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Novel *gyrA* Point Mutation in a Strain of *Escherichia coli* Resistant to Fluoroquinolones but Not to Nalidixic Acid

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We have previously described a clinical isolate of *Escherichia coli* (Q2) that is highly resistant to fluoroquinolones (MIC of ciprofloxacin, 16 μg/ml) but susceptible to nalidixic acid (MIC of nalidixic acid, 4 μg/ml) (N. Moniot-Ville, J. Guibert, N. Moreau, J. F. Acar, E. Collatz, and L. Gutmann, Antimicrob. Agents Chemother. 35:519–523, 1991). Transformation of strain Q2 with a plasmid carrying the wild-type *gyrA* gene from *E. coli* K-12(pAFF801) resulted in a 32-fold decrease in the MIC of ciprofloxacin, suggesting that at least one mutation in *gyrA* was involved in the resistance of Q2. Intragenic *gyrA* fragments of 668 and 2,500 bp from strain Q2 were amplified by the polymerase chain reaction. We sequenced the 668-bp fragment and identified a single novel point mutation (transition from G to A at position 242), leading to an amino acid substitution (Gly-81 to Asp) in the gyrase A subunit. We constructed hybrid plasmids by substituting either the 668-bp fragment or the 2,500-bp fragment from Q2 DNA, both of which contained the *gyrA* point mutation, for the corresponding fragments in wild-type *gyrA* (2,625 bp) of *E. coli* K-12. When introduced into *E. coli* KNK453 (*gyrA* temperature sensitive), both plasmids conferred an eightfold increase in the MIC of ciprofloxacin, but only a twofold increase in the MIC of nalidixic acid. When introduced into *E. coli* Q2, neither plasmid conferred any change in the MICs of ciprofloxacin or nalidixic acid, suggesting that only the point mutation found in *gyrA* was involved in the resistance that we observed.

Quinolones are among the most potent antibacterial agents (1, 2) developed so far for use in humans. On the basis of their structures and antimicrobial spectra, quinolones have been separated into two groups; the original agents include nalidixic acid, flumequine, and oxolinic acid, while the newer fluoroquinolones include all the recently developed molecules, such as norfloxacin, ofloxacin, pefloxacin, and ciprofloxacin. Besides the antimicrobial activities of the older quinolones, which are essentially directed against enterobacteria, the newer quinolones have broader spectra of activity, including activities against nearly all gram-negative species (bacilli and cocci), some gram-positive species (staphylococci), and intracellular bacterial species, such as rickettsii and mycobacteria (2). The chemical structures of the two groups differ by a piperazine ring at position C-7 and a fluorine atom at position C-6 in the second group (5). Quinolones have been shown to inhibit DNA gyrase activities such as DNA supercoiling and DNA replication (8). DNA gyrase, an A_2B_2 tetrameric enzyme which is the target of the quinolones (11), is a type II topoisomerase that is essential for replication and gene expression and that is also involved in recombination and conjugation (16, 32, 33). Until now, chromosomal mutations are the only observed genetic changes which can lead to quinolone resistance (9). The mechanisms involved in quinolone resistance include a decrease in membrane permeability, increased efflux of quinolones, or a decreased affinity of DNA gyrase for these antibiotics (9). Several investigators (10, 13, 26, 36, 38) showed that point mutations in the structural gene for gyrase A subunit (*gyrA*) are responsible for the quinolone resistance acquired by different strains of *Escherichia coli* selected either in vitro or in vivo. The point mutations responsible for resistance were located in a very small region of the 2,625-kb *gyrA* gene, i.e., between nucleotides 200 and 350. The resistance patterns associated with these mutations were always characterized by the higher MICs of nalidixic acid (8- to 400-fold increase in the MICs) than those of the newer quinolones such as ciprofloxacin (26, 36, 38).

