SURVEILLANCE OF INVASIVE MENINGOCOCCAL DISEASE IN ITALY, 2011-2012

C. Fazio*[1], F. D'Ancona[2], A. Neri[1], M.G. Caporali[2], G. Renna[1], P. Mastrantonio[1] and P. Stefanelli[1]

[1]Department of Infectious, Parasitic & Immune-mediated Diseases; [2]National Centre for Epidemiology, Surveillance and Health Promotion
Istituto Superiore di Sanità, Rome, Italy
phone +39 06 49902126 - e.mail: cecilia.fazio@iss.it

INTRODUCTION AND PURPOSE

In Italy, the National Surveillance System of Invasive Meningococcal Diseases (IMD), collects, since 1994, isolates and case reports. Since 2007, notifications are reported in a dedicated website (http://www.simi.iss.it/meningite_batterica.htm). Here, we described all cases of IMD occurred from 2011 through 2012. In particular, we investigated the clinical features of patients with IMD and the molecular characteristics of meningococci.

MATERIALS AND METHODS

Patients and bacterial strain characterization

From 2011 through 2012, a total of 290 IMD cases have been reported. The website record reports for each case information on both clinical status and microbiological investigations. Analysis was carried out by EpiInfo version 3.3.2.
During the study period, 160 meningococci have been sent to NRL. All the isolates were subcultured to confirm the serogroup by standard methods. For 4 clinical samples (blood and CSF) molecular identification of serogroups has been performed. Susceptibilities to penicillin G, rifampicin, ciprofloxacin and ceftriaxone were determined by Etest method (bioMérieux SA - France). The breakpoints were those recommended by the European Committee on Antimicrobial Susceptibility Testing - EUCAST version 3.1, 2013-02-11 (http://www.eucast.org/).

Molecular characterization

All the samples have been completely characterized for MLST, PorAVR1, PorAVR2 and FetA, (http://neisseria.org/).

RESULTS

Epidemiological and clinical data

As shown in Figure 1, IMD incidence decreased from 0.56/100,000 inhabitants in 2004 to 0.3 in 2006. A further decrease has been observed since 2010; the incidence remained stable over the last three years.

In the years 2011 and 2012, the annual incidence in the general population was 0.25/100,000 and 0.23/100,000, respectively. The highest incidence has been observed in children < 1 year (2.8 in 2011 and 2.4/100,000 in 2012), followed by 1-4 year age group (1.0 and 1.1/100,000, respectively); in the 15-24 year age group it was 0.54/100,000 and 0.4/100,000, respectively.

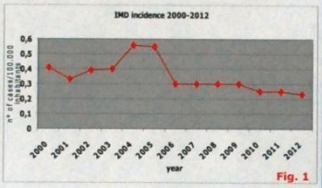


Fig. 1

From 2011 to 2012, the incidence of cases due to serogroup B, decreased from 0.12 to 0.09/100.000 in the general population.
Differently, IMDs due to serogroup C, increased from 0.03 to 0.06, even if within the same range of the most recent years. In particular, this increase was due to cases occurred mostly in adolescents (15-24 year old) and adults (25-64 year old) (Fig.2).

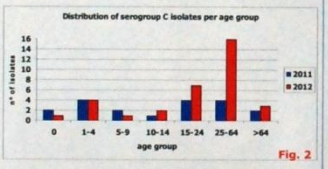


Fig. 2

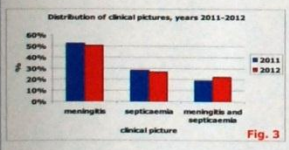


Fig. 3

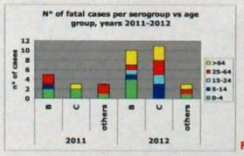


Fig. 4

Meningitis represented the main clinical picture, characterizing more than 50% of cases in both years (Fig.3).

An alarming increase of case fatality rate from 9.6% to 24.2% has been observed in all serogroups. The mean age of dead patients increased from 25 in 2011 to 37 years in 2012 (Fig.4).

Serogroups and antimicrobial susceptibility

From 2005 to 2011, an increase in the proportion of serogroup B strains and a decrease of serogroup C strains have been observed. Conversely, from 2011 to 2012, the proportion of serogroup B decreased from 64% to 48% and the proportion of serogroup C increased from 16% to 32%. Among the others groups, we observed an increase of serogroup Y from 14% in 2011 to 19% in 2012 (Fig.5).

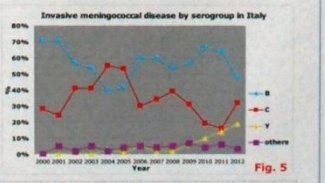


Fig. 5

All meningococci analyzed were susceptible to the antimicrobials tested, except for two isolates resistant to rifampicin (MIC 0.38 and 2 mg/L) and for the several meningococci with a decreased susceptibility to penicillin G (PenI isolates).

