Swiss National Reference Center for Meningococci

> 2015 Annual Report <

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Introduction

Invasive strains of *Neisseria meningitidis* are a life-threatening cause of bacterial sepsis and meningitis, 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 2015.

Materials and Methods

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). Confirmation of identification is done by biochemical profiling (apiNH, bioMérieux) and PCR of the *N. meningitidis*-specific targets *ctrA* (Corless et al., 2001) and *sodC* (Dolan Thomas et al., 2011).

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 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. Azithromycin MICs are interpreted according to breakpoints proposed by the Clinical and Laboratory Standards Institute (CLSI). 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.

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 2015, the CNM has received a total of 40 invasive isolates of *N. meningitidis*. These strains were isolated from blood specimens (N=30) and cerebrospinal fluids (N=10).

The strains received were isolated from 21 female and 19 male patients and represented 100% of all cases of invasive meningococcal diseases (N=40) reported to Swiss public health authorities in 2015 (Figure 1).

Five strains were recovered from the age group representing very young children aged 0-2 years, and five strains were isolated from toddlers and young children (3-9 years). Ten strains were isolated from teenagers (10-19 years) and young adults (20-29 years) combined; the remaining strains (N=20, 50.0%) were from patients up to 90+ years old.

Serotyping by agglutination revealed that the majority of isolates belonged either to serogroup B (N=14, equivalent to 35.0%) or serogroup W (N=14, 35.0%). The remaining isolates were positive for serogroup Y (N=7, 17.5%), serogroup C (N=4, 10.0%), and serogroup X (N=1, 2.5%) (Figure 2).

Serogroup B and W strains were found predominantly in patients aged 0-29 years old whereas no particular serogroup(s) prevailed 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 Y was not detected in the French speaking part (western areas). No serogroups B, W and C were isolated in the Italian speaking part (southern areas) which in 2015 reported only one single case of invasive meningococcal disease due to a serogroup Y strain.

The diverse variety of *porA* and *fetA* alleles found in serogroup B strains is depicted in Figure 3 (*porA*-VR1), Figure 4 (*porA*-VR2) and Figure 5 (*fetA*-VR). Serogroup W strains revealed a total of two different allele combinations as follows (*porA*-VR1, *porA*-VR2, *fetA*-VR): 5, 2, F1-1 (N=13 isolates) and 18-1, 3, F5-5 (N=1). Serogroup Y strains exhibited a total of four different allele combinations: 5-2, 10-1, F4-1 (N=4); 5-1, 10-4, F3-6 (N=1); 5-1, 2-2, F5-8 (N=1); and 18-1, 3, F3-47 (N=1). Serogroup C strains showed three different allele combinations: 5, 2, F3-3 (N=2); 5-1, 10-8, F3-6 (N=1); and 16-11, F1-5 (N=1).

Molecular characterization using MLST revealed the occurrence of at least 8 different clonal complexes (CC) encompassing at least 18 different sequence types. The most frequent complexes were CC11 (N=16 isolates), CC41/44 (N=6), CC23 (N=4), CC213 (N=4), and CC269 (N=4). CC11 mainly correlated with serogroup C and W isolates, CC23 with serogroup Y, and CC41/44, CC213, and CC269 with serogroup B (Table 1).

Applying EUCAST and CLSI breakpoints, all invasive *N. meningitidis* strains tested were found to be susceptible to azithromycin, ceftriaxone, chloramphenicol, ciprofloxacin, meropenem, minocycline, and rifampicin. However, only 70% of these isolates were considered susceptible to penicillin using EMGM criteria (Table 2).

A total of 27 uncultured 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=22), EDTA-anticoagulated blood samples (N=4), and one pericardial aspiration. We received a concomitant meningococcal isolate for two CSF specimens. No DNA extract showed PCR inhibition and *N. meningitidis* DNA was found in four CSF samples, one blood sample, and the pericardial aspiration; for each of these specimens both PCR assays (*ctrA* target and *sodC* target) were

positive. Subsequent PCR to assess the serogroup by molecular means revealed that four samples contained serogroup B and one sample serogroup W; the remaining sample could not be subjected to subsequent PCR serotyping due to the lack of material. As for the two CSF specimens with a concomitant isolate, the PCR-based serotyping results correlated with the conventional agglutination of the isolates (one case was positive for serogroup B, the other case positive for serogroup W).

