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Annual Report of the Swiss National Reference Center for Meningococci, 2022

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1. Introduction

Neisseria meningitides nasopharynx colonization of healty people is about 1/10. Colonized people spread the bacteria to others by respiratory secretions (e.g., saliva). When the conditions are met in at risk-people, the transmitted bacteria invade the body and cause different types of illnesses. Invasive meningococcal infection is an overwhelming disease that is leading to substantial mortality and morbidity. The case fatality rate of *N. meningitidis* is about 7% in high income countries. However, in low income countries, the fatality rate can reach up to 50%. Developmental disorders, and hearing loss remain among the main neurological sequelae observed in the survivors of the disease (Asturias et al., 2022).

Invasive strains of *N. meningitidis* 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.

The last decade has witnessed considerable changes in the epidemiology of invasive meningococcal infections in Europe and Switzerland with the increase in the prevalence of Y and W serogroups. Arthritis, pharyngitis, and pneumonia represent some of the atypical clinical manifestations related to these serogroups.

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 performs 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 2022.

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. The identification is confirmed 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 assessed 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]).

In addition, multilocus sequence typing (MLST) is carried out 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).

The antimicrobial susceptibility testing is performed for each isolate using Mueller-Hinton agar + 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F, bioMérieux). Minimum inhibitory concentrations (MICs) are measured for penicillin, ceftriaxone, meropenem, ciprofloxacin, minocycline and rifampicin by E-test strips (AB Biodisk, bioMérieux). The MICs are interpreted according to the current breakpoints recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, www.eucast.org).

In case of no growth of the strain, clinical specimens are analyzed by qPCR to screen for *N. meningitidis* DNA , and if present, we assess the occurrence of the main serogroups by amplifying their 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 performed to assign *N. meningitidis*-positive specimens to serogroups B, C and Y/W135; assignment to serogroup A is achieved by qPCR targeting the *sacC* gene (Mölling et al., 2002). Finally, differentiation between serogroups Y and W135 is asessed 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 2022). 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 recently 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 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 highlighted 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.

Acoording to our analyses, the WGS represents the only technique that can allow to capture a detailed epidemiological picture, nation-wide, of a complex species like *Neisseria meningitidis*.

6. Additional meningococcal research

Our recent work on meningococci was submitted for publication to Microbiol Res. Announcement.

F. MAUFFRAY, N. GAIA, C. CHAABANE, G. RENZI, A. FISCHER, A. CHERKAOUI, **J. SCHRENZEL**, and V. LAZAREVIC: Draft genome sequence of a mixed serogroup W/Y invasive *Neisseria meningitidis* strain.

7. Advisory service and Networking

7.1 Advisory service

Molecular testing:

We systematically conduct molecular assays to define the serogroups using isolates or directly from clinical specimens when the bacterial growth is not possible (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 further analyze 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 2022, the CNM has received a total of 12 invasive isolates of *N. meningitidis*. These strains were isolated from blood specimens (n=10) and cerebrospinal fluid (n=2) (Figure-1).

Figure-2 depicts the number of *N. meningitidis* strains isolated in 2022 according to gender and serougroups.

Since 2014, the number of invasive meningococci isolated was increasing (Figure 3). However, in 2021 and 2022, the number of invasive *N. menigitidis* isolates was very low compared to previous years (1.7 fold less strains isolated; Figure 1). Similar to 2020, this downward trend already observed in 2019 was deeply magnified by the sanitary situation linked to Sars-CoV-2.
Due to the low number of meningococci isolated in 2021 and 2022, epidemiological analysis should be considered with caution. Trends observed in 2021 and 2022 might not reflect a real change in meningococcal epidemiology, but rather the consequences of the sanitary measures deployed during the pandemics.

Among invasive meningococci, serogroup B was the most frequently isolated (9/12; 75%), followed by serogroups W and Y (2/12; 17%) and serogroup C (1/12, 8%) (Figure 4). The small number of invasive strains isolated in 2022 makes irrevelevant any comparison of serogroups evolution between 2020 and 2021 (Figure 5).

In 2022, the serogroup B was isolated in all age groups as depicted in Figure-6. However, the serogroups W and Y were isolated only in patients aged 50 and more.

