Swiss National Reference Center for Meningococci

> 2014 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 subjected to antimicrobial susceptibility testing for surveillance purposes.

This annual report describes the methods used and results obtained at the CNM in calendar year 2014.

Materials and Methods

On behalf of the SFOPH, the CNM is investigating isolates of *N. meningitidis* as well as native 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).

Sequence analysis is performed on each isolate on two variable regions in the gene encoding the antigenic outer membrane protein porin A (*porA*-VR1 and *porA*-VR2) and on 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/).

In addition, multilocus sequence typing (MLST) is done on each isolate according to protocols recommended by the EMGM (Harrison et al., 2011; http://emgm.eu). This approach is targeting variable regions of 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. 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 (http://pubmlst.org/neisseria/).

Isolates are 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 agar supplemented with horse blood (X-factor) and NAD (V-factor) (bioMérieux) using E-test strips (AB Biodisk, bioMérieux) containing azithromycin, ceftriaxone, ciprofloxacin, chloramphenicol, meropenem, minocycline, penicillin, and rifampicin, respectively. Minimum inhibitory concentrations (MICs) are interpreted according to current breakpoints recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, www.eucast.org) except for azithromycin and penicillin which are interpreted according to breakpoints proposed by the Clinical and Laboratory Standards Institute (CLSI) and the EMGM, respectively.

Native 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 acid extraction from clinical specimens such as cerebrospinal fluid and EDTA anticoagulated 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 2014, the CNM has received a total of 35 invasive isolates of *N. meningitidis*. These strains were isolated from blood specimens (N=25), cerebrospinal fluids (N=7), a joint fluid (N=1), a middle ear aspirate (N=1), and a tubo-ovarian abscess (N=1).

The strains received were isolated from 17 female and 18 male patients and represented 94.6% of all cases of invasive meningococcal diseases (N=37) reported to Swiss public health authorities in 2014 (Figure 1).

2 strains were recovered from the age group representing very young children aged 0-2 years. 15 strains were isolated from teenagers (10-19 years) and young adults (20-29 years) combined; the remaining strains were from patients up to 80+ years old.

Serotyping by agglutination revealed that most isolates belonged to serogroup B (N=13, equivalent to 37.1%), followed by serogroup C (N=10, 28.6%), serogroup Y (N=6, 17.1%), and serogroup W (N=6, 17.1%) (Figure 2).

Serogroup B strains were predominant (40% of cases) in teenagers (10-19 years) and young adults (20-29 years) combined but no predominance was observed for a particular serogroup in any other age group.

All main serogroups (B, C, Y and W) were recovered in the German speaking (central, northern and eastern) areas of Switzerland, however, serogroup W was not

detected in the French speaking part (western areas), and no serogroups B and Y were found in the Italian speaking part (southern areas).

Sequencing of *porA* and *fetA* gene variable regions of serogroup C strains revealed a total of five different allele combinations as follows (*porA*-VR1, *porA*-VR2, *fetA*-VR): 5, 2, F3-3 (N=6 isolates); 5-1, 10-8, F3-6 (N=1); 7-1, 1, F5-5 (N=1); 18-1, 3, F3-6 (N=1); and 19, 15-1, F1-50 (N=1). Serogroup Y strains exhibited a total of four different allele combinations: 5-2, 10-1, F4-1 (N=3); 5, 2, F5-8 (N=1); 18-1, 3, F3-4 (N=1); and 18-1, 3, F4-1 (N=1). Serogroup W strains exhibited two different allele combinations: 5, 2, F1-1 (N=5); and 1-5, 2-2, F1-1 (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).

Molecular characterization using MLST revealed the occurrence of at least 9 different clonal complexes (CC) encompassing at least 16 different sequence types. The most frequent complexes were CC11 (N=8 isolates), CC41/44 (N=5), CC23 (N=3), CC32 (N=3), and CC213 (N=3). CC11 and CC23 mainly correlated with serogroups C and W, and serogroup Y agglutination, respectively. CC32, CC41/44 and CC213, and most other CCs, were mainly found in serogroup B strains (Table 1).

Using CLSI and EUCAST breakpoints, susceptibility testing to azithromycin, ceftriaxone, chloramphenicol, ciprofloxacin, meropenem, minocycline, and rifampicin showed that all isolates were susceptible to these agents (Table 2). 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, 60% 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).

