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Quinolone-Resistant *Neisseria gonorrhoeae*: Correlation of Alterations in the GyrA Subunit of DNA Gyrase and the ParC Subunit of Topoisomerase IV with Antimicrobial Susceptibility Profiles

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Fifty-five clinical strains of *Neisseria gonorrhoeae* were examined for mutations in the *gyrA* and *parC* genes and for antimicrobial susceptibility profiles. The MICs of quinolones for 31 strains with alterations in GyrA were significantly higher than the MICs for 24 strains without such alterations. Eleven strains with alterations in both GyrA and ParC were significantly more resistant to fluoroquinolones than those with alterations in GyrA alone. The MICs of cephalosporins for these strains were also significantly higher than those for other strains.

Several mechanisms of resistance to quinolones in various bacterial species, including *Neisseria gonorrhoeae*, have been identified and characterized (1, 3, 9, 10). In laboratory mutants of *N. gonorrhoeae*, amino acid changes associated with quinolone resistance have been identified in the A subunit (GyrA) (1) and the B subunit (GyrB) (9) of DNA gyrase and in the ParC subunit of topoisomerase IV (1). In a preliminary study (3) using a small number of clinical isolates of *N. gonorrhoeae*, amino acid changes in GyrA identical to those in laboratory mutants were found. However, alterations in ParC in clinical isolates have not been studied, nor have the antimicrobial susceptibility profiles of strains with quinolone resistance mutations been assessed. In this study, we examined 55 clinical strains of *N. gonorrhoeae* for alterations in GyrA and ParC and correlated these changes with the antimicrobial susceptibility profiles for other agents used in the treatment of gonorrhea.

Fifty-five clinical strains of *N. gonorrhoeae* were used in this study. Ten of these strains had been examined only for the presence of mutations in the *gyrA* gene (3); 45 were newly chosen at random for this study. All strains had been isolated between 1991 and 1993 from Japanese patients with gonococcal urethritis who were treated at several independent hospitals in Japan. None of the patients had received antibiotic treatment before visiting a clinic. The isolates were not epidemiologically related.

To analyze alterations in the region from amino acid 75 to 114 in GyrA, corresponding to the quinolone resistance-determining region of *Escherichia coli* GyrA (11) and the analogous region of ParC (1, 4), DNA fragments of the *gyrA* gene (nucleotides 174 to 398) and the *parC* gene (nucleotides 196 to 357) were amplified from chromosomal DNAs of the strains by PCR and then their sequences were determined as reported previously (1, 3). β-Lactamase activities of the strains were tested with nitrocefin disks. The presence of the TEM-1 β-lactamase gene was determined by a PCR-based assay specific for TEM-1-carrying plasmids of penicillinase-producing *N. gonorrhoeae* (8). The presence of the *tetM* determinant, which is responsible for plasmid-mediated tetracycline resistance, was also determined by a PCR-based assay (6). The susceptibilities of the strains to nalidixic acid, norfloxacin, ofloxacin, ciprofloxacin, penicillin G, tetracycline, doxycycline, cefotiam, ceftriaxone, cefotaxime, ceftizoxime, cefixime, erythromycin, clarithromycin, and azithromycin were determined as described previously (3).

Statistical analysis was conducted by using the Wilcoxon rank sum test. All statistical comparisons were two tailed and were performed with the significance set at $P < 0.05$.

Of the 55 strains, 31 (56.4%) had nucleotide changes in the *gyrA* gene resulting in amino acid changes, and 11 (20.0%) of these also had mutations in the *parC* gene (Table 1). Three strains had double amino acid changes in GyrA, and all of these had single changes in ParC. Of the 28 strains carrying single amino acid changes in GyrA, only 8 (28.6%) simultaneously had ParC alterations. Of the 24 strains which did not have nucleotide changes in the *gyrA* gene, 2 had silent mutations at Tyr-104 in ParC but none had mutations resulting in amino acid changes in the regions analyzed in this study. Among the GyrA alterations, a substitution of tyrosine for serine at position 91 (Ser-91→Tyr) was newly identified in *N. gonorrhoeae* and an Asp-95→Asn was also identified as a novel single amino acid change. Among ParC alterations, Asp-86→Asn, Ser-87→Ile, and Glu-91→Gly substitutions were newly identified in this study. These alterations were present in the quinolone resistance-determining region of GyrA or the analogous region of ParC, particularly in the amino acid of GyrA or ParC equivalent to Ser-83 of *E. coli* GyrA and in its vicinity (1, 4, 11). Therefore, it was postulated that these alterations would be associated with quinolone resistance, though it was not concluded from this study that they could actually give rise to the resistance phenotypes in *N. gonorrhoeae.*

β-Lactamase activity was detected enzymatically in 14 of the 55 strains, and the presence of the TEM-1 gene in these 14 strains was confirmed by the PCR-based assay. Six and three of these penicillinase-producing *N. gonorrhoeae* strains had single amino acid changes of Ser-91→Phe and Asp-95→Asn, respectively, in GyrA; none had alterations in ParC. The *tetM* determinant was not detected by PCR in any of these strains.

