Annual Report of the National Center for Meningococci 2012

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Introduction

Invasive strains of *Neisseria meningitidis* remain a life-threatening cause of bacterial meningitis and sepsis, mainly in infants, adolescents and young adults (Thigpen et al., 2011). However, 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 Center for Meningococci (CNM, Centre National des Méningocoques) at the University Hospital in Geneva.

The CNM provides reference testing of invasive *N. meningitidis* isolates since 1990 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 data, each strain is classified and data are integrated in national (SFOPH) and international epidemiological databases (European Meningococcal Epidemiology in Real Time [EMERT] database; http://emgm.eu/emert) in order to share information and monitor trends about meningococcal populations. In addition, each isolate is tested against a panel of generally recommended antibiotics against *N. meningitidis*. Antimicrobial susceptibility data are also used for surveillance purposes.

This annual report describes the methods used and results gathered at the CNM in calendar year 2012.

Materials and Methods

On behalf of the SFOPH, the CNM is investigating isolates of *N. meningitidis* as well as clinical specimens derived from normally sterile body sites.

Isolates are subcultured 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 (Pasteurex Meningitidis, Bio-Rad). Additional agglutination may include serogroups X, Y, W135 and Z (Difco Neisseria meningitidis antisera, Becton Dickinson) and a confirmation of identification (apiNH, BioMérieux).

Multilocus sequence typing (MLST) is performed on each isolate according to protocols recommended by the EMGM (http://emgm.eu). The current approach includes seven housekeeping genes (*abcZ*, encoding a putative ABC transporter; *adk*, adenylate kinase; *aroE*, shikimate dehydrogenase; *fumC*, fumurate; *gdh*, glucose-6-phosphate dehydrogenase; *pdhC*, pyruvate deshydrogenase 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, 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/).

In addition, molecular characterization includes sequencing of two variable regions in the gene encoding the antigenic outer membrane protein A (*porA*-VR1 and *porA*-VR2) and 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 (http://pubmlst.org/neisseria/).

Each isolate is then classified based on a combination of serotyping and molecular typing data according to the following scheme:

Serogroup : PorA-VR1, PorA-VR2 : FetA-VR : ST (or MLST clonal complex)

Isolates are tested for antimicrobial susceptibility on Muller-Hinton sheep blood agar using E-test strips (AB Biodisk, BioMérieux) containing penicillin, cefuroxime, ceftriaxone, minocycline, rifampicin, erythromycin, azithromycin, ciprofloxacin, and chloramphenicol, respectively. Minimum inhibitory concentrations (MICs) are interpreted according to current breakpoints recommended by the EMGM, the British Society for Antimicrobial Chemotherapy, and the Clinical and Laboratory Standards Institute (CLSI).
Conventional susceptibility testing is complemented via sequencing of the *penA* gene in order to detect mutations associated with altered penicillin-binding proteins which may confer reduced susceptibility to penicillin (Taha et al., 2007; http://pubmlst.org/neisseria/).

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 acids extraction from clinical specimens such as cerebrospinal and joint fluids is performed using the MagNAPure Compact system (Roche Diagnostics). DNA is amplified by real-time PCR to screen for the presence of the *N. meningitidis*-specific *ctrA* capsule gene (Corless et al., 2001).

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 *mynB* 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

In calendar year 2012, the CNM has received a total of 34 isolates of *N. meningitidis*. These strains were mainly derived from normally sterile body sites including blood (N=22), cerebrospinal fluid (N=7), and joint fluid (N=2); one strain was isolated from a sinus specimen and the two remaining strains were referred without corresponding information.

The strains received were isolated from 20 male and 14 female patients and represented 73.9% of all cases of invasive meningococcal diseases (N=46) reported to Swiss public health authorities in 2012 (Figure 1).

Most patients belonged to one of the following age groups: very young children aged 0-2 years (N=8), teenagers (10-19 years) (N=11), and young adults (20-29 years) (N=6). In any other age group (up to 80+), there were only sporadic cases.

