Swiss National Reference Center for Meningococci

> 2016 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. It can evolve as epidemics and requires a continuous surveillance, especially nowadays with the appearance of a hypervirulent serogroup W clone in Europe (Ladhani et al., 2015). Also, 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 into 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. This methodology is evolving rapidly towards Next Generation Sequencing (NGS) (Mustapha et al., 2016), a method that we used for sporadic cases in 2016 but that we would like to introduce as regular testing in the near future. 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 2016.

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 performed 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 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 2016, the CNM has received a total of 46 invasive isolates of *N. meningitidis*. These strains were isolated from blood specimens (N=35), cerebrospinal fluid (N=10) and joint (N=1).

The strains received were isolated from 20 female and 26 male patients and represented 92% of all cases of invasive meningococcal diseases (N=50) reported to Swiss public health authorities in 2016 (Figure 1).

Five strains were recovered from the age group representing very young children aged 0-1 years, and three strains were isolated from children aged 1-14 years. Of these eight strains, all but one were of serogroup B! (Figure 2). Ten strains were isolated from teenagers (15-19 years) and young adults (20-24 years) combined, half of them belonged to serogroup W. Eight strains were isolated from adults aged between 25-49 years, but no single strain was from serogroup W. The remaining strains (N=20, 43%) were from patients from 50 up to 90+ years old, with more than half of them belonging to serogroups Y or W.

Serotyping of all strains by agglutination or PCR revealed that the majority of isolates belonged to serogroup B (N=19, equivalent to 41%). Serogroup W was the second most frequent (N=12, 26%), followed by serogroup C (N=9, 20%) and serogroup Y (N=6, 13%) (Figure 3).

All main serogroups (B, C, Y and W) were recovered in all regions of Switzerland except for the Italian speaking area where serogroup Y was not detected. Figure 4 shows the geographical distribution of serogroups.

Molecular characterization using MLST revealed that the 19 serogroup B strains were distributed in 17 different sequence types, but 3 main clonal complexes (CC41/44, CC213, CC269). Serogroup W strains were essential of CC11, as was the case for serogroup C. Serogroup Y were almost all from CC 23 (Table 1).

Applying EUCAST and CLSI breakpoints, all invasive *N. meningitidis* strains tested were found to be susceptible to ceftriaxone, chloramphenicol, ciprofloxacin, meropenem, minocycline, and rifampicin. However, only 80% of these isolates were considered susceptible to penicillin using EMGM criteria, and 98% to azithromycin (Table 2). Interestingly, all the penicillin non-susceptible were from serogroup B (N=7) and serogroup C (N=2), which is also the serogroup of the strain resistant to azithromycin.

A total of 35 invasive specimens (33 from GE, 1 from VD and 1 from SG) were processed for direct detection of *N. meningitidis* DNA by PCR because culture was negative. These specimens consisted of cerebrospinal fluids (CSF, N=28) or DNA extracts of sterile fluid (N=1), EDTA-anticoagulated blood samples (N=4), and articular fluids (N=2). No DNA extract showed PCR inhibition and *N. meningitidis* DNA was found in only two (2/35 = 6%) specimens: an EDTA blood from GE and an extract of a “sterile fluid” from VD (CHUV). For each of these specimens, both PCR assays (*ctrA* target and *sodC* target) were positive.

Summary of key observations

- Serogroup B was the most frequently determined in invasive strains of meningococcus (41%), followed by serogroup W (26%) and serogroup C (20%). The remaining cases were associated with serogroup Y (13%).
- Predominant MLST profiles were CC41/44, CC213 and CC269 in serogroup B isolates, CC11 in serogroups C and W, and CC23 in serogroup Y.
- Of the 12 serogroups of W strains, 9 were of the exact same finetype: PorA 5,2:FetA 1-1:ST-11, suggesting the possibility of a clonal distribution. Of the last two, one is same finetype but ST-9446, and one is of a different finetype.
- Susceptibility of *N. meningitidis* to antibiotics recommended for prophylaxis (rifampicin and ciprofloxacin) and treatment (ceftriaxone) remained 100%.