Summary of key observations

- Serogroups B and W were determined each in 35% of all cases; the remaining cases were associated with serogroups C, Y, and X.
- Predominant MLST profiles were CC41/44, CC213 and CC269 in serogroup B isolates, 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) remained 100%.

Discussion

In 2015, a total of 40 cases of invasive meningococcal diseases were reported to the SFOPH, and for all of these cases corresponding isolates were referred to the CNM for reference testing (Figure 1). Compared to 2014, there was a slight increase (+8%) in cases but the total number still remained well below the annual average of about 60 cases reported in Switzerland during the previous ten years.

Serogroup B strains were isolated in one third (35%) of cases and remained most prevalent compared to all other capsular groups with the notable exception of serogroup W. This group was determined in another third of cases and continued to follow an expanding trend which started around 2012 to 2013 (Figure 2). The rate of serogroup W among invasive *N. meningitidis* strains referred to the CNM increased from less than 5% per year (period 2005-2011) to 9% in 2012, 7% in 2013, 17% in 2014, and 35% in 2015.

Molecular typing of the serogroup W strains isolated in 2015 by sequencing of *porA*-VR1, *porA*-VR2, *fetA*-VR and MLST revealed the predominance of the finetype 5,2:F1-1:ST-11 (thirteen out of fourteen strains). The same finetype was already prevalent among serogroup W strains in the previous year (2014 Annual Report). In Switzerland, it was first detected in 2012, and corresponding invasive strains have since been isolated in all parts of the country (central, northern, eastern, southern and western areas). During the same period, no such expansion has been observed for any other meningococcal subpopulation.

A comparable upsurge of invasive serogroup W strains has recently been described for England and Wales (Ladhani et al., 2015) where the incidence of such isolates has risen from historically low 1-2% per year (pre-2000) to 15% in 2014, and 24% in 2015. In addition, most of the more recent serogroup W isolates from England and Wales exhibited the same finetype (5,2:F1-1:ST-11) like the one currently prevailing among Swiss serogroup W strains. However, the routine finetyping scheme proposed by the EMGM does not allow to assess clonal relatedness between ST-11 complex strains. Therefore, in order to detect and compare individual clonal lineages, whole genome sequence analysis is used increasingly for meningococcal typing. The genomic relatedness of current and historical invasive serogroup W strains (including isolates from England and Wales) has been investigated by two scientific teams (Lucidarme et al., 2015; Mustapha et al., 2015). It was concluded that the recent serogroup W expansion in England and Wales was due to a lineage closely related to, but distinct from, the serogroup W, ST-11 “Hajj outbreak” lineage which caused an epidemic among Hajj pilgrims and contacts in 2000.

Invasive meningococcal disease by serogroup W strains is potentially preventable through vaccination with the quadrivalent MenACWY (capsular antigen) conjugate vaccine. Additional protective immunity against serogroup W may be induced by the novel protein-based multicomponent vaccine Bexsero (4CmenB; GSK Vaccines). This vaccine was initially developed to induce immunity against serogroup B strains, however, its non-capsular antigen composition (NHBA, neisserial heparin binding antigen; NadA, *Neisseria* adhesin A; fHbp, factor H-binding protein; meningococcal outer membrane vesicles from an outbreak-related serogroup B strain) may also lead to immunity against non-B serogroups (Read et al., 2014). Regarding serogroup W, a

recent study demonstrated potent serum bactericidal anti-W antibody activity in serum samples from infants vaccinated with Bexsero (Ladhani et al., 2016).

Conclusions and Perspectives

In 2015, the CNM observed an expansion of *N. meningitidis* serogroup W strains (finetype 5,2:F1-1:ST-11), and susceptibility of invasive meningococci isolated in Switzerland to antibiotics recommended for prophylaxis (rifampicin and ciprofloxacin) and treatment (ceftriaxone) remained 100%.

