SURVEILLANCE OF INVASIVE MENINGOCOCCAL DISEASE IN ITALY, 2011-2012

C. Fazio1*, F. D’Ancona2, A. Neri3, M.G. Caporali3, G. Renna3, P. Mastrantonio3 and P. Stefanelli3

1Department of Infectious, Parasitic & Immune-mediated Diseases, National Centre for Epidemiology, Surveillance and Health Promotion, University of S. Calogero, Rome, Italy

* Corresponding author. E-mail: cesare.fazio@iss.it

INFECTION AND PURPOSE

In Italy, the National Surveillance System of Invasive Meningococcal Diseases (IMD), collects, since 1994, isolates and case reports. Since 2007, most IMD cases have been reported through the internet (http://www.sism.iss.it/meningite_batterica.htm). In total, 7,681 IMD cases were reported in Italy in 2011 through 2012. It is important to investigate the clinical features and the molecular characteristics of meningococci.

RESULTS

Molecular analyses

As shown in Figure 1, IMD incidence decreased from 0.30/100,000 in 2007 to 0.07/100,000 in 2011. Further decrease has been observed in 2012: the incidence remained stable over the past three years.

In the years 2011 and 2012, the incidence was higher in the general population, with an increase in the incidence in the general population. The highest incidence has been observed in children <1 year (2.8 in 2011 and 3.4/100,000 in 2012), followed by 1-4 year age group (1.0 and 1.1/100,000, respectively) and in the 5-14 year age group (0.34/100,000 and 0.4/100,000, respectively).

From 2011 to 2012, the incidence of cases due to serogroup B increased from 0.12/100,000 in the general population. The increase was observed in children <1 year and adults (15-64 year old) and adults (15-24 year old) (Fig. 2).

Meningococci represented the main cause of IMD in both years (Fig. 3).

Serogroup A

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Serogroup B

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Meningococci represented the main cause of IMD in both years (Fig. 3).

Serogroup C

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Meningococci represented the main cause of IMD in both years (Fig. 3).

CONCLUSIONS

Although the incidence of IMD remained low and stable in the last three years, meningococcal serogroups showed a dynamic pattern, likely responsible for the increased case fatality rate in the year 2012.

Dr Snežana Delić, Davor Ćulić
Center for Microbiology, Meningococcus and Haemophilus Reference Laboratory, Institute for Public Health – Sombor, Serbia

During the three-year period, Serbian MHRL collected sixteen *N. meningitidis* isolates. The number and percentage of isolates regarded on the serogroup were: serogroup B – 14 isolates (87.5%), serogroup C – 2 isolates (12.5%).

The origins of received isolates during this period were: blood 4 (25%), CSF 12 (75%). The total numbers of processed blood cultures were 82475 (28663/2009, 29780/2010, 24032/2011) and 5210 CSF (1746/2009, 1866/2010, 1598/2011).

Fifteen patients (96%) survived sepsis, meningitis or sepsis and meningitis, and for one patient (4%) disease was fatal. The gender distribution was: male 12 (75%), female 4 (25%); in conclusion male to female ratio was 3:1, and the ratio per year was 4:1 in 2009, 1:1.5 in 2010 and 5:0 in year 2011.

Regarding the clinical outcome of the meningococcal disease, the case fatality ratio was 1:16 (6.25%)

Antibiotic susceptibility testing was performed for all confirmed 16 isolates, and fenotyping (porA) and genotyping (fet A) methods for 15 isolates. Molecular methods were performed in The National Centre of Epidemiology „Johan Bela“ – Budapest, Hungary and in Meningococcal Reference Laboratory, Austrian Agency for Food and Health Safety – Graz, Austria.

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Bad Loipersdorf
Austria, 2013
Prevalence and phase variable expression status of two autotransporters, MspA and NaIP, in carriage and disease isolates of Neisseria meningitidis

Neil J. Oldfield, Suzan Matar, Fadil A. Bidmos, Mohamed Alamro, Keith Neal, David P.J. Turner, Christopher D. Bayliss, and Diawar A.A. Ali’Aleed

Background:
- Phase variation (PV) is the reversible switching ON or OFF of molecules; a process mediated by slipped-strand mispairing at repeat tracts during DNA replication.
- Many meningococcal surface structures undergo PV; this may allow the evasion of adaptive immune responses and enhance persistence.
- NaIP and MspA are outer membrane/secreted autotransporters. Expression of both is modulated by PV via polyC tracts within their coding sequences.
- The function of MspA is unclear; NaIP is a cell-surface protease which cleaves several putative vaccine candidates including NhBA. Its expression may therefore influence isolate susceptibility to vaccine-induced immune responses.