Discussion

In 2016, a total of 50 cases of invasive meningococcal diseases were reported to the SFOPH, and for 46 of these cases corresponding isolates were referred to the CNM for reference testing (Figure 1). Compared to 2015, there was a slight increase (+10%) 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 remained the most prevalent (41%) followed by serogroup W (26%) that rose around 2013 (Figure 2) and is somehow worrisome for Switzerland, due to its tendency to be clonal and of the same finetype (PorA 5,2:FetA 1-1:ST-11) as the newly described hypervirulent strain (Ladhani et al., 2015). Importantly, no such expansion has been observed for any other meningococcal subpopulation.

However, as shown by Mustapha (Mustapha et al., Vaccine 2016), the routine finetyping scheme proposed by the EMGM does not allow precisely assessing clonal relatedness between ST-11 complex strains. Therefore whole genome sequence (WGS) analysis is used increasingly for meningococcal typing. This was performed in the Genomic Research Laboratory of Prof. Schrenzel in 2016 to compare meningococcal strains of two ethnically linked patients who suffered from invasive meningococcal disease at 2-3 weeks interval in Geneva. It showed only 36 SNPs

differences which is very few for meningococcus and could therefore be considered as clonal. 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 “Hajj outbreak” lineage that caused an epidemic among Hajj pilgrims and contacts in 2000. We plan to determine the WGS of all the serogroup W strains identified since 2014 in the CNM, to determine the % of clonal strains and correlate our strains with the hypervirulent “UK strain”. WGS will also help us to better apprehend the epidemic potential of this serogroup W meningococcus in Switzerland.

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). Due to the evolution of the prevalence of the diverse serogroups in Switzerland and the availability of a conjugated quadrivalent vaccine, the SFOPH reviewed the recommendation for vaccination in march 2015 (https://www.bag.admin.ch/bag/fr/home/themen/mensch-gesundheit/uebertragbare-krankheiten/infektionskrankheiten-a-z/meningokokken-erkrankungen.html).

Conclusions and Perspectives

In 2016, the CNM observed that serogroup B remained the most prevalent in Switzerland particularly in neonates and children less than 15 years old. Serogroup W has the potential to be of clonal expansion, but its incidence has not risen in Switzerland in 2016 as compared to 2015. However it appears particularly frequent in

adolescent and young adults as well as in adults over 50 years. As it is more prone to present unusually with a pneumonia and only invasive cases are reported, its real prevalence might be underestimated. Susceptibility of meningococci to antibiotics recommended for prophylaxis (rifampicin and ciprofloxacin) and treatment (ceftriaxone) remained 100%.

Acknowledgements

The authors thank Gisela Cristina GETAZ-JIMENEZ and Isabelle JAN for excellent assistance, and the SFOPH for financial and scientific support.

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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 2009 to 2016.