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TABLE 1. Amino acid changes in GyrA and ParC inferred by nucleotide changes in the *gyrA* and *parC* genes of *N. gonorrhoeae*

No. of strains	Amino acid (codon) at indicated position in[a]:														
	GyrA							ParC							
	90	91	92	93	94	95	96	86	87	88	89	90	91	92
WT[b]	Asp (GAT)	Ser (TCC)	Ala (GCA)	Val (GTT)	Tyr (TAC)	Asp (GAC)	Thr (ACC)	Asp (GAC)	Ser (AGT)	Ser (TCC)	Ala (GCC)	Tyr (TAT)	Glu (GAG)	Ala (GCG)
1	—	Phe (TTC)	—	—	—	Gly (GGC)	—	—	—	—	—	—	Gly (GGG)	—
2	—	Phe (TTC)	—	—	—	Asn (AAC)	—	—	—	Pro (CCC)	—	—	—	—
2	—	Phe (TTC)	—	—	—	—	—	—	Ile (ATT)	—	—	—	—	—
6	—	Phe (TTC)	—	—	—	—	—	Asn (AAC)	—	—	—	—	—	—
14	—	Phe (TTC)	—	—	—	—	—	—	—	—	—	—	—	—
2	—	Tyr (TAC)	—	—	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	Asn (AAC)	—	—	—	—	—	—	—	—
24	—	—	—	—	—	—	—	—	—	—	—	—	—	—

[a] —, identical to wild type.
[b] WT, wild type.

Figure 1 presents the distribution of MICs of quinolones for the strains and the association of types of amino acid changes in GyrA and ParC with the MICs. The clinical strains could be clearly assigned to two categories by the MICs of quinolones. For this study, the 31 strains for which the quinolone MICs were higher were referred to as quinolone resistant whereas the remaining 24 strains, for which the MICs were lower, were referred to as quinolone susceptible. All strains in the quin-

