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Annual Report of the Swiss National Reference Center for Meningococci, 2020

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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 2020.

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 2020). 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).

We have then further analyzed the molecular epidemiology of *N. meningitidis* W135 (NmW) between 2017 and 2018 in Switzerland. In this period, we reported the circulation of three main NmW lineages: the Hajj-related, South American and ST-9316. While the first two lineages are part of the same clonal complex 11 and were already present in Switzerland, ST-9316 was new and emerged in 2018 in the canton of Vaud.

We showed that the distribution of cc11 lineages is quite heterogenous without a precise geographical localization. We identified several outbreaks that occurred in 2017-2018 due to cc11 lineages. In particular, we observed that some of these outbreaks were sub-variants of already circulating strains. Monitoring the current situation by WGS is strongly recommended as the heterogeneity of circulating lineages detected so far can favor the evolution and emergence of new strains.

With our analyses, we further confirm that WGS represent the only technique that can allow to capture a detailed epidemiological picture, nation-wide, of a complex species like *Neisseria meningitidis*. A manuscript is in preparation.

6. Additional meningococcal research

Our work on meningococci was also partially presented (during lectures) at the following meetings:

02. **J. SCHRENZEL**: Value of Rapid Diagnostics: Critical Integration with Clinical Care. Invited speaker at the 30th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID). Paris, France, April 2020.

01. **J. SCHRENZEL**: Digitalization and machine learning in clinical microbiology. Digitalization and Infectious Diseases. Basel, Switzerland, January 2020.

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 2020, the CNM has received a total of 12 invasive isolates of *N. meningitidis*. These strains were isolated from blood specimens (n=10), cerebrospinal fluid (n=1) and synovial fluid (n=1).

The strains received were isolated from 8 female and 4 male patients, representing 60% of all cases of invasive meningococcal diseases (n=20) reported to Swiss public health authorities in 2020 (SFOPH; Figure 1).

Since 2014, the number of invasive meningococci isolated was increasing (Figure 1). However, in 2020, this number drastically dropped in Switzerland as compared to 2019 (2.8 fold less strains isolated; Figure 1). This downward trend was already observed in 2019 but was deeply magnified by the sanitary situation linked to Sars-CoV-2. Among invasive meningococci, serogroups W and B were equally frequently isolated (n=4, 33.5%, each), followed by serogroup Y (n=3, 25%) and serogroup C (n=1, 8%) (Figure 2). The amount of invasive strains isolated in 2020 related to the epidemiological situation make irrevelevant any comparision of serogroup evolution between 2019 and 2020 (Figure 3).

All serogroup B strains were isolated from young patients <15 years old (Figure 4). Serogroup W strains were collected from all patients' groups (from 1 to >65 yo), serogroup

C from one adult (20-24 years old) and serogroup Y was isolated from adults and senior patients (20 to >65 years old).

All serogroups (B, C, Y and W135) were mostly recovered from German speaking regions of Switzerland (67%), although some strains were isolated in the French (25%, W and Y) and Italian (8%, B) speaking regions. Figure 5 shows the geographical distribution of the serogroups, expected based on the Swiss demography.

Molecular characterization using MLST (Table 1 and Figure 6) revealed that ST-11 (33%) was the most prevalent sequence type present in Switzerland in 2020, with 100% of the serogroup W strains. All other serogroups identified were unique (n= 8; 67%). One ST could not be identified (ND). When looking in more details, almost all serogroup W strains (75%, 3/4) were of the same finetype (PorA 5,2:FetA 1-1:ST-11) inside the ST-11. The fourth W strain was very closely related with the same PorA but a different FetA (PorA 5,2:FetA 1-94:ST-11).

Applying EUCAST breakpoints (v10; 2020), all invasive *N. meningitidis* strains tested were found to be susceptible to ceftriaxone, ciprofloxacin, meropenem, minocycline, and rifampicin. However, only 50% of these isolates were considered fully susceptible to penicillin (Table 2). Penicillin non-susceptible strains were not associated to a specific serogroup. MIC50 and MIC90 determined in 2020 were identical to 2019 except for Meropenem and Minocyclin which both revealed a higher MIC50, but only Minocycline had a higher MIC90.

