Swiss National Reference Center for Meningococci

> 2013 Annual Report <

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Introduction

Invasive strains of *Neisseria meningitidis* are a life-threatening cause of bacterial meningitis and sepsis, mainly in infants, adolescents and young adults. However, sporadic cases may occur in any age group and every effort must be undertaken to optimize the prevention, diagnosis and treatment of such infections.

In Switzerland, invasive meningococcal diseases must be reported to the Swiss Federal Office of Public Health (SFOPH), and corresponding isolates should be referred to the Swiss National Reference Center for Meningococci (CNM, Centre National des Méningocoques; http://www.meningo.ch) at the University Hospital in Geneva.

The CNM provides reference testing of invasive *N. meningitidis* isolates in collaboration with the SFOPH, and currently employs serotyping and molecular typing following protocols recommended by the European Meningococcal Disease Society (EMGM) (http://emgm.eu). Based on a combination of serogroup and

molecular typing data, each strain is classified and data are integrated in national (SFOPH) and international epidemiological databases (European Meningococcal Epidemiology in Real Time [EMERT] database; http://emgm.eu/emert) in order to monitor and share information about trends in meningococcal populations. In addition, each isolate is tested against a panel of generally recommended antibiotics against *N. meningitidis*. Antimicrobial susceptibility data are also used for surveillance purposes.

This annual report describes the methods used and results obtained at the CNM in calendar year 2013.

Materials and Methods

On behalf of the SFOPH, the CNM is investigating isolates of *N. meningitidis* as well as clinical specimens derived from normally sterile body sites.

Isolates are subcultured overnight on chocolate agar to determine their serogroup using fresh colonies and commercial agglutination kits. The initial test panel includes serogroups A, B, C, and Y/W135 (Pastorex Meningitis, Bio-Rad). Additional agglutination may include serogroups W135, X, Y, Z and Z' (Difco Neisseria Meningitidis Antisera, Becton Dickinson) and a confirmation of identification (apiNH, bioMérieux).

Multilocus sequence typing (MLST) is performed on each isolate according to protocols recommended by the EMGM (Harrison et al., 2011; http://emgm.eu). The current approach includes seven house keeping genes (*abcZ*, encoding a putative ABC transporter; *adk*, adenylate kinase; *aroE*, shikimate dehydrogenase; *fumC*, fumurate dehydrogenase; *gdh*, glucose-6-phosphate dehydrogenase; *pdhC*, pyruvate dehydrogenase subunit, and *pgm*, phophoglucomutase). Each isolate is classified according to its multilocus genotype designated as sequence type (ST), which is the combination of its alleles over the seven genetic loci tested (http://pubmlst.org/neisseria/). STs can be further grouped into clonal complexes

(CC), which are defined in the *Neisseria* MLST profile database as a group of STs that share at least four of the seven loci in common with a central ST.

In addition, molecular characterization includes sequencing of two variable regions in the gene encoding the antigenic outer membrane protein porin A (*porA*-VR1 and *porA*-VR2) and one variable region in the *fetA* gene (*fetA*-VR) encoding another antigenic outer membrane protein exhibiting sequence data which can be useful in tracing clones emerging or circulating in local populations (World Health Organization Manual – Laboratory Methods for the Diagnosis of Meningitis caused by *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* [2nd edition]; http://pubmlst.org/neisseria/).

Each isolate is then classified based on a combination of serotyping and molecular typing data according to the following scheme:

Serogroup : *porA*-VR1, *porA*-VR2 : *fetA*-VR : MLST (ST or CC)

Isolates are tested for antimicrobial susceptibility on Mueller-Hinton sheep blood agar using E-test strips (AB Biodisk, bioMérieux) containing penicillin, ceftriaxone, minocycline, rifampicin, azithromycin, ciprofloxacin, and chloramphenicol, respectively. Minimum inhibitory concentrations (MICs) are interpreted according to current breakpoints recommended by the EMGM (for penicillin) and the Clinical and Laboratory Standards Institute (for the remaining antimicrobial agents). Conventional susceptibility testing is complemented via sequencing of the *penA* gene in order to detect five characteristic mutations associated with an altered penicillin-binding protein (PBP2) which may confer reduced susceptibility to penicillin (Taha et al., 2007; http://pubmlst.org/neisseria/).