From 2011 to 2012, an increase of MIC50 and MIC90 values for Penicillin G has been observed (Fig.6).

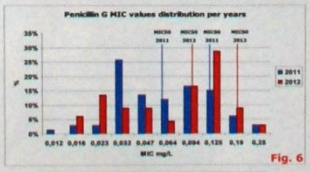


Fig. 6

The PenI isolates increased from 41% in 2011 to 58% in 2012. This increase involved all principal serogroups. In particular, serogroup C isolates showed the highest percentages of PenI (Fig.7).

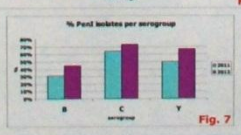


Fig. 7

Molecular analyses

Serogroup B

Among serogroup B isolates, MLST showed 11 already known clonal complexes (CC). The majority of isolates belonged to CC41/44 (31.2%), CC32 (16.9%), CC162 (14.3%), CC269 (11.7%).
The CC41/44 was the predominant in the two years, although the percentage slightly decreased from 31% (2011) to 28% (2012) (Fig.8). In the same period, an increase was observed for CC269 (from 8% to 20%), for CC32 (from 10% to 24%) and for CC162 (from 10% to 16%). Differently, three clonal complexes (CC865, CC167, CC198) disappeared in 2012.

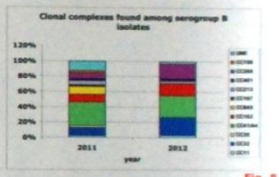


Fig. 8

Among serogroup B CCs, 49 different STs have been detected. The CC41/44 was characterized by 10 STs in 2011 and by 6 in 2012; among these only the ST-1403 was present in both of the years (Fig.9).

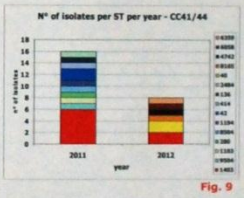


Fig. 9

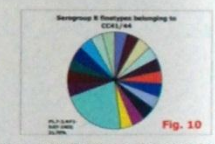


Fig. 10

Among 19 finetypes detected in the CC41/44, the most common was B:P1.7-2,4:F1-5:ST-1403(cc41/44) (22%) (Fig.10).

Serogroup C

Among serogroup C isolates, MLST showed 5 known clonal complexes and 11 different STs. Clonal complexes CC11 and CC334 were the most common and accounted for the 61.5% and 25.6%, respectively.
During the two years, the CC11 was the most frequently isolated and the percentage increased from 43.8% in 2011 to 73.9% in 2012. Differently, the CC334 decreased from 43.8% to 13% (Fig.11).

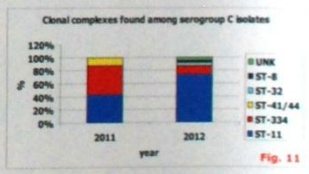


Fig. 11

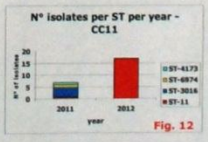


Fig. 12

The CC11 was characterized by four STs (ST-11, ST-3016, ST-4173, ST-6974) in 2011 and by only one in 2012 (ST-11) (Fig.12).

Among the 5 finetypes detected in the CC11, the most common were C:P1.7-4,14-6:F3-9:ST-1031(cc334) in 2011 (78%) and C:P1.5-1,10-8:F3-6:ST-11(cc11) in 2012 (62%) (Fig.13).

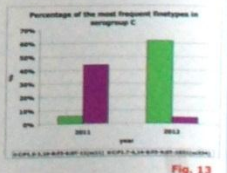


Fig. 13

Serogroup Y

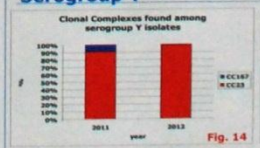


Fig. 14

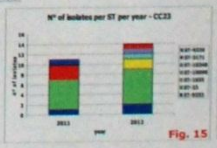


Fig. 15

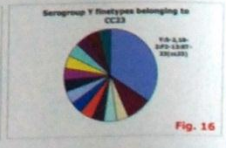


Fig. 16

Among serogroup Y isolates, MLST analysis showed 2 known clonal complexes and 7 different STs.
Clonal complex CC23 was the most common and accounted for the 92% in 2011 and 100% in 2012 (Fig.14). This clonal complex was characterized by 4 STs in 2011 and by 6 in 2012 (Fig.15).

Among 16 finetypes detected in the CC23, the most common was Y:P1.5-2,10-2:F2-13:ST-23(cc23) (33%) (Fig.16).

CONCLUSIONS

✓ Although the incidence of invasive meningococcal disease (IMD) remained low and quite stable during the last two years, meningococcal serogroups showed a dynamic distribution. It is noteworthy the increased case fatality rate in the year 2012.

