SHORT COMMUNICATION

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Antibiotic susceptibility of invasive *Neisseria meningitidis* isolates from 1995 to 2008 in Sweden—the meningococcal population remains susceptible

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Abstract

The susceptibility to 7 antibiotics was determined for all Swedish invasive *Neisseria meningitidis* isolates from 1995 to 2008 ($N = 717$). In general, these remain highly susceptible to the antibiotics recommended for use. Accordingly, penicillin G remains effective for the treatment of invasive meningococcal disease and ciprofloxacin appropriate for prophylaxis.

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Introduction

The continuing increase in antibiotic resistance in many bacterial pathogens is a serious public health threat worldwide. *Neisseria meningitidis*, causing meningitis and septicaemia, has been an exception, in that it has generally remained susceptible to the antibiotics used for treatment and prophylaxis. However, during the last decade, there have been several reports of *N. meningitidis* isolates with intermediate susceptibility to penicillins [1–4], and earlier, exceedingly rare β -lactamase producing isolates were described [5,6]. Intermediate susceptibility/resistance to ciprofloxacin [7–12] and resistance to rifampicin [13–15] have also been reported from several countries. Furthermore, although rare, there have been reports of chloramphenicol-resistant *N. meningitidis* isolates from Australia, France and Vietnam [16,17]. Resistance to ceftriaxone is claimed to have been identified and was reported from India [18]. These strains have, however, not been further examined, comprehensively phenotypically and genetically characterized, and/or confirmed by an independent laboratory [19].

During recent decades, Sweden has had a low incidence of invasive meningococcal disease, i.e. an annual incidence of 0.5 to 1.1 cases per 100,000 inhabitants during 1995 to 2008 (Swedish Institute for Infectious Disease Control; <http://smittskyddsinstitutet.se/in-english/statistics/menin-gococcal-infection>).

Any comprehensive description and analysis of the antibiotic susceptibility of Swedish *N. meningitidis* isolates over a longer time period has hitherto never been performed.

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The aims of the present study were to describe and analyse the antibiotic susceptibility of all Swedish invasive *N. meningitidis* isolates from 1995 to 2008, to identify any longitudinal trends in the susceptibility and/or resistance, and to briefly discuss effective antibiotics for treatment and prophylaxis.

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Materials and methods

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All Swedish invasive *N. meningitidis* isolates cultured and characterized by the Swedish Reference Laboratory for Pathogenic *Neisseria*, Örebro University Hospital, Sweden, from 1995 to 2008 ($N = 717$; from blood (438), cerebrospinal fluid (266), joint fluid (12), unspecified (1)) were included in the study. The corresponding patients were 52% males (mean age 24 y, median age 18 y, range 0–91 y) and 48% females (mean age 32 y, median age 20 y, range 0–96 y). The isolates were of serogroup B ($n = 391$; 55%), C ($n = 204$; 28%), Y ($n = 79$; 11%), W-135 ($n = 33$; 5%), 29E ($n = 2$), A ($n = 1$), X ($n = 1$), Z ($n = 1$), and non-groupable (NG; $n = 5$). The minimum inhibitory concentrations (MICs) of

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0 Table I. Susceptibility of all Swedish invasive *Neisseria meningitidis* isolates from 1995 to 2008 ($N = 717$) to 7 different antibiotics.

Antibiotic	Breakpoint S≤/R>	MIC range (mg/l)	MIC ₅₀ (mg/l)	MIC ₉₀ (mg/l)	Susceptible %	Intermediate susceptible %	Resistant %
Penicillin G	0.094/1 ^a	0.006–1	0.047	0.094	91.4	8.6	0
Penicillin V	1/1 ^b	<0.016–6	0.25	0.75	98	0	2
Cefotaxime	0.12/0.12 ^b	<0.002–0.047	0.003	0.006	100	0	0
Chloramphenicol	2/4 ^c	0.094–6	0.75	1.5	99.6	0.3	0.1
Ciprofloxacin	0.03/0.25 ^c	<0.002–0.012	0.004	0.006	100	0	0
Rifampicin	0.25/1 ^c	<0.002–0.38	0.012	0.047	99.9	0.1	0
Sulfadiazine ^d	1/4 ^c	0.19–>256	256	>256	8	25.5	66.5

MIC, minimum inhibitory concentration.

^aBreakpoints previously described by Taha et al. [2], based on a combination of identified penicillin G MIC and presence/absence of *penA* mosaic allele.

^bBreakpoints in accordance with the Swedish Reference Group for Antibiotics (<http://www.srga.org>).

^cBreakpoints proposed by the European Meningococcal Disease Society [25].

^dBreakpoints for sulfisoxazole were used, which when compared to sulfadiazine, using Etest, displayed similar MIC distribution (data not shown).

penicillin G, penicillin V, cefotaxime, chloramphenicol, ciprofloxacin, rifampicin, and sulfadiazine (used as an epidemiological marker) were determined for all isolates using the Etest method (AB Biodisk, Solna, Sweden) on Mueller–Hinton agar supplemented with 5% heated ('chocolated') defibrinated horse blood, at 37°C in 5% CO₂ for 16–18 h. The breakpoints used are shown in Table I. All isolates were also tested for β -lactamase production using nitrocefin discs (AB Biodisk, Solna, Sweden).