We previously described (23) a clinical strain, *E. coli* Q2, which expressed high-level resistance to ciprofloxacin but which remained susceptible to nalidixic acid and flumequine. Although a decrease in quinolone uptake associated with abnormal porin and lipopolysaccharide patterns were observed, it was concluded that a modification in DNA gyrase was the major resistance mechanism involved. In order to assess whether subunit A or B of the gyrase of *E. coli* Q2 was modified, genetic analyses by complementation tests and sequencing of part of the *gyrA* and *gyrB* genes were carried out. One point mutation in the *gyrA* gene that was different from those described previously was found and was thought to play a role in this unusual resistance phenotype.

(This work was presented in part at the 92nd General Meeting of the American Society for Microbiology [4].)

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in the present study are listed in Table 1. All *E. coli* strains were grown in brain heart infusion (BHI) broth or on BHI agar (Difco).

Antibiotics and MICs. The following antibiotics were kindly provided by the indicated manufacturers: nalidixic acid, Sterling-Winthrop, Clichy, France; cinoxacin, Lilly France, Saint-Cloud, France; flumequine, 3M Pharma, Malakoff, France; oxolinic acid, Parke-Davis, Courbevoie,

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TABLE 1. *E. coli* strains and plasmids used in the present study

E. coli strain or plasmid	Genotype and derivation	Relevant phenotype[a]	Source (reference)
Strains			
Q1		Nal^s Cip^s	Clinical strain (23)
Q2		Nal^s Cip^r	Clinical strain (23)
KNK453	*gyrA43*(Ts[b]) *polA thiA uvrA thx*	Nal^s Cip^s	R. Menzel (19)
RFM386	*strA galK* Δ*recAsrl srl*::dTn*10*/pRM386	Nal^s Cip^s	R. Menzel (27)
RFM876	*strA galK2 gyrB221*(*couR*) *gyrB203* (Ts)/pJB11	Nal^s Cip^s	R. Menzel (35)
DH5α	F$^-$ φ80 d *lacZ* ΔM15, Δ(*lacZYA argF*)U169, *endA1 recA1 hsdR17 deoR supE44 thi-1, gyrA96 relA1*	Nal^r Cip^s	Clontech (14)
JM101	F′ *traD36 proAB lacI*^{q} Z ΔM15 *thi strA endA hsdR sbcB15 supE*		Clontech
Plasmids			
pRM386	*gyrA* from *E. coli* K-12 cloned in pBR322		R. Menzel (27)
pAZ13	Central *Pvu*II fragment of Tn*903* containing *aphA-1* inserted at the *Pvu*II site into pBR322	Km^r	This study
pAFF801	*aphA-1* inserted at the *Sca*I site of pRM386	Km^r	This study
pAFF802	Derivative of pRM386 conferring resistance to ceftazidime	Caz^r	This study
pAFF803	Derivative of pRM386 with a 668-bp fragment of *gyrA* from Q2	Ap^r	This study
pAFF804	Derivative of pAFF803 with *aphA-1* inserted at the *Sca*I site of pAFF803	Km^r	This study
pAFF805	Derivative of pRM386 with a 2,500-bp fragment of *gyrA* from Q2	Ap^r	This study
pAFF806	Derivative of pAFF805 with *aphA-1* inserted at the *Sca*I site of pAFF805	Km^r	This study
pJB11	*gyrB* from *E. coli* KL16 cloned in pBR322	Ap^r	R. Menzel (35)
pAFF811	*aphA-1* inserted at the *Sca*I site of pJB11	Km^r	This study

[a] Nal, nalidixic acid; Cip, ciprofloxacin; Km, kanamycin; Caz, ceftazidime; Ap, ampicillin; s, susceptible; r, resistant.
[b] Ts, temperature sensitive.