Aim: To determine the presence and phase-variable expression status of NaIP & MspA in recent isolates to help understand the contribution of these proteins to meningococcal-host interactions.

Methods:
- Carriage isolates (n=127) were obtained from students at Nottingham University, UK during 2008-09. Presence of NaIP and mspA was determined by PCR. Length of repeat tracts was examined by DNA sequencing and gene scanning. Expression in representative strains was confirmed by immunoblot. For invasive strains, we examined the sequences in the MRPR meningococcal Genome Library database (2010-11 isolates; n=514).

Results:
- High prevalence of NaIP and mspA
  - Overall, 98.1% of strains examined possessed mspA and 88.8% possessed NaIP.
  - No strains in either collection lacked both genes and there was no significant difference in the frequency of NaIP or mspA in disease versus carriage isolates.
  - NaIP-negative strains from different lineages were examined further. Deletion was due to replacement by an IS element or recombination between repetitive elements leaving behind different numbers of residual dRS3 repeats & Correia-like sequences (Fig. 1).
  - Most NaIP-negative isolates were from the ST-461 & ST-269 clonal complexes. ST-461 strains typically had NaIP2-class deletions, whilst ST-269 strains commonly harbored NaIP3-class deletions.

Conclusions:
- Two phase-variable expressed autotransporters, NaIP and MspA, are highly prevalent in carriage and disease isolates.
- Strains lacking NaIP were detected; characterization of the deletion loci showed multiple independent deletion events. Similar events led to the deletion of fetA, porA, and hpuAB.
- Differences in the proportions of strains in ON/OFF expression states were apparent between carriage & invasive collections, and between serogroups, indicating differential requirements for MspA & NaIP expression in different niches and in different lineages.
- One target for NaIP-mediated cleavage is NhBA; NaIP expression may therefore reduce the susceptibility of MenB isolates to 4CMenB-induced immune responses. We suggest that this will not be a universal effect as NaIP was absent in 16% of invasive MenB strains, whilst ~60% of NaIP-positive MenB strains were not expressing NaIP.

For more details, see Oldfield et al 2013 PLoS One 8, e69746.
Introduction

National, laboratory-based surveillance for invasive meningococcal disease (IMD) was established in 1999. Prior to 2006, serogroup W (MenW) was rare in South Africa (SA) and accounted for 5% of invasive meningococcal disease (IMD). Expansion of the ST-11 complex caused MenW to increase, replacing serogroup A as the predominant serogroup. In 2006, MenW represented 67% of IMD, subsequently declining to 50% in 2012.

Aim

We analysed clonality amongst selected MenW isolates at the whole genome sequence (WGS) level over a 10-year period.

Methods

IMD cases were reported through national surveillance from 2003 through 2012. One MenW isolate per year (N=10) was randomly chosen for WGS. DNA was extracted using the Wizard Genomic DNA purification kit (Promega) and quantified using the Qubit instrument (Invitrogen). Sequence data were generated using the Illumina platform and assembled using Velvet and VelvetOptimiser. Assembled sequences were uploaded to the Neisseria PubMLST database, which runs the BigSdb platform. The BigSdb database was used to analyse ST-11 complex isolates using MLST, rMLST and 1,975 defined loci (whole genome MLST) in the FAM15 reference genome. ST-11 complex isolates from SA were compared to ST-11 complex genomes from the UK: 6 from the Southampton outbreak in 1997 and 37 from endemic ST-11 complex circulating during 2010-2012 (MRF Genome Library). Phylogenetic networks were constructed using the NeighborNet algorithm.

Conclusion

ST-11 complex isolates from South Africa were closely related to each other at the WGS level and represented a distinct WGS group compared to current endemic ST-11 meningococcal isolates circulating in the UK, although two endemic UK isolates were identical to the SA isolates.
Characterisation of pilE antisense RNA in Neisseria meningitidis

Felicia Tan, Edmund Loh, Christopher M. Tang and Rachel M. Exley
Sir William Dunn School of Pathology, University of Oxford, Oxford, OX1 3RE, UK.

