ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1997, p. 175–179 Vol. 41, No. 1
0066-4804/97/$04.00+0

Quinolone Resistance Locus *nfxD* of *Escherichia coli* Is a Mutant Allele of the *parE* Gene Encoding a Subunit of Topoisomerase IV

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Received 8 August 1996/Returned for modification 30 September 1996/Accepted 29 October 1996

The locus *nfxD*, which contributes to high-level quinolone resistance in *Escherichia coli* KF111b (*gyrA*^{r} *nfxB nfxD*), is only expressed in the presence of a *gyrA* mutation, and maps to the region of the *parC* and *parE* genes, was outcrossed into strain KF130, creating strain DH161 (*gyrA*^{r} *nfxD*). DNA sequence analysis of DH161 revealed no changes in the topoisomerase IV *parC* quinolone resistance-determining region but did identify a single T-to-A mutation in *parE* at codon 445, leading to a change from Leu to His. Full-length cloned *parE*$^+$ partially complemented the resistance phenotype in KF111b and DH161, but did not complement the resistance phenotype in strain KF130 (*gyrA*^{r}). No complementation was seen with cloned, truncated *parE*$^+$. To confirm these findings, *gyrA*^{r} was first outcrossed from KF130 into *E. coli* W3110parE10 [*parE* temperature sensitive(Ts)] and KL16. The transduced strains KL16 and W3110parE10 were subsequently transformed with plasmids containing cloned *parE* from DH161 or KL16. Cloned *parE* from DH161 increased norfloxacin resistance in the *parE*(Ts) background twofold at 30°C and fourfold at 42°C compared to those for cloned *parE* from KL16. The same experiment with a non-Ts background revealed a twofold increase in the norfloxacin MIC at both 30 and 42°C. These data identify the *nfxD* conditional resistance locus as a mutant allele of *parE*. This report is the first of a quinolone-resistant *parE* mutant and confirms the role of topoisomerase IV as a secondary target of norfloxacin in *E. coli*.

DNA gyrase, a type 2 topoisomerase, is composed of two subunits encoded by *gyrA* and *gyrB* and functions in *Escherichia coli* in the maintenance of chromosomal superhelical tension via the introduction of negative supercoils (15, 21). Fluoroquinolones target DNA gyrase by locking the enzyme in a cleavable complex with DNA (23), ultimately leading to cell death (3). Single-step mutations in DNA gyrase effect a 100-fold increase in the MICs of older quinolones such as nalidixic acid or 16- to 32-fold increases in the MICs of newer quinolones such as norfloxacin, ciprofloxacin, and ofloxacin (8). Serial passage in media containing increasing concentrations of quinolones can select for resistant strains with multiple mutations and for which MICs are even higher.

Topoisomerase IV, an essential enzyme of *E. coli*, the primary function of which is chromosomal segregation following DNA replication, is composed of two subunits encoded by *parC* and *parE*, which are homologous to *gyrA* and *gyrB* of DNA gyrase, respectively (12). Mutations in *parC* at positions similar to those seen in the quinolone resistance-determining region of *gyrA* (28, 30) have been found in highly resistant strains, but interestingly, they only appear in the presence of a primary *gyrA* mutation (7, 14, 16, 26).

E. coli KL16 was serially passaged on norfloxacin, and strain KF111b was isolated. The MIC for strain KF111b is 125-fold greater than that for the parent strain (10). It was determined that KF111b had, in addition to the expected *gyrA* mutation, at least two other resistance loci, termed *nfxB* and *nfxD*. Previous work in this laboratory has shown *nfxB* to be associated with reduced *ompF* expression and reductions in cellular drug accumulation (9), while *nfxD* mapped to the region near the *parC* and *parE* genes and was only conditionally expressed in resistant *gyrA* mutants (25). Wild-type *gyrA*, cloned on a plasmid, fully complemented the resistance phenotype of a *gyrA*^{r} *nfxD* double mutant (25).