Summary of key observations

- The number of *Neisseiria meningitidis* isolated from invasive infection was drastically decreased compared to 2019, most probably related to the COVID19 epidemiological situation and its strongly enforced social contact measures.
- Serogroups B and W were the most frequently determined in invasive strains of meningococci (34% each), followed by serogroup Y (25%). The remaining case was associated with serogroup C (8%) 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.
- 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 (v10; 2020), was only 50%.

9. Discussion

In 2020, a total of 20 cases of invasive meningococcal diseases were reported to the SFOPH. According the the SFOPH and for the second time since 2014, the incidence in 2020 was lower as compared to 2019 (0.23 in 2020 and 0.51 in 2019 for 100 000 inhabitants). Mean incidence for the last 10 years is 0.57 with a standard deviation of 0.18, suggesting that the incidence is beginning to decrease (mean incidence for 10 years in 2019 was 0.61 with SD=0.13). The main change in meningococcal epidemiology in Switzerland in 2020 (similar to 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 to the strain described in the UK (Ladhani et al., 2015) that spreaded 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).

Distribution of serogroup B in Switzerland in 2020 is lower as compared to 2019 but remains higher as compared to 2018 (33.5% in 2020 vs 52% in 2019 vs 26% in 2018). However, the absolute number of serogroup B invasive meningococci decreased (n=4 in 2020 vs n=17 in 2019), most likely due to COVID-19 sanitary measures enforced in Switzerland.

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

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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 2020.

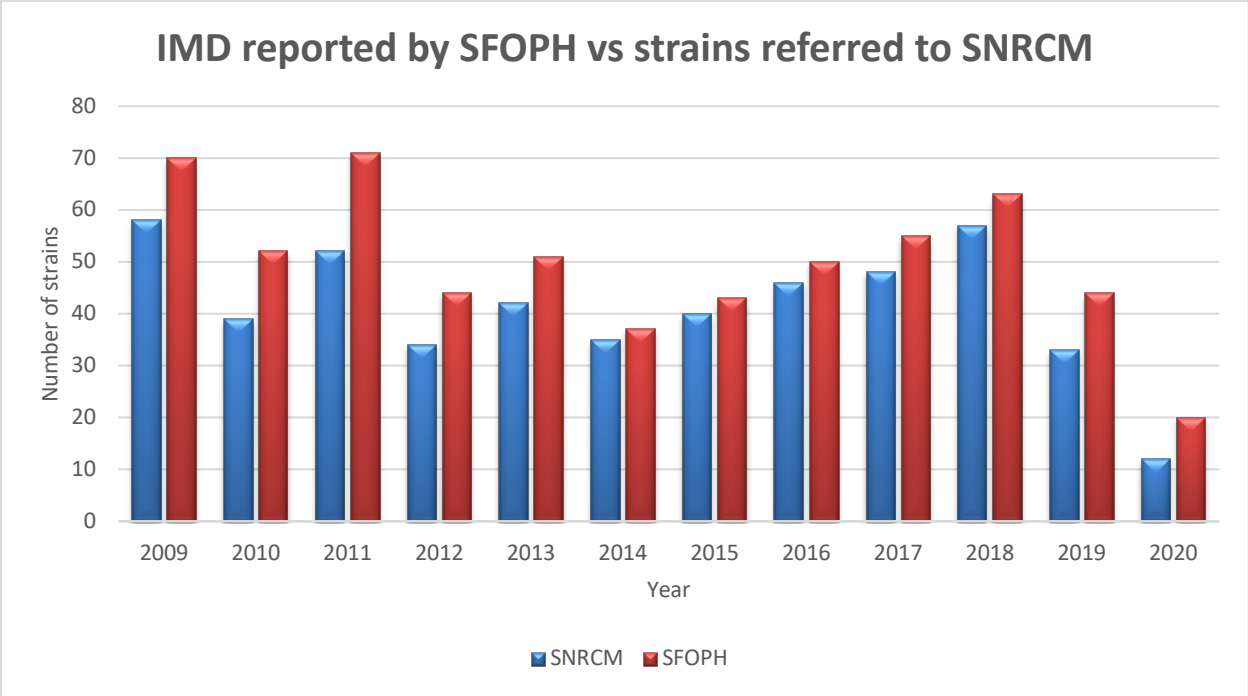


Figure 2. Serogroups distribution in 2020.

