+ T-cell proliferation. Although non-fractionated PBMC are used in the proliferation assay, the design of the assay makes it possible to detect CD4+ T-cell responses against the antigens tested. Antigen-driven polyclonal B-cell responses generally peak at day 2-3, whereas the detection of proliferation is performed from day 6 to 7, which generally is corresponding to the peak in T-cell proliferation. We have confirmed that CD4+ T-cells predominate the proliferation by two different experimental approaches. Firstly, by using flow cytometry, we have shown that the blastoid and dividing cell population present at day 6 consisted of more than 90% CD4+ T-cells. Secondly, the predominant contribution of CD4+ T-cells

was confirmed by the observation that anti-MHC class II antibodies (anti-HLA-DR) inhibited the proliferative responses to meningococcal antigens in a dose-dependent manner.

The number of meningococcal outer membrane proteins so far studied with respect to T-cell responses is limited and include the major porins PorA and PorB and class 5 proteins (Opc and Opa) and TspA (1,2,3). The recently identified auto-transporter A protein has also been shown to elicit T-cell responses (4). A study of T-cell proliferative responses after meningococcal disease has been performed in children from whom cytokine responses were also measured (5). Studies of T-cell responses after vaccination have been undertaken for the Dutch hexavalent PorB vaccine (6) and for the Norwegian serogroup B meningococcal outer membrane vesicle (OMV) vaccine. Both systemic and nasal vaccination with the Norwegian OMV vaccine induced antigen-specific T-cell proliferation against the PorA and PorB antigen, in addition to the OMV vaccine antigen. The T-cell response against the PorA antigen was considerably higher than measured against PorB and correlated with mucosal IgA, serum IgG, and with bactericidal activity against meningococci (7,8).

Conclusions

The ability to differentiate between Th1 and Th2 T-cell responses is also important in order to evaluate the vaccine potential of meningococcal antigens. It is well established that Th2 cells, producing IL4 and IL5, play a major role in B-cell differentiation and antibody production. Cytokine responses after vaccination with meningococcal antigens has not yet been systematically analyzed, but the introduction of flow cytometric methods for detection of Th1/Th2 cytokines produced at the single cell level will now make this more feasible.

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Future meningococcal vaccines

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Since polysaccharide conjugate vaccines including *N .meningitidis* group C conjugate vaccines are shown to be highly efficacious in preventing invasive disease, additional conjugate vaccines for group A, W135 and Y are expected to be available in the near future.

Various group B meningococcal vaccines are under development. Vaccines with **PorA outer membrane protein** of the epidemic type are most advanced. At the RIVM a hexavalent PorA vaccine is developed that contains the 6 most predominant sero-subtypes in Europe (P1.7,16; P1.5-1,2-2; P1.19,15-1; P1.5-2,10; P1.12-1,13; P1.7-2,4, embedded in two outer membrane vesicles, each containing three PorA's. Class 2/3 protein (PorB), as well as the B polysaccharide are not expressed due to gene deletions. In addition, a monovalent P1.4 vaccine was developed similarly for epidemic control in New Zealand. The vaccines were shown to be safe and immunogenic, and to induce immunological memory. Recently, lipopolysaccharide mutations were introduced in the vaccine to reduce inflammatory reactions by the vaccine without affecting adjuvant activity.

In addition to the PorA **several other outer membrane proteins** and **lipopolysaccharide** are being studied as vaccine candidates. Finally, **genomics** offer new possibilities for new unexpected vaccine candidates.

Demonstration of web research with the MLST database

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Multi Locus Sequence Typing is an increasingly popular method both for typing meningococci and for epidemiological studies. The MLST website (www.mlst.net) is a central part of this success and enables comparative studies and research online. This presentation, which will be a live internet demonstration of the web resources, will be both an update on ongoing activities and an introduction to new features. We aim to focus on three aspects.

Firstly, by using an example of real questions one may wish to address with the database, we will demonstrate the features of the interactive website, especially the potential for data analysis.

Secondly, we will introduce the interface, which is used by curators to manage the database.

Thirdly, the design of the database, and method for setting up a database for a new MLST species will be discussed.

As well as showing the facilities available on the web, one aim of this session will be to give participants a more in depth understanding of the MLST databases and their management.

The session is intended to be interactive, and participants are welcome to bring their data for discussion. We are also happy to advise those who wish to set up new MLST databases.

LST database

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Assessing the risk of laboratory acquired meningococcal disease

Stuart JM[1], **Gray SJ**[2] and **Kaczmarski EB**[2].

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Introduction

A small number of possible laboratory acquired cases of meningococcal disease have been reported within the UK and from laboratories around the world, but the actual risk is not known.

Design of statistical risk assessment

Cases of meningococcal infection in laboratory workers in England and Wales between 1985 and 1999 were identified by a retrospective survey of NHS microbiology and Public Health Laboratories and by literature review. Absolute and relative risks of secondary meningococcal disease in laboratory workers were calculated from the number of identified secondary cases, estimates of numbers of laboratory workers at risk and incidence of meningococcal disease in the general population. Sensitivity analysis was done to allow for uncertainty around these parameters.

Risk assessment results

Five secondary cases were identified in laboratory workers. All 5 had been exposed to cultures of *N. meningitidis* in the 10 days before onset, 4 were confirmed by culture from a normally sterile clinical site and one by demonstrating seroconversion. They were all associated with introduction of a procedure involving manipulation of meningococcal suspensions outside a safety cabinet (UK Class I). The absolute risk was estimated as 1 case per 8000 exposed laboratory workers, a relative risk of 318 (95%CI 103 - 744).

Risk assessment conclusions

The risk of meningococcal disease in laboratory workers who prepare suspensions of meningococci outside safety cabinets are at increased risk. Safety cabinets (UK Class I) should always be used for such procedures and chemoprophylaxis administered in case of inadvertent exposure. Vaccination should be considered for laboratory staff that work regularly with these organisms.

Practical risk reduction within PHLS MRU

Currently *N.meningitidis* are considered a UK ACDP (Advisory Committee on Dangerous Pathogens) hazard group 2 organism. To minimise infection risks all operations likely to produce aerosols are carried out within a class I safety cabinet (UK). Our presentation will illustrate practical considerations in reducing risks with a view to provoking discussion.

Meningococci isolated from cases of meningococcal disease in Israel – National laboratory data for the period 1983 - 2000

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Introduction

Meningococcal disease has not been a major public health problem in Israel in recent years. Despite a rapidly rising population, associated with significant immigration from Russia and her neighbours and from Africa, the incidence of meningococcal disease has, if anything, decreased. This paper summarises data gathered at the National Centre for Meningococci at Tel Hashomer.

Materials and Methods

All laboratories isolating meningococci from cases of meningococcal disease are required to submit all such isolates to the National Center for Meningococci. Internal audit has shown that more than 95% are actually received. Serogroup determination is carried out by slide agglutination. Susceptibility testing is carried out using the Etest, on Mueller-Hinton agar with 5% sheep blood.

Results

Since the last peak incidence of 1.5/100,000 residents/year recorded in 1993, the incidence of meningococcal disease in Israel as based on laboratory confirmed cases has shown a sustained reduction, dropping to 0.8/100,000/year in 2000 when 51 isolates were received. This despite a population increase of 25% since 1990. Serogroup B, which comprised >80% of isolates in the mid-1980s, now represents about 57%. Group C hovered between 20% and 30% throughout the 1990s, and dropped to 6% in 2000. In contrast, the proportion of serogroup Y isolates has been steadily increasing, now representing over 20% of clinical isolates. This group has increased in incidence from 0.02 to 0.09/100,000/year in the 1980s, reaching 0.18/100,000/year in 2000. There has been no increase in the proportion of strains with reduced susceptibility to penicillin, and all isolates were highly susceptible to ceftriaxone and ciprofloxacin. On the other hand, there has been a minor increase in ofloxacin MICs, several recent isolates reaching MICs of 0.023 mg/L.

Comment

Despite interesting changes in relative proportions of meningococcal serogroups in recent years, the overall incidence of disease has remained encouragingly low. No clinically worrisome changes have occurred in antibiotic susceptibilities.

Meningococcal Disease in the Republic of Croatia in the Year 2000

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[2] Croatian National Institute of Public Health, Zagreb, Croatia
[3] Centers for Disease Control and Prevention, Atlanta, USA

Aim

The aim of this study was to present national data for invasive meningococcal disease in Croatia in the year 2000.

Introduction

N. meningitidis is one of the leading causes of bacterial meningitis and sepsis in Croatia. The rates of meningococcal disease over the past ten years in Croatia ranged from 0.7-1.5/100,000. First identification of *N. meningitidis* intermediately resistant to penicillin in Croatia was reported in 1998 (1). For the year 2000, susceptibility testing results and serogroup distribution of *N. meningitidis* isolates are available for the whole country.

Materials and methods

Clinical and epidemiological data are based on clinical notification and were collected by the Croatian National Institute of Public Health.

All *N. meningitidis* isolates from blood and cerebrospinal fluid where transported in silica gel packages (2) to the Laboratory for Microbiology at the University Hospital for Infectious Diseases "Dr. Fran Mihaljević", Zagreb.

The serogroup of *N. meningitidis* isolates was identified by using serogroup-specific antisera (Sanofi Diagnostics Pasteur, Marnesa-la-Coquette, France). Antibiotic susceptibility was determined by the Etest (AB Biodisk, Solna, Sweden).

Results

Based on clinical notifications, there were 41 patients with invasive meningococcal disease in Croatia in 2000, resulting in the incidence rate of 0.8/100,000. There were 28 (68%) females and 13 (32%) males.

Number of cases and incidence rates for each age group is presented in Table 1. Two patients died resulting in lethality of 5%: one patient was in the age group 10-14 (mortality 0.003/1000) and the other in the age group 65+ (mortality 0.001/1000). No clusters of cases were recorded.

Twenty-six of those 41 (63%) cases were culture-confirmed with serogroup distribution as follows: 20 B, 4 C, 1Y and 1 W135. The difference in number of

cases, incidence, mortality and patients' age in 24 culture-confirmed cases caused by serogroups B and C is shown in Table 2.

Disease attack rates differ in various parts in Croatia.

All 26 *N. meningitidis* isolates were susceptible to broad spectrum cephalosporins, ciprofloxacin and chloramphenicol. Resistance to rifampicin was determined in a single isolate (4%). Six (23%) *N. meningitidis* isolates were intermediately resistant to penicillin.

Table 1. Number of cases and incidence for each age group for invasive meningococcal disease in Croatia in 2000.

	Age Groups								
	0-4	**5-9**	**10-14**	**15-19**	**20-29**	**30-39**	**40-49**	**50-59**	**60+**
No cases	24*	4	4	4	0	0	2	0	3
Incidence	8.6	1.3	1.2	1.2	0	0	0.3	0	0.3

*9 cases ≤ 1 year

Table 2. Number of cases, incidence, mortality and patients' age in 24 meningococcal disease cases caused by *N. meningitidis* serogroup B and C.

	Serogroup B	**Serogroup C**
No cases	20	4
Incidence	0.8	0.3
Mortality	0	0
Age	1.5 mo- 79 y	4 –18 y

Conclusions

Based on clinical notifications, incidence rate for invasive meningococcal disease in Croatia in 2000 did not differ from incidences recorded during the last decade, and ranged in different Croatian counties from 0-1.8/100,000. It ranged from 0 in age groups 20-39 and 50-59, to 8.6/100,000 in age group 0-4. Likewise, there was no significant deviation in mortality either (0.04/100,000). The majority of isolates (20 or 77%) belonged to serogroup B, resulting in incidence of 0.8/100,000.

All four *N. meningitidis* serogroup C isolates from this study were intermediately resistant to penicillin. This data supports previous observations that *N. meningitidis* serogroup C strains are about three times more common among intermediately penicillin resistant strains than among the total population of meningococcal strains (3,4).

Acknowledgement
Members of the Croatian Study Group for Bacterial Meningitis: Vlasta Gilić, Vlatka Janeš-Poje, Marina Payerl-Pal, Volga Punda-Polić, Nevenka Tkalec-Makovec, Marina Vodnica.

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Epidemiology of invasive meningococcal disease in the Republic of Ireland: A report on laboratory confirmed cases, July 1997 – June 2000

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Aims

The epidemiology of IMD for the three epidemiological years, 1997/98, 1998/99 and 1999/00 with regard to overall incidence, incidence by age group, mortality, geographical variation in attack rates, serogroup differences and details of antibiotic susceptibility of isolates are described.