The CNM has started a scientific collaboration with the group of Dr. Taha (Unité des infections bactériennes invasives) at the Institut Pasteur, Paris, France, with the aim to assess the potential coverage of Bexsero on invasive meningococci isolated in Switzerland. A collection of about 100 serogroup B strains collected from 2010 to 2015 is currently being investigated through MATS testing (ELISA-based meningococcal antigen typing system) (Vogel et al., 2012, 2013) and whole genome sequencing.

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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 2005 to 2015.

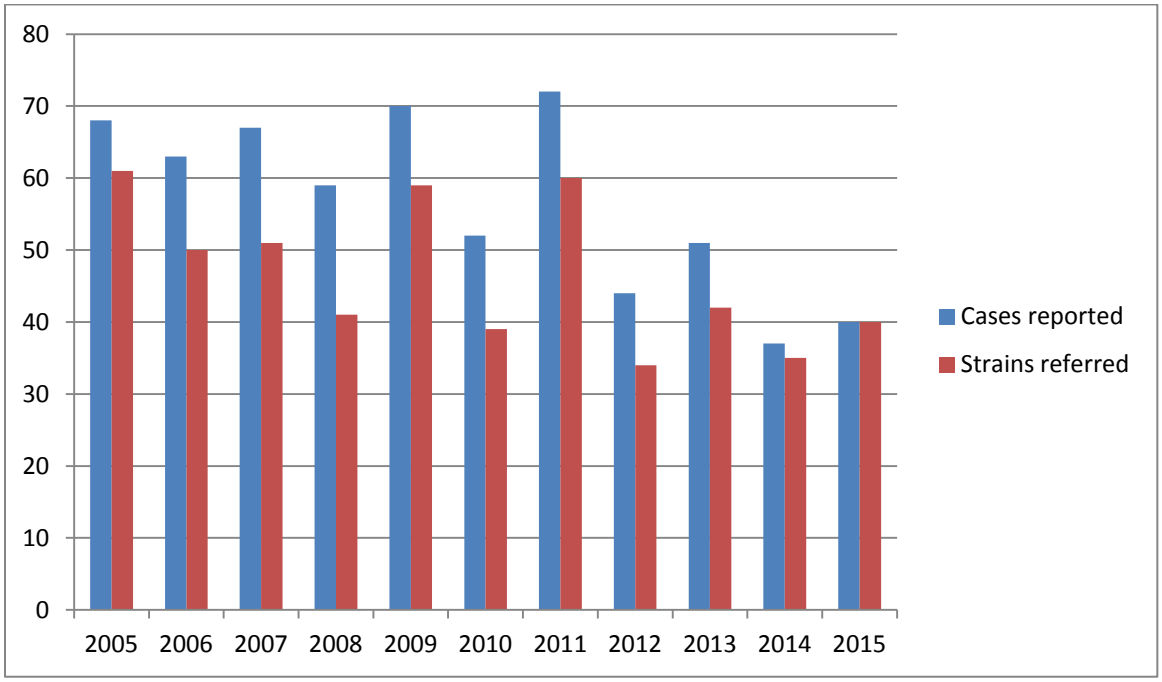

Figure 2. Annual number of strains representing main serogroups B, C, Y and W of invasive *N. meningitidis* as determined by agglutination at the Swiss National Reference Center for Meningococci from 2005 to 2015.

