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Antimicrobial Susceptibilities of *Neisseria gonorrhoeae* Strains Representing Five Distinct Resistance Phenotypes

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The susceptibilities of 109 strains of *Neisseria gonorrhoeae* to penicillin G, tetracycline, amoxicillin-clavulanic acid, cefotetan, cefoxitin, ceftriaxone, ciprofloxacin, and fleroxacin were determined. The activities of cefmetazole, cefuroxime, cefixime, and ofloxacin were also determined against 62 of these strains. Strains represented penicillin-susceptible (Pen^s) *N. gonorrhoeae*; penicillinase-producing *N. gonorrhoeae* (PPNG) possessing 2.9-, 3.05-, 3.2-, or 4.4-MDa β-lactamase plasmids; strains with high-level, plasmid-mediated tetracycline resistance (TRNG); strains with plasmid-mediated resistance to penicillin and tetracycline; and strains with chromosomally mediated resistance to penicillin and tetracycline (CMRNG). Ceftriaxone, cefixime, and ciprofloxacin were the most active agents tested against all strains. Pen^s, TRNG, and PPNG strains possessing a 3.2-MDa β-lactamase plasmid were more susceptible to amoxicillin-clavulanic acid, extended- and broad-spectrum cephalosporins, and quinolones than were either PPNG strains possessing a 2.9-, a 3.05-, or a 4.4-MDa β-lactamase plasmid or CMRNG strains.

Antimicrobial resistance in *Neisseria gonorrhoeae* is an increasing and costly public health problem. Despite an overall decline in the number of reported cases of gonorrhea in the United States in the past decade, the prevalence of antimicrobial agent-resistant strains has steadily increased; in some geographic areas, more than 20% of cases of gonorrhea are caused by antimicrobial agent-resistant strains (3, 13). Increasing resistance to inexpensive therapeutic antimicrobial agents, such as penicillin and tetracycline, led the Centers for Disease Control (CDC) to recommend alternative but more costly regimens, such as ceftriaxone, for the primary treatment of uncomplicated gonorrhea (1, 2). Newer β-lactams and quinolones have been introduced as potentially alternative therapies for gonorrhea. Many of these agents appear to be promising, but in vitro and clinical trial data are often limited to the evaluation of efficacy in populations in which the prevalence of resistant strains may be limited. In vitro surveillance data on gonococcal strain populations and antimicrobial agent resistance in the United States have shown that the temporal and geographic distributions of plasmid and resistance phenotypes vary and that strain populations are dynamic (9, 13). In this in vitro study, we assessed more thoroughly the in vitro susceptibilities of strains representative of five distinct resistance phenotypes of *N. gonorrhoeae* to selected β-lactams and quinolones, including agents currently recommended by the CDC for the treatment of uncomplicated gonorrhea.

A panel of 109 strains representing five major resistance phenotypes of *N. gonorrhoeae*, based on published National Committee for Clinical Laboratory Standards resistance definitions and CDC surveillance definitions for gonococcal resistance, were selected from the CDC reference collection of strains obtained from recent international and national surveillance studies (11, 14). The strains included (i) penicillin-susceptible (Pen^s) *N. gonorrhoeae* (42 strains) (MICs, <2.0 μg/ml), (ii) penicillinase-producing *N. gonorrhoeae* (PPNG; 34 strains) (β-lactamase positive), (iii) *N. gonorrhoeae* with high-level, plasmid-mediated tetracycline resistance (TRNG; 9 strains) (TetM determinant present), (iv) *N. gonorrhoeae* with chomosomally mediated resistance to both penicillin and tetracycline (CMRNG; 16 strains) (MICs, ≥2.0 μg/ml), and (v) *N. gonorrhoeae* with plasmid-mediated resistance to both penicillin and tetracycline (PPNG/TRNG; 8 strains) (β-lactamase positive and TetM positive). The PPNG strains possessed the following β-lactamase plasmids: 2.09-MDa plasmid, 5 strains; 3.05-MDa plasmid, 5 strains; 3.2-MDa plasmid, 13 strains; and 4.4-MDa plasmid, 11 strains. All PPNG/TRNG strains possessed a 3.2-MDa β-lactamase plasmid. Strains were stored frozen at −70°C in Trypticase soy broth (Difco Laboratories, Detroit, Mich.) containing 20% glycerol.

