Annual Report of the National Center for Meningococci 2004

Béatrice Ninet, Peter Rohner, Jacques Schrenzel

Hôpitaux Universitaires de Genève

Laboratoire Central de Bactériologie

24 rue Micheli-du-Crest

1211 Genève 14

Phone : 022 37 29 252 Fax : 022 37 27 304

Introduction

Meningococcal diseases are an important cause of morbidity and mortality worldwide. They remain a leading cause of bacterial meningitis and sepsis in infants and adolescents. Preventive measures have been implemented to avoid the spread of the germ in certain populations at risk. The epidemiological surveillance by the National Center for Meningococci (NCM) contributes to implement these measures. The Central Bacteriology Laboratory of the University Hospitals Geneva has performed this task since 1990 in collaboration with the Swiss Federal Office of Public Health (SFOPH), Bern.

Materials and methods:

During the year 2004, the NCM has received 59 strains of *Neisseria meningitidis* isolated from normally sterile specimens like cerebrospinal fluid (CSF) (n=19) or blood samples (n= 39) corresponding to 71% of severe meningococcal cases notified to the SFOPH. One strain of respiratory origin was also analysed because it was isolated from the father of a new-born child with documented meningitis.

The serogroups (A, B, C, W135-Y) were determined with the latex agglutination kit (Biorad-Pasteur, Paris) . 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. 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 (1). In order to detect epidemic clones, the 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) (2). Determined sequences were subsequently introduced into the web site http://mlst.zoo.ox.ac.uk in order to obtain the MLST type number.

The NCM has also implemented a PCR technique 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*) (3)).

Results:

The 59 strains received in 2004 represented 71% of the 83 cases of invasive meningococcal diseases notified to the Swiss Federal Office of Public Health (Figure 1). The number of cases has continuously decreased since 2000, reaching a similarly low incidence as in 2003, i.e. 1.2 annual cases per 100’000 inhabitants. Further epidemiological data are published on the web site of the Swiss Federal Office of Public Health http://www.bag.admin.ch/infreporting.

Like in other European countries, serogroup B strains were predominant with about the same percentage as in 2003 representing 58% of strains, followed by serogroup C (34%), serogroup Y (3%) and W135 (3%) (Table 1, Figure 2). The epidemic peak

related to serogroup C strains from 1999 to 2002 has remarkably decreased (Figure 3).

Similarly to 2003, the MLST 11 has been most frequently identified (19%) followed by the MLST 8 (8%) and various other MLST types (Table 2). No epidemic clone has been detected with this typing method.

Of the 59 strains tested in 2004, 86% were susceptible to penicillin, 10% were resistant and 4% were intermediate. Other tested antimicrobial agents were active against meningococci, with the exception of erythromycin (only 8% of susceptible strains) and azithromycin (one strain I and one strain R).

Discussion:

The repartition of strains (serogroups and MLST types) has not varied in 2004, as compared to 2003. Serogroup C strains remain at the same level as the year before, corresponding to about one third of all strains analysed . This 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.

Direct detection of meningococci by real time PCR was implemented for clinical specimens (CSF and blood) and yielded a good sensitivity. In our assay, 1-2 bacteria were detectable in CSF samples and 10 bacteria in blood samples. This approach allows the confirmation of suspected cases of invasive meningococcal disease (IMD). In England the number of confirmed cases has doubled after the introduction of PCR. A project protocol was established together with the Swiss Federal Office of Public Health to explore the potential role of PCR in the diagnosis and surveillance of IMD and to estimate the distribution of true incidence in Swiss children. This project was submitted and approved by the Pediatric Infectiology Group of Switzerland (PIGS). It consists in analyzing CSF and blood samples drawn from children suspected of IMD. For 2005, we have also planed to develop the direct determination of serogroups by nucleic acid amplification coding for specific sialic acids of serogroups B, C, Y. W135 strains and a non sialic acid for serogroup A strains (4).

In collaboration with the SFOPH, the NCM has published the epidemiological data on severe meningococcal infections in Switzerland (5, 6).

Finally, we have also created a website CNM and provide updated protocols (http://www.meningo.ch/).

