Annual Report of the National Center for Meningococci 2011

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Introduction

Meningococcal infections carry a heavy burden of morbidity and mortality, deserving maximal efforts for prevention and treatment. Meningococcal meningitis harboured over 75% mortality in the eighteenth century. In 1913, however, Flexner published a study on 1300 cases of meningococcal meningitis treated with intrathecal anti-meningococcal serum, with a drop of mortality to 30% [1]. In 1937, Schwentker reported the effect of the first antibiotic treatment of meningococcal meningitis (sulphanilamide) with a drop of mortality to 10% [2]. In a recent study from the USA [3], the global mortality rate of meningococcal meningitis was 15% and had not changed in-between 1997 and 2007. In the subgroup of meningococcal meningitis, the mortality rate is 10%, unchanged since 1937! This historical overview gives us a clear

message: the best way to further diminish the mortality of meningococcal infection lies clearly in the prevention!

However, they are different strains of *N. meningitidis*, harbouring different capsular polysaccharides (serogroups), so that there is no single vaccine covering all the circulating strains. Therefore, in order to take decisions for prevention measures, such as the type of vaccine or prophylaxis to be used, it is crucial to follow closely the epidemiology of this disease in (and around) our country. The National Center for Meningococci (NCM) is a key player for this epidemiological surveillance, by an efficient biological characterisation of all strains and a surveillance of their resistance patterns. The bacteriology laboratory of the University Hospital of Geneva has performed this task since 1990 in collaboration with the Swiss Federal Office of Public Health (SFOPH). Since 2009, the NCM is also active in the European Meningococcal Epidemiology in Real-Time (EMERT) network directed by the European Meningococcal Disease Society (EMGM).

Classification and characterization of clinical isolates have been based on phenotypic characteristics of variable outer membrane-expressed structures such as the capsule (serogroups), major outer membrane proteins (serotypes) and other outer membrane proteins (sero-subtypes). For many studies however, serological methods suffer a number of limitations, including an incomplete coverage of the antibody panels employed and inconsistent correspondence with genetic relationships. Many methods based on DNA technology have been proposed including ribotyping, random amplified polymorphic DNA (RAPD), fluorescent amplified fragment length polymorphism (AFLP) and pulsed-field gel electrophoresis (PFGE) but the comparison of these techniques among laboratories remains difficult. The methods of

choice selected by the EMGM, and applied in the NCM to characterize meningococcal strains are:

- The Multi Locus Sequence Typing (MLST)
- The sequencing of two regions of the *porA* gene (porA-VR1 and porA-VR2 corresponding to sero-subtypes)
- The sequencing of the *fetA* gene (coding for an iron repressible outer-membrane protein).

The NCM performs also the surveillance of antibiotic resistance in *N. meningitidis* invasive infections. The spread of antibiotic resistance must be monitored to insure best practices in therapeutic and prophylactic treatments. It was recently emphasised by the emergence of ciprofloxacin-resistant isolated strains in India, France [4] and in the USA [5]. Luckily, the development of resistances of *N. meningitidis* to antimicrobial agents is not particularly efficient, but the decrease in penicillin susceptibility observed over the last 10 years by alterations in penicillin-binding proteins (PBPs) has underscored the importance of such systematic analysis.

In many cases, as early antibiotic treatment is recommended before lumbar puncture when bacterial meningitis is suspected, cultures remain sterile. Therefore, molecular methods for non-culture diagnosis of *N. meningitidis* and its serogroup, as well as its susceptibility to Penicillin have been developed and are currently used in the NCM.

Materials and Methods

When a strain is received at the CNM, its serogroup is immediately determined with latex agglutination kits. The serogroups A, B, C Y and W135 are assessed with the Pastorex^{TM} meningitis kit (Bio-Rad, Pasteur, Paris, France). In absence of agglutination, the identification of *N. meningitidis* is verified and serogroups X and Z are tested. As soon as a fresh culture is available (usually the day after reception of the strain), the isolate is tested for its antimicrobial susceptibility profile (Minimal Inhibitory Concentration = MIC) to the following nine antimicrobial agents: penicillin, cefuroxime, ceftriaxone, minocycline, rifampicin, erythromycin, azithromycin, ciprofloxacin, and chloramphenicol. Values of the E-test (AB Biodisk, Sweden, distributed in Switzerland by Biomérieux) on Mueller-Hinton 5% sheep blood agar are interpreted according to the CLSI and EMGM recommendations. Since 2008, an analysis of *penA* gene sequences of all Swiss strains was established in our laboratory to detect the expression of altered forms of PBP2.The PCR and sequencing method have been previously described by Taha and *al*. [6]. Sequences were analysed against a European database accessible at the following URL: http://neisseria.org/nm/typing/penABlast.

Until 2010, serogroups, serotypes and sero-subtypes were determined with a dot-ELISA technique based on monoclonal antibodies. This method has been substituted by new DNA typing sequencing methods as recommended by the EMGM:

Serogroup : PorA (vr1) : PorA (vr2) : FetA (vr1) : clonal complex (MLST)

Multi Locus Sequence Typing (MLST sequence type and clonal complex) reflects the variation present in the nucleotide sequence of 400-500 bp internal fragments from seven housekeeping-genes [7]. The following loci are examined by sequencing: *abcZ* (putative ABC transporter), *adk* (adenylate kinase), *aroE* (shikimate deshydrogenase), *fumC* (fumarate), *gdh* (glucose-6-phosphate deshydrogenase), *pdhC* (pyruvate deshydrogenase subunit) and *pgm* gene (phophoglucomutase). The

porA VRs and *fetA* VR sequencing methods have been established according to the published data in the web site www.Neisseria.org. All allele determinations are then accessible in the different databases via the following URL: http://pubmlst.org/neisseria/ .