✓ From 2011 to 2012, serogroup B meningococci were predominant in Italy, although in 2012 a decrease has been noted. The rise of serogroup Y persists, as also reported in other European Countries.

✓ After the introduction of MenC vaccination in 2005, serogroup C declined mainly among infants and children in our Country. On the contrary, an increase of meningococccal serogroup C cases was observed in 2012 in adolescents and young adults.
The re-emergence of the ST-11 among serogroup C meningococci after two years has been noted.

✓ The number of PenI isolates increased in 2012. In particular, serogroup C was characterized by the highest proportion of PenI strains.

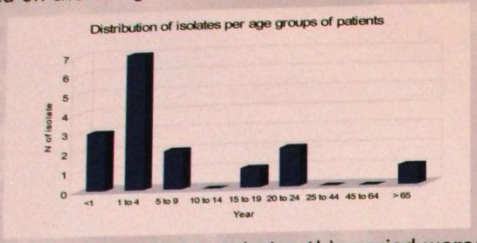
✓ Updated phenotypic and genotypic characterizations of meningococci responsible of invasive diseases are of great interest due to their implication on vaccine policies for the use of old and new vaccine formulations.

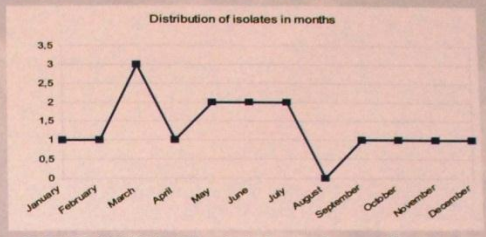
Report on Meningococcus invasive isolates collected in Serbia in a three-year period (2009-2011)

dr Snežana Delić, Davor Čulić

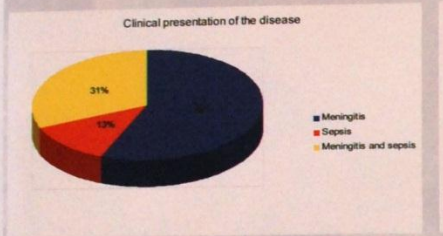
Center for Microbiology, Meningococcus and Haemophilus Reference Laboratory, Institute for Public Health – Sombor, Serbia

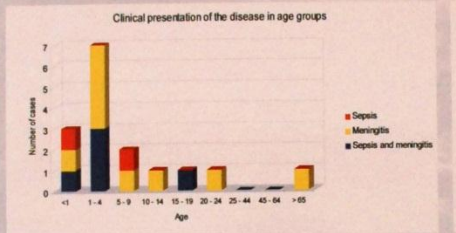
During the three-year period, Serbian MHRL collected sixteen *N. meningitidis* isolates. The number and percentage of isolates regarded on the serogroup were: serogroup B – 14 isolates (87.5%), serogroup C – 2 isolates (12.5%).

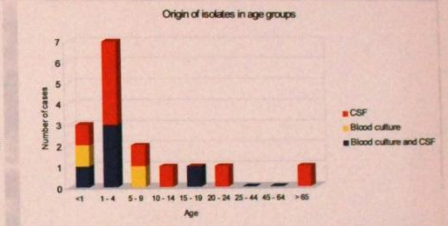




The origins of received isolates during this period were: blood 4 (25%), CSF 12 (75%). The total numbers of processed blood cultures were 82475 (28663/2009, 29780/2010, 24032/2011) and 5210 CSF (1746/2009, 1866/2010, 1598/2011).

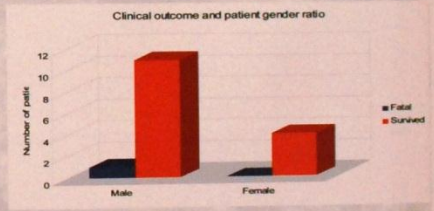




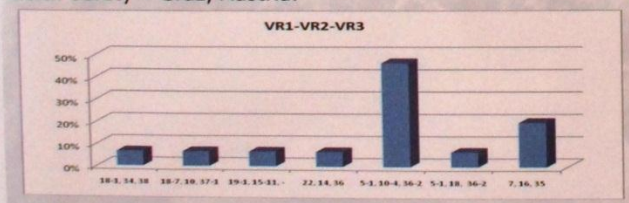


Fifthteen patients (96%) survived sepsis, meningitis or sepsis and meningitis, and for one patient (4%) disease was fatal. The gender distribution was: male 12 (75%), female 4 (25%); in conclusion male to female ratio was 3:1, and the ratio per year was 4:1 in 2009, 1:1.5 in 2010 and 5:0 in year 2011.