References:

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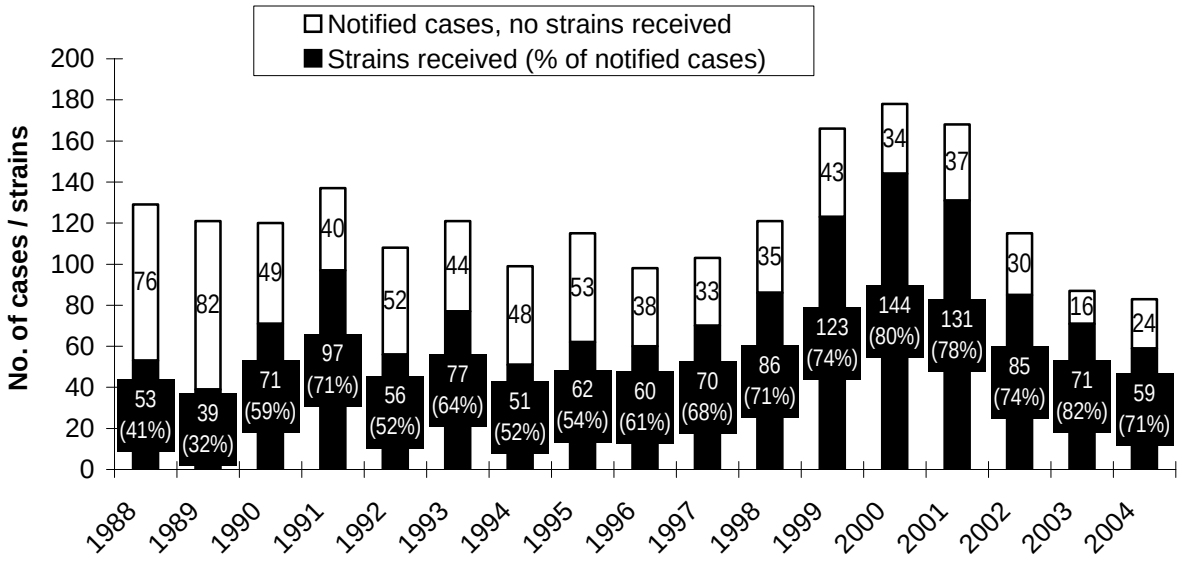

Figure 1 : Comparison of annual number of *N. meningitidis* strains received at the national center for meningococci in Geneva and the number of invasive meningococcal infections notified to the Swiss Federal Office of Public Health from 1988 to 2004

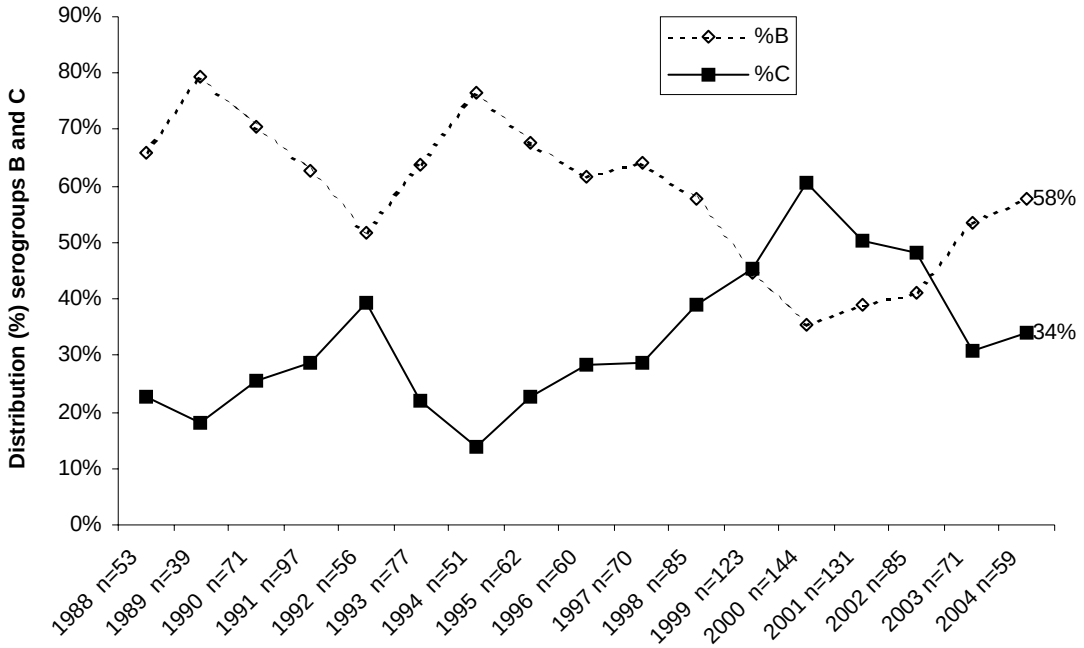

Figure 2 : Distribution of serogroups B and C of *N. meningitidis* from 1988 to 2004

