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Emerging In Vitro Resistance to Quinolones in Penicillinase-Producing *Neisseria gonorrhoeae* Strains in Hawaii

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The susceptibilities of 37 penicillinase-producing strains of *Neisseria gonorrhoeae* (PPNG), isolated in Hawaii from December 1991 through January 1994, were determined to ciprofloxacin and ofloxacin, fluoroquinolone agents currently recommended by the Centers for Disease Control and Prevention as alternative regimens for the treatment of uncomplicated gonorrhea. Nine isolates (24.3%) exhibited decreased susceptibilities (MICs, ≥0.06 μg/ml) to ciprofloxacin and ofloxacin. Ciprofloxacin MICs for three isolates (8.1%) were 2.0 μg/ml; these isolates belonged to the auxotype/serovar class Pro/IB-7 and possessed the 3.2-MDa β-lactamase and the 24.5-MDa conjugative plasmids. Six strains for which ciprofloxacin MICs were 0.06 to 0.125 μg/ml belonged to a variety of gonococcal phenotypes. Strains for which ciprofloxacin MICs were 2.0 μg/ml were isolated from persons who had traveled to, or were sexual contacts of persons who had recently traveled to, Southeast Asia. Persons infected with these isolates had been treated with ceftriaxone (250 mg intramuscularly, single dose); therefore, none of these cases were associated with clinical failure following the use of fluoroquinolone therapy. Further studies are needed to confirm the clinical and public health significance of increased in vitro resistance to ciprofloxacin and ofloxacin in *N. gonorrhoeae*.

Following the emergence, in the early 1980s, of strains of *Neisseria gonorrhoeae* that exhibited increased in vitro resistance to penicillins and tetracyclines, the Centers for Disease Control and Prevention (CDC) recommended that broad-spectrum cephalosporins be used for the primary therapy of uncomplicated gonorrhea (1). Recently, CDC also has recommended the use of fluoroquinolones as an alternative primary therapy for uncomplicated gonorrhea (2, 3). To monitor the susceptibilities of strains of *N. gonorrhoeae* to a variety of antimicrobial agents, CDC implemented the Gonococcal Isolate Surveillance Project (15). Until recently, gonococcal isolates in the United States have been highly susceptible to fluoroquinolone agents (6). However, a number of gonococcal isolates for which MICs are 0.125 to 0.25 μg/ml have been detected sporadically from several sentinel sites and, recently, have been isolated repeatedly in Cleveland, Ohio (6, 10). In this report, we expand documentation of the detection in Hawaii of penicillinase-producing isolates of *N. gonorrhoeae* (PPNG) which exhibited not only decreased susceptibilities similar to those of isolates from Cleveland, Ohio, but also exhibited ciprofloxacin and ofloxacin MICs of 2.0 μg/ml (4).

From December 1991 through February 1994, a total of 37 PPNG isolates were submitted to CDC for routine screening and confirmation of susceptibilities to penicillin, tetracycline, ceftriaxone, and spectinomycin; isolates were from private physicians' offices and public health clinics in Honolulu. CDC initiated routine screening of these isolates for susceptibility to fluoroquinolones because of increased use of these agents for primary therapy of gonorrhea. Subsequent to the recommendation of fluoroquinolones as alternative regimens for the treatment of gonorrhea, isolates with increased in vitro resistance to ciprofloxacin and ofloxacin have been detected. Isolates were stored frozen at −70°C in trypticase soy broth (Difco Laboratories, Detroit, Mich.) containing 15% glycerol. Isolates were characterized by auxotype, serovar, and plasmid content as described previously (9, 11, 16); requirements for proline, arginine, hypoxanthine, uracil, and methionine were determined. Antimicrobial susceptibilities to penicillin, tetracycline, erythromycin, spectinomycin, ceftriaxone, cefixime, ciprofloxacin, and ofloxacin were determined by the agar dilution method as described previously (7, 12). Antimicrobial agents for agar dilution testing were obtained as standard powders for in vitro susceptibility testing from the following sources and reconstituted according to the manufacturer's instructions: penicillin G, tetracycline, and cefixime (Lederle Laboratories, Pearl River, N.Y.); cefoxitin (Merck Sharp & Dohme Laboratories, Rahway, N.J.); ceftriaxone (Hoffmann-La Roche, Nutley, N.J.); ofloxacin (Ortho Pharmaceutical Corp., Raritan, N.J.); and ciprofloxacin (Miles, 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. Inocula were prepared by suspending growth, from overnight cultures on supplemented GC base medium, in Mueller-Hinton broth to a density equivalent to a 0.5 McFarland standard, and then diluted 1:10 in Mueller-Hinton broth. Media were inoculated with a multipoint replicator (Cathra Systems; AutoMed, Arden Hills, Minn.), which delivered an inoculum of approximately 10^4 CFU (7, 12). A control plate without antibiotics was included to control for growth of all isolates. Plates were incubated for 24 h at 35 to 36.5°C in a CO_2-enhanced (5%) atmosphere. All susceptibility test runs were quality controlled with *N. gonorrhoeae* ATCC 49226, which met the acceptable control ranges