In this study, quinolone resistance above that attributable to *gyrA* and associated with the *nfxD* locus was determined to be caused by a single point mutation in the coding sequence of topoisomerase IV *parE*. Complementation was only partial in the presence of wild-type *parE*$^+$, indicating incomplete dominance over *parE*^{r}. These findings were confirmed by outcrossing *gyrA*^{r} into a temperature-sensitive (Ts) *parE* background and augmenting resistance with *parE*^{r} cloned on a plasmid. Our results support topoisomerase IV as a direct but secondary target of current quinolone antimicrobial agents in *E. coli.*

MATERIALS AND METHODS

Bacterial strains, media, growth conditions, DNA purification, and plasmids. The *E. coli* strains and plasmids used in the study and their sources are described in Table 1. Bacterial cells were grown either in Luria broth (LB) or on LB supplemented with agar. Transformed cells were always grown in the presence of ampicillin (AMP; Sigma) at 100 μg/ml. Ts strains were grown at 30°C (permissive) or 42°C (nonpermissive). All other strains were grown at 37°C. Plasmid DNA was isolated by either the Wizard Minipreps DNA Purification System (Promega), Plasmid Midi (Qiagen), or Plasmid Maxi (Qiagen) protocols.

Determination of MICs. The MICs for plasmid complementation assays were determined by the gradient plate method. Cells were grown overnight in Mueller-Hinton (MH) broth (BBL) and were diluted to approximately 2×10^7 CFU/ml in MH broth. Gradient plates were constructed by using a bottom layer of MH agar containing AMP (100 μg/ml) and norfloxacin (Sigma) at 0, 2.5, 5, or 10 μg/ml (depending on the strain to be tested) and a top layer of MH agar containing AMP (100 μg/ml). Diluted cells were then streaked onto the surface with a sterile cotton swab. MICs were determined by multiplying the ratio of the

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

Strain or plasmid	Genotype or characteristic	Source or reference
Strains		
KL16	Hfr *thi-1 relA spoT1* λ^-	B. Bachmann
KF111b	KL16 *gyrA nfxB nfxD*	10
DH161	KL16 *gyrA nfxD*	25
KF130	KL16 *gyrA*	10
DB130	KF130 *gyrA zei-723*::Tn*10*	This study
RM3691	*gyrA zei-723*::Tn*10*	R. Maurer
W3110parE10	*parE*(Ts)	13
EJ812	C600*parC*1215(Ts)	12
DB230-1	W3110parE10 *gyrA zei-723*::Tn*10*	This study
DB232-6	KL16 *gyrA zei-723*::Tn*10*	This study
Plasmids		
pEN160	*parE*, KL16	This study
pEN161	*parE*, DH161	This study
pEN260	*parC*, KL16	This study
pEN261	*parC*, DH161	This study
pEN262	*parC*, KF111b	This study
pET3c-parE	$parE^+$	20
pET3c-parEt	*parE*, truncated	20
pET3c-parC	$parC^+$	20
pET3c-parCt	*parC*, truncated	20

total distance of growth (in millimeters from 0 μg/ml) to plate length by the maximum concentration of norfloxacin per plate. All plates were freshly prepared, and assays were performed on 3 separate days. Other MICs were determined by the agar dilution method with L agar supplemented with AMP at 100 μg/ml (10).

Transformation and transduction. Transformation of cells used in the gradient plate analysis was performed by the method of Chung et al. (2), with the exception that the culture medium was supplemented with $MgSO_4$ (20 mM). Other transformations were performed by the method of electroporation. Approximately 5 μg of plasmid DNA was added to 150 μl of cells (made electrocompetent following the manufacturer's protocol; Bio-Rad) in a 0.2-cm Gene Pulser cuvette (Bio-Rad). Electrotransformation was performed in a Gene Pulser (Bio-Rad) set at 25 μF, 247 kV, and 200 Ω. Selection by both methods was on L agar supplemented with AMP (100 μg/ml). P1*cmclr* (Table 1) transduction was performed by the method of Silhavy et al. (24), with slight modification. Cells were grown in LB supplemented with 10 mM $MgSO_4$ and 10 mM $CaCl_2$. Selection for transposon Tn*10* was performed in the presence of tetracycline (TET; 30 μg/ml) at 30°C.

