Annual Report of the National Center for Meningococci 2007

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Introduction

The incidence of meningococcal disease varies geographically in Europe but it remains a major childhood infection with substantial psychological impact in the population. The rapid onset of disease, the high rate of fatality and the high proportion of surviving patients with severe complications ensure that the disease remains prominent on the public health agenda throughout Europe. Immediate management of invasive meningococcal infections requires prompt measures to confirm the diagnosis, to administer appropriate antimicrobial chemotherapy and to prevent secondary cases in close contacts (vaccination and/or chemoprophylaxis). Classification and characterization of clinical isolates are essential to follow their diversity and their spreading in the country or between the continents. This characterization has 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 (1). 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. For this reason, the MultiLocus Sequence Typing (MLST) which is a reproducible method is now considered as the method of choice by the European meningococcal network. In Switzerland, all tasks of characterization are performed at the Central Laboratory of Bacteriology located at the University Hospitals of Geneva (National Center of Meningococci = CNM) with the aim to rapidly detect a potential outbreak and to facilitate national communication of the Swiss Federal Office of Public Health (SFOPH). The CNM performs also the surveillance of antibiotic resistance profiles. The development of resistances of *N. meningitidis* to antimicrobial agents is not particularly efficient but the decrease in penicillin susceptibility observed over the last 5 years by alterations in penicillin-binding proteins (PBPs) has underscored the importance of such analysis. The spread of antibiotic resistance is also a predominant problem and it must be analyzed to monitor best practices in therapy. To manage accurately and rapidly invasive meningococcal infections, the confirmation of *N. meningitidis* by culture remained essential. As early antibiotic treatment is recommended when invasive meningococcal infection is suspected, isolation of viable organism is however often compromised. We have developed molecular methods for non-culture diagnosis and laboratory confirmation of such bacterial infections. These techniques allow aetiological diagnosis and treatment but also preventive measures to be applied rapidly, even in cases culture remain negative. These PCR methods allow the identification of bacterial DNA from CSF and whole blood and also the genogrouping of strains by segregating them to serogroups A, B, C, Y or W135.

Materials and Methods

During the year 2007, the NCM has received 51 strains of *Neisseria meningitidis* isolated from normally sterile specimens like blood (n=32), CSF (n=13), blood and

CSF (n=5) and blood and joint fluid (n=1). These strains correspond to 67% of the invasive meningococcal cases notified to the SFOPH (n=76).

When a strain is received by the CNM, its serogroup is immediately determined with two types of latex agglutination kits. The serogroups A, C or Y-W135 are performed with the Pastorex^{TM} meningitis kit (Bio-Rad, Pasteur, Paris) and the serogroup B with the Welcogen *N. meningitidis* B/*E. coli* K1 reagent (Remel). When the fresh culture is available (normally the second day after the 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, azythromycin, ciprofloxacin, and chloramphenicol. Values of the E-test (AB Biodisk, Sweden, distributed in Switzerland by Bio-Rad) on Mueller-Hinton 5% sheep blood agar are interpreted according to the American CLSI recommendations (2). But for cefuroxime and erythromycin, no value is provided by the CLSI and therefore the criteria proposed by the British Society for Antimicrobial Chemotherapy (3) are applied. Serogroups, serotypes and sero-subtypes are determined with a dot-ELISA technique based on a set of 27 monoclonal antibodies purchased from the National Institute for Biological Standards and Controls, Herdfordshire, Great Britain (4). As explained in the introduction, the DNA typing method chosen in all countries of Europe is now MultiLocus Sequence Typing (MLST) due to its reproducibility, reliability, affordable cost and throughput. MLST indexes the variation present in the nucleotide sequence of 400-500bp internal fragments from seven housekeeping-genes (5). 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 MLST profile is then accessible in the MLST database via the Internet (http://pubmlst.org/ neisseria/) as developed by Keith Jolley and Man-Suen Chan and operated at the University of Oxford (6).