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TABLE 1. Phenotypic characteristics of penicillinase-producing strains of *N. gonorrhoeae* exhibiting decreased susceptibilities to ciprofloxacin[a]

Ciprofloxacin MICs (μg/ml) and no. of isolates	Plasmid content (MDa)[b]	A/S class	Range of zone inhibition sizes (mm)	
			Ciprofloxacin	Ofloxacin
2.0 (3)	3.2, 24.5	Pro/IB-7	22–24	18–20
0.06				
1	3.05, 24.5	Proto/IB-2	NT[c]	NT
1	3.05, 24.5	Proto/IB-4	NT	NT
1	4.4	Proto/NT	NT	NT
0.125				
1	3.2	Proto/IB-2	NT	NT
1	3.2, 24.5	Pro/IB-7	NT	NT
1	3.05, 24.5	Proto/IB-2	NT	NT

[a] Abbreviations: Pro., proline requiring; Proto, no requirements for proline, arginine, hypoxanthine, uracil, or methionine; NT, not tested.
[b] All strains possessed a 2.6-MDa cryptic plasmid.
[c] Strains not tested by disk diffusion; disk diffusion susceptibilities of strains from Cleveland, Ohio, with 0.125 to 0.25 μg of ciprofloxacin per ml and 0.125 to 0.5 μg of ofloxacin per ml had zone inhibition sizes of 31 to 39 mm and 28 to 35 mm, respectively.

recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (7, 12), and with three additional *N. gonorrhoeae* strains: F-28 (spectinomycin-resistant), F-45 (a strain with chromosomally mediated resistance to penicillin and tetracycline), and 76.061782 (PPNG) or P681E (a strain with plasmid-mediated resistance to penicillin and tetracycline. The susceptibility of a strain to an agent was defined as the MIC, the lowest concentration inhibiting growth to ≤1 CFU. Susceptibilities were interpreted according to the recommendations of NCCLS (7, 12). Disk diffusion susceptibilities to ciprofloxacin and ofloxacin (Becton Dickinson; 5-μg mass) were determined as recommended by NCCLS (7, 13).

A total of 37 isolates were characterized by antimicrobial susceptibility profile, auxotype, serovar, and plasmid profiles (Table 1). Nine isolates had decreased susceptibilities to ciprofloxacin: MICs for three isolates were 2.0 μg/ml (4), and MICs for six isolates were 0.06 to 0.125 μg/ml. The phenotypic characteristics of these isolates are shown in Table 1. Isolates for which ciprofloxacin MICs were 2.0 μg/ml belonged to the same auxotype/serovar (A/S) class, Pro/IB-7, and all possessed the 3.2-MDa β-lactamase, the 24.5-MDa conjugative plasmid, and the 2.6-MDa cryptic plasmid. Isolates for which ciprofloxacin MICs were 0.06 to 0.125 μg/ml belonged to a variety of A/S classes and possessed different β-lactamase plasmids (Table 1).