Figure-7 shows the distribution of serogroups by geographical regions in 2022. No isolate was referred from the Italian speaking region in 2022.

Molecular characterization using MLST (Table 1 and Figure 8) revealed that there was no prevalent sequence type among the 12 isolates analyzed in 2022. One ST could not be identified (ND).

Applying EUCAST breakpoints (v12; 2022), all invasive *N. meningitidis* strains tested were susceptible to penicillin, ceftriaxone, ciprofloxacin, meropenem, minocycline, and rifampicin. Table-2 depicts the MICs ranges by drugs, with the MIC50 and MIC90.

Summary of key observations

- The number of *Neisseiria meningitidis* isolated from invasive infections was drastically decreased compared to 2020, most probably related to the COVID19 epidemiological situation and its strongly enforced contact measures.
- Serogroup B was the most frequent (9/12; 75%), followed by serogroups W and Y (2/12; 17%); and serogroup C (1/12, 8%).
- There was no prevalent sequence type among the 12 isolates analyzed in 2022.
- Susceptibility of *N. meningitidis* to antibiotics recommended for prophylaxis (rifampicin and ciprofloxacin) and treatment (ceftriaxone) remained 100%.

9. Discussion

In 2022, a total of 12 cases of invasive meningococcal diseases were reported to the SFOPH. According the the SFOPH and for the third time since 2014, the incidence in 2022 was lower as compared to 2020 (0.09 in 2021, 0.23 in 2020 and 0.51 in 2019 for 100 000 inhabitants). Average incidence for the last 10 years was 0.49 with a standard deviation (SD) of 0.19, suggesting that the incidence is indeed decreasing (average incidence for 10 years in 2020 was 0.57 with a SD of 0.18). The main change in meningococcal epidemiology in Switzerland in 2022 (similarly to 2021 and 2020) is the decrease in the development of the 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 patients over 50 years old). Therefore, Swiss recommendations for vaccination against meningococcal disease are adapted with the use of the quadrivalent MenACWY (capsular antigen) conjugate vaccine (See SFOPH website for the latest recommendations).

In Switzerland the proportion of serogroup B among the invasive strains in 2022 is higher than in 2021; 75% (9/12) versus 29% (2/7), reflecting the increase in absolute number of serogroup B invasive meningococci identified in 2022, yet with small numbers related to the sanitary measures.

10. Acknowledgements

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11. References

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Figures

Figure 1. Number of *N. meningitidis* strains isolated in 2022 according to age of the patients and specimen types.

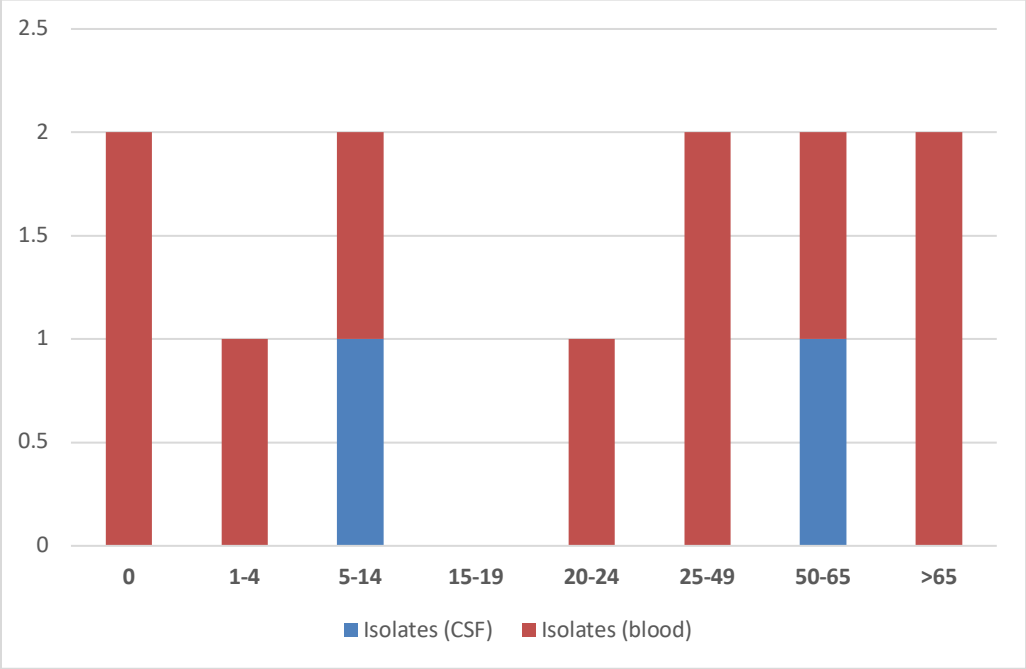


Figure 2. Number of *N. meningitidis* strains isolated in 2022 according to gender and serougroups.

