Annual Report of the National Center for Meningococci 2006

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Introduction

Neisseria meningitidis is a leading cause of bacterial meningitis and severe sepsis. In cases of invasive infection, measures must be taken promptly to administer appropriate antimicrobial chemotherapy and to prevent secondary cases by vaccination and/or chemoprophylaxis. Meningococcal infection is a major public health problem in European countries particularly in infants and adolescents with a great emotional impact in the population. A good characterization of all isolated strains in Switzerland is important to set off preventive measure in certain populations at risk like the recommendations of vaccination in November 2005 (1). The National Center for Meningococci (NCM) is a key for this epidemiological surveillance by an efficient biological characterisation of all strains and a surveillance of their resistance patterns. The Central 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), Bern. Since three years, molecular biology detection of *N. meningitidis* has also allowed an increase of the laboratory confirmation rate of meningococcal infections with a non laboratory-based culture method.

Materials and methods:

During the year 2006, the NCM has received 49 strains of *Neisseria meningitidis* isolated from normally sterile specimens like blood samples (n= 25), cerebrospinal fluid (CSF) (n=15), blood and LCR (n=8) and blood and one joint fluid (n=1). These specimens corresponded to 64% of severe meningococcal cases notified to the SFOPH (n=77). One additional strain of respiratory origin was also analysed because it was isolated from a child with a diagnosis of meningitis and septicemia but in whom no culture revealed positive from any of these specimens.

The serogroups (A, C, Y-W135) were determined with the latex agglutination kit (Biorad-Pasteur, Paris). The Welcogen *N. meningitidis* B/*E. coli* K1 reagent kit (Remel) was used to perform the serogroup B determination. Furthermore, these strains were also tested for their antimicrobial susceptibility (Minimal Inhibitory Concentration = MIC) to 9 antimicrobial agents (penicillin, cefuroxime, ceftriaxone, minocycline, rifampicin, erythromycin, azithromycin, ciprofloxacin, chloramphenicol) with the E-test method (AB Biodisk, Solna, Sweden) on Mueller-Hinton 5% sheep blood agar. The values for the determination of inhibitory concentrations were interpreted according to the criteria proposed by the British Society for Antimicrobial Chemotherapy (2) but for the penicillin, ceftriaxone, rifampicin, azithromycin, ciprofloxacin and chloramphenicol, values of the American CLSI recommendations were applied (3). Serogroups, serotypes and subtypes were determined with a dot-ELISA technique based on a set of 27 monoclonal antibodies purchased from the National Institute for Biological Standards and Controls, Hertfordshire, Great Britain (4). In order to detect epidemic clones, multilocus sequence typing method (MLST) was also performed on all strains. This genotyping method is based on the sequencing of seven housekeeping-genes *abcZ* (putative ABC transporter), *adk* (adenylate kinase), *aroE* (shikimate deshydrogenase), *fumC* (fumarate), *gdh* (glucose-6-phosphate deshydrogenase) pdhC (pyruvate déshydrogénase subunit) and *pgm* (phosphoglucomutase) (5). Determined sequences were subsequently introduced into the web site http://mlst.zoo.ox.ac.uk in order to obtain the corresponding MLST type number.

The NCM has implemented in 2004 a PCR assay to directly detect meningococci in specimens such as whole blood and CSF. Starting from two hundred microliters of

sample, nucleic acids were extracted with the nucleic acid isolation kit I and the blood protocol of the MagNa Pure Compact system (Roche Diagnostic Ltd.) after a short pre-treatment using enzymatic and buffer lysis. A real-time PCR amplification was then performed to detect the *ctra* target gene (capsular transport gene of *Neisseria meningitidis*) (6). These molecular techniques were first evaluated with the precious help of a Swiss study in collaboration with the SFOPH and the PIGS (Pediatric Infectious Group of Switzerland) (7). During the year 2005, a serogroup determination (A, B, C, Y-W135) was then developed using similar molecular methods (8). In 2006, different samples of blood or CSF were sent to Geneva by other Swiss laboratories where we performed PCR-based identification of the pathogens and PCR-based serogrouping on all PCR positive samples.

Results:

The 50 strains received in 2006 represented 64% of the 77 cases of invasive meningococcal diseases notified to the Swiss Federal Office of Public Health (Figure 1). The number of cases has decreased since 2000 with an incidence close to the incidence of the last year (1 annual case per 100'000 inhabitants). Therefore Switzerland belongs to the European countries with a low incidence. Further epidemiological data are published on the web site of the Swiss Federal Office of Public Health http://www.bag.admin.ch/infreporting.

