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Alterations in the GyrA Subunit of DNA Gyrase and the ParC Subunit of Topoisomerase IV in Quinolone-Resistant Clinical Isolates of *Klebsiella pneumoniae*

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We determined a partial sequence of the *Klebsiella pneumoniae parC* gene, including the region analogous to the quinolone resistance-determining region of the *Escherichia coli gyrA* gene, and examined 26 clinical strains of *K. pneumoniae* for an association of alterations in GyrA and ParC with susceptibilities to quinolones. The study suggests that in *K. pneumoniae* DNA gyrase is a primary target of quinolones and that ParC alterations play a complementary role in the development of higher-level fluoroquinolone resistance.

Klebsiella pneumoniae is susceptible to fluoroquinolones (7, 12); thus, urinary tract infections caused by this pathogen have successfully been treated with the fluoroquinolones. Recently, however, we have observed fluoroquinolone treatment failures in urinary tract infections in which *K. pneumoniae* strains exhibited decreased susceptibilities to the agents.

Among the mechanisms of quinolone resistance, alteration of the GyrA subunit of DNA gyrase has a central role in conferring high-level quinolone resistance in gram-negative bacteria such as *Escherichia coli* and *Neisseria gonorrhoeae* (1, 2, 6, 14, 15). In these species, alteration of the ParC subunit of DNA topoisomerase IV seems to play a complementary role in increasing resistance to fluoroquinolones (1, 3, 9, 10). In *Staphylococcus aureus*, however, topoisomerase IV is a primary target of quinolones, and alteration of the GrlA subunit of topoisomerase IV, equivalent to ParC, plays a major role in the development of fluoroquinolone resistance (5, 11, 13). In *K. pneumoniae*, the association of alterations in GyrA and ParC with quinolone resistance is not well understood. In this study, we initially sought to determine a partial sequence of the *parC* gene of *K. pneumoniae* and then examined clinical strains of *K. pneumoniae* for the association of alterations of GyrA and ParC with quinolone resistance.

The type strain of *K. pneumoniae*, strain ATCC 13883, was purchased from the American Type Culture Collection. Twenty-six clinical strains of *K. pneumoniae* were isolated from Japanese patients with urinary tract infections from 1991 through 1993. No patients were given quinolones when the strains were isolated, and the isolates were not epidemiologically related.

To determine a partial sequence of the *parC* gene of *K. pneumoniae* including the region analogous to the quinolone resistance-determining region (QRDR) of the *gyrA* gene (14), a DNA fragment was amplified from a chromosomal DNA of the type strain *K. pneumoniae* ATCC 13883 by PCR with two primers (primers EC-PARC-A and EC-PARC-B), and the PCR product was sequenced. Primers EC-PARC-A and EC-PARC-B were identical to nucleotide positions 185 to 204 and 353 to 372, respectively, of the *E. coli parC* gene (8). Isolation of chromosomal DNAs, PCR amplification, and sequencing of the PCR products were performed as reported previously (2).

To analyze mutations in the region of the *K. pneumoniae gyrA* gene corresponding to the QRDR of the *E. coli gyrA* gene (14), DNA fragments of the *gyrA* gene were amplified from chromosomal DNAs of type and clinical strains by PCR with two primers, primers KP-GYRA-A and KP-GYRA-B, and their sequences were determined. Primers KP-GYRA-A and KP-GYRA-B were identical to nucleotide positions 139 to 162 and 360 to 381, respectively, of the *K. pneumoniae gyrA* gene (4). Mutations in the analogous region of the *parC* genes of the clinical isolates were also detected by similar procedures by using PCR with the primers of EC-PARC-A and EC-PARC-B.

Susceptibilities to nalidixic acid and ciprofloxacin were determined by the twofold agar dilution method with an inoculum of 10^4 CFU per spot. MICs were defined as the lowest concentrations of drug that completely inhibited visible growth of the inoculum after incubation for 18 h at 37°C.

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

The primers EC-PARC-A and EC-PARC-B amplified a DNA fragment with the expected size of 188 bp from the chromosomal DNA of the type strain, and the PCR product was sequenced (Fig. 1). The 49-amino-acid sequence deduced from the nucleotide sequence of the amplified 148-bp DNA fragment excluding the primers exhibited 73% similarity with the nucleotide sequence of the *K. pneumoniae* GyrA subunit (4) and was identical to the sequence of the *E. coli* ParC subunit (8).

Table 1 presents a summary of the analysis of 26 clinical isolates of *K. pneumoniae* for an association of alterations in GyrA and ParC with susceptibilities to quinolones. In the GyrA of type strain ATCC 13883, for which the nalidixic acid MIC is 6.25 μg/ml and the ciprofloxacin MIC is ≤0.025 μg/ml, a codon at amino acid position 83 was TCC, encoding serine, although the equivalent codon was previously reported to be ACT, encoding threonine, in *K. pneumoniae* M5a1 (4). Ten clinical isolates had TCC (Ser) at codon 83, and 16 strains had TTC (Phe) or TAC (Tyr) at this position, which seemed to be changed from TCC (Ser) by a point mutation within codon 83. Other amino acid changes were observed at Asp-87. All the alterations in GyrA were analogous to those frequently found