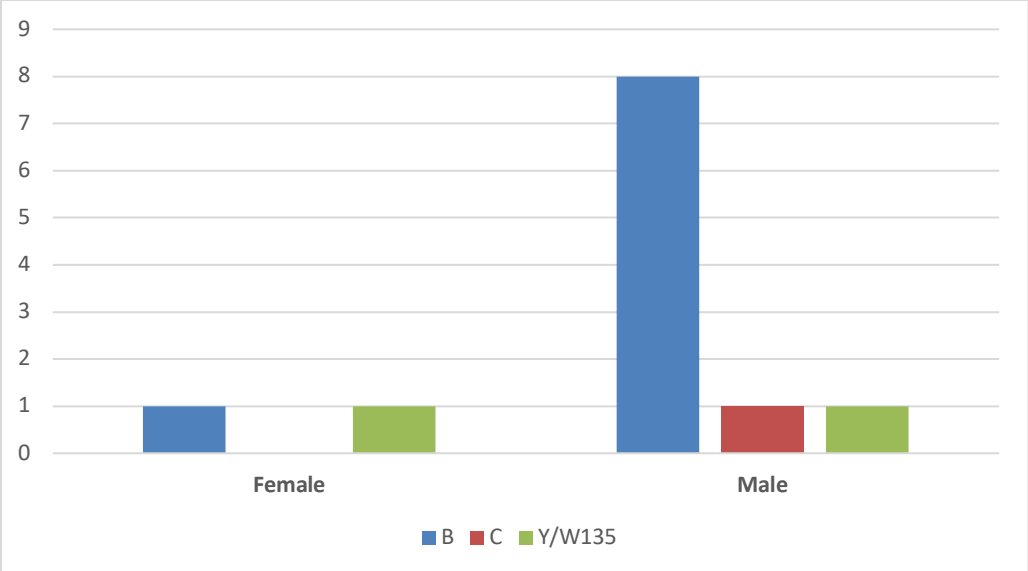


Figure 3. Annual number of cases of invasive meningococcal diseases reported to the Swiss Federal Office of Public Health (SFOPH) and number of *N. meningitidis* strains referred to the Swiss National Reference Center for Meningococci (SNRCM) from 2009 to 2022.