The CNM provides to all Swiss laboratories or hospitals a rapid determination of meningococcal infection by PCR amplification. The CNM has used a rapid and sensitive nucleic acid amplification method to detect a meningococcal infection and to identify the serogroup at the gene level. Nucleic acids of various clinical samples are automatically extracted with the MagNAPure Compact system (Roche Diagnostic Ltd.). DNA is amplified with a real-time PCR to detect the *ctrA* gene (capsular transport gene specific of *Neisseria meningitidis*) [8] and, whenever this first PCR assay is positive, we perform a second amplification to detect the genes encoding the specific polysialyltransferase (*siaD* gene) for B, C, Y/W135 serogroups and *mynB* gene for A serogroup, respectively [9]. In 2010, a new PCR method was also developed to characterize specifically the serogroup Y (*synF* gene) or W135 (*synG* gene), a useful alternative for culture-negative samples [10]. In 2012, we plan to implement a second PCR targeting the *sodC* gene of *N. meningitidis*. As described earlier, *ctrA* can exceptionally be polymorphic [11,12] and *sodC* targeting will be positive also for non-encapsulated *N. meningitidis* [13]. Therefore, when *ctrA* is negative but the clinical suspicion high (or the phenotypic test positive), we will perform the *sodC* PCR.

Results

During the year 2011, the CNM has received 60 strains (or DNA) of *Neisseria meningitidis* isolated from normally sterile specimens like blood (n=40), CSF (n=19), and joint fluid samples (n=1). These strains/DNA correspond to 64% of the invasive meningococcal cases notified to the SFOPH (n=79) (Figure 1). The incidence of IMD remains low in Switzerland (1/100'000 in 2011). Similarly to the other European countries, the serogroup B remains predominant (52%), especially in children aged less than 2 years (85%) followed by serogroup C (23%) and serogroup Y (22%) (Figures 2, 3 and 4). Please note that serogroup Y is absent in children aged less

than 10 years (Figure 5) and appears to be limited to the German-speaking area of our country (Figure 6). This may be explained by the highest prevalence of this serogroup in Northern than Southern European countries, according to the data available on EMERT. All strains of serogroup Y that could be typed were of Sequence Type (ST) 23. Moreover, there was very limited variation of the *porA* subtyping, which is consistent with a new clonal complex establishing in our country.

When looking for the most predominant genotype circulating in Switzerland by MLST (Figure 7), we can identify this new clonal complex (ST23) from the Y serogroup, as well as ST11, with most isolates corresponding to serogroup C, but some to serogroup B ("switching capsule") (Table 1). Among serogroup B strains, MLST types were much more variable, with ST41 as leader (Figure 7 and 8). In 2011, the direct PCR detection allowed diagnosing 8 culture-negative invasive cases of *Neisseria meningitidis* infections.

New recommendations were published by the European Monitoring Group on Meningococci for AST in 2007 [14]. These recommendations were updated in 2010 for penicillin but a divergence was shown in the breakpoints for penicillin between CLSI and the European group. The European group has chosen a value of 0.094 corresponding to several mutations in the *penA* gene and the CLSI has a breakpoint value of 0.06 (unchanged in 2012). We applied the European recommendations and with this new breakpoint, few strains were characterized as susceptibility decreased strains. No strain was defined as a penicillin-resistant isolate (CMI ≥ 0.5) and *penA* gene mutations were well correlated with the AST (figure 9). As described in the European data project (EU.MenNet project), five polymorphic sites might differentiate penicillin susceptible from intermediate strains and those five specific positions are the main keys for the definition of intermediate resistance to penicillin. In our experience, the same mutations responsible for antimicrobial resistance were also observed and a very good correlation existed between the MIC values and the presence of mutations in the *penA* gene. Seven strains showed mutations in the trans- peptidase-encoding region of the *penA* gene. All strains with a MIC<0.125 showed no mutation in the *penA* gene. In 2011, all strains were susceptible to

Ceftriaxone, Rifampicine and Ciprofloxacine, confirming the actual prophylactic and therapeutic regimens used in Switzerland (Table 2).

Summary of key observations:

- A stability of the number of invasive isolates since 2004
- Almost ¼ of the patients suffering from invasive meningococcal infection were over 50 years old, and 1/5 were 65 years old or more.
- Serogroup Y strains seems to have established in Switzerland, as frequent as serogroup C strains but more uniform in its genetic make-up.
- In Switzerland, susceptibility of meningococcus to the antibiotics used for prophylaxis (rifampicin and ciprofloxacin) remains 100%.

Discussion

In Switzerland invasive meningococcal infections (IMI) remain seldom with an incidence of 1/100'000 in 2011. However, this incidence is much higher than in the USA (0.2/100'000 in 2007) [3]. The installation of a new strain of serogroup Y (ST-23) since 2010 is somehow worrisome, especially when realizing that it is essentially constituted by one clone:
Serogroup Y:PorA(vr1) 5-2:PorA(vr2) 10-1 : FetA (vr1) F4-1 : clonal complex ST-23.

Surprisingly, in 2011, all IMI due to serogroup Y took place in the German-speaking part of Switzerland (Figure 6). This serogroup spared children aged less than 10 years, hitting preferentially adolescents and people over 65 years old, without gender specificity. The proportion of serogroup Y isolates have the potential to grow further in our country, as was the case in the USA since the 1990th [15] and other European countries since the 2000th [16,17]. However, in 2011, the conjugated quadrivalent vaccine MCV-ACWY (Menveo ®) has been authorized in Switzerland and incorporated in the vaccination schemes for patients at risk in the new recommendations of the SFOPH. Depending on the evolution of this epidemiology, the indications for use of this new conjugated vaccine will be adapted by the SFOPH.