Clinical specimens are investigated using PCR to screen for *N. meningitidis* DNA, and if present, to assess the occurrence of the main serogroups by amplifying corresponding genetic targets.

Nucleic acids extraction from clinical specimens such as cerebrospinal fluid and blood is performed using the MagNAPure Compact 2.0 System (Roche Diagnostics).

DNA is amplified by real-time PCR to screen for the presence of the *N. meningitidis*-specific *ctrA* gene (Corless et al., 2001) and *sodC* gene (Dolan Thomas et al., 2011). PCR assays targeting the polysialyltransferase (*siaD*) gene are employed to assign *N. meningitidis*-positive specimens to serogroups B, C and Y/W135; assignment to serogroup A is achieved by PCR targeting the sacC gene (Molling et al., 2002). Finally, differentiation between serogroups Y and W135 is done by amplification of the *synF* gene (Y) and *synG* gene (W135) (Fraisier et al., 2009).

Results

During the calendar year 2013, the CNM has received a total of 42 invasive isolates of *N. meningitidis*. 40 strains were isolated from normally sterile body sites including blood (N=34) and cerebrospinal fluid (N=6); two strains were referred without further information.

The strains received were isolated from 23 male and 19 female patients and represented 80.8% of all cases of invasive meningococcal diseases (N=52) reported to Swiss public health authorities in 2013 (Figure 1).

15 cases were reported for the age group representing very young children aged 0-2 years. 10 cases were found in teenagers (10-19 years) and young adults (20-29 years) combined; the remaining cases occurred in patients up to 80+ years old, without accumulation in any particular age group.

Serotyping by agglutination revealed that most isolates belonged to serogroup B (N=23, equivalent to 54.8%), followed by serogroup Y (N=8, 19.0%), serogroup C (N=7, 16.7%) and serogroup W135 (N=3, 7.1%) (Figure 2). In addition, one single isolate was assigned to serogroup X (2.4%).

Serogroup B strains were predominant in children aged 0-2 years (92.3% of cases) but no predominance was observed for other serogroups in any other age group.

Serogroups B, W135 and Y were recovered with comparable frequencies in all areas of Switzerland, however, serogroup C strains were mainly isolated from cases in German speaking districts.

Molecular characterization using MLST revealed the occurrence of at least 11 different clonal complexes (CC) encompassing 23 different sequence types (ST). The most frequent complexes were CC11 (N=9 isolates), CC41/44 (N=8), CC23 (N=6), CC213 (N=5), and CC32 (N=4). CC11 and CC23 mainly correlated with serogroup C and Y agglutination, respectively. CC32, CC41/44 and CC213, and most other CCs and STs, were found in serogroup B strains (Table 1).

Sequencing of *porA* and *fetA* gene variable regions of serogroup C strains revealed a total of three different allele combinations as follows (*porA*-VR1, *porA*-VR2, *fetA*-VR): 5, 2, F3-3 (N=3 isolates); 5-1, 10-8, F3-6 (N=3); and 5-1, 10-46, F1-62 (N=1). Serogroup Y strains exhibited a total of six different allele combinations: 5-2, 10-1, F4-1 (N=3); 5-2, 10-2, F2-13 (N=1); 5-1, 10-1, F3-9 (N=1); 5-1, 10-4, F4-1 (N=1); 5-1, NA, F4-1 (N=1); and 21, 16, F3-7 (N=1). The diverse variety of alleles found in serogroup B strains is depicted in Figure 3 (*porA*-VR1), Figure 4 (*porA*-VR2) and Figure 5 (*fetA*-VR).

