Annual Report of the National Center for Meningococci 2003

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Introduction

Neisseria meningitidis remains a leading cause of meningitis worldwide. Other invasive infections caused by meningococci like bacteremia may be even more severe (1). In order to establish reliable treatment and prophylaxis recommendations, a surveillance of invasive meningococcal diseases has been implemented in Switzerland, similarly to many other countries (2). The central bacteriology laboratory of the university hospital Geneva has been designated since 1990 as Swiss national center for meningococci in collaboration with the Swiss Federal Office of Public Health, Bern.

Materials and Methods

During the year 2003, the national reference laboratory for meningococci received 97 viable *N. meningitidis* strains, of which 71 were isolated from blood cultures, cerebrospinal fluid and other normally sterile specimens. These strains were typed with a dot-ELISA technique using monoclonal antibodies purchased from the National Institute for Biological Standards and Controls, Hertfordshire, Great Britain (3). This set of antibodies, used also by many national centers for meningococci, consists of 27 monoclonal antibodies directed against the following antigens :

Serogroups : A, B, C, W135, Y,
Serotypes : 1, 2a, 2b, 2c, 4, 14, 15, 21,
Subtypes : P1.1, P1.2, P1.3, P1.4, P1.5, P1.6, P1.7, P1.9, P1.10, P1.12, P1.13, P1.14, P1.15, P1.16.

For the 71 strains isolated from normally sterile body fluids, the multilocus sequence type (MLST) was also determined. This technique is based on the sequencing of the 7 housekeeping-genes, *abcZ* (putative ABC transporter), *adk* (adenylate kinase), *aroE* (shikimate dehydrogenase), *fumC* (fumarate), *gdh* (glucose-6-phosphate dehydrogenase), *pdhC* (pyruvate dehydrogenase subunit), et *pgm* (phosphoglucomutase) (4). The determined sequences were subsequently introduced into the web site http://mlst.zoo.ox.ac.uk/dbqry/mening/ in order to obtain the MLST type number.

Minimal inhibitory concentrations of 9 antimicrobial agents were determined with the E-test (AB Biodisk, Solna, Sweden) on Mueller-Hinton 5% sheep blood agar, and the values were interpreted according to available criteria proposed by the British Society for Antimicrobial Chemotherapy (5).

Results

The 71 viable strains isolated from normally sterile specimens received in 2003 represented 82% of the 87cases of invasive meningococcal diseases notified to the Swiss Federal Office of Public Health (figure 1). After the peak of 178 cases notified in 2000 the number of cases has continuously decreased to a remarkably low incidence of 1.2 annual cases per 100'000 inhabitants (6). Further epidemiological are published by the Swiss Federal Office of Public Health on the web site http://www.bag.admin.ch/infreporting.

Since 2000 we observed a decrease of serogroup C strains causing severe infections (figure 2). In 2003 serogroup B was predominant (54%), followed by serogroup C (31%), Y (7%) and W135 (6%) (table 1, figure 2). The epidemic from 1999 to 2002 was caused by an increasing number of severe cases caused serogroup C meningococci (figure 3). A similarly increasing number of serogroupe C has also been reported in France (7).

Similarly to 2002, the MLST 11 has been most frequently identified (15%) in 2003, predominantly with the antigenic formula C:2a:P1.2,5, followed by the MLST 8 mostly serotype C:2b:P1.2,5 (table 2).

Of the 71 strains tested in 2003, 86% were fully susceptible to penicillin (MIC <= 0.12 µg/mg), similarly to previous years. The other tested antimicrobial agents were

highly active against the meningococci with the exception of erythromycin and azithromycin, where the MICs were close to the breakpoint (table 3).

Discussion

Reliable serotyping results have been essential for vaccine recommendations. In particular, since the recent availability of efficient meningococcal serogroup C conjugate vaccines (8). The MLST results have been useful for a better understanding of the epidemiology. This technique allows a reliable comparison of inter-laboratory results and related strains can be followed more accurately. Due to the reliability of this method, sero subtyping with monoclonal antibodies may be simplified with influencing negatively the epide.

Antimicrobial agents remained highly active against Swiss meningococci isolated in 2003, in particular ceftriaxone, ciprofloxacin, and rifampicin. Surveying antimicrobial susceptibility remains essential, particularly since ciprofloxacin resistant isolates were recently reported from Spain (9).