Introduction and Methods

In the Republic of Ireland, (population 3.6 million), statutory notifications of "Bacterial Meningitis including Meningococcal Septicaemia" have continued to increase since the early 1990s, rising from 382 notifications in 1995, to 589 in 1999. A system of enhanced notification was introduced during 1996, and the National Meningococcal Reference Laboratory (MRL) was established in late 1996. The MRL provides a diagnostic and confirmatory service for IMD using polymerase chain reaction (PCR) testing of blood and spinal fluid samples from suspected cases. Laboratories are encouraged to submit all isolates of *N. meningitidis* to the MRL. The laboratory-confirmed IMD database held at the MRL includes culture and/or PCR positive cases and cases with compatible acute and convalescent phase serology. Details are communicated to and crosschecked with each regional Public Health Department, and with the National Disease Surveillance Centre, monthly.

Results

Between July 1997 and June 1998, a total of 351 cases of IMD were identified, increasing to 405 cases in 1998/99 and 449 in 1999/00. Group B and Group C together accounted for 96.6% of cases diagnosed. The increase in 98/99 was primarily with Group B and in 99/00 was primarily with Group C. The total number of cases of Group C was similar in the first two EY. There were 119 cases in 98/99, (34% of total laboratory diagnosed IMD) and 113 cases in 99/00, (28% of total). The number of Group C cases increased in the third year to 167 cases, 37% of the total. Overall, the highest age specific incidence rates for both Group B and Group C were in the youngest age group (≤4 years) and, in the

case of Group C, there was a second peak in the 15-19 year old age group.

Epidemiological Year	**IMD Incidence /100,000**	**Group B /100,000**	**Group C /100,000**
97/98	9.68	5.98	3.28
98/99	11.17	7.6	3.12
99/00	12.38	7.47	4.61

In all, 42.7% of cases were diagnosed by culture, 53.5% by PCR alone, and 3.8% by serology. However the percentage diagnosed by culture declined from 47.6% in the first EY to 39% in the third EY with a corresponding increase in cases diagnosed by PCR alone, from 48.4% in 97/98 increasing to 57.7% in 99/00. The incidence rate in one Health Board (NEHB) was significantly above the national rate each year. Fifty-one deaths due to IMD occurred over the three years (CFR 4.1%), 24 with Group B and 17 with Group C. The CFR for Group B disease was highest in the 20-24 year old age group whilst the CFR for Group C was highest in the 15-19 year olds. One serosubtype, 4:p1.4 accounted for 30% of Group B isolates submitted, whilst 2a:p1.5,p1.2 was the most common Group C serosubtype (51% of typed isolates). Penicillin MICs increased over the three years and, in 99/00, 25.8% of isolates had a penicillin MIC of >0.1mg/L.

Conclusions

A high rate of laboratory confirmed IMD was identified in the ROI in the last three completed epidemiological years rising to 12.38/100,000 in 1999/00.The incidence of Group C disease increased in the EY 99/00. The increasing contribution of PCR alone to diagnosis of IMD is a worrying trend resulting in fewer isolates available for typing. Group C disease accounted for 17 deaths in the three years. The introduction of Group C conjugate vaccine should significantly reduce the incidence of Group C IMD. Ongoing surveillance will be necessary to monitor the effects of the vaccine.

Early impact of introduction of meningococcal group C conjugate vaccines on the epidemiology of invasive meningococcal disease in the Republic of Ireland

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Background

Invasive meningococcal disease (IMD) is hyperendemic in the Republic of Ireland at this time with a laboratory confirmed incidence rate of 12.38/100,000 in the epidemiological year 1999/00. Group B and Group C together accounted for 96.6% of cases diagnosed in the last three EYs. In all, the highest age specific incidence rates were in the youngest age group (≤4 years) with, in the case of Group C, a second peak in the 15-19 year old age group. Mortality from Group B and Group C disease was similar overall, at 4.4% and 4.2% respectively. The highest age-specific mortality from Group C disease (8.6%) was in 15-19 year olds. There were 17 deaths due to Group C in the last three EYs, and 15 of these (88%) were in individuals <22 years of age.

Interim Results

In the current epidemiological year 00/01, the usual winter peak of Group C disease activity did not occur. In the 6 month period October 2000 – Mar 2001, there were 35 laboratory-confirmed cases of Group C IMD. This represents a 65% reduction from the number diagnosed in the same time period in 99/00, a 46% reduction over the 98/99 total and a 49% reduction from the 87/98 total. In August and September 2000, Group C accounted for 35.4% and 36.8% respectively, of all laboratory-diagnosed cases of IMD. This had dropped to 20% and 8.7% respectively in February and March 2001. Group B disease in Oct-Mar 00/01 showed a reduction of 5.5% *versus* with 99/00, a 14% reduction from 98/99, and a 12% increase on 97/98.

Oct – Mar	Total C cases	Total B cases
00/01	35	137
99/00	100	145
98/99	65	160
97/98	69	122

There was a similar reduction in numbers of cases diagnosed throughout all age groups. Group C meningitis developed in one 19 year old who had been immunised 23 days previously, this was considered to be a partial vaccine failure on the basis of a high SBT on Day 4 of the illness. None of the other 34 cases had received MenC vaccine.

Conclusion

The preliminary indications are that introduction of MenC vaccine has reduced the incidence of IMD. The reduction observed across all age groups is interesting and suggests a herd effect. However it may alos reflect opportunistic immunisation "out of phase", this will be clarified when full details of vaccine administered are submitted to the Health Boards. In contrast to the findings in the UK, the incidence of Group B disease also declined but this fall has been less dramatic than that seen with Group C. It may be that the rate of Group B IMD has reached a plateau.

Editors remarks: When was the vaccine introduced and which ages were vaccinated?

Laboratory surveillance of meningococcal disease in Belgium 2000

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In 2000 the National Meningococcal Reference Laboratory (I. P. H., Brussels) received 267 strains of *N. meningitidis* isolated from patients with invasive meningococcal disease (34% meningitis alone, 30% septicaemia, 29% combination of both, 7% not specified or other) for confirmation, serogrouping, serotyping and susceptibility testing. This represents an annual incidence of 2.6 cases per 100,000 inhabitants (compared with previous year, crude incidence decreased from 2,9 to 2,6 per 100.000 population). The reported case fatality rate was 4.9%. Most cases occurred in the winter months and were observed in Flanders. Of the 267 laboratory cases, sex was specified in 266: 55% were male. Over 40% of the cases were among children less than 5 years of age; the age group of 15-19 years represented 15% of all cases. Serogroup B predominant accounted for 64% of cases. Serogroup C was responsible for 33% of cases and for 7 of the 13 fatal meningococcal infections. During the past few years, fatality case was associated with serogroup B disease. One of the 4 serogroup W135 cases (1.6% of cases) was linked with the Haj pilgrimage. Serotype 4 (64%) was the most commonly identified for serogroup B . Predominant serotypes among serogroup C isolates were 2a (47%) and 2b (36%).

Serogroup Distribution

	A	B	C	Y	W135	Other	Unk.
N	0	165	85	2	4	1	10
%	0	64.2	33	0.8	1.6	0.4	-

Age Distribution

Age Group	Cases (N)	Cases %	Male (N)	Female (N)	Group B (N)	Group C (N)	Deaths (N)
0-11 m.	35	13,2	16	19	27	7	1
1-4 y.	74	27,9	40	34	45	25	6
5-9 y.	40	15	23	16	25	14	0
10-14 y.	23	8.6	17	6	11	10	2
15-19 y.	41	15.4	21	20	26	10	0
20-24 y.	15	5.6	8	7	8	6	1
25-44 y.	14	5.3	6	8	10	4	1
45-64 y.	12	4.5	8	4	9	2	0
65+ y.	12	4.5	6	6	4	6	2
unknown	1	-	1	0	0	1	0
Total	**267**	**100**	**146**	**120**	**165**	**85**	**13**

Trends in meningococcal disease

Since the beginning of the 1990s an increase in the incidence of invasive meningococcal disease has been noted in Belgium. The incidence calculated from the submission of meningococcal isolates to the Reference Laboratory has gradually increased from 1 to 3 cases per 100,000 inhabitants between 1991 and 1999. Until 1996 this increase in the incidence of the disease was closely associated with group B meningococci. The rise in group B disease was accompanied by a change in serotype distribution: B:4:P1.4 emerged as the principal serotype and accounted for 50% of all group B isolates (91%) in 1996.

Since 1997 an increasing number of invasive disease due to serogroup C meningococci is observed: 13% of all isolates in 1997, 21% in 1998, 28% in 1999 and 33% in 2000.

Automated methods for the laboratory confirmation of meningococcal disease

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Aims

To automate many of the procedures involved in the laboratory confirmation of meningococcal disease within a national reference laboratory.

Materials and Methods

The laboratory confirmation of meningococcal disease and the typing of meningococcal isolates using PCR and DNA sequencing are laborious and prone to user inaccuracy. In addition, the end-point of both methods requires user intervention for data handling. A robotic liquid handler and automated DNA sequencer were therefore purchased to enable the automation of PCR and DNA sequencing.

Results

The robotic liquid handler and automated DNA sequencer were successfully integrated into the routine service of the reference laboratory. A higher throughput of PCR testing was possible and DNA sequencing was introduced as an additional service.

Discussion

Automated methods provide both meningococcal antigen detection in bodily fluids and also full MLST typing of meningococcal isolates. These methods, along with standard culture phenotyping and antibody testing, provide Scotland with an excellent reference service for the confirmation of meningococcal disease. This is even more important since the introduction of the meningococcal C (MenC) immunisation programme in the UK.

Non-culture confirmation of meningococcal disease by *por*A PCR and DNA sequencing using automated methods

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Aims

To provide non-culture confirmation of meningococcal disease (MD) using the *por*A polymerase chain reaction (PCR) and DNA sequencing by automated methods.

Materials and Methods

A prospective study was performed to evaluate the procedure necessary for PCR automation to enable the rapid and accurate laboratory confirmation of meningococcal disease. One thousand sera received from patients with clinically-suspected MD were investigated. The results of the PCR tests were compared with clinical information, meningococcal antibody testing and blood culture isolates.

Results

One thousand sera were tested for the presence of IS1106 and *por*A by PCR using an automated liquid handling system. The system automated all the liquid handling steps required for setting up the PCR reaction, automated the thermocycling procedure, and automated the gel-loading operation. In addition, the procedure was less labour intensive, accurate and highly reproducible. The method required very few consumable items and was therefore very cost-effective.

Discussion

It was shown that both methods were highly effective when automated for the confirmation of MD. However, the *por*A method was less sensitive but at the same time more useful as it could be used for additional DNA sequencing analysis and therefore provide a subtype of the infecting organism. Automated PCR is a cost-effective method for the laboratory confirmation of MD when a high-throughput is required. The method is less labour-intensive than the equivalent manual procedure and is less prone to errors associated with liquid handling.

Laboratory confirmation of Neisseria meningitidis: past, present and future

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Aims

To highlight the constant evolution of techniques required for the laboratory confirmation of meningococcal disease and future prospects for the role of new technologies in a National Reference Laboratory.

Materials and Methods

A retrospective and prospective overview of the traditional techniques and the more recent molecular advances used in the laboratory confirmation of meningococcal disease (MD) was performed.

Results

The SMPRL provides a national service for the laboratory confirmation of MD in Scotland. The ability for the SMPRL to type micro-organisms to a sub-species level plays an essential role in the diagnosis, treatment and control of infection. Traditionally, the differentiation of micro-organisms has involved analysis of phenotypic markers such as capsule and outer membrane proteins using latex agglutination, co-agglutination and ELISA methods. Recent developments in DNA analysis have resulted in a natural evolution towards genotypic procedures. These are based on DNA analysis, such as PCR for IS*1106*, *sia*D, *ctr*A and *por*A, and more recently the incorporation of an automated multi-locus sequence typing (MLST) service for the full identification of meningococcal isolates in Scotland.

Discussion

The potential application of recent genotypic methods indicate that there is a trend towards the use of DNA sequencing as a typing tool, along with other methods such as DNA chip technology. These raise the issue of the long-term provision of reference facilities because such methods could provide a service for typing all human pathogens.

Epidemiology of meningococcal disease in Germany, 2000

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In 2000, 757 cases of meningococcal disease were notified in Germany, corresponding to an incidence of 0.92 cases per 100 000 inhabitants. Twenty-one percent of invasive isolates originated from children aged 1 to 4 years, 18% from infants and 20% from adolescents aged 15 to 19 years. The case fatality rate was 6% (28 of 457 invasive isolates submitted).

Serogoup B isolates were responsible for 70.9% of cases of invasive disease, 21.4% were caused by group C, 3.5% by group Y and 3.3% by group W135 strains. In infants, the proportion of group B disease was 88% and in this age group 22% of all invasive group B strains were isolated. In the age group of 15 to 19 years, the proportion of C disease was 30% and 29% of all invasive C isolates derived from adolescent patients. Especially in the southern regions of Germany group C proportion was above average (e.g. Baden-Württemberg 39%, Bavaria 27%).