Serotyping by agglutination revealed that most isolates belonged to serogroup B (N=14, equivalent to 41.2%), followed by serogroup C (N=9, 26.5%), serogroup Y (N=5, 17.7%) and serogroup W135 (N=3, 8.8%) (Figure 2). One isolate each was assigned to serogroup A (2.9%) and X (2.9%), respectively, and one isolates could not be classified by any agglutination assay.

Serogroup B strains were predominant in children aged 0-2 years (75% of cases). The most prominent serogroups in teenagers (10-19 years) were C (36%), Y (27%) and B (18%) whereas in young adults aged 20-29 years serogroups B (50%) and C (50%) were equally distributed.

Serogroup B, C and W135 strains were recovered with comparable frequencies in both the French and German speaking (Western and Eastern) areas of Switzerland.

In contrast, all serogroup Y strains except one were isolated in German speaking districts.

Molecular characterization using MLST revealed the occurrence of 14 different sequence types, however, there were only three types found in more than two isolates, namely ST-11 (N=9), ST-23 (N=6) and ST-32 (N=4); all other types remained sporadic. ST-11 and ST-23 correlated with serogroup C and Y agglutination, respectively. All ST-32 occurred in serogroup B strains which also exhibited most of the sporadic STs many of which being assigned to the ST-41/44 complex (Table 1).

Subtyping by partial sequence analysis of genes encoding outer membrane proteins further substantiated the above molecular findings in that all serogroup C strains harbored *porA*-VR1 type 5, *porA*-VR2 type 2 and *fetA*-VR type F3-3 alleles. All but one serogroup Y strains showed *porA*-VR1 type 5-2, *porA*-VR2 type 10-1, and *fetA*-VR type F4-1 alleles; the remaining serogroup Y strain differed from this combination only by its *fetA*-VR allele which was of type F5-12. In contrast, many different types of *porA*-VR1, *porA*-VR2 and *feta*-VR alleles were detected among the serogroup B isolates (Figures 3).

For susceptibility testing of penicillin, we applied the breakpoint recommended by the EMGM ($\leq$ 0.094 µg/ml) which is slightly above the corresponding CLSI value ($\leq$ 0.064 µg/ml). Using the European breakpoint, 76% of all isolates were found susceptible to this agent. All of the remaining isolates were intermediate; no isolate exhibited a MIC suggesting resistance to this substance (values $\geq$ 0.5 µg/ml).
The molecular assessment of susceptibility via *penA* gene analysis correlated with the MICs observed for penicillin. All isolates with MICs $\geq$ 0.19 µg/ml harboured the characteristic five mutations in their *penA* gene sequence, however, one isolate considered susceptible (MIC 0.094) also contained these mutations, and two apparently intermediate isolates (MIC 0.125) showed no mutation in that gene (Table 2).

Susceptibility testing to cefuroxime, ceftriaxone, minocycline, rifampicin, azithromycin, ciprofloxacin, and chloramphenicol showed that all isolates were

susceptible to these agents. Reduced susceptibility to erythromycin was observed in 35% of isolates tested (Table 3).

In calendar year 2012, the CNM has received a total of 39 clinical specimens for direct detection of *N. meningitidis* DNA by PCR. All these specimens consisted of cerebrospinal fluids or DNA extracts thereof. No DNA extract showed PCR inhibition and *N. meningitidis* DNA was found in five samples (13%). Subsequent PCR to assess the serogroup revealed that three samples contained serogroup B and one sample serogroup C; the remaining sample was not included in serogroup testing by PCR since its serogroup (B) had been determined previously via agglutination of the corresponding isolate.

Summary of key observations

- Most isolates received originated from either very young children (0-2 years), teenagers (10-19) or young adults (20-29); isolates from any other age group (up to 80+) were sporadic.
- B remains the most prevalent (and genetically heterogeneous) serogroup followed by serogroups Y and C, both representing a clonal lineage.
- In Switzerland, susceptibility of *N. meningitidis* to antibiotics recommended for prophylaxis (rifampicin and ciprofloxacin) remains 100%.