In 2006, all strains were serogrouped. Serogroup B strains were predominant (n=32) with still a small increase as compared to previous years representing 64% of strains, followed by serogroup C (13 strains, 26%), serogroup Y (3 strains, 6%) and W135 (2 strains, 4%) (Table 1, Figure 2). The epidemic peak related to serogroup C strains observed between 1999 and 2002 has continued to decline (Figure 3). This decreasing trend of in group C-related meningitis and septicaemia is probably due to the recent availability of the MenC vaccine and its use according to the national recommendations. Public health authorities recommended vaccination for children and teenagers if the incidence increases in this group of age (over 2.8 cases per 100 000 inhabitants), in the immunodeficiency population and for the laboratory workers (1).

Similarly to 2005, the MLST 11 has been most frequently identified (26%) followed by various and other new MLST types (Table 2). A clone with an MLST 2816 has been detected with this typing method. All these patients lived in Luzern and the importance of this observation of a new epidemic strain will be investigated.

Interestingly one strain of serotype B (by agglutination, dot Elisa and PCR specific to the serogroup) revealed of MLST type 11. All our other strains with this MLST type 11 belonged of serogroup C. Such rare strains of MLST type 11 and serogroup B have been described recently in Europe, and attributed to a phenomenon of switching capsule (9-10).

Of the 50 strains tested in 2006, an important decrease of susceptibility for penicillin was detected. Only 6% remained susceptible, 4% were resistant and 90% were intermediate with the proposed breakpoints by CLSI. The MIC range (Table 3) was not different to the values reported during the previous years (range 0.03 to 0.5) and to the data published in the literature (11). One strain was exceptionally resistant with a MIC of 0.75. Other tested antimicrobial agents were active against meningococci, with the exception of erythromycin (only 22% of susceptible strains). For the first time, two strains were intermediate to Azythromycin (CMI of 3 and 4). Like during previous years, no resistance against ciprofloxacin was detected in the isolates.

During 2006, 40 suspected clinical specimens (18 CSF, 18 bloods and 4 other specimens) of 37 different patients were prospectively analysed. The CSF and/or blood specimens were positive for 17 specimens of 15 patients. The serogroup determination by PCR have been performed and defined for all the cases. Three patients were infected with a *N. meningitidis* of serogroup C, twelve patients with a serogroup B. Among the 17 samples positive for *N. meningitidis* by PCR, 9 were culture negative (53%).

Discussion:

The repartition of strains (serogroups and MLST types) has not really changed in 2006, as compared to 2005. Serogroup B strains were predominant, encompassing two thirds of the analysed strains. The serogroup determination is essential for vaccine recommendations from a public health standpoint and for the secondary prophylaxis of contacts. The determination of serotypes and subtypes remains

valuable allowing a reliable comparison of inter-laboratory results. The MLST type 11 was predominant and corresponded exclusively to strains of serogroup C except for one strain of serogroup B which appears to be the first strain described in Switzerland with such a switching capsule mechanism. Capsule switching in *Neisseria menigitidis* is thought to occur by horizontal DNA exchange between meningococcal strains. Antigenic variants may be generated by allelic replacement of *siaD* gene and selected by specific immunity against the capsular antigen. The Swiss strain analysed was probably in this case because it was of serogroup B by PCR and the target of this PCR was the sialic acids (*sia D*). Serogroup B and C with MLST 11 isolates both express similar virulence (12). This justifies an enhanced system of surveillance by molecular typing of such isolates, particularly after serogroup-specific vaccination. All other strains of serogroup B displayed different MLST (9 different MLST strains for 31 strains). This method is useful because a same strain of serogroup B with a MLST type of 2816 was detected in six patients living in the canton of Luzern, thus conducting to analyse more precisely this cluster in central Switzerland. The OFSP will be in charge of this investigation in order to understand if this strain was epidemic or not.

The proportion of strains with a reduced sensitivity to penicillin has increased. Forty five strains were intermediate to this antimicrobial agent and 40% of these strains had a CMI of > 0.125mg/L suggesting a polymorphism in the *penA* gene encoding penicillin-binding protein 2. A direct correlation between a change in the expression of this gene and the reduced sensitivity has been described. All other currently used antibiotics remain very active against the Swiss strains of meningococci.

Direct detection of meningococci by real-time PCR was performed in the Swiss pediatric population and yielded a good sensitivity. This approach has allowed the confirmation of suspected cases of invasive meningococcal disease (IMD). Altogether, the implementation of this molecular assay yielded an increase in detection of infection by 52% (suspicion of infection but culture negative). All samples positive for *N. meningitidis* by PCR could also be rapidly tested for direct molecular serogroups. All cases were from paediatric origin, 20% infected with serogroup C strains and 80% with serogroup B. The major advance was this molecular serogroup determination because it provides a rationale to a rapid decision from public health

authorities concerning additional contact vaccination to the administration of a prophylactic antibiotic.