The predominant phenotype of group B isolates was B:15:P1.7,16 (43 strains, 14% of B isolates). Totally, strains of serotype 15 with subtypes P1.7; P1.16; P1.7,16 and NST were responsible for 20% of group B disease. The second most common B phenotype was B:NT:NST (8% of B strains) and the third most common B:4:P1.4 (7% of B strains).

Among group C strains serotype 2a predominated (38% of C isolates), serotype 2b amounted to 16% of C disease and C:NT strains (subtypes P1.2; P1.5; P1.2,5 and NST, respectively) were second most common (33% of C isolates). In 2000, 27% of C strains examined using pulsed-field gel electrophoresis (PFGE) belonged to ET-15. One third of C strains examined in PFGE showed macrorestriction patterns corresponding to A4- cluster.

Eleven (73%) of the 15 invasive W135 strains isolated in 2000 and one invasive B:2a:P1.2 strain of a pilgrim showed macrorestriction profiles (PFGE) identical to the "Mecca strain".

In 2000, 9% of total meningococcal isolates were moderately sensitive to penicillin G, whereas 18% of serogroup C isolates showed a reduced penicillin sensitivity (medium: GC agar base with 1% IsoVitaleX and 1% hemoglobin, MIC for moderate sensitivity = 0,25 μg/ml - 1 μg/ml). No strain was resistant to penicillin G. Five isolates (0.6%, 4 invasive and 1 carrier strain) were resistant to rifampicin (MIC >= 32 μg/ml).

Characterization of invasive *Neisseria meningitidis* serogroup C strains of Austria, Germany and Hungary with serological and molecular methods

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Aims

Invasive *Neisseria meningitidis* serogroup C strains isolated in 1998 in the 3 European countries Austria, Germany and Hungary were compared using serological and molecular methods.

Introduction

Serogroup C is the second most common serogroup of invasive meningococcal isolates in Europe. During the last years an average of 60 – 65% of European strains belonged to group B and 30 – 35% to group C.

Material and Methods

Determination of serogroup was performed with poly- or monoclonal antibodies using slide agglutination and monoclonal antibodies using Whole cell ELISA. Serotypes and serosubtypes were determined in Whole cell ELISA with monoclonal antibodies (NIBSC, Hertfordshire, U.K.). For molecular characterization pulsed-field gel electrophoresis (PFGE) was done using the restriction endonuclease *Nhe*I.

Results

In Austria, 1998 25% of invasive disease was caused by serogroup C. Six (38%) of the 16 group C isolates examined belonged to serotype 2a, 25% to serotype 2b and 31% were not serotypeable (NT, serosubtypes P1.2; P1.2,5 and non-serosubtypeable NST).

In Germany, 1998 the proportion of invasive group C strains was 18.8% (69 isolates). Serotype 2a was found in 54%, serotype 2b in 16% and NT strains with subtypes P1.2; P1.5; P1.2,5 and NST in 15% of them.

In 1998 during a year of low incidence rate (0.3/100 000) the percentage of group C strains in Hungary was 23 (3 of 13 isolates examined). Two of the 3 C strains showed serotype 2a and one of them was serotype 2b.

All C:2b isolates of Germany 1998 showed clonally related fingerprints which corresponded to the A4-cluster. Eightynine percent of them had one of the two prevalent C:2b restriction profiles which differ in one band. Among German C:2a strains ET-37 complex predominated. Nineteen percent of invasive group C and 35% of invasive C:2a isolates belonged to ET-15. The fingerprints of 4 of the 10 C:NT isolates corresponded to the restriciton patterns of A4-cluster and 1 C:NT isolate belonged to ET-37 complex.

All 4 Austrian C:2b isolates had an identical macrorestriction pattern which corresponds to one of the two most common C:2b restriction pofiles of German isolates. In Austria, ET-37 complex also predominated among invasive C:2a strains. Half of serotype 2a isolates and 1 C:NT:P1.2,5 isolate belonged to ET-15. The fingerprint of 3 further C:NT:P1.2,5 strains corresponded or were clonally related to the C:2b pattern.

The 2 C:2a strains of Hungary belonged to ET-37 complex (non ET-15). The 1 C:2b isolate had the same macrorestriction profile as the Austrian and the German serotype 2b isolates.

Conclusion

In 1998, in Austria 25% and in Germany 19% of invasive C isolates belonged to ET-15. All C:2b strains of Austria, Germany and Hungary examined belonged to cluster A4 and had corresponding macrorestriction patterns.

First results of a longitudinal study of meningococcal carriership in teen-agers

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Aims

There are only few data concerning carriership of meningococci in Germany. Therefore meningococcal carrier rates and the dynamics of carriage in adolescents in combination with its risk factors were examined.

Methods

In total, 4783 throat swabs of 1910 pupils aged 14 to 19 years were cultured for meningococci on Martin-Lewis-Medium. The teenagers attended 19 different schools of various types, located in 6 different health departments of Northrhine-Westfalia, Germany. The participants were examined for colonization with *Neisseria meningitidis* three times in intervals of approximately 10 weeks between February and September 2000. 1910 pupils participated in the first round, 1677 in the second, and 1196 in the third round. The isolated meningococcal strains were characterized serologically and using macrorestriction analysis (PFGE, restriction endonuclease *Nhe*I). Questionnaires covering demographic data, living conditions, leisure-time activities, state of health etc. were completed by the teenagers at every sampling date. Analysis of the longitudinal study for assessing acquisition and its risk factors included 981 students with complete data (pharyngeal swabs, questionnaires) from all 3 rounds only. For determining carriage rates all throat swabs taken were utilized.

Results

The overall carriage rate was 18.8%. The proportion of students from whom meningococci had been isolated was 17.5% in the first round. In the second round the proportion had increased to 20.6% and in the third round 18.4% of the participants were colonized with meningococci. The carriage rates considerably varied between schools: the highest rate found was 44.0%, the lowest 5.0%. But substantial variation in carriership occurred in one and the same school, too.

The predominant serogroup among serologically groupable meningococcal strains was B (an average of 12.3% during the 3 sampling periods), the second most common was Y (an average of 9.0%). The proportion of group C strains was an average of 3.6%. Approximately 61% of isolates were serologically not groupable (non-groupable: 19.3%, polyagglutinable: 30.6% and auto-

agglutinable: 10.7%). A predominance of different serogroups was seen in the different health departments. In total, the carrier isolates were phenotypically very heterogeneous (305 different phenotypes of 901 carrier strains). 29E:NT:P1.2,5 and Y:14:NST were the predominant phenotypes of serologically groupable isolates. In one health department C:2a:P1.2,5 was the most common phenotype (5.8% of carrier isolates). All C:2a:P1.2,5 strains belonged to ET-15.

7.2% of participants were carriers during the whole 6-month study period, 5.0% were colonized during the first and second sampling and 2.3 % had lost carrier strain after the first. According serological typing results of strains, 6.8% of students acquired meningococci between the first and the second and 5.3% between the second and the third sampling period. In 0.8% of participants carriage was detected at the first and the third, but not at the second sampling.

Regarding risk factors, primary results indicate that in univariate analysis, acquisition was associated with active smoking (OR 3.7, 95% CI 1.8-7.1), passive smoking (OR 2.1, 95% CI 1.1-4.4) and visits to discotheques (OR 1.6, 95% CI 1.1-2.3).

Conclusions

In Germany, too, the meningococcal carrier rate in teenagers, which amounted to an average of 18.8% is high. There was no seasonal variation of average carrier rate in contrast to disease. However, considerable variation in carriership was found in one and the same school and between different schools. Carriage of ET-15 strains could at least persist for 10 weeks. 12.2% of students acquired meningococci during the study period. Some risk factors for acquisition as active as well as passive smoking and visits to discotheques could be found.

Invasive meningococcal disease in Sweden year 2000

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In year 2000 a total of 59 cases of invasive meningococcal disease were identified in Sweden via a compilation of the compulsory laboratory reports and the clinical notifications (incidence rate 0.67), which is the lowest rate since the mid fifties.

The number of cases per age group, the corresponding serogroup and mortality is presented in the table.

2000		Serogroup				Mortality			
age	number	B	C	W-135	Y	B	C	unknown	total
0-4*	12	4	1	3	-	1	-	-	1
5-9	1	-	-	-	-	-	-	-	-
10-14	0	-	1	-	-	-	-	-	-
15-19	17	8	6	1	-	-	1	-	1
20-24	9	2	2	-	-	1	-	1	2
25-44	5	2	1	-	-	-	-	-	-
45-64	10	5	2	1	-	-	1	-	1
65+	5	2	2	-	1	1	1	-	2
	59	**23**	**15**	**5**	**1**	**3**	**3**	**1**	**7**

* 2 children <1 year

It can be noted that there were no group A isolates and that group B dominated. There were no epidemiological situations or clusters during the year known to the reference bodies. Two cases had W-135 epidemiology for connection with the Hajj 2000 problems (see separate presentation).

The total case fatality rate was 12 %. One of these cases was a small child and one a teenager.

Vaccination against meningococcal disease was not used in the general public. Single persons travelling to "risk" areas were vaccinated.

With a low incidence and mortality rate in children/young people general vaccinations against group C has at present little general support.

Problems identified include requests for more support with non-culture methods for diagnoses and characterisation.

ɔport with non-culture

Development of surface-labelling and opsonic assays for assessment of serogroup B meningococcal vaccines

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Introduction

Meningococcal serogroup C polysaccharide conjugate vaccines have been shown to provide excellent efficacy in the UK with a dramatic reduction in disease levels in vaccinated groups. Serum bactericidal activity (SBA) in vaccinees appears to reflect this protective immunity. For assessment of protective responses to protein-based serogroup B vaccines, assays in addition to SBA will be required. It is important that subunit vaccines elicit antibodies that bind to the surface of meningococcal cells. Surface-labelling of bacteria by antibody detected by flow cytometry has been used. However, growth conditions and fixation of the bacteria must be carefully considered if informative data are to be generated. It is also recognised that opsonophagocytosis is an important host defence mechanism against meningococcal disease. We are developing an opsonic assay using the human pro-monocytic cell line U937 and fixed, fluorescently-labelled meningococci.

Materials and Methods

Bacterial culture: *Neisseria meningitidis* isolate K454 (B15:P1:7,16) was grown in Muller Hinton broth with or without the addition of 5µg/ml EDDHA. Frozen stocks were revived on blood agar in 5%CO_2 at 37°C overnight. Liquid cultures were incubated at 37°C with agitation for 4h.

Bacterial preservation: Bacteria were preserved by the addition of 2% (w/v) formaldehyde, 2% (w/v) paraformaldehyde or 0.2% (w/v) sodium azide.

Transferrin-binding dot blots: Small volumes of bacteria (5µL) were dried onto nitrocellulose. The strips were blocked in PBS containing 1% (w/v) skimmed milk powder and 0.05%(v/v) Tween 20 for 1h and incubated with human transferrin-horseradish-peroxidase (Tf-HRP) (1µg/ml, Jackson) and developed using 4-chloronaphthol.

Antibodies: Monoclonal antibodies were obtained from NIBSC (UK). Polyclonal antibodies to recombinant transferrin binding proteins were generated at CAMR in NZW rabbits.

Surface-labelling: Killed bacteria were incubated with either FITC-labelled human transferrin (Tf-FITC) or antibody. Antibody binding was detected by the addition of a secondary anti-mouse-IgG-FITC conjugate. FITC labelling was then assessed using flow cytometry.

Cell culture: U937 cells (supplied by ECCAC) were grown in RPMI medium supplemented with 20% (v/v) FCS and 2µM L-glutamine.

Opsonophagocytosis assay: Bacteria were fluorescently labelled by several methods. FITC was conjugated to the bacteria using succinimidyl ester linkage. Propidium iodide was also used at 3µg/ml to label killed bacteria. Antibody and bacteria were preincubated for 30min at 37°C in 5%CO_2, U937 cells were added, and the mixture incubated for 15-30 minutes at 37°C in 5%CO_2. The assay was stopped by the addition of 2% paraformaldehyde and analysed using a flow cytometer (Beckton Dickenson).

Results and Discussion

Dot blots and surface-labelling of *Neisseria meningitidis* indicated that transferrin binding was impared in formaldehyde-killed bacteria but retained in azide-killed organisms. Azide-killed bacteria were also found to be more suitable for determination of antibody surface-labelling. Fluorescent labelling via protein conjugation also resulted in masking or denaturing of meningococcal transferrin binding proteins (Tbp). However, the use of propidium iodide enabled fluorescent labelling without surface protein disruption in azide killed meningococci. U937 cells were able to phagocytose fluorescent beads which were coated in rabbit antiserum, in preference to uncoated beads. The cell line did not require differentiation for this activity. Rabbit anti-TbpA and TbpB antisera increased uptake of azide-killed, PI-stained meningococci. This uptake was further enhanced if bacteria had first been grown under iron-limited conditions.