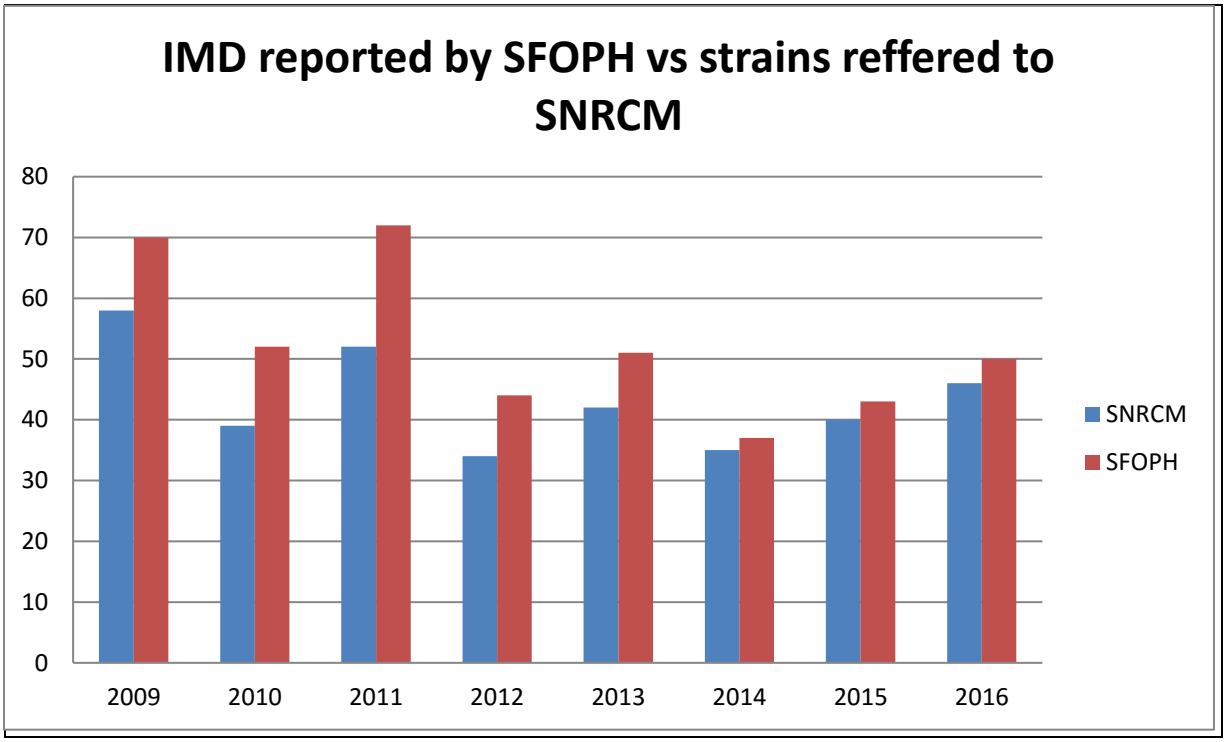

Figure 2. Annual number of strains representing main serogroups B, C, Y and W of invasive *N. meningitidis* as determined by agglutination at the Swiss National Reference Center for Meningococci from 2009 to 2016.