The susceptibilities of isolates to a variety of antimicrobial agents are shown in Table 2. All strains were resistant to penicillin (MIC ≥ 2.0 μg/ml). Six of nine (66%) strains that exhibited decreased susceptibilities to ciprofloxacin were resistant to tetracycline (MIC ≥ 2.0 μg/ml), as were 13 of 28 (46.4%) isolates for which ciprofloxacin MICs were ≤0.008 μg/ml. Most strains (35 of 37; 94.6%) were susceptible to cefoxitin (MIC ≤ 2.0 μg/ml), and all strains were susceptible to ceftriaxone (MIC ≤ 0.25 μg/ml), cefixime (MIC ≤ 0.25 μg/ml), and spectinomycin (MIC ≤ 128.0 μg/ml). Disk diffusion zone sizes of isolates from Hawaii for which ciprofloxacin MICs were 2.0 μg/ml were determined. Zone inhibition sizes were 22 to 24 mm for ciprofloxacin and 18 to 20 mm for ofloxacin (Table 1).

To our knowledge, this is the first documentation, in the United States, of *N. gonorrhoeae* isolates for which ciprofloxacin and ofloxacin MICs are 2.0 μg/ml. These isolates were PPNG belonging to a single A/S class introduced into Honolulu by travelers returning from different, rural destinations in the Republic of the Philippines in May 1993 and January 1994. There was no evidence, based on epidemiologic investigation, that the strains were spread by secondary transmission to more than one person in Hawaii. All patients were treated successfully with ceftriaxone (250 mg intramuscularly); thus, we have no data that would document that persons infected with these strains would fail therapy with the currently recommended doses of ciprofloxacin or ofloxacin. One gonococcal isolate for which the ciprofloxacin MIC was 2.0 μg/ml has been reported associated with a gonococcal infection in Thailand (5). In addition, two isolates identical in phenotypic characteristics to those isolated in Hawaii have been isolated in Canada, one in Vancouver, British Columbia, in October 1992 and the second in Winnipeg, Manitoba, in June 1993 (18). Infection with one of these strains was acquired in Asia; the geographic origin of the second infection was not identified.

Previous reports have documented *N. gonorrhoeae* isolates for which ciprofloxacin or ofloxacin MICs were 0.125 to 0.25 μg/ml associated with gonococcal infections acquired in Southeast Asia (8, 14); the lack of phenotypic characterization prevents comparison of previously described strains with those in the current study. Strains with these MICs have also been isolated sporadically in the United States (6), but appear to have become endemic only recently in Cleveland, Ohio (10). The strains identified in Hawaii were phenotypically different from the recently described Cleveland, Ohio, case (10) or from other sporadically occurring cases (6; unpublished data).

On the basis of the distribution of MICs, strains for which MICs were 0.125 μg/ml appear to belong to a cluster of strains which include those for which ciprofloxacin MICs were 0.06 μg/ml, which are interpreted as susceptible by NCCLS criteria (12). Because we have no treatment efficacy data that confirm that infections with such isolates are treated successfully with the currently recommended doses of ciprofloxacin or ofloxacin, we cannot impartially recommend that such isolates be interpreted as susceptible. We suspect that the majority of infections caused by these isolates will be treated successfully, although factors other than the susceptibility of the isolate (such as failure of an infected individual to adequately absorb the drug or metabolic impairment) may result in clinical treatment failures. However, treatment failure to 500 mg of ciprofloxacin has been reported in a patient in Sydney, Australia, who was infected with an isolate similar to those isolated in North America (PPNG: Pro/IB-5,7; ciprofloxacin MIC, 1.0 μg/ml) (17).

Each patient infected by ciprofloxacin-resistant PPNG isolates had only one sexual contact identified in Hawaii with no documented evidence of transmission beyond these individuals, and all were treated effectively with ceftriaxone. Thus, these strains do not appear to have become endemic in Hawaii or to have been spread from Hawaii to other geographic locations in the continental United States at this time. As gonococcal strains with decreased susceptibility to fluoroquinolones are isolated more frequently in the United States, it is important that we monitor the susceptibilities of isolates from patients for whom therapy with ciprofloxacin or ofloxacin has failed to identify isolates that may be clinically resistant to these regimens, particularly in light of increasing intercontinental travel and the risk of future introductions to the continental United States. This is particularly important for effective therapy and management of persons reporting recent

TABLE 2. Agar dilution susceptibilities, by ciprofloxacin (Cip) susceptibility category and β-lactamase plasmid content, of 37 strains of PPNG from Hawaii, 1991–1994

