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# Annual Report of the Swiss National Reference Center for Meningococci, 2019

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## 1. Introduction

Invasive strains of *Neisseria meningitidis* constitute 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 spread of a hypervirulent serogroup W clone in Europe (Knol et al., 2017; 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 have to 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 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 during the calendar year 2019.

## 2. Materials and Methods

The CNM is investigating invasive 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 and C (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 penicillin, ceftriaxone, meropenem, ciprofloxacin, minocycline 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).

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 MagPurix 12 Nucleic Acid Extraction System (Zinexts Life science; Taiwan). 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 (Mölling et al., 2002). Finally, differentiation between serogroups Y and W135 is performed 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 2019). Previous strains were also stored but their recovery by culture cannot be guaranteed (n=1'914 isolates between 1989 and 2009).

## 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. 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 as published last year in the Journal of Infection (Leo et al., 2019). This project was made possible through a specific grant from SFOPH (Decision 16.928412).

## 6. 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 has been published in 2019 in Frontiers in Cardiovascular Medicine (Choutko et al., 2019).

This work was also presented (during lectures) at the following meetings:

07. **J. SCHRENZEL**: Métagénomique clinique : du rêve…. Invited speaker to the 39ème Réunion interdisciplinaire de chimiothérapie anti-infectieuse (RICAI). Paris, France, December 2019.

06. **J. SCHRENZEL**: Clinical Metagenomics: Developments, applications, position, limitations. Keynote speaker and Chaiman at the 12th European Meeting on Molecular Diagnostics. Noordwijk aan Zee, The Netherlands, October 2019.

05. **J. SCHRENZEL**: Microbiome analysis in clinical medicine: hope or hype? Invited speaker by the 11th ISABS Conference on Forensic and Anthropological Genetics and Mayo Clinic Lectures in Individualized Medicine. Split, Croatia, June 2019.

04. **J. SCHRENZEL**: Infectious endocarditis: molecular diagnosis including Next Generation Sequencing (NGS). Chair and invited speaker to the 15th International Society of Cardiovascular Infectious Diseases (ISCID). Lausanne, June 2019.

03. S. LEO, V. LAZAREVIC, M. GIRARD, L. ANSON, N. GAIA, G. RENZI, A. CHERKAOUI, R. BORN, S. BASLER, and **J. SCHRENZEL**: Genomic epidemiology of *Neisseria meningitidis* serogroup W isolates collected in Switzerland between 2010 and 2016. 15th EMGM (European Meningococcal and Haemophilus Disease Society) Congress, Lisbon, Portugal, May 2019.

02. **J. SCHRENZEL**: Endocardite infectieuse : quoi de neuf ? Invited speaker by Congrès des Quatre Saisons Cardiovasculaires Genevoises. Geneva, March 2019.

01. **J. SCHRENZEL**: Complications (non)-infectieuses du méningocoque. Colloque de médecine, Geneva, February 2019.

# 7. Advisory service and Networking

## 7.1 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.

## 7.2 Networking

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

## 7.3 Website

The dedicated website (www.meningo.ch) was fully rebuilt in 2018, and is available in French, German, Italian and English. We are currently updating it to better display the information.

# 8. Results

During the calendar year 2019, the CNM has received a total of 33 invasive isolates of *N. meningitidis*. These strains were isolated from blood specimens (n=26) and cerebrospinal fluids (n=7).

The strains received were isolated from 15 female, 17 male patients and 1 whose gender was not determined, representing 77% of all cases of invasive meningococcal diseases (n=43) reported to Swiss public health authorities in 2019 (SFOPH; Figure 1).

Since 2014, the number of invasive meningococci isolated is increasing (Figure 1). However, in 2019, this number drastically dropped in Switzerland as compared to 2018 (1.7 fold less strains isolated; Figure 1). Among invasive meningococci, serogroup B was the most frequently isolated (n=17, 52%), followed by serogroup W135 (n=8, 24%) (Figure 2). The relative amount of serogroup B isolated in 2019 was smilar to 2018 (n=15 in 2018 and n=17 in 2019), but for the first time since 2014, the proportion of serogroup W decreased; we observed a 3-fold reduction as compared to 2018 (n=8 in 2019 and n=23 in 2018). Similarly to 2018, serogroup C represented 12% (n=4) of all invasive meningococci (Figure 2) but serogroup Y distribution decreased to 9% (n=3; compared to 21% in 2018). In addition, in 2019, one serogroup X was identified (the last invasive serogroup X was isolated in 2015, Figure 2 and Figure 4).

