

Antibiotic Susceptibility Patterns of *Neisseria meningitidis* Isolates from Patients and Asymptomatic Carriers

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The activities of seven antimicrobial agents used for treatment and prophylaxis of meningococcal disease was investigated against 901 *Neisseria meningitidis* isolates, 112 of which were recovered from patients and 789 of which were recovered from asymptomatic carriers. The proportions of isolates with decreased susceptibility to penicillin were 55.3 and 39.0%, respectively. Penicillin- and ampicillin-intermediate strains were more common among serogroup C meningococci than among non-serogroup C meningococci from both patients and carriers.

Meningococcal infections are usually treated with penicillin, ampicillin, or a combination of penicillin and chloramphenicol. Isolates of *Neisseria meningitidis* with increased levels of resistance to penicillin have been reported in the last few years, particularly from Spain and the United Kingdom (17, 20). Resistance is due, at least in part, to the development of altered forms of the penicillin-binding protein PBP 2 (11). MICs for penicillin-intermediate isolates (Penⁱ) (0.12 to 1 µg/ml) are 2- to 20-fold higher than those for the susceptible ones (≤ 0.06 µg/ml). The production of a β -lactamase as a mechanism of penicillin resistance in meningococci has been reported in only five isolates (3, 6, 7, 19).

The scientific literature contains a wealth of susceptibility data for clinical meningococci isolates associated with a variety of medical conditions. There is, however, a lack of data that describe the antimicrobial susceptibilities of *N. meningitidis* strains that colonize the nasopharynx. Knowledge of susceptibility patterns and of trends in the resistance of colonizing strains may be of great value in establishing a policy for empirical antimicrobial treatment of meningococcal disease (MD) and in developing appropriate prophylactic regimens for eradication of the carrier state in persons at high risk of developing serious infection.

The aim of this study was to compare the levels of sensitivities to antimicrobial drugs commonly used for treatment and prophylaxis of MD for isolates obtained from patients with those for isolates obtained from asymptomatic carriers. We would also like to determine if serogroup C meningococcal strains isolated during an epidemic wave from patients and carriers were more resistant to those drugs than the other serogroups and nongroupable strains.

A total of 901 meningococci isolates were used for this study: (i) 112 clinical isolates isolated from cerebrospinal fluid and/or blood from patients with meningococcal disease in Galicia, a region of Spain, between 1994 and 1997 and (ii) 789 isolates obtained from a study of asymptomatic carriers (between December 1996 and January 1997).

All strains were identified as *N. meningitidis* by standard

methods (14). The serogroup of each meningococcus was determined by slide agglutination with polyclonal sera produced in our laboratory (14).

Sensitivities to penicillin, ampicillin, cefotaxime, ceftriaxone, rifampin, ciprofloxacin, and sulfadiazine were determined by the agar dilution method in Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) with a final inoculum of 10^5 CFU/spot. The MIC doubling dilution ranges tested were 0.007 to 2 µg/ml for penicillin, ampicillin, and rifampin; 0.0003 to 0.03 µg/ml for cefotaxime; 0.00007 to 0.06 µg/ml for ceftriaxone; and 0.0003 to 0.012 µg/ml for ciprofloxacin. For sulfadiazine, 1, 5, 10, 25, 50, and 100 µg/ml were the dilutions tested.

Cultures were incubated for 24 h at 37°C under a 5% CO₂ atmosphere. The plates were read manually, and the MIC was defined as the lowest concentration at which no growth was visible on agar plates.

The breakpoints used for penicillin, cefotaxime, ceftriaxone, and ciprofloxacin were those recommended by the National Committee for Clinical Laboratory Standards (NCCLS) for *Neisseria gonorrhoeae*: for penicillin, susceptible, ≤ 0.06 µg/ml; intermediate, 0.12 to 1 µg/ml; and resistant, ≥ 2 µg/ml; for cefotaxime, susceptible, ≤ 0.5 µg/ml; for ceftriaxone susceptible, ≤ 0.25 µg/ml; for ciprofloxacin, susceptible, ≤ 0.06 µg/ml, intermediate, 0.12 to 0.5 µg/ml; and resistant, ≥ 1 µg/ml (13). For ampicillin we used the same criteria used for penicillin. Since no MIC breakpoints are approved by NCCLS for rifampin, those proposed by the Spanish antibiogram committee (MENSURA group) (sensitive, ≤ 1 µg/ml; resistant, ≥ 4 µg/ml) were used (2). For sulfadiazine the breakpoint was ≥ 10 µg/ml. *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were used as quality control organisms and were included each time that a set of isolates was tested.

