Prof. Jacques Schrenzel
Laboratoire de bactériologie
Laboratoire de recherche génomique
Service des maladies infectieuses
Département des spécialités de médecine
Service de médecine de laboratoire
Département de médecine génétique, de laboratoire et de pathologie
Hôpitaux Universitaires de Genève
Rue Gabrielle-Perret-Gentil 4
CH-1211 Genève 14
web www.genomic.ch
Tél. (4122) 372 73 08
Fax (4122) 372 73 12
Courriel Jacques.Schrenzel@hcuge.ch

Annual Report of the Swiss National Reference Center for Meningococci, 2017

Address

National Reference Center for Meningococci

Hôpitaux Universitaires de Genève

Laboratoire de Bactériologie

Rue Gabrielle-Perret-Gentil 4

1211 Genève 14

Phone: 022 372 73 01; Fax: 022 372 73 12; Jacques.Schrenzel@hcuge.ch

Website: French: http://www.meningo.ch/

Website: German: http://www.meningo.ch/index_DE.html

Website: Italian: http://www.meningo.ch/index_IT.html

Website: English: http://www.meningo.ch/index_EN.html

Table of contents

Hôpitaux Universitaires de Genève Rue Gabrielle-Perret-Gentil 4 CH-1211 Genève 14
Tél. 022 372 73 01 Fax 022 372 73 12 E mail: jacques.schrenzel@hcuge.ch Internet : www.hug-ge.ch

1. Introduction

Invasive strains of *Neisseria meningitidis* are a life-threatening cause of bacterial sepsis and meningitis, mainly in infants, adolescents and young adults. They can cause outbreaks and therefore require 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 a selection of cases collected between 2010 and 2016, to determine the clonality of the meningococcal strains of serogroup W finetype (PorA 5,2:FetA 1-1:ST-11). This was executed as a separate subproject supported by the SFOPH (Decision 16.928412).

This annual report describes the methods used and results obtained at the CNM in the calendar year 2017.

2. 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 sub-cultured overnight on chocolate agar plates. Confirmation of identification is performed by PCR using the *N. meningitidis*-specific targets *ctrA* (Corless et al., 2001), *sodC* (Dolan Thomas et al., 2011), *tauE, metA*, and *shlA* (Diene et al., 2016). Serogroups are determined by PCR as well as by commercial agglutination kits: A, B, C, and Y/W135 (Pastorex Meningitis, Bio-Rad) and W135, X, Y, Z and Z' (Difco Neisseria Meningitidis Antisera, Becton Dickinson).
Sequence analysis is performed on each isolate in two variable regions of the gene encoding the antigenic outer membrane protein porin A (*porA*-VR1 and *porA*-VR2) and in one variable region of the *fetA* gene (*fetA*-VR) encoding another outer membrane protein exhibiting sequence data which can be useful for 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 performed 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 a 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 groups 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 also tested for antimicrobial susceptibility on Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F, bioMérieux) using E-test strips (AB Biodisk, bioMérieux) containing azithromycin, ceftriaxone, ciprofloxacin, chloramphenicol, meropenem, minocycline, penicillin, and rifampicin. 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. Azithromycin MICs are interpreted according to breakpoints proposed by the Clinical and Laboratory Standards Institute (CLSI).

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 targets described above (panel has been completed based on Diene et al, 2016). 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).

3. Strain collection

The CNM stores all the received invasive meningococcal isolates at -80°C. The collection currently includes more than 500 isolates (between 2009 and 2018). Previous strains were also stored but their recovery by culture is not fully guaranteed.

4. National and International quality assurance

There is currently no international quality assurance pertaining to meningococci. We are actively scouting whether this service would become available.

5. Development of new diagnostic tools

- Meningococcal W135 molecular subtypes identified using Whole Genome Sequencing.

We have determined a wet-lab protocol for WGS of *N. meningitidis* strains as well as a dedicated bioinformatics analysis to assess whether the apparently clonal W135 strains (undistinguishable using the finetype methods) could be better characterized, with the objective to analyse potential clonal relationship.

- Additional molecular targets for *N. meningitidis* detection by qPCR

Bacterial identification by qPCR can be affected by genetic polymorphisms within the assay target. To prevent false-negative PCR results, we implemented in 2017 the following three molecular targets, *tauE*, *metA*, and *shlA*, based on the work of Diene et al., 2016.