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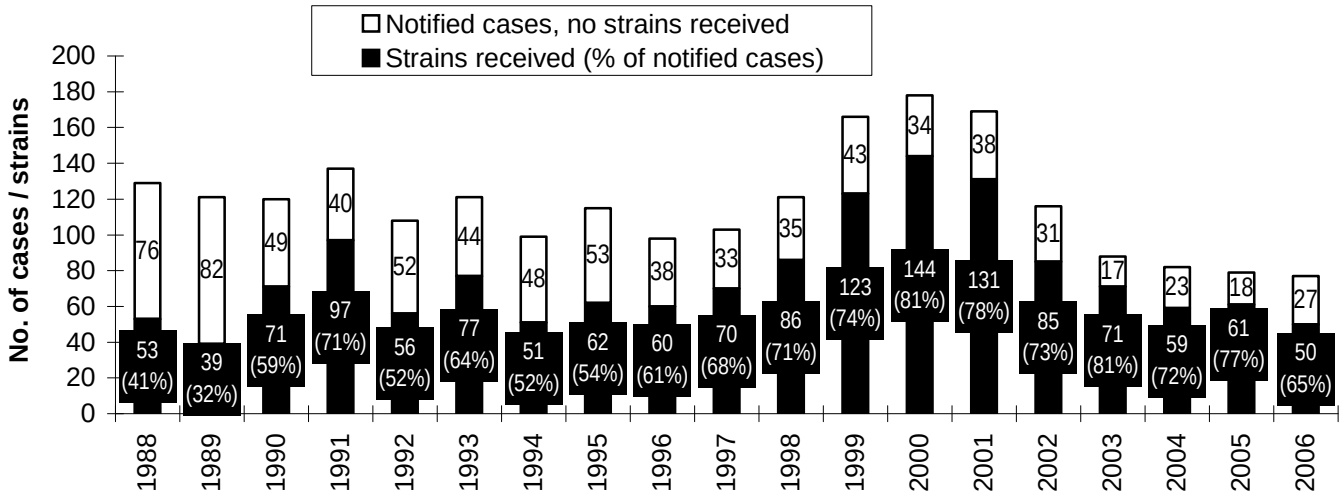

Figure 1 : Comparison of annual number of *N. meningitidis* strains received at the national center for meningococci in Geneva and the number of invasive meningococcal infections notified to the Swiss Federal Office of Public Health from 1988 to 2006

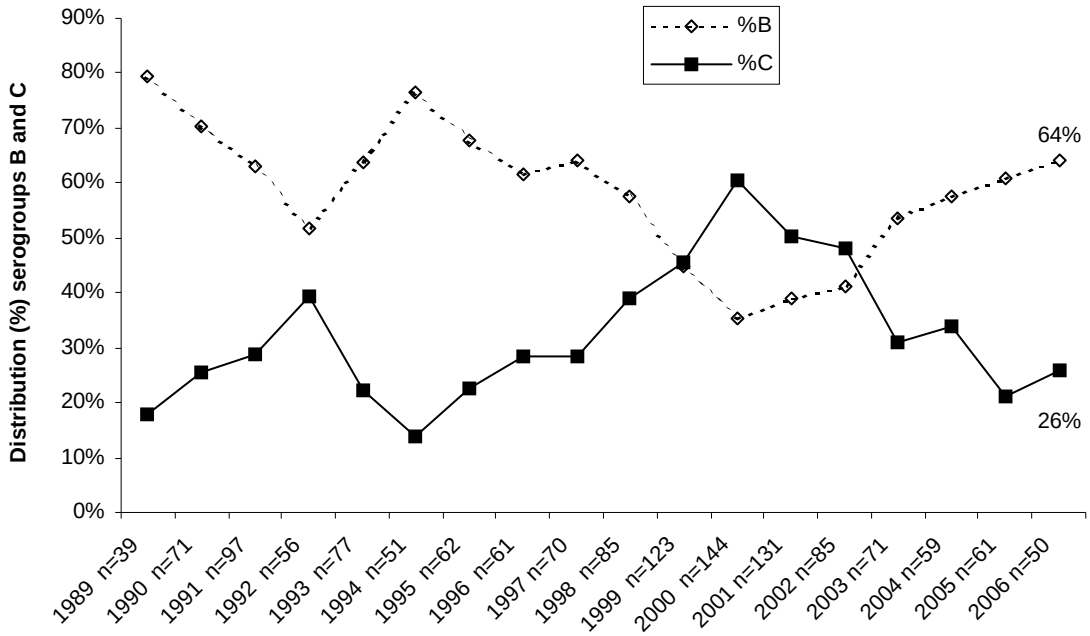

Figure 2 : Distribution of serogroups B and C of *N. meningitidis* from 1988 to 2006

