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Neisseria gonorrhoeae with Decreased Susceptibility to Ciprofloxacin: Pulsed-Field Gel Electrophoresis Typing of Strains from North America, Hawaii, and the Philippines

Ciprofloxacin and ofloxacin are among the antibiotics recommended by the Centers for Disease Control and Prevention (1) and are widely used to treat uncomplicated gonorrhea in adults. Strains of *Neisseria gonorrhoeae* which are less susceptible or resistant to fluoroquinolones have been reported in several countries. Criteria for classifying gonococcal ciprofloxacin susceptibilities have been proposed as follows: MIC ≥ 1.0 μg/ml, resistant; MIC of 0.125 to 0.5 μg/ml, decreased susceptibility; and MIC ≤ 0.06 μg/ml, susceptible (2).

Sixteen isolates of *N. gonorrhoeae* belonging to auxotype/serovar classes of either Pro/IB-5,7 or Pro/IB-1 for which ciprofloxacin MICs ranged from 0.008 to 4.0 μg/ml were examined by pulsed-field gel electrophoresis (PFGE) (4) with *Nhe*I, *Spe*I, and *Xba*I (Promega, Madison, Wis.) (Table 1.) Genomic similarities were assessed on the basis of all DNA bands seen after digestion with the three enzymes but differed from those of the first nine Pro/IB-5,7 isolates by >2 bands with each enzyme, and these two isolates are likely to be less closely related to the first nine Pro/IB-5,7 isolates. One susceptible isolate (93-026609) had the same *Xba*I pattern as isolates 93-0914 and 93-1223 but differed in *Nhe*I and *Spe*I PFGE patterns, suggesting that 93-026609 may be genetically more distinct from all other Pro/IB-5,7 isolates examined.

Two of the three Pro/IB-1 isolates (93-0202 and C-259) shared two PFGE patterns and had very similar *Xba*I patterns (differing by one band at the 50-kb range). The Pro/IB-5,7 isolate 92-032894 did not have the same pattern as any of the other Pro/IB-5,7 isolates but shared an *Nhe*I pattern (Nd) with 93-0202 and C-259. The third Pro/IB-1 isolate, C-140, did not share any PFGE patterns with the other isolates examined in

TABLE 1. PFGE patterns of isolates of *N. gonorrhoeae* used in this study

Isolate no.	City	Country or U.S. state	Yr	Auxotype/ Serotype	Ciprofloxacin MIC (μg/ml)	β-Lactamase plasmid[a]	PFGE pattern		
							*Nhe*I[b]	*Spe*I[c]	*Xba*I[d]
11417	Vancouver	Canada	1992	Pro/IB-5,7	2.0	+	Na	Sa	Xa
C-139	Cebu City	Philippines	1994	Pro/IB-5,7	1.0	+	Na	Sa	Xa
HNL-93-0801	Honolulu	Hawaii	1993	Pro/IB-5,7	0.25	+	Na	Sa	Xa
93-026468	Honolulu	Hawaii	1993	Pro/IB-5,7	0.25	−	Na	Sa+1	Xa
93-022567[e]	Honolulu	Hawaii	1993	Pro/IB-5,7	2.0	+	Na	Sa+1	Xa
93-022566[e]	Honolulu	Hawaii	1993	Pro/IB-5,7	2.0	+	Na	Sa+1	Xa+1
12963	Winnipeg	Canada	1993	Pro/IB-5,7	2.0	+	Na	Sa	Xa+1
94-007726	Honolulu	Hawaii	1994	Pro/IB-5,7	2.0	+	Nb	Sa	Xa+1*
M-106	Manila	Philippines	1994	Pro/IB-5,7	4.0	+	Nc	Sa	Xa+1
93-1223	Seattle	Washington	1993	Pro/IB-5,7	0.25	−	Nf	Sf	Xf
93-0914	Portland	Oregon	1993	Pro/IB-5,7	0.25	−	Nf	Sf	Xf
93-026609	Honolulu	Hawaii	1993	Pro/IB-5,7	0.03	+	Ng	Sg	Xf
92-032894	Honolulu	Hawaii	1992	Pro/IB-5,7	0.008	+	Nd	Sh	Xc-1
93-0202	Honolulu	Hawaii	1993	Pro/IB-1	2.0	−	Nd	Sb	Xc
C-259	Cebu City	Philippines	1994	Pro/IB-1	4.0	−	Nd	Sb	Xc*
C-140	Cebu City	Philippines	1994	Pro/IB-1	0.5	−	Ne	Se	Xe

[a] +, present; −, absent.
[b] PFGE patterns NA, Nb, and Nc differed from each other by ≥2 bands.
[c] PFGE pattern Sa+1 had one band at 120 kb more than PFGE pattern Sa.
[d] PFGE pattern Xa+1 had one band at 200 kb more than PFGE pattern Xa; PFGE pattern Xa+1* had one band at 200 kb more than PFGE pattern Xa but was missing a band at 150 kb that Xa+1 carries; PFGE pattern Xc-1 had one band less at 80 kb than Xc; there was a small difference at 50 kb between Xc and Xc*.
[e] Isolates recovered from sexual partners.

in ethidium bromide-stained agarose gels.

We noticed that two isolates recovered from sexual partners (93-02567 and 93-02566) differed by a single 200-kb band after *Xba*I digestion. Our previous work with eight sexual partner pairs or groups showed indistinguishable PFGE patterns within each pair or group (4). We assumed that isolates from the partner pair having the Xa and Xa+1 patterns and identical *Nhe*I and *Spe*I patterns were closely related. By this reasoning, the first nine isolates in Table 1 were considered a genetic cluster, even though they were recovered from distinct locations separated by thousands of miles over a 2-year period.

The PFGE patterns of two other North American Pro/IB-5,7 isolates, 93-1223 and 93-0914, were identical to each other the study.

In this study, we assumed that isolates having PFGE patterns which differed by the presence or absence of a single band were closely related while those that had PFGE patterns which differed by ≥2 bands were likely to be less closely related. This is a more stringent criterion for interpreting PFGE patterns than what has been proposed (3). We found genetic relatedness as well as diversity among Pro/IB-5,7 and Pro/IB-1 *N. gonorrhoeae* isolates recovered over a 2-year time period from various geographic locations. Phenotypic properties such as auxotype/serotype class, presence of β-lactamase or plasmids, and antibiotic susceptibility have been used to group *N. gonorrhoeae* isolates. In this study, the PFGE groups did not always corre-

late with groups based on phenotype. An example is the first nine isolates in Table 1, which share common PFGE patterns but differed in their susceptibility to ciprofloxacin and carriage of the β-lactamase plasmid. Similarly, 92-032894 (Pro/IB-5,7) shared PFGE patterns with Pro/IB-1 isolates rather than Pro/IB-5,7. This suggests that PFGE analysis can be a useful addition in grouping isolates into genetic clusters which can be used to trace the movement of particular genotypes over time and into different social and geographical settings.

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