Since 2004, the NCM has developed a rapid and sensitive nucleic acid amplification method to detect a meningococcal infection and one year later, to identify the serogroup at the genetic level. Nucleic acids of CSF or blood-EDTA are automatically extracted with the MagNa pure 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*) (7) and in a second time, whenever the first PCR

assay is positive, to detect the genes encoding the specific polysialyltransferase (*siaD* gene) for B, C, Y and W135 serogroups and *mynB* gene for A serogroup, respectively (8). This laboratory assay is offered to all Swiss laboratories or hospitals with a rapid response, especially for all paediatric patients.

Results

The 51 strains received in 2007 represented 67% of the 76 cases of invasive meningococcal diseases (IMD) notified to the Swiss Federal Office of Public Health (Figure 1). The number of IMD is almost identical as in 2006 with an incidence below 1 case per 100'000 inhabitants. This incidence is low: among European countries, the overall incidence ranges between < 1 per 100 000 up to 5 per 100 000 inhabitants. Other epidemiological data were recently published by the SFOPH (9) and available on its web site, http://www.bag.admin.ch. All strains except one were serogrouped. Like in the other European countries, the serogroup B is always predominant (49%) but the proportion of serogroup C (37%) has increased as compared to the last two years (Figure 2). The number of strains is relatively low and it is difficult to derive a trend based on these data. The two serogroups, B and C, encompass all serogroups detected in Switzerland in 2007 with the exception of one additional case of serogroup Y (Figure 3, Table 1). As observed during previous years, MLST type 11 was the most predominant genotype. It represents 35% of all MLST and all strains except one that belongs to serogroup C. Like in 2006, one strain of serotype B (B:2a:P1.5) and typed as MLST 11 has been detected, this strain corresponding to the known phenomenon of capsular switching (10). The second MLST most frequently identified was the MLST type 41 (16%), with all isolates corresponding to serogroup B. Various and new other MLST types were also present in one or two instances. (Table 2).

In 2007 new breakpoints for different antibiotics were defined by CLSI (2) and new recommendations were published by the European Monitoring Group on Meningococci (11). With these new breakpoints the decrease of susceptibility for penicillin described in 2006 was not confirmed, 53% of strains were susceptible and 47% were intermediate. The degree of resistance is in accordance with the data of other European countries. It is quite stable with the "intermediate" resistance (Pen^I)

isolates showing minimal inhibitory concentrations of 0.094 or 0.38mgL^{-1}. No strain was defined as a resistant isolate (CMI ≥ 0.5). Resistance to rifampicin which is occasionally observed following chemoprophylaxis was not detected in strains tested in 2007. The classical susceptibility of meningococci to quinolones appears to evolve these last few years (12) and has been monitored with care. In Switzerland, no intermediate or resistant strains have been detected so far. Other tested antimicrobial agents were active against meningococci (Table 3).
During 2007, 38 suspected clinical specimens (24 CSF, 11 blood samples and 4 other specimens) of 34 different patients were prospectively analyzed by amplification of the *ctra* gene. Sixteen PCR were positive for *N.meningitidis* and 22 were negative. For all PCR positive results except three patients, a second amplification was performed. Nine patients could be reported infected by the serogroup B, 3 by the serogroup Y or W135 and one by the serogroup C. The CNM has received only 6 strains corresponding to the 15 patients tested positive by PCR elsewhere. Most of the time, cultures remained negative.

Discussion

The number of cases of IMD was unchanged in 2007 as compared to 2006 with about two thirds of the strains corresponding to these infections being shipped to the CNM. The repartition of serogroups was exactly the same as in other European countries with a predominance of the serogroup B followed by serogroups C and Y. The proportion of serogroup C has slightly increased in 2007 and this serogroup determination remains essential to establish the recommendations of vaccination. In Switzerland, serogroup C meningococcal immunization is recommended for certain risk groups (immunodeficient persons, laboratory personnel, military conscripts). Supplementary meningococcal immunization is now also recommended for healthy children aged from 1 to 4 years as well as healthy adolescents aged from 11 to 19 years. The determination of serotypes must be followed with care to exclude any increase of serogroup C strains like that was witnessed in 2000 where the incidence topped to 2.5 cases for 100 000 inhabitants with 61% of the strains belonging to

serogroup C. Consequently, a large campaign of immunization was carried out in the Canton of Fribourg.