A manuscript entitled "Epidemiological surveillance of meningococcal disease in Switzerland, 1999-2002" prepared by the Swiss Federal Office of Public Health, Bern has been submitted in 2003.

For 2004 the national reference laboratory will focus on the application of direct detection of meningococcal DNA by PCR with the primary aim to confirm suspected cases with negative blood cultures and CSF cultures.The specific detection will be performed by quantitative Taqman amplification of the *ctrA* gene (capsular transport gene) according to the primers and probe previously described (10). Samples positive with this first amplification will be further analyzed to determine the serogoups. Serogroup B, C, Y and W135 strains contain gene cassettes that control the expression of polysaccharide capsules of different sialic acids and the *siaD* gene has been determined as a specific target for genogrouping (11). The biosynthesis of the serogroup A capsular polysaccharide is controlled by another gene cassette containing four open reading frames (ORFs) *sac*A to-D. Therefore serogroup A strains are not detected with the method described above. However ORF3 (*sacC*) has been reported a specific target for these strains (12).

Reference List

(1) Rosenstein NE, Perkins BA, Stephens DS, Popovic T, Hughes JM. Meningococcal disease. N Engl J Med 2001; 344(18):1378-1388.

(2) Office fédéral de la santé publique. Prévention des infections invasives à méningocoques. Bull OFSP 2001; 46:893-901.

(3) Frasch CE, Zollinger WD, Poolman JT. Serotype antigens of *Neisseria meningitidis* and a proposed scheme for designation of serotypes. Rev Infect Dis 1985; 7(4):504-510.

(4) Maiden MC, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R et al. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci U S A 1998; 95(6):3140-3145.

(5) British Society for Antimicrobial Chemotherapy. BSAC standardized disc sensitivity testing method. BSAC 1998.

(6) Office fédéral de la santé publique. Evolution des infections à méningocoques en Suisse: juillet 1999-juin 2002. Bull OFSP 2003;(4):48-50.

(7) Antignac A, Ducos-Galand M, Guiyoule A, Pires R, Alonso JM, Taha MK. *Neisseria meningitidis* strains isolated from invasive infections in France (1999-2002): phenotypes and antibiotic susceptibility patterns. Clin Infect Dis 2003; 37(7):912-920.

(8) Ramsay ME, Andrews N, Kaczmarski EB, Miller E. Efficacy of meningococcal serogroup C conjugate vaccine in teenagers and toddlers in England. Lancet 2001; 357(9251):195-196.

(9) Alcala B, Salcedo C, De La FL, Arreaza L, Uria MJ, Abad R et al. *Neisseria meningitidis* showing decreased susceptibility to ciprofloxacin: first report in Spain. J Antimicrob Chemother 2004; 53(2):409.

(10) 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(4):1553-1558.

(11) Tzanakaki G, Tsolia M, Vlachou V, Theodoridou M, Pangalis A, Foustoukou M et al. Evaluation of non-culture diagnosis of invasive meningococcal disease by polymerase chain reaction (PCR). FEMS Immunol Med Microbiol 2003; 39(1):31-36.

(12) 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(12):4531-4535.

Figure 1 : Comparison of annual number of viable *N. meningitidis* strains received at the national center for meningococci in Geneva and number of invasive meningococcal infections notified to the Swiss Federal Office of Public Health from 1988 to 2003

