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In Vitro Activities of Ciprofloxacin, Cefotaxime, Ceftriaxone, Chloramphenicol, and Rifampin against Fully Susceptible and Moderately Penicillin-Resistant *Neisseria meningitidis*

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Moderately penicillin-resistant *Neisseria meningitidis* was responsible for an outbreak of meningococcal disease in Saskatoon, Saskatchewan, Canada in 1993. We tested fully susceptible and moderately resistant strains of *N. meningitidis* against ciprofloxacin, cefotaxime, ceftriaxone, chloramphenicol, penicillin, and rifampin. Eighteen percent of the isolates were moderately resistant to penicillin (MIC, ≥0.06 μg/ml) whereas susceptibility was 100% for the other agents tested.

Neisseria meningitidis with decreased susceptibility to penicillin (moderate resistance) was recognized in Spain in 1985 (9, 10) and subsequently in other countries (10) including Canada (8) and the United States (3, 4). In 1993, an outbreak of meningococcal disease occurred in Saskatoon, Saskatchewan, Canada, and the majority of the isolates (serogroup C, enzyme electrophoretic type 15) were found to have decreased susceptibility to penicillin (2). Pulsed-field gel electrophoresis showed that all isolates with decreased susceptibility had the same genomic fingerprints following digestion with any of three separate restriction enzymes and that the genomic fingerprints of isolates not showing decreased susceptibility were different.

Ciprofloxacin is a 4-fluoroquinolone antimicrobial agent that has broad-spectrum activity against gram-positive and gram-negative bacteria. For *N. meningitidis*, MICs were reported to range from <0.002 to 0.012 μg/ml (3). Previously, Pugsley et al. (6) and Renkonen et al. (7) evaluated ciprofloxacin for the treatment of nasopharyngeal carriage of *N. meningitidis* in studies that showed ciprofloxacin to be highly efficacious for this purpose. With the discovery of the Saskatoon isolates showing decreased susceptibility to penicillin, we were interested in determining if this change had any effects on the in vitro activity of ciprofloxacin. We undertook a study to investigate the in vitro activity of ciprofloxacin against *N. meningitidis* isolates that were either fully susceptible or showing reduced susceptibility to penicillin and compared the results with those for penicillin, cefotaxime, ceftriaxone, chloramphenicol, and rifampin.

A total of 78 *N. meningitidis* isolates from three different geographical regions of Canada were tested: 30 from British Columbia, provided by J. A. Smith; 32 from Nova Scotia, provided by K. Forward and D. Haldane; and 16 from Saskatchewan. Ten of the isolates from Saskatchewan were identical by pulsed-field gel electrophoresis, and the MICs for these isolates ranged 0.12 to 0.25 μg/ml. One of these ten isolates was included in testing to determine the susceptibility rates.

The MICs of ciprofloxacin for *N. meningitidis* were determined by three separate methods: Microscan MIC Plus (Baxter Diagnostics, Inc., Deerfield, Ill.) type 2 panels, agar dilution, and E test (AB Biodisk, Solna, Sweden). The MICs of cefotaxime, ceftriaxone, chloramphenicol, penicillin, and rifampin were determined by using only the E test. The Microscan MIC panels were used according to the manufacturer's instructions, and interpretation of the results was done in accordance with the MIC interpretative standards of the National Committee for Clinical Laboratory Standards (5). Briefly, 4 or 5 large or 5 to 10 small well-isolated colonies from 18- to 24-h noninhibitory agar plates were transferred to 3 ml of inoculum water with a wooden applicator stick. The final concentration was equivalent to a McFarlane barium sulfate turbidity standard of 0.5. After the mixture was vortexed, 0.1 ml (100 μl) of the standard suspension was added to 25 ml of inoculum water with pluronic-D. Following mixing, a Renox inoculator was used to deliver 0.115 ml of this final suspension to each well of the MIC plate, and the mixtures were incubated for 18 to 24 h at 35°C. The plates were read manually, and the MIC was recorded as less than or equal to the lowest concentration at which no growth was observed. For the E test, a 0.5-McFarlane-standard suspension of the test organism was prepared and 150-mm-diameter Mueller-Hinton agar plates were inoculated by using a nontoxic swab to produce a confluent growth. E test strips containing the antimicrobial agent were placed on the inoculated plates with sterile forcepts. The plates were incubated for 18 to 24 h at 35°C in 5% CO_2. MICs were recorded at the point at which growth of the microorganism intersected the E test strip. For agar dilution, Mueller-Hinton plates containing ciprofloxacin (antimicrobial agent concentrations ranged from 0.015 to 4 μg/ml) were purchased from Automed, Inc. (Arden Hills, Minn.). The plates were inoculated with a 0.5-McFarlane-standard suspension with a Steers replicator. The plates were incubated for 18 to 24 h in 5% CO_2. The MIC was interpreted as less than or equal to the lowest concentration at which no growth was shown.

The control strains used were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213 and ATCC 25923.