Like in 2006, we have detected one isolate of serogroup B but with the genotype of a serogroup C (MLST11) strain. This strain changing its polysaccharidic capsule is probably due to the pressure of immunization (capsular switching). The MLST type 11 was yet predominant with a majority of strains belonging to serogroup C followed by the MLST type 41 corresponding exclusively to serogroup B strains. The MLST method is powerful to detect rapidly a cluster of strains and to identify a clone particularly associated with disease. The geographical distribution of the clonal complexes found in Switzerland is similar to the European-wide dispersion: the genetic characterization of isolates reveals the spread of the major disease-associated clonal complexes. No epidemic was detected in 2007 but some MLST were particularly controlled for example the MLST type 269 associated with serogroup B disease and which has recently emerged in Québec, Canada. During periods of endemic meningococcal disease (2004-2005), this serogroup B strain was identified as a cluster of cases responsible of IMD (13).

The proportion of strains with a reduced sensitivity to penicillin has not increased. No strain was defined as resistant to this antibiotic but 21% of all isolates had a CMI of >0.125mg/L suggesting a polymorphism in the *penA* gene encoding penicillin binding protein. In 2008, a systematic sequencing of the *penA* gene for all new isolates will be established and a retrospective study of strains isolated during the last five years will be developed. The analysis of the sequence of the *penA* gene, in both susceptible and intermediate strains, is now becoming an important task within the Eu-MenNet project. This assay will be developed to compare our data with that of other European countries, the analysis of the DNA sequences being an important step for defining the breakpoints using solid genetic grounds. Overall, all other antibiotics currently used against meningococci were very active but a new problem of resistance appeared in other countries (12), in relationship with fluoroquinolones. Ciprofloxacin or levofloxacin may be used only for prophylaxis of meningococcal contacts, but for surveillance purposes, the MIC of the nalidixic acid has been advised by the CLSI. A nalidixic acid MIC ≥ 8µg/ml may correlate with diminished fluoroquinolone susceptibility. We will therefore implement the testing of this antibiotic in 2008.

PCR is a very efficient method to detect the presence of *Neisseria meningitidis* in a clinical specimen. We have received samples (blood or CSF) from different places in Switzerland with a good feed-back of the paediatric doctors. The molecular assays decrease the time of identification of *N. meningitidis* but also improve case ascertainment by confirmation of infections in culture-negative clinical samples. As it is difficult to evaluate the exact input of this method in Switzerland, it will be very interesting to compare cases with only a PCR positive result with cases detected by other methods (clinical, microscopy, culture). In 2008, the OFSP will probably have these results because in October 2007, a new recommendation was promulgated to declare all patients with a positive microscopic examination. In Geneva, we perform the majority of the molecular detection assays for IMD suspicion cases from all over Switzerland. Thus, we will send the file of our database to the OFSP in order to compare the results with microscopy, culture or clinical suspicion.

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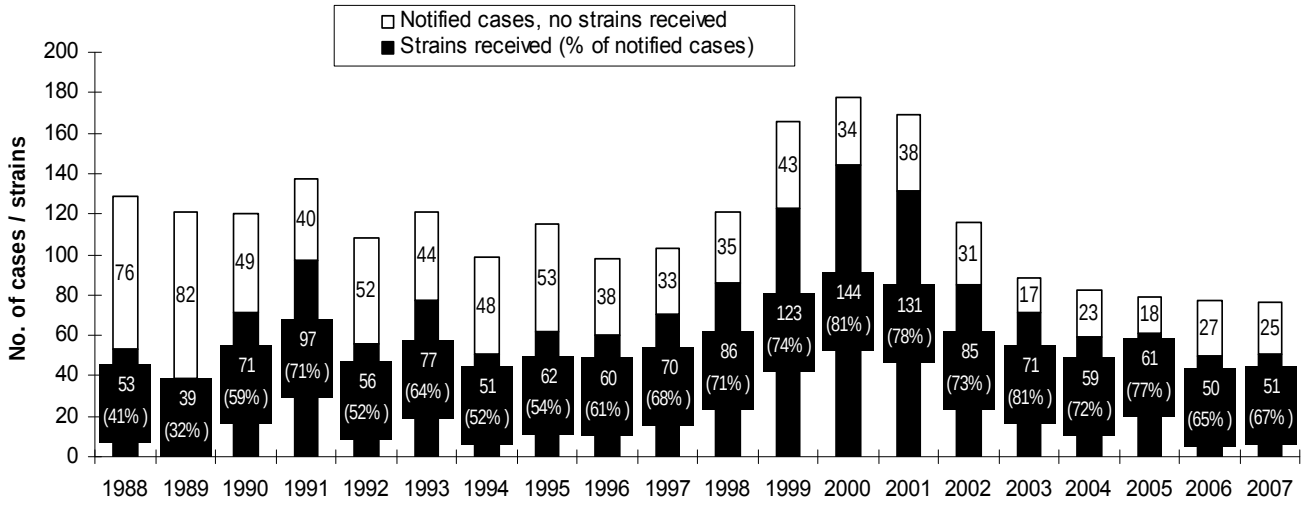