For susceptibility testing to penicillin, we applied the breakpoints recommended by the EMGM: susceptible, MIC $\leq$ 0.094 µg/ml; intermediate, MIC = 0.125 to 1 µg/ml; resistant, MIC > 1 µg/ml. Using these breakpoints, 73.8% of all isolates were found susceptible. All of the remaining isolates were intermediate; no isolate exhibited a MIC suggesting resistance to this substance (Table 2).
The molecular assessment of susceptibility to penicillin via *penA* gene analysis correlated well with the corresponding MICs observed. All isolates harbouring the characteristic mutations in their *penA* sequences also exhibited penicillin MICs > 0.094 µg/ml, however, four phenotypically intermediate but unrelated isolates (MIC = 0.125 µg/ml [N=3 isolates] and MIC = 0.25 µg/ml [N=1]) showed no such mutations (Table 2).

Using CLSI breakpoints, susceptibility testing to ceftriaxone, minocycline, rifampicin, ciprofloxacin, and chloramphenicol showed that all isolates were susceptible to these

agents. Reduced susceptibility to azithromycin was observed in 4.8% of isolates tested (Table 3).

In calendar year 2013, the CNM has received a total of 27 clinical specimens for direct detection of *N. meningitidis* DNA by PCR. These specimens consisted of cerebrospinal fluids (CSF) or DNA extracts thereof (N=23), EDTA blood samples (N=3), and one native skin biopsy specimen. We did not receive any concomitant isolate for any of these specimens except for one CSF aliquot which was submitted together with a blood culture-derived strain. No DNA extract showed PCR inhibition and *N. meningitidis* DNA was found in five cerebrospinal fluid samples; for each of these specimens both PCR assays (*ctrA* target and *sodC* target) were positive. Subsequent PCR to assess the serogroup revealed that three samples contained serogroup B and one sample serogroup C; the remaining sample could not be subjected to subsequent PCR typing due to the lack of material. The PCR-based serotyping result for the CSF aliquot correlated with the conventional agglutination of the concomitant isolate (serogroup B).

Summary of key observations

- Serogroup B represents about half of all cases and remains most prevalent followed by serogroups Y and C.
- Predominant MLST profiles are CC32, CC41/44 and CC213 in serogroup B, CC23 in serogroup Y, and CC11 in serogroup C.
- Susceptibility of *N. meningitidis* to antibiotics recommended for prophylaxis (rifampicin and ciprofloxacin) and treatment (ceftriaxone) remains 100%.

Discussion

In 2013, a total of 52 cases of invasive meningococcal diseases were reported to the SFOPH, and in 42 cases (80.8%) corresponding isolates were referred to the CNM for reference testing (Figure 1). Compared to 2012, there was an increase in cases, however, the total number remained below the annual average of about 60 cases reported in Switzerland during the past ten years.

As in previous years, serogroup B was determined in about half of the isolates, and most of the remaining strains exhibited serogroup C and Y, respectively (Figure 2). Interestingly, serogroup Y seemed to re-emerge while serogroup C appeared to further decrease. Nonetheless, the small number of isolates and short time period covered does not permit any reliable trend projection.

Molecular analysis of the serogroup Y strains by MLST and sequencing of *porA*-VR1, *porA*-VR2, and *fetA*-VR revealed six different lineages and no evidence for any particular clonal expansion. The number of strains harboring the previously noticed lineage Y:5-2,10-1:F4-1:ST-23 remained unchanged compared to last year. The absence of a predominant clone was also observed for the serogroup C strains which belonged to three different lineages. As expected, serogroup B strains were found to exhibit many different MLST profiles (Table 1) as well as very diverse *porA* and *fetA* sequence data (Figures 3-5). Overall, at least five different MLST clonal complexes, nine *porA*-VR1, twelve *porA*-VR2, and eleven *fetA*-VR allele types were observed.