Most serogroup B strains were isolated from young patients <15 years old (Figure 3). No other serogroup was identified in these patients. Serogroup W strains were collected from all patients > 15 years old, serogroup C from adults only (20-65 years old) and serogroups X and Y were only isolated in senior patients (>65 years old).

All main serogroups (B, C, Y and W) were recovered in all regions of Switzerland except for the Italian speaking area where only serogroups B and W were detected. Serogroup B and W were essentially found in the German part of Switzerland. The only serogroup X strain was isolated in the French speaking part of Switzerland. Figure 5 shows the geographical distribution of the serogroups.

Molecular characterization using MLST (Table 1 and Figure 6) revealed that ST-11 (37%) was the most prevalent sequence type present in Switzerland in 2019, with 100% of the serogroup W strains and 75% of serogroup C strains harbouring a sequence type 11 profile. Distribution of ST in serogroup B is higly heterogeneous with almost half strains belonging to unique ST (41%). When looking in more details, almost all serogroup W strains (87.5%, 7/8) were of the same finetype (PorA 5,2:FetA 1-1:ST-11) inside the ST-11.

Applying EUCAST breakpoints, all invasive *N. meningitidis* strains tested were found to be susceptible to ceftriaxone, ciprofloxacin, meropenem, minocycline, and rifampicin. However, only 62.5% of these isolates were considered fully susceptible to penicillin

(this value is similar to that reported in 2018, Table 2). Penicillin non-susceptible strains were not associated to a specific serogroup.

**Summary of key observations**

- Serogroup B was the most frequently determined in invasive strains of meningococci (52%), followed by serogroup W (24%). The remaining cases were associated with serogroup C (12%), Y (9%) and X (3%) strains.
- Predominant MLST profile was ST-11.
- All but 1 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 (analysis is ongoing).
- Susceptibility of *N. meningitidis* to antibiotics recommended for prophylaxis (rifampicin and ciprofloxacin) and treatment (ceftriaxone) remained 100%. However, susceptibility to penicillin, according to EUCAST breakpoints, was only 62.5%.

## 9. Discussion

In 2019, a total of 43 cases of invasive meningococcal diseases were reported to the SFOPH. According the the SFOPH and for the first time since 2014, the incidence in 2019 was lower as compared to 2018 (0.50 in 2019 and 0.73 in 2018 for 100 000 inhabitants). However, mean incidence for the last 10 years is 0.61 with a standard deviation of 0.13, suggesting that the incidence remained stable over the last decade. The main change in meningococcal epidemiology in Switzerland in 2019 is the decrease in the development of serogroup W135 hypervirulent strain. This serogroup W135 is mostly of clonal origin, some of the isolates were linked with the strain described in the UK (Ladhani et al., 2015) and spreading into other European countries (like Switzerland until 2018) as described in the Netherlands by Knol and colleagues in 2017 (Knol et al., 2017). Importantly, no such expansion has been observed for any other meningococcal subpopulation since the emergence of serougroup C between 1994-1996 (Gray et al., 2006).

This particular strain of meningococcus (W135) is associated with unusual clinical presentations (especially pneumonia, more often bacteriemic or along with purpura fulminans), and affects 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).

Despite increased distribution of serogroup B in Switzerland in 2019 as compared to previous years (52% in 2019 vs 26% in 2018), the absolute number of serogroup B invasive meningococci remained stable (n=17 in 2019 vs n=15 in 2018).

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.