Antimicrobial agents were obtained as standard powders for in vitro susceptibility testing from the following sources and reconstituted according to manufacturer's instructions: penicillin G, tetracycline, and cefixime (Lederle Laboratories, Pearl River, N.Y.); amoxicillin-clavulanic acid (Beecham Laboratories, West Point, Pa.); cefmetazole (The Upjohn Co., Kalamazoo, Mich.); cefotetan (Stuart Pharmaceuticals, Wilmington, Del.); cefoxitin (Merck Sharpe & Dohme Laboratories, Rahway, N.J.); cefuroxime (Glaxo Pharmaceuticals, Research Triangle Park, N.C.); ceftriaxone and fleroxacin (Hoffmann-La Roche); ofloxacin (Ortho Pharmaceutical Corp., Raritan, N.J.); and ciprofloxacin (Miles Laboratories, West Point, Conn.). Susceptibilities were determined on GC II agar base medium (Becton Dickinson, Cockeysville, Md.) supplemented with 1% IsoVitaleX (Becton Dickinson) and containing serial twofold dilutions of each agent. Media were inoculated with 10^4 CFU by use of a multipoint replicator (Cathra Systems, AutoMed, Arden Hills, Minn.) (11). The susceptibility of a strain to an agent was defined as the MIC, the lowest concentration inhibiting growth to ≤1 CFU. The susceptibilities of all strains to penicillin G, tetracycline, amoxicillin-clavulanic acid, cefotetan, cefoxitin, ceftriaxone, ciprofloxacin, and fleroxacin were determined; the susceptibilities of 61 of

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TABLE 1. Susceptibilities of strains of *N. gonorrhoeae*, by resistance phenotype, to selected antimicrobial agents used in the treatment of gonorrhea

Agent and resistance phenotype	MIC (μg/ml)		
	Range	50%	90%
Penicillin G[a]			
PPNG	8.0–≥64.0	32.0	≥64.0
PPNG/TRNG	4.0–8.0	8.0	16.0
TRNG	0.125–0.5	0.25	0.5
CMRNG	1.0–16.0	2.0	8.0
Susceptible[b]	0.015–2.0	0.25	1.0
Amoxicillin-clavulanic acid[a]			
PPNG	1.0–16.0	4.0	16.0
PPNG/TRNG	1.0–4.0	2.0	4.0
TRNG	0.5–1.0	0.5	1.0
CMRNG	0.5–4.0	2.0	4.0
Susceptible	0.06–4.0	0.5	1.0
Cefmetazole[c]			
PPNG	0.5–16.0	2.0	16.0
PPNG/TRNG	0.5–1.0	0.5	1.0
TRNG	1.0–4.0	1.0	4.0
CMRNG	8.0–32.0	16.0	32.0
Susceptible	0.06–16.0	1.0	4.0
Cefotetan[a]			
PPNG	0.25–8.0	1.0	8.0
PPNG/TRNG	0.25–1.0	0.5	1.0
TRNG	0.5–1.0	0.5	1.0
CMRNG	2.0–8.0	8.0	8.0
Susceptible	0.06–8.0	0.5	2.0
Cefoxitin[a]			
PPNG	0.25–2.0	1.0	2.0
PPNG/TRNG	0.25–1.0	0.25	1.0
TRNG	0.25–1.0	0.5	1.0
CMRNG	1.0–4.0	2.0	4.0
Susceptible	0.125–2.0	0.5	1.0
Cefuroxime[c]			
PPNG	0.03–2.0	0.25	1.0
PPNG/TRNG	0.03–0.125	0.03	0.125
TRNG	0.06–0.25	0.125	0.25
CMRNG	0.25–8.0	2.0	4.0
Susceptible	0.008–1.0	0.125	0.5
Ceftriaxone[a]			
PPNG	≤0.002–0.03	0.008	0.03
PPNG/TRNG	0.004–0.015	0.004	0.015
TRNG	0.004–0.008	0.008	0.008
CMRNG	0.015–0.25	0.03	0.125
Susceptible	≤0.002–0.125	0.008	0.03
Cefixime[c]			
PPNG	0.008–0.03	0.015	0.015
PPNG/TRNG	0.004–0.015	0.008	0.015
TRNG	0.008–0.015	0.008	0.015
CMRNG	0.008–0.125	0.03	0.125
Susceptible	≤0.002–0.03	0.008	0.03
Ciprofloxacin[a]			
PPNG	≤0.002–0.25	0.008	0.015
PPNG/TRNG	≤0.002–0.004	≤0.002	0.004
TRNG	≤0.002	≤0.002	≤0.002
CMRNG	0.004–0.03	0.008	0.03
Susceptible	≤0.002–0.125	≤0.002	0.008
Ofloxacin[c]			
PPNG	≤0.002–1.0	≤0.002	0.008
PPNG/TRNG	0.008–0.015	0.008	0.015
TRNG	0.008–0.015	0.015	0.015
CMRNG	0.03–0.06	0.03	0.06
Susceptible	0.008–0.25	0.008	0.03
Fleroxacin[c]			
PPNG	0.015–1.0	0.06	0.5
PPNG/TRNG	0.015–0.03	0.015	0.03
TRNG	0.015–0.03	0.015	0.03
CMRNG	0.06–0.125	0.06	0.125
Susceptible	0.015–1.0	0.015	0.125
Tetracycline[a]			
PPNG	0.5–4.0	1.0	4.0
PPNG/TRNG	16.0	16.0	16.0
TRNG	8.0–32.0	16.0	32.0
CMRNG	2.0–8.0	2.0	4.0
Susceptible	0.25–4.0	0.5	2.0