The extensive molecular diversity among serogroup B strains is of particular interest in the context of the very recent introduction of the protein-based vaccine 4CMenB (Bexsero®, Novartis) in neighbouring European countries. This vaccine contains four subcapsular components of *N. meningitidis*, i.e. porin A (PorA, presented as part of an outer membrane vesicle), factor H-binding protein (fHbp), neisserial heparin-binding antigen (NHBA), and neisserial adhesin A (NadA). 4CMenB is projected to induce a protective immune response against serogroup B strains, however, its efficacy varies depending on the individual antigenic composition of a meningococcal clone or lineage (Vogel et al., 2012, 2013). Analysis of the prevalence and sequence variations of the genes encoding the antigens included in this novel vaccine is therefore recommended to complement serologic and other vaccine coverage assays (Bambini et al., 2013). In addition, ongoing molecular typing of *N. meningitidis* is essential to monitor the impact of 4CMenB on the circulation of serogroup B as well as non-B sub-populations. Finally, some nonpathogenic members of the genus *Neisseria* inhabiting the nasopharynx may also be affected directly by the vaccine or indirectly by a shift in neisserial populations since some of the antigens targeted are relatively conserved between *N. meningitidis* and other neisseriaceae (Lucidarme et al., 2013; Muzzi et al., 2013).

During the past year, susceptibility of *N. meningitidis* to penicillin remained relatively constant with about 25% of isolates exhibiting an intermediate phenotype which was usually associated by characteristic mutations in the *penA* gene; only four intermediate strains lacked these alterations and might resist to the tested drug through other means (Table 2).
All strains were fully susceptible to ceftriaxone, minocycline, rifampicin, ciprofloxacin, and chloramphenicol (Table 3). Only two isolates showed reduced susceptibility to azithromycin (MIC = 3 µg/ml). Our findings therefore support the prophylactic use of rifampicin or ciprofloxacin in post-exposure situations.

Conclusions and Perspectives

In 2013, serogroup B was determined in half of all *N. meningitidis* isolates referred, and no resistant MICs to currently recommended antibiotics for prophylaxis or treatment of invasive meningococcal diseases were observed.

In order to assess the meningococcal population in Switzerland in the context of the introduction of the novel vaccine 4CMenB targeting serogroup B, the CNM will collaborate with European reference partners.

The CNM will also implement additional molecular assays to complement antigenic characterization of selected serogroup B isolates, and develop a bioinformatic platform to gather, analyze and manage corresponding data.

References

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Figure 1. Annual number of cases of invasive meningococcal diseases reported to the Swiss Federal Office of Public Health and number of *N. meningitidis* strains referred to the Swiss National Reference Center for Meningococci from 2003 to 2013.