However, in 2011, strains from the serogroup B remain the major strains circulating, especially in children under 2 years. The situation with serogroup B strains is very different: many different clones (ST-41 and ST-1161 being the most frequent ones, see Figure 7) and no vaccine available yet. This is due to the capsular polysaccharide of serogroup B that is almost identical to polysialic acid of human glycoproteins, thus non-immunogenic even when conjugated. The PorA protein of the external membrane is prolific and very immunogenic, but extremely variable, making it impossible to create a vaccine covering all strains. Luckily, in recent years, several candidates for an efficient vaccine for serogroup B *N. meningitidis* have been developed. One of the most promising ones is a vaccine combining recombinant proteins with outer membrane vesicle [18], which seems safe with good immunogenicity and should soon be FDA-approved[19,20].
The arrival of an efficient vaccine for serogroup B *N. meningitidis* is a major step and, depending on its use, it could significantly influence the epidemiology of IMI. Indeed, it is problematic to have one of the major serogroups not covered with an efficient vaccine, since strains of meningococcus are able to switch capsule [21]. In 2011, we identified 2 strains from clone ST-11, that probably switched from serogroup C to serogroup B (Table 1), as previously described [22]. Capsular switching is a good example of the importance of fine typing of circulating clones such as performed by the National Center for Meningococci (NCM).

Contrary to historical knowledge, reflected in antibiotic guides such as the Stanford antibiotic guide 2012, IMI does happen in patients over 50 years old [3]. In Switzerland, in 2011, almost ¼ of IMI concerned patients over 50 years. This is another example of the utility of the epidemiological surveillance of IMI in our country.

In June 2009, the EMGM group achieved consensus for the laboratory methods and variables to be used for high discrimination of circulating meningococcal strains. It was confirmed that laboratory surveillance should rely only on molecular and sequence-based typing data. The proposed scheme was as follow:

Serogroup: PorA(vr1): PorA(vr2): FetA (vr1): clonal complex (MLST).

In Geneva, we have implemented and validated these methods to retrospectively analyse all invasive strains (isolates of 2009) as well as for our prospective surveillance (all isolates received in our CNM since January 2010). For this reason we removed the previous database to communicate the results at the SFOPH and proposed a new database representing this molecular typing scheme. A European collection of data was established in 2010 (EMERT = European Meningococcal Epidemiology in Real Time). EMERT collects data about the strains causing meningococcal disease throughout Europe in real time. As we had the approval of SFOPH, we introduced our data by batches and several times a year in order to compare our epidemiologic situation with other European countries. This contributes to centralize data and helps to increase our visibility to other countries.

For the antibiotic susceptibility testing, the results are very similar to the previous years. The proportion of strains with reduced sensitivity to penicillin is low (Table 2) and only these strains display mutations in the *penA* gene (values of MIC of 0.125, 0.19, 0.25). The correlation between the detected mutations and the susceptibility to penicillin is excellent. Up to now, resistance to ciprofloxacin or rifampicin has not been encountered in strains isolated from IMI in Switzerland.

Conclusions:

In 2011 the incidence of invasive meningococcal infections in Switzerland has been stable (1/100000 population). Serogroup Y remains as prevalent as serogroup C, with ST-23, ST-11 and ST-41 constituting the main clones circulating in our country.

The CNM has been very prompt to adapt his work to the molecular requirements introduced by the EMEG and is one of the most “complete” participants of the EMERT. Close follow-up of the epidemiological evolution of the different clones in and around Switzerland will be of major importance in the next years, with the arrival of a conjugated vaccine for serogroup B, and the progressive use of Menveo®.
For the PCR detection, we plan to introduce a second PCR targeting *sodC*, in order to complement our *ctrA* gene PCR.

In order to improve the quality of our statistics, we need to receive > 95% of the strains of IMI, instead of 65%. We hope that the SFOPH will have the possibility to require the referral of invasive strains of meningococcus to the NCM, as an obligatory procedure in the next years.

References:

1. Flexner S. The Results of the Serum Treatment in Thirteen Hundred Cases of Epidemic Meningitis. J Exp Med 1913;17:553-76.
2. Schwentker FF, Gelman S, Long PH. Landmark article April 24, 1937. The treatment of meningococcic meningitis with sulfanilamide. Preliminary report. By Francis F. Schwentker, Sidney Gelman, and Perrin H. Long. Jama 1984;251:788-90.
3. Thigpen MC, Whitney CG, Messonnier NE, et al. Bacterial meningitis in the United States, 1998-2007. N Engl J Med 2011;364:2016-25.
4. Skoczynska A, Alonso JM, Taha MK. Ciprofloxacin resistance in Neisseria meningitidis, France. Emerg Infect Dis 2008;14:1322-3.
5. Wu HM, Harcourt BH, Hatcher CP, et al. Emergence of ciprofloxacin-resistant Neisseria meningitidis in North America. N Engl J Med 2009;360:886-92.
6. Taha MK, Vazquez JA, Hong E, et al. Target gene sequencing to characterize the penicillin G susceptibility of Neisseria meningitidis. Antimicrob Agents Chemother 2007;51:2784-92.
7. Jolley KA, Chan MS, Maiden MC. mlstdbNet - distributed multi-locus sequence typing (MLST) databases. BMC Bioinformatics 2004;5:86.
8. Corless CE, Guiver M, Borrow R, Edwards-Jones V, Fox AJ, Kaczmarski EB. 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.
9. Molling P, Jacobsson S, Backman A, Olcen P. Direct and rapid identification and genogrouping of meningococci and porA amplification by LightCycler PCR. J Clin Microbiol 2002;40:4531-5.
10. Fraisier C, Stor R, Tenebray B, Sanson Y, Nicolas P. 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.
11. Jaton K, Ninet B, Bille J, Greub G. False-negative PCR result due to gene polymorphism: the example of Neisseria meningitidis. J Clin Microbiol 2010;48:4590-1.
12. Cavrini F, Liguori G, Andreoli A, Sambri V. Multiple nucleotide substitutions in the Neisseria meningitidis serogroup C ctrA gene cause false-negative detection by real-time PCR. J Clin Microbiol 2010;48:3016-8.