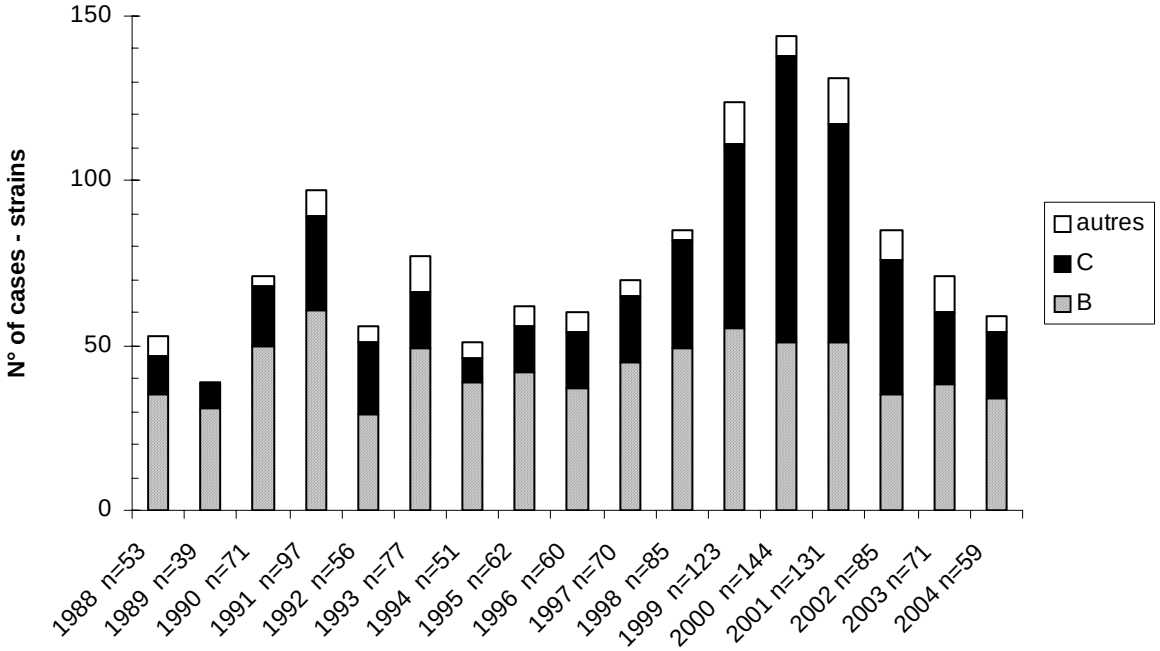

Figure 3 : Serogroup distribution of invasive meningococcal isolates 1988-2004

	Serotype								
Serogroup	1	2a	2b	4	14	15	unknown	total	
A									0%
B	3			14		7	10	34	58%
C		11	6				3	20	34%
W135							2	2	3%
Y					2			2	3%
unknown				1				1	2%
Total	3	11	6	15	2	7	15	59	100%
	5%	19%	10%	25.5%	3%	12%	25.5%	100%	

Table 1 : Distribution of serogroups and serotypes of *N. meningitidis* strains isolated in Switzerland 2004

	MLST					
Complete serotype	**11**	**8**	**ND***	**Other[2]**	**Total**	
C:2a:P1.2,5	3				3	5%
C.2a :P1.-	2				2	3%
C2a :P1.2	1				1	2%
C2a :P1.5	5				5	8%
C:2b:P1.2,5		4			4	7%
C:2b:P1.2		1			1	2%
Other[1]			7	36	43	73%
Total	11	5	7	36	59	100%
	19%	8%	12%	61%	100%	

Table 2: Distribution of most frequent serotypes and MLST types of meningococci in Switzerland 2004.

*: Not defined MLST

[1] :Other serotype: 36 different complete serotypes

[2] :Other MLST: 27 different MLST