[a] All 109 strains were tested: PPNG, 34; TRNG, 9; PPNG/TRNG, 8; CMRNG, 16; and susceptible, 42.

[b] Isolates were assigned to the susceptible category if they were β-lactamase negative and were found to require an MIC of <2.0 μg/ml in previous testing. Thus, some strains with CMRNG-like susceptibility profiles, including decreased susceptibility to cephalosporins and quinolones, are included in this category.

[c] A total of 61 strains were tested: PPNG, 16; TRNG, 5; PPNG/TRNG, 5; CMRNG, 11; and susceptible, 24.

these strains (PPNG, 16 strains; PPNG/TRNG, 5 strains; TRNG, 5 strains; susceptible, 24; and CMRNG, 11 strains) to cefmetazole, cefuroxime, cefixime, and ofloxacin were determined. Susceptibilities were interpreted according to the recommendations of the National Committee for Clinical Laboratory Standards or currently proposed criteria (11). Interpretive criteria were not available for amoxicillin-clavulanic acid.

The susceptibilities of the strains are summarized by distinct resistance phenotypes in Table 1. Among strains that lacked plasmid-mediated resistance, there was overlap between the Pen^s and CMRNG categories that has been observed previously (5). In this study, we assigned strains to the CMRNG category only if they were resistant to both penicillin and tetracycline (MICs, ≥2.0 μg/ml). Consequently, if the strict definition of an MIC of ≥2.0 μg/ml is used to define a strain as resistant, strains for which the MIC of penicillin or tetracycline was 2.0 μg/ml and for which the MIC of the other agent was 1.0 μg/ml may have been assigned to the susceptible category even though their overall susceptibility profile, with decreased susceptibility to ceftriaxone or quinolones, indicated that the strains were CMRNG. The assignment of such "borderline" strains—including one strain which was assigned to the Pen^s category on the basis of previous testing but which was found resistant to penicillin in the testing done for this study—to the CMRNG category would produce more natural groupings of strains (particularly since MICs for some strains may vary by one or two dilutions in repeated testing) that would also reduce the overlap observed between the results for Pen^s and CMRNG strains.

When the susceptibility results for the β-lactamase-producing strains, PPNG and PPNG/TRNG, were combined, the susceptibilities to all β-lactams except for cefixime were distributed bimodally. The MICs of penicillin G for all PPNG

TABLE 2. Susceptibilities, by β-lactamase plasmid size in megadaltons, of strains of PPNG to penicillin, ceftriaxone, and ciprofloxacin

Agent and β-lactamase plasmid size (MDa)	No. of strains for which the MIC (μg/ml) was:																MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
	≤0.002	0.004	0.008	0.015	0.03	0.06	0.125	0.25	0.5	1.0	2.0	4.0	8.0	16.0	32.0	≥64.0		
Penicillin G																		
2.9	—[a]	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	≥64.0	≥64.0
3.05	—	—	—	—	—	—	—	—	—	—	—	—	—	1	2	2	32.0	≥64.0
4.4	—	—	—	—	—	—	—	—	—	—	—	—	—	4	3	4	32.0	≥64.0
3.2	—	—	—	—	—	—	—	—	—	—	—	—	7	2	—	4	8.0	≥64.0
Ceftriaxone																		
2.9	—	1	—	3	1	—	—	—	—	—							0.015	0.03
3.05	—	1	1	2	1	—	—	—	—	—							0.015	0.03
4.4	—	3	3	5	—	—	—	—	—	—							0.008	0.015
3.2	2	5	2	1	3	—	—	—	—	—							0.004	0.03
Ciprofloxacin																		
2.9	—	—	1	4	—	—	—	—	—	—							0.015	0.015
3.05	3	—	1	—	—	—	—	1	—	—							≤0.002	0.25
4.4	3	—	5	1	—	—	1	1	—	—							0.008	0.25
3.2	8	1	2	2	—	—	—	—	—	—							≤0.002	0.015