13. Dolan Thomas J, Hatcher CP, Satterfield DA, et al. sodC-based real-time PCR for detection of Neisseria meningitidis. PLoS One 2011;6:e19361.
14. Vazquez JA. Resistance testing of meningococci: the recommendations of the European Monitoring Group on Meningococci. FEMS Microbiol Rev 2007;31:97-100.
15. Krauland MG, Dunning Hotopp JC, Riley DR, et al. Whole Genome Sequencing to Investigate the Emergence of Clonal Complex 23 Neisseria meningitidis Serogroup Y Disease in the United States. PLoS One 2012;7:e35699.
16. Fazio C, Neri A, Starnino S, Sofia T, Mastrantonio P, Stefanelli P. Characterization of invasive serogroup Y meningococci in Italy: prevalence of ST-23 Complex/Cluster A3. New Microbiol 2008;31:467-72.
17. Hedberg ST, Toros B, Fredlund H, Olcen P, Molling P. Genetic characterisation of the emerging invasive Neisseria meningitidis serogroup Y in Sweden, 2000 to 2010. Euro Surveill 2011;16.
18. Su EL, Snape MD. A combination recombinant protein and outer membrane vesicle vaccine against serogroup B meningococcal disease. Expert Rev Vaccines 2011;10:575-88.
19. Gossger N, Snape MD, Yu LM, et al. Immunogenicity and tolerability of recombinant serogroup B meningococcal vaccine administered with or without routine infant vaccinations according to different immunization schedules: a randomized controlled trial. Jama 2012;307:573-82.
20. Santolaya ME, O'Ryan ML, Valenzuela MT, et al. Immunogenicity and tolerability of a multicomponent meningococcal serogroup B (4CMenB) vaccine in healthy adolescents in Chile: a phase 2b/3 randomised, observer-blind, placebo-controlled study. Lancet 2012;379:617-24.
21. Harrison LH, Shutt KA, Schmink SE, et al. Population structure and capsular switching of invasive Neisseria meningitidis isolates in the pre-meningococcal conjugate vaccine era--United States, 2000-2005. J Infect Dis 2010;201:1208-24.
22. Lancellotti M, Guiyoule A, Ruckly C, Hong E, Alonso JM, Taha MK. Conserved virulence of C to B capsule switched Neisseria meningitidis clinical isolates belonging to ET-37/ST-11 clonal complex. Microbes Infect 2006;8:191-6.

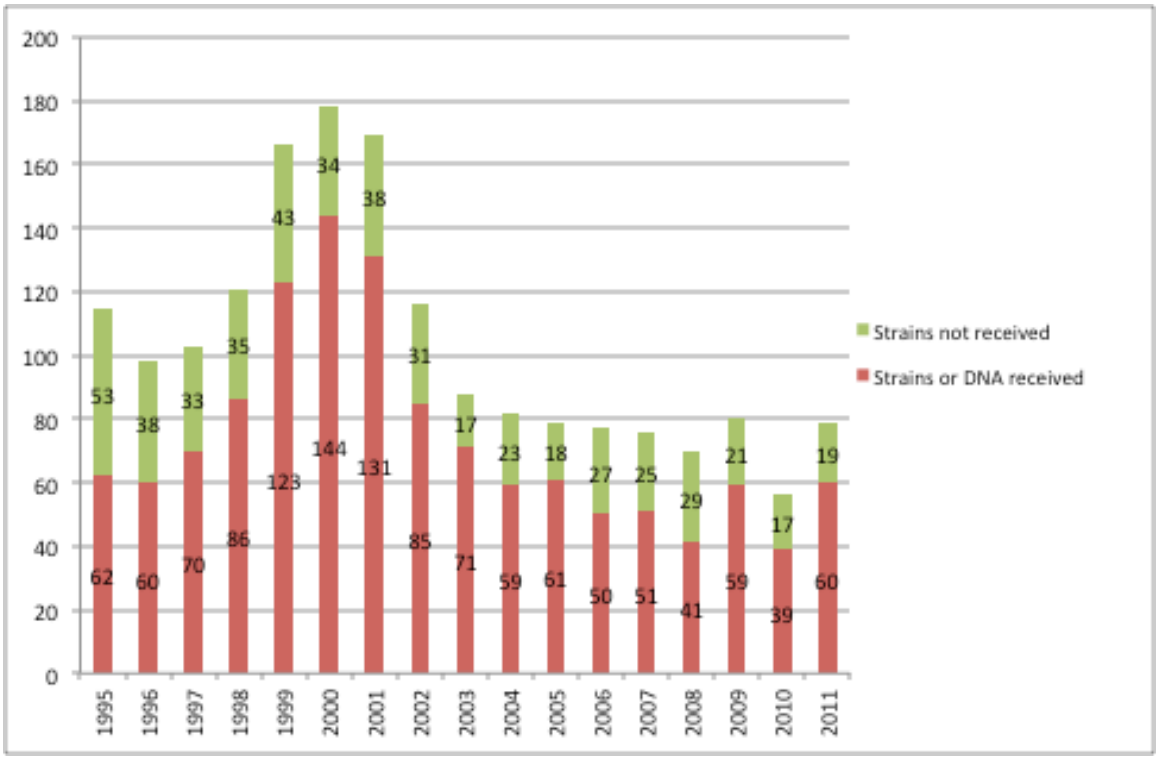

Figure 1: Comparison of the annual number of *N. meningitidis* strains received at the National Center for Meningococci in Geneva and the number of invasive meningococcal infections notified to the Swiss Federal Office of Public Health from 1995 to 2011