A total of 28 clinical specimens were processed for direct detection of *N. meningitidis* DNA by PCR. These specimens consisted of cerebrospinal fluids (CSF) or DNA extracts thereof (N=21), blood samples (N=4), two joint fluids, and one skin vesicle (blister) aspirate. We received a concomitant isolate from blood for two CSF specimens. No DNA extract showed PCR inhibition and *N. meningitidis* DNA was found in two CSF samples, two joint fluids , and two blood specimens; for each of

these specimens both PCR assays (*ctrA* target and *sodC* target) were positive. Subsequent PCR to assess the serogroup revealed that two samples contained serogroup B, one sample serogroup C, and one sample serogroup Y; the remaining samples could not be subjected to subsequent PCR typing due to the lack of material. As for the two CSF specimens with a concomitant isolate, the PCR-based serotyping result for one CSF correlated with the conventional agglutination of the isolate (serogroup Y), however, for the other sample pair the CSF was PCR-negative.

Summary of key observations

- Serogroup B is determined in about 40% of all cases and remains most prevalent followed by serogroups C, Y and W.
- Predominant MLST profiles are CC32, CC41/44 and CC213 in serogroup B, CC11 in serogroups C and W, and CC23 in serogroup Y.
- Susceptibility of *N. meningitidis* to antibiotics recommended for prophylaxis (rifampicin and ciprofloxacin) and treatment (ceftriaxone) remains 100%.

Discussion

In 2014, a total of 37 cases of invasive meningococcal diseases were reported to the SFOPH, and in 35 cases (94.6%) corresponding isolates were referred to the CNM for reference testing (Figure 1). Compared to 2013, there was a decrease (-27%) in cases and the total number remained well below the annual average of about 60 cases reported in Switzerland during the previous ten years.

In almost 40% of cases, serogroup B was determined, and the other serogroups present (C, Y and W) remained less frequent as in previous years (Figure 2). Among these, however, an increase of serogroups C and W, and a decrease of serogroup Y was observed. Compared to the previous years, serogroup W which is rare in Switzerland seemed to steadily increase, but it remains to be seen whether this trend will continue in the coming years.

Molecular typing of the six serogroup W strains by sequencing of *porA*-VR1, *porA*-VR2, *fetA*-VR and MLST revealed two different lineages with W:5,2:F1-1:ST11 being the predominant type (five strains). This type was isolated in northern, central as well as southern areas of Switzerland. Nonetheless, the rather small number of serogroup W strains does not allow for a reliable conclusion as per a particular clonal expansion.

Molecular typing data of the strains belonging to the serogroups C, Y, and B, in combination with residential information of patients, did not reveal any clonal trends in these meningococcal subpopulation referred to the CNM in 2014. A total of seven and four different lineages were observed among the serogroup C and Y strains, respectively. Most isolates belonging to serogroup B exhibited an individual lineage as summarized in Table 1 (MLST profiles) and Figures 3 to 5 (*porA*-VR1, *porA*-VR2, and *fetA*-VR alleles).

The introduction of the novel anti-meningococcal serogroup B vaccine 4CMenB (Bexsero, Novartis) in Europe, and in Switzerland in the foreseeable future, may impact the evolution of serogroup B also as well as non-B populations as has been reported for a large cohort of students (Read et al., 2014). In this study, a phase 3 randomised clinical trial involving almost 3’000 participants, it has been found that 4CMenB significantly lowers carriage of any meningococcal strain, irrespective of detectable production of the capsule. This effect beyond serogroup B strains is expected and explained by the meningococcal protein targets (PorA, porin A; fHbp, factor H-binding protein; NhbA, neisserial heparin-binding antigen; NadA, neisserial adhesin A) of the 4CMenB vaccine which is not directed against particular capsular polysaccharides.

In order to assess and monitor the impact of the 4CMenB vaccine on meningococci isolated in Switzerland, in 2014 the CNM has expanded its panel for molecular typing of serogroup B strains and upgraded the bioinformatic platform to integrate data from MLST, *porA*, *fetA*, *fHbp, nhba,* and *nadA* sequence analysis (Bambini et al., 2013). Further, the CNM has initiated a collaboration with the French National Reference Center for Meningococci to investigate the potential coverage of the 4CMenB vaccine on ‘Swiss’ meningococci through MATS testing (ELISA-based meningococcal antigen typing system) (Vogel et al., 2012, 2013).