Results

The results of the antibiotic susceptibility testing are summarized in Table I. In brief, all isolates (100%) were β -lactamase-negative and highly susceptible to cefotaxime (MIC ≤ 0.047 mg/l) and ciprofloxacin (MIC ≤ 0.012 mg/l). Mainly all isolates (99.9%) were susceptible to rifampicin, i.e. only 1 serogroup B isolate from 2001 displayed intermediate susceptibility (MIC 0.38 mg/l). With regard to chloramphenicol, 1 isolate (0.1%) was resistant (serogroup B from 1996; MIC 6 mg/l) and 2 isolates (0.3%) displayed intermediate susceptibility (one serogroup C from 2000 and 1 serogroup B from 2003). In total, intermediate susceptibility to penicillin G (pen^I) was observed in 8.6% of the isolates, and 59% of the pen^I isolates were of serogroup B. Overall, the pen^I phenotype was most prevalent in serogroup W-135 isolates (18% of all W-135 isolates), followed by serogroup B (10%) and C (7%). Two percent of the isolates were resistant to penicillin V, and 50% of these were serogroup B isolates. The proportion of isolates displaying intermediate susceptibility to penicillin G varied between 4% and 18% during the examined y, however, even studying the evolution of penicillin MICs, there was no obvious longitudinal

trend towards a less susceptible meningococcal population (Table II). The percentage of sulfadiazine resistance varied from 52% to 81% per y.

Discussion

The Swedish population of invasive *N. meningitidis* isolates remains highly susceptible to the antibiotics recommended, both for treatment and prophylaxis. For penicillin G, in some y an increase of isolates with intermediate susceptibility could be observed, however there was no obvious longitudinal trend towards a less susceptible meningococcal population for penicillin G or any of the other antibiotics. Compared to studies from other European countries, such as France [20], Portugal [21] and Italy [22], the antibiotic susceptibility patterns were similar for cefotaxime, chloramphenicol, ciprofloxacin, rifampicin, and sulfadiazine. However, in France and Portugal the proportions of isolates with intermediate susceptibility to penicillin G were substantially higher than in Sweden: 31.7% in France (MIC ≥ 0.125 mg/l) [20] and 24.6% in Portugal (MIC > 0.06 mg/l) [21]. A very high percentage of pen^I isolates was also reported in a Spanish study (55.3%) [23]. In all these 3 countries the prevalence of pen^I isolates was significantly higher among serogroup C isolates than serogroup B isolates. Accordingly, the high prevalence of pen^I in these countries is at least partly due to the high prevalence of serogroup C isolates belonging to serotype 2b:P1.5,2, which is a phenotype previously shown to be associated with intermediate penicillin susceptibility [2]. In fact, in Italy the proportion of pen^I isolates significantly increased (from 7.5% to 27.4%) as a result of the introduction of this serogroup C clone [24]. Thus, in Sweden the relatively low level of the pen^I phenotype in general, and in serogroup C in particular, might at

0 Table II. Susceptibility to penicillin G among all Swedish invasive *Neisseria meningitidis* isolates recovered during 1995–2008 (*N* = 717). 57

	Y	MIC range (mg/l)	MIC ₅₀ (mg/l)	MIC ₉₀ (mg/l)	Susceptible % (≤0.094 mg/l) ^a	Intermediate susceptible %	Resistant % (>1 mg/l) ^a	
5	1995	0.012–0.19	0.032	0.094	94	6	0	60
	1996	0.012–0.25	0.032	0.094	95	5	0	
	1997	0.016–0.25	0.047	0.094	90	10	0	
	1998	0.006–0.5	0.047	0.094	90	10	0	
	1999	0.016–0.19	0.047	0.064	96	4	0	
10	2000	0.023–0.25	0.047	0.094	93	7	0	65
	2001	0.008–0.25	0.047	0.094	95	5	0	
	2002	0.016–0.25	0.047	0.19	86	14	0	
	2003	0.016–0.19	0.047	0.125	88	12	0	
	2004	0.012–0.38	0.064	0.19	82	18	0	
15	2005	0.012–0.19	0.064	0.094	94	6	0	70
	2006	0.016–1	0.064	0.125	89	11	0	
	2007	0.016–0.25	0.047	0.19	88	12	0	
	2008	0.012–0.125	0.047	0.064	95	5	0	

MIC, minimum inhibitory concentration.

^aBreakpoints previously described by Taha et al. [2], based on a combination of identified penicillin G MIC and presence/absence of *penA* mosaic allele. 75

least partly be explained by the low prevalence of C:2b:5,2 meningococci.

An additional explanation for the lower level of intermediate susceptibility and resistance observed among Swedish invasive *N. meningitidis* isolates might be the relatively low usage of antibiotics in Sweden compared to countries in Southern Europe (data from European Surveillance of Antimicrobial Consumption, available at: http://www.esac.ua.ac.be/main.aspx?c=*ESAC2&n=50103). Most important, no treatment failure of invasive meningococcal disease using the recommended penicillin G treatment has yet been identified in Sweden. Also globally these are extremely rare, most of them have not been appropriately clinically and microbiologically confirmed and accordingly cannot be considered as conclusive. Thus, in Sweden intravenous penicillin G remains effective for the treatment of invasive meningococcal disease. Furthermore, ciprofloxacin remains appropriate for prophylaxis of invasive meningococcal disease. However, antibiotic susceptibility testing remains crucial to perform, i.e. in order to confirm adequate choice of antibiotic(s) for the treatment of invasive meningococcal disease as well as to timely identify and monitor any emergence of new resistance in *N. meningitidis*. In addition, extended-spectrum third-generation cephalosporins, such as ceftriaxone and cefotaxime (if *Listeria monocytogenes* is suspected or cannot be excluded, e.g. intravenous amoxicillin needs to be administered in addition) and meropenem, which at present are also investigated at the Swedish Reference Laboratory for Pathogenic *Neisseria*, are effective and especially useful when the etiological agent of the bacterial meningitis has not been species-confirmed.

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Declaration of interest: The authors have no conflict of interest to declare. 85

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