An immortalised cell line will aid standardisation of opsonophagocytosis assays provided it has the appropriate properties. U937 cells were selected because, unlike HL60, they express the high affinity IgG Fc receptor (CD64) and function without the need for lengthy chemically-induced differentiation.

Conclusion

The selection of assays required for assessment of meningococcal group B vaccines will require careful consideration before implementation. Standard techniques used in pneumococcal and serogroup C meningococcal polysaccharide vaccine assessment may not be directly transferable to studies involving protein-based serogroup B vaccines. We have developed an opsonophagocytosis assay using azide-killed, propidium iodide-labelled bacteria using an immortalised human cell line expressing the high affinity IgG Fc receptor (CD64). We are continuing evaluation of this assay with candidate protein antigens for possible utility in meningococcal group B vaccines.

Epidemiology of meningococcal disease AUSTRIA 2000

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In 2000 a total of 88 laboratory-confirmed cases of meningococcal disease were reported. The incidence rate was 1.09. Five deaths were registered which gives a case-fatality rate of 5.7%. Overall the clinical presentation was 48% meningitis alone, 23% septicaemia alone, and 26% a combination of both.

Serogroup B und C disease together accounted for 92% of the 88 cases, with serogroup B predominant (serogroup B 74%, serogroup C 18%, serogroup W135 1.1%, serogroup unknown 6.8%). The most commonly reported antigen formula was B:15:P1.7,16 with 18.6% of all infection isolates followed by B:nt:nst (8.5%). 25 different antigen formulas were found by serogroup B infections. 23% of the serogroup B infections were caused by B:15:P1.7,16. From the 5 different serogroup C antigen formulas C:2a:P1.2, C:2a:P1.5 and C:2b:P1.2 were predominant. Each of the three represent 25% of all serogroup C infections and 5.1% of all infection isolates. One W135:2a:P1.2,5 strain was isolated.

No isolates were resistant to penicillin, rifampicin, ofloxacin, or ciprofloxacin.

Epidemiology of meningococcal infection in England and Wales 1999-2001

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There have been three main features of meningococcal disease (MCD) epidemiology since the last EMGM:

i) Overall decreased serogroup C activity following introduction of meningococcal C conjugate (MCC) vaccine to the infant schedule in November 1999 with a catch-up programme including all children and young people aged up to 18 years and polysaccharide vaccine to all students who started at university and college in October 1999. Overall disease activity has fallen by 56% from 756 in the first 43 weeks (July to March) of epiyear 1998-99 to 336 in the same period in 2000-01. The fall has been evident in successive age cohorts as they were vaccinated. Disease activity in older agegroups has increased by about 20%.
ii) An increase in serogroup B disease activity has occurred in all agegroups. The overall number of cases in the first 43 weeks of epiyear 1998-99 was 1139 while in the comparable period in 2000-01 the total was 1407 – an increase of 23.5%.
iii) Increased serogroup W135 disease activity from 35 cases in 1998-99 to 104 in 2000-01 has been in most part accounted for by introduction and spread of an ET37/ST11 strain following the 2000 Hajj pilgrimage to Mecca boosted by further introduction following the 2001 Hajj.

Non-culture diagnosis continues to provide a substantial number of laboratory proven cases – in 2000, 42% were confirmed by PCR while in 2001 the proportion is 48%.

Strain characterization has been enhanced. All strains are phenotyped using NIBSC reagents however since August 2000 a randomly selected 50% additionally have porA sequencing performed and 1 in 5 of these has a full MLST determination.

Strains which form parts of clusters have MLST performed in addition to those examined using the randomized algorithm. MLST on non-culture proven cases has been developed so that the great majority of PCR confirmed cases for which serogroup can be determined can be fully sequence typed.

PCR diagnosis of invasive disease caused by *Neisseria meningitidis, Haemophilus influenzae* b and *Streptococcus pneumoniae* in the Czech Republic

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Introduction

The incidence of invasive meningococcal disease increased in the Czech Republic after 1993 when *Neisseria meningitidis* C:2a:P1.2,P1.5 belonging to the complex ET-15/37 emerged. The National Reference Laboratory for Meningococcal Infections started a nation-wide active surveillance of invasive meningococcal disease in collaboration with microbiologists, epidemiologists and clinicians. This active surveillance resulted (among other positive outcomes) in higher rates of laboratory confirmation of invasive meningococcal disease (using culture and non-culture methods), which varied between 56.7 % and 93.1 % in the Czech Republic in the period from 1993 to 2000. However, we have introduced a further non-culture, PCR method for diagnosis of invasive meningococcal disease in addition to those currently used i.e., direct microscopy, latex agglutination and serology, to increase the percentage of laboratory confirmed cases. When this target was achieved, we started to introduce PCR diagnosis of invasive disease caused by *Haemophilus influenzae* b and *Streptococcus pneumoniae.*

Materials and Methods

1. *Standardisation of the PCR method on strains*

The PCR method was optimised on *N. meningitidis* B and *N. meningitidis* C strains, which were harvested into distilled water after 18 hours of incubation and the suspension was heated at 90°C for 10 min. DNA was extracted by the Iso Quick kit (ORCA Research Inc., USA) and QIAamp kit (QIAGEN, Germany). The polymerase chain reaction (PCR)-based diagnostic assay of Zambardi was modified and standardised under our conditions with kind advice of Dr. Taha, Dr. Tzanakaki, Dr. Olcén and Dr. Bäckman.

Oligonucleotides used in the study are summarised in the Table:

Oligonucl.	Sequence	Target
98-19	5′-ggatcatttcagtgttttccacca-3′	*N. meningitidis* B
98-20	5′-gcatgctggaggaataagcattaa-3′	
98-17	5′-tcaaatgagtttgcgaatagaaggt-3′	*N. meningitidis* C
98-18	5′-caatcacgatttgcccaattgac-3′	
98-6	5′-gctggcgccgctggcaacaaaattc-3′	*N. meningitidis*
98-10	5′-cttctgcagattgcggcgtgccgt-3′	
ru8	5′-aaggaggtgatcca(g/a)ccgca(g/c)(g/c)ttc-3′	*H. influenzae* b
HI	5′-cctaagaagagctcagag-3′	
ru8	5′-aaggaggtgatcca(g/a)ccgca(g/c)(g/c)ttc-3′	*S. pneumoniae*
SP	5′-gctgtggcttaaccatagtag-3′	
NM	5′-tgttgggcaacctgattg-3′	*N. meningitidis*
ru8	5′-aaggaggtgatcca(g/a)ccgca(g/c)(g/c)ttc-3′	
ru8	5′-aaggaggtgatcca(g/a)ccgca(g/c)(g/c)ttc-3′	*Bacteria*
U3	5′-aact(c/a)cgtgccagcagccgcggtaa-3′	

Amplification was performed in a Amplitrone II thermocycler and optimal conditions for different primers were tested.

2. *PCR detection of N. meningitidis B and C from cerebrospinal fluid*
Sensitivity and specificity of the PCR method were assessed in 195 cerebrospinal fluid (CSF) samples from patients admitted to 5 hospitals in the Czech Republic with the following diagnoses:

- invasive meningococcal disease – 80 CSF samples
- bacterial non-meningococcal meningitis – 49 CSF samples
- viral meningitis – 66 CSF samples

The study was retrospective and the CSF samples obtained within a complex research project from 1997 to 1999 were investigated. The CSF was frozen after sampling and kept at –80°C in the laboratories of the collaborating hospitals. The frozen CSF samples were transported on dry ice to the National Reference Laboratory for Meningococcal Infections in Prague. DNA was extracted from the CSF by Chelex-100 (Sigma). Recently PCR detection of *N. meningitidis* serogroup B and C from serum and blood has been introduced in our laboratory using IsoQuick kits (ORCA Research Inc., USA) for DNA extraction.

The sensitivity, specificity, positive predictive value and negative predictive value were calculated.

Results and Discussion

The following parameters of the PCR detection of *N. meningitidis* serogroup B and C from the CSF were calculated: sensitivity = 0.85, specificity = 0.95, positive predictive value = 0.88, negative predictive value = 0.93. In our conditions, PCR gave laboratory confirmation in nearly 50% (47.4%) of patients with invasive meningococcal disease where the etiology was not confirmed by classical methods. The semi-nested PCR strategy and an extended PCR for detection of other species has been introduced in our laboratory at present and the first results of the detection of *H. influenzae* b and *S. pneumoniae* are encouraging.

Conclusions

The PCR method for non-culture diagnosis of invasive disease was introduced in the National Reference Laboratory for Meningococcal Infections in Prague for detection of *N. meningitidis* , *H. influenzae* b and *S. pneumoniae.*

Acknowledgement

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Multilocus sequence typing directly from clinical material

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Introduction

Two new molecular methods were introduced in the National Reference Laboratory for Meningococcal Infections in Prague in 2000:

- PCR for non culture diagnosis of invasive meningococcal disease
- MLST for sequence typing of *Neisseria meningitidis*

Recently, the death of a 13 year old girl caused most probably by *N. meningitidis* occured, however all classical laboratory methods gave negative results. The only positive method was PCR, which revealed *N. meningitidis* C from cerebrospinal fluid and serum. Faced with this emergency situation, we decided to try MLST typing directly from clinical material.

Materials and Methods

DNA was extracted by the QIAamp kit (QIAGEN, Germany). First amplification of seven alleles (*abcZ*, *adk*, *aroE*, *fumC*, *gdh*, *pdhC* and *pgm*) was performed in a Amplitrone II thermocycler using Hot start. The primer pairs used for the PCR amplification of internal fragments of these genes were identical to those presented on the MLST website (http://mlst.zoo.ox.ac.uk) and were prepared by GENERI BIOTECH, Czech Republic. Second amplification of the same alleles was performed from 1 μl of amplified products and after this a purification of the product was performed with 20% PEG.

The sequencing reactions were performed in PCR tubes with the BigDye terminator cycle sequencing kit (PE Biosystems) and subsequently analysed with an ABI PRISM 377 automated DNA sequencer (Perkin Elmer). The final sequence of each locus was determined with the LASERGENE software package (DNASTAR, Madison, Wisconsin). Housekeeping alleles and sequence types (STs) were assigned by reference to the MLST website (http://mlst.zoo.ox.ac.uk).

Results and Discussion

The amplification and sequencing of the *pgm* allele failed, however interrogation of the MLST database (http://mlst.zoo.ox.ac.uk) allowed us to assign ST-11. This finding is in correlation with the actual

epidemiological situation in the Czech Republic, where the majority of deaths by invasive meningococcal disease is caused by group C meningococci belonging to the ET-15/37 complex, showing ST-11.

These encouraging results motivate us for the strategy of MLST typing of *N. meningitidis* directly from clinical material of suspected meningococcal deaths, which will contribute to better epidemiological monitoring. We are in the process of performing MLST on a sample from cerebrospinal fluid of a Danish girl who died during her visit to the Czech Republic and PCR revealed *N. meningitidis* B.

Conclusion

MLST typing of *N. meningitidis* directly from clinical material was introduced in our laboratory and will be performed in suspected meningococcal deaths routinely.

Acknowledgement

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Analysis of the epidemiological situation in invasive meningococcal disease in the Slovak Republic in 2000

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Introduction

As we previosly reported invasive meningococcal disease (IMD) occured in the past in the Slovak Republic only sporadically. The epidemiological and clinical situation changed in 1996 when a new meningococcal clone ET-15/37 appeared in the Slovak Republic. Since this period Slovak Republic belongs to countries with higher occurence of IMD caused by invasive clone C:2a: P1.2,P1.5,ET-15/37. Up to 72 % (59/82) of IMD was caused by serogroup C in 1998 from which 53 % was represented by the invasive clone. This clone was the cause of 66 % of the mortality. Since this period the number of IMD caused by serogroup C slowly decreased but this serogroup still remains the predominant ethiologic agent of these diseases.

Material and Methods

Active surveillance of invasive meningococcal disease in the Slovak Republic started in 1994 in the Reference Laboratory for Meningococci (RLM). Microbiological laboratories established at district and regional State Institute of the Health sent strains isolated from patients, contacts and healthy carriers to the RLM where they are identified. The *N. meningitidis* strains were classified for serogroup based on capsular polysaccharides by glass or slide latex agglutination (Sanofi Diagnostic Pasteur) and serotyped and subtyped by whole-cell ELISA using the monoclonal antibodies from NIBSC.