Of the 69 *N. meningitidis* isolates, 19 were recovered from blood cultures, 14 were from cerebral spinal fluid cultures, and 7 were from respiratory tract cultures. Data on the origin of 29 isolates were not available. The serotype distribution was as follows: 21, type B; 38, type C; 5, type Y; and 5, type information not available.

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TABLE 1. Susceptibility testing results for 68 *N. meningitidis* isolates with six antimicrobial agents

Antimicrobial agent	Method[a]	MIC (μg/ml)[b]			% of isolates susceptible (μg/ml)[c]
		50%	90%	Range	
Ciprofloxacin	Agar dilution	≤0.015	≤0.015	0.015	100 (≤1.0)
	Microscan	≤0.25	≤0.25	0.25	100 (≤1.0)
	E test	0.004	0.006	0.002–0.008	100 (≤1.0)
Penicillin	E test	0.032	0.064	0.006–0.25	82 (≤0.06)
Cefotaxime	E test	0.003	0.006	≤0.002–0.016	100 (≤0.25)
Ceftriaxone	E test	≤0.002	≤0.002	≤0.002–0.002	100 (≤0.25)
Chloramphenical	E test	0.75	1.0	0.38–1.0	100 (≤8.0)
Rifampin	E test	0.016	0.032	0.004–0.25	100 (≤1.0)

[a] E test results were obtained with Mueller-Hinton agar.
[b] 50% and 90%, MICs at which 50 and 90%, respectively, of the isolates were inhibited.
[c] Breakpoint MIC for susceptibility (5).

One isolate repeatedly (six attempts) failed to grow well enough to produce reliable results and was excluded from further study. Susceptibility testing of ciprofloxacin, by three different methods, against meningococcal isolates showed that the E test gave the most consistent, reliable, and reproducible results. By the E test, 68 of 68 isolates had measurable, easily definable end points compared with 31 of 68 isolates by agar dilution and 59 of 68 isolates by Microscan MIC panels. For this reason, the E test was subsequently used for testing cefotaxime, ceftriaxone, chloramphenicol, penicillin, and rifampin.

Table 1 shows the results of susceptibility testing of 68 *N. meningitidis* isolates to six antimicrobial agents. As measured by the E test, the penicillin MIC was greater than 0.1 μg/ml only for the isolate from the Saskatoon outbreak. For 11 isolates, the MICs of penicillin were >0.06 and ≤0.094 μg/ml.

The MICs of cefotaxime were as follows: <0.002 to 0.008 μg/ml for 65 isolates, 0.012 μg/ml for 2 isolates, and 0.016 μg/ml for 1 isolate. The MICs of ceftriaxone were <0.002 μg/ml (66 isolates) and 0.002 μg/ml (2 isolates). The MICs of chloramphenicol were <0.05, 0.75, 1.0 μg/ml for 16, 38, and 14 isolates, respectively. The MICs of rifampin were ≤0.008 μg/ml for 21 isolates, >0.008 to <0.1 μg/ml for 42 isolates, and >0.1 to ≤0.25 μg/ml for 5 isolates. Ciprofloxacin MICs by the E test were ≤0.004, 0.006, and 0.008 μg/ml for 34, 33, and 1 isolate, respectively. The MICs of chloramphenicol and rifampin were not different between fully susceptible and moderately resistant isolates.

Table 2 shows the correlation between penicillin susceptibility patterns and the activities of other antimicrobial agents for the 68 *N. meningitidis* isolates in this study. There was an increase in the MIC values of cefotaxime and ciprofloxacin, but not of ceftriaxone, at which 50% of the isolates were inhibited when the MICs for fully penicillin-susceptible and moderately penicillin-resistant isolates were compared.

We have previously described *N. meningitidis* isolates with decreased susceptibility to penicillin that were responsible for an outbreak of meningococcal disease in Saskatoon, Saskatchewan (4). All isolates with decreased susceptibility to penicillin had identical genomic fingerprints when their DNAs were compared by pulsed-field gel electrophoresis.