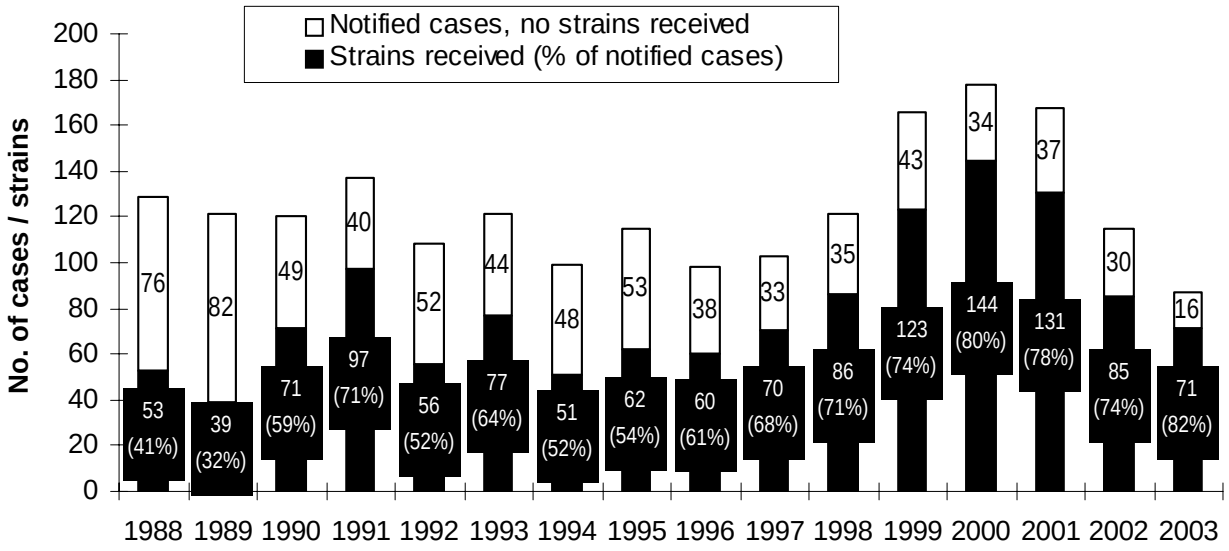

Table 1 : Distribution of serogroups and serotypes of *N. meningitidis* strains isolated in Switzerland 2003

	Serotype									
Serogroup	1	2a	2b	4	14	15	21	unknown	total	
A										0%
B	2		1	4		7	1	23	38	54%
C		11	9					2	22	31%
W135								4	4	6%
Y					4			1	5	7%
unknown				1				1	2	3%
Total	2	11	10	5	4	7	1	31	71	100%
	3%	15%	14%	7%	6%	10%	1%	44%	100%	

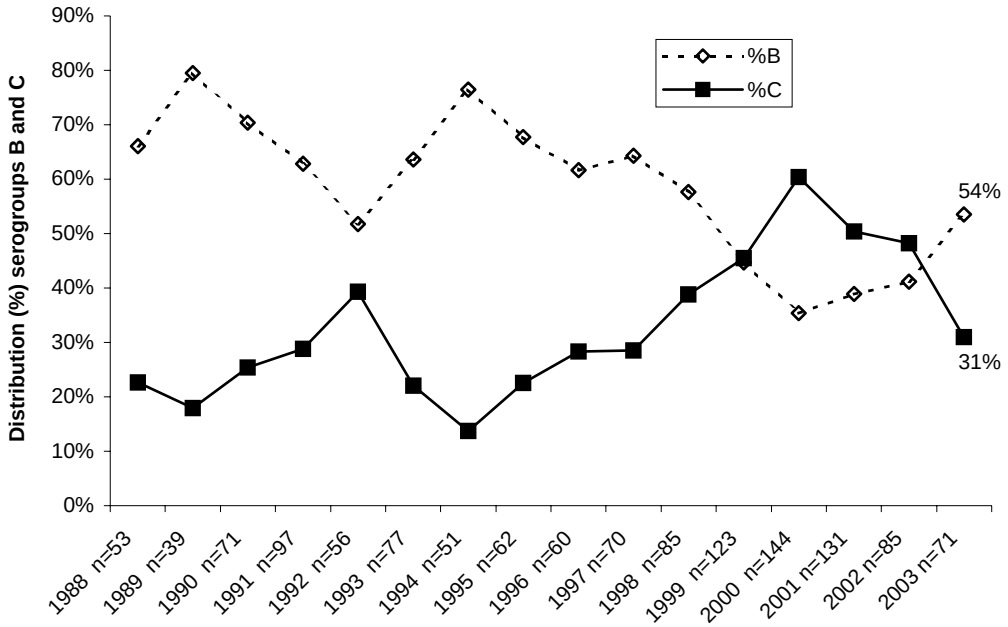

Figure 2 : Proportional distribution of serogroups B and C of *N. meningitidis* from 1988 to 2003.