France; norfloxacin, Merck Sharp & Dohme-Chibret, Paris, France; ofloxacin, Roussel, Paris, France; ciprofloxacin, Bayer Pharma, Sens, France; pefloxacin, Roger Bellon, Neuilly, France; tosufloxacin, Lederle, Rungis, France; temafloxacin, Abbott-France, Rungis, France; and sparfloxacin, Rhône Poulenc Rorer, Vitry, France. The MICs of the quinolones were determined on BHI agar plates by using a Steers replicator device that delivered ca. 10^4 bacteria per spot. MICs were read after 18 h of incubation at 37°C.

Preparation of chromosomal and plasmid DNAs. Chromosomal DNA was prepared as described by Maniatis et al. (20). Plasmid DNA was prepared by the alkaline lysis method of Birnboim and Doly (3).

Amplification of chromosomal DNA from *E. coli* Q2 by PCR. Two fragments of *gyrA* and one fragment of *gyrB* were amplified from the chromosomal DNA of *E. coli* Q2 by the polymerase chain reaction (PCR). The different sets of primers used for amplification are described in Table 2, along with the lengths of the amplified gene fragments. Oligonucleotides were synthesized by Bioprobe System, Montreuil-sous-bois, France. Plasmid DNAs (pRM386 or pJB11) were used as controls. PCR was carried out with *Taq* polymerase from Boehringer (Mannheim, Federal Republic of Germany) as recommended by the manufacturer. Each reaction mixture contained 2.5 U of *Taq* polymerase per 100-μl reaction mixture, 50 ng of DNA template, Tris · HCl (10 mM; pH 8), KCl (50 mM), $MgCl_2$ (1.5 mM), gelatin (0.1%), 200 μM (each) dATP, dCTP, dGTP, and dTTP, and the primers at approximately 0.5 μM each. The reaction mixtures were incubated for 40 cycles in a programmable heat block (Techne Instruments, Ltd.) for 1 min at 93°C, 1 min at the annealing temperature (55°C), and 2 min at 72°C.

Construction of hybrid plasmids and complementation tests. After purification, the amplified 668-bp *gyrA* fragment (PCR product 1; Table 2) was digested with *Sst*I and *Hin*dIII and the amplified 2,500-bp *gyrA* fragment (PCR product 2; Table 2) was digested with *Sst*I and *Sst*II. These fragments, which were amplified from the chromosome of *E. coli* Q2, were used to replace the corresponding fragments of the wild-type *gyrA* gene present in pRM386, which generated plasmids pAFF803 and pAFF805, respectively. To allow complementation in *E. coli* Q2, which was resistant to ampicillin, the *aphA-1* gene from Tn*903* (25) coding for kanamycin resistance was inserted into pRM386, pAFF803, pAFF805, and pJB11. The small *Pvu*II-*Pvu*II fragment of pAZ13 was isolated from the agarose gel and ligated into the *Sca*I site of the thus inactivated ampicillin resistance gene (bla_{Tem-1}) present in the plasmids. The kanamycin resistance plasmids derived

TABLE 2. PCR products

PCR product	Gene	Amplified fragment length (bp)	Sequence of primer I (5′ → 3′)[a]	Sequence of primer II (5′ → 3′)[a]
1	*gyrA*	668 (40 to 707[b])	GAGGAAGAGCTGAAGAGCTCCT *Sst*I	CCGGTACGGTAAGCTTCTTCAA *Hin*dIII
2	*gyrA*	2,500 (40 to 2,578)	GAGGAAGAGCTGAAGAGCTCCT *Sst*I	CATCGTCACCTTCCGCGGCACT *Sst*II
3	*gyrB*	435 (952 to 1,386)	GGCCTGATTGAGCTCGTTTCCG *Sst*I	GAGAAGCTTATCGAGGCGCGCT *Hin*dIII

[a] The restriction sites chosen for cloning are underlined. All primers were 22-mers.
[b] Values in parentheses are the positions in the gene of the first and last nucleotides from the amplified fragment.