Discussion

In Switzerland, invasive meningococcal infections are rare with an annual incidence rate of < 1 case per 100'000 residents. In 2012 a total of 46 cases were reported to the SFOPH, and in 34 cases (74%) corresponding isolates were referred to the CNM for characterization using reference methods (Figure 1).

Agglutination was performed on all isolates and the results were plotted together with corresponding data from previous years (Figure 2). As in the past, the main serogroups, namely B, C, Y, and W135 encompassed > 90% of all isolates tested with serogroup B still being the most prevalent type. However, the plot indicated that the recent expanding trend of serogroup Y seemed to reverse. This serogroup was

emerging in Switzerland during the last two years and it was speculated that such an expansion may continue due to a particularly invasive clone of *N. meningitidis* (Krauland et al., 2012).

Molecular typing using MLST and comparative sequence analysis of outer membrane proteins (*porA*-VR1, *porA*-VR2, and *fetA*-VR) showed that all serogroup Y strains exhibited the same MLST sequence type as well as identical *porA* alleles. Further, all but one serogroup Y strain harboured the same *fetA* allele, and consequently, it is assumed that most serogroup Y isolates referred to the CNM in 2012 represent a clonal lineage (Y : 5-2, 10-1 : F4-1 : ST-23). Whether this particular lineage may re-emerge as a dominant clone remains to be seen, however, it has been limited essentially to German speaking (Eastern) areas of Switzerland as of to date.
A clonal distribution covering both French (Western) and German speaking areas of Switzerland was found for the serogroup C strains which were all identical in all genetic loci investigated (C : 5, 2 : F3-3 : ST-11). In contrast, serogroup B strains were shown to be very heterogeneous in MLST data (Table 1) as well as *porA* and *fetA* sequence data (Figure 3).

The selective pressure on particular lineages within serogroups of *N. meningitidis* will certainly increase in the near future as new vaccines are currently being introduced in Switzerland and neighbouring countries. The Swiss authorities have authorized in 2011 the conjugated quadrivalent vaccine MCV-ACWY (Menveo®) covering serogroups A, C, W135, and Y. The European Union will approve very soon a market authorization for a vaccine directed against serogroup B strains (Bexsero®). This novel vaccine is protein based and has been shown to induce a protective immune response also in children less than two years old, an age group at particular risk for a serogroup B infection. However, probably not all invasive strains will be covered, and hypothetically, uncovered variants might emerge and spread. In order to monitor eventual trends in the meningococcal lineages, molecular genetic and antigen typing analysis using standardized approaches will be of outmost importance (Vogel et al., 2012, 2013).

The susceptibility of *N. meningitidis* isolates to penicillin remained comparable to previous years as most strains were fully susceptible and no strain was found resistant to this substance. Applying the EMGM breakpoint ($\leq$ 0.094 µg/ml), only few

strains showed intermediate susceptibility to penicillin, usually correlating with the presence of characteristic mutations in their *penA* gene. However, one strain with a MIC of 0.094 was found to harbour these same mutations (Table 2). This apparent discrepancy might be explained by reduced expression of the altered penicillin-binding protein.

The majority of strains were fully susceptible to all other antimicrobial substances tested (cefuroxime, ceftriaxone, minocycline, rifampicin, erythromycin, azithromycin, ciprofloxacin, and chloramphenicol); only some strains showed reduced susceptibility to erythromycin (Table 3). These results support the current recommendation of rifampicin or ciprofloxacin as prophylactic antibiotic in (potential) post-exposure situations.

Conclusions and Perspectives

In 2012 the proportion of serogroup Y among invasive *N. meningitidis* isolates did not further increase, and no resistant MICs to currently recommended antibiotics for the treatment of invasive meningococcal infection were observed.
It remains to be seen how the meningococcal population in Switzerland will evolve during and after the introduction of vaccines covering all endemic serotypes.
The CNM will continue to provide a microbiological follow-up of this anticipated evolution using established methodology in close collaboration with referring laboratories and Swiss Public Health authorities.
The CNM will also implement a real-time PCR assay targeting the *sodC* gene (Dolan Thomas et al. 2011) to complement the molecular test panel for clinical specimens potentially containing *N. meningitidis* DNA lacking the *ctrA* capsule gene.