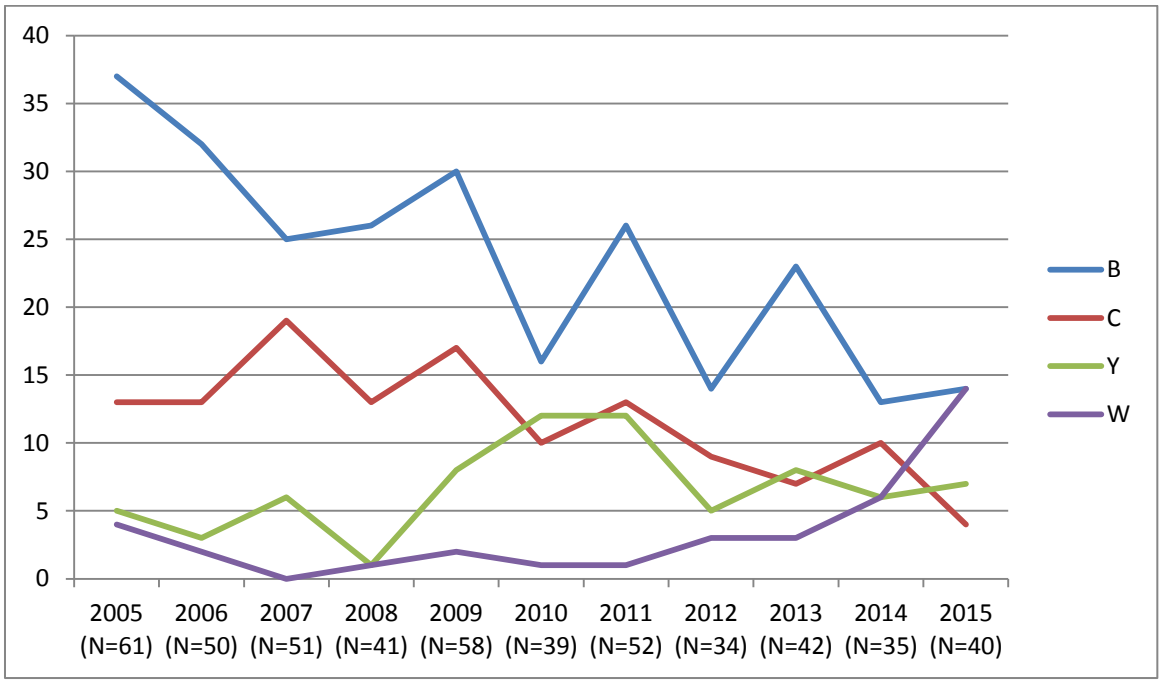

Figure 3. Distribution of *porA*-VR1 alleles in serogroup B isolates of *N. meningitidis* referred to the Swiss National Reference Center for Meningococci in 2015.