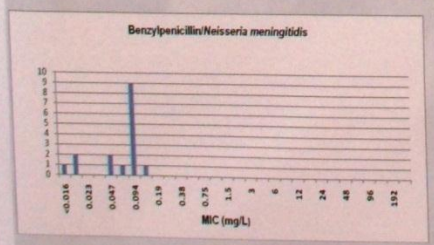
Regarding the clinical outcome of the meningococcal disease, the case fatality ratio was 1:16 (6.25%)

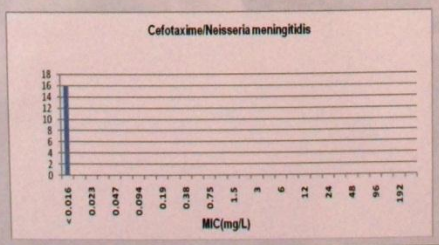


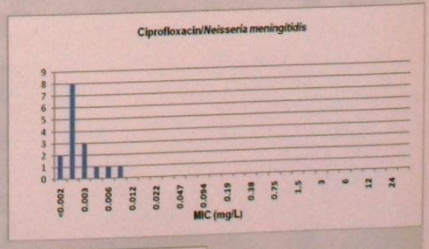
Antibiotic susceptibility testing was performed for all confirmed 16 isolates, and fenotyping (porA) and genotyping (fet A) methods for 15 isolates. Molecular methods were performed in The National Centre of Epidemiology „Johan Bela" - Budapest, Hungary and in Meningococcal Reference Laboratory, Austrian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

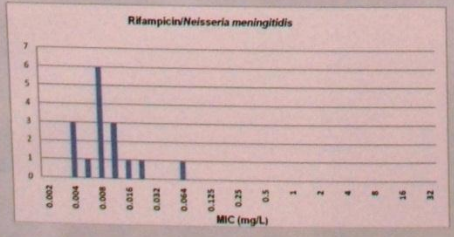


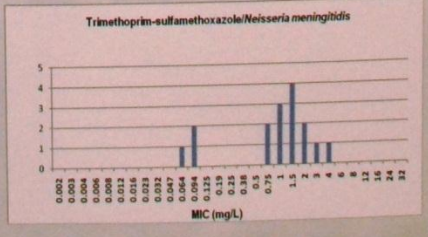
PorA VR1	N	%	PorA VR2	N	%	FetA	N	%
5-1	8	53.3	10-4	7	46.7	5-8	5	33
7	3	20	16	3	20	3-3	2	13
18-1	1	6.7	10	1	6.7	1-14	1	7
18-7	1	6.7	34	1	6.7	1-5	1	7
19	1	6.7	15	1	6.7	1-7	1	7
22	1	6.7	14	1	6.7	3-5	1	7
			18	1	6.7	5-1	1	7
						5-2	1	7
						5-5	1	7
						5-75	1	7











Bad Loipersdorf
Austria, 2013

Prevalence and phase variable expression status of two autotransporters, MspA and NalP, in carriage and disease isolates of *Neisseria meningitidis*

Neil J. Oldfield[1], Suzan Matar[1,2], Fadil A. Bidmos[3], Mohamed Alamro[3], Keith Neal[1], David P.J. Turner[1], Christopher D. Bayliss[3] & Dlawer A.A. Ala'Aldeen[1]

[1]*Molecular Bacteriology and Immunology Group, School of Life Sciences, University of Nottingham, UK (www.nottingham.ac.uk/mbig);* [2]*University of Jordan, Jordan;* [3]*University of Leicester, UK*

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

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.

➢ NalP 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; NalP 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 NalP & 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 *nalP* 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 MRF Meningococcus Genome Library database (2010–11 isolates; n=514).

Results:

❖ High prevalence of *nalP* and *mspA*

➢ Overall, 98.1% of strains examined possessed *mspA* and 88.8% possessed *nalP*.

➢ No strains in either collection lacked both genes and there was no significant difference in the frequency of *mspA* or *nalP* in disease versus carriage isolates.

➢ *nalP*-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 *nalP*-negative isolates were from the ST-461 & ST-269 clonal complexes. ST-461 strains typically had Δ*nalP*2-class deletions, whilst ST-269 strains commonly harbored Δ*nalP*3-class deletions.

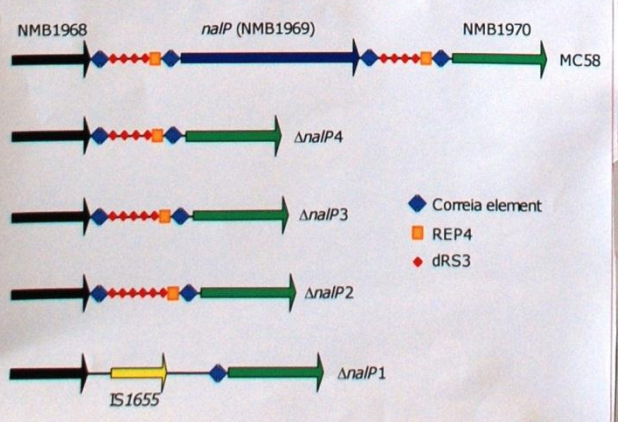


Fig. 1. Genetic arrangement of the nalP locus in N. meningitidis. Unidirectional arrows represent ORFs. Repetitive elements are represented by different symbols. Other repetitive sequences are not shown for clarity.