References

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Figure 1. Annual number of cases of invasive meningococcal infections reported to the Swiss Federal Office of Public Health and number of *N. meningitidis* strains referred to the Swiss National Center for Meningococci from 2003 to 2012.

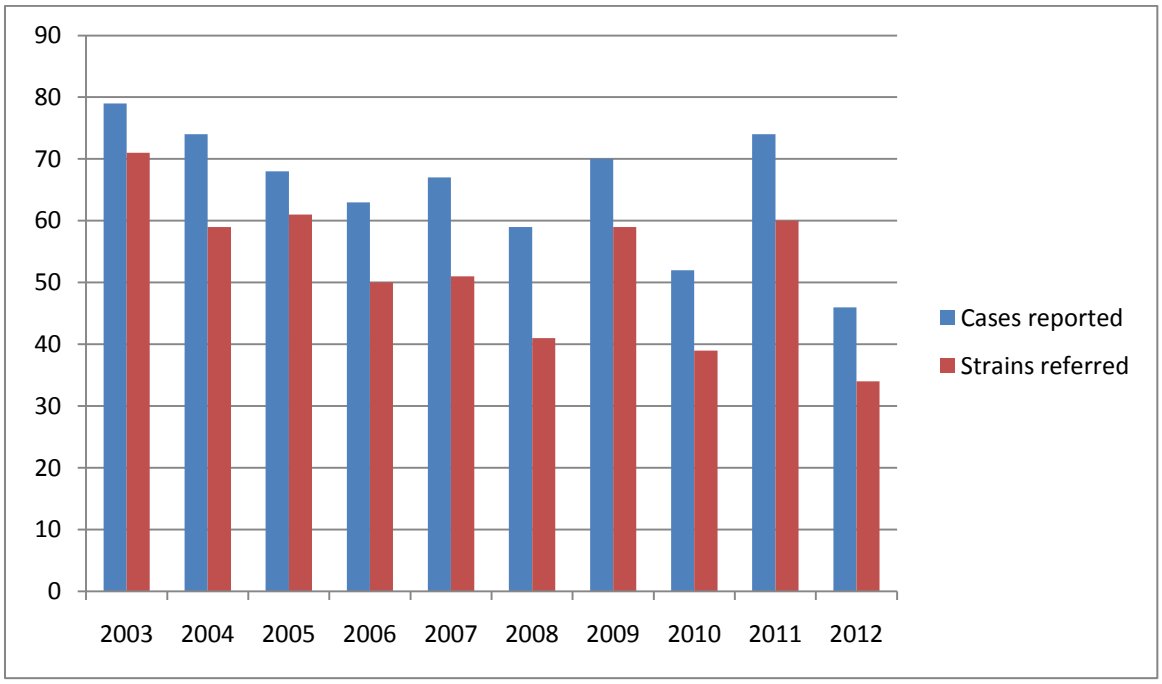

Figure 2. Annual number of strains representing main serogroups B, C, Y and W135 of *N. meningitidis* as determined by agglutination at the Swiss National Center for Meningococci from 2003 to 2012.