[a] —, zero.

possessing a 2.9-, a 3.05-, or a 4.4-MDa plasmid were ≥16.0 μg/ml (Table 2). In contrast, the penicillin G susceptibilities of PPNG possessing a 3.2-MDa plasmid were distributed bimodally (Table 2); for seven strains, the MICs were ≤16.0 μg/ml, and for four strains, the MICs were ≥64.0 μg/ml. For all PPNG/TRNG, the penicillin G MICs were 4.0 to 16.0 μg/ml. Similar bimodal distributions were noted for the combined susceptibilities of β-lactamase-producing (PPNG and PPNG/TRNG) strains to other β-lactams and quinolones (Table 2). Thus, when susceptibilities of strains were compared by resistance phenotype and plasmid profile, Pen^s, TRNG, and most PPNG strains possessing a 3.2-MDa β-lactamase plasmid, as a group, were more susceptible to both β-lactams and quinolones than were CMRNG and PPNG strains possessing a 2.9-, a 3.05-, or a 4.4-MDa β-lactamase plasmid; for the latter strains, as a group, the MICs of each class of agents were higher.

Regardless of plasmid content, however, all 42 β-lactamase-producing strains were uniformly resistant to penicillin G (MICs, ≥2.0 μg/ml). In the absence of formal criteria for the interpretation of susceptibilities to amoxicillin-clavulanic acid, we interpreted these data by using the criteria for *Haemophilus* spp. For 24 of 42 (57.1%) β-lactamase-producing strains, the MICs of amoxicillin-clavulanic acid were ≥4.0 μg/ml, and for 16 of 42 (38.1%), the MICs of amoxicillin-clavulanic acid were ≥8.0 μg/ml (Table 1). All TRNG, CMRNG, and Pen^s strains were susceptible to ≤4.0 μg of amoxicillin-clavulanic acid per ml. The MICs for 90% of strains (MIC_{90}s) of PPNG, PPNG/TRNG, and CMRNG were higher (16.0, 4.0, and 4.0 μg/ml, respectively) for amoxicillin-clavulanic acid than were those for 90% of either Pen^s or TRNG strains (1.0 μg/ml); 14 of 16 (87.5%) strains were also less susceptible to amoxicillin-clavulanic acid (MIC_{90}, 4.0 μg/ml).

Overall, ceftriaxone and cefixime were the most active cephalosporins against all strains tested, followed by the other cephalosporins in order of decreasing in vitro activities: cefuroxime, cefoxitin, cefotetan, and cefmetazole (Table 1). Although all strains were susceptible to ceftriaxone and cefixime (MICs, ≤0.25 μg/ml), the MIC_{90}s of cefixime and ceftriaxone were consistently higher for CMRNG strains than for strains belonging to any other resistance phenotype, including PPNG possessing a 2.9-, a 3.05-, or a 4.4-MDa β-lactamase plasmid (Table 1). However, the median ceftriaxone MICs were consistently lower for Pen^s, CMRNG, and PPNG strains (0.004, 0.008, and 0.03 μg/ml, respectively) than were the median cefixime MICs for these strains (0.015, 0.015, and 0.06 μg/ml, respectively).

Cefoxitin was the most active of the extended-spectrum cephalosporins; for all strains, the MICs of cefoxitin were <8.0 μg/ml, although three CMRNG strains showed intermediate susceptibilities (MIC, 4.0 μg/ml) to this agent. A total of 2 of 62 (3.2%) strains (2 CMRNG strains) were resistant to cefuroxime (MICs, ≥4.0 μg/ml), and 5 strains (1 PPNG and 4 CMRNG strains) showed intermediate susceptibilities (MIC, 2.0 μg/ml) to this agent. In contrast, 15 of 109 (13.8%) strains (4 PPNG, 9 CMRNG, and 2 susceptible strains) were resistant to cefotetan (MICs, ≥8.0 μg/ml). A total of 21 of 62 (33.3%) strains tested were resistant to cefmetazole. All CMRNG strains were resistant to cefmetazole, as were seven PPNG strains (β-lactamase plasmid content: 2.9 MDa, one strain; 3.2 MDa, three strains; and 4.4 MDa, three strains) and three susceptible strains, each of which was CMRNG-like. In addition, one PPNG strain (4.4-MDa plasmid) and one susceptible strain, which was CMRNG-like, showed intermediate susceptibilities to cefmetazole.