Results

In 2000 we reported decrease of IMD occurence in comparison to previous years in the Slovak Republic. In 1997 morbidity reached the highest value during last 20 years (129 cases) and mortality was 18.6 %, in 2000 only 68 cases of IMD was recorded. The mortality of 8.8 % was still higher as compared to long period data (5%). Higher mortality is still associated with an increase of isolation frequency of serogroup C strains and invasive clone C:2a: P1.2,P1.5 of the ET-15/37 complex. In 2000 *N.meningitidis* serogroup C occured in 35 cases (52%) of IMD. Only 20 cases (30%) of IMD was caused by serogroup B. Occurence of other serogroups was not detected. Percentage of strains belonging to the invasive clone C:2a:P1.2,P1.5 of the ET-15/37 complex showed increasing trend. This clone caused 74% (26/35) cases of IMD.

Phenotypes B:2a:P1.2,P1.2, B:22:NST and B:4:P1.15 occured most frequently (3x) in serogroup B.

The most handicapped group was the age group of 1-4 years old (25 cases). The age distribution for serogroup C showed distinct peak in the 1-4 year age group (21 %). The highest proportion of group B isolates occured in 0-11 month old children.

The highest occurence of IMD in 2000 was recorded in the eastern region of the Slovak Republic. In the district of Michalovce, 10 cases were reported and morbidity reached the highest value from all 79 districts in the Slovakia (10 times higher than slovak mean value). All cases were caused by serogroup C. Therefore vaccination of 5000 gipsy children between 2-15 years was carried out.

Penicillin susceptibility data indicate that penicillin based regimens still remain suitable for the treatment of IMD in the Slovak Republic.

Conclusion

Since 1997 the invasive meningococcal disease morbidity in the Slovak Republic showed a decrease in both reporting systems (i.e. routine notification and active surveillance), nevertheless the fatality rate and percentage of invasive clone C:2a:P1.2,P1.5 of ET-15/37 complex causing IMD remains high. For this reason the active surveillance and detailed investigation of meningococci have to be continued.

Invasive meningococcal disease in the Czech Republic in 2000

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Introduction

An emergency epidemiological situation of invasive meningococcal disease started in the Czech Republic in 1993, when a new complex, ET-15/37, occurred. Strains belonging to this complex had not been found in the Czech Republic at least since 1970. Since 1993 the ET-15/37 strain caused increased morbidity and case fatality rates in the country. This situation peaked in 1995 and after this year a decreasing trend in morbidity of invasive meningococcal disease was noticed. However, the case fatality rate persisted at relatively high figures, even when the attention of medical doctors and the public was given to this disease.

Materials and Methods

Epidemiological data came from active surveillance performed by the NRL for Meningococcal Infections in collaboration with microbiologists and epidemiologists from the whole country from 1993 onwards.

Meningococcal strains isolated from patients and healthy carriers were sent from the field microbiological laboratories to the NRL, where the identification of the following characteristics was performed: phenotypes (slide agglutination and WCE), susceptibility to antibiotics and sulphonamide (MIC), ET-types (MLEE) and PFGE analysis in selected strains.

Results and Discussion

The results for the year 2000 are presented.

The total number of cases was 74 = 0.7/100000 population. The percentage of laboratory confirmed cases was 77.7 % (by cultivation 75.7 %, by PCR 2 %).

The highest morbidity was in the youngest age group, where the disease was caused mainly by serogroup B. The year 2000 was the first in which we have noticed also the prevalence of serogroup B in teenagers, where serogroup C prevailed significantly in the period 1993-1999.

In 7 of 74 cases a fatal outcome was noticed (case fatality rate = 9.4%): 4 deaths were in the age group 15-19 years (case fatality rate = 21%). Three deaths were caused by serogroup C, two by serogroup B.

After seven years of high prevalence of serogroup C among strains isolated from patients, which was caused by the ET-15/37 complex, we have noticed the

prevalence of serogroup B in 2000 (58.1%), which is typical for the endemic situation. Serogroup C reached only 14.9% in 2000. We expect this trend to remain, i.e. high prevalence of serogroup B among patients′ strains in coming years. Active surveillance contributed to the decrease in the percentage of patients′ strains where the serogroup was not identified (24.3% in 2000 compared to 43.3% in 1993) and to the increase of strains sent to the NRL (64.9 % in 2000 compared to 48.5 % in 1993).

We found a decrease of strains belonging to the ET-15/37 complex in 2000 (only 27.6%) compared to its high prevalence which culminated in 1997 (75 %). The prevailing phenotype of strains of the ET-15/37 complex was C:2a:P1.2, P1.5 and only four strains presented B variation of this phenotype. Serogroup W135 used to be rare among strains from the patients with invasive meningococcal disease. In 2000 we found two of these strains only and they were not epidemiologically related to Mecca pilgrimage and did not belong to the ET-37 complex.

Monitoring of the susceptibility continued in 2000 and confirmed the previous finding of good susceptibility of Czech meningococci to antibiotics and sulphonamides.

Guidelines concerning epidemiological measures are followed nation-wide and no secondary case of invasive meningococcal disease was found in 2000.

A strategy of targeted vaccination (using A+C polysaccharide vaccine) of part of the population at highest risk was adopted after 1993. This targeted vaccination is realised less frequently now, as we have noticed a decreasing incidence of invasive meningococcal disease and a decreasing percentage of group C. For this reason a massive vaccination with meningococcal conjugate C vaccine is not indicated.

Conclusion

The emergency situation in invasive meningococcal disease caused by strains of the ET-15/37 complex seems to be over in the Czech Republic. However, the case fatality rate remains relatively high. Continuing active surveillance of invasive meningococcal disease is planned with the aim of recognizing any epidemiological change as soon as possible.

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Multilocus sequence typing performed in the Czech Republic

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Introduction

Over the last three decades a large number of *Neisseria meningitidis* strains isolated in the Czech Republic from patients with invasive meningococcal disease have been collected and analysed using Whole-Cell ELISA (WCE) and multilocus enzyme electrophoresis (MLEE). We found that the Czech meningococcal population is different from those in western countries: a higher proportion of non-typeable (NT) and/or non-subtypeable (NST) strains and a higher proportion of serotype 22 strains are seen. These differences could be the result of the isolation of the human population, and consequently the microbial population, during the four decades up to the late 1980´s as a consequence of the Cold War. Our project is focused on meningococcal strains with unusual or uncharacterised phenotypes to be investigated by multilocus sequence typing (MLST) among which identification of new sequence types (STs) is expected. Finding large and significant ST clone(s) among NT/NST meningococcal strains causing invasive meningococcal disease could be important for new meningococcal vaccine development. MLST gives 100% characterisation of *N. meningitidis* and provides the most valuable epidemiological marker, ST (sequence type). For this reason we are introducing the method in our laboratory for routine use in epidemiological investigation of meningococcal strains isolated in various epidemiological conditions.

Materials and Methods

Neisseria meningitidis strains

The collection of strains isolated from cerebrospinal fluid (CSF) or blood of patients with invasive meningococcal disease in the Czech Republic from 1971 to 2001 (March) represents 1224 isolates, which are stored lyophilized or frozen (at -80°C). In all strains serogroups were determined, and in 1119 strains serotypes and subtypes were determined. In 1990 we started to investigate the strains by MLEE, and ET-types were identified for 444 strains. We started to identify ST-types recently

and strains with unusual or non-characterised phenotypes were investigated first.

ST identification

STs were identified by MLST developed at Oxford University. MLST was introduced to our laboratory thanks to the kind help and advice of Dr. Martin Maiden and Dr. Keith Jolley (Oxford University, UK). DNA was extracted from meningococcal cultures grown on chocolate modification of Mueller-Hinton agar (37°C, 5% CO_2, 18 hours incubation). An opaque cell suspension was prepared in 1 ml deionised water and meningococcal DNA was extracted from 100µl of the suspension with the IsoQuick Nucleic Acid Extraction kit (Orca Research Inc). Nucleotide sequences were determined from PCR products, which were verified in 2% gel electrophoresis. According to the protocols used in Oxford University (http://mlst.zoo.ox.ac.uk), the meningococcal MLST scheme uses internal fragments of the following seven house-keeping genes: *abcZ* (putative ABC transporter), *adk* (adenylate kinase), *aroE* (shikimate dehydrogenase), *fumC* (fumarate hydratase), *gdh* (glucose-6-phosphate dehydrogenase), *pdhC* (pyruvate dehydrogenase subunit), *pgm* (phosphoglucomutase). The primer pairs used for the PCR amplification of internal fragments of these genes were identical to those presented on the MLST website (http://mlst.zoo.ox.ac.uk) and were prepared by GENERI BIOTECH, Czech Republic. The sequencing reactions were performed in PCR tubes with the BigDye terminator cycle sequencing kit (PE Biosystems) and subsequently analysed with an ABI PRISM 377 automated DNA sequencer (Perkin Elmer). The final sequence of each locus was determined with the LASERGENE software package (DNASTAR, Madison, Wisconsin). Housekeeping alleles and sequence types (STs) were assigned by reference to the MLST website.

Results and Discussion

For validation of MLST under our conditions, we made our first MLST characterisation on known *N. meningitidis* C:2a:P1.2,P1.5, ET-15, where ST-11 was expected and successfully identified. After this, meningococcal strains isolated from patients with invasive meningococcal disease and showing unusual and/or uncharacterised phenotypes were selected for MLST. The first MLST results confirm our working hypothesis: The Czech meningococcal population is different compared to the western populations and in Czech *N. meningitidis* strains causing invasive meningococcal disease new STs could be identified.

We consider MLST as one of the most valuable methods for the definition of epidemiological markers, STs, enabling understanding of the epidemiology of meningococcal infections and the development of the meningococcal population.

Conclusions
The genotypes of the Czech meningococcal population have started to be investigated by a new, advanced method, MLST. The first results are suggestive of the genetic dissimilarity of the Czech and the western meningococcal populations.

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Meningococcal disease in Romania 1995 – 2000
Epidemiological and bacteriological studies

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Aims

The aim of our study was to review the epidemiological status of meningococcal disease in Romania and the characterization of isolates received by the National Reference Center for Meningococci from 1995 to 2000.

Introduction

In Romania, meningococcal meningitis usually evolves endemo-sporadic, sometimes having endemo-epidemic aspects. Periodical, at irregular intervals (5, 10, 15 years) epidemic outbreaks were registered. These were characterized by an annual incidence of morbidity between 5.2 (1970) and 11.4 (1987) per 100,000 inhabitants.

Unlike countries from Europe and America where serogroups B or C have been the predominant cause of meningococcal disease, both the Romanian epidemics were due to serogroup A (isolated in the epidemic of the 1970 in 96% and in 1987 in 84.5%).

Materials and Methods

We investigated 95 *Neisseria meningitidis* strains received by the National Reference Center for Meningococci. The strains were isolated from CSF and blood (84 cases) and 10 were isolated from nasopharynx and sputum. Approximately half of strains were obtained from patients hospitalized in Clinics of Infectious Diseases from Bucharest.

The meningococci were identified according to classical criteria, including Gram staining, oxidase test and carbohydrates degradation tests.

Meningococci serogrouping was performed by slide agglutination using antisera, produced by Cantacuzino Institute, for serogroups A, B, C, X, Y, Z, W-135 and 29E.

Sensitivity to antimicrobial drugs was tested by the agar diffusion and Minimum Inhibitory Concentration by the agar dilution.

The data about the notification cases were obtained from the Medical Statistics Center.

Results and Discussions

* Incidence: Starting with 1995, the incidence of meningococcal meningitis increased from 0.58 (131 cases) to 1.76 (396 cases) in 1999.

* Seasonal distribution: Meningococcal diseases in Romania showed a clear seasonal distribution most cases occuring in the first quarter of each year (40%).

* Distibution by age and gender: The highest incidence was in children under 1 year (19%) and about 37% of cases were under 5 years of age.

Overall there was a higher percentage of cases in males than in females (62.7% males).

* Geographic distribution: The regions having a lower socio-economical level had an incidence higher than the average per country (4.12 in Vaslui district, comparative to 1.19 the average per country in the same year).

* Distribution by serogroup: The analysis of *N. meningitidis* serogroups evolution during 1995 – 2000 proved a great mobility of groups. Most cases of meningococcal diseases were caused by group B (37.8%) and group A (34.7%). Non-groupable strains were frequently isolated in this period (25%). The frequence of group C and Y was very low (1%).

* Antibiotic resistance: 46 strains from patients and carriers were tested by the agar dilution method to the following antibiotics: penicillin, ciprofloxacin, cefotaxime and sulphamethoxazole.

Regarding penicillin, 19.5% of strains were relatively resistant (MIC >0.125 mg /l). 97.8% of strains were resistant to sulphamethoxazole (MIC >8mg/l). Ciprofloxacin and cefotaxime: All strains were fully susceptible. Susceptibility of rifampicin was tested by the agar diffusion method. All strains were susceptible.