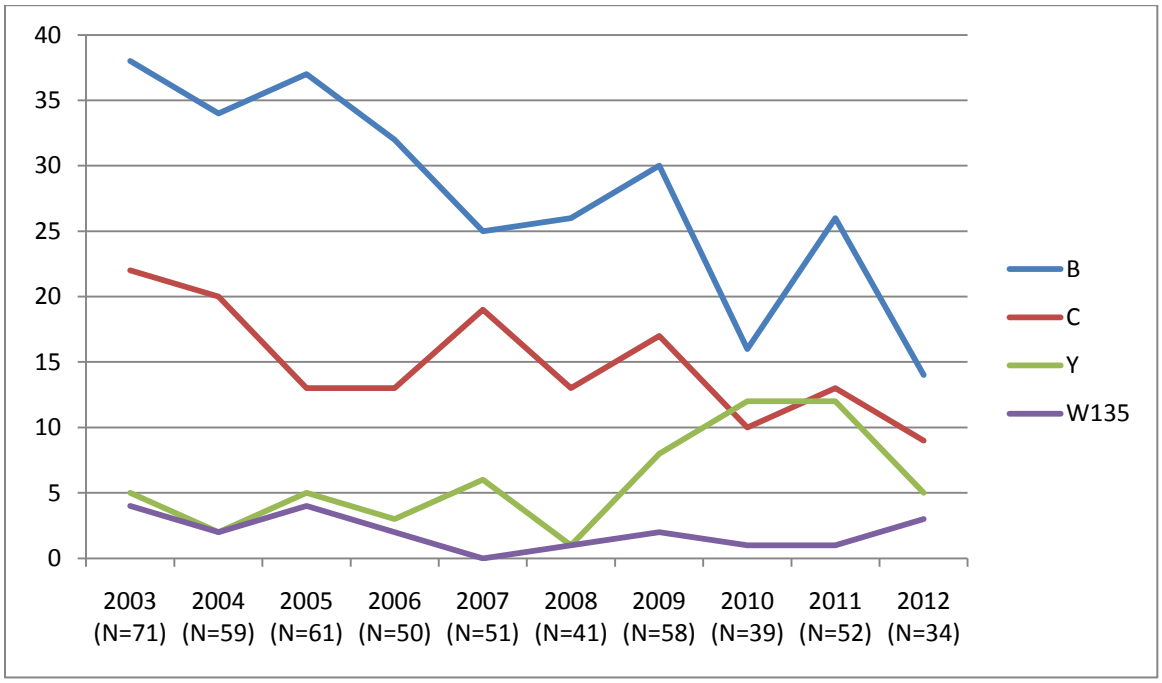

Figure 3. Distribution of *porA*-VR1, *porA*-VR2 and *fetA*-VR alleles among serogroup B isolates of *N. meningitidis* in Switzerland in 2012.