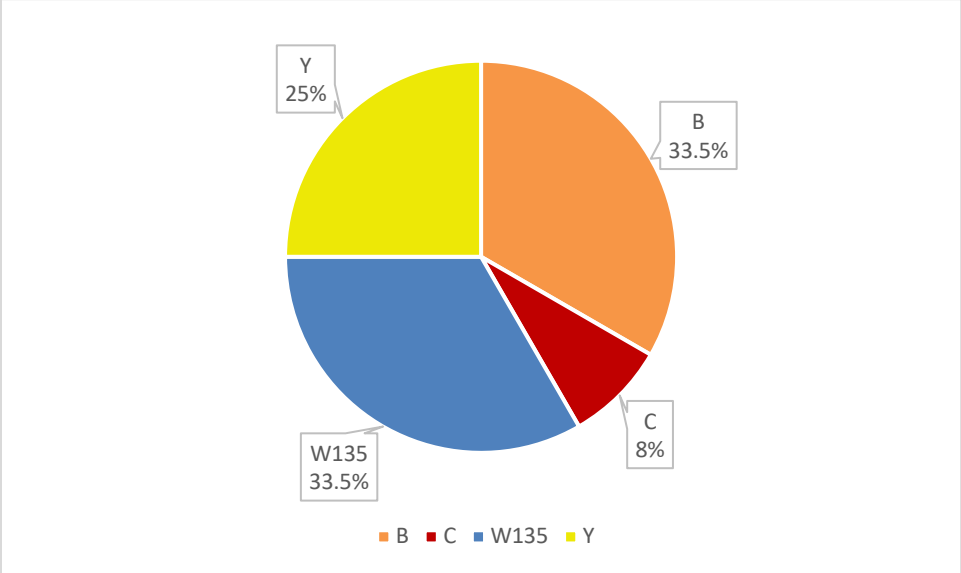


Figure 3. Annual number of strains representing the main serogroups B, C, X, Y and W135 of invasive *N. meningitidis* as determined at the Swiss National Reference Center for Meningococci from 2009 to 2020.