❖ PV status of *mspA* and *nalP*

➢ Tract lengths ranged from 6–14 bp (mode= 9, phase ON) in *mspA* and 6–15 bp (mode = 10; phase ON) in *nalP* (Fig. 2).

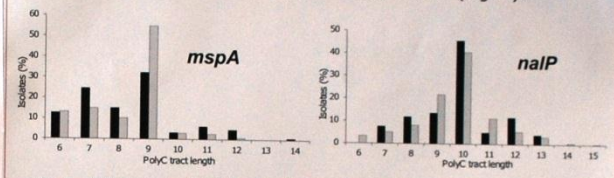


Fig. 2. Distribution of tract lengths for *mspA* and *nalP* in invasive and carriage isolates. Black bars, carriage; grey bars, invasive. An ON PV state is produced by 6, 9 or 12Cs for *mspA* and by 7, 10 or 13Cs for *nalP*.

❖ Influence of serogroup on PV status

➢ For *nalP* harbored by MenY strains, there was a significant difference between the frequency of the phase ON phenotype during carriage and invasive disease (p=0.0259; Table 1).

➢ The frequency of the ON phenotype was significantly higher in invasive MenY strains than invasive MenB strains (p<0.0001), whilst the frequency of the ON phenotype was not statistically different between carried MenY and MenB strains (Table 1).

Serogroup	Carriage		Invasive		P value
	Phase ON	Phase OFF	Phase ON	Phase OFF	
B	58.8%[a]	41.2%	40.1%[b]	59.9%	0.1366
Y	69.2%[a]	30.8%	86.3%[b]	13.7%	0.0259
Others	44.9%	55.1%	51.1%	48.9%	0.6797
Total	57.6%	42.4%	49.1%	50.9%	0.1188

Table 1. Distribution of phase ON and OFF *nalP* genes isolates by serogroup.
[a] No statistically significant difference; [b] Statistically significant difference (p<0.0001).

➢ For MenB strains, the vast majority of carriage and invasive isolates, were *mspA* phase ON (Table 2). Only 32.7% of carriage and 13.7% of invasive MenY strains were *mspA* phase ON, with the shift to an *mspA* OFF phenotype in MenY invasive strains being statistically significant (p=0.015).

➢ For both carriage and invasive isolates, the frequency of the *mspA* phase ON phenotype was significantly higher in MenB strains than MenY strains (p<0.0001 for both comparisons). This difference resulted from a difference in mode tract length; 9Cs (phase ON) for MenB, 7Cs (phase OFF) for MenY strains (not shown).

Serogroup	Carriage		Invasive		P value
	Phase ON	Phase OFF	Phase ON	Phase OFF	
B	89.5%[a]	10.5%	86.1%[b]	13.9%	1.0000
Y	32.7%[a]	67.3%	13.7%[b]	86.3%	0.0150
Others	52.7%	47.3%	17.4%	82.6%	0.0004
Total	50.0%	50.0%	69.2%	30.8%	<0.0001

Table 2. Distribution of phase ON and OFF *mspA* genes by serogroup.
[a] Statistically significant difference (p<0.0001); [b] Statistically significant difference (p<0.0001).

Conclusions:

➢ Two phase-variably expressed autotransporters, NalP and MspA, are highly prevalent in carriage and disease isolates.

➢ Strains lacking *nalP* were detected; characterisation of the deletion loci showed multiple independent deletion events. Similar events lead 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 & NalP expression in different niches and in different lineages.

➢ One target for NalP-mediated cleavage is NhbA; NalP 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 *nalP* was absent in 16% of invasive MenB strains, whilst ~60% of *nalP*-positive MenB strains were not expressing NalP.

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

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

Whole genome resolution of selected *Neisseria meningitidis* serogroup W strains, South Africa, 2003-2012

Mignon du Plessis[1], Shanil Govindpershad[1], Martin C. J. Maiden[2], Keith A. Jolley[2], Stephen D. Bentley[3], Julian Parkhill[3], Cheryl Cohen[1], Keith P. Klugman[4], Linda de Gouveia[1], Nicole Wolter[1] and Anne von Gottberg[1]

[1] National Institute for Communicable Diseases (NICD), a division of the National Health Laboratory Service (NHLS), Johannesburg, South Africa [2] Department of Zoology, University of Oxford, UK [3] Wellcome Trust Sanger Institute, Hinxton, UK [4] Hubert Department of Global Health, Rollins School of Public Health, and Division of Infectious Diseases, School of Medicine, Emory University, Atlanta, USA

Corresponding author: mignond@nicd.ac.za www.nicd.ac.za

Introduction

National, laboratory-based surveillance for invasive meningococcal disease (IMD) was instituted 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 BIGS database was used to analyse ST-11 complex isolates using MLST, rMLST and 1,975 defined loci (whole genome MLST) in the FAM18 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[1,2], 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 sub-group compared to current endemic ST-11 meningococcal isolates circulating in the UK, although two endemic UK isolates were identical to the SA isolates.