Figure 1: Comparison of the annual number of *N. meningitidis* strains received at the National Center for Meningococci in Geneva and the number of invasive meningococcal infections notified to the Swiss Federal Office of Public Health from 1988 to 2007

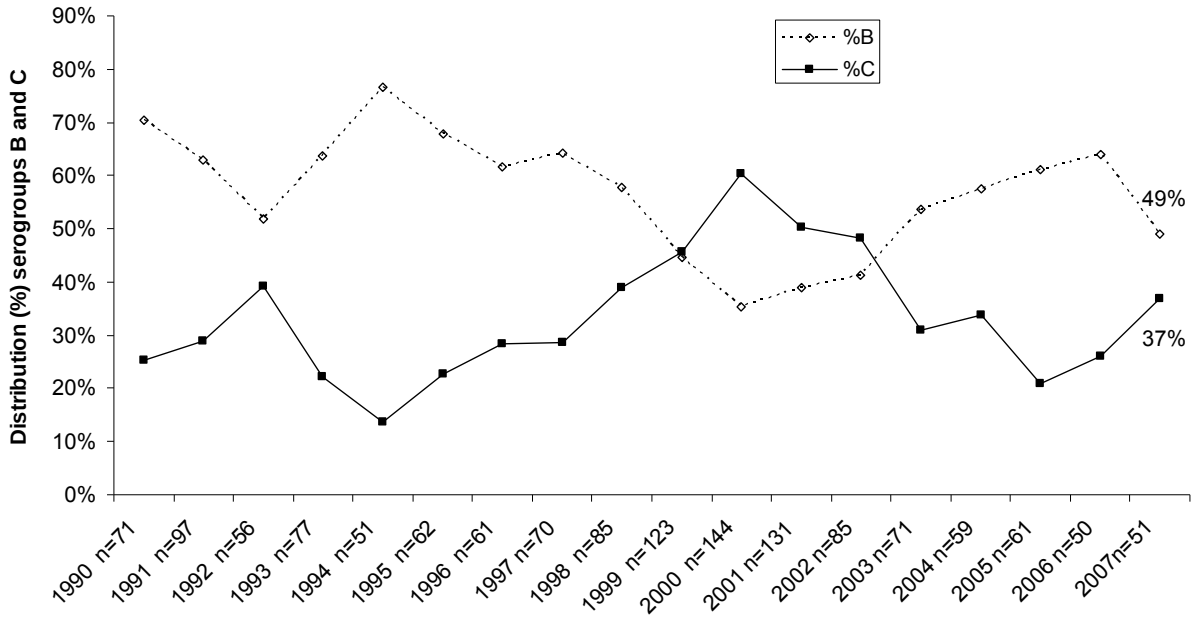

Figure 2: Distribution of serogroups B and C of *N. meningitidis* from 1990 to 2007