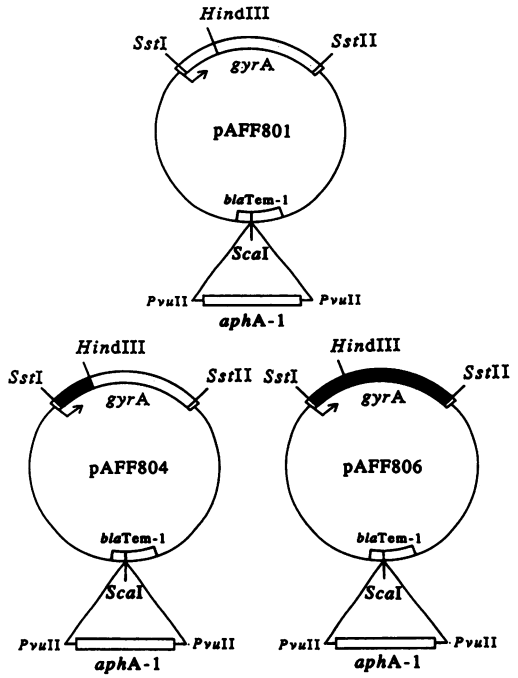

FIG. 1. Construction of plasmids from pRM386. Insertion of the kanamycin resistance gene (*aphA-1* present in the *Pvu*II-*Pvu*II fragment of pAZ13) into the *Sca*I site of the β-lactamase gene (bla_{Tem-1}) generated plasmids pAFF801, pAFF804, and pAFF806 derived from plasmids pRM386, pAFF803, and pAFF805, respectively. The heavy bars indicate the *gyrA* fragments of *E. coli* Q2.

from those described above were named pAFF801, pAFF804, pAFF806, and pAFF811, respectively. pAFF802, a derivative of pRM386 which expressed ceftazidime resistance instead of ampicillin resistance because of a point mutation in the β-lactamase gene, was obtained as described previously (6). Transformation was carried out as described previously (20).

Sequencing. The amplified *Sst*I-*Hin*dIII *gyrA* fragments (668 bp) and the *Sst*I-*Hin*dIII *gyrB* fragments (435 bp) from *E. coli* Q2 and *E. coli* K-12 were ligated into M13mp18 and M13mp19 (22) and introduced into *E. coli* JM101 by transformation. DNA sequencing was carried out by the dideoxy chain termination method of Sanger et al. (28).

RESULTS

Complementation tests and construction of chimeric *gyrA* genes. Since it was previously shown (23) that modification of DNA gyrase might in part explain the resistance pattern of *E. coli* Q2, and since it is now well established that most of the mutations leading to high-level quinolone resistance occur in the subunit A of DNA gyrase, we first complemented *E. coli* Q2 by transformation with the wild-type *gyrA* gene of *E. coli* K-12 carried on the kanamycin resistance plasmid pAFF801 (Fig. 1). As shown in Table 3, complementation with the wild-type *gyrA* gene which is known to be dominant over the *gyrA* gene for quinolone resistance (15) resulted in 16- to 128-fold decreases in the MICs of oxolinic acid, cinoxacin, and the newer fluoroquinolones but caused a 4-fold increase in the MIC of nalidixic acid and no change in the MIC of flumequine. These results suggested that at least one mutation occurred in the *gyrA* gene of *E. coli* Q2. To confirm this hypothesis and to determine the position of the mutation(s), chimeric genes were constructed.

Two amplified fragments generated from the chromosomal *gyrA* gene of *E. coli* Q2 were substituted for the homologous fragments of the wild-type *gyrA* gene present on pRM386 (27). The first fragment was 668 bp in length (PCR product 1; Table 2) and contained the so-called quinolone resistance-determining *gyrA* region (36), where most of the point mutations involved in quinolone resistance have been mapped. Substitution of the homologous fragment by the 668-bp fragment in pRM386 and insertion of the *aphA-1* gene at the *Sca*I site resulted in plasmid pAFF804 (Fig. 1). The second amplified fragment was 2,500 bp in length (PCR product 2), which covers all of the *gyrA* gene except 65 bp. Substitution of the homologous fragment by the 2,500-bp fragment in pRM386 and insertion of the *aphA-1* gene at the *Sca*I site resulted in plasmid pAFF806 (Fig. 1). Complementation of *E. coli* Q2 with pAFF804 or pAFF806 resulted in no change in the MIC of any quinolone (Table 3). If the