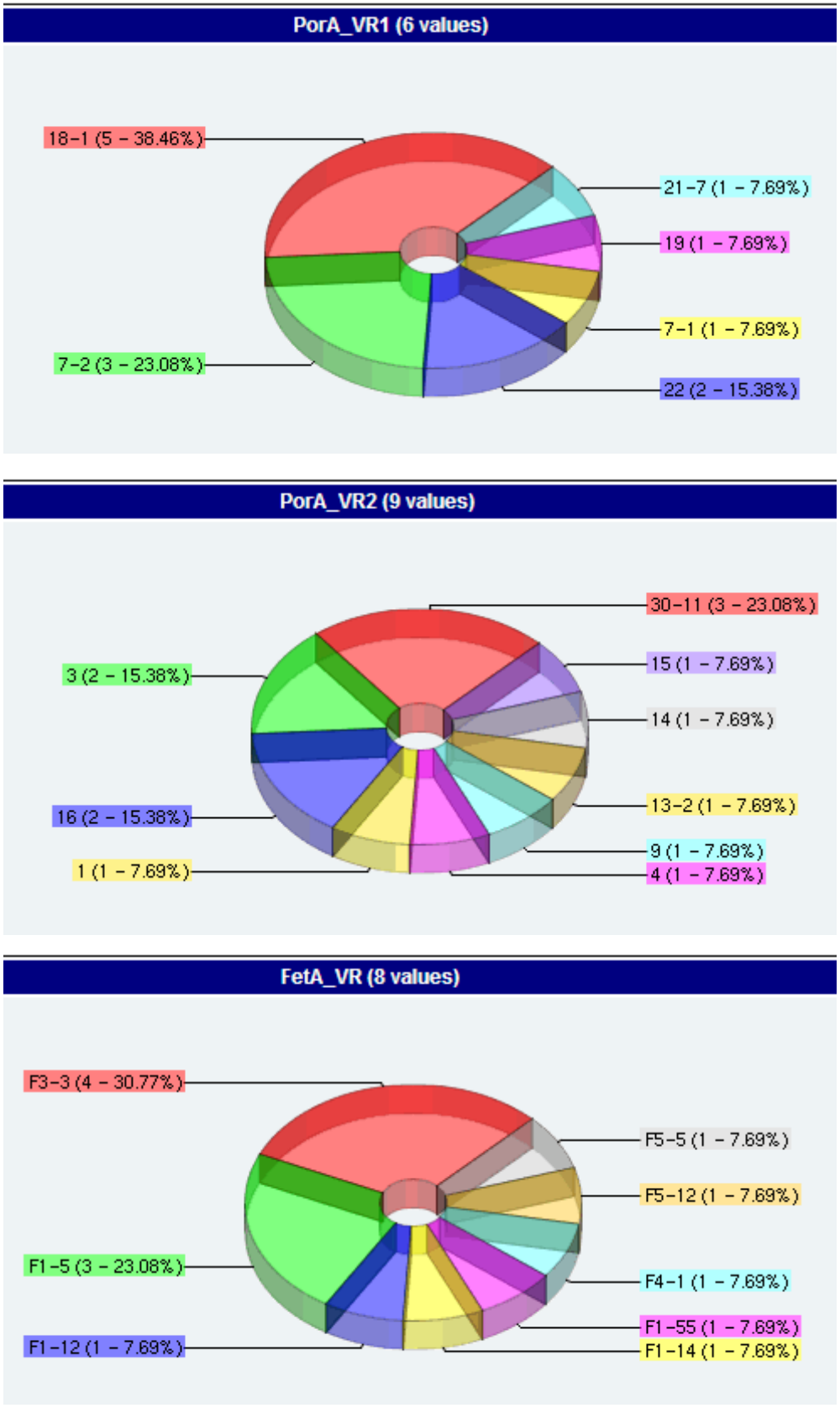

Table 1. Distribution of MLST clonal complexes and sequence types of *N. meningitidis* in Switzerland in 2012.

MLST		Serogroup (number of isolates)			
Clonal complex designation	Sequence type designation	B	C	Y	Other [serogroup]
1	75				1 [A]
11	11		9		1 [W135]
22	184				1 [W135]
22	1221				1 [W135]
23	23			5	
32	32	4			
32	6387	1			
41/44	41	1			
41/44	1194	2			
41/44	4064	1			
53	53				1 [NA]*
181	5789				1 [X]
213	NA*	1			
269	6416	1			
1157	1157	1			

*NA, no assignment available

Table 2. Correlation between susceptibility to penicillin and occurrence of mutations in the *penA* gene in strains of *N. meningitidis* isolated in Switzerland in 2012.

	Minimum inhibitory concentration of penicillin (µg / ml)							
	0.012	0.032	0.047	0.064	0.094	0.125	0.19	0.25
Category*	S	S	S	S**	S***	I	I	I
Total number of strains	1	7	5	11	2	2	4	2
Number of strains with *penA* mutations	0	0	0	0	1	0	4	2

*S, susceptible; I, intermediate

**Breakpoint of the Clinical and Laboratory Standards Institute (≤ 0.064 µg/ml)

***Breakpoint of the European Meningococcal Disease Society (≤ 0.094 µg/ml)

Table 3. Inhibitory activity of nine antimicrobial agents on 34 strains of *N. meningitidis* isolated in Switzerland in 2012.

Agent	Minimum inhibitory concentration of penicillin (µg / ml)			Breakpoint susceptible (≤ µg/ml)	% susceptible
	Range	50%	90%		
Penicillin	0.012 - 0.25	0.064	0.19	0.094*	76
Cefuroxime	0.047 - 0.75	0.125	0.75	1**	100
Ceftriaxone	<0.002 - 0.012	0.003	0.006	0.12***	100
Minocycline	0.094 – 0.5	0.19	0.38	2***	100
Rifampicin	0.003 - 0.125	0.012	0.047	0.5***	100
Erythromycin	0.19 – 1	0.38	0.75	0.5**	65
Azithromycin	0.19 – 1	0.5	1	2***	100
Ciprofloxacin	0.003 - 0.012	0.006	0.008	0.03***	100
Chloramphenicol	0.25 – 1	0.75	1	2***	100

*European Meningococcal Disease Society

**British Society for Antimicrobial Chemotherapy

***Clinical and Laboratory Standards Institute