Construction of strains DB230-1 and DB232-6. DNA from *E. coli* RM3691 (Table 1) was first transduced into KF130 by using bacteriophage P1*cmclr*. Transductants (DB130) were selected on TET and were screened for norfloxacin resistance and the ability to grow at 42°C. We found that 18 of 23 transductants were resistant to norfloxacin and TET and able to grow at 42°C, indicating 78% linkage of *gyrA* to transposon *zei-723*::Tn*10*. P1*cmclr* was grown on DB130, and lysogeny was selected by chloramphenicol. A P1*cmclr* lysate of DB130 was used to transduce strains W3110parE10 and KL16, creating strains DB230-1 and DB232-6, respectively. Transductants were selected on TET and were screened for quinolone resistance and temperature sensitivity.

PCR. For each allele of *parE* and *parC*, chromosomal DNA was prepared from strain KL16, DH161, or KF111b as described previously (25). To clone *parE*, DNA was subjected to PCR with a 29-mer, 5′-GTGAATTCCGATCGTCGATTTTCTTGGTC, and a 35-mer, 5′-GTGAATTCCCTTTAAACCTCAATCTCCGCCATGTC, both of which contain an *Eco*RI restriction site engineered into the 5′ end. To clone *parC*, DNA was subjected to PCR with a 35-mer, 5′-GTGGATCCATGAGCGATATGGCAGAGCGCCTTGCG, and a 29-mer, 5′-CAGGATCCTTACTCTTCGCTATCACCGCT, both of which contain a *Bam*HI restriction site engineered into the 5′ end. Reactions were performed in a DNA thermal cycler with 2.0 U of Vent DNA polymerase (New England Biolabs), 1× Vent DNA buffer (New England Biolabs), 2 mM $MgSO_4$, 250 μM (each) deoxynucleotide triphosphates, 10 μl of 200 nM stock (each) oligonucleotide primer, and the bacterial DNA (final volume, 100 μl). Twenty-nine cycles with the following temperature profiles were used for both reactions: 94°C for 2 min and 30 s, 94°C for 1 min, 70°C for 45 s, and 75°C for 2 min and 20 s. PCR products were either digested with *Eco*RI or *Bam*HI and ligated into similarly digested pGEM7-zf(+).

DNA sequence analysis. The entire KL16 and DH161 *parE* alleles were independently sequenced from plasmids pEN160 and pEN161, respectively, by using the ABI Fluorescent System and *Taq* Dye terminators (Qiagen). The following primers for *parE*, based on the sequence determined by Kato et al. (12) and Peng and Marians (20), were used: in the forward reading frame, 5′-GTGAATTCCGATCGTCGATTTTCTTGGTC, 5′-GTACCGGCGGTTGAACTGAT, 5′-ATTGTGCCCTGGCGTTGAGA, 5′-TGTACCGCGCAGGACCTTAA, 5′-CATTTCCGCGCGTTGGTGAA, 5′-ATGCAGGGCGGTACCCATGTTAA, and 5′-TTGCCGGGCAGACGAAAGA, and in the reverse reading frame, 5′-CTCTCCGGATCCAAGCTTA (pEN161 only), 5′-GTCGCATGCTCCTCTAGA (pEN160 only), 5′-TCCGTCAGCGCGTAATA, 5′-TAAGGTCCTGCGCGGTACAAT, 5′-ATGGGTACCGCCCTGCATCGTT, 5′-TTCATCCGGCCAGAAGT, and 5′-ATGCAGGCCGCCAGAGAA. Sequencing of *parC* was performed by the method of Sanger et al. (22) with cloned *parC* on plasmids pEN260, pEN261, and pEN262 and the following primers: 5′-GTATGCGATGTCTGAACT and 5′-AAGCCACATTGCGTAACG.

RESULTS

Cloning and sequencing of *parC* and *parE* alleles. Chromosomal DNA from *E. coli* KL16, DH161, and KF111b was extracted, and *parC* was amplified by PCR as described in Materials and Methods. In all three cases a band corresponding to 2.2 kb was cut with *Bam*HI and was cloned into pGEM7-zf(+), creating plasmids pEN260, pEN261, and pEN262, respectively (Table 1). The putative quinolone resistance-determining region of each of the three *parC* alleles, corresponding to amino acids 59 through 107, was sequenced. The *parC* sequence of KL16 was identical to those published previously (12, 20), and the *parC* sequences of DH161 and KF111b were also identical to that of KL16 (data not shown).