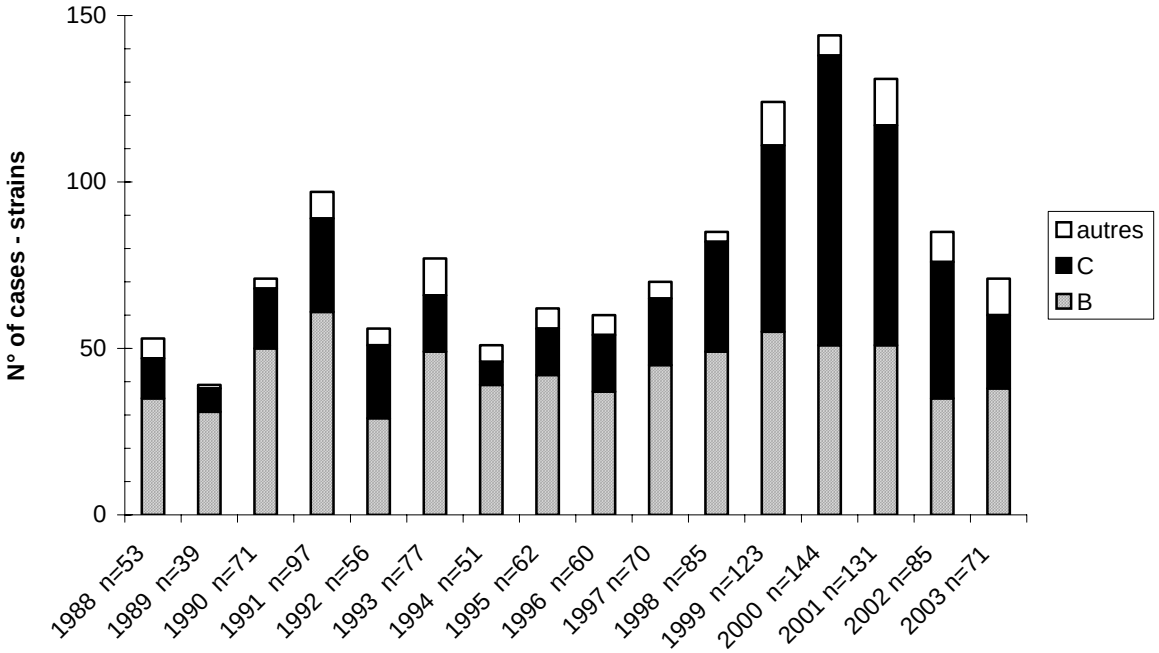

Figure 3 : Serogroup distribution of invasive meningococcal isolates 1988-2003.

Table 2 : Distribution of most frequent serotypes and MLST types of meningococci in Switzerland 2003

	MLST												
Complete serotype	11	8	41	23	1909	22	32	213	283	869	other	Total	
B:-:P1.-										1	6	7	10%
C:2a:P1.2,5	6											6	8%
C:2b:P1.2,5		4									2	6	8%
B:-:P1.5					3						1	4	6%
Y:14:P1.-				3							1	4	6%
B:-:P1.3,9											3	3	4%
B:-:P1.4			2								1	3	4%
B:15:P1.16											3	3	4%
B:15:P1.7,16							2				1	3	4%
C:2a:P1.5	3											3	4%
B:-:P1.6									2			2	3%
B:4:P1.-											2	2	3%
C:2b:P1.2		1									1	2	3%
W135:-:P1.3,6											2	2	3%
other	2	1	3			2		2		1	10	21	30%
Total	11	6	5	3	3	2	2	2	2	2	33	71	100%
	15%	8%	7%	4%	4%	3%	3%	3%	3%	3%	46%	100%	

Table 3 : Inhibitory activity of 9 antimicrobial agents on 71 meningococci isolated in Switzerland in 2003

Agent	Minimal Inhibitory Concentration (µg / ml) range	50%	90%	Breakpoint sensitive ≤ µg/ml	% sensitive
Penicillin	0.03-0.38	0.06	0.25	0.12*	86%
Cefuroxime	0.03-1	0.06	0.38	1	100%
Ceftriaxone	<0.01	<0.01	<0.01	1	100%
Minocycline	0.06-1	0.25	0.5	4	100%
Rifampicin	0.01-0.25	0.01	0.09	1*	100%
Erythromycin	0.38-2	1	1	0.5*	31%
Azithromycin	0.25-4	1	2	2	99%
Ciprofloxacin	<0.01	<0.01	<0.01	1	100%
Chloramphenicol	0.5-2	1	1	2*	100%

* According to the British Society for Antimicrobial Chemotherapy (5).