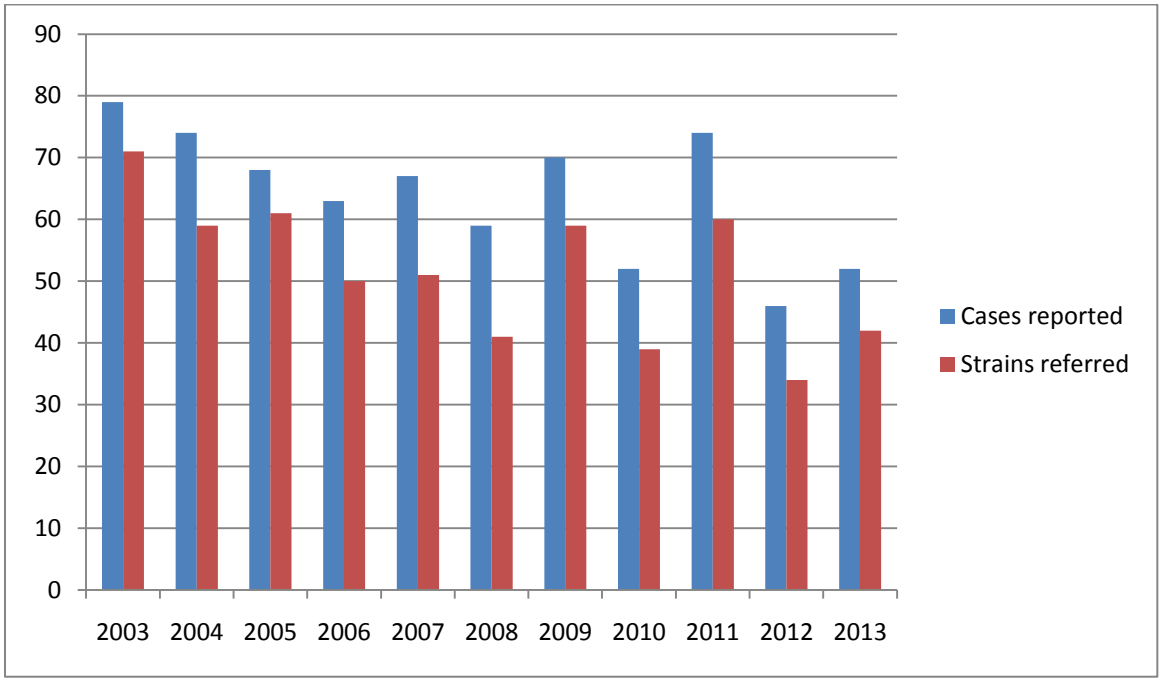

Figure 2. Annual number of strains representing main serogroups B, C, Y and W135 of *N. meningitidis* as determined by agglutination at the Swiss National Reference Center for Meningococci from 2003 to 2013.

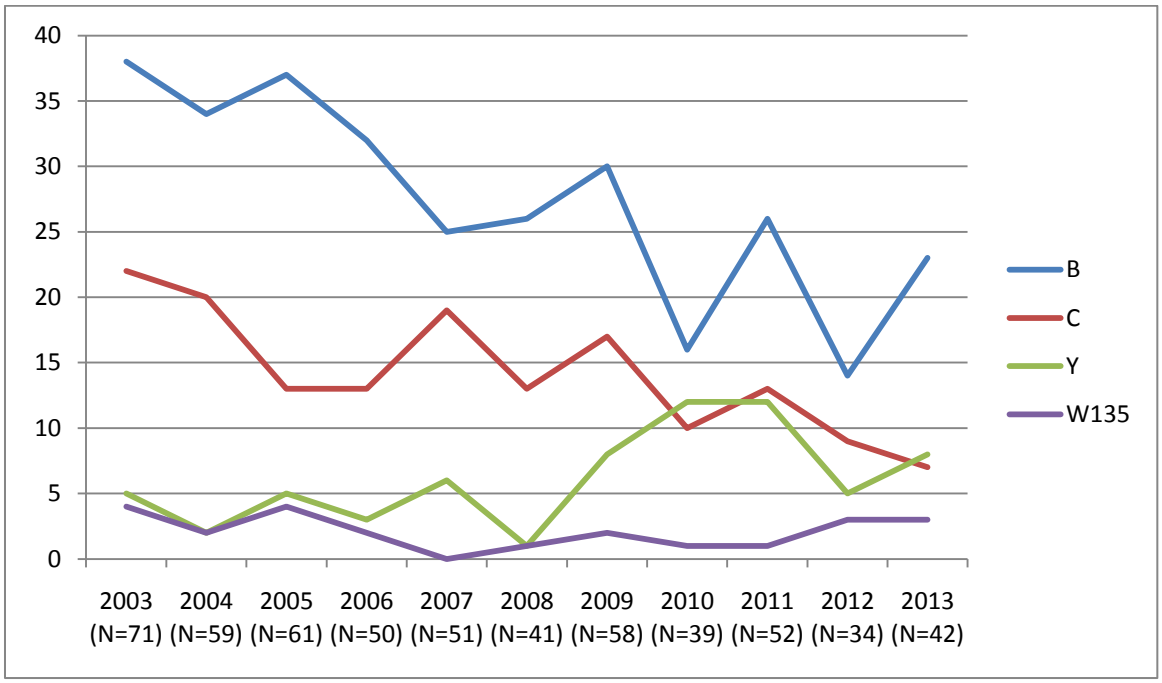

Figure 3. Distribution of *porA*-VR1 alleles in serogroup B isolates of *N. meningitidis* referred to the Swiss National Reference Center for Meningococci in 2013.