Conclusions

- The incidence of meningitis cases decreased dramatically from 11.4 in 1987 (epidemic year in Romania) to 0.58 (1995) and 1.76 (1999).
- The frequency of serogroup A, very low during 1990 – 1995, will increase from 1996.
- Serogroup B, prevailed during 1991 – 1994, decreased in 1996 – 1998.
- What has to be specified is that there is a great difference between the number of cases registered at the Statistics Center and the number of strains that were received by the National Reference Center.

New Zealand's continuing epidemic of meningococcal disease

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Introduction

New Zealand is in its tenth year of a serogroup B meningococcal disease epidemic. In that time serogroup B isolates have increased in frequency from 48% of case isolates in 1990 to 94% in 2000, largely attributable to the epidemic strain serologically defined as B:4:P1.4, and belonging to the ST41 complex.

Methods

Meningococcal disease surveillance in New Zealand uses a combination of notifiable disease and laboratory data. Meningococci isolated from cases are serologically typed. For cases confirmed by PCR testing of patient specimens only, and for serologically non-typeable isolates, the DNA sequence encoding the subtype is determined. Population census data from 1991 and 1996 is used for calculating disease rates.

Results

The annual incidence of notified cases of meningococcal disease increased from 53 (1.6 per 100,000 population) in 1990 to a peak of 613 (16.9 per 100,000) in 1997. From 1998 through 2000 the number of cases has plateaued at an average of 475 cases per year (13.1 per 100,000). There is no sign of the epidemic waning in 2001 with 146 cases and six deaths reported to the beginning of May compared with 109 cases and two deaths reported for the same period in 2000. To the end of 2000, the epidemic involved 3547 cases; approximately 3000 in excess of the number expected based on the pre-epidemic disease incidence of 53 cases. The overall case fatality rate is 4.5% (158/3547).

Meningococcal disease continues to occur most frequently in the northern region of the North Island, with a rate in 2000 of 21.3 per 100 000 occurring in the Northern region compared with 14.0 in Midland, 8.2 in Central, and 5.8 in the Southern region. These rates are higher than those recorded in most European countries. Age-standardised rates in Maori are almost three times higher than in the European population, while rates in Pacific people are almost six times higher than in the European population. Rates of disease in all ethnic groups are particularly high among those under five years of age. In 2000, the highest rate observed was in Pacific children under one year of age (576.9 per 100 000). In children under 10 years of age, the rates in Maori and Pacific people were significantly higher ($p<0.001$) than those in the European population.

From 1992 through 1994 the culture-confirmation rate for cases averaged 76% and only 19% were judged probable cases on clinical criteria alone. The campaign, implemented in 1995, to ensure antibiotics are administered to suspected cases of meningococcal disease prior to admission to hospital, resulted in an immediate reduction of culture-confirmed cases. Culture-confirmation reached its lowest rate of 48% in 1999. That year, 34% were judged probable cases. PCR testing was introduced in mid-1998. Of the 60 PCR-confirmed cases in 2000 whose antibiotic status was known, 47.6% (28/60) received antibiotics prior to admission. This emphasises the value of PCR testing, even after antibiotics. The 2000 overall confirmed case rate of 72.5% was the highest since 1994 and PCR contributed to this increase. The major PCR test used is the *porA*-PCR test with the subtype determined using either sequence-specific probes, or direct sequencing. By combining results from the serosubtyping of case isolates with the subtype information obtained by sequence analysis of DNA in patient specimens, we showed that in 2000 meningococci with the P1.7b,4 PorA protein were responsible for 84.6% (269/318) of all meningococcal cases able to be determined in this way.

Discussion

Meningococcal disease rates in New Zealand continue to be high and comparison with the Norwegian epidemic curve suggests that they are likely to remain elevated for some time. A vaccine that induces immunity to the epidemic strain could have a vital role in controlling this epidemic. Efforts are underway to obtain such a vaccine. Overcrowded living conditions have been shown to be an important risk factor for meningococcal disease in South Auckland children. This has led to a Government initiative to improve living conditions. The surveillance data, showing that the case-fatality rate is significantly lower for patients seen by a doctor and given antibiotics prior to hospitalisation, supports the effectiveness of this programme. The marked reduction in ability to confirm cases by culture of a meningococcus following pre-hospital administration of antibiotics is being offset by the use of PCR testing. Such testing provides additional information on the meningococcal strain subtype which is vital in determining the overall burden of disease caused by our epidemic 'strain' and for control strategies.

The epidemiology of meningococcal disease in Malta, 2000

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Objective
To describe the occurrence of meningococcal disease in Malta with respect to demographic variables and characteristics of the isolated strains of *Neisseria meningitidis*.

Design
Data collection from routine notifications and laboratory reports in 2000.

Setting
The Maltese islands.

Subjects
Maltese residents developing meningococcal disease in 2000.

Methods
Population-based surveillance for meningococcal disease is routinely carried out by the Disease Surveillance Branch of the Department of Public Health based on physician reporting by notification. The adopted clinical case definition is that recommended by the WHO Recommended Surveillance Standards[1]. All pathological samples from cases with meningococcal disease are sent to the Department of Pathology at the main state hospital for laboratory investigations. Microbiological investigations are carried out at the Microbiology Laboratory of the same department. Culture positive cases are sent to the Public Health Laboratory Service – Meningococcal Reference Unit, in Manchester, for serotyping and subtyping. Fatal cases attributed to meningococcal disease are also notified to the Disease Surveillance Branch through the Department of Health Information, which processes all death certificates.

Results
Thirty-one cases of meningococcal disease were reported in 2000 (8.12 per 100,000 population). Cases were reported throughout the year, mostly in the winter season. All cases were sporadic with the exception of one secondary case that occurred in a household contact despite antibiotic chemoprophylaxis. The highest proportion of cases was in the 10-14 year age group. The main clinical presentation of nine cases was meningitis; septicaemia occurred in 10 cases and 12 cases had features of both meningitis and septicaemia. Serogroup B continued to predominate with 94% of positively identified cases followed by

serogroup C (6%). There were three deaths attributed to meningococcal disease; all were cases of septicaemia giving an overall case fatality rate of 9.7%.

Conclusion

During the year 2000, Malta continued to be hyper-endemic for meningococcal disease with increasing incidence rates reported since 1996. Indeed, the reported incidence of meningococcal disease in Malta reached its highest point in 2000 since 1942. The importance of close surveillance, particularly to monitor any changes in the serogroup of the meningococci causing disease, cannot be overemphasised. With the availability of vaccines against serogroup B meningococci, vaccination will become of major importance in preventing meningococcal disease in Malta. Meanwhile, the main methods of control remain the early treatment of cases and the prevention of secondary cases.

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[1] World Health Organization. WHO Recommended Surveillance Standards, 2nd Edition, October 1999. WHO/CDS/CSR/ISR/99.2.

Changing shape of clonal distribution of Czech *Neisseria meningitidis* isolates related to declining prevalence of the ET-37 complex strain in the early 2000's

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By the vast prevalence of serogroup B and C organisms among isolates from preceding decades (together >97%), Czech *N. meningitidis* population suggests general similarities to that of the Atlantic regions of Europe. Our laboratory assesses continually the basic clonal origin of the majority of *N. meningitidis* isolates collected from invasive ethiologies over the Czech Republic by the means of MLEE. In relation to the hyperendemic increase of the disease incidence linked to the spread of ET-37 complex organisms of prevailing ET-15 clonal variant and C:2a:P1.5,2 phenotype over the country, representation of serogroup C prevailed over serogroup B for a temporary period from 1993 till 1999. MLEE analyses revealed, that in parallel to the natural descent of the ET-37 entity, the endemic occurence of two distinct complexes of serogroup B organisms has been increasing since the late 1990's. The ET-5 complex together with the major clonal complex related to serotype 22 meningococci have been responsible for the clonal replacement detected. Representation of ET-37 complex organisms among invasive isolates belonging to major hypervirulent clonal complexes of Czech meningococci (i.e. to the ET-37, ET-5, or serotype 22-related complex) has dropped from almost the 90% level in 1995 to the 46% level in 2000 and this process is still going on. Within the same period, the respective 4% or 7% representation of the ET-5 complex and the serotype 22-related complex among hypervirulent clonally-related invasive isolates have increased to the 25% or 29% level, respectively. As a consequence, the attribution of invasive isolates to the major clonal complexes of Czech meningococci has further maintained a significant level of >60%. The presence of the complex of serotype 22-related organisms is continuous in Central Europe and was in minor observed retrospectively in the 1970's and the 1980's already. In spite of the partial heterogeneity of antigenic and MLEE patterns of isolates of the serotype 22-related complex, general linkage to the ST-18 complex defined by MLST was verified in a multiple. The minor, or even lacking, occurence of cluster A4 and lineage III isolates among Czech isolates has remained unchanged till the present time.

Conclusions

During recent period, natural clonal replacement of the ET-37 complex strain was detected within *N. meningitidis* population of the Czech Republic. The still increasing occurence of both the ET-5 complex and serotype 22-related

organisms indicate the outset of an overall endemic spread of serogroup B-related populations. In spite of the mutual similarity of serogroup distribution of Czech and West-European meningococci, their overal clonal distribution is still diverse.

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Meningococcal disease in Russia and in Moscow in 1999-2000

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The general incidence of systemic meningococcal disease (SMD) in Russia decreased in 1995-1999. In 2000 the small increase was reported; the annual incidence per 100,000 (AI) reached 2.1 that corresponded to 3097 cases (Table 1). This AI was about twofold less than in 1973 (the peak of serogroup A epidemic) or in 1984 (the peak of serogroup B epidemic). 2179 cases of SMD occurred in children less that 15 years of age; the AI in this group was 8.2. Some regions were more affected (Figure), first of all, the regions in South-Eastern part of Russia adjacent to the borders with China (the Khabarovsk Region) and Mongolia (Republic Tuva). The epidemic in South-Eastern Russia was caused by serogroup A meningococci and coexisted with serogroup A epidemic in Mongolia (1994-95). Another regions of stable high incidence were the North-Western part of Russia adjacent to the White and Baltic seas (the Murmansk Region is given as an example in Table) and the narrow European meningitis belt parallel to the latitude 50°N (the Lipezk Region is given as an example). Data about prevailing serogroup in these regions were scarce; probably serogroup A, B, and C meningococci presented in approximately the same proportion as in Moscow City.

The AI in Moscow fluctuated from 2 to 4/100,000 in 1990s. In 1996, an outbreak of serogroup A disease occurred, initially associated with the Vietnamese community. During 1997 to 1999, hundreds of thousands of Muscovites were immunized with A polysaccharide vaccine. The incidence rate and the proportion of serogroup A isolates decreased and have remained stable through 2000. Molecular analysis showed that a 1996 outbreak was part of the pandemic spread from Asia of genocloud 8 of subgroup III (1). The AI of serogroup B disease was stable, but in 1999-2000 the incidence of group C disease reached 0.7/100,000 in comparison to 0.3-0,4 in 1993-98 (Table 2). The age distribution was as expected, with the primary peak in infancy, which was most prominent for serogroup B disease (Table 3). In contrast, the second peak in teenagers and young adults was more typical for serogroup C and serogroup A disease. Case fatality rate fluctuated near 8% and was slightly less for serogroup C than for serogroup B or serogroup A disease. All meningococcal isolates were sensitive to penicillin.

In conclusion, epidemic situation in Russia remains stable in 1999-2000 with a small increase in several regions and in the whole country. This may turn to

worse after the possible introduction of epidemic serogroup A strains from the South-Eastern part and group C strains from North-Western part into the central part of country.

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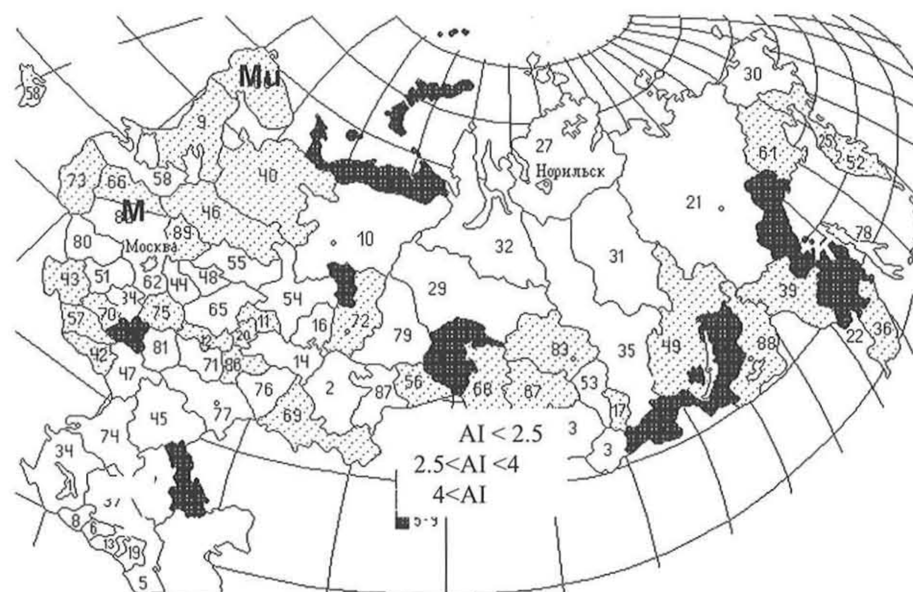
Figure 1. The distribution of meningococcal disease in Russia in 1995-1999. Annual incidence is coded by shading.