Because the *nfxD* locus maps on the *E. coli* chromosome in the region of the linked *parC* and *parE* genes, we next cloned and sequenced *parE* from KL16 and DH161. Chromosomal DNAs from both of these strains were extracted, and *parE* was amplified by PCR as described in Materials and Methods. In both cases, the amplified product was a single 1.9-kb band with engineered *Eco*RI sites at each end. *parE* was cloned into the *Eco*RI site of pGEM7-zf(+), creating plasmids pEN160 (KL16) and pEN161 (DH161) (Table 1). The sequences of both strands of the entire *parE* gene plus approximately 50 bp upstream of *parE* from pEN160 and pEN161 were determined. The sequences generated were compared to each other and the published *parE* sequence of *E. coli* W3110 (12, 20). The *parE* sequence of KL16 was identical to that of the published *parE* sequence (data not shown). When comparing *parE* of DH161 to that of KL16, a single T-to-A change was detected, resulting in the replacement of a leucine with a histidine at codon 445 (Fig. 1).

Complementation of norfloxacin resistance. To determine the relationship of the *parE* mutation found in the *nfxD* mutant to the resistance conferred by the *nfxD* locus, we performed complementation experiments (Table 2). *nfxD*-containing and control strains were transformed with a plasmid containing the full-length $parE^+$ gene (pET3c-parE). As controls, strains were

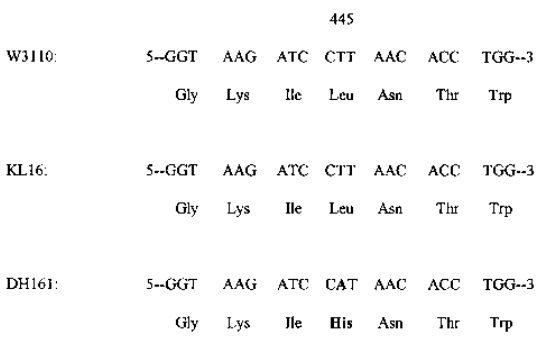

FIG. 1. Partial nucleotide sequences of cloned *parE* from KL16 and DH161 compared to that of W3110 *parE* (12, 20). The single amino acid difference at codon 445 is indicated in boldface.

TABLE 2. Complementation of norfloxacin resistance in *E. coli* KL16, KF130, KF111b, and DH161 via transformation by plasmids containing native or truncated *parC* or *parE*

Strain and plasmid	Norfloxacin gradient (μg/ml)	Maximum growth (mm)	Norfloxacin MIC (μg/ml)
KL16			
pET3c-parC	0–0.6	30.0 ± 0.0	0.2
pET3c-parCt	0–0.6	30.0 ± 0.0	0.2
pET3c-parE	0–0.6	30.0 ± 0.0	0.2
pET3c-parEt	0–0.6	30.0 ± 0.0	0.2
KF130			
pET3c-parC	0–2.5	43.8 ± 2.5	1.2
pET3c-parCt	0–2.5	55.5 ± 1.7	1.5
pET3c-parE	0–2.5	36.3 ± 3.0	1.0
pET3c-parEt	0–2.5	59.3 ± 2.6	1.6
KF111b			
pET3c-parC	0–10.0	75.0 ± 10.0	8.3
pET3c-parCt	0–10.0	87.5 ± 2.5	9.7
pET3c-parE	0–10.0	31.3 ± 1.6	3.5
pET3c-parEt	0–10.0	81.3 ± 5.4	9.0
DH161			
pET3c-parC	0–10.0	38.5 ± 5.5	4.3
pET3c-parCt	0–10.0	40.5 ± 4.7	4.5
pET3c-parE	0–10.0	19.8 ± 4.7	2.2
pET3c-parEt	0–10.0	41.5 ± 3.8	4.6