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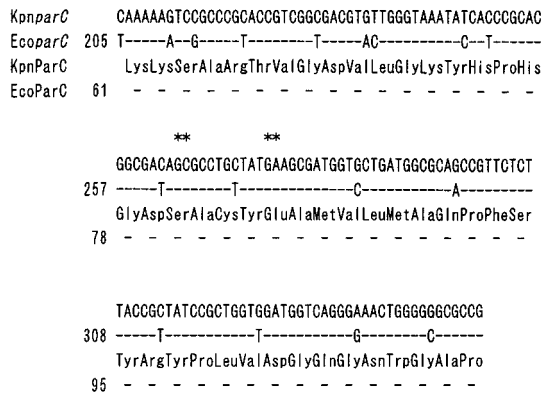

FIG. 1. Comparisons of the nucleotide sequence of the DNA fragment amplified from the chromosomal DNA of *K. pneumoniae* ATCC 13883 (Kpn*parC*) with the corresponding sequence of the *E. coli parC* gene (Eco*parC*) and of the deduced amino acid sequence (KpnParC) with the corresponding sequence of the *E. coli* ParC protein (EcoParC). Parts of the primers are excluded from the sequence. The sites in which nucleotide changes were observed in this study are denoted by asterisks. Dashes on the Eco*parC* and EcoParC lines indicate nucleotides and amino acids identical to nucleotides in Kpn*parC* and amino acids in KpnParC, respectively.

and previously demonstrated to be responsible for quinolone resistance in *E. coli* (6, 14, 15). In ParC, four types of single amino acid changes were identified. These alterations were present at the amino acid positions corresponding to Ser-80 and Glu-84 of *E. coli* ParC (8) and *S. aureus* GrlA (5). In *E. coli* strains with alterations in GyrA, ParC alterations at these amino acid positions have been observed to give rise to increments in quinolone resistance (9, 10). In *S. aureus* strains, the anologous alterations have primarily been responsible for quinolone resistance (5, 11, 13).

The strains with alterations in GyrA were more resistant to nalidixic acid than those without an alteration in GyrA ($P < 0.001$). The nalidixic acid MICs for the strains having single amino acid changes in GyrA ranged from 100 to >800 μg/ml, and those for the strains having double amino acid changes in GyrA with or without alterations in ParC were >800 μg/ml. For ciprofloxacin, the strains with single amino acid changes in GyrA were significantly more resistant than those without a GyrA alteration ($P < 0.005$), the strains with double amino acid changes in GyrA but without a ParC alteration were more resistant than those with single amino acid changes in GyrA ($P < 0.05$), and the strains with double amino acid changes in GyrA and single acid amino changes in ParC were significantly more resistant than those with double amino acid changes in GyrA alone ($P < 0.005$). In this study, no strains having alterations in ParC without the simultaneous presence of alterations in GyrA were found. These findings suggest that in *K. pneumoniae* DNA gyrase is a primary target of quinolones, that only a single amino acid change at Ser-83 or Asp-87 in GyrA is sufficient to generate high-level resistance to nalidixic acid and to decrease susceptibility to ciprofloxacin, and that the accumulation of alterations in GyrA and the simultaneous presence of alterations in ParC play a complementary role in developing higher-level fluoroquinolone resistance.

Nucleotide sequence accession number. The partial sequence of the *K. pneumoniae parC* gene reported here appears in the DDBJ, EMBL, and GenBank nucleotide sequence databases with the accession number D86602.

TABLE 1. Alterations in GyrA and ParC in quinolone-resistant clinical isolates of *K. pneumoniae*

Strain	Amino acid (codon) at the indicated position in:				MIC (μg/ml)[a]	
	GyrA		ParC		NA	CPFX
	83	87	80	84		
Type strain	Ser (TCC)	Asp (GAC)	Ser (AGC)	Glu (GAA)	6.25	≤0.025
102	—[b]	—	—	—	3.13	≤0.025
193, 600	—	—	—	—	6.25	≤0.025
424	—	—	—	—	6.25	0.05
544	—	—	—	—	12.5	≤0.025
743	—	—	—	—	12.5	≤0.025
413	—	—	—	—	25	0.2
196	—	—	—	—	50	0.39
377	—	Gly (GGC)	—	—	100	0.1
166	Tyr (TAC)	—	—	—	200	0.39
338	Phe (TTC)	—	—	—	400	0.39
430	Phe (TTC)	—	—	—	>800	0.39
227	Tyr (TAC)	—	—	—	>800	0.39
017	Tyr (TAC)	—	—	—	>800	0.78
397	Tyr (TAC)	—	—	—	>800	1.56
555	Phe (TTC)	—	—	—	>800	3.13
356	—	Gly (GGC)	—	—	>800	3.13
143	Phe (TTC)	Gly (GGC)	—	—	>800	1.56
596	Phe (TTC)	Asn (AAC)	—	—	>800	6.25
467	Phe (TTC)	Asn (AAC)	—	—	>800	12.5
237	Phe (TTC)	Asn (AAC)	—	Gly (GGA)	>800	6.25
043	Phe (TTC)	Ala (GCC)	Ile (ATC)	—	>800	12.5
802	Phe (TTC)	Asn (AAC)	Ile (ATC)	—	>800	25
211	Phe (TTC)	Asn (AAC)	Arg (AGA)	—	>800	50
300	Phe (TTC)	Gly (GGC)	—	Lys (AAA)	>800	50
199	Phe (TTC)	Gly (GGC)	—	Gly (GGA)	>800	400

[a] NA, nalidixic acid; CPFX, ciprofloxacin.
[b] —, identical to the type strain.

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