M = Moscow City; T = Republic Tuva; K = Khabarovsk Region; L = Lipezk Region; Mu = Murmansk Region.

Table 1. Annual incidence rate per 100,000 for Russia and some Russian regions.

	1973	1984	1995	1996	1997	1998	1999	95-99, mean	2000
Russia	4.9	3.9	2.6	2.6	2.4	2.0	2.0	2.3	2.1
Moscow City	15.3	8.4	1.7	3.8	1.9	1.9	1.7	2.2	2.6
Republic Tuva			27.7	13.0	6.8	2.6	1.3	9.8	
Khabarovsk Region			7.9	6.4	5.1	4.9	5.5	6.0	
Lipezk Region			3.5	4.6	6.1	3.4	2.6	4.0	
Murmansk Region			3.5	3.3	2.9	3.3	2.4	3.1	

Table 2. Meningococcal disease by years and serogroup, Moscow City.

Year	1973	1984	1995	1996	1997	1998	1999	2000
Serogroup A	>11.5	3.4	0.6	2.4	0.6	0.6	0.6	1.0
Serogroup B	NK	4.3	0.8	1.1	1.0	0.9	0.6	0.8
Serogroup C	NK	0.5	0.3	0.3	0.3	0.4	0.6	0.8

Table 3. Age distribution of meningococcal disease by serogroup, Moscow City.

Age, years	< 1	1-4	5-9	10-14	15-19	20-24	25-44	45-64	65+
Serogroup A	10%	14%	8%	5%	19%	8%	20%	14%	3%
Serogroup B	30%	26%	6%	1%	2%	3%	10%	15%	7%
Serogroup C	14%	14%	9%	15%	20%	8%	12%	8%	2%

Differential genodiagnostics of meningitis in Moscow

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Background

About thousand cases of bacterial and viral meningitis occurred annually in Moscow. The number of positive culture obtained from patients with bacterial meningitis is low because of wide, officially recommended prehospital use of antibiotics. Virological diagnosis of aseptic meningitis is not performed as a routine. Non-cultural techniques for differential diagnosis and typing of meningitis in Moscow were required and developed.

Methods

A set of PCR-based diagnostic assays included: 1) Multiplex 16S RNA gene-specific assay for Neisseria, Streptococcus, and Haemophilus (M-N/S/H). 2) Multiplex assay for serogroup A, B, and C meningococci using *mynA* and *siaD* genes (M-A/B/C assay). 3) Assay for *H. influenzae* type b using *bexA* gene (Hib assay). 4) Assay for enteroviruses (EV) using 5'-untranslated region; these primers did not discriminate the species within entoroviruses but had to interact with all of them (EV-assay). 5) Specific assays for human herpes viruses (HHV6, HSV1/2, VZV, CMV, EBV). This set was applied to the CSF samples obtained from patients with bacterial and aseptic meningitis in Moscow. In parallel, laboratory diagnosis was made by culture from the CSF and by latex agglutination tests (LA) using Slidex-5 kit (BioMerieux).

Trial

At the developmental and trial stage, all methods were applied to any sample. No false-positive (in comparison to culture) or double-positive cross-reactive result was observed. All samples positive in M-A/B/C assay were also positive for Neisseria in M-N/S/H assay. All identified *H. influenzae* belonged to serotype b and the results of Hib-specific *bexA* assay and 16S RNA Haemophilus assay coincided. Approximately a half of aseptic meningitis was caused by enteroviruses. These findings suggested the simplified diagnostic approach. To minimise the expenses and labour time, the current routine procedure is following: all samples are tested by culture, M-N/S/H, and EV assays. LA test is applied only to the CSF samples with the WBC count > 10 cells/μl. The samples positive for Neisseria in M-N/S/H assay are then tested in M-A/B/C assay. The samples positive for Haemophilus in M-N/S/H assay are tested also in Hib assay. The samples, which were negative both in M-N/S/H assay and in EV assay, are tested additionally in assay for human herpes viruses.

Results

According to PCR diagnosis, 58% of aseptic meningitis cases were enteroviral, which was consistent with clinical and epidemiological findings in these patients. Human herpes viruses were found occasionally (HSV1/2 in 8% of cases, HHV6 and EBV in 2% of samples), but this did not definitely suggested the herpes etiology of meningitis. 30% of aseptic meningitis cases remained undiagnosed.

47% of bacterial meningitis cases were diagnosed by culture (and PCR), 48% of cases were diagnosed by LA (and PCR); if the data of culture and LA were combined, this gave the possibility to diagnose 70% of cases. Only 17% of specimens/cases were negative in M-N/S/H assay, but 10% of them were caused by "other" bacteria not included in the M-N/S/H assay (*K. pneumoniae, S. aureus,* etc.) and were diagnosed by culture. Thus, 93% of bacterial meningitis cases were diagnosed when both culture and PCR was applied (54% of cases were caused by *N .meningitidis*, 25% by *S pneumoniae*, 9% by *H. influenzae* type b, and 10% by "other" bacteria). In the group of patients, who received antibiotics before admission, culture gave 28% of positive diagnostic results; this figure reached 58% in the group of patients, who did not receive antibiotics before the CSF sampling. PCR results were not sensitive to the prehospital use of antibiotics. PCR assays detected bacterial DNA in 34% of specimens taken after 7 days of intensive antibiotic treatment in hospital, whereas these samples were bacteriologically sterile.

Conclusion

PCR-based assays provide a substantial improvement in diagnostic capabilities, especially if patients receive antibiotics before lumbar puncture.

Meningococcal disease in Denmark 2000

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Introduction

In Denmark both manifestations of meningococcal disease, meningitis and septicaemia, are notifiable. The notification system for communicable diseases is administered by the Department of Epidemiology and the Neisseria Reference Laboratory System at the Neisseria Unit. The Neisseria Unit continuously refers information about each laboratory confirmed case to the Department of Epidemiology. If the case has not yet been notified, the clinical department is requested to notify the case.

Materials and methods

The material comprised all notified cases of meningococcal disease from 01.01. to 31.12.2000. It was necessary to request the clinical departments for 37 % of the notifications. Figures are subject to amendment due to late notifications and the receipt of further data.

Results

During the year 2000 163 cases were notified of which 121 (74 %) were confirmed by culture of *Neisseria meningitidis*, 31 cases (19 %) were confirmed by other laboratory methods, nine cases (6 %) were diagnosed on clinical grounds, and two cases are still under investigation.

The overall incidence of meningococcal disease was 3.1 per 100,000 population. The mortality rate was 9 %. The age-specific incidences per 100,000 population were as follows: below 1 year 34.7; 1-4 years 16.0; 5-9 years 5.9; 10-14 years 3.7; 15-19 years 8.5; 20-24 years 1.5; 25-44 years 0.8; 45-64 years 0.6; and for 65 years and above 1.9.

A total of 101 cases (83 %) were due to serogroup B, and 19 cases (16 %) were due to serogroup C. The incidence of serogroup B disease was 1.9 per 100,000 population, and of serogroup C disease 0.3 per 100,000 population. There was one case due to serogroup A with relation to the Hajj, Mekka, and one case due to serogroup W135 without any relation to the Hajj, Mekka. The dominating clone was B:15:P1.7,16, sulphonamide resistant.

There were no major differences in the geographical distribution. Only two small clusters were registered, both with two persons each.

Conclusion

The incidence of meningococcal disease in Denmark remained at the same low level in 2000 as in the previous two years, 3.1-3.5 per 100,000 population. The incidence of serogroup C was low. At the moment there is no plan to incorporate a serogroup C conjugate vaccine in the childhood vaccination programme.

Meningococcal meningitis in Poland in 2000

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Objectives

The aim of the study was to characterise invasive meningococcal disease (IMD) in Poland in 2000.

Material and Methods

In 2000 the National Reference Centre for Bacterial Meningitis (NRCBM) collected 44 strains of *Neisseria meningitidis*. The strains were identified to the species level by standard methods. Serogroups, serotypes and serosubtypes were determined by whole cell ELISA method. Minimal inhibitory concentrations (MICs) of the following antimicrobial agents were evaluated by agar dilution method according to NCCLS: penicillin, ceftriaxone, cefotaxime, chloramphenicol, spiramycin, rifampin, ciprofloxacin on Mueller-Hinton agar with 5% of sheep blood and cotrimoxazole on Mueller-Hinton agar with 5% of lysed horse blood.

Results

In 2000 ninety-eight cases of meningococcal meningitis (annual attack rate 0.25/100,000) based on clinical picture were reported. A total of 34 isolates (35.1%) from cerebrospinal fluid (CSF) were received by the NRCBM. Other strains were isolated from blood (n=8) and sputum (n=2). Most cases took place from January to March (n=18; 41%). Higher morbidity was observed among male patients (n=26; 62%). The highest incidence of IMD was in children under 5 years of age (n=24, 55%), with the majority of cases (n=14, 32% among all patients) under 1 year. Among all meningococci the most predominant was serogroup B (n=34; 77%), followed by group C (n=7; 16%) and W135 (n=2; 4.5%). One isolate from sputum was nongroupable. Subsequent subtyping revealed that the most predominant type in Poland was 22 (n=15, 44% among group B isolates) and phenotype B:22:P1.14 (n=8, 53% among type 22 isolates). Fifty percent of meningococcal strains were nontypeable. Accept 70% of strains that were nonsusceptible to cotrimoxazole, all meningococci were susceptible to other antimicrobial agents tested.

Conclusions

- In 2000 *N. meningitidis* was still the most common etiologic agent of bacterial meningitis in Poland.
- The most prevalant was serogroup B, being responsible for 80% of the cases.
- No epidemic cases of meningococcal meningitis were identified in 2000.
- Contrary to previous years, no single isolate showed decreased susceptibility to penicillin.

FcγRIIa and FcγRIIIb allotypes were not associated with contraction or severity of systemic meningococcal disease in Norwegian teenagers and adults

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Aim

Different Fcγ-receptor (FcγR) allotypes may influence the extent of phagocytosis and elimination of invading meningococci[1-3]. A predominance of certain FcγR allotypes have been found in patients with meningococcal disease[4-6]. The aim of this study was to investigate whether certain FcγRIIa and FcγRIIIb allotypes were associated with contraction and severity of meningococcal disease in Norwegian teenagers and adults.

Methods

Fifty patients with bacteriologically confirmed meningococcal disease, and 100 healthy controls, all Caucasian living in Western Norway, were genotyped for FcγRIIa and FcγRIIIb polymorphisms using polymerase chain reaction.

The distribution of the FcγRIIa and FcγRIIIb allotypes did not differ significantly between the patients and the controls. The most severely ill patients, i.e. those with septic shock without meningitis on admission, were selected in one group. The FcγRIIa-R131 allele frequency was 0.54 in this group and 0.58 in the remaining patients, not differing significantly ($P=0.64$). The allele frequency of FcγRIIIb-Na2 was 0.54 for both the patient groups. One patient with fatal outcome was genotyped H/H131 and Na1/Na1.

Previous studies have found a higher proportion homozygote for FcγRIIIb-Na2 and/ or FcγRIIa-R131 in late complement deficient meningococcal disease patients and a higher proportion homozygote for FcγRIIa-R131 in children with fulminant meningococcal septic schock [4-6]. However, the contraction and the severity of meningococcal disease in Norwegian teenagers and adults, were not associated with these FcγRIIa and FcγRIIIb allotypes.

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A 20-year population based study of meningococcal disease in a Danish county with emphasis on meningococcal phenotypic and genotypic markers and case fatality rate

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Introduction

Previous studies addressing the association between meningococcal phenotypic and genotypic markers and outcome of meningococcal disease (MD) have come to contradictory results. We tested the hypothesis that meningococcal phenotypic and genotypic markers of invasive strains are associated with case fatality rate (CFR) and we assessed associations of certain types with changes in incidence and CFR of MD over time.

Patients and Methods

During 1980-99 all patients hospitalized with MD in the County of North Jutland (appr. 490,000 inhabitants) have been registered in The North Jutland Meningococcal Research Database. Patient charts were reviewed in order to ensure that all patients fulfilled a standard set of criteria for MD. Meningococcal phenotypic markers included serogroup, serotype and -subtype; a subset of isolates were selected for further analysis by multi-locus enzyme electrophoresis (MEE) including 8 enzymes. Growth of meningococci in blood and cerebrospinal fluid (CSF) was used as proxy measures for septicaemia and meningitis, respectively. Time trends were studied using five 4-year periods.