also transformed with plasmids containing a truncated (pET3c-parEt) *parE*$^+$ and full-length (pET3c-parC) and truncated (pET3c-parCt) *parC*$^+$ genes. For wild-type strain KL16, there were no differences in the MIC of norfloxacin among transformants containing any of the four plasmids. In contrast, MICs for KF111b and DH161, *nfxD gyrA* mutants containing the *parE*(Leu445His) allele, were reduced 2.6- and 2.1-fold, respectively, when transformants with pET3c-parE were compared with transformants with pET3c-parEt. There was, however, also a smaller (1.6-fold) but reproducible reduction in the MIC of norfloxacin when the pET3c-parE transformant of KF130 *gyrA* was compared with the pET3c-parEt transformant of the same strain, suggesting that the effect of pET3c-parE was not completely specific for the *nfxD* mutants. The pET3c-parC plasmids had even less of an effect on the MIC of norfloxacin for the mutant strains. Plasmid pET3c-parEt or pET3c-parE was used to transform *E. coli* W3110parE10 [*parE*(Ts)], and plasmid pET3-parCt or pET3c-parC was used to transform *E. coli* EJ812 [*parC*(Ts)]. We confirmed that pET3c-parE and pET3c-parC complemented the temperature sensitivities of W3110parE10 and EJ812, respectively (data not shown), and thus were expressing their respective functional subunits. In contrast, pET3c-parEt did not complement the growth of W3110parE10 at 42°C, indicating that it was a suitable control plasmid for comparison with pET3c-parE in the resistance complementation experiments.

Contribution of *parE*(Leu445His) to quinolone resistance. Because of possible nonspecific effects in the *parE*$^+$ complementation experiments, we attempted to define further the role of *parE*(Leu445His) in the resistance of *nfxD* mutants by demonstrating the ability of this *parE* allele to confer resistance in a *parE*(Ts) mutant. Because *nfxD* resistance is expressed only in a *gyrA*^{r} background, it was first necessary to construct strain DB230-1 *gyrA*^{r} *parE*(Ts) as described in Materials and Methods. The *parE*$^+$ gene was cloned from strain KL16(pEN160), and the *parE*(Leu445His) allele was cloned from DH161(pEN161) in plasmid pGEM7-zf(+). Each of these plasmids and the vector plasmid alone were used to transform DB230-1 [*gyrA*^{r} *parE*(Ts)], DB232-6 (*gyrA*^{r} *parE*$^+$), W3110parE10 [*gyrA parE*(Ts)], and KL16 (*gyrA*$^+$ *parE*$^+$), and transformants were tested for their resistance to norfloxacin at permissive and nonpermissive temperatures (Table 3). At the permissive temperature, pEN161 caused a twofold increase in the norfloxacin MIC for DB230-1 [*gyrA parE*(Ts)]. At the nonpermissive temperature, however, there was a fourfold increase in the MIC of norfloxacin for a pEN161 transformant relative to that for the pEN160 transformant of DB230-1. Furthermore, both plasmids complemented the thermosensitive phenotype of the *parE*(Ts) strains DB230-1 and W3110parE10, but only in DB230-1 did pEN161 cause an increase in resistance, consistent with the conditional expression of the *nfxD* resistance phenotype previously reported. Interestingly, there was also an apparent slight effect of pEN161 in DB232-6, increasing resistance twofold at both temperatures. For all strains, growth at the higher temperature was associated with a two- to fourfold increase in the norfloxacin MIC.

DISCUSSION

E. coli KF111b was selected for quinolone resistance on increasing concentrations of norfloxacin, and the MIC for strain KF111b was 125-fold greater than that for the parent strain, KL16 (10). Previous analysis of KF111b in this laboratory revealed mutations in *gyrA* and *nfxB*, a locus affecting expression of OmpF, which together accounted for only a portion of the total resistance profile of KF111b (9, 10). A third resistance locus was subsequently mapped to 65 min on the *E. coli* chromosome by linkage to *zgh-3075*::Tn*10* and was termed *nfxD* (25). The *parC* and *parE* genes encoding topoisomerase IV also map in this region of the *E. coli* chromosome (12), and thus, *nfxD* was considered to be a possible mutant allele of *parC* or *parE*. *nfxD* was the first quinolone resistance locus to be found to be conditionally expressed in *gyrA* mutants. Subsequently, several groups have identified mutations in *parC* in strains with *gyrA*^{r} mutations (7, 14, 16). *parC* resistance mutations were also found to be expressed only in the presence of *gyrA*^{r} mutations (14). Our sequence analysis of cloned *parC* from DH161 and KF111b included the region