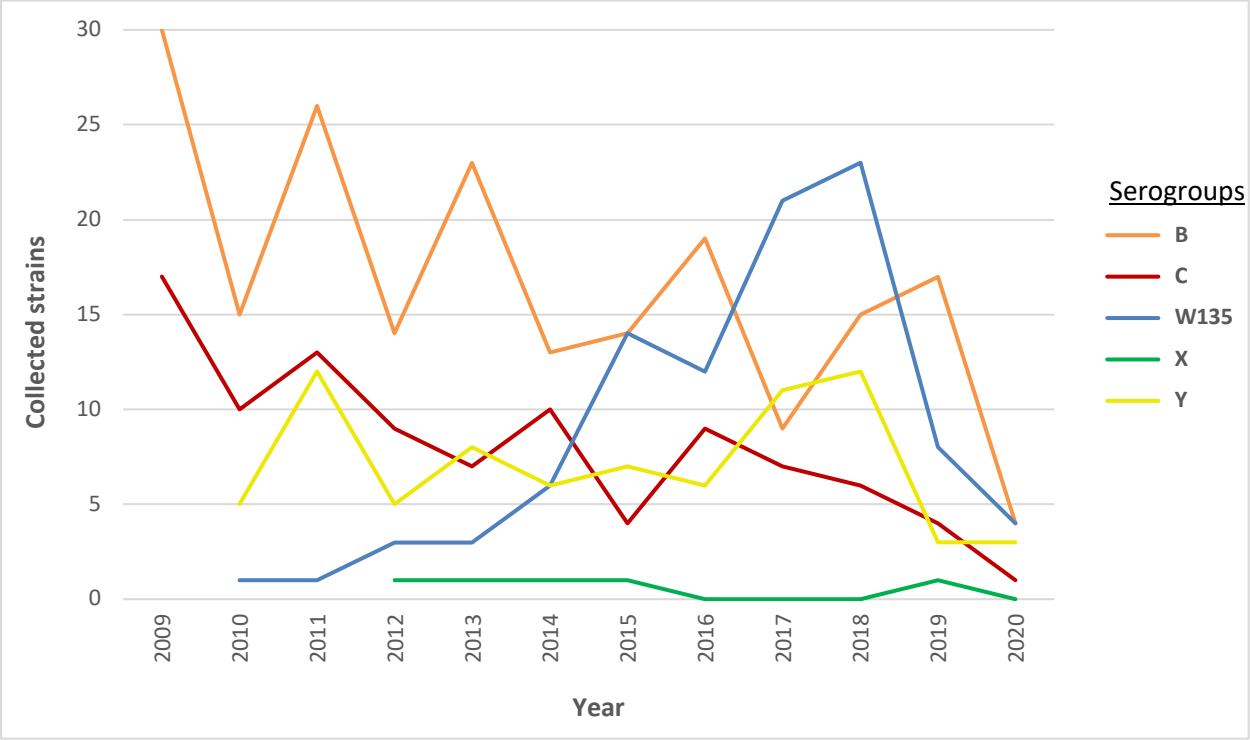


Figure 4. Number of isolates in 2020, by serogroups and age groups.