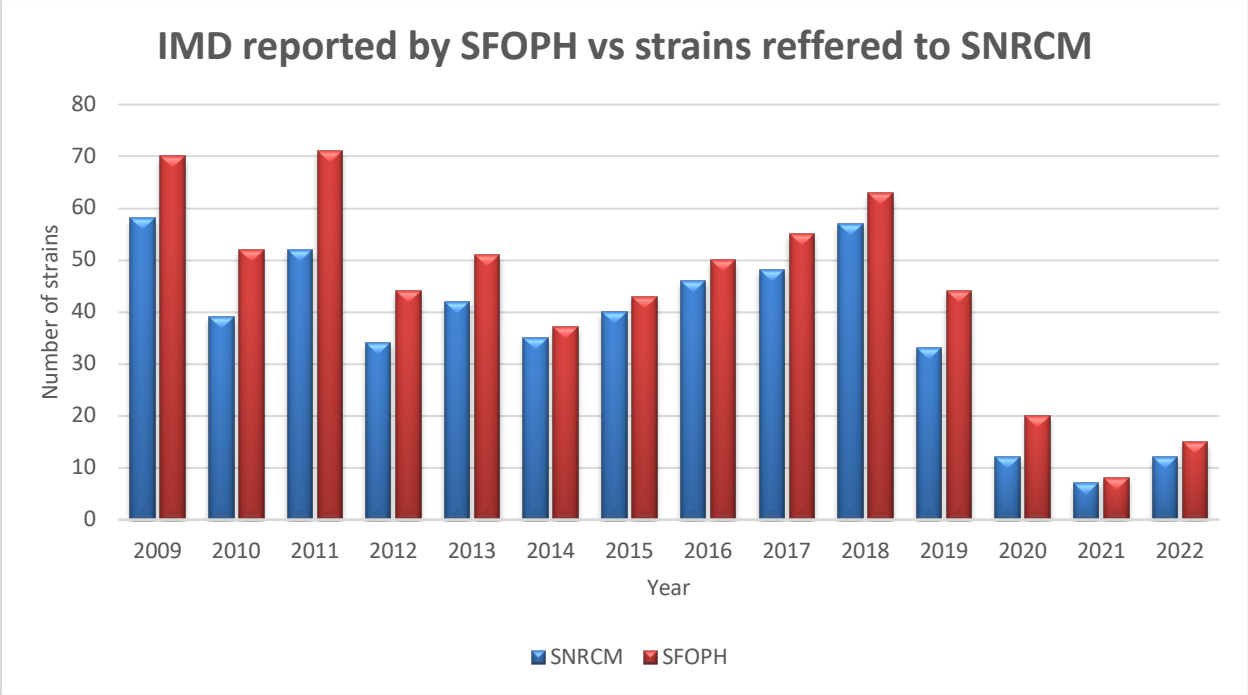


Figure 4. Serogroups distribution in 2022 (n=12).