## 10. Acknowledgements

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

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## Figures

**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 2019.

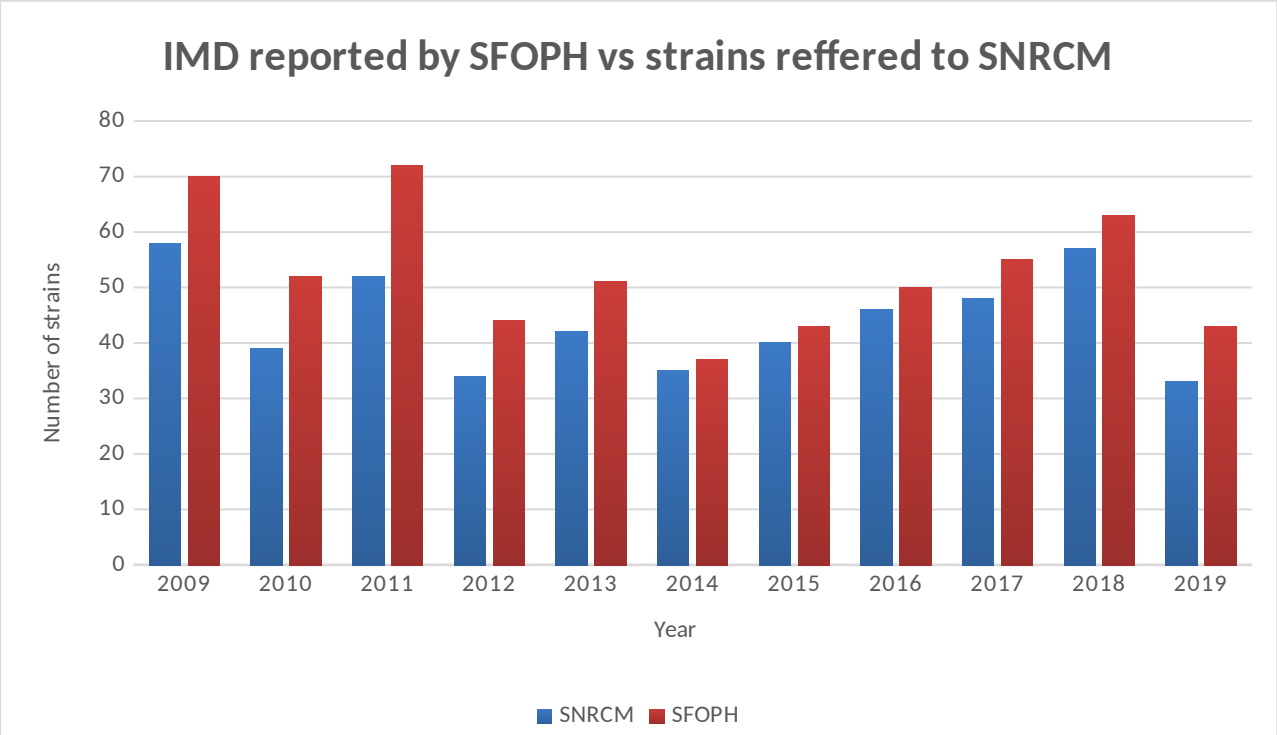


**Figure 2.** Serogroup distribution in 2019

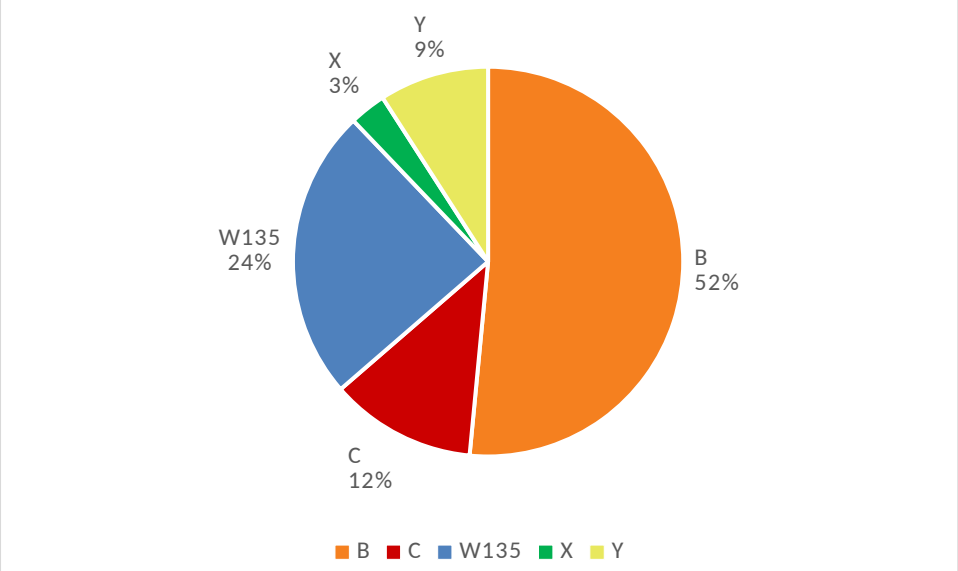


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**Figure 3.** Number of isolates in 2019, by serogroups and age groups.