Results

- In SA, 4537 cases were reported; 3327 (73%) were viable and assigned a serogroup, of which MenW represented 51% (1710/3327). MenW increased in incidence from 0.06/100,000 population in 2003 to 0.64/100,000 in 2006, and declined to 0.09/100,000 in 2012 (p<0.0001) **(Figure 1)**.
- 8/10 of the randomly chosen SA MenW isolates were ST-11, with identical porA and FetA antigens. The remaining two isolates belonged to ST-22 (ST-184) and ST-865 (ST-8608) complexes **(Table 1)**.
- The application of rMLST to the SA and UK isolates demonstrated that 'ET-15' variants of the ST-11 complex responsible for the Southampton outbreak were distinct. The SA isolates were not closely related to 'ET-15' Southampton isolates but clustered more closely with the 'non-ET-15' endemic UK isolates **(Figure 2)**.
- Whole genome analysis further resolved the UK endemic and SA strains into two groups. Exceptions were two UK isolates which remained within the SA cluster and one SA isolate which was not closely related to any of the SA or UK isolates **(Figure 3)**

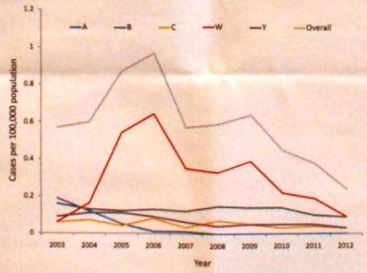


Figure 1. Incidence of invasive meningococcal disease in South Africa, 2003-2012, by serogroup

Table 1. Characteristics of South African serogroup W meningococcal isolates selected for whole genome analysis, 2003-2012 (N=10)

Isolate ID	Year	Specimen	Patient age	Patient sex	Sequence type	Clonal complex	PorA (VR1,VR2)	FetA
SA_NM1	2008	Blood	3 months	Male	11	ST-11/ET-37	5,2	F1-1
SA_NM2	2009	CSF	10 months	Male	8608	ST-865	7-1,1	F3-6
SA_NM4	2012	Blood	8 years	Male	184	ST-22	18-1,3	F3-9
SA_NM6	2004	Blood	9 months	Male	11	ST-11/ET-37	5,2	F1-1
SA_NM9	2007	CSF	3 years	Male	11	ST-11/ET-37	5-1,2	F1-1
SA_NM10	2010	CSF	1 month	Female	11	ST-11/ET-37	5,2	F1-1
SA_NM11	2011	CSF	1 year	Male	11	ST-11/ET-37	5,2	F1-1
SA_NM12	2006	Blood	1 year	Male	11	ST-11/ET-37	5,2	F1-1
SA_NM13	2003	Blood	18 years	Male	11	ST-11/ET-37	5,2	F1-1
SA_NM16	2005	CSF	17 years	Female	11	ST-11/ET-37	5,2	F1-1

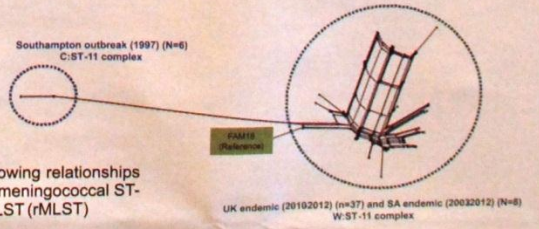
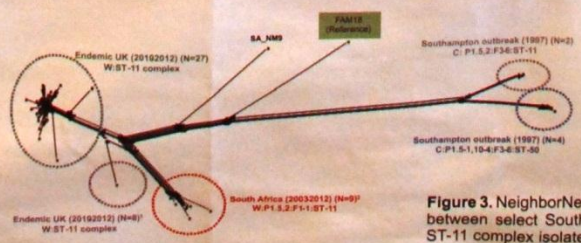


Figure 2. NeighborNet joining tree showing relationships between select South African and UK meningococcal ST-11 complex isolates using ribosomal MLST (rMLST)



[1] Includes SA_NM15 (2003)
[2] Includes two UK endemic isolates (2006 and 2011)

Figure 3. NeighborNet joining tree showing relationships between select South African and UK meningococcal ST-11 complex isolates using 1,975 defined loci (whole genome MLST)

References

Characterisation of *pilE* antisense RNA in *Neisseria meningitidis*

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

UNIVERSITY OF OXFORD

Background

Neisseria meningitidis has evolved specialised virulence factors that enable it to successfully colonise and cause disease in humans. Type four pili (Tfp) 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 pilin 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*.