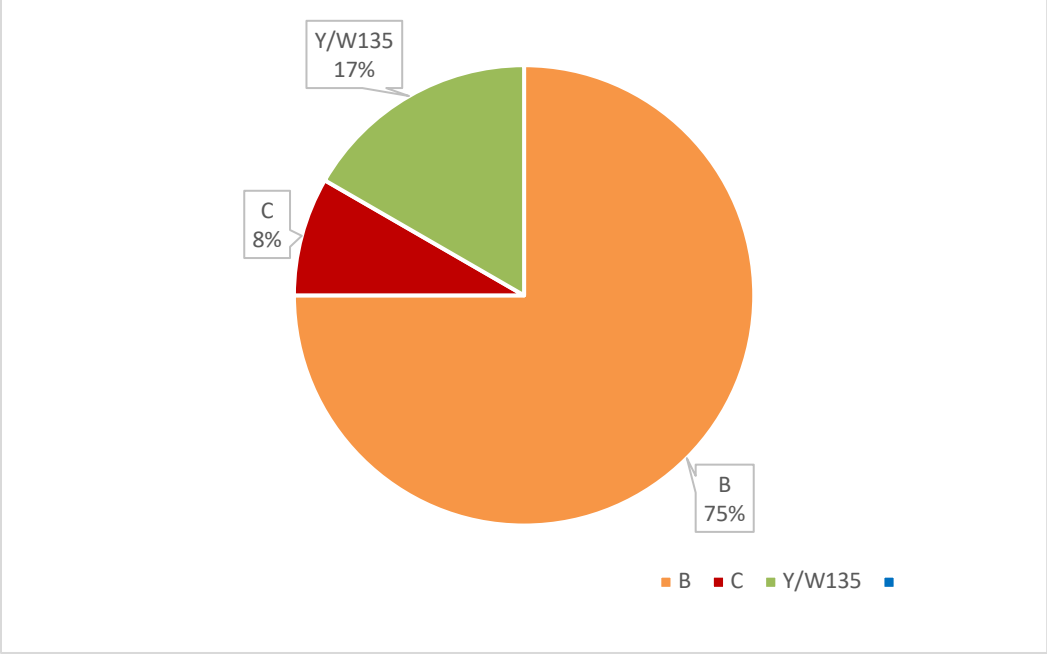
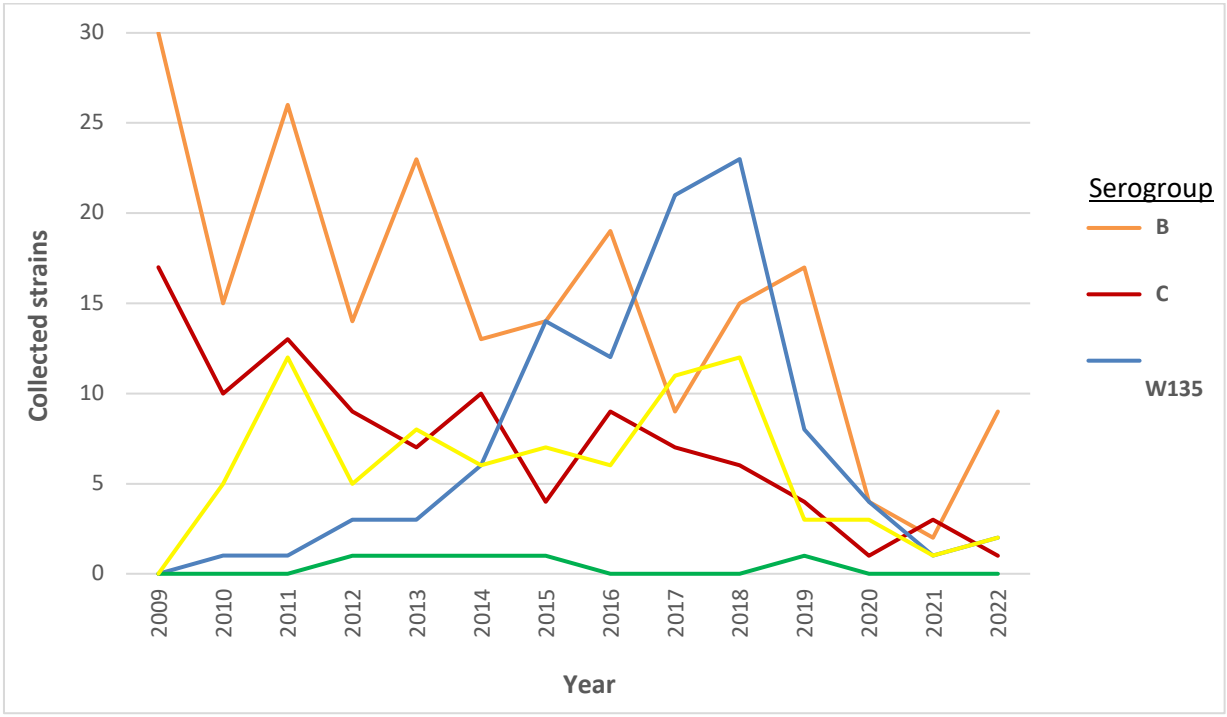