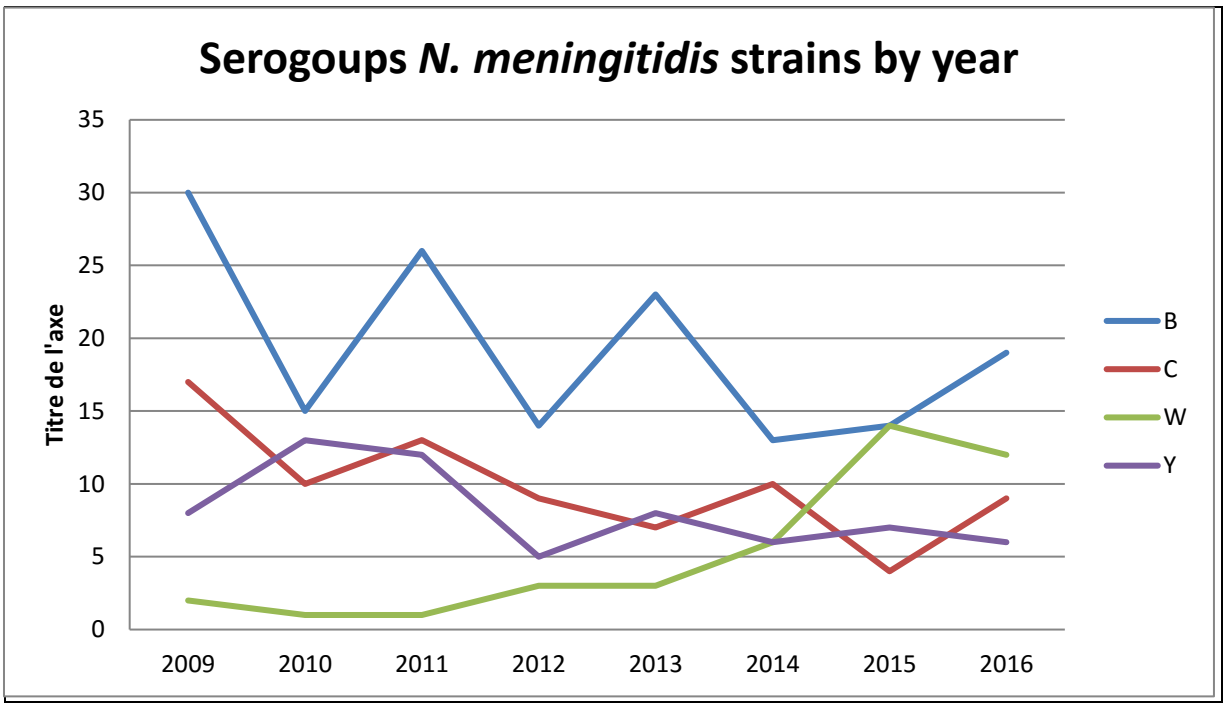

Figure 3. Prevalence of the different serogroup according to age group

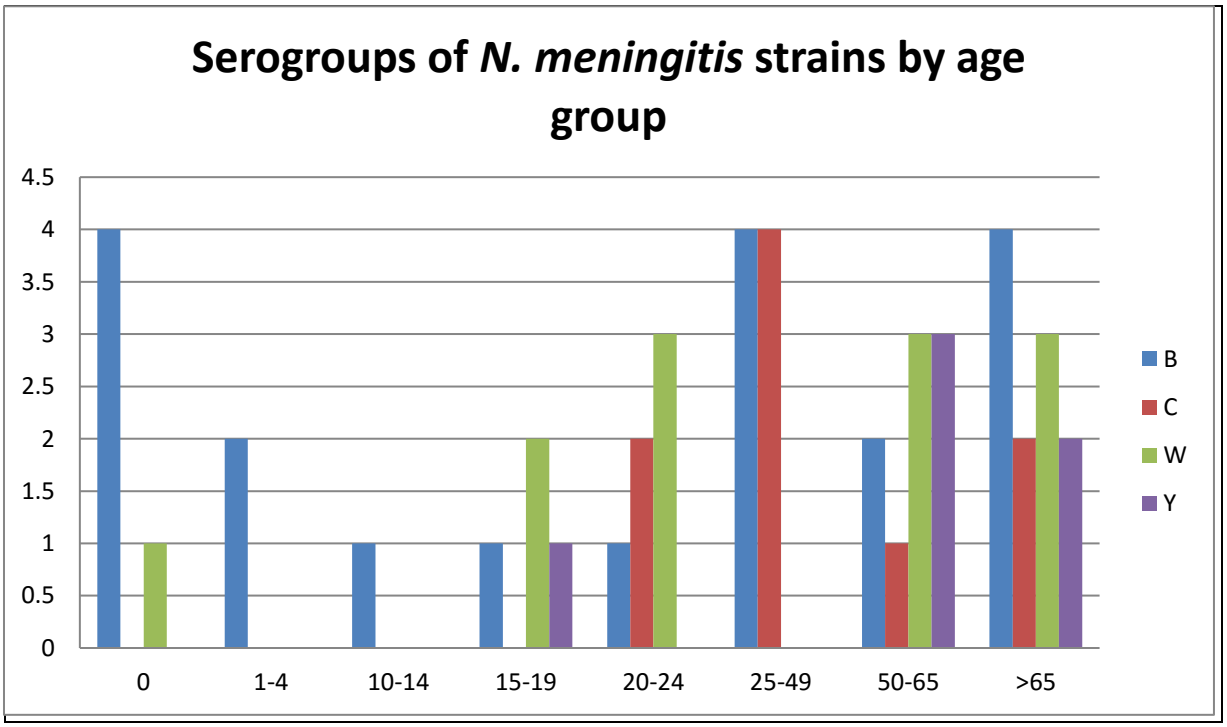

Figure 4. Distribution of serogroups by geographical regions in 2016

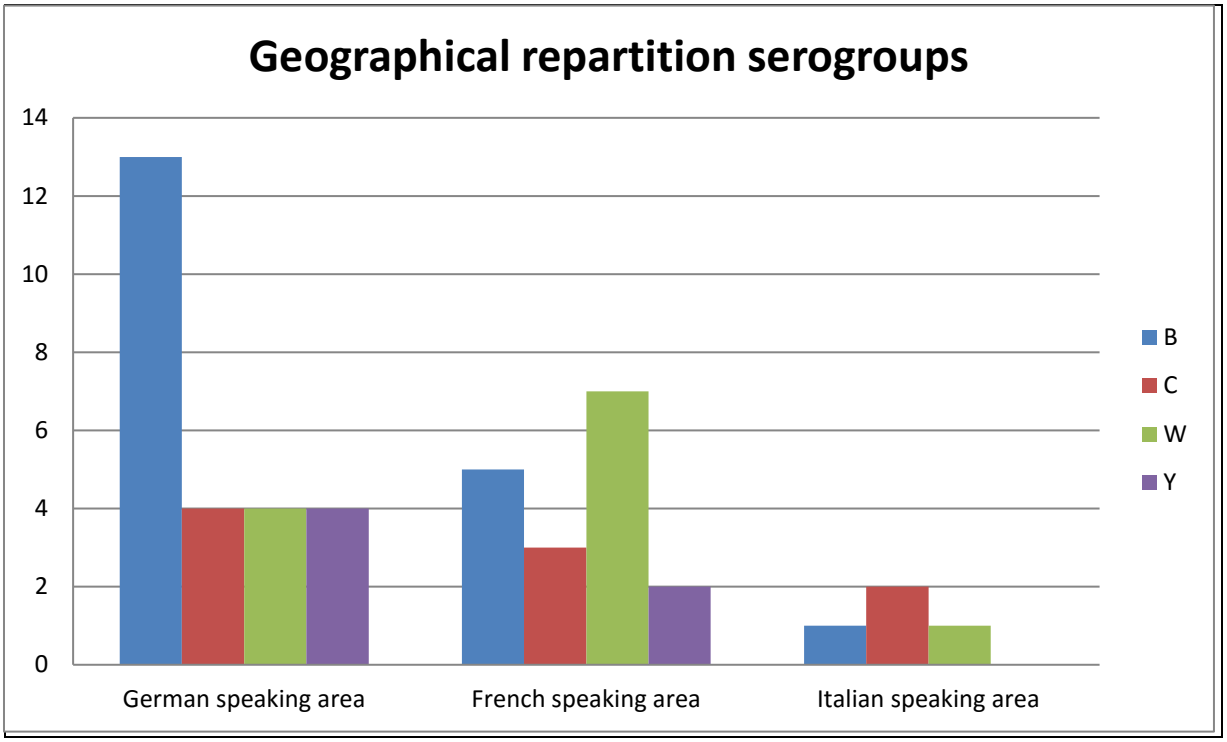
Geographical repartition serogroups
14
12
10
8
6
4
2
0
German speaking area
French speaking area
Italian speaking area
B
C
W
Y

Table 1. Synopsis of MLST profiles and serogroups of invasive *N. meningitidis* strains referred to the Swiss National Reference Center for Meningococci in 2016.

MLST Profile[a]		Serogroup (number of isolates)				
Clonal complex	Sequence type	B	C	W	Y	Other [serogroup]
11	11	1	6	10		
23	23				5	
41/44	11063	1				
41/44	1111	1				
41/44	340	1				
41/44	3496	1				
213	213	2				
213	278	1				
213	1946	1				
269	269	2				
269	1161	1				
35	35			1		
9446	9446			1		
114	114				1	

[a]MLST, multilocus sequence typing

Table 2. Inhibitory activity of antimicrobial agents on 40 invasive *N. meningitidis* strains referred to the Swiss National Reference Center for Meningococci in 2016.

Antimicrobial agent	Minimum inhibitory concentration (µg/ml)			Breakpoint susceptible (≤ µg/ml)	% of strains considered susceptible
	Range	50%	90%		
Azithromycin	0.032-3	0.5	1.5	2[a]	98
Ceftriaxone	0.002-0.008	0.003	0.004	0.12[b]	100
Chloramphenicol	0.38-1.5	1	1.5	2[b]	100
Ciprofloxacin	0.003-0.023	0.006	0.012	0.03[b]	100
Meropenem	0.004-0.19	0.023	0.094	0.25[b]	100
Minocycline	0.047-0.5	0.25	0.5	1[b]	100
Penicillin	0.0016-0.5	0.047	0.25	0.094[c]	80
Rifampicin	0.002-0.125	0.012	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)