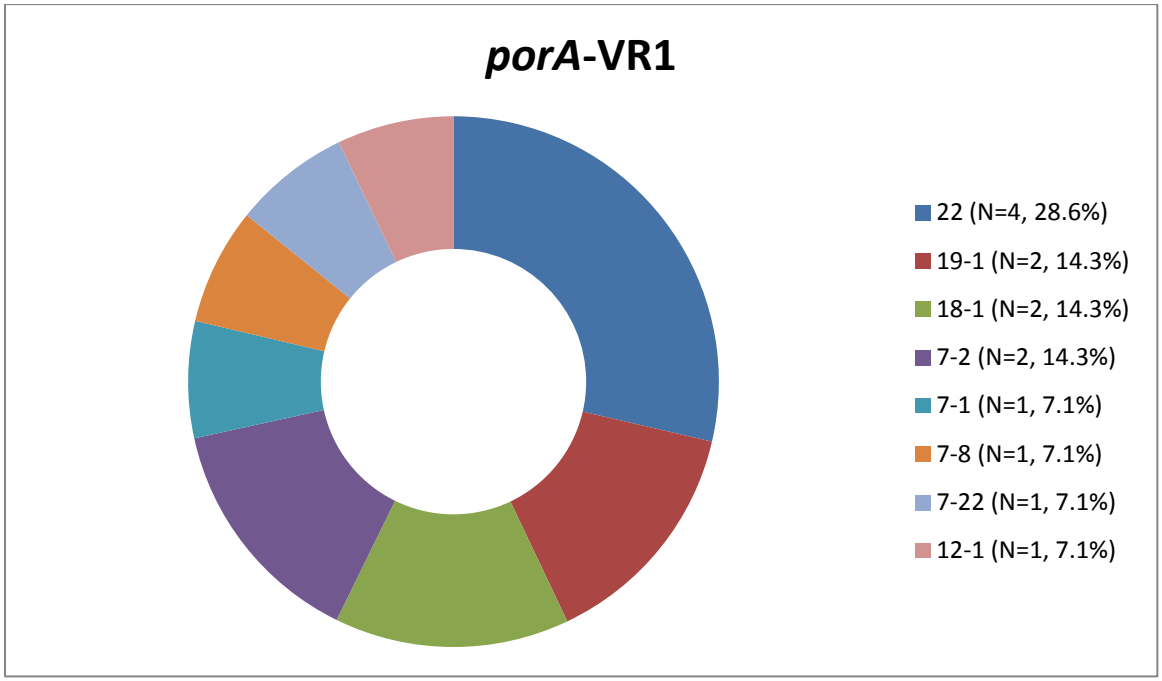

Figure 4. Distribution of *porA*-VR2 alleles in serogroup B isolates of *N. meningitidis* referred to the Swiss National Reference Center for Meningococci in 2015.

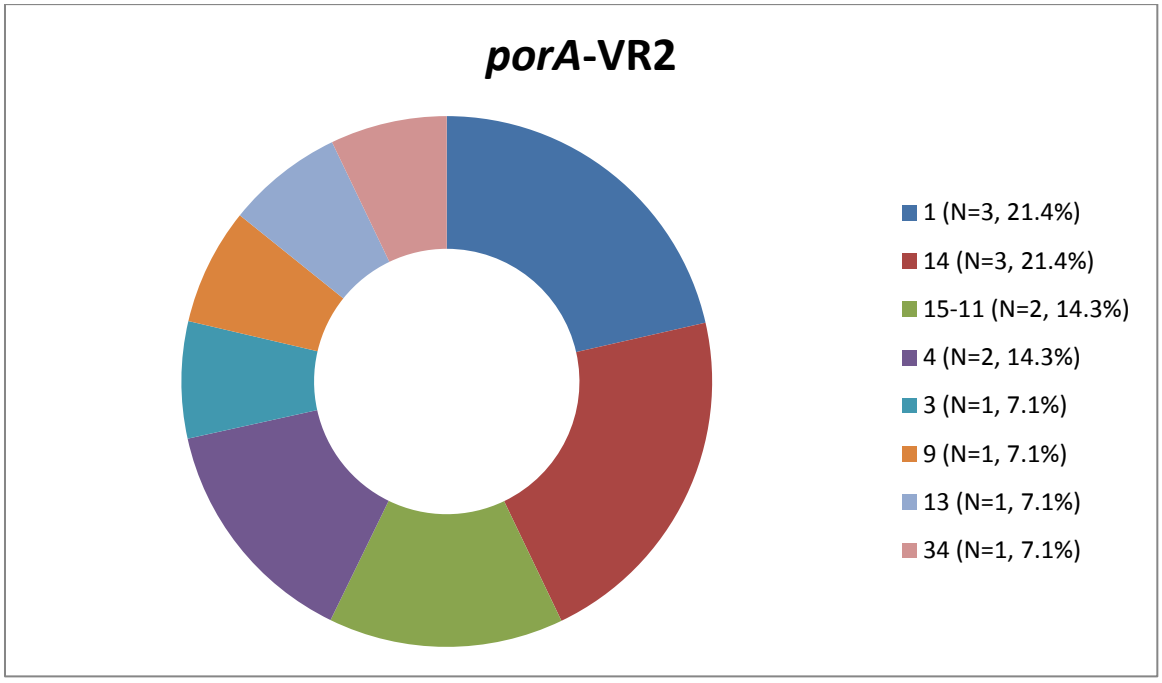

Figure 5. Distribution of *fetA*-VR alleles in serogroup B isolates of *N. meningitidis* referred to the Swiss National Reference Center for Meningococci in 2015.