The interpretation of data for the activity of quinolones against these strains was complicated by the occurrence of strains with decreased susceptibility to these agents. On the basis of the interpretive criteria for susceptibility, ciprofloxacin and ofloxacin appeared to be equally active against the strains tested, followed by fleroxacin. A total of 106 of 109 (97.2%) strains were susceptible to ciprofloxacin (MICs, ≤0.06 μg/ml); for 3 strains (2 PPNG and 1 CMRNG), the MICs were ≥0.125 μg/ml. A total of 108 of 109 (99.1%) strains were susceptible to ofloxacin; for 1 PPNG strain, the ofloxacin MIC was 1.0 μg/ml. In contrast, two strains (one PPNG and one CMRNG) were resistant to fleroxacin (MICs, ≥1.0 μg/ml), and two strains (one PPNG and one CMRNG) showed intermediate susceptibility (MIC, 0.5 μg/ml) to this agent. However, the median MICs of ciprofloxacin for each

resistance phenotype were usually at least two and three concentrations lower than the corresponding median MICs of ofloxacin and fleroxacin, respectively. These data indicate that, in vitro, ciprofloxacin is more active than either ofloxacin or fleroxacin.

In the absence of efficacy data, it is not possible to assess the clinical significance of isolates with decreased susceptibility to quinolones, which were identified not only in this study but in several geographic areas in the United States (5). It is recommended that posttreatment isolates from persons treated with a quinolone be tested for susceptibility to that agent and that isolates with decreased susceptibility be sent to a reference laboratory for further evaluation. Until the clinical significance of these isolates is understood, it would not be prudent to raise the criterion for the interpretation of susceptibility to these agents, although this may be appropriate after a thorough evaluation of treatment efficacy and antimicrobial susceptibility data.

A total of 35 of 109 (32.1%) strains were resistant to tetracycline (MICs, ≥2.0 μg/ml) (Table 1). The resistance of the PPNG/TRNG and TRNG strains was predictable. However, in addition, 17 PPNG strains, 16 CMRNG strains, and 7 susceptible strains were also resistant to tetracycline.

These data confirmed previous observations that PPNG strains possessing a 2.9-, a 3.05-, or a 4.4-MDa β-lactamase plasmid and CMRNG strains are less susceptible to both β-lactams and quinolones than are PPNG strains possessing a 3.2-MDa β-lactamase plasmid (8a, 15). Of equal concern is the observation that gonococci with chromosomally mediated resistance to penicillin and tetracycline, including PPNG strains with a 2.9-, a 3.05-, or a 4.4-MDa β-lactamase plasmid, also remained less susceptible to amoxicillin-clavulanic acid. In contrast, TRNG strains were usually susceptible to β-lactams and quinolones and could be grouped with susceptible strains for in vitro susceptibility evaluations. However, PPNG/TRNG, some PPNG, TRNG, CMRNG, and some "susceptible" strains could be grouped for similar evaluations of tetracycline-class agents.

A few studies from the Far East have reported a high prevalence of PPNG strains that possess a 3.05- or a 4.4-MDa β-lactamase plasmid and that show chromosomally mediated resistance and CMRNG strains (4, 8, 12). These studies reported the existence of gonococcal strains with decreased susceptibilities to several newer quinolone agents. Most evaluations of promising new agents, however, have been conducted in North America, where the gonococcal strain populations typically have been susceptible to both cephalosporins and quinolones, partly because these populations have a low proportion of CMRNG strains (6, 7). Therefore, the prevalence of PPNG infections is not, in itself, an indicator of the prevalence of strains with chromosomally mediated resistance in a specific geographic location. In the United States, most PPNG strains possess a 3.2-MDa β-lactamase plasmid and are characteristically susceptible to both cephalosporins and quinolones. Outbreaks caused by PPNG strains that possess a 4.4- or a 3.05-MDa plasmid and that also exhibit chromosomally mediated resistance have occurred very sporadically in the United States (10, 15). In addition, most centers in the United States have reported a low frequency of infections caused by CMRNG strains (5); however, a few centers recently isolated an increased number of CMRNG strains (8a).

These data emphasize the importance of conducting a periodic surveillance of the in vitro activities of currently recommended and potentially useful new antimicrobial agents in geographic locations in which strain populations are representative of the spectrum of antimicrobial agent resistance in *N. gonorrhoeae*. Thus, it is essential that future in vitro and clinical studies of newer agents be carried out in communities in which the prevalence of strains with both chromosomally mediated and high-level, plasmid-mediated resistances to the class of agents under study can be properly evaluated.

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