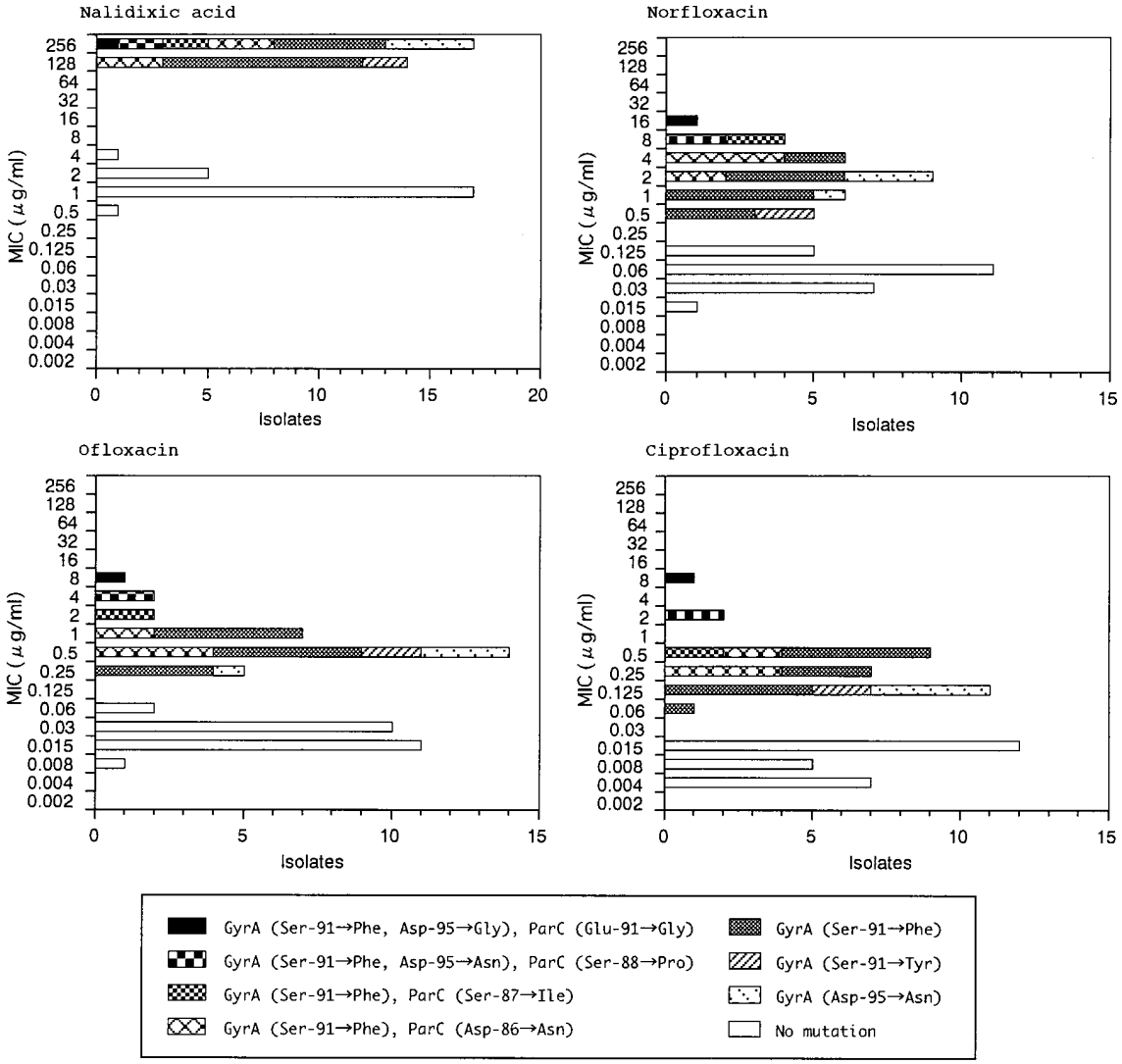

FIG. 1. Distribution of MICs of nalidixic acid, norfloxacin, ofloxacin, and ciprofloxacin for strains with various types of alterations in GyrA and/or ParC and for those without mutations. The types of mutations are indicated in the box.

TABLE 2. Susceptibilities of 55 strains of *N. gonorrhoeae*, classified by quinolone resistance phenotypes associated with alterations in GyrA and ParC, to various antimicrobial agents

Antimicrobial agent	Quinolone resistance category of strain[a]	No. of strains	MIC (μg/ml)[b]			Statistical difference (P)[c] in susceptibility to drug listed	
			Range	50%	90%	vs moderately FQ resistant	vs Q susceptible
Penicillin G[d]	Highly FQ resistant	11	1.0–8.0	1.0	4.0	NS[e]	<0.01
	Moderately FQ resistant	11	0.5–2.0	1.0	2.0		<0.05
	Q susceptible	19	0.125–2.0	0.5	1.0		
Tetracycline	Highly FQ resistant	11	1.0–4.0	2.0	4.0	NS	NS
	Moderately FQ resistant	20	1.0–4.0	2.0	4.0		<0.05
	Q susceptible	24	0.25–8.0	1.0	4.0		
Cefotiam	Highly FQ resistant	11	0.025–2.0	1.0	2.0	<0.05	<0.01
	Moderately FQ resistant	20	0.25–2.0	0.5	1.0		NS
	Q susceptible	24	0.06–2.0	0.5	1.0		
Cefotaxime	Highly FQ resistant	11	0.015–0.25	0.125	0.25	<0.01	<0.01
	Moderately FQ resistant	20	0.03–0.125	0.06	0.12		<0.01
	Q susceptible	24	0.008–0.125	0.03	0.12		
Ceftizoxime	Highly FQ resistant	11	0.008–0.25	0.06	0.25	<0.01	<0.01
	Moderately FQ resistant	20	0.015–0.06	0.03	0.03		NS
	Q susceptible	24	0.004–0.06	0.015	0.06		
Ceftriaxone	Highly FQ resistant	11	0.008–0.125	0.06	0.06	<0.01	<0.01
	Moderately FQ resistant	20	0.015–0.06	0.015	0.03		NS
	Q susceptible	24	0.004–0.06	0.015	0.03		
Cefixime	Highly FQ resistant	11	0.008–0.25	0.06	0.25	<0.05	<0.01
	Moderately FQ resistant	20	0.015–0.06	0.03	0.06		NS
	Q susceptible	24	0.008–0.06	0.015	0.06		
Doxycycline	Highly FQ resistant	11	0.5–2.0	1.0	2.0	NS	NS
	Moderately FQ resistant	20	0.5–2.0	1.0	2.0		NS
	Q susceptible	24	0.25–2.0	1.0	2.0		
Erythromycin	Highly FQ resistant	11	0.25–2.0	0.25	2.0	NS	NS
	Moderately FQ resistant	20	0.25–2.0	1.0	2.0		NS
	Q susceptible	24	0.03–2.0	0.5	2.0		
Clarithromycin	Highly FQ resistant	11	0.125–2.0	0.25	1.0	NS	NS
	Moderately FQ resistant	20	0.25–2.0	1.0	1.0		NS
	Q susceptible	24	0.03–2.0	0.5	1.0		
Azithromycin	Highly FQ resistant	11	0.03–0.25	0.06	0.25	NS	NS
	Moderately FQ resistant	20	0.03–0.25	0.125	0.25		NS
	Q susceptible	24	0.03–0.25	0.125	0.25		