TABLE 3. MICs for *E. coli* DB230-1, DB232-6, W3110parE10, and KL16 transformed by plasmids pEN160, pEN161 or pGEM7-zf(+)

Strain	Plasmid	Norfloxacin MIC (μg/ml)	
		30°C	42°C
DB230-1	pEN160	0.64	1.25
	pEN161	1.25	5.0
	pGEM7-zf(+)	1.25	NG[a]
DB232-6	pEN160	0.64	2.5
	pEN161	1.25	5.0
	pGEM7-zf(+)	0.64	2.5
W3110parE10	pEN160	0.08	0.16
	pEN161	0.08	0.16
	pGEM7-zf(+)	0.08	NG
KL16	pEN160	0.04	0.16
	pEN161	0.04	0.16
	pGEM7-zf(+)	0.04	0.16

[a] NG, no growth.

predicted to contain resistance mutations, based on analogy to *gyrA* (12, 20) and in which previously reported *parC* mutations were identified. No *parC* mutation was found, however. The entire *parE* gene from DH161 and KL16 was then sequenced, and a single change from T to A was detected in codon 445, encoding a change from Leu to His.

Several lines of evidence support the role of *parE* (Leu445His) in quinolone resistance in KF111b and DH161. First, *nfxD* was conditionally outcrossed from KF111b via *zgh-3075*::Tn*10* into KF130 (*gyrA*^{r}) [creating strain DH161 (*gyrA*^{r} *nfxD*)] but could not be outcrossed into KL16. Furthermore, cloned *gyrA*$^+$ fully complements norfloxacin resistance in DH161 (25). Our previous findings led us to the conclusion that *nfxD* is silent in the absence of a *gyrA*^{r} mutation, a finding which is consistent with that seen for *parC* quinolone resistance mutations (14). Second, only full-length *parE*$^+$, cloned on a plasmid, partially complemented *nfxD* in KF111b and DH161. A slight reduction was, however, also seen in the MICs for KF130 and DB230-1, but not that for KL16 transformed with full-length *parE*$^+$, compared to those for cells transformed with truncated *parE*. Thus, in the presence of *gyrA*^{r}, plasmid-encoded ParE may have effects on cellular growth or other factors that confound possible specific complementation of *parE*(Leu445His) by *parE*$^+$.

Third, to clarify the role of *parE*(Leu445His) in resistance, cloned *parE* from DH161 or KL16 was transformed into a *parE*(Ts) background with (in DB230-1) and without (in W3110parE10) a *gyrA*^{r} mutation. At the permissive temperature, a twofold increase in the MIC of norfloxacin was seen for strains with the DB230-1 and DB232-6 (*gyrA*^{r}) backgrounds with *parE*^{r} compared to the MICs for *parE*$^+$ or the plasmid vector control strains. At the nonpermissive temperature in the DB230-1 background, a further increase in the MIC of norfloxacin to fourfold was seen for strains with *parE*^{r} compared to that for strains with *parE*$^+$. These results are consistent with the codominance of the plasmid-encoded alleles when both resistant and susceptible alleles are fully functional. The absence of an effect of *parE*^{r} in strains W3110parE10 and KL16 at either temperature is further consistent with the dependence of resistance on a mutant *gyrA*^{r} gene, as was the resistance seen for the *nfxD* locus.