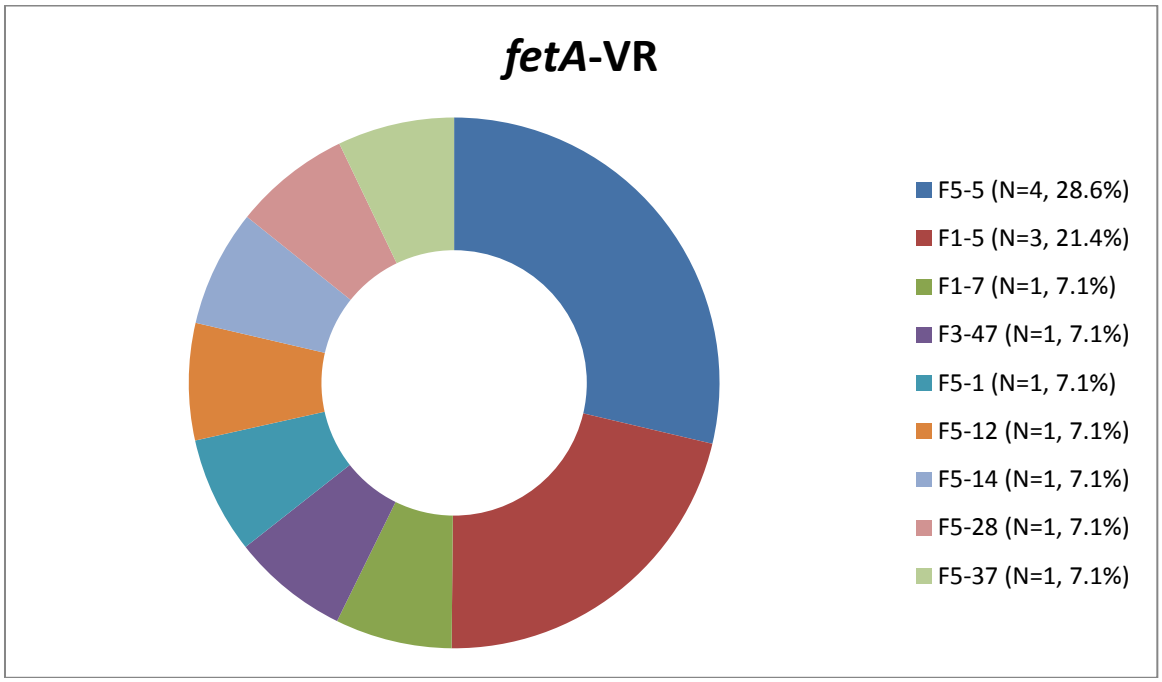

Table 1. Synopsis of MLST profiles and serogroups of invasive *N. meningitidis* strains referred to the Swiss National Reference Center for Meningococci in 2015.

MLST Profile[a]		Serogroup (number of isolates)				
Clonal complex	Sequence type	B	C	W	Y	Other [serogroup]
11	11		3			
11	11			13		
22	1286			1		
23	23				4	
41/44	41	1				
41/44	280	1				
41/44	340	1				
41/44	2487	1				
41/44	3532	1				
41/44	4064	1				
167	168				1	
213	213	2				
213	3496	2				
269	978	1				
269	1214	1				
269	1214				1	
269	10544	1				
269	NA	1				
1157	1157					1 [X]
NA	3015				1	
NA	11506		1			

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

Table 2. Inhibitory activity of antimicrobial agents on 40 invasive *N. meningitidis* strains referred to the Swiss National Reference Center for Meningococci in 2015.

Antimicrobial agent	Minimum inhibitory concentration (µg/ml)			Breakpoint susceptible (≤ µg/ml)	% of strains considered susceptible
	Range	50%	90%		
Azithromycin	0.047-2	0.75	1.5	2[a]	100
Ceftriaxone	0.002-0.023	0.003	0.006	0.12[b]	100
Chloramphenicol	0.38-2	1	1.5	2[b]	100
Ciprofloxacin	0.004-0.016	0.008	0.012	0.03[b]	100
Meropenem	0.008-0.125	0.016	0.064	0.25[b]	100
Minocycline	0.006-0.75	0.38	0.75	1[b]	100
Penicillin	0.023-2	0.064	0.19	0.094[c]	70
Rifampicin	0.002-0.25	0.012	0.032	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)