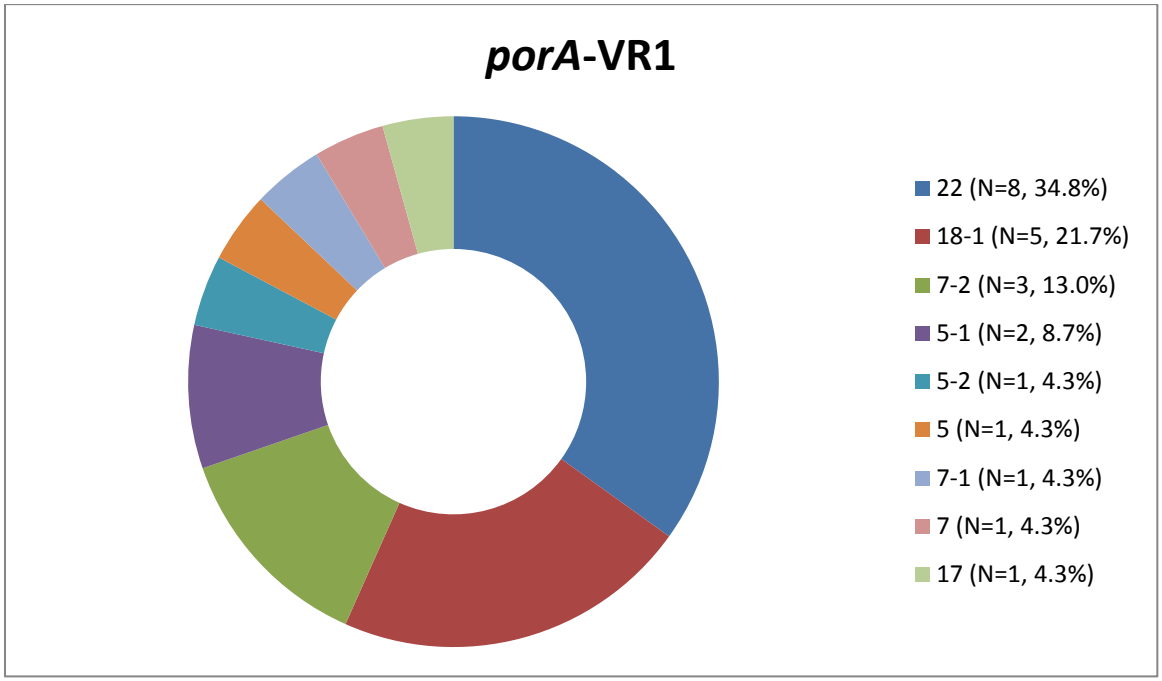

Figure 4. Distribution of *porA*-VR2 alleles in serogroup B isolates of *N. meningitidis* referred to the Swiss National Reference Center for Meningococci in 2013.