The Leu445His mutation that we have identified is in the region of ParE that is homologous to the region of GyrB in which quinolone resistance mutations have been found and which is thought to be a domain involved in the interactions between the GyrB and GyrA subunits (11, 17, 29). This region is highly conserved between the ParE and GyrB subunits. Of the 52 amino acids aligned between positions 395 and 446 of ParE and the homologous positions 401 and 452 of GyrB, 37 are identical and another 6 are conservatively substituted, representing together 83% of the amino acids in the region. This region is also one of those most highly conserved among ParE subunits of different species, including *Staphylococcus aureus* (4) and *Streptococcus pneumoniae* (19). Quinolone resistance mutations in *E. coli* GyrB have been found in the middle portion of this region at positions 426 (Asp is replaced by Asn) and 447 (Lys is replaced by Glu). A possible quinolone resistance mutation in *Salmonella typhimurium* GyrB has been described, but it is located outside the conserved region at position 463 (Ser is replaced by Tyr) (5). No changes in the residues in ParE (Asp420 and Lys441, respectively) homologous to those in GyrB (Asp426 and Lys 447) were found in the *nfxD* mutant. Our sequence alignment revealed an alanine at position 457 in ParE (unchanged in the *nfxD* mutant) which corresponded to Ser463 in GyrB. The amino acid at position 445 which is mutated in ParE^r is located at the C-terminal side of the conserved domain and is retained in GyrB as a wild-type Leu at position 451. Interestingly, the substitution of His for Leu at position 445 in ParE^r produces a change from a neutral to a positive charge. The electrostatic effects of a similar positive charge generated by the Asp426Asn resistance mutation in GyrB have been postulated to cause resistance by repulsion of the positively charged piperazinyl group of fluoroquinolones (29). Thus, our findings are consistent with such a model that has now been extended to resistance mediated by altered ParE. Recently, the crystal structure of part of yeast topoisomerase II has been determined, and Leu480 of this enzyme, which corresponds to Leu445 of ParE, is distant from the active-site tyrosine and the amino acids that are homologous to those in GyrA that, when mutated, cause quinolone resistance (1). Therefore, we must also consider more strongly other plausible models in which resistance is mediated by altered ParE or GyrB by conformational changes in the respective cognate subunits that secondarily alter the binding of fluoroquinolones to putative contact points on ParC or GyrA, which are in proximity to the active-site tyrosine (1). In either case, our findings provide further support for the notion that the sites and functional requirements for the interactions of quinolones with DNA gyrase and topoisomerase IV are similar.

The apparent codominance of the plasmid-encoded *parE*$^+$ or *parE*(His445) over their chromosomal counterparts in merodiploids is similar to that seen with multicopy alleles of either *parC*$^+$ or *parC*^{r} (14). These findings are in contrast to the dominance of *gyrA*$^+$ over both *gyrA*^{r} (6) and *parE*(His445) (25) and the dominance of *gyrB*$^+$ over *gyrB*^{r} (17). These differences imply that although the structural requirements for binding of quinolones to gyrase-DNA complexes or topoisomerase IV–DNA complexes may be similar, the consequences of these interactions differ. The finding of codominance of *parC*$^+$ and *parC*$^+$ based on gene dosage led others to postulate that DNA strand breaks generated by the interactions of quinolones with sensitive topoisomerase IV may be more readily repaired than those generated by the interaction of quinolones with DNA gyrase, because topoisomerase IV, in contrast to DNA gyrase, acts at a distance from the replication fork (14). This model then implies that movement of a replication fork through a topoisomerase-quinolone-generated DNA break generates a lethal or poorly repairable lesion or is necessary to trigger subsequent lethal events. Additional factors, however, may be involved in the interaction of quinolones with topoisomerase IV in other species. In *S. aureus*, in particular, these differences include the following: (i) topoisomerase IV is the principal quinolone target (4, 18, 27), (ii) the dominance of *grlA* (*parC*) by gene dosage requires the additional presence of plasmid-encoded *grlB* (*parE*) (27), and (iii) *grlA* (*parC*) single mutants are less effectively killed compared to the effectiveness with which *gyrA* single mutants are killed (18).

We conclude from these results that the norfloxacin resistance locus *nfxD* is a mutant allele of the *parE* gene of *E. coli* topoisomerase IV and that there is, like for *parC*, codominance of *parE* alleles encoded on a plasmid. In addition, these findings provide further support for the concept that topoisomerase IV is a secondary target of current quinolones in *E. coli.*

ACKNOWLEDGMENTS

We thank H. Peng and K. Marians for providing *parC*$^+$ and *parE*$^+$ plasmids and R. Maurer, J. Kato, and B. Bachmann for providing bacterial strains.

This work was supported in part by U.S. Public Health Service grant AI23988 from the National Institutes of Health.

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