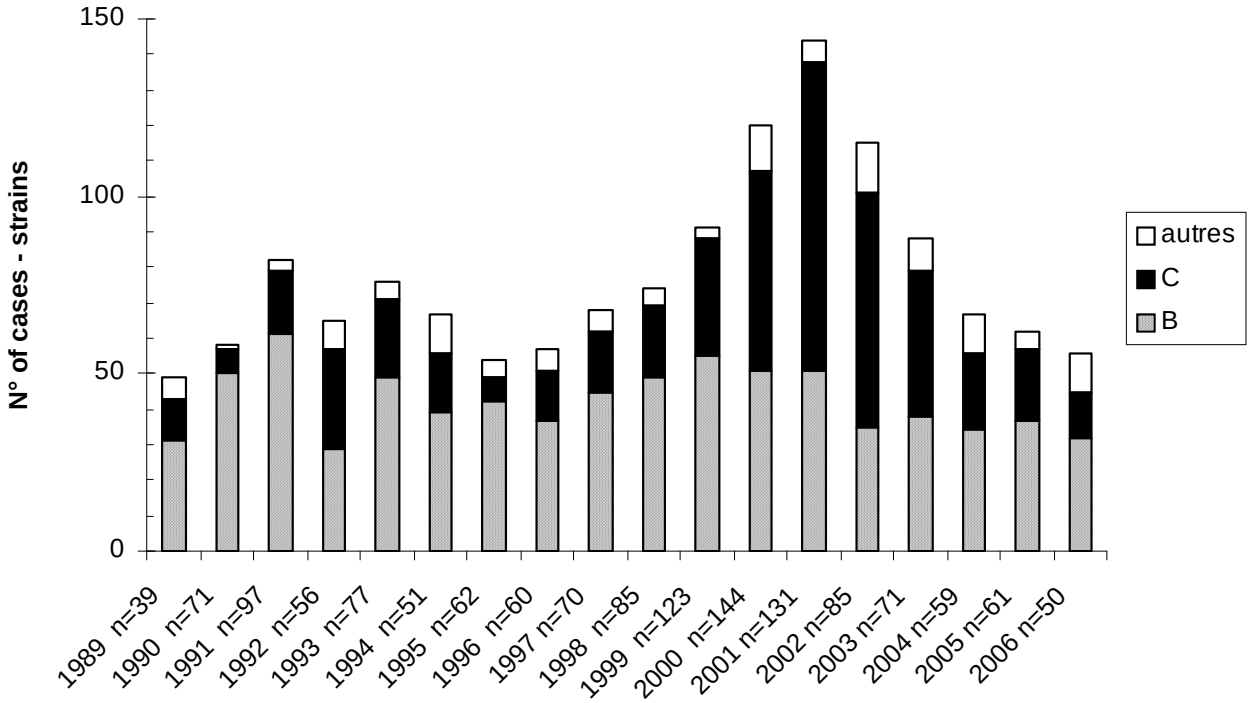

Figure 3 : Serogroup distribution of invasive meningococcal isolates 1988-2006

Serogroup	1	2a	4	14	15	unknown	total	
A								0%
B	3	1	7	2	7	12	32	64%
C	1	12					13	26%
W135						2	2	4%
Y	1			2			3	6%
Total	5	13	7	4	7	14	50	100%
	10%	26%	14%	8%	14%	28%	100%	

Table 1 : Distribution of serogroups and serotypes of *N. meningitidis* strains isolated in Switzerland 2006

Table 2: Distribution of most MLST types of meningococci in Switzerland 2006.

	11	ND*	41	2816	Other MLST**	Total
Number of strains	13	12	5	6	14	50
	26%	24%	10%	12%	28%	100%

*: Not defined MLST

**:Other MLST: 11 different MLST

Table 3:

Inhibitory activity of 9 antimicrobial agents on 61 meningococci isolated in Switzerland in 2006

Agent	Minimal Inhibitory Concentration (µg / ml) range	50%	90%	Breakpoint sensitive ≤ µg/ml	% sensitive
Penicillin	0.023-0.75	0.094	0.25	0.06*	6%
Cefuroxime	<0.02-0.75	0.064	0.19	1**	100%
Ceftriaxone	<0.01	<0.01	<0.01	0.1*	100%
Minocycline	0.064-0.75	0.25	0.5	4**	100%
Rifampicin	<0.01-0.5	0.023	0.125	0.5*	100%
Erythromycin	0.094-3	0.75	1	0.5**	22%
Azithromycin	0.125-4	1	1.5	2*	96%
Ciprofloxacin	<0.01	<0.01	<0.01	<0.03*	100%
Chloramphenicol	0.5-2	1	1.5	2*	100%

* CLSI/NCCLS 2006 (3)
**According to the British Society for Antimicrobial Chemotherapy (5).