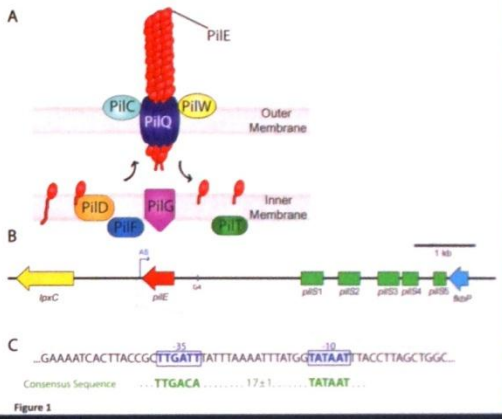


Figure 1

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).

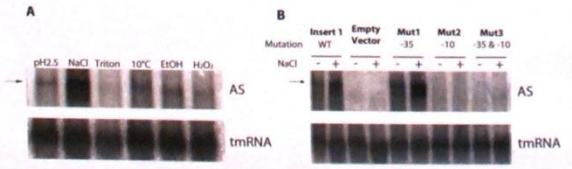


Figure 2

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

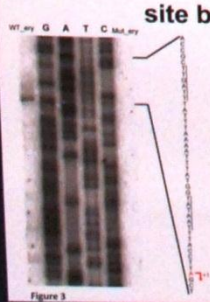


Figure 3

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*.

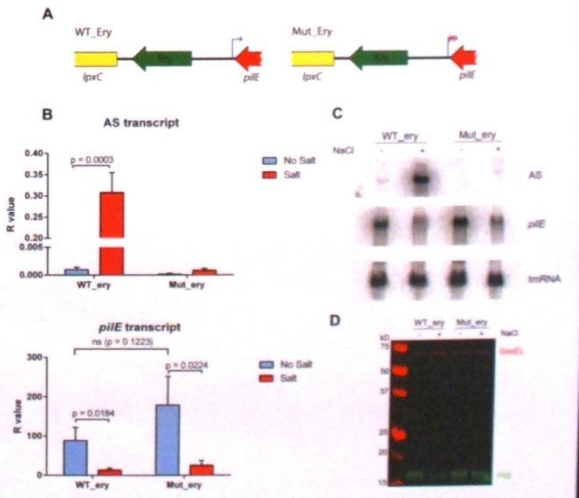


Figure 4

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 qRTPCR (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

[1] Stephens DS, McGee Z A. *The Journal of infectious diseases* 1981, **143**:525–32.
[2] Merz a J, So M, Sheetz MP. *Nature* 2000, **407**:98–102.
[3] Aas FE, Wolfgang M, Frye S, Dunham S, Løvold C, Koomey M. *Molecular microbiology* 2002, **46**:749–60.
[4] Vink C, Rudenko G, Seifert HS. FEMS microbiology reviews 2011, **36**:917–948.

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

Pana M.[1], Zota L [2], Claus H.[3], Vogel U. [3], Frosch M. [3], Elias J.[3],Korman-Klement A.[4], Reichl B.[4], Orendi U.[4], Taghawi-Donjai P.[4], Mikula C.[4], and Heuberger S.[4]

1:National Institute for Research & Development for Microbiology & Immunology "Cantacuzino", Bucharest, Romania,
2:National Institute of Public Health, Bucharest, Romania, 3: Institute fur Hygiene und Mikrobiologie,Wurzburg,Germany,
4:Institute fur Med. Mikrobiologie und Hygiene, Graz, Austria

Abstract

Background: IBD labnet – ECDC funded laboratory network for the surveillance of invasive bacterial diseases (2008).
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 Reference Laboratory from Cantacuzino Institutand analysed at anslysed at the Inst.fur Hygiene & Mikobiologie, in Wurzburg and at Inst.fur Med.Mikrobiologie und Hygiene, in Graz.
The strains were tested by phenotypical and PCR/RT-PCR method for species identification, serogrouping was performed by agglutination with Remel sera, and susceptibility to antibiotics (penicillin, ceftriaxone, rifampicin, ciprofloxacin) by E test.
Por A was also performed for 39 strains and fet A for 37 strains.
Results: analysis of culture revealed 58 % group B, 35 % group C and 7 % group W135.
The susceptibility testing results were analyzed according to EUCAST v.1.3. and showed 10.2 % resistant,64 % intermediate resistant and 25.8 % susceptible. Concerning Por A, P.22 was predominant (30.7 %) belonging to serogroup B. Concerning fet A the results revealed the following aspects: F3-9 was 21.6 %, F5-8 and F1-5 were 13 % each.
Conclusions: 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.