In 2014, the CNM added meropenem to its antimicrobial susceptibility test panel for *N. meningitidis*, and applied EUCAST breakpoints whenever available and recommended (Table 2). All strains were found susceptible to azithromycin, ceftriaxone, chloramphenicol, ciprofloxacin, meropenem, minocycline, and rifampicin. Only 60% of the strains exhibited susceptibility to penicillin compared to 73.8% in the previous year, however, this apparent reduction might be a bias due to the relatively small study population.

Conclusions and Perspectives

In 2014, the CNM did not observe any particular clonal expansion of *N. meningitidis*, and susceptibility of meningococci to antibiotics recommended for prophylaxis (rifampicin and ciprofloxacin) and treatment (ceftriaxone) remained 100%.

The CNM will collaborate with the French National Reference Center for Meningococci at the Institut Pasteur in Paris to characterize 'Swiss' *N. meningitidis* serogroup B isolates using the MATS approach, and complement this information with corresponding antigen sequence data obtained at the CNM.

Acknowledgements

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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 2004 to 2014.

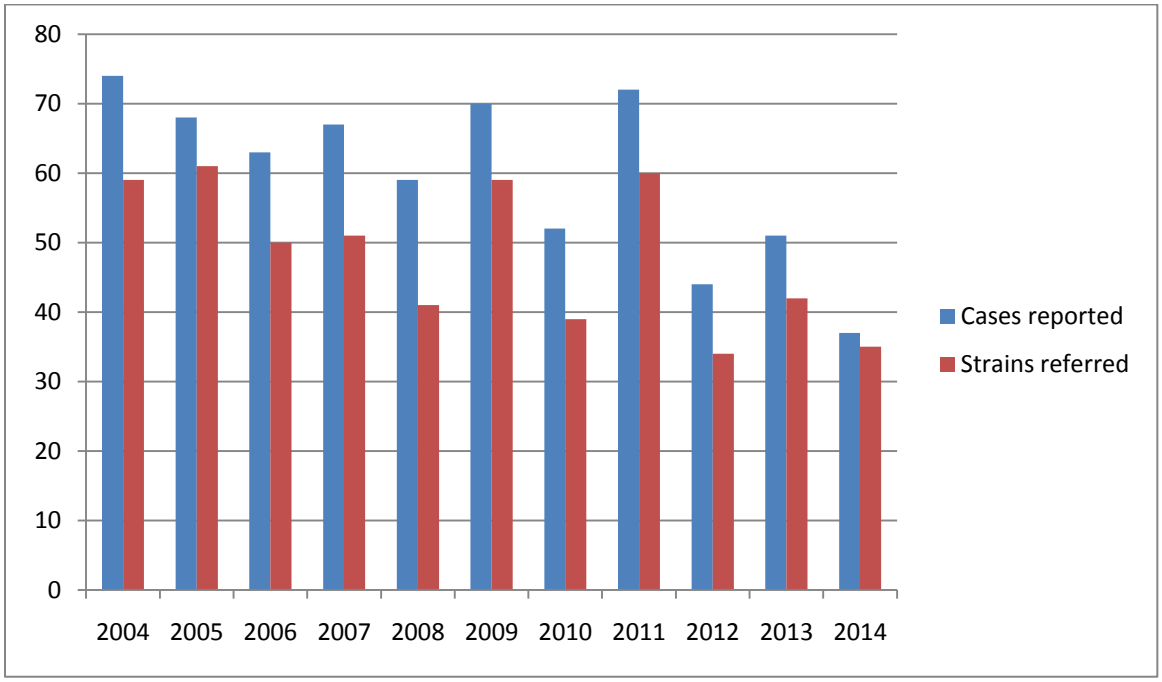

Figure 2. Annual number of strains representing main serogroups B, C, Y and W of *N. meningitidis* as determined by agglutination at the Swiss National Reference Center for Meningococci from 2004 to 2014.