6. Epidemiological research

The precision of NGS permitted us to identify several independent monoclonal outbreaks related to *N. meningitidis* W135 that occurred between 2010 and 2016 in Switzerland. Our meta-analyses included samples from other previously published works and allowed establishing connections between Swiss MenWs and other European outbreaks (paper in preparation).

This project was made possible through a grant from SFOPH (Decision 16.928412).

7. Additional meningococcal research

The molecular expertise developed in the framework of the CNM permitted us to decipher a very intriguing case of left-heart failure due to acute aortic valve endocarditis. The combination of qPCR assays for molecular detection and typing, as well as the use of NGS on the valve material permitted to assess the diagnosis of an acute meningococcal endocarditis, due to a serotype B strain, despite the impossibility to cultivate the organism. This exceedingly rare diagnosis, likely underestimated due to rapid pre-emptive therapy, underlines the need to obtain an etiological diagnosis. This work will be presented as an abstract and published.

8. Advisory service and Networking

8.1 Advisory service

Molecular testing: We systematically conduct molecular assays to determine the serotype directly from clinical invasive *N. meningitidis* specimens (or suspicion thereof). As mentioned above, it is likely that the true incidence of invasive *N. meningitidis* infection is missed by rapid empiric therapy (precluding successful cultivation), nor to mention the new clinical presentations related to W135 such as pneumoniae (typically undetected and not referred to the CNM unless presenting with a bacteraemia and thus fulfilling the current definition of invasive infection). Our current molecular approach covers the most frequent serotypes and a result can usually be communicated to the clinicians.

8.2 Networking

We will contact the Italian reference center for meningococci to analyse further our peculiar W135 epidemics, in conjunction with their national epidemiology.

8.3 Website

The dedicated website (www.meningo.ch) will be fully rebuilt in 2018.

9. Results

During the calendar year 2017, the CNM has received a total of 48 invasive isolates of *N. meningitidis*. These strains were isolated from blood specimens (N=44) and cerebrospinal fluids (N=4).

The strains received were isolated from 29 female and 19 male patients and represented 91% of all cases of invasive meningococcal diseases (N=53) reported to Swiss public health authorities in 2017 (Figure 1).

There was no real pattern of serogroup associated to the patient's age, except for seniors that were almost exclusively affected by serogroups W and Y (Figure 2).

Serotyping of all strains by agglutination or PCR revealed that the majority of isolates belonged to serogroup W (N=21, equivalent to 44%) and serogroup Y (N=11, 23%), a major change as compared to former years, mainly due to bacteriemic episodes in seniors. Serogroup B (N=9, 19%) and C (N=7, 14%) were mainly seen in adolescent and young adults. (Figure 3).

All main serogroups (B, C, Y and W) were recovered in all regions of Switzerland except for the Italian speaking area where only serogroups Y and W were detected. Serogroup B was essentially found in the German speaking part of Switzerland. Figure 4 shows the geographical distribution of the serogroups.

Molecular characterization using MLST revealed that ST-11 was the most prevalent sequence type present in Switzerland in 2017, with 100% of the serogroup W strains and 71% of serogroup C strains harbouring a sequence type 11 profile. ST-23 is the second most frequent and represents more than half of serogroup Y strains (Table 1). When looking in more details, all serogroup W strains were also of the same finetype (PorA 5,2:FetA 1-1:ST-11) inside the ST-11. Importantly, NGS from the strains collected between 2010 and 2016 showed several independent monoclonal outbreaks that occurred in Switzerland.

Applying EUCAST breakpoints, all invasive *N. meningitidis* strains tested were found to be susceptible to ceftriaxone, chloramphenicol, ciprofloxacin, meropenem, minocycline, and rifampicin. However, only 50% of these isolates were considered fully susceptible to penicillin, and 96% to azithromycin using CLSI breakpoints (Table 2). Penicillin non-susceptible strains were not associated to a specific serogroup.