European legislation
- EC Decision 2119/98/EC changed by EC Decision 2007/875/EC, sets the need to set up an European network for the surveillance of communicable diseases;
- EC Decision 2000/96/EC changed by EC Decision 2007/875/EC contains the list of diseases that present mandatory surveillance and reporting;
- EC Decision 2000/57/EC, 28/IV/2008 replaced by EC Decision concerning (on) the events to be reported in the early warning and rapid response for the prevention and control of communicable diseases;
- 28/IV/2008 EC decision in order to change Decision 2002/253/EC, established case definitions for reporting communicable diseases to the Community network under Decision No. 2119/98/EC of the European Parliament and of the European Council.

Romanian legislation
- Government Decision no. 589/2007, concerning the establishing of the methodology for data collection and reporting for communicable disease surveillance;
- Ministry of Health Order no. 1466/2008, for approving the informational circuit of single sheet reporting communicable diseases;
- Ministry of Health Order no. 883/2005, concerning the approving the methodology for early warning and rapid response to communicable diseases;
- Ministry of Health Order no. 860/2004, concerning the approving of the list of priority diseases in Romania stipulates that meningococcal disease is priority transmissible disease, surveillance based on supervisory methodology;
- Ministry of Health Order no. 588/2000, concerning establishing of certain categories of diseases, for which laboratory testing is mandatory reporting, requires that all medical laboratories, regardless (of) the system they belong to, will report at 6 months, starting with (beginning with) 01.08.2000, at the National Institute for Research & Development for Microbiology & Immunology "Cantacuzino", the number of microorganisms isolated and identified, as well as the pathogen antimicrobial susceptibility results (tests performed).

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).

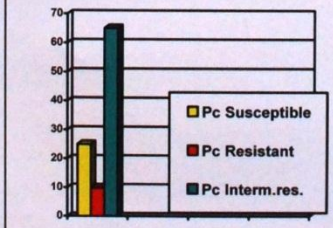
County	No.of isolates		
Bucuresti	12	Brasov	1
Covasna	9	Valcea	1
Galati	5	Salaj	1
Dolj	3	Ilfov	1
Bihor	2	Cluj	1
Timis	1	Braila	1
Maramures	1		

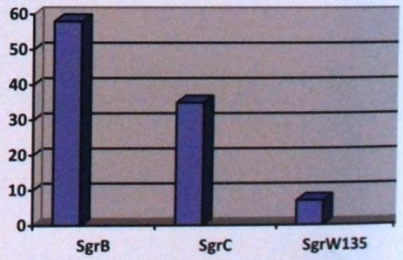
Aim

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

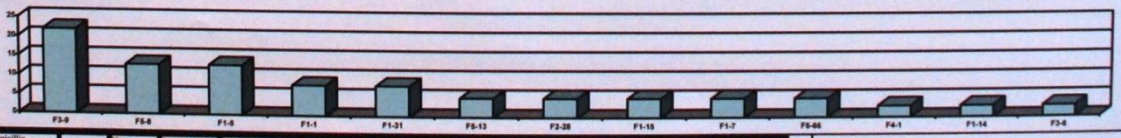
Methods:

39 *N.meningitidis* strains coming from CSF, were collected at the National Reference Laboratory from Cantacuzino Institute and analysed at the Inst.fur Hygiene & Mikobiologie, in Wurzburg and at Inst.fur Med.Mikrobiologie und Hygiene, in Graz.
The strains were tested by phenotypical and PCR/RT-PCR method for species identification, serogrouping was performed by agglutination with Remel sera,, and susceptibility to antibiotics (penicillin, ceftriaxone, rifampicin, ciprofloxacin) by E test. Por A was also performed for 39 strains and fet A for 37 strains.





Antigen sequence types (%)Fet-A



No	Serogr	Penicillin (mg/l)	No	Serogr	Penicillin (mg/l)	No	Serogr	Penicillin (mg/l)	No	Serogr	Penicillin (mg/l)
1	C	0.25	192	C	0.19	328	B	0.125	27	B	0.064
2	B	0.19	244	C	0.25	86	B	0.19	19	W135	0.047
3	C	0.25	429	B	0.064	148	B	0.19	26	B	0.125
5	C	0.125	118	C	0.25	117	B	0.25	136	B	0.125
9	C	0.25	164	C	0.064	9	B	0.25			
10	B	0.19 (I)	163	C	0.19	306	B	0.19			
11	B	0.25 (I)	185	B	0.5	321	B	0.125			
419	C	0.094	315	B	0.5	10	B	0.25			
157	C	0.064	29	B	0.25	159	C	0.047			
79	C	0.094	28	B	0.19	4	B	0.064			
121	C	0.19	348	W135	0.064	237	B	0.125			

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.