

## Decreased Susceptibility to Penicillin G and Tet M Plasmids in Genital and Anorectal Isolates of *Neisseria meningitidis*

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**Genital and anorectal isolates of *Neisseria meningitidis* were characterized, and their antimicrobial susceptibilities were determined. Twelve of 43 isolates demonstrated moderate susceptibility to penicillin G (MIC range, 0.125 to 0.5 µg/ml). Two isolates were resistant to tetracycline (MIC, ≥8 µg/ml) and contained plasmids of 25.2 MDa.**

The isolation of *Neisseria meningitidis* from genital and anorectal sites has been described for the past 60 years. Reports have documented the isolation of *N. meningitidis* from the cervix, the urethra, and the anal canal (1, 3, 5, 7-9, 14, 19). The antimicrobial susceptibilities of genital and anorectal isolates of *N. meningitidis* and the mechanism of their resistance continue to be of clinical and epidemiologic interest.

Isolates of *N. meningitidis* with decreased susceptibility to penicillin G from patients with meningococcal disease have been reported in recent years from South Africa (2), Spain (28, 30), the United Kingdom (31, 32), Greece (34), and Canada (22) and have also been described from unpublished observations in the United States (33). Most of these moderately susceptible strains were β-lactamase negative with MICs of penicillin ranging from 0.1 to 1.2 µg/ml. Strains of *N. meningitidis* showing β-lactamase activity have been reported from Spain (6), South Africa (2), and Canada (4). Three high-level tetracycline-resistant (Tc<sup>r</sup>; MIC, ≥16 µg/ml) oropharyngeal isolates and a single urethral isolate of *N. meningitidis* carrying the Tet M-containing conjugative plasmid have also been described (10, 12, 13, 31). Conjugation is thought to be the most common way in which plasmids are transferred in the genus *Neisseria* (18). The role of *Neisseria gonorrhoeae* as a possible reservoir for the mobilization of resistance plasmids into *N. meningitidis* has been suggested (26).

In this study, genital and anorectal isolates of *N. meningitidis* were systematically collected and characterized. Their antimicrobial susceptibilities were determined and their mechanism of tetracycline resistance was examined.

During the 3-year period from August 1989 through July 1992, the Seattle King County Health Department Public Health Laboratory evaluated approximately 162,000 cultures on selected media for the presence of *N. gonorrhoeae*. In this period, 44 cultures demonstrating bacterial growth other than *N. gonorrhoeae* were presumptively identified as *N. meningitidis* by colonial morphology, oxidase reaction, Gram stain, and carbohydrate utilization. Isolates were subcultured to chocolate agar and provided to the *Neisseria* Reference Laboratory, University of Washington, Seattle.

At the *Neisseria* Reference Laboratory, isolates were again

confirmed as *N. meningitidis* by the identification scheme in the 5th edition of the *Manual of Clinical Microbiology* as described by Morello et al. (17) and maintained on GC II agar base (Becton Dickinson, Cockeysville, Md.) supplemented with 1% IsoVital X (Prepared Media Laboratories, Tualatin, Ore.). Organisms were incubated in a humidified CO<sub>2</sub> incubator at 36.5°C with 6.5% CO<sub>2</sub> and stored at -70°C in sterile tryptic soy broth (Becton Dickinson) containing 20% (vol/vol) glycerol. The isolates of *N. meningitidis* (n = 44) were serogrouped by slide agglutination with antisera obtained from Difco Laboratories (Detroit, Mich.) (17) and tested for β-lactamase production by a chromogenic cephalosporin assay (21). Antibiotic MICs were determined by the reference agar dilution method recommended by the National Committee for Clinical Laboratory Standards (20). The following antimicrobial agents used for the agar dilution study were obtained as standard powders from the indicated manufacturers: penicillin G (Eli Lilly & Co., Indianapolis, Ind.), tetracycline, erythromycin, rifampin, and ceftriaxone (Sigma Chemical Corporation, St. Louis, Mo.), and ciprofloxacin (Miles Pharmaceuticals, West Haven, Conn.). All tests were performed with the inclusion of the following recommended quality control strains: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853.

Plasmid profiles were determined by agarose gel electrophoresis (16) on isolates considered resistant to tetracycline (MIC, ≥8 µg/ml) with representative tetracycline-susceptible (Tc<sup>s</sup>) isolates as controls. Testing for the Tet M determinant was performed as described previously with a <sup>32</sup>P-radiolabeled pJ13 probe (27). Conjugation experiments were performed by the filter-mating technique (18) with a genital isolate of *N. gonorrhoeae* and pharyngeal isolates of *N. meningitidis* and commensal *Neisseria* spp. as the recipients.