TABLE 3. MICs of quinolones for *E. coli* strains harboring plasmids carrying different *gyrA* alleles[a]

E. coli strain	MIC (μg/ml)										
	Nal	Flu	Ofl	Pef	Nor	Oxo	Cin	Tos	Tem	Spa	Cip
Q1	2	1	0.06	0.125	0.06	1	4	0.03	0.06	0.03	0.015
Q2	4	2	16	16	8	16	1,024	8	4	8	8
Q2(pAFF801)[b]	16	2	0.5	0.5	0.5	1	16	0.125	0.25	0.06	0.125
Q2(pAFF804)	4	2	16	16	8	16	1,024	16	8	8	8
Q2(pAFF806)	4	2	16	16	8	16	1,024	16	8	8	8
Q2(pAFF811)	8	4	8	4	2	16	1,024	4	4	2	2
Q2(pAFF802-pAFF811)	4	ND[c]	ND	0.5	ND	ND	ND	ND	ND	ND	0.125
KNK453	4	1	0.125	0.25	0.125	0.5	8	0.06	0.125	0.03	0.06
KNK453(pRM386)	8	2	0.125	0.5	0.125	0.5	8	0.06	0.125	0.06	0.06
KNK453(pAFF803)	16	4	1	2	0.5	4	64	0.25	0.5	0.25	0.25
KNK453(pAFF805)	16	4	1	2	0.5	4	64	0.125	0.5	0.25	0.25
DH5α	32	1	0.03	0.125	0.03	0.5	8	0.015	0.007	0.007	0.007
DH5α(pAFF801)	2	0.25	0.015	0.007	0.03	0.125	1	0.015	0.007	0.007	0.007
DH5α(pAFF804)	2	1	0.06	0.125	0.03	1	8	0.03	0.03	0.03	0.015

[a] MICs were determined on BHI agar plates containing 100 μg of kanamycin per ml. Nal, nalidixic acid; Flu, flumequine; Ofl, ofloxacin; Pef, pefloxacin; Nor, norfloxacin; Oxo, oxolinic acid; Cin, cinoxacin; Tos, tosufloxacin; Tem, temafloxacin; Spa, sparfloxacin; Cip, ciprofloxacin.

[b] Identical MICs were obtained in the presence of pAFF802 (see Table 1).

[c] ND, not done.

resistance mutation(s) present in the *gyrA* gene of strain Q2 was not included in the small or the large fragment cloned in the hybrid plasmids, the chimeric genes so constructed should be dominant over the chromosomal *gyrA* gene of Q2 and should make the strain susceptible to fluoroquinolones. Because there was no change in the MIC of any quinolone after transformation of Q2 with the hybrid plasmid pAFF804, we concluded that the small fragment contained the essential mutation present in the *gyrA* gene of *E. coli* Q2 and that no additional mutation interfering with resistance was carried by the larger fragment.