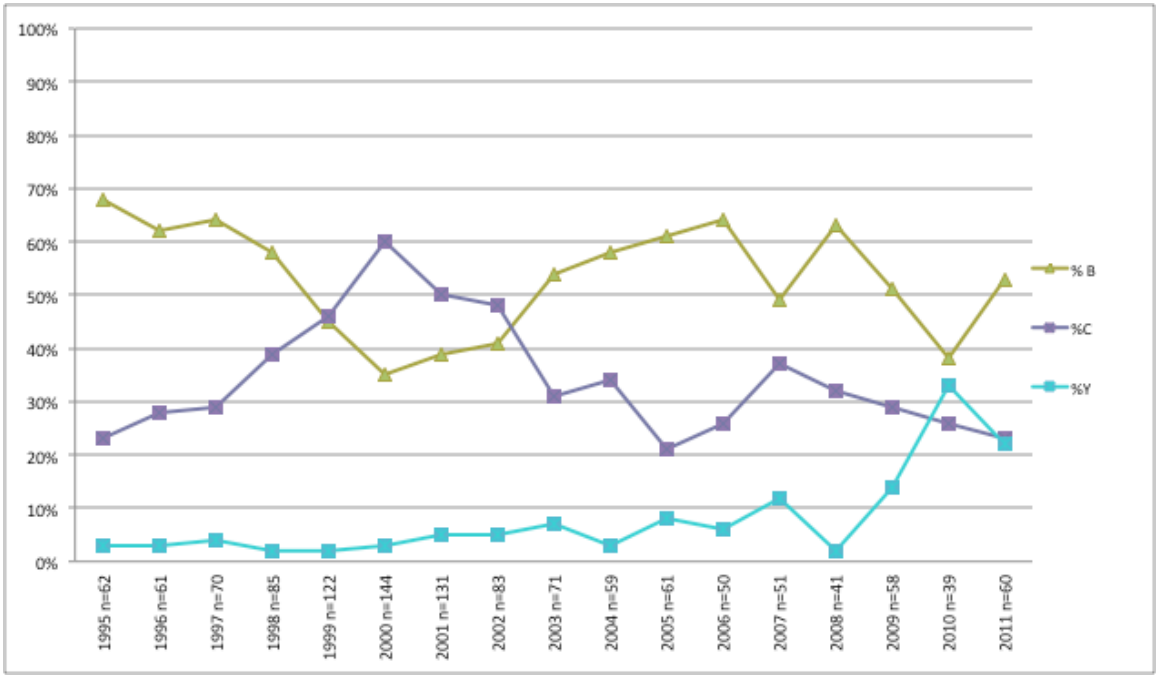

Figure 2: Distribution of serogroups B, C and Y of *N.meningitidis* from 1995 to 2011