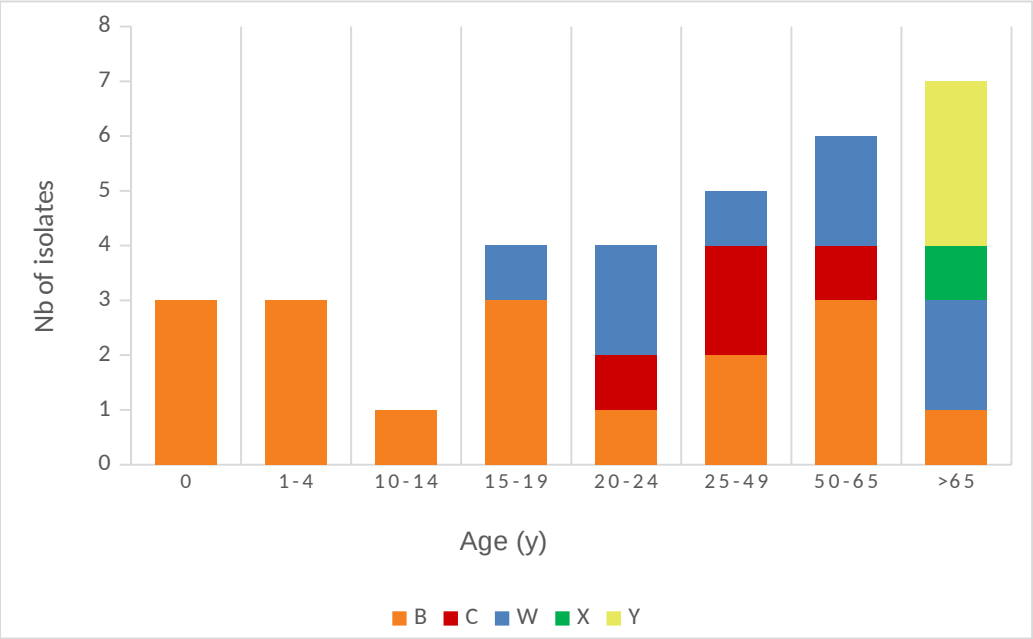



**Figure 4.** Annual number of strains representing main serogroups B, C, X, Y and W135 of invasive *N. meningitidis* as determined at the Swiss National Reference Center for Meningococci from 2009 to 2019