Statistical analyses of categorical data were performed with the chi-square test or Fisher's exact test (two-tailed hypothesis). Continuous data were analyzed with the Student *t* test (normally distributed data) or the Kruskal-Wallis test (data not normally distributed).

A total of 44 *N. meningitidis* isolates from 43 patients were characterized. All biochemical results for each isolate were consistent with expected reactions for *N. meningitidis*. Isolates from the cervix and anorectum of one patient were phenotypically indistinguishable by methods utilized in this study; analyses are thus based on unduplicated isolates from the 43 patients.

Isolates were more often recovered from men (30 of 32,000),

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TABLE 1. Genital and anorectal isolates of *N. meningitidis* by sex of the patient, anatomic site, and serogroup

Site	Sex <sup>a</sup>	No. of isolates	No. of groupable isolates	Serogroup(s) (no. of isolates in group)
Cervix	f	8	4	B, C (2), X
Rectum	f	5	1	C
Urethra	m	20	7	B (2), C (2), Y (2), Z
Rectum	m	10	9 <sup>b</sup>	B (2), C (3), Y (2), Z' (2)

<sup>a</sup> f, female; m, male.

<sup>b</sup>  $P = <0.01$  (proportion of rectal isolates from men that were groupable, versus proportion of other isolates that were groupable).

than women (13 of 130,000) ( $P \leq 0.001$ ). No differences between the sexually transmitted disease (STD) clinic and non-STD clinic populations as to sex, anatomic sites of isolation, antimicrobial susceptibilities, or serogrouping results (data not shown) were observed. Isolates from 21 of 43 patients were groupable; anorectal isolates from males were more often groupable than were isolates from other site and sex combinations ( $P < 0.01$ ) (Table 1).

The MICs were determined for the 43 isolates of *N. meningitidis* by agar dilution. This method classified all isolates as susceptible to ceftriaxone (MIC range, 0.00025 to 0.002  $\mu\text{g/ml}$ ), ciprofloxacin (MIC,  $\leq 0.0005$   $\mu\text{g/ml}$ ), and rifampin (MIC range, 0.001 to 0.03  $\mu\text{g/ml}$ ) (Table 2).

Approximately one-half (21 of 43) of the isolates exhibited intermediate susceptibilities to erythromycin (MIC range, 1 to 4  $\mu\text{g/ml}$ ); the remainder were susceptible (MIC,  $\leq 0.5$   $\mu\text{g/ml}$ ) to erythromycin. Most isolates were susceptible to penicillin G (MIC,  $\leq 0.06$   $\mu\text{g/ml}$ ); 12 isolates (28%) were moderately susceptible (MIC range, 0.125 to 0.5  $\mu\text{g/ml}$ ). All control strain results were within acceptable ranges.

Anorectal isolates were more likely than urogenital isolates to be moderately susceptible to penicillin G (Table 3). However, there was no statistically significant relationship between moderate susceptibility to penicillin G and serogroup. Isolates moderately susceptible to penicillin G were less susceptible to other antimicrobial agents than were penicillin-susceptible strains, although only ceftriaxone differences were statistically significant (Table 4). All isolates were  $\beta$ -lactamase negative.

Two isolates (one group Y and one group Z) were resistant to tetracycline (MIC,  $\geq 8$   $\mu\text{g/ml}$ ), and both had decreased susceptibility to penicillin G (MICs, 0.125 and 0.5  $\mu\text{g/ml}$ ). The remainder of the meningococcus strains were relatively susceptible to tetracycline (MIC,  $\leq 1$   $\mu\text{g/ml}$ ). One of the high-level tetracycline-resistant isolates (MIC,  $> 8$   $\mu\text{g/ml}$ ) demonstrated moderate susceptibility to erythromycin (MIC = 2  $\mu\text{g/ml}$ ). The Tc<sup>r</sup> strains contained plasmids of 25.2 MDa which hybridized with the Tet M probe and were both transferred along with the

TABLE 2. Antimicrobial susceptibilities of genital and anorectal *N. meningitidis* isolates ( $n = 43$ )

Agent	MIC ( $\mu\text{g/ml}$ ) <sup>a</sup>			Geometric mean
	Range	50%	90%	
Penicillin G	0.015–0.5	0.03	0.125	0.0344
Tetracycline	0.125–16.0	0.25	1.0	0.3753
Ceftriaxone	0.00025–0.002	0.0005	0.002	0.0005
Ciprofloxacin	$< 0.0005$	0.0005	0.0005	0.0005
Erythromycin	0.125–2.0	0.5	2.0	0.657
Rifampin	0.001–0.03	0.002	0.015	0.0037

<sup>a</sup> 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.