Figure 5. Annual number of strains representing the main serogroups B, C, X, Y and W135 of invasive *N. meningitidis* as determined at the Swiss National Reference Center for meningococci from 2009 to 2022.



Rem : in 2022 2 strains of *N. meningitidis* had both W135 and Y capsule. Therefore, 2 strains for W135 and 2 strains for Y are represented on this graph.

Figure 6. Number of isolates in 2022, by serogroups and age groups.

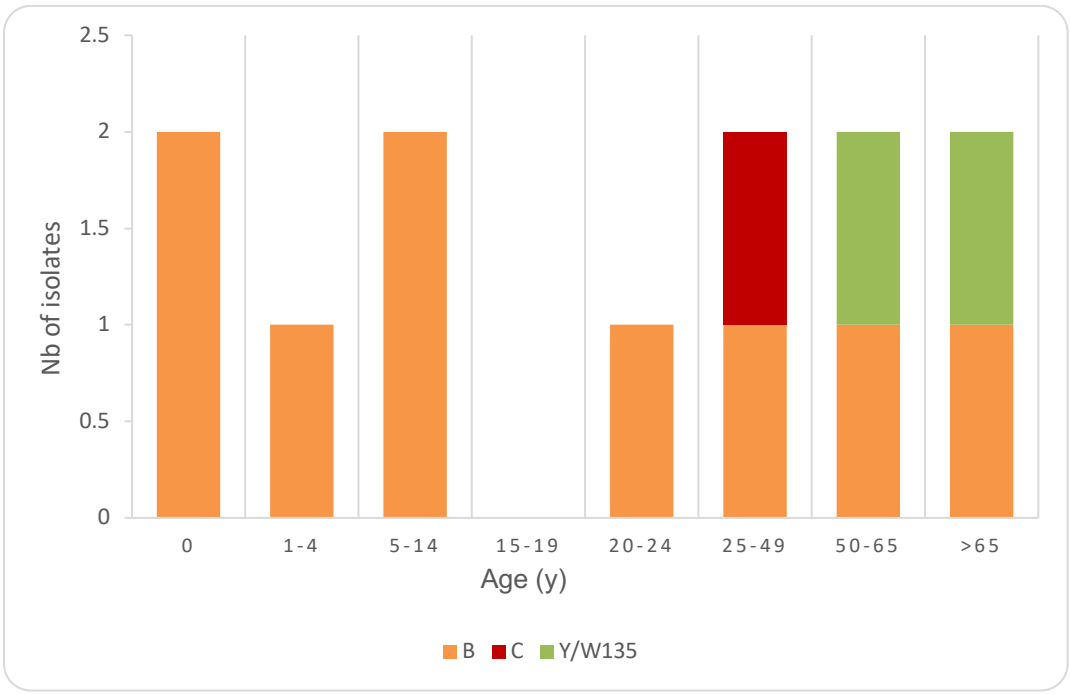


Figure 7. Distribution of serogroups by geographical regions in 2022

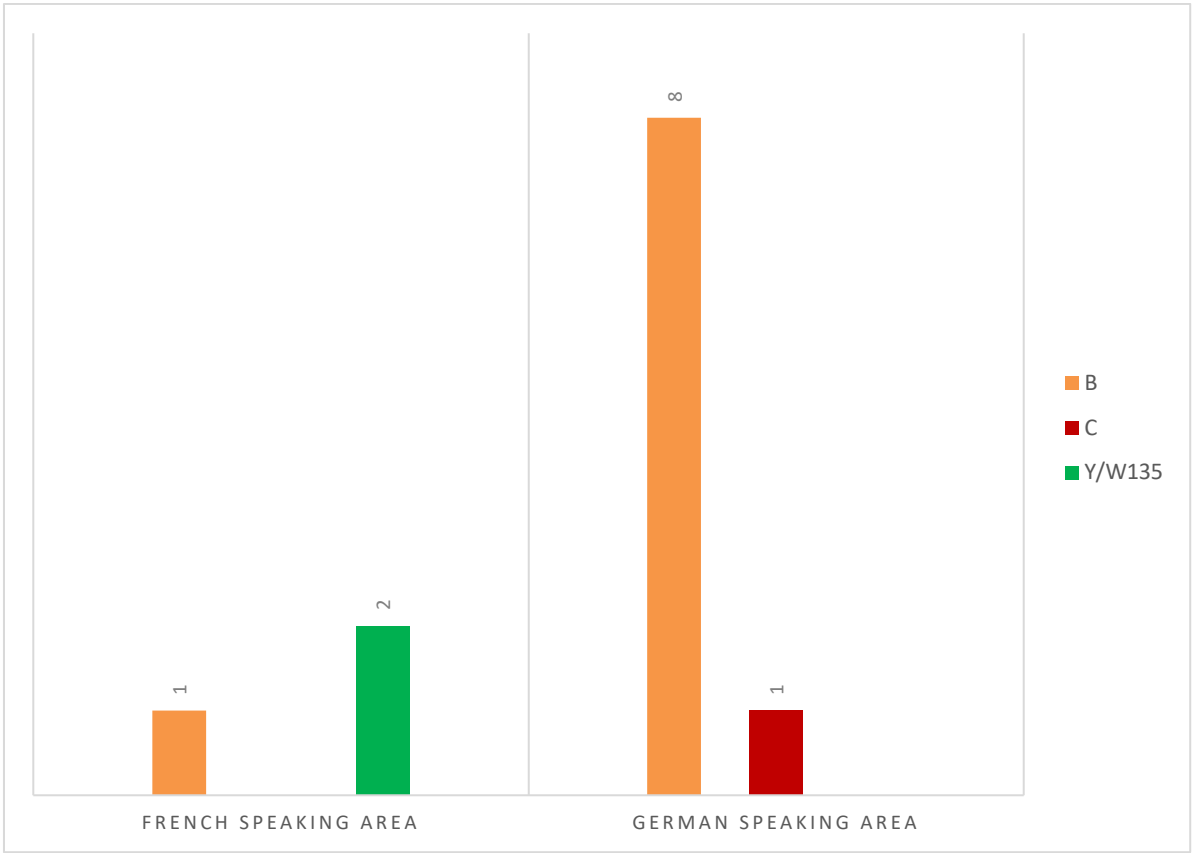


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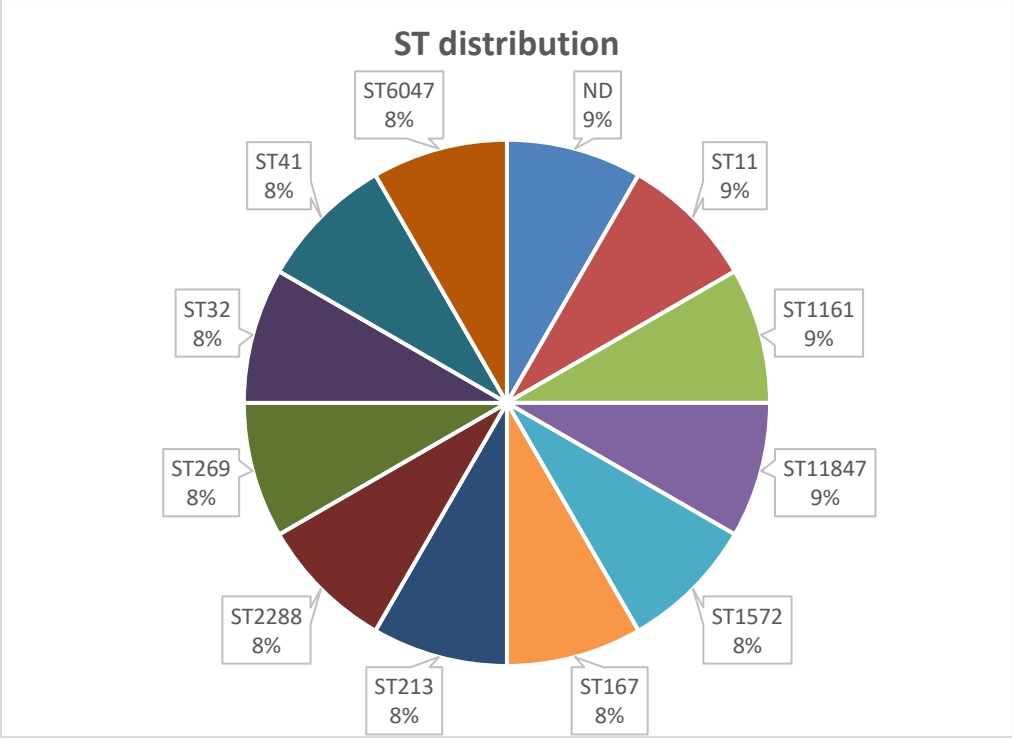
Figure 8. Distribution of sequence types in 2022



Tables

Table 1. Synopsis of MLST profiles and serogroups of invasive *N. meningitidis* strains referred to the Swiss National Reference Center for meningococci in 2022.

Serogroups	Sequence type (MLST)
B	8 ST diffrents (89%) + 1 ST ND (11 %)
C	1 ST11 (100%)
Y/W135	1 ST11847 (50%) + 1 ST167 (50%)

Table 2. Antimicrobial susceptibility testing (EUCAST breakpoints) of the 12 invasive *N. meningitidis* strains referred to the Swiss National Reference Center for meningococci in 2022.

	Minimum inhibitory concentration (MIC)			Breakpoint susceptible (≤ µg/mL)	% of strains considered susceptible
	Range	MIC50	MIC90		
Penicillin	0.032-0.25	0.094	0.25	0.25	100
Ceftriaxone	0.002-0.016	0.002	0.002	0.12	100
Meropenem	0.004-0.047	0.008	0.032	0.25	100
Ciprofloxacin	0.002-0.006	0.003	0.004	0.03	100
Minocycline	0.032-0.5	0.25	0.5	1	100
Rifampicin	0.003-0.38	0.023	0.19	0.25	100

Red: increased (resistance) vs 2021
Green: decreased (resistance) vs 2021
Black: identical to 2021