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gyrA and *gyrB* Mutations in Quinolone-Resistant Strains of *Escherichia coli*

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The proportion of *gyrA* and *gyrB* mutations in quinolone-resistant *Escherichia coli* strains was examined by introducing cloned wild-type *gyrA* and *gyrB* genes. In 25 spontaneous mutants of strain KL16, 13 had *gyrA* and 12 had *gyrB* mutations. In eight clinical isolates, five had *gyrA* mutations and one had a *gyrB* mutation; mutations in two isolates remained unidentified.

Recently developed quinolone antibacterial agents have broad and potent antibacterial activity and are not cross resistant with antibiotics. They are well absorbed when administered orally and have been used clinically against various kinds of infections. As they are used more frequently, quinolone-resistant organisms have begun to appear. At least three mechanisms of quinolone resistance have been recognized: mutations in the *gyrA* gene (2, 4, 5, 11, 14), mutations in the *gyrB* gene (13), and mutations causing poor drug transport (1, 4–6, 9). However, which kind(s) of mutation is major is still unknown. We have cloned *gyrA* and *gyrB* genes from the chromosome of wild-type *Escherichia coli* KL16 and its quinolone-resistant mutants and determined the sites of the mutations in the *gyr* genes (13, 14). Using the cloned genes, we examined the frequency of *gyrA* and *gyrB* mutations in quinolone-resistant *E. coli* by transformation.

A plasmid carrying the wild-type *gyrA* gene, pAW012, was constructed by inserting a 4.5-kilobase filled-in *Stu*I-*Spl*I fragment of the *gyrA* gene from pAW011 (14) into an *Eco*RV site of pBR322. A plasmid carrying a 3.4-kilobase fragment of the wild-type *gyrB* gene, pJB11, was constructed as described previously (13). The authentic *gyrA* and *gyrB* mutants N-51, N-89, P-10, P-18, N-24, and N-31 have been proved to possess a mutation in gene *gyrA* or *gyrB* (13, 14). A transport mutant, KEA13 (4), was kindly supplied by K. Hirai. Spontaneous quinolone-resistant mutants of *E. coli* KL16 were isolated on LB agar (8) containing nalidixic acid or enoxacin at four times the MICs. Quinolone-resistant clinical isolates were obtained from urine cultures. The quinolones used were synthesized in our laboratories. Plasmid DNA was isolated by the method of Wilkie et al. (10). Transformation was done by the $CaCl_2$ method (7), and transformants were selected on LB agar containing ampicillin at 25 μg/ml.

As the wild-type *gyrA* and *gyrB* genes are known to be dominant over the corresponding quinolone-resistant alleles (2, 3, 13, 14), it was anticipated that quinolone-resistant strains having a *gyrA* or *gyrB* mutation would become quinolone susceptible when they were transformed with a plasmid carrying the wild-type *gyrA* or *gyrB* gene. This was first confirmed by transformation of authentic quinolone-resistant *gyrA* and *gyrB* mutants of *E. coli* KL16 with pAW012 and pJB11 (Table 1). Every *gyrA* and *gyrB* mutant became fully susceptible to nalidixic acid when it was transformed with a corresponding wild-type *gyr* gene, while the transport mutant, KEA13, did not. Next, we checked the proportion of *gyrA* and *gyrB* mutations among spontaneous quinolone-resistant mutants of *E. coli* KL16 selected by nalidixic acid or enoxacin (Table 2). Of 20 mutants selected by nalidixic acid, 10 had *gyrA* and 10 had *gyrB* mutations. In *gyrA* mutants, five showed a relatively low nalidixic acid MIC (50 μg/ml) and a few exhibited high MICs (200 to ≥400 μg/ml), while the MIC for the parent strain KL16 was 3.13 μg/ml. Most *gyrB* mutants showed relatively low nalidixic acid MICs (25 to 50 μg/ml), and none had MICs as high as 200 to ≥400 μg/ml. Three of the five *gyrB* mutants with low MICs (50 μg/ml) were hypersusceptible to amphoteric quin-

TABLE 1. Quinolone susceptibility of authentic *gyr* mutants of *E. coli* KL16 transformed with a plasmid having the wild-type *gyrA* or *gyrB* gene

Strain	Mutation in chromosome	MIC of nalidixic acid (μg/ml) for:		
		Nontransformant	Transformant with pAW012[a]	Transformant with pJB11[b]
KL16	Wild type	3.13	3.13	3.13
N-51	*gyrA*	400	3.13	400
P-18	*gyrA*	400	3.13	400
P-10	*gyrA*	25	3.13	25
N-89	*gyrA*	12.5	3.13	12.5
N-24	*gyrB*	25	25	3.13
N-31	*gyrB*	50	50	3.13
KEA13	34 min[c]	12.5	12.5	12.5

[a] A plasmid having the wild-type *gyrA* gene.
[b] A plasmid having the wild-type *gyrB* gene.
[c] A mutation causing loss of OmpF protein.

TABLE 2. *gyrA* and *gyrB* mutations in spontaneous quinolone-resistant mutants of *E. coli* KL16

Selective agent (concn)	Mutation	No. of strains with nalidixic acid MIC (μg/ml) of:				
		25	50	100	200	≥400
Nalidixic acid (12.5 μg/ml)	*gyrA*	0	5	2	2	1
	gyrB	4	5 (3[a])	1	0	0
Enoxacin (0.4 μg/ml)	*gyrA*	2	0	1	0	0
	gyrB	1	1	0	0	0

[a] Mutants resistant to nalidixic acid but hypersusceptible to amphoteric quinolones.

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TABLE 3. *gyrA* and *gyrB* mutations in quinolone-resistant clinical isolates of *E. coli*

Mutation	No. of strains with nalidixic acid MIC (μg/ml) of:		
	100	200	≥400
gyrA	0	2	3
gyrB	1	0	0
Unidentified	0	0	2

olones such as pipemidic acid, norfloxacin, enoxacin, ofloxacin, and ciprofloxacin (data not shown) as reported previously in the *nal-31* mutant N-31 (12). Of five mutants selected by enoxacin, three possessed *gyrA* and two had *gyrB* mutations. These results demonstrate that the frequency of spontaneous mutations is practically the same in both *gyrA* and *gyrB* genes.

Next, we examined the proportion of *gyrA* and *gyrB* mutations in quinolone-resistant clinical isolates of *E. coli* obtained from urine. Although 38 isolates were tested for transformation with pAW012 and pJB11, transformants appeared with only eight strains (Table 3). Unsuccessful transformation may be due to DNA restriction. Of the eight strains, five had *gyrA* mutations and one had a *gyrB* mutation; the other two had unidentified mutation(s), because they did not change with respect to nalidixic acid-resistance when transformed with either plasmid. The five *gyrA* mutants all showed high nalidixic acid MICs (200 to ≥400 μg/ml), while that for the *gyrB* mutant was relatively low (100 μg/ml). Why *gyrA* mutants are major in clinical isolates might be because some *gyrA* mutants are more resistant to quinolones than *gyrB* mutants and therefore have a selective advantage. The strains with unidentified mutations may have both *gyrA* and *gyrB* mutations or may be transport mutants, but further study is required to clarify this.

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