Ciprofloxacin, a 4-fluoroquinolone, has been available in Canada since 1989. Previously, Pugsley et al. (6) and Rekonen et al. (7) found ciprofloxacin to be highly efficacious for erradication of nasopharyngeal carriage of *N. meningitidis*. Unfortunately, not all serogroups were represented or detailed in the reports, nor were the susceptibility rates of the isolates to penicillin. We investigated the in vitro activity of ciprofloxacin against penicillin-susceptible and moderately penicillin-resistant strains of meningococcus to determine if the mechanism of decreased susceptibility to penicillin had any effect on the activities of ciprofloxacin and other compounds. We suspect that the mechanism of reduced susceptibility to penicillin in our isolates involves altered penicillin-binding proteins (studies to prove this are ongoing). This finding would be consistent with the findings by other investigators for similar strains recovered in Spain (10). Ciprofloxacin activity against moderately penicillin-resistant strains of *N. meningitidis* was slightly different from that of strains fully susceptible to penicillin (Table 2). This result needs to be interpreted with caution since the difference between the MICs at which 50 and 90% of the isolates were inhibited (Table 1) was determined by using only one isolate. A larger study may help to resolve this observation. Of the two mechanisms associated with ciprofloxacin resistance (altered membrane permeability and altered DNA gyrase) we were concerned that cell wall changes may have affected the diffusion of the antimicrobial agent into the bacterial cell. The changes that have resulted in the decreased susceptibility to penicillin do not appear to have changed the diffusion of ciprofloxacin into the cell since activity against all of the strains tested does not appear to be significantly altered. Bellete et al. (1) reported on the susceptibility of meningococcal isolates recovered during an outbreak of meningococcal disease in Malawi. All 77 meningococcal isolates were susceptible to chloramphenicol, ciprofloxacin, and rifampin. Six isolates were resistant to penicillin and all but one was resistant to sulfonamides. Tzanakaki et al. (11) found that 48, 19, and 36% of meningococcal isolates from patients with meningitis, from school children, and from recruits, respectively, in Greece were not susceptible to penicillin. All but one isolate was susceptible to ciprofloxacin. Resistance to rifampin was not detected in meningococcal isolates from patients with meningitis; however, resistance was detected in 6% of the isolates recovered from carriers. Finally, Hughes et al. (3) evaluated the E test for susceptibility testing of *N. meningitidis*. Of 102 isolates tested, 84.3% were fully susceptible to penicillin, while 100% were susceptible to ciprofloxacin.

The results obtained from testing cefotaxime, ceftriaxone, chloramphenicol, and rifampin were, for the most part, similar to those of other investigators. Saez-Nieto et al. (10) found that there was a very slight increase in the MICs of cefotaxime and ceftriaxone for penicillin-resistant *N. meningitidis*. In our study, there was a slight increase in the MICs of cefotaxime when tested against moderately resistant strains. It is interesting that of 11 isolates for which the penicillin MIC was 0.047 μg/ml, the cefotaxime MICs were 0.006 μg/ml for 4 isolates and 0.016 μg/ml for 1 isolate. For 10 of 11 isolates the ceftriaxone MIC

TABLE 2. Correlation of penicillin susceptibility patterns and activities of other antimicrobial agents in this study

Antimicrobial agent	MIC_{50} by penicillin susceptibility pattern for[a]:	
	Susceptible isolates	Relatively resistant isolates
Cefotaxime	0.002	0.006
Ceftriaxone	≤0.002	≤0.002
Ciprofloxacin	0.004	0.006

[a] Penicillin MICs were <0.06 μg/ml (n = 56 isolates) and >0.06 to 0.25 μg/ml (n = 12 isolates) for penicillin-susceptible and relatively penicillin-resistant strains. MIC_{50}, MIC at which 50% of the isolates were inhibited.

was ≤0.002 μg/ml, and for 1 isolate the MIC was 0.002 μg/ml. These data are consistent with those of others (10) and indicate different levels of cross-resistance to other beta-lactam antimicrobial agents. Hughes et al. (3) found that 97.1% of meningococcal isolates were susceptible to rifampin. Higher rates of rifampin resistance (6.4%) were found in isolates from meningococcal carriers in Greece (11). We did not find any rifampin-resistant isolates in our study; however, a more widespread geographical sampling may help to determine the true incidence of rifampin-resistant isolates in both Canada and the United States. Finally, chloramphenicol resistance was not detected in any of our isolates, and this finding is consistent with the findings of Saez-Nieto et al. (9).

The use of the E test for determining the susceptibility of antibiotics against *N. meningitidis* was investigated previously (3). Hughes et al. (3) found that E test MICs were within ±1 $\log_2$ dilution of the MICs by agar dilution. Ease of use, clear end points, rapid results, and clearly defined detection of high-level resistance were all cited as favorable characteristics of the E test method. Our experience with this methodology for susceptibility testing of meningococci is consistent with that of Hughes et al. (3), and as such we highly recommend it as the preferred method for *N. meningitidis* susceptibility testing. Microscan MIC panels and the agar dilution method gave less-consistent results than did the E test. We are not sure of the reason for this observation. By agar dilution, the interpretation of results was impossible for 37 isolates because the organisms did not grow on the control plates in three separate attempts. Similarly for the MIC panels, poor or no growth in the control wells made interpretation of the results for nine isolates (two or three separate attempts) impossible. On the basis of these results and those from the E test, we prefer the E test for susceptibility testing of *N. meningitidis.*

The results from this study indicate that ciprofloxacin is highly active against *N. meningitidis* isolates that are fully susceptible and moderately resistant to penicillin. Previous investigations has shown ciprofloxacin to be highly effacacious for the erradication of nasopharyngeal carriage of *N. meningitidis* (6, 7). Given that meningococcal resistance to rifampin has been recognized and given that ciprofloxacin activity is not diminished in isolates resistant or moderately resistant to penicillin, fluoroquinolones will likely be useful agents for treating nasopharyngeal carriage of this microorganism. Finally, our results suggest that rifampin, cefotaxime, ceftriaxone, and chloramphenicol are highly active against *N. meningitidis* and, as such, remain valuable agents for prophylaxis or treatment for *N. meningitidis* infections.

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