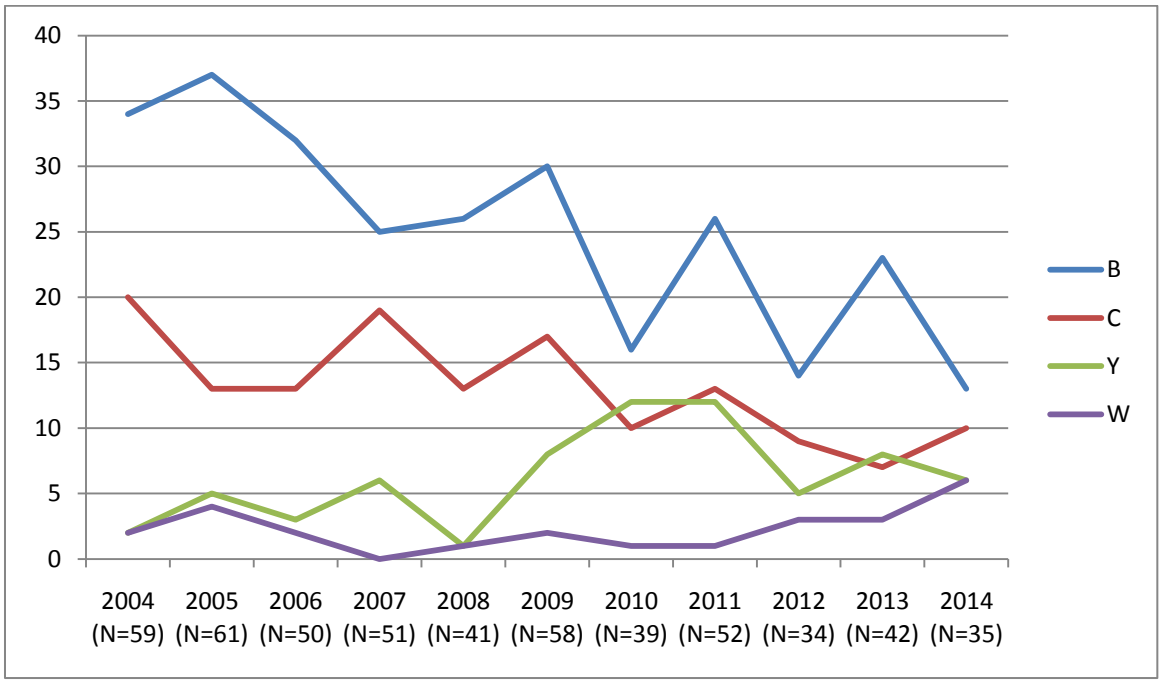

Figure 3. Distribution of *porA*-VR1 alleles in serogroup B isolates of *N. meningitidis* referred to the Swiss National Reference Center for Meningococci in 2014.