TABLE 3. Characteristics of patients and their isolates by penicillin G susceptibility

Characteristic	No. of isolates (%) with indicated susceptibility to penicillin G	
	Susceptible ( $n = 31$ ; MIC, $\leq 0.06$ $\mu\text{g/ml}$ )	Moderately susceptible ( $n = 12$ ; MIC, $\geq 0.125$ $\mu\text{g/ml}$ )
Male	21 (68)	9 (75)
Rectal	8 (26)	7 (58) <sup>a</sup>
STD clinic <sup>b</sup>	17 (55)	8 (67)
Groupable	14 (45)	7 (58)

<sup>a</sup>  $P = 0.06$  (Fisher's exact test).

<sup>b</sup> STD, sexually transmitted disease.

Tc<sup>r</sup> phenotype to recipients during conjugation. The frequencies of Tet M transfer were  $10^{-3}$  for *N. gonorrhoeae*,  $10^{-3}$  to  $10^{-4}$  for *N. meningitidis*, and  $10^{-5}$  to  $10^{-7}$  with commensal *Neisseria* recipients. Recipients contained plasmids of 25.2 MDa as visualized by agarose gel.

Infection or colonization of genital and anorectal sites with *N. meningitidis* is infrequent. During the 3-year period of our study, *N. meningitidis* strains were isolated from 43 (0.03%) of approximately 162,000 patients tested for genital or anorectal gonococcal infection. This rate agrees with a similar study reported by Faur and colleagues in New York City during the 1970s (5).

The proportion of isolates that were groupable in this study (48%) was lower than in the New York City study (86%) (5) and in an STD clinic-based study in Denver (90%) (11).

However, proportionally more genital isolates were evaluated in this study, and genital isolates in our study were less likely to be groupable than were anorectal isolates from presumably homosexual men (Table 1).

Moderate susceptibility to penicillin G was found in 28% of these genital and anorectal isolates of  $\beta$ -lactamase-negative *N. meningitidis*. Although this level of moderate susceptibility to penicillin G has not been reported in the United States, Spanish investigators have described moderate susceptibility to penicillin G in up to 40% of isolates from patients with meningococcal disease (28). The mechanism of this moderate susceptibility is believed to be the decreased affinity of penicillin-binding proteins (15, 29).

The broad-spectrum cephalosporin, ceftriaxone, tested in our study appeared to have high activity against the strains of *N. meningitidis* which showed moderate susceptibility or resistance to penicillin G, tetracycline, or erythromycin. This observation has also been reported from Spain (29).

The tetracycline resistance determinant Tet M, carried on the 25.2-MDa conjugative plasmid, has been described in several species of genital tract microorganisms, including *N. gonorrhoeae* (24). The Tc<sup>r</sup> strains of *N. meningitidis* from our

TABLE 4. Susceptibility of isolates to tetracycline, erythromycin, and ceftriaxone according to susceptibility to penicillin G

Antimicrobial agent	MICs (range, geometric mean [ $\mu\text{g/ml}$ ]) for isolates with indicated susceptibility to penicillin G	
	Susceptible ( $n = 31$ ; MIC, $\leq 0.06$ $\mu\text{g/ml}$ )	Moderately susceptible ( $n = 12$ ; MIC, $\geq 0.125$ $\mu\text{g/ml}$ )
Tetracycline	0.125–16, 0.313	0.125–1.0, 0.606
Erythromycin	0.25–2.0, 0.636	0.125–2.0, 0.717
Ceftriaxone	0.0005–0.002, 0.0004	0.0025–0.002, 0.0009 <sup>a</sup>

<sup>a</sup>  $P < 0.01$  (Kruskal-Wallis test).

study were isolated from anorectal and urethral anatomic sites in Seattle during an epidemic of Tc<sup>f</sup> *N. gonorrhoeae* from 1989 to 1991. It has been shown that donor strains of *N. gonorrhoeae* carrying the 25.2-MDa plasmids can transfer these plasmids as well as  $\beta$ -lactamase plasmids to *N. meningitidis* (23, 25). The presence of conjugal plasmids in two of these *N. meningitidis* isolates may suggest the potential for transfer of plasmids in nature between *Neisseria* spp. in the genital tract. Our study did not identify any  $\beta$ -lactamase-containing strains of *N. meningitidis*.

The existence, characterization, and antimicrobial susceptibilities of urogenital and anorectal meningococci in Seattle has been discussed. These strains exhibit one or more determinants of resistance or moderate susceptibility to penicillin G, tetracycline, and erythromycin. The presence of decreased susceptibility to penicillin G in these meningococci could contribute to therapeutic complications in the treatment of meningococcal disease when penicillin G is the therapy of choice.

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