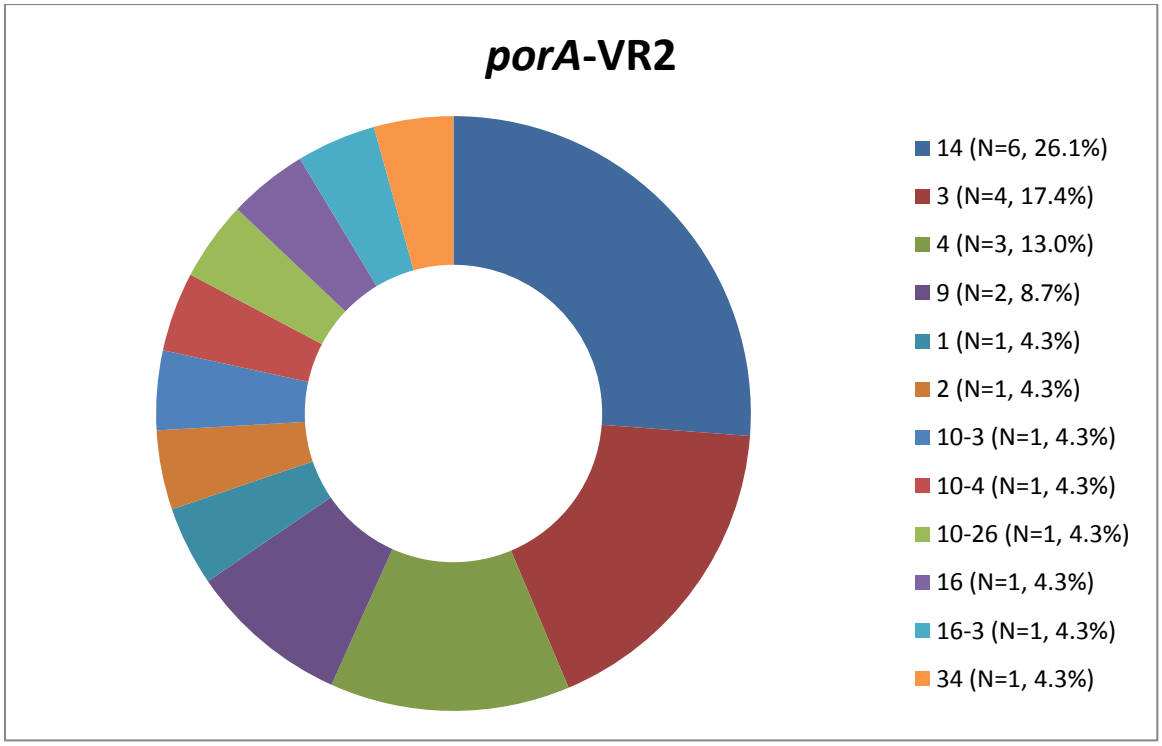

Figure 5. Distribution of *fetA*-VR alleles in serogroup B isolates of *N. meningitidis* referred to the Swiss National Reference Center for Meningococci in 2013.