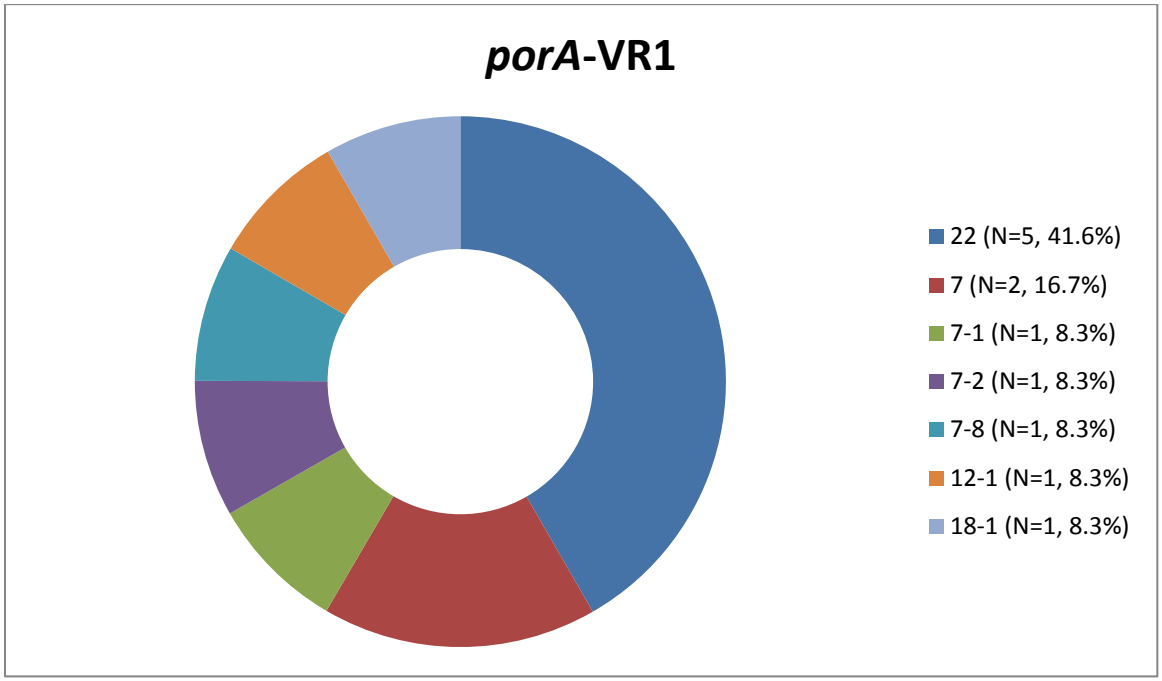

Figure 4. Distribution of *porA*-VR2 alleles in serogroup B isolates of *N. meningitidis* referred to the Swiss National Reference Center for Meningococci in 2014.

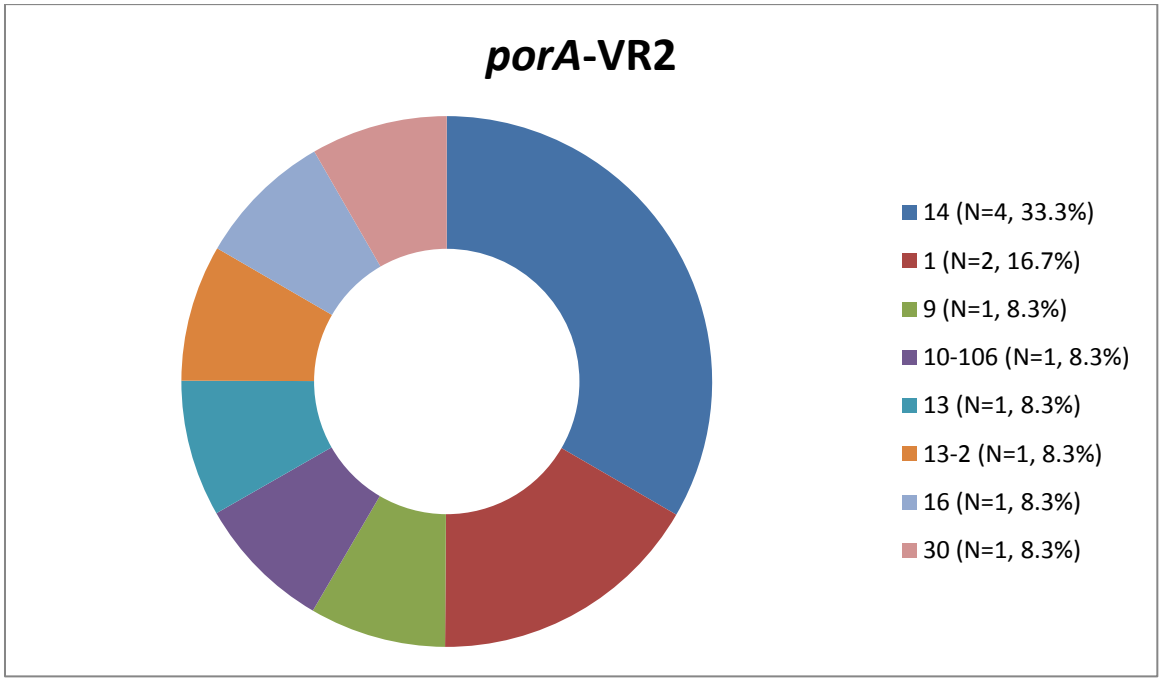

Figure 5. Distribution of *fetA*-VR alleles in serogroup B isolates of *N. meningitidis* referred to the Swiss National Reference Center for Meningococci in 2014.

