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Contribution of a New Mutation in *parE* to Quinolone Resistance in Extended-Spectrum-β-Lactamase-Producing *Escherichia coli* Isolates▽

Antonio Sorlozano,[1] Jose Gutierrez,[1]* Antonio Jimenez,[2] Juan de Dios Luna,[3] and José Luis Martínez[2]

Departments of Microbiology[1] and Biostatistics,[3] School of Medicine, University of Granada, and Department of Urology, San Cecilio University Hospital,[2] Granada, Spain

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Mutations in the quinolone resistance-determining regions of *gyrA*, *gyrB*, *parC*, and *parE* were studied in 30 fluoroquinolone-resistant clinical isolates of *Escherichia coli* producing extended-spectrum β-lactamases. Ten isolates showed a mutation in *parE* that was significantly associated with an increase in the MIC for fluoroquinolones.

Spain has one of the highest rates of resistance to quinolones among European countries. Queipo-Zaragoza et al. (11) reported a significant increase in resistance to quinolones among clinical isolates of *E. coli* from 1990 (3%) to 1998 (23%). In 2003, resistance to ciprofloxacin in clinical isolates of *E. coli* obtained from blood cultures reached 19% in Spain, exceeded only by the reported rate in Portugal (9). However, these data are very likely biased (1).

There has also been an increase in the use of fluoroquinolones, especially cephalosporins, to treat enterobacterial infections, which has in turn increased the appearance of extended-spectrum β-lactamases (ESBLs) among these infections. The association between ESBL production and fluoroquinolone resistance is well documented (8) and may be due in part to the joint transfer of both mechanisms via plasmids (10). Nevertheless, this explains only a minimal proportion of the coresistance found.

The objective of this study was to investigate the presence of mutations in regions that code for quinolone resistance in the chromosomal genes *gyrA*, *gyrB*, *parC*, and *parE* in ESBL-producing clinical isolates of *Escherichia coli*.

Thirty previously characterized (13–15) ESBL-producing clinical isolates of *E. coli* were characterized for fluoroquinolone resistance using a microdilution assay for ciprofloxacin and levofloxacin (2). Reference strains *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 served as controls in all MIC determinations. PCR and direct DNA sequencing were used to identify mutations. The oligonucleotide primers 5′-ACGTACTAGGCAATGACTGG-3′ and 5′-AGAAGTCGC CGTCGATAGAAC-3′ were used to amplify nucleotides of *gyrA*, generating a fragment of 189 bp; 5′-TGTATGCGATGTC TGAACTG-3′ and 5′-CTCAATAGCAGCTCGGAATA-3′ were used for *parC* (264-bp fragment); and 5′-TACCGAG CTGTTCCTTGTGG-3′ and 5′-GGCAATGTGCAGACCAT CAG-3′ were used for *parE* (266-bp fragment), following a previously described procedure (4). Finally, the oligonucleotide primers 5′-CTCCTCCCAGACCAAAGACA-3′ and 5′-TCACGACCGATACCACAGCC-3′ were used for *gyrB* (447-bp fragment), following a previously described procedure (17). Amplified DNA products were resolved by electrophoresis in agarose (1.6%, wt/vol) gels containing ethidium bromide. The PCR product was purified (Wizard PCR Preps DNA purification system; Promega, Madison, WI), processed with a DNA sequencing kit, and analyzed in an automatic DNA se-

TABLE 1. MIC (in μg/ml) for ciprofloxacin and levofloxacin and mutations detected in each gene of *E. coli* isolates

Isolate	MIC (μg/ml) of:		Mutation in:[a]	
	Ciprofloxacin	Levofloxacin	*parC*	*parE*
1	16	8	S80I	—
25	8	4	E84K	—
26	32	16	S80I	—
41	32	8	S80I	S458A
46	64	16	S80I	S458A
58	4	4	S80I	—
65	8	8	S80I	—
101	8	8	S80I	—
102	8	8	S80I	—
115	8	4	S80I	—
140	4	4	S80R	—
141	8	4	S80R	—
153	16	8	S80I	—
159	64	16	S80I	—
179	64	16	S80I, E84V	—
180	32	16	S80I, E84G	—
185	32	16	S80I	—
189	128	16	S80I	S458A
191	32	16	S80I, A108V	—
195	64	16	S80I	S458A
200	128	32	S80I	S458A
207	64	16	S80I	S458A
217	16	8	S80I, A108V	—
219	16	8	S80I	—
224	128	8	S80I	S458A
226	32	8	S80I	S458A
232	64	16	S80I	S458A
237	32	32	S80I	—
245	32	16	S80I	S458A
255	8	8	S80I	—

[a] —, absence of mutation. All isolates had mutations resulting in S83L and D87N in *gyrA* and no mutation in *gyrB*.