[a] Strains were assigned to the following categories: highly fluoroquinolone-resistant strains with alterations in both GyrA and ParC (highly FQ resistant), moderately fluoroquinolone-resistant strains with alterations in GyrA alone (moderately FQ resistant), and quinolone-susceptible strains without alterations in the region of GyrA or ParC analyzed here (Q susceptible).
[b] 50% and 90%, MICs at which 50 and 90% of the isolates, respectively, are inhibited.
[c] Analyzed by the Wilcoxon rank sum test.
[d] Penicillinase-producing *N. gonorrhoeae* strains were excluded from the analysis of the susceptibility to penicillin G.
[e] NS, not significant.

olone-resistant category had alterations in GyrA with or without alterations in ParC, but the quinolone-susceptible strains had no alterations in the regions of GyrA and ParC sequenced. The MIC of nalidixic acid for all the quinolone-resistant strains was 128 or 256 μg/ml. With regard to the susceptibilities of the strains to fluoroquinolones, the fluoroquinolone MICs for the strain with a double amino acid change of Ser-91→Phe and Asp-95→Gly in GyrA and a single amino acid change of Glu-91→Gly in ParC were the highest, followed by the MICs for the strains with the other double amino acid change in GyrA and a single amino acid change of Ser-88→Pro in ParC. Among the MICs of fluoroquinolones for the strains with single amino acid changes in GyrA, those for the strains which also had alterations in ParC were higher than those for strains without alterations in ParC ($P < 0.01$ for norfloxacin, $P = 0.058$ for ofloxacin, and $P < 0.05$ for ciprofloxacin). Overall, the MICs of quinolones for the quinolone-resistant strains were significantly higher than those for the quinolone-susceptible strains. There were no significant differences in the MICs of nalidixic acid for the strains with alterations in both GyrA and ParC and those with alterations only in GyrA ($P = 0.151$), but the strains with alterations in both GyrA and ParC were

significantly more resistant to fluoroquinolones than those with alterations only in GyrA ($P < 0.01$ for norfloxacin, $P < 0.05$ for ofloxacin, and $P < 0.01$ for ciprofloxacin). These results are in agreement with the previous findings of studies using laboratory mutants of *N. gonorrhoeae* as well as the DNA transformation analyses directly showing that DNA gyrase is the primary target of quinolones (1).

To characterize the susceptibilities of the quinolone-resistant strains to other agents, we divided them into highly fluoroquinolone-resistant strains, with alterations in both GyrA and ParC, and moderately fluoroquinolone-resistant strains, with alterations only in GyrA, and then compared their susceptibilities with those of the quinolone-susceptible strains (Table 2). Among the MICs of penicillin G for the non-penicillinase-producing *N. gonorrhoeae* strains, those for the highly fluoroquinolone-resistant and moderately fluoroquinolone-resistant strains were significantly higher than those for the quinolone-susceptible strains. The MICs of tetracycline for all the quinolone-resistant strains were higher than those for the quinolone-susceptible strains ($P < 0.05$). The MICs of cephalosporins were low for all the strains, which were assigned to a cephalosporin-susceptible strain category (5, 7). However, the MICs of cephalosporins for the highly fluoroquinolone-resistant strains were significantly higher than those for the moderately fluoroquinolone-resistant and the quinolone-susceptible strains. The susceptibilities of the quinolone-resistant strains to other agents were similar to those of the quinolone-susceptible strains. There were no differences in the susceptibility profiles for other agents between the highly fluoroquinolone-resistant strains and the moderately fluoroquinolone-resistant strains.

In this study, most patients infected with the quinolone-resistant strains were treated successfully with broad-spectrum cephalosporins. At the moment, treatment with broad-spectrum cephalosporins, including ceftriaxone, or with alternative regimens (2) is efficacious against gonococcal infections caused by strains showing decreased susceptibilities to quinolones. However, this study demonstrated that the cephalosporin-susceptible, highly fluoroquinolone-resistant strains with multiple quinolone resistance mutations (5, 7) had significantly reduced susceptibilities to cephalosporins compared with other strains. Therefore, in view of the increase in the number of highly fluoroquinolone-resistant strains, as well as the potential for emergence of cross-resistance to cephalosporins or other agents in fluoroquinolone-resistant strains, it is important to monitor the susceptibilities of gonococcal isolates, particularly post-treatment isolates from patients treated unsuccessfully with fluoroquinolones.

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