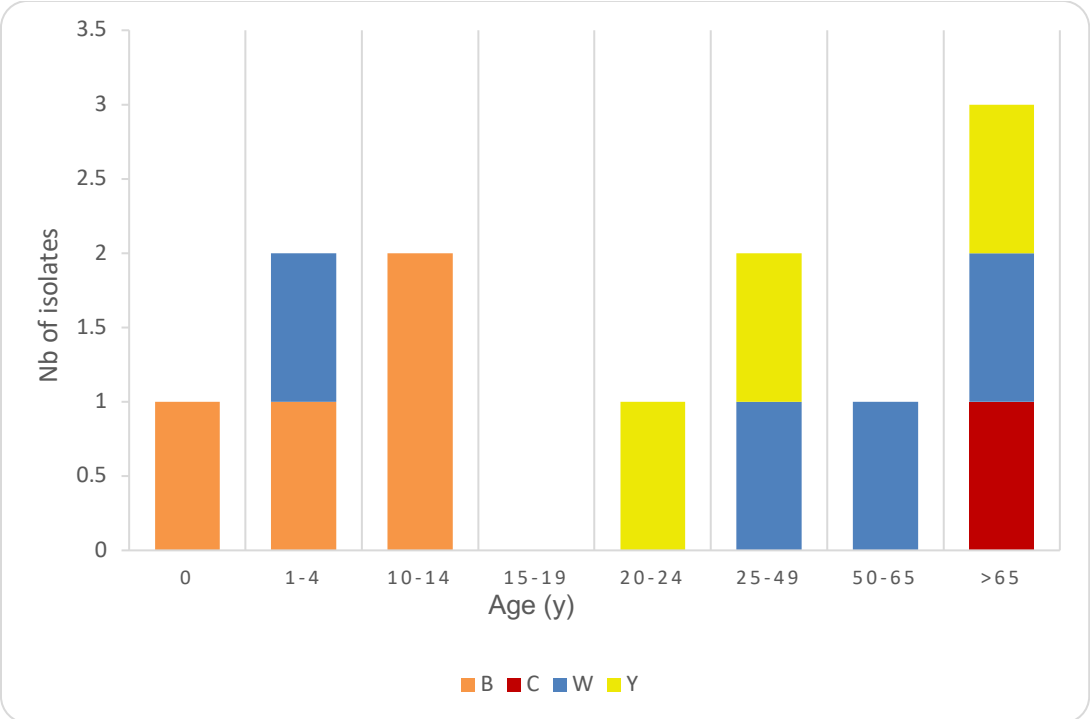


Figure 5. Distribution of serogroups by geographical regions in 2020

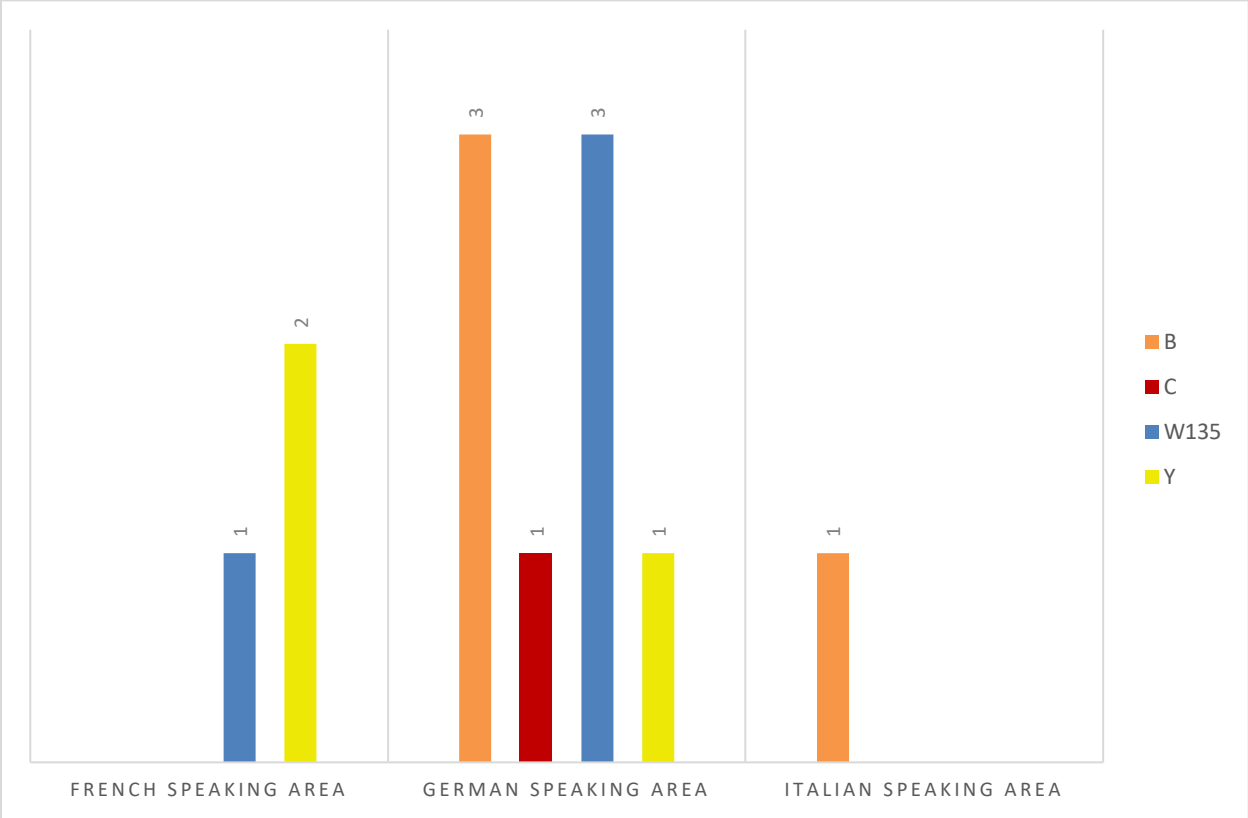
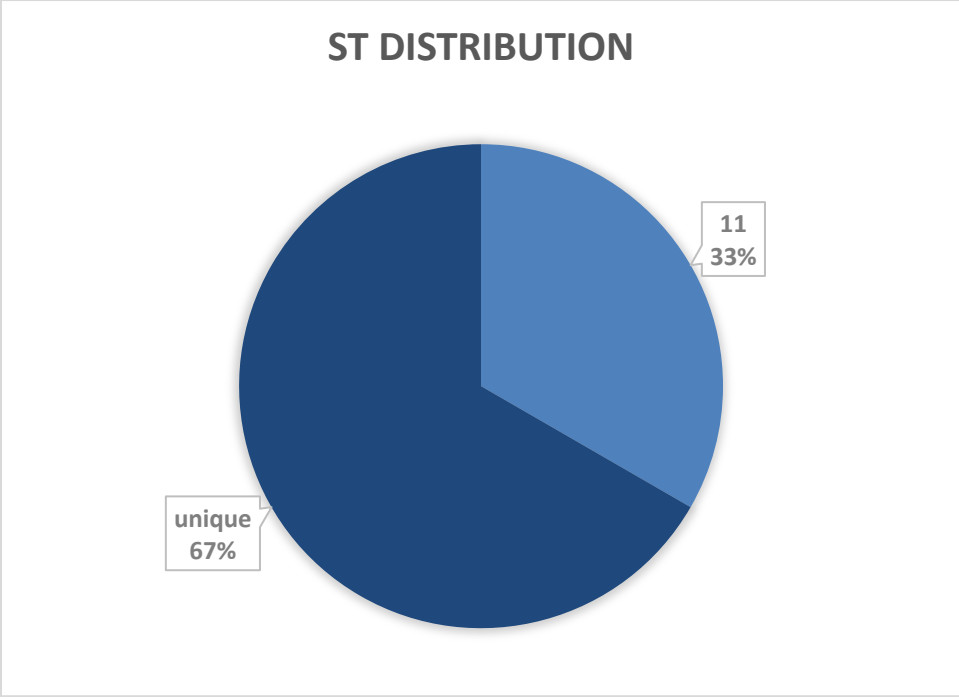