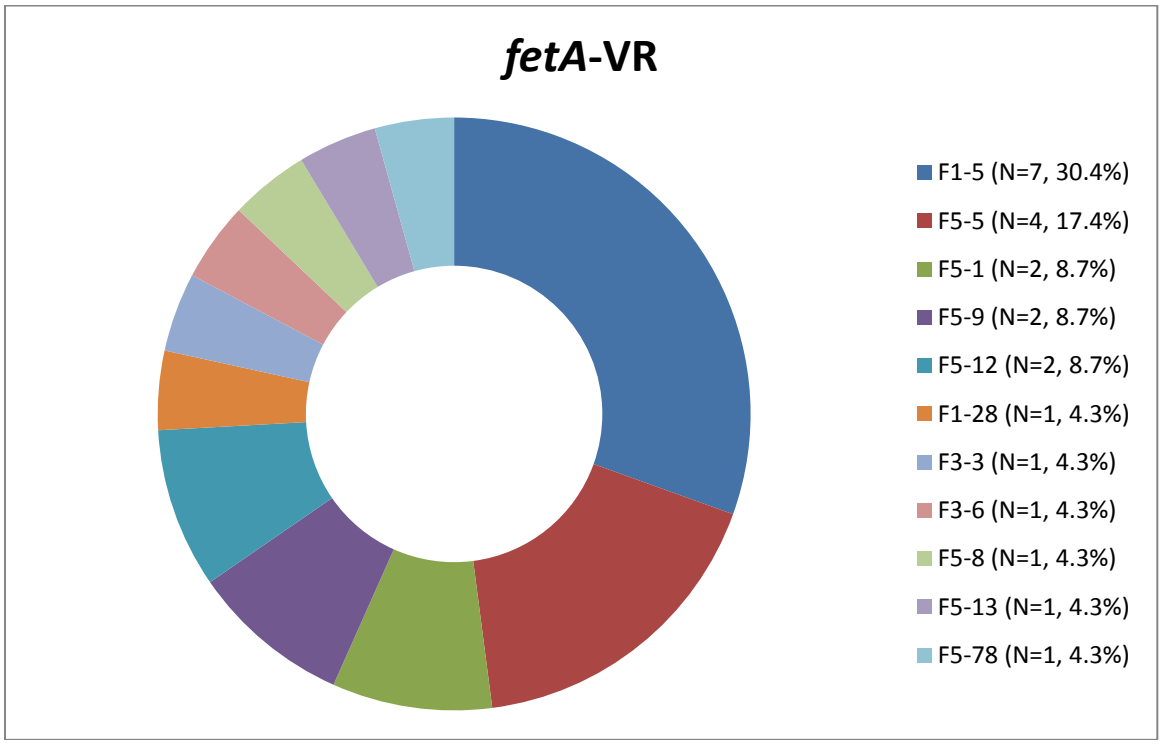

Table 1. MLST clonal complexes and sequence types of *N. meningitidis* strains referred to the Swiss National Reference Center for Meningococci in 2013.

MLST[a]		Serogroup (number of isolates)			
Clonal complex designation	Sequence type designation	B	C	Y	Other [serogroup]
11	11		4		1 [W135]
11	5752		1		
11	9927		1		
11	NA		1		1 [W135]
22	184				1 [W135]
23	23			3	
23	1655			1	
23	NA			2	
32	34	1			
32	1130	1			
32	5782	1			
32	7460	1			
41/44	41	1			
41/44	280	2			
41/44	1194	1			
41/44	1403	1			
41/44	NA	3			
103	103			1	
162	162	1			
174	1466			1	
213	213	3			
213	6765	1			
213	9812	1			
269	1161	1			
269	1163	1			
1157	1157				1 [X]
NA	NA	3			

[a]NA, no assignment available

Table 2. Correlation between susceptibility to penicillin and occurrence of characteristic mutations in the *penA* gene in strains of *N. meningitidis* referred to the Swiss National Reference Center for Meningococci in 2013.

	Minimum inhibitory concentration of penicillin (µg / ml)							
	0.032	0.047	0.064[a]	0.094[b]	0.125	0.19	0.25	0.38
Category[c]	S	S	S	S	I	I	I	I
Total number of strains	2	4	13	12	4	2	3	2
Number of strains with characteristic *penA* mutations	0	0	0	0	1	2	2	2

[a]Breakpoint of the Clinical and Laboratory Standards Institute (CLSI)
[b]Breakpoint of the European Meningococcal Disease Society (EMGM)
[c]Interpretation according EMGM: S, susceptible; I, intermediate.

Table 3. Inhibitory activity of selected antimicrobial agents on 42 *N. meningitidis* strains referred to the Swiss National Reference Center for Meningococci in 2013.

Antimicrobial agent	Minimum inhibitory concentration (µg/ml)			Breakpoint susceptible (≤ µg/ml)	% of strains considered susceptible
	Range	50%	90%		
Penicillin	0.032 – 0.38	0.094	0.25	0.094[a]	73.8
Ceftriaxone	<0.002 – 0.008	0.003	0.006	0.12[b]	100
Minocycline	0.094 – 0.5	0.19	0.38	2[b]	100
Rifampicin	0.002 – 0.25	0.016	0.064	0.5[b]	100
Azithromycin	0.032 – 3	0.38	1	2[b]	95.2
Ciprofloxacin	0.003 – 0.012	0.006	0.012	0.03[b]	100
Chloramphenicol	0.38 – 1.5	0.75	1	2[b]	100

[a]Breakpoint of the European Meningococcal Disease Society (EMGM)
[b]Breakpoint of the Clinical and Laboratory Standards Institute (CLSI)