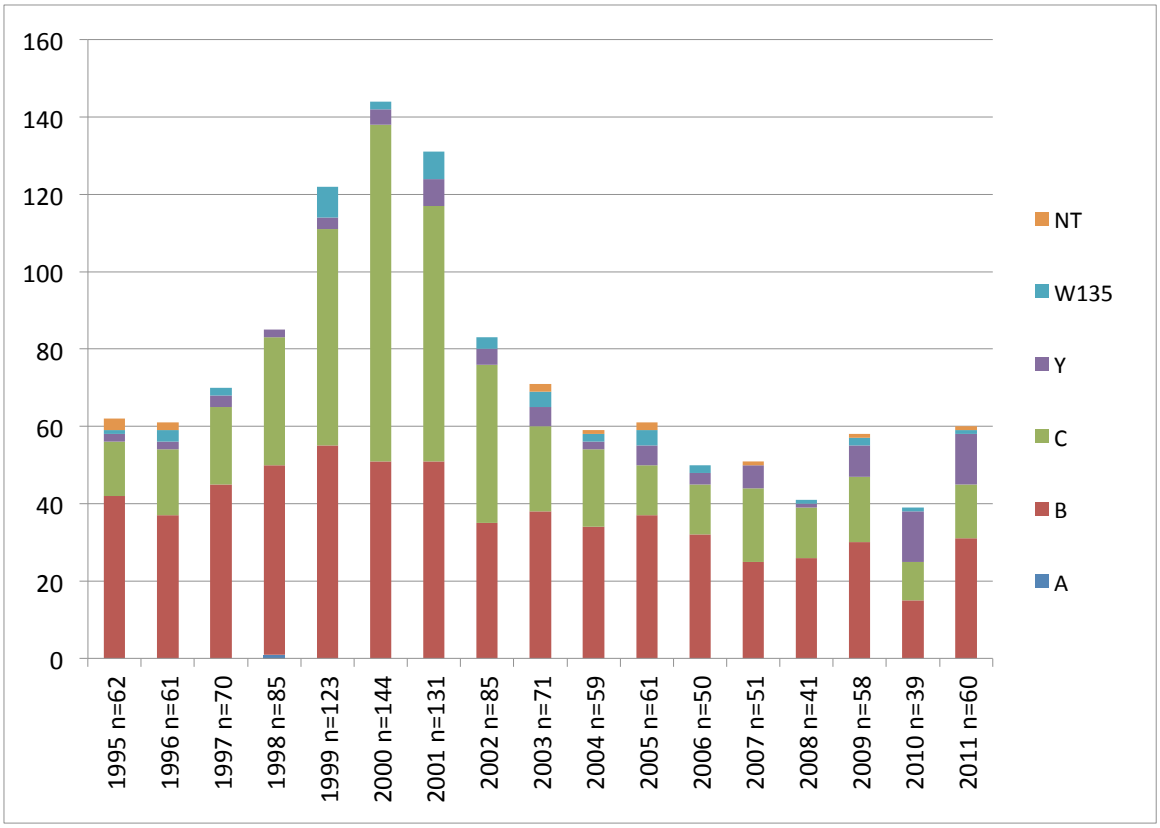

Figure 3: Serogroup distribution of invasive meningococcal isolates 1995-2011

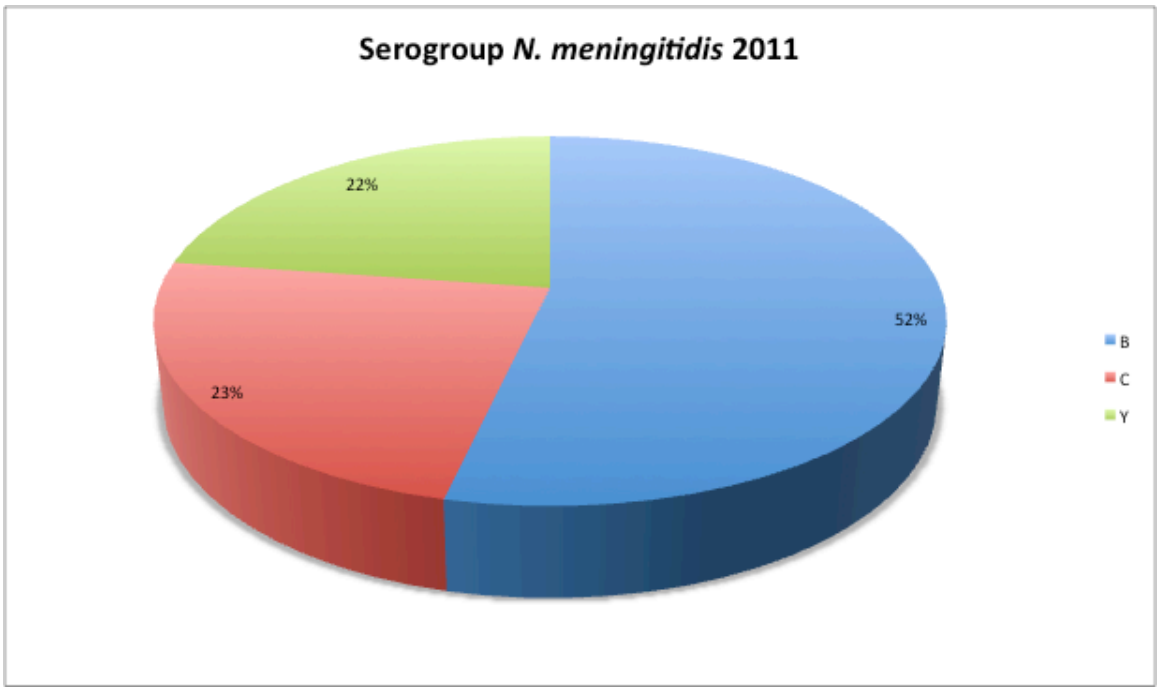

Figure 4: Distribution of serogroups of *N. meningitidis* in Switzerland in 2011

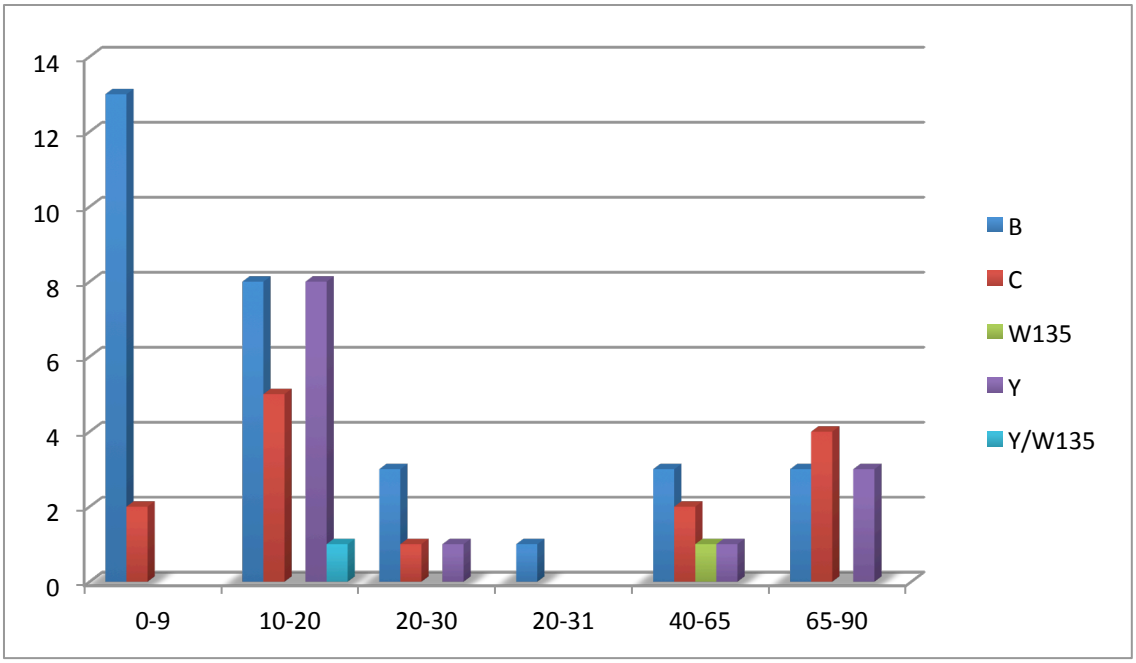

Figure 5: Distribution of serogroups B, C and Y of *N. meningitidis* by age group in Switzerland in 2011