Agent (Cip MIC [μg/ml])[a]	No. of strains for which MICs (μg/ml) were:															MIC (μg/ml)[b]	
	≤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	50	90
Penicillin																	
≤0.008																	
3.2	—[c]	—	—	—	—	—	—	—	—	—	2	5	1	2	—	4.0	16.0
4.4	—	—	—	—	—	—	—	—	—	—	1	3	2	1	11	≥32.0	≥32.0
0.06	—	—	—	—	—	—	—	—	—	—	—	—	—	1	2		
0.125	—	—	—	—	—	—	—	—	—	—	—	—	—	1	2		
2.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3		
Tetracycline																	
≤0.008																	
3.2	—	—	—	—	—	—	1	—	6	—	1	—	—	2	—	0.5	2.0
4.4	—	—	—	—	—	—	2	1	1	4	4	3	1	2	—		
0.06	—	—	—	—	—	—	—	—	—	1	2	—	—	—	—		
0.125	—	—	—	—	—	—	—	—	—	2	1	—	—	—	—		
2.0	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—		
Ciprofloxacin																	
≤0.008																	
3.2	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	≤0.002	≤0.002
4.4	8	5	5	—	—	—	—	—	—	—	—	—	—	—	—	0.004	0.008
0.06	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—		
0.125	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—		
2.0	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—		
Ofloxacin																	
≤0.008																	
3.2	—	3	4	2	1	—	—	—	—	—	—	—	—	—	—	0.008	0.015
4.4	1	2	3	7	5	—	—	—	—	—	—	—	—	—	—	0.015	0.03
0.06	—	—	—	—	—	—	2	1	—	—	—	—	—	—	—		
0.125	—	—	—	—	—	—	1	2	—	—	—	—	—	—	—		
2.0	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—		
Cefoxitin																	
≤0.008																	
3.2	—	—	—	—	—	—	—	4	5	—	1	—	—	—	—	0.5	0.5
4.4	—	—	—	—	—	—	—	4	2	3	7	2	—	—	—	1.0	4.0
0.06	—	—	—	—	—	—	1	—	1	1	—	—	—	—	—		
0.125	—	—	—	—	—	—	—	2	—	—	1	—	—	—	—		
2.0	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—		
Ceftriaxone																	
≤0.008																	
3.2	3	4	2	1	—	—	—	—	—	—	—	—	—	—	—	0.004	0.008
4.4	3	4	8	3	—	—	—	—	—	—	—	—	—	—	—	0.008	0.015
0.06	—	1	2	—	—	—	—	—	—	—	—	—	—	—	—		
0.125	1	—	2	—	—	—	—	—	—	—	—	—	—	—	—		
2.0	—	—	3	—	—	—	—	—	—	—	—	—	—	—	—		
Cefixime																	
≤0.008																	
3.2	—	1	3	3	2	1	—	—	—	—	—	—	—	—	—	0.015	0.03
4.4	—	1	3	4	9	1	—	—	—	—	—	—	—	—	—	0.03	0.03
0.06	—	—	1	1	1	—	—	—	—	—	—	—	—	—	—		
0.125	—	—	—	2	1	—	—	—	—	—	—	—	—	—	—		
2.0	—	—	—	—	3	—	—	—	—	—	—	—	—	—	—		

[a] 3.2 and 4.4, 3.2- and 4.4-MDa β-lactamase plasmids, respectively.
[b] 50 and 90, MICs for 50 and 90% of strains, respectively.
[c] —, the indicated MIC was not found for any strains.

travel to Asia or sexual contact with persons who have traveled to Asia.

In the past decade, fluoroquinolone regimens have been used increasingly in the United States for therapy of uncomplicated gonorrhea, as well as for genitourinary tract infections and other bacterial infections. The detection of strains of *N. gonorrhoeae* for which ciprofloxacin and ofloxacin MICs are 2.0 μg/ml is of serious medical concern and public health significance. Recognition of strains exhibiting these MICs among gonococci in the United States poses a potential threat to the current and future efficacy of fluoroquinolones for primary therapy of gonorrhea. Additional studies are needed to ascertain the geographic distribution and frequency and the clinical significance of isolates with increased in vitro resistance to ciprofloxacin and ofloxacin.

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