Statistical analysis of data was performed by the chi-square test with the Mantel-Haenszel correction. The data were analyzed with Epi-Info software, version 6.04 (5).

We analyzed the patterns of susceptibility of the *N. meningitidis* isolates stratified according to their origin (carriers or patients). The proportion of Penⁱ clinical isolates was 55.3%; 82.1% were ampicillin intermediate (Ampⁱ). Thirty-nine percent of *N. meningitidis* isolates from carriers were penicillin intermediate, and 65.5% were Ampⁱ. It has been suggested that the source of these intermediate strains may be the commensal *Neisseria* species. *N. meningitidis* is a transformable bacterium, and horizontal genetic exchange has influenced the emergence

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TABLE 1. Antimicrobial susceptibilities of serogroup C and meningococci of other serogroups or nongroupable meningococci from patients and carriers

Isolate group and antibiotic	MIC ($\mu\text{g/ml}$)			% Strains ^a		
	Range	50%	90%	S	I	R
Clinical isolates ($n = 112$)						
Serogroup C ($n = 72$)						
Penicillin	0.015–0.5	0.12	0.25	33.3	66.7	0.0
Ampicillin	0.03–0.5	0.25	0.5	5.6	94.4	0.0
Cefotaxime	≤ 0.0003 –0.015	0.003	0.007	100.0	— ^b	—
Ceftriaxone	0.0003–0.0015	0.0007	0.0015	100.0	—	—
Rifampin	≤ 0.007 –0.12	0.03	0.12	100.0	—	0.0
Ciprofloxacin	0.003–0.006	0.006	0.006	100.0	0.0	0.0
Sulfadiazine	25–>100	100	>100	0.0	—	100.0
Non-serogroup C ($n = 40$)						
Penicillin	≤ 0.007 –0.12	0.06	0.12	65.0	35.0	0.0
Ampicillin	0.015–0.5	0.12	0.25	40.0	60.0	0.0
Cefotaxime	≤ 0.0003 –0.015	0.003	0.007	100.0	—	—
Ceftriaxone	0.0003–0.0015	0.0007	0.0015	100.0	—	—
Rifampin	≤ 0.007 –0.12	0.03	0.12	100.0	—	0.0
Ciprofloxacin	0.003–0.006	0.006	0.006	100.0	0.0	0.0
Sulfadiazine	≤ 1 –>100	25	100	15.0	—	85.0
Carrier isolates ($n = 789$)						
Serogroup C ($n = 89$)						
Penicillin	≤ 0.007 –0.5	0.12	0.25	36.0	64.0	0.0
Ampicillin	0.03–1	0.25	0.5	13.5	86.5	0.0
Cefotaxime	0.0007–0.015	0.003	0.007	100.0	—	—
Ceftriaxone	0.00015–0.007	0.0015	0.0015	100.0	—	—
Rifampin	≤ 0.007 –0.25	0.03	0.12	100.0	—	0.0
Ciprofloxacin	0.0015–0.006	0.006	0.006	100.0	0.0	0.0
Sulfadiazine	5–>100	>100	>100	4.5	—	95.5
Non-serogroup C ($n = 700$)						
Penicillin	≤ 0.007 –0.5	0.06	0.25	64.1	35.9	0.0
Ampicillin	≤ 0.007 –1	0.12	0.5	37.1	62.9	0.0
Cefotaxime	≤ 0.0003 –0.03	0.003	0.007	100.0	—	—
Ceftriaxone	≤ 0.00007 –0.015	0.0007	0.0015	100.0	—	—
Rifampin	≤ 0.007 –2	0.03	0.12	100.0	—	0.0
Ciprofloxacin	≤ 0.0003 –0.012	0.006	0.006	100.0	0.0	0.0
Sulfadiazine	≤ 1 –>100	100	>100	7.7	—	92.3

^a S, I, and R, susceptible, intermediate, and resistant, respectively.

^b —, category not determined.

and spread of Penⁱ strains by mosaic gene formation (10). In our study all isolates from patients and carriers were inhibited by concentrations of penicillin and ampicillin that are readily achieved by standard dosing regimens. Penicillin was more active than ampicillin. The percentage of Penⁱ and Ampⁱ strains was higher among isolates from patients than among isolates from carriers ($P < 0.05$).