Figure 6. Distribution of sequence types in 2020



Tables

Table 1. Synopsis of MLST profiles and serogroups of invasive *N. meningitidis* strains referred to the Swiss National Reference Center for Meningococci in 2020.

Serogroups	Sequence type (MLST)
B	4 ST unique (100%)
C	1 ST6928 (100%)
W135	4 ST11 (100%)
Y	2 ST unique (66.7%) + 1 ST ND (33.3%)

Table 2. Antimicrobial susceptibility testing (EUCAST breakpoints) of 12 invasive *N. meningitidis* strains referred to the Swiss National Reference Center for Meningococci in 2020.

	Minimum inhibitory concentration (MIC)			Breakpoint susceptible (≤ µg/mL)	% of strains considered susceptible
	Range	MIC50	MIC90		
Penicillin	0.047-0.38	0.064	0.38	0.06*	50**
Ceftriaxone	<0.002-0.002	0.002	0.002	0.12	100
Meropenem	0.006-0.032	0.012	0.032	0.25	100
Ciprofloxacin	0.002-0.006	0.004	0.006	0.03	100
Minocycline	0.125-0.5	0.25	0.38	1	100
Rifampicin	0.004-0.064	0.016	0.047	0.25	100

* Penicillin susceptibility breakpoint has been changed in the new EUCAST clinical breakpoint version (v11; 2021) and is now (in 2021) at 0.25mg/L. This modification will be integrated in the future CNM report and will have important consequences on Penicillin susceptibility evolution between 2020 and 2021. If this new breakpoint was used for 2020 data, 83% of invasive *Neisseria meningitidis* would be susceptible to Penicillin (instead of 50%).This will be discussed in the next CNM report (CNM 2021).

** Penicillin susceptibility was intermediate for 16.7% of the isolates

Red: increase (resistance) vs 2019
Green: decrease (resistance) vs 2019
Black: identical to 2019