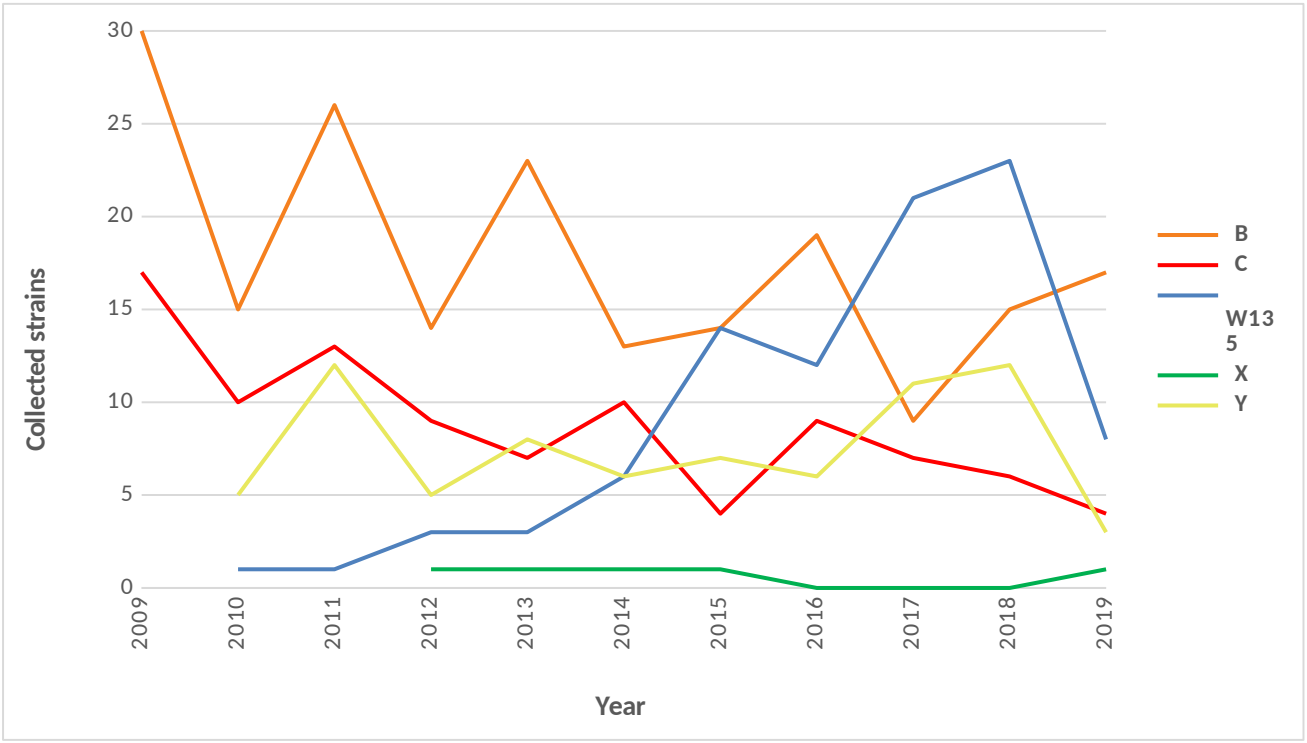



**Figure 5.** Distribution of serogroups by geographical regions in 2019

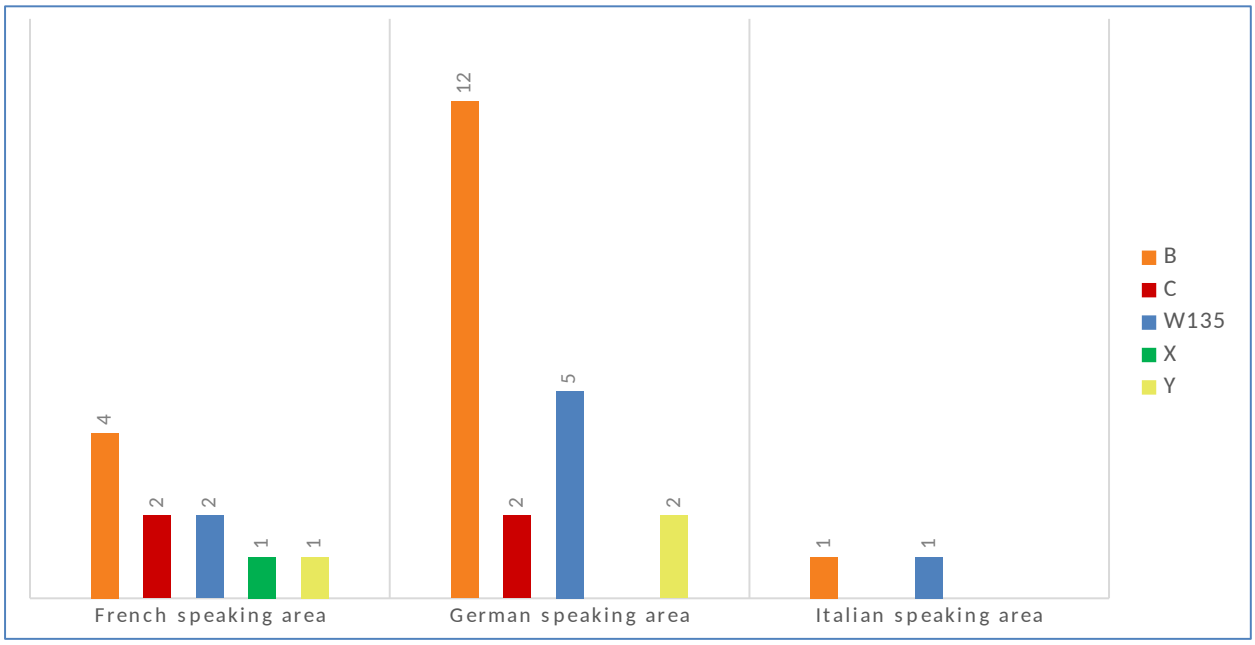
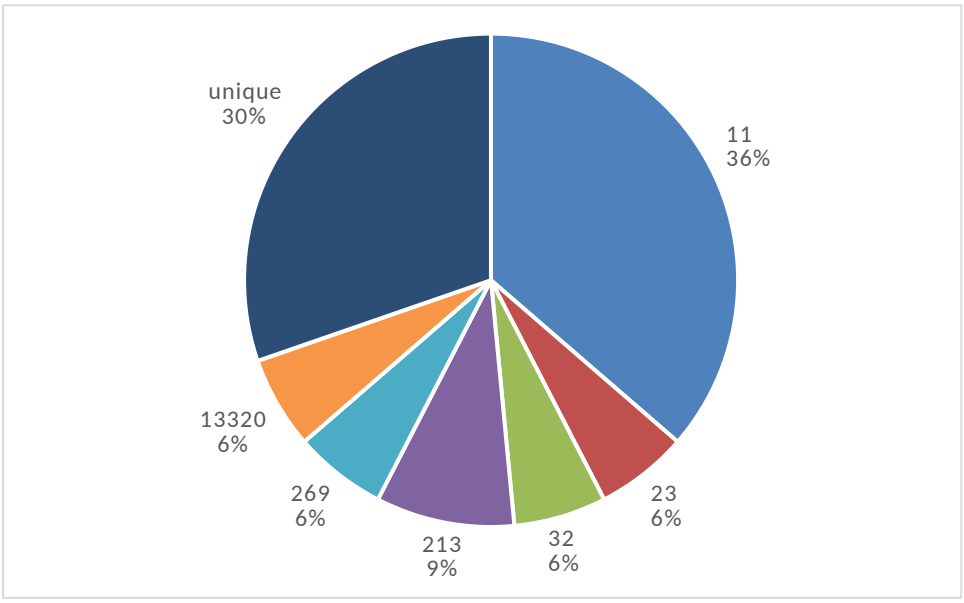



**Figure 6.** Distribution of sequence types in 2019

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# Tables

**Table 1.** Synopsis of MLST profiles and serogroups of invasive *N. meningitidis* strains referred to the Swiss National Reference Center for Meningococci in 2019.

| Serogroups | Sequence type (MLST) |
|---|---|
| **B** | **3 ST213** (17%) + **7 ST uniques*** (41%) + 2 ST32 (12%) + 2 ST269 (12%) + 2 ST13320 (12%) + 1 ND (6.0%) |
| **C** | **3 ST11** (75%) + 1 ST5133 (25%) |
| **W135** | **8 ST11** (100%) |
| **Y** | 2 ST23 (67%) + 1 ST3582 (33%) |
| **X** | 1 ST9126 (100%) |

*: includes one ST11

**Table 2.** Antimicrobial susceptibility testing (EUCAST breakpoints) of 33 invasive *N. meningitidis* strains referred to the Swiss National Reference Center for Meningococci in 2019.

| | Minimum inhibitory concentration (MIC) | | | Breakpoint susceptible (≤ µg/mL) | % of strains considered susceptible |
|---|---|---|---|---|---|
| | Range | MIC50 | MIC90 | | |
| **Penicillin** | 0.016-1 | 0.064 | 0.38 | 0.06 | 62.5 |
| **Ceftriaxone** | <0.002-0.006 | 0.002 | 0.002 | 0.12 | 100 |
| **Meropenem** | 0.003-0.064 | 0.006 | 0.032 | 0.25 | 100 |
| **Ciprofloxacin** | 0.002-0.006 | 0.004 | 0.006 | 0.03 | 100 |
| **Minocycline** | 0.094-0.38 | 0.19 | 0.25 | 1 | 100 |
| **Rifampicin** | 0.004-0.19 | 0.016 | 0.094 | 0.25 | 100 |

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