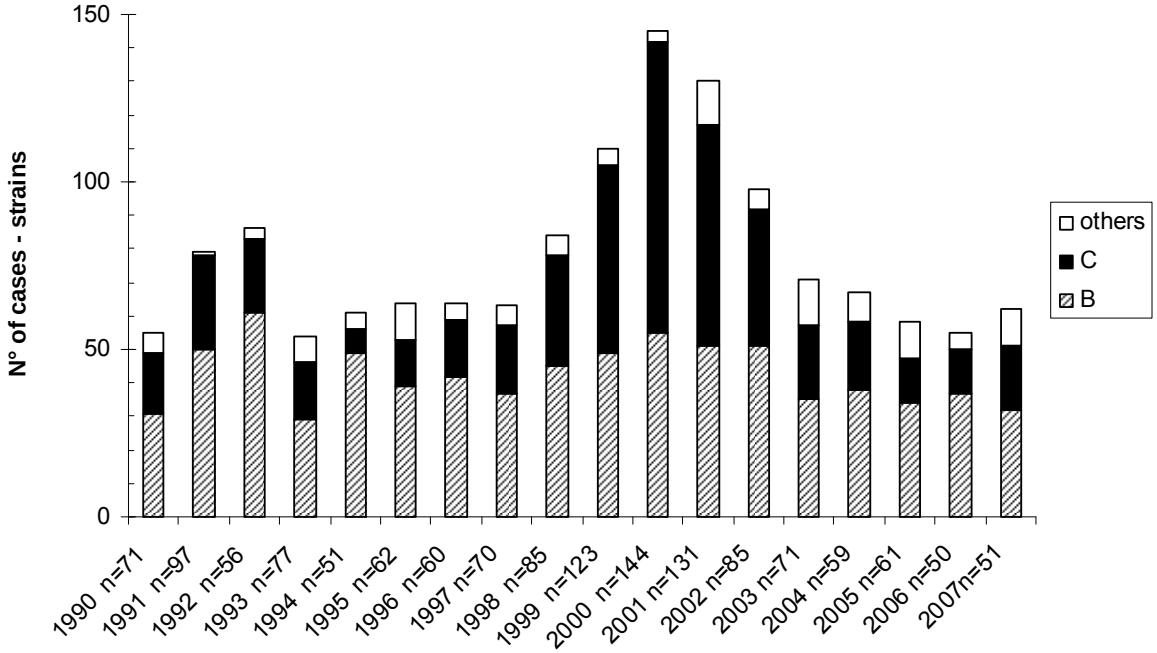

Figure 3: Serogroup distribution of invasive meningococcal isolates 1990-2007

Table 1: Distribution of serogroups and serotypes of *N. meningitidis* strains isolated in Switzerland 2007

Serotype	4	2a	14	15	1	2c	unknown	Total	
Serogroup									
A									0%
B	7	1	1	2	3		11	25	49%
C		17					2	19	37%
W135									0
Y	1		4			1		6	12%
unknown					1			1	2%
Total	8	18	5	2	4	1	13	51	100%
	16%	35%	10%	4%	8%	2%	25%	100%	

Table 2: Distribution of the most frequent MLST types for meningococci in Switzerland, during 2007

	11	41	ND*	Other MLST**	Total
Number of strains	18	7	5	21	51
	35%	14%	10%	41%	100%

*: Not defined MLST

**: Other MLST: 17 different MLST types

Table 3:

Inhibitory activity of 9 antimicrobial agents on 51 meningococci isolated in Switzerland during 2007

Agent	Minimal Inhibitory Concentration (µg / ml) range	50%	90%	Breakpoint sensitive ≤ µg/ml	% sensitive
Penicillin	0.032-0.38	0.064	0.125	0.06*	53%
Cefuroxime	0.032-1.5	0.094	0.25	1**	98%
Ceftriaxone	<0.01	<0.01	<0.01	0.12*	100%
Minocycline	0.125-0.5	0.25	0.5	2***	100%
Rifampicin	<0.01-0.25	0.023	0.094	0.5***	100%
Erythromycin	0.38-2	0.75	1.5	0.5**	20%
Azithromycin	0.38-1.5	0.75	1	2***	100%
Ciprofloxacin	<0.01-0.01	<0.01	<0.01	<0.03*	100%
Chloramphenicol	0.38-2	1	1.5	2*	100%

*CLSI/NCCLS 2007 (3) and EMGM working group
**British Society for Antimicrobial Chemotherapy (5).
*** CLSI/NCCLS 2007