To assess the effect of the hybrid plasmids containing the small or the large fragment generated from the *gyrA* gene of strain Q2 on quinolone resistance in a background in which the wild-type *gyrA* gene of the recipient strain would not be dominant, the chimeric plasmids pAFF803 and pAFF805 were transferred into *E. coli* KNK453 (*gyrA* temperature sensitive) (Table 3). Compared with the nontransformed strain, for *E. coli* KNK453 transformed with pAFF803 or pAFF805, the MICs of the newer quinolones like ofloxacin, pefloxacin, and ciprofloxacin increased eightfold and the MICs of nalidixic acid and flumequine increased only fourfold. Moreover, the increase in the MICs of the latter compounds was only twofold when compared with that for KNK453 harboring plasmid pAFF801 or pRM386, which contain the wild-type *gyrA* gene. The MICs of the quinolones for the transformed strain KNK453 were the same at 37 and 42°C. A similar observation was made previously (10, 38) and suggested that this mutant of the *gyrA* gene of KNK453 is not only temperature sensitive but also has a less functional gyrase A protein whose expression appears to be recessive (19). The MICs of the quinolones for KNK453 transformed with pAFF803 or pAFF805 were identical. The similar change in MICs obtained for KNK453 transformed with the two hybrid plasmids suggested that the putative mutation(s) was present in the 668-bp fragment.

Nucleotide sequence analysis. Nucleotide sequencing of the 668-bp fragment revealed only one transition (G to A) at nucleotide 242, leading to the replacement of the glycine at position 81 in the wild-type gyrase A protein by aspartic acid in the altered gyrase A protein. This mutation has not been described previously and results in the substitution of a negatively charged amino acid for a neutral one. This would be expected to change the secondary and probably the tertiary structures of the protein.

Complementation by the *gyrB* gene. To assess whether a *gyrB* mutation was associated with the *gyrA* mutation in *E. coli* Q2, this strain was transformed with pAFF811 carrying the wild-type *gyrB* gene (Table 3). While no significant changes in the MICs of nalidixic acid and flumequine were observed, two- to fourfold decreases in the MICs of some of the other quinolones were found. To assess whether these results were only due to a gene dosage effect or whether there was actually a mutation in the *gyrB* gene, three types of experiments were performed. First, the MICs of novobiocin were determined and were found not to be different for *E. coli* Q1 and *E. coli* Q2 (data not shown). Second, a fragment of 435 bp (nucleotides 952 to 1,386) of the *gyrB* gene that is known to contain the sites where two different mutations of *gyrB* associated with quinolone resistance have been localized (34, 35, 37) was amplified from the chromosome of *E. coli* Q2 by PCR (PCR product 3; Table 2). The nucleotide sequence of the 435-bp fragment of *gyrB* of *E. coli* Q2 was identical to that of *gyrB* of *E. coli* KL16 cloned into pJB11. Third, to assess whether a mutation outside of this fragment was involved, both the wild-type *gyrA* and *gyrB* genes were introduced into *E. coli* Q2 by successive transformation with pAFF811 (kanamycin resistant) and pAFF802 (ceftazidime resistant), respectively. The presence of the two plasmids in *E. coli* Q2 was verified by the extraction of the plasmids, retransformation of *E. coli* DH5α, and selection of separate clones expressing resistance to kanamycin or ceftazidime. The presence of the two wild-type genes did not change the MICs of the quinolones for *E. coli* Q2 compared with those for *E. coli* Q2 containing the wild-type *gyrA* gene alone. This suggested to us that no mutation was present in the *gyrB* gene of *E. coli* Q2.

Finally *E. coli* DH5α, which is resistant to nalidixic acid (MIC, 32 μg/ml) and which has an unspecified mutation in *gyrA*, when transformed with pAFF804 became susceptible to nalidixic acid (MIC, 2 μg/ml) and remained susceptible to the fluoroquinolones. This would indicate that the susceptible character of the gyrase is dominant, independently of whether its gene is chromosome or plasmid borne.