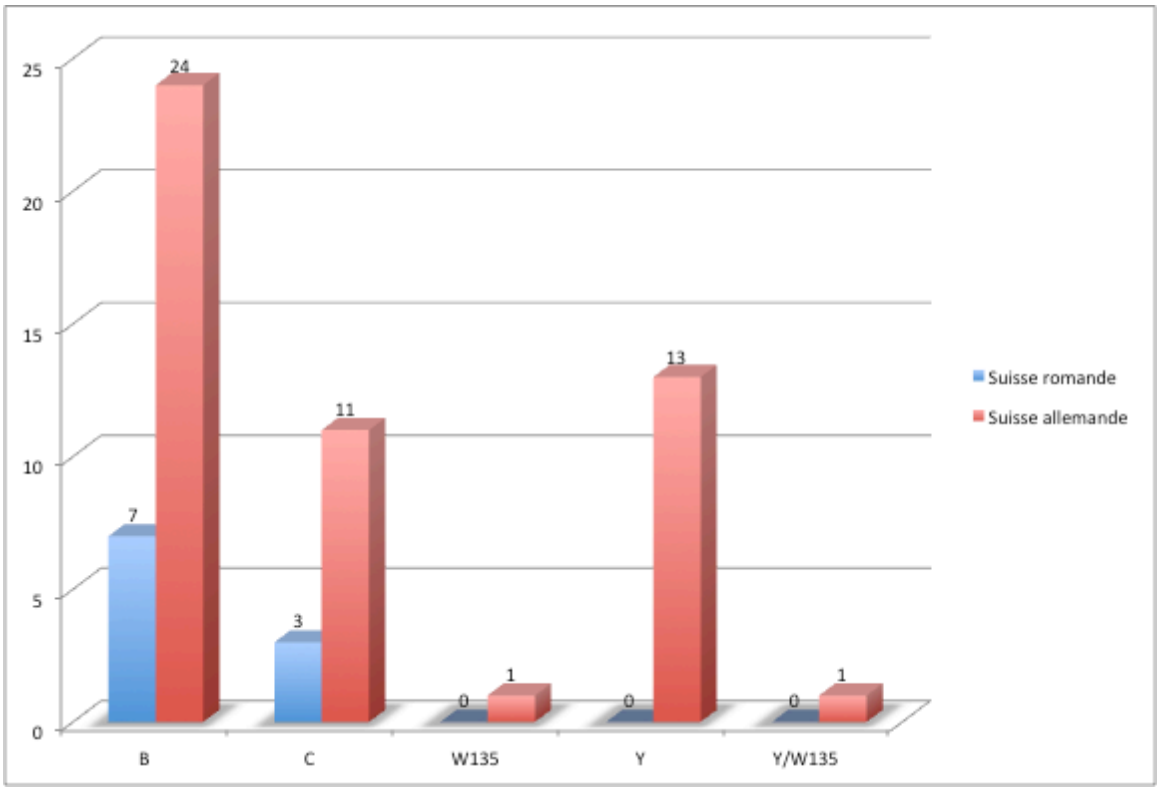

Figure 6: Geographical distribution of serogroups B, C and Y of *N. meningitidis* in Switzerland in 2011

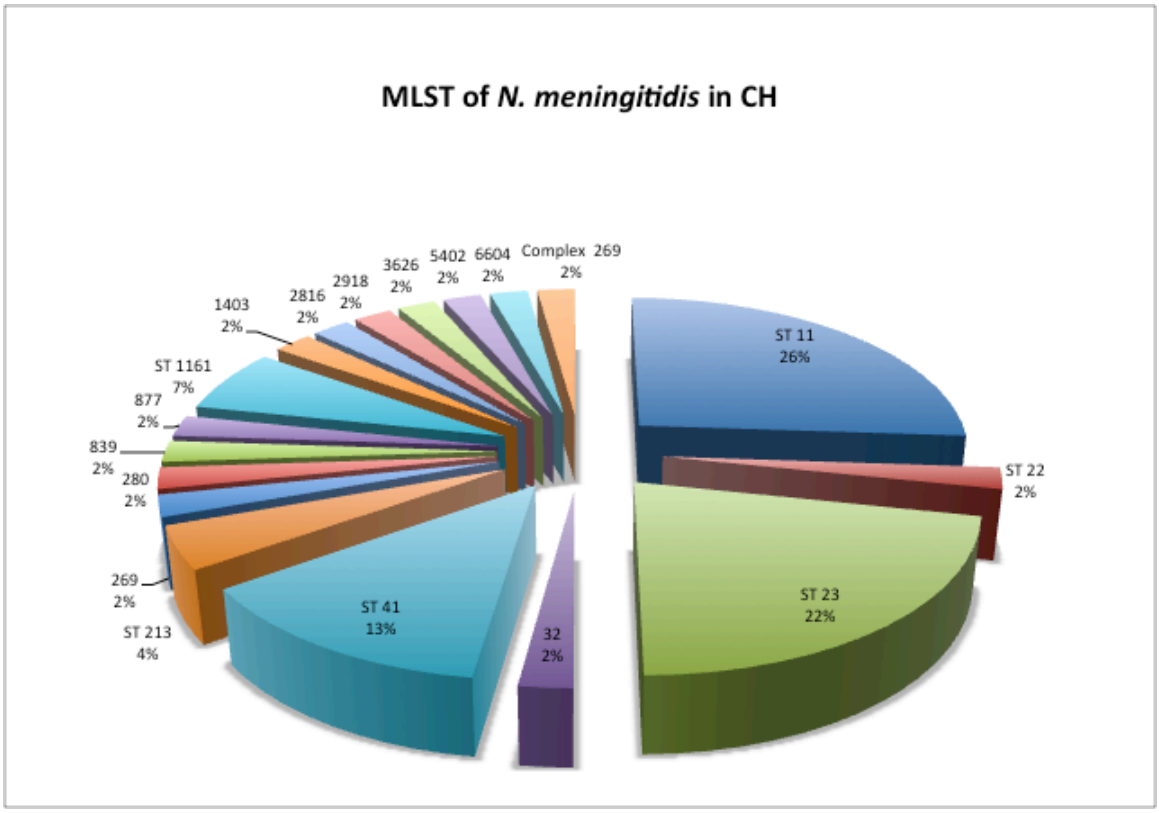

Figure 7: Distribution of MLST clones of *N. meningitidis* in Switzerland in 2011