* Corresponding author. Mailing address: Departamento de Microbiología, Facultad de Medicina, Avda de Madrid 11, E-18012 Granada, Spain. Phone: 34 958 24 35 50. Fax: 34 958 24 61 19. E-mail: josegf@ugr.es.

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TABLE 2. Spearman's coefficient (rho) values and significance for the relationship between MICs for ciprofloxacin or levofloxacin and the number of accumulated mutations for each isolate[a]

Drug	*parC*		*parE*		All	
	Spearman's coefficient (rho)	Significance (bilateral)	Spearman's coefficient (rho)	Significance (bilateral)	Spearman's coefficient (rho)	Significance (bilateral)
Ciprofloxacin	0.104	0.584	0.689	0.000	0.722	0.000
Levofloxacin	0.199	0.292	0.370	0.044	0.485	0.007

[a] Determinations were not made for *gyrA* and *gyrB* because the number of mutations was the same for all isolates (two in *gyrA* and none in *gyrB*).

quencer (Applied Biosystems, Foster City, CA). *E. coli* strain 1-319, supplied by Jordi Vila of the University of Barcelona, was used as a control in the sequencing procedure (16). The sequence obtained for each of the genes in each of the 30 isolates was compared with the nucleotide sequence of the quinolone resistance-determining region (QRDR) of the reference strain *E. coli* K-12.

The Spearman correlation coefficient was used to explore relationships between the MICs of ciprofloxacin or levofloxacin and the numbers of mutations in each gene and in all four genes and to establish the direction of any relationship found.

Table 1 shows MICs for ciprofloxacin and levofloxacin obtained by microdilution and lists the mutations detected in each gene.

Table 2 displays the results of the statistical analysis, showing that the only correlation coefficients significantly different from 0 were those for the number of mutations in *parE* and the total number of mutations in relation to the MICs of both antibiotics.

The frequency of ESBL-producing strains detected in our region in 2002 (15) was much higher than that found in the same area by a Spanish multicenter study in 2000 (5). A similar finding was reported for quinolone resistance (3, 15). Besides the passage of time, the precise reasons for this increase are not clear.

Two mutations, encoding Ser83Leu and Asp87Asn, were detected in the QRDR of *gyrA* in all 30 *E. coli* isolates studied. The additional mutation in the Asp87 codon of *gyrA* is associated with a greater increase in fluoroquinolone resistance (17). However, since all isolates possessed these two mutations, it cannot be concluded that these changes are responsible for the differences in MICs for ciprofloxacin and levofloxacin among these isolates.

Six different mutations were observed in the QRDR of *parC* in these *E. coli* isolates: they encoded Ser80Ile (76.7% of isolates), Ser80Arg (6.7%), Glu84Lys (3.3%), Ser80Ile and Glu84Gly (3.3%), Ser80Ile and Glu84Val (3.3%), and Ser80Ile and Ala108Val (6.7%). Even though four isolates possessed two mutations and the rest showed a single substitution, from a statistical standpoint, it cannot be stated that these changes were in themselves responsible for the difference in MICs for fluoroquinolones among the different isolates.

Regarding *parE*, a similar study in Spain found no changes in the QRDR, although the authors did not rule out the possibility of other changes outside this region (12). In fact, 10 (33.3%) of the present *E. coli* isolates showed a single mutation in *parE*, encoding Ser458Ala. A different mutation outside the QRDR, found in the same codon as that for Ser458Thr, was previously reported by Komp et al. (7). Statistical analysis of the relationship between the increase in MICs for ciprofloxacin and levofloxacin among the studied isolates and the presence of the Ser458Ala mutation showed significance, i.e., the increase in MICs was significantly related to the presence of this change. Seven mutations in *parE* have been described to date (6). The DNA sequence of *parE* is very similar to that of *gyrB*; therefore, *parE* mutations may also be associated with quinolone resistance. As all of these mutations have been detected outside the QRDR of *parE*, their contribution to quinolone resistance requires further investigation (6).

Other mechanisms implicated in fluoroquinolone resistance were not considered in the present study and may be responsible for the differences in MICs for fluoroquinolones observed among the isolates with the same types of mutation in all genes. Mutations in DNA gyrase, whether associated with mutations in topoisomerase IV or not, are an important determining factor for high levels of fluoroquinolone resistance. Changes in permeability, active expulsion of the antibiotic, and, to a lesser degree, the presence of plasmids are complementary mechanisms that cannot be excluded and may enhance development of resistance and contribute to the selection of fluoroquinolone-resistant isolates in the course of treatment with these antibiotics.

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