DISCUSSION

A modification of the DNA gyrase was thought to play a major role in the particular phenotype of acquired quinolone resistance in the clinical strain *E. coli* Q2 (23). We therefore searched for possible mutations of the *gyrA* and *gyrB* genes. The PCR technique was used to amplify intragenic fragments of the two genes that are known to comprise the quinolone resistance-determining regions (36, 37), and the nucleotide sequences of these fragments determined. Such a strategy has previously been used to study quinolone resistance in *E. coli* (26) and *Staphylococcus aureus* (12). In *E. coli* Q2, it led to the demonstration that a transition of G to A at position 242 of the *gyrA* gene caused the substitution of Asp for Gly-81. The construction of chimeric genes and their expression in a temperature-sensitive *gyrA* strain helped us to ascertain that the G-to-A mutation at position 242 was responsible for the particular pattern of quinolone resistance that we observed. The introduction of the wild-type *gyrA* gene into *E. coli* Q2 reversed the phenotype; however, the MICs of the fluoroquinolones remained four- to eightfold greater than those of the susceptible strain *E. coli* Q1. This probably resulted from a decrease in uptake of these compounds, which has been observed in strain Q2 (23). Similarly, introduction of the hybrid plasmid pAFF803 harboring the mutated *gyrA* gene of Q2 into the temperature-sensitive *gyrA* strain *E. coli* KNK453 increased the MICs of newer quinolones 8-fold, although their levels remained 16-fold lower than those observed for strain Q2. The reason for this discrepancy is not known, but it might be explained by the decreased uptake of the newer fluoroquinolones but not of nalidixic acid and flumequine into Q2 mentioned above (23). In this strain, the high MICs of the newer fluoroquinolones conferred by the mutation described in *gyrA* appeared to be enhanced by the reduced uptake of these compounds in strain Q2.

Until now, 10 point mutations (in 18 strains) leading to eight amino acid substitutions related to quinolone resistance have been described in the *E. coli gyrA* gene (10, 13, 26, 36, 38). These mutations are all located within a 130-bp fragment of the *gyrA* gene called the quinolone resistance-determining region, which is centered around codon 83 (36). Thus, it is not surprising that the novel mutation in strain Q2 was located in the same region. The most frequent amino acid substitutions found in the mutants of *E. coli* described previously are those of Trp or Leu for Ser-83 (10, 26, 38). One point mutation at the same position as that described in

this work has been reported previously (36), with a substitution of Cys for Gly-81, both amino acids being neutral. This change resulted in an 8-fold increase in the MICs of the newer fluoroquinolones and, in contrast to what we observed for strain Q2, produced a 16-fold increase in the MIC of nalidixic acid. In *E. coli* and other species, quinolone resistance mutations (10, 12, 13, 26, 31, 36, 38) occurred in the highly conserved quinolone resistance-determining domain of the gyrase A subunit (7, 17, 21, 24), suggesting that this domain has an important role in the interaction between quinolones and gyrase. However, until now no model has been presented in which this domain, which is very close to Tyr-122, the DNA attachment site of subunit A (18), would function as a binding site for certain quinolones. The mutation described in this report would lend some support to that hypothesis. The difference between the substitution of Asp-81 for Gly and the mutations described previously is that it confers a different resistance pattern, i.e., resistance to fluoroquinolones but not to nalidixic acid and flumequine. The result of this substitution is the replacement of a neutral amino acid by a negatively charged amino acid and the juxtaposition of two Asp residues (81 and 82) in the vicinity of Ser-83, which seems to play a major role in the quinolone-gyrase interaction. When attempting to relate the structures of quinolones and the resistance phenotype of *E. coli* Q2 (or that conferred by the chimeric *gyrA* gene constructed with the G-to-A mutation at position 242), it appears that two molecules, nalidixic acid and flumequine, are in a category apart from the other agents. The main structural difference is the absence of a cyclic substituent at position C-7 of the two compounds (5). Shen and colleagues (29, 30) suggested that quinolones bind first to DNA and that the substituents at positions C-6 and C-7 then help to stabilize the quinolone-DNA complex, while the piperazine moiety at position C-7 possibly interacts with gyrase. It is then conceivable that a modification of gyrase, such as that observed in strain Q2, could create a steric obstruction and decrease the binding of molecules with a bulky group at position C-7, while no change in binding would occur with molecules like nalidixic acid and flumequine, which have a linear substituent at that position.

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