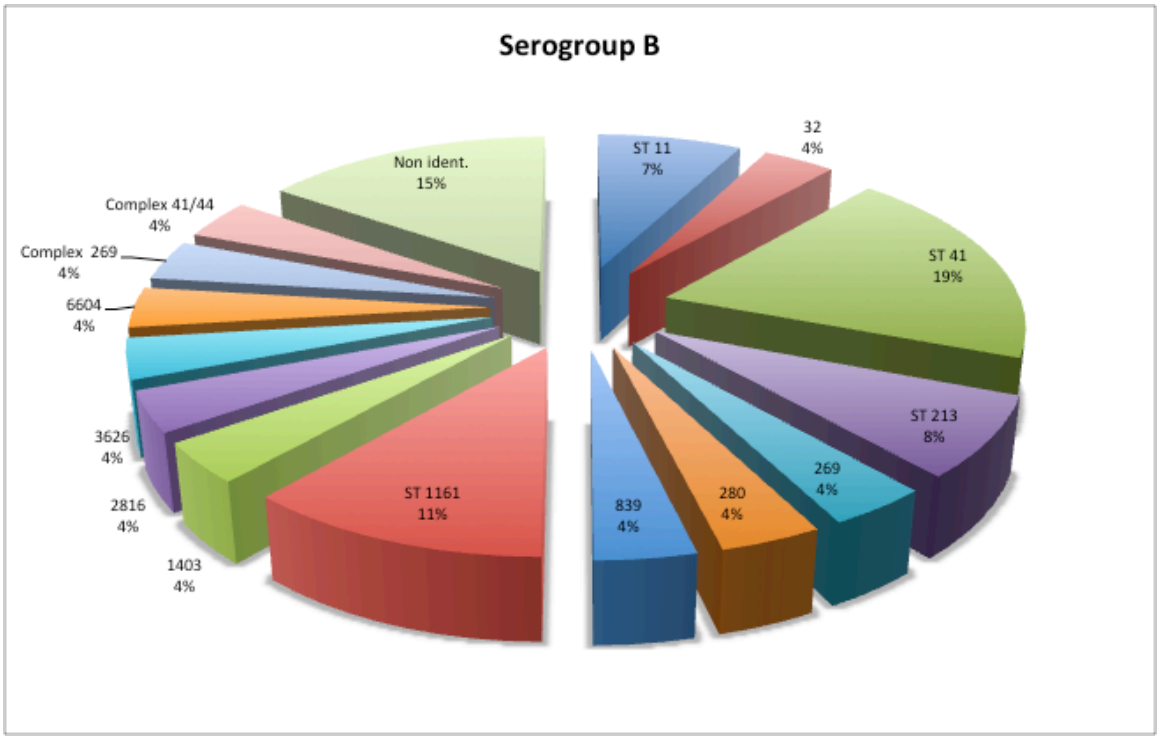

Figure 8: Distribution of MLST of serogroups B *N. meningitidis* in Switzerland in 2011

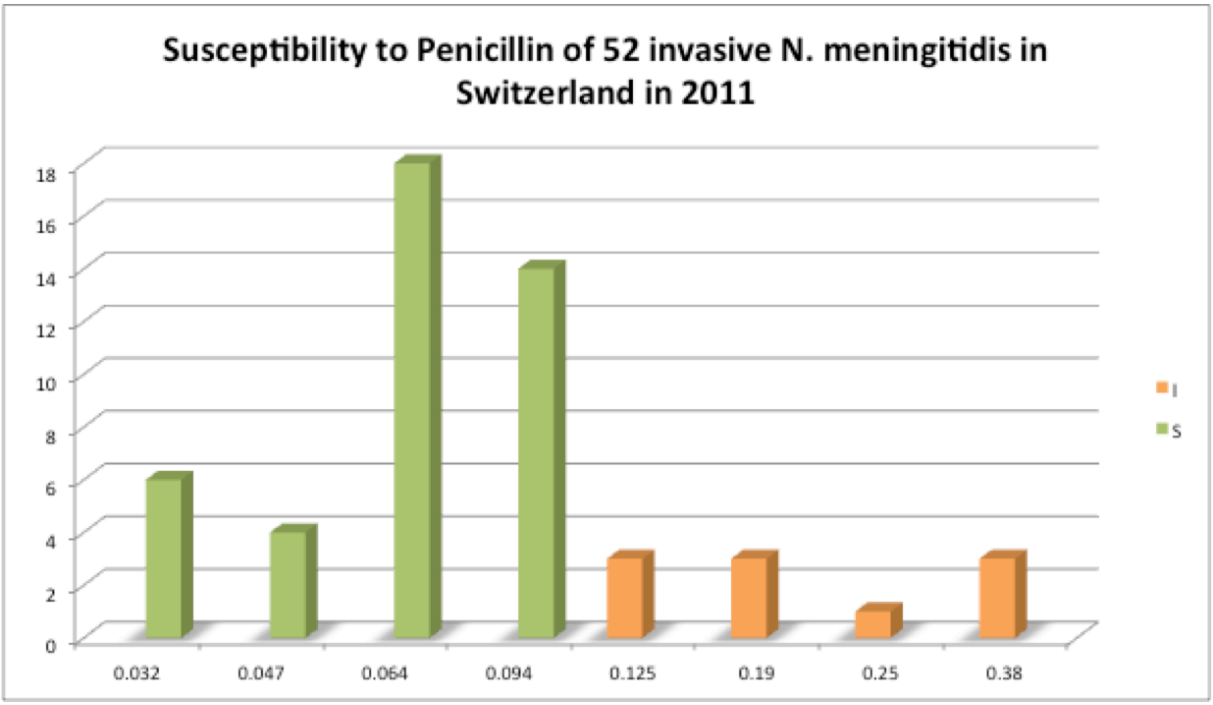

Figure 9: Susceptibility to Penicillin of invasive strains of meningococcus in 2011

ST	Serogroup B	Serogroup C	Serogroup W135	Serogroup Y
11	2	8		
22			1	
23				9
32	1			
41	5			
213	2			
269	1			
280	1			
839	1			
877		1		
1161	3			
1403	1			
2816	1			
2918		1		
3626	1			
5402		1		
6604	1			
Complex 11		1		
Complex 23				1
Complex 269	1			
Complex 11		1		
Complex 41/44	1			
Non ident.	4			2

Table 1: Detailed MLST repartition of different serogroups of invasive N. meningitidis in Switzerland in 2011

Agent	Minimal Inhibitory Concentration (µg / ml) range	50%	90%	Breakpoint sensitive ≤ µg/ml	% sensitive
Penicillin	0.032-0.28	0.064	0.19	0.094*	81%
Cefuroxime	0.016-1	0.25	0.38	1**	100%
Ceftriaxone	<0.002	0.003	0.008	0.12*	100%
Minocycline	0.065-0.75	0.25	0.5	2***	100%
Rifampicin	0.003-0.25	0.012	0.064	0.5***	100%
Erythromycin	0.19-3	0.38	0.75	0.5**	79%
Azithromycin	0.25-3	0.5	1.5	2***	98%
Ciprofloxacin	0.002-0.012	0.006	0.008	<0.03*	100%
Chloramphenicol	0.5-2	0.75	1.5	2*	100%

*CLSI/NCCLS 2011 (3) and EMGM working group
**British Society for Antimicrobial Chemotherapy (5).
*** CLSI/NCCLS 2011

Table 2: Inhibitory activity of 9 antimicrobial agents on 52 meningococci isolated in Switzerland during 2011