Results

Epidemiology: A total of 413 patients with MD were identified of which 224 (54%) were male and 189 (46%) were female. 11% of patients were <1 year, 30% 1-4 years, 12% 5-9 years, 9% 10-14 years, 17% 15-19 years, 5% 20-29 years and 16% ≥30 years.

The overall incidende rate (IR) was 4.3 per 100,000 inhabitants per year. From 1980-83 to 1996-99, the age-standardized IR changed from 3.6 to 4.8 /100,000 with a peak of 6.3 in 1992-95. During the study period the proportion of patients ≥30 years changed from 7% to 21%, the proportion of patients with septicaemia without meningitis increased from 9% to 40%, and the proportion of patients with symptoms <24 hours before admission increased from 42% to 70%.

Phenotypic and genotypic markers: From 320 patients (77%) an isolate of *N. meningitidis* was obtained from blood or cerebrospinal fluid. A complete phenotype was available for 315 of these isolates. 226 (72%) were serogroup B and 75 (24%) serogroup C. The two major phenotypes were B:15:P1.7,16 (n=100, 32%) and C:2a:P1.2,5 (n=31, 10%). 181 isolates were selected for further analysis by MEE including all B:15:P1.7,16 and C:2a:P1.2,5 isolates. Among B:15:P1.7,16 isolates 81 were ET-4 and 15 were ET-23. Among C:2a:P1.2,5 isolates 16 were ET-15, 8 were ET-25, and 5 were ET-3.

From 1980-83 to 1996-99 the proportion of B:15:P1.7,16 isolates changed from 29% to 50%. C:2a:P1.2,5 isolates changed from 5% to 4% with a peak of 19% in 1984-87. ET-4 was predominant among B:15:P1.7,16 isolates throughout the study period whereas ET-type 23 appeared in 1989 and increased in prevalence thereafter.

Outcome: CFR increased from 1.4% to 13% with a peak of 17% in 1988-91. From 1980-87 to 1988-99 CFR increased from 3.4% to 20% in patients with B:15:P1.7,16 infection, changed from 21% to 24% in patients with C:2a:P1.2,5 infection, and increased from 0 to 13% in patients with other phenotypes. For patients with B:15:P1.7,16, ET-4 infection, CFR was 3.7% during 1980-87 and 20% during 1988-99; for patients with B:15:P1.7,16, ET-23 infection CFR was also 20%.

In patients <30 years (n=256) B:15:P1.7,16 and C:2a:P1.2,5 isolates were associated with an increased risk of death as compared to any other phenotype (RR 6.4, (95% CI 1.8-22) for B:15:P1.7,16 and RR 9.4 (95% CI 2.4-37) for C:2a:P1.2,5). Stratification by septicaemia and duration of disease before admission did not change these estimates substantially. Among patients ≥30 years (n=59) there was no association between phenotypes and death.

By stratified analysis we found age, septicaemia, and duration of disease to be independently associated with increased risk of fatal outcome.

Conclusions

We identified phenotypes B:15:P1.7,16 and C:2a:P1.2,5 as independent risk factors for death among patients below the age of 30 years. During the study period there was a marked increase in CFR among patients with B:15:P1.7,16 infection and although it coincided with the appearance of ET-type 23, this seemed not to be an explanatory factor in itself.

The increased prevalence of MD caused by the B:15:P1.7,16 phenotype contributed to the increased CFR during the late part of the study period. Other factors associated with this increase were increased proportions of patients ≥30 years, patients with septicaemia, and patients with a short duration of disease before admission.

Meningococcal meningitis in Italy in the years 1999-2000

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Aims

To estimate the incidence of meningococcal meningitis per year and to characterise *N. meningitidis* strains isolated in Italy during the last two years.

Material and Methods

Notifications of bacterial meningitis and meningococci isolated from cerebrospinal fluid (CSF) and blood (BL) were sent to the National Reference Centre at the Istituto Superiore di Sanità. The strains were characterised by serotyping, antimicrobial susceptibility testing and molecular typing by PFGE and/or PCR-RFLP for *por*A and *por*B. Epidemiological data were analysed according to the different parameters set by the European Surveillance Network.

Results

Two hundred and seventy-four and 203 cases of meningococcal meningitis were reported in the year 1999 and 2000, respectively. However only 103 isolates in 1999 and 100 in 2000 were sent to the reference lab. The most predominant serogroup was serogroup B (74%) followed by serogroup C (25%). Only one strain was typed as W135 but there was no link with pilgrims returning from The Haj. A decreased susceptibility to penicillin was detected in 6.2 % of the strains. No outbreak occurred during the two years. A low degree of genetic relatedness was found even among the most frequent serotypes except for those belonging to serogroup C and strains typed as B:14:P1.13 isolated mainly in the area of Bolzano in Northern Italy.

Conclusion

N. meningitidis is the second most common agent of bacterial meningitis in Italy, the first being *S. pneumoniae*. All cases occurring over the last years have been sporadic and mainly due to serogroup B strains. The wide distribution of different molecular types confirms the absence of localised clusters except for serotype B:14:P1.13 during 1999.

National Epidemiological Data - Australia
1 January to 31 December 2000

National Neisseria Network (NNN) of Australia*
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Background

Australia has a decentralised system of health care delivery by which health issues are dealt with by autonomous regional government departments. Laboratory surveillance of invasive meningococcal disease (IMD) is undertaken by nationally by a network of reference laboratories from all States and Territories*.

Methods

Laboratory confirmation of IMD is by culture, NAA and serology. Non-culture based diagnostic tests were progressively introduced in different regions and not all tests were available in all centres in 2000. The patient age, the site of isolation and outcome are recorded for laboratory confirmed cases where known. Standardised methods and QA are used to produce uniform data on the phenotype and antibiotic susceptibility of isolates from cases of IMD. Data are collated in a central database.

Results

Case numbers: Nationally there were 537 laboratory-confirmed cases of IMD in Australia in 2000 for a disease incidence of 2.89/100,000 population. 390 (73%) cases were culture confirmed; 98 (18%) were diagnosed by PCR; and 49 (9%) by serology. Case rates varied by region from 1.6 to 5.3/100,000; and nationally by age with rates in those 1 year or less of 18.6; 1 – 4y 7; 15 –19y 6; and 20 – 24y 3.5/100,000. Age specific disease rates also varied between jurisdictions.

Serogroup distribution: Of the culture confirmed cases, nationally 218 (56%) were serogroup B and 143 (37%) serogroup C. There were however significant differences in serogroup distribution between the various jurisdictions. Serogroup C strains were found disproportionately in the two most populous States: 80% of all Group C strains were isolated in New South Wales and Victoria but only 50% of the serogroup B isolates. In these two States serogroup

C strains were concentrated amongst adolescents and young adults. In other jurisdictions serogroup B strains comprised between 66 and 100% of isolates. Serogroup B accounted for most infections in those aged 4y or less. There were 10 (2.5%) culture-confirmed serogroup W135 infections, approximately the same proportion as in previous years. None were linked epidemiologically to the Hajj.

Serotype/serosubtype distribution. B:4:P1.4(7) was frequently encountered in Eastern States and B:15:P1.7 was also prominent. The 2a serotype continued to be found extensively in serogroup C strains with combinations of serosubtypes P1.5 and P1.2. In the State of Victoria phenotype C:2a:P1.4(7) was again prominent and accounted for a significant proportion of disease concentrated in the late adolescent/young adult population. This phenotype was rarely encountered elsewhere in Australia.

Outcome data was available from 141 serogroup B infections (9 deaths) and 102 serogroup C infections (12 deaths). 11 deaths in serogroup B and C infections occurred in those aged 4y or less and 6 in those aged 15 to 24.

Antibiotic susceptibility data: Penicillin - 253 of 369 strains tested (68%) were 'less sensitive" to the penicillins with the highest MICs recorded as 0.5 mg/L. All isolates tested were sensitive to ceftriaxone, ciprofloxacin and rifampicin.

Conclusions

There were significant differences in disease rates, affected age groups and infecting organisms in the different regions of Australia. Valid comparisons of basic data are best made through a networked system using standardised methods.

Diversity of *Neisseria meningitidis* type 22 strains isolated in Poland

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Introduction

Characterisation of *N. meningitidis* strains isolated in Poland showed that type 22 is the predominant type among meningococcal isolates from patients with meningococcal diseases (1, 2). It has also been shown, that this serotype is frequently isolated from patients living in Central-Eastern part of Europe (3, 4). The aim of this study was to investigate the diversity of *N. meningitidis* serotype 22 strains collected in Poland from 1995 to 1999.

Materials and Methods

All together 84 N. meningitidis type 22 strains (68/166 from blood/ cerebrospinal fluid, 3/14 from sputum, 1 from nose swab of patients and 12/271 from pharyngeal swabs of carriers /19-22 years/), were analysed using serotyping and different genotyping methods.

Serological grouping as well as typing and subtyping (by whole-cell ELISA) were performed. Multilocus Enzyme Electrophoresis (MEE) /isoenzymes: ME, IDH, G6P, GD1, GD2, FUM, ALP and ADK/ and Pulsed Field Gel Electrophoresis (PFGE) after treatment of meningococcal DNA with *Bgl* II or *Spe* I restriction enzymes were applied together with Du-Pont Co. automated ribotyping system, by which DNA fragments obtained after treatment with *EcoR* I restriction enzyme were analysed.

Results and Comments

Almost 95% of *N. meningitidis* type 22 strains belonged to serogroup B. About 80% of serotype 22 invasive isolates were recovered from children 5 years of age or younger. Among *N. meningitidis* type 22 meningococcal strains, 15 different subtypes were detected and subtype P1.14 was predominant (32/84-38%). However, 17 strains were nonsubtypable.

MEE data classified 70 *N. meningitidis* type 22 isolates into all 27 types. However, classification performed on the basis of data obtained from PFGE was quite different. Comparative PFGE analyses of 32 *N. meningitidis* 22: P.1.14, all but one serogroup B strains, using two different restrictions enzymes showed different patterns of homology between strains. Using the second genotyping method - ribotyping, the 71 *N. meningitidis* type 22 isolates were classified into 31 different ribogroups. The highest number of isolates belonging to one ribogroup was 15, all but one serogroup B, and belonging to 5 different serosubtypes. Within this ribogroup, two NST strains were also present. Thirty-

two type 22:P1.14 strains were also very heterogeneous and belonged to 17 different ribogroups.

Strains belonging to *Neisseria meningitidis* type 22 were most frequently recovered from severe cases of meningitis and septicaemia in Poland. The type 22 strains were separated into clusters by serosubtyping, electrophoretical typing and DNA analyses by PFGE and ribotyping. However, the grouping according to serosubtypes was quite different from that obtained by remaining 3 methods. Furthermore, these methods seldom identified the same clusters. Our results indicate that *N. meningitidis* type 22 isolates were extremely heterogeneous when different techniques of characterisation were applied. The genetic variation among type 22 strains has recently been analysed (5). Sequence analysis of *porB* genes from *N. meningitidis* type 22 showed the presence of seven distinct *porB* sequences. This could explain our observation of pronounced genetic as well as antigenic diversity.

Conclusion

The present study has demonstrated a considerable heterogeneity of *N. meningitidis* type 22 strains, which are the most common cause of meningococcal disease in Poland. Depending of the typing method applied different clusters of strains were obtained.

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The Bavarian meningococcal carriage study: genetic typing and cluster analysis

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Aims

The Bavarian meningococcal carriage study was undertaken to better understand the population structure of carried meningococci; to establish a MLST data-set for German meningococcal strains from healthy carriers; to evaluate DNA island typing.

Introduction

Genetic analyses of meningococcal carrier strains have been performed before by MLEE (1) and MLST (2). These studies demonstrated that the population structure of carried meningococci differs strikingly from that of disease isolates. We now typed by MLST 830 carriage isolates collected in Bavaria. Furthermore, the presence of DNA islands, which are differentially distributed among hypervirulent isolates (3,4), was assessed.

Results and discussion

We found more than 280 sequence types and 38 complexes. The composition of clones differed considerably to that of Czech isolates (2). Hypervirulent sequence types were found in less than 10% of the carriers. Genetic serogrouping revealed two frequent complexes lacking the *ctrA* gene. Furthermore, rates of serogroup switching were determined for the serogroups B, C, W135 and Y. An excellent correlation of DNA islands (3,4) with clonal groupings was found. By DNA island typing the major clonal groupings of carried meningococci could be grouped into three to four clusters of related clonal groupings.

Conclusion

A large carriage collection extensively characterized by MLST, capsular genotyping, and DNA island typing has been set up. This collection, and the Czech and UK collections of meningococcal carriage isolates provide the basis for comparative epidemiology of meningococcal carriage in Europe.

References

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