Summary of key observations

- Serogroup W was the most frequently determined in invasive strains of meningococcus (44%), followed by serogroup Y (23%) and serogroup B (19%). The remaining cases were associated with serogroup C (14%).
- Predominant MLST profiles were ST-11 and ST-23.
- All but 2 of our serogroup W strains were of the exact same finetype: PorA 5, 2:FetA 1-1:ST-11, suggesting the possibility of a clonal distribution. This observation warrants further NGS-based investigation, as suggested by our 2010-2016 analysis. The objective would be to assess whether the increasing incidence of such strains results from an ongoing monoclonal outbreak.
- Susceptibility of *N. meningitidis* to antibiotics recommended for prophylaxis (rifampicin and ciprofloxacin) and treatment (ceftriaxone) remained 100%. However, susceptibility to penicllin, according to EUCAST breakpoints, was only 50%.

10. Discussion

In 2017, a total of 53 cases of invasive meningococcal diseases were reported to the SFOPH, an incidence that remains stable across the last few years. The main change in meningococcal epidemiology in Switzerland is the development of serogroup W (44%) hypervirulent strain that is mostly of clonal origin, some of them linked with the strain described in the UK (Ladhani et al., 2015). Importantly, no such expansion has been observed for any other meningococcal subpopulation.

This particular strain of meningococcus is associated with unusual clinical pictures (especially pneumonia, more often bacteriemic or with purpura fulminans), and an

unusual target population (more often seen in patients over 50 years old). Therefore, Swiss recommendations for vaccination against meningococcal disease have been and will be further adapted, with the use of the quadrivalent MenACWY (capsular antigen) conjugate vaccine.
(See SFOPH website for last updated recommendations).

Finally, our surveillance of antimicrobial susceptibilities of *N. meningitidis* strains involved in invasive diseases in Switzerland speaks against the use of penicillin as first line empirical treatment of meningococcal disease. Ceftriaxone remains the drug of choice in these situations.

11. Acknowledgements

The authors thank the Laboratory of Bacteriology for excellent assistance, and the SFOPH for financial and scientific support.

12. References

Corless CE, Guiver M, Borrow R et al. Simultaneous detection of *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* in suspected cases of meningitis and septicemia using real-time PCR. J Clin Microbiol 2001;39:1553-8.

Diene SM, Bertelli C, Pillonel T, et al. Comparative genomics of Neisseria meningitidis strains: new targets for molecular diagnostics. Clin Microbiol Infect. 2016;22:568.e1-7

Dolan Thomas J, Hatcher CP, Satterfield DA, et al. sodC-based real-time PCR for detection of *Neisseria meningitidis*. PLOS ONE 2011;6:e19361.

Fraisier C, Stor R, Tenebray B, et al. Use of a new single multiplex PCR-based assay for direct simultaneous characterization of six *Neisseria meningitidis* serogroups. J Clin Microbiol 2009;47:2662-6.

Harrison OB, Brueggemann AB, Caugant DA, et al. Molecular typing methods for outbreak detection and surveillance of invasive disease caused by *Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae*. Microbiology 2011;157:2181-95.

Ladhani SN, Beebeejaun K, Lucidarme J, et al. Increase in endemic *Neisseria meningitidis* capsular group W sequence type 11 complex associated with severe invasive disease in England and Wales. Clin Inf Dis 2015;60:578-85.

Molling P, Jacobsson S, Backman A, et al. Direct and rapid identification and genogrouping of meningococci and porA amplification by LightCycler PCR. J Clin Microbiol 2002;40:4531-5.

Mustapha MM, Marsh JW, Harrisson LH Global epidemiology of capsular group W meningococcal disease (1970–2015): Multifocal emergence and persistence of hypervirulent sequence type (ST)-11 clonal complex – Vaccine 34 (2016) 1515–1523

World Health Organization. Laboratory Methods for the Diagnosis of Meningitis caused by *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* [2nd edition]. 2011. WHO Press, World Health Organization, Geneva.

Figure 1. Annual number of cases of invasive meningococcal diseases reported to the Swiss Federal Office of Public Health (SFOPH) and number of *N. meningitidis* strains referred to the Swiss National Reference Center for Meningococci (SNRCM) from 2009 to 2017.