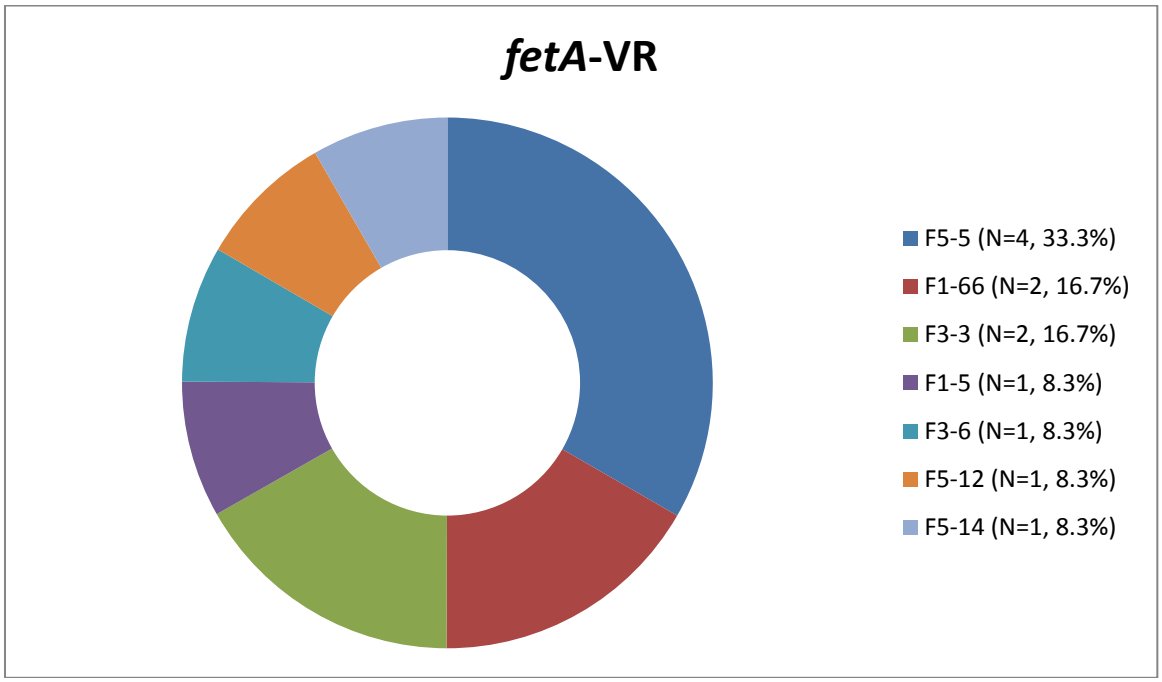

Table 1. Synopsis of MLST profiles and serogroups of *N. meningitidis* strains referred to the Swiss National Reference Center for Meningococci in 2014.

MLST Profile[a]		Serogroup (number of isolates)			
Clonal complex	Sequence type	B	C	Y	Other [serogroup]
11	11		3		5 [W]
11	5752		3		
11	10054		1		
11	NA		1		1 [W]
22	184			1	
23	23			1	
23	NA			2	
32	32	1			
32	2816	1			
32	NA	1			
41/44	7034	1			
41/44	10407		1		
41/44	NA	2	1		
60	60			1	
198	9196	1			
213	213	1			
213	7258	1			
213	NA	1			
269	1161	1			
269	NA	1			
NA	1111	1			
NA	5436			1	

[a]MLST, multilocus sequence typing; NA, no assignment available

Table 2. Inhibitory activity of selected antimicrobial agents on 35 *N. meningitidis* strains referred to the Swiss National Reference Center for Meningococci in 2014.

Antimicrobial agent	Minimum inhibitory concentration (µg/ml)			Breakpoint susceptible (≤ µg/ml)	% of strains considered susceptible
	Range	50%	90%		
Azithromycin	0.125-2	0.38	1.5	2[a]	100
Ceftriaxone	<0.002-0.012	0.003	0.008	0.12[b]	100
Chloramphenicol	0.5-2	0.75	1.5	2[b]	100
Ciprofloxacin	0.004-0.023	0.008	0.012	0.03[b]	100
Meropenem	0.004-0.094	0.012	0.032	0.25[b]	100
Minocycline	0.032-1	0.25	0.5	1[b]	100
Penicillin	0.032-0.38	0.094	0.19	0.094[c]	60
Rifampicin	0.002-0.25	0.023	0.094	0.25[b]	100

[a]Clinical and Laboratory Standards Institute (CLSI)
[b]European Committee on Antimicrobial Susceptibility Testing (EUCAST)
[c]European Meningococcal Disease Society (EMGM)