Background

Neisseria meningitidis has evolved specialised virulence factors that enable it to successfully colonise and cause disease in humans. Type four pili (T4p) are particularly important for colonisation. Pili are hair-like projections that are comprised primarily of the major pilin protein encoded by the pilE gene. The pilus subunits are arranged in a helical configuration to form the pilus fibre (Figure 1A). Pili mediate adhesion to host surfaces, microcolony formation, twitching motility, natural transformation, and are subject to phase and antigenic variation [1-4].

Sequence analysis of the N. meningitidis pilE locus of strain 8013 has resulted in the identification of a putative promoter for a cis-encoded RNA on the antisense strand of pilE (Figure 1B). The putative promoter contains sequences with homology to the E. coli promoter consensus sequence (Figure 1C).

Cis-encoded antisense (AS) RNAs are noncoding regulatory RNAs that modulate the stability or the translation rate of their target RNAs. Noncoding RNAs are increasingly recognized as important regulators of gene expression in bacteria.

The aims of this project are to determine if the putative AS promoter is functional and to abolish its activity in N. meningitidis in order to study its function and to understand the contribution of the AS RNA to pilE expression in N. meningitidis.

Results

1. Transcription from the AS promoter is upregulated during NaCl stress in an E. coli system.

The AS promoter region was introduced into a reporter vector in E.coli and bacteria were subjected to acid, osmotic, temperature, envelope or oxidative stress. The presence of the AS transcript was detected by Northern Blot. Higher levels of the AS transcript suggest that transcription from the AS promoter is induced by NaCl stress (Figure 2A).

Site directed mutagenesis of the -10 and -35 sequences was carried out to identify mutation(s) that would abolish promoter activity. Northern Blot analysis indicates that transcription was abolished upon mutation of the -10 sequence (Figure 2B).

2. Identification of the AS transcriptional start site by primer extension.

Total RNA was extracted from N. meningitidis strain 8013 and primer extension was performed to determine the transcriptional start site of the AS transcript. The start site (denoted by +1) was identified to be 8 nt downstream of the -10 sequence of the putative promoter.

3. The cis-encoded AS RNA is transcribed in N. meningitidis.

AS promoter mutant strains of N. meningitidis and the corresponding control strains were generated via double-crossover recombination (Figure 4A).

The strains were subjected to NaCl stress at mid-log phase. Strand-specific qRT-PCR (Figure 4B) and Northern Blot (Figure 4C) indicate that the AS transcript is upregulated in NaCl stress and the pilE transcript is downregulated upon NaCl stress, however this seems to occur even in the absence of AS transcription.

A small but consistent increase in pilE transcript levels was observed in the Mut, ery strain compared to WT, ery. However, no differences in PilE protein levels were observed (Figure 4D).

Conclusions

We have identified a cis-encoded RNA on the antisense strand of pilE in N. meningitidis. We have shown that this AS is expressed upon NaCl stress but this has no significant effect upon pilE transcript levels. We are currently investigating the function of the AS RNA in N. meningitidis.

References

**Molecular methods in the surveillance of invasive meningococcal disease in Romania, as part of ECDC/IBD – labnet activities**


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**Background**

*Neisseria meningitidis* strains isolated from meningococcal infections sent to the National Reference Center for *N*. meningitidis - Lab. Bacterial Resp. Infections, are essential elements in meningococcal disease surveillance in each country, contributing to the European database and meningococcal vaccine development. IBD labnet – ECDC funded laboratory network for the surveillance of invasive bacterial diseases (2008).

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**Aim**

To study Romanian meningococci circulating strains, in the IBD-labnet training program, supported by ECDC.

**Methods:**

39 *N*. meningitidis strains coming from CSF, were collected at the National Referent Laboratory from Cantacuzino Institute and analyzed at the Institut für Hygiene und Mikrobiologie, Wurzburg and at the Institut für Med. Mikrobiologie und Hygiene, Graz.

The strains were tested by phenotypical and PCR/RT-PCR method for species identification, serogrouping was performed by agglutination with Rtiler sera, and susceptibility to penicillin (penicillin, oxacillin, rifampicin, cephalosporins) by E test. Por A was also performed for 39 strains and for A for 37 strains.

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**Antigen sequence types (%) Fet-A**

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**Conclusions**

1. The study was limited by possible underestimation of the incidence of invasive meningococcal disease in Romania and susceptibility to penicillin in meningococci should be monitored in the future.
2. There is an urgent need:
   - For a close collaboration between clinical medicine and the institutions of public health concerning the surveillance of meningococcal disease and antibiotic consumption.
   - Further partnerships between countries.
   - Development of new meningococcal vaccines.