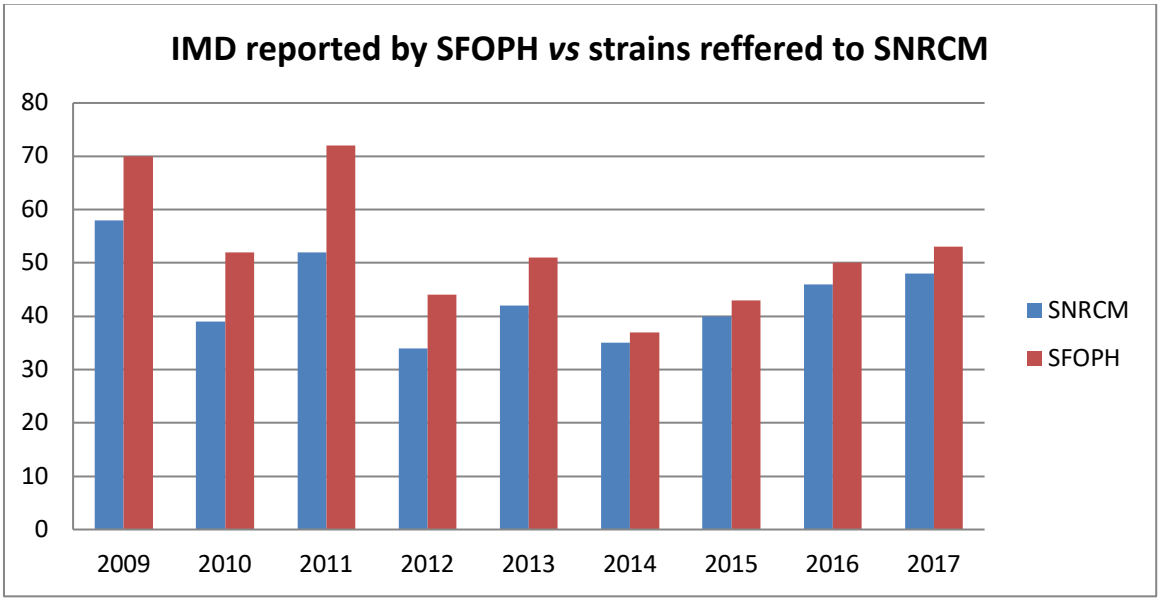
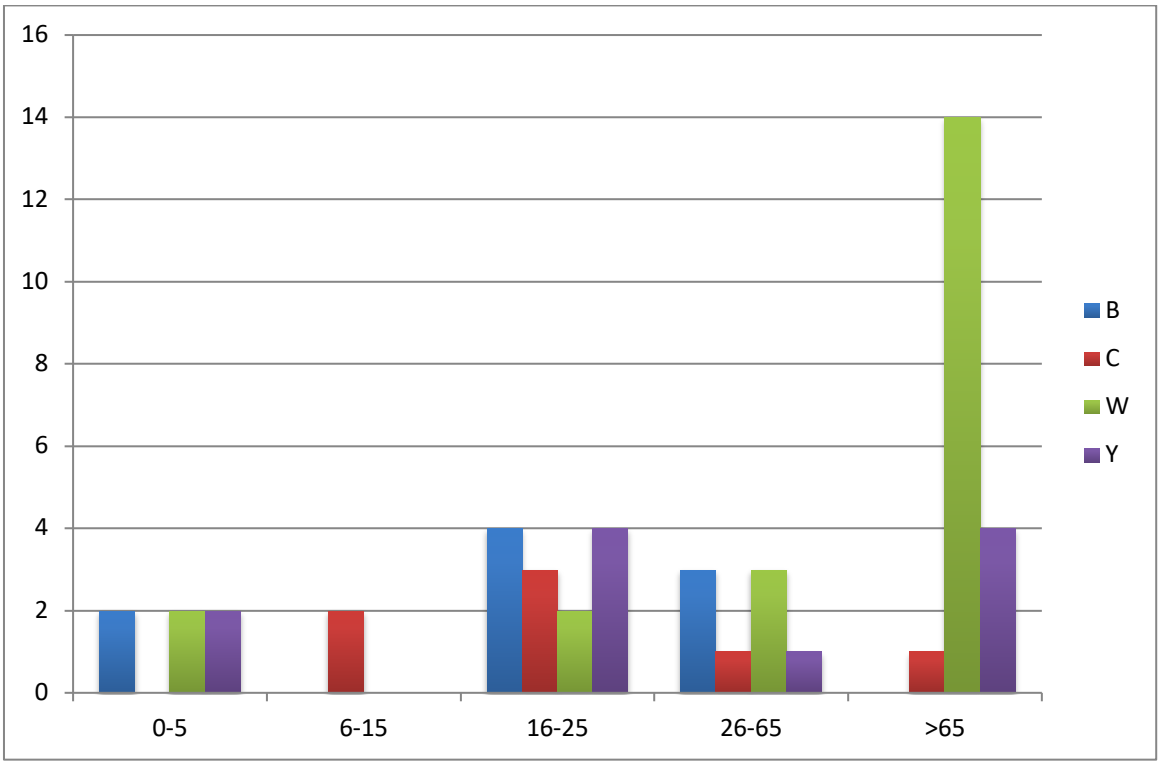