Because most of the MD cases in recent years in Spain were produced by serogroup C strains, we compared the antibiotic susceptibilities of serogroup C isolates with those of non-serogroup C isolates from patients and carriers. MIC ranges, the MICs at which 50% of isolates are inhibited (MIC₅₀s), and the MIC₉₀s are shown in Table 1. It has been shown previously that serogroup C strains are more common among Penⁱ strains than among the total population of meningococcal strains (15). In our study the proportion of serogroup C clinical strains that were Penⁱ was 66.7%, while 35.0% of other serogroups and nongroupable strains revealed this level of resistance to penicillin ($P < 0.05$). The proportion of Ampⁱ isolates varied between 94.4% (serogroup C strains) and 60.0% (non-serogroup

C strains) ($P < 0.05$). The situation for isolates from carriers was similar.

Continuous surveillance of the penicillin-resistant strains of meningococci could be very important for detection of the emergence of strains for which penicillin MICs are greater than 1 $\mu\text{g/ml}$, which could be a serious problem in the treatment of meningococcal infections. However, good alternatives for therapy are the broad-spectrum cephalosporins (12). Cefotaxime and ceftriaxone demonstrated excellent in vitro effectiveness against the meningococci in our series and other reports (15). On the basis of the MICs, ceftriaxone was more active than cefotaxime (cefotaxime MIC₉₀, 0.007 $\mu\text{g/ml}$; ceftriaxone MIC₉₀, 0.0015 $\mu\text{g/ml}$). The susceptibility pattern was similar for clinical and carrier isolates. However, it would appear to be important to monitor clinical and carrier isolates for any changes in susceptibility patterns because the emergence of expanded-spectrum cephalosporin resistance would be of considerable concern as it further will limit the options available for the treatment of serious meningococcal infections, as has occurred for *Streptococcus pneumoniae* (4, 16).

All serogroup C clinical isolates were sulfadiazine resistant (S^r); however, 15% of non-serogroup C were sensitive to this drug. The percentage of S^r carrier isolates ranged between 92.3 to 95.5% (non-serogroup C and serogroup C strains, respectively). Because of the early introduction of sulfonamides, resistance to these compounds is widespread (9). Our findings demonstrate that the sulfonamide susceptibilities of *N. meningitidis* strains that colonize the nasopharynx are similar to those of clinical isolates, with a high proportion of S^r strains. For this reason, at present the relative susceptibility of meningococci to sulfonamides is mainly used as an epidemiological marker.

In Galicia an important increase in the incidence of MD took place in 1995-1996, so we might also expect an increase in the use of rifampin. However, the rate of susceptibility to this antibiotic was high, which could reflect a limited use of it in Galicia, as meningococci may develop resistance to rifampin during prophylactic treatment (1), and confirms that rifampin is an antibiotic to be used for prophylaxis of MD in this region. The MIC_{50} and MIC_{90} were equal for clinical and carrier isolates (0.03 and 0.12 $\mu\text{g/ml}$, respectively).

Ciprofloxacin MICs were reported to range from 0.003 to 0.006 $\mu\text{g/ml}$ for clinical isolates and from ≤ 0.0003 to 0.012 $\mu\text{g/ml}$ for carrier strains. In view of the present trends in development of ciprofloxacin resistance in *N. gonorrhoeae* (8, 21), it would be likely that the development of ciprofloxacin resistance in meningococci will follow the path already taken by their close relatives, gonococci. Although we did not find any clinical isolate for which the ciprofloxacin MIC was ≥ 0.12 $\mu\text{g/ml}$, it would appear to be important to monitor isolates for any changes in susceptibility patterns because changes in therapeutic measures (not necessarily against meningococcal infections, perhaps as therapy for respiratory tract infections) would lead to a rapid appearance of meningococcus strains for which ciprofloxacin MICs are ≥ 0.12 $\mu\text{g/ml}$.

Among the bacteria that cause serious infections, *N. meningitidis* is one of the least problematic in terms of antibiotic resistance. Although the prevalence of resistance in meningococci is still low, continued surveillance is necessary to monitor trends in their susceptibilities to antimicrobial drugs and so to advise clinicians on appropriate empirical therapy and chemoprophylaxis.

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