Figure 2. Prevalence of the different serogroups according to age groups in 2017.



Hôpitaux Universitaires de Genève Rue Gabrielle-Perret-Gentil 4 CH-1211 Genève 14
Tél. 022 372 73 01 Fax 022 372 73 12 E mail: jacques.schrenzel@hcuge.ch Internet : www.hug-ge.ch

Figure 3. Annual number of strains representing main serogroups B, C, Y and W of invasive *N. meningitidis* as determined at the Swiss National Reference Center for Meningococci from 2009 to 2017

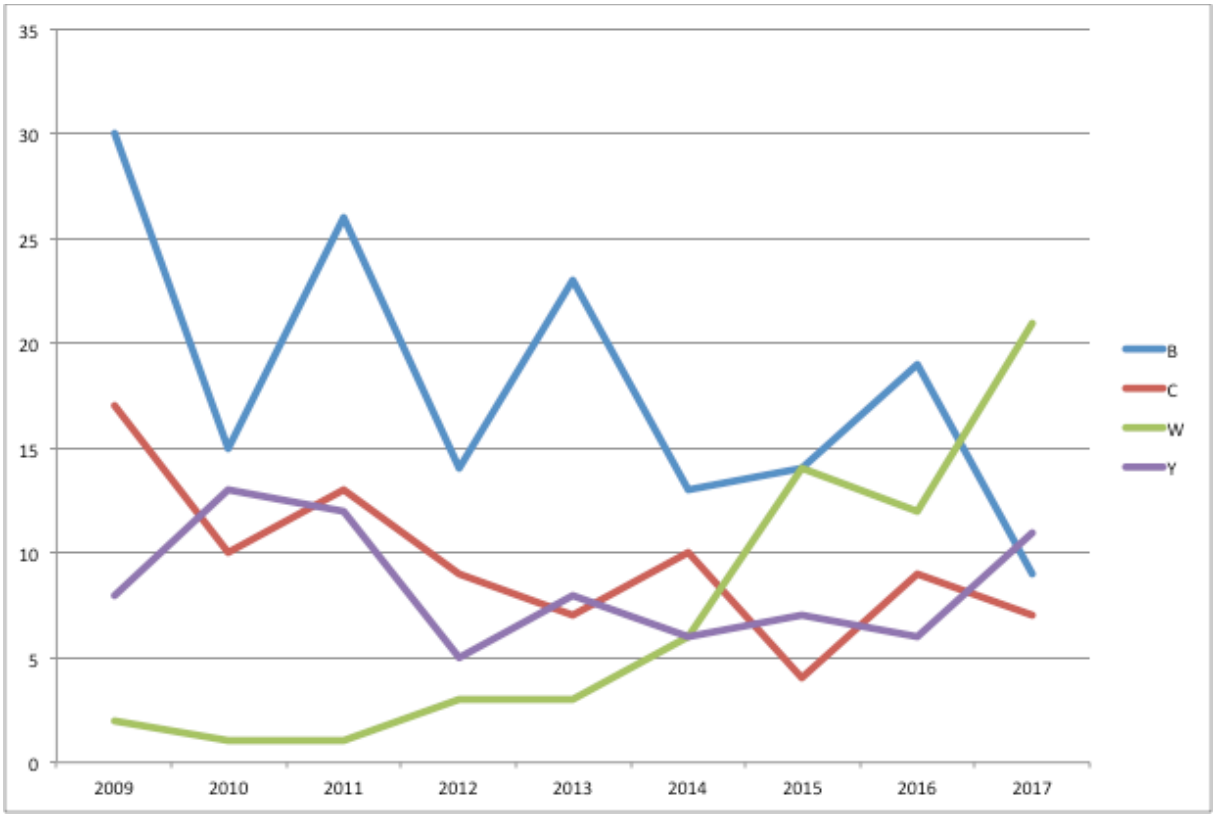


Figure 4. Distribution of serogroups by geographical regions in 2017

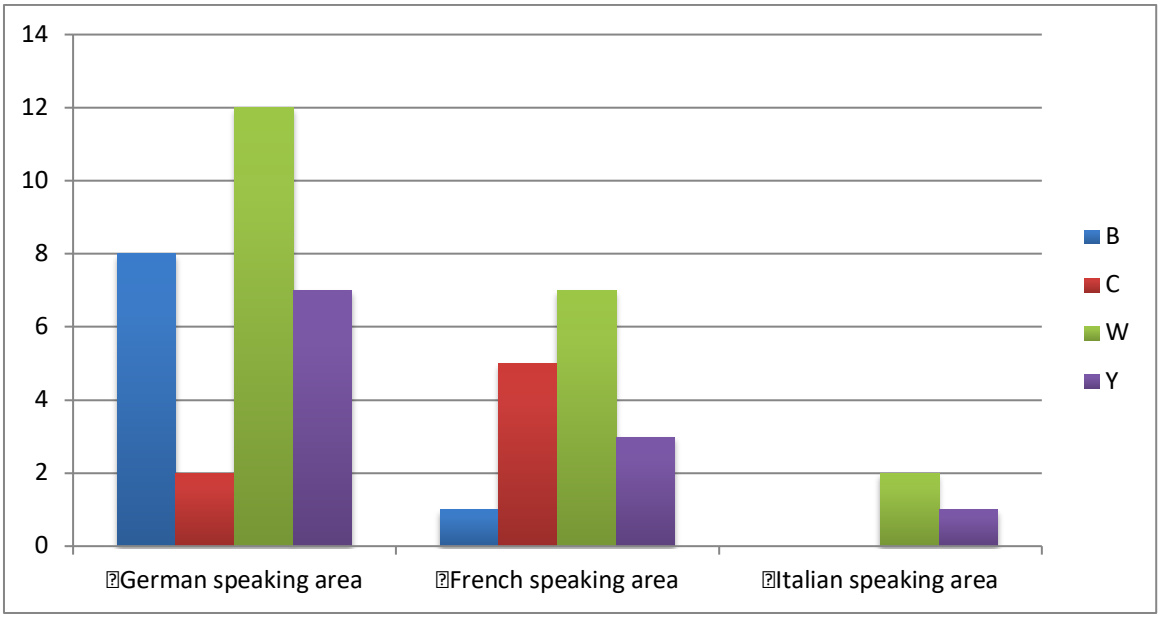


Table 1. Synopsis of MLST profiles and serogroups of invasive *N. meningitidis* strains referred to the Swiss National Reference Center for Meningococci in 2017.

Serogroups	**Sequence type (MLST)**
B	7 different ST
C	ST-11 (71%), 1 ST3327, 1 ND
W	ST-11 (100%)
Y	ST-23 (55%), 4 other ST

Table 2. Antimicrobial susceptibility testing (EUCAST breakpoints) of 48 invasive *N. meningitidis* strains referred to the Swiss National Reference Center for Meningococci in 2017.

Antimicrobial agent	Minimum inhibitory concentration (µg/ml)			Breakpoint susceptible (≤ µg/ml)	% of strains considered susceptible
	Range	50%	90%		
Azithromycin	0.038-4	1	1.5	2[a]	96
Ceftriaxone	<0.002-0.032	0.002	0.004	0.12	100
Chloramphenicol	0.06-2	1	1.5	2	100
Ciprofloxacin	0.002-0.012	0.004	0.008	0.03	100
Meropenem	0.003-0.094	0.008	0.047	0.25	100
Minocycline	0.032-0.75	0.25	0.5	1	100
Penicillin	0.0094-1	0.064	0.75	0.06	50
Rifampicin	0.002-0.19	0.016	0.047	0.25	100

[a]Clinical and Laboratory Standards Institute (CLSI) 2016