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Use of a Broad-Host-Range *gyrA* Plasmid for Genetic Characterization of Fluoroquinolone-Resistant Gram-Negative Bacteria

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The *gyrA* genotypes of ciprofloxacin-resistant clinical isolates of *Escherichia coli* (*n* = 3), *Klebsiella pneumoniae* (*n* = 4), *Providencia stuartii* (*n* = 2), *Pseudomonas aeruginosa* (*n* = 1), and *Acinetobacter calcoaceticus* (*n* = 1) were analyzed in a dominance test. This test is based on the dominance of a wild-type *gyrA* gene (*gyrA*$^+$) over the quinolone resistance allele (*gyrA*) in a heterodiploid strain. Plasmid pBP515, developed to carry the *gyrA*$^+$ gene of *E. coli* K-12 on a broad-host-range vector derived from pRSF1010, was used to obtain heterodiploid strains. Plasmid pBP515 encodes kanamycin and gentamicin resistance and is transferable via mobilization by a pRP1-derived helper plasmid (pRP1H) to strains of several gram-negative species. After the introduction of pBP515, single-cell MICs (as measured by reduction of the viable cell count) of ciprofloxacin and nalidixic acid decreased by 4- to >8,000-fold for all strains tested, and 8 of the 11 strains regained ciprofloxacin susceptibilities similar to those of the respective wild types. The results indicate that (i) high-level fluoroquinolone resistance in clinical isolates of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. calcoaceticus* can result from mutational alteration of the *gyrA* gene, and (ii) *gyrA* mutations are involved in high levels of fluoroquinolone resistance in *P. stuartii*. Additional mutations outside the *gyrA* locus may contribute to resistance in *K. pneumoniae* and *P. stuartii*.

Fluorinated quinolones are broad-spectrum antibacterial agents (11, 38, 42) that inhibit the bacterial type II topoisomerase DNA gyrase (45). This enzyme consists of two homodimers, subunits A and B (23, 34), which are coded for by two unlinked genes, *gyrA* and *gyrB*, respectively (18, 19). DNA gyrase is essential for replication, recombination, repair, and transcription of DNA (for reviews, see references 17 and 48).

Two types of quinolone-resistant mutants have been described: permeability mutants and gyrase mutants. Permeability mutants show reduced accumulation of quinolones and either reduced or enhanced permeability to unrelated drugs (for a review, see reference 39). Clinical resistance to fluoroquinolones arising solely from permeability alterations has been demonstrated only for *Pseudomonas aeruginosa* (52).

Mutations in the target enzyme, gyrase, have been found in both gram-negative (12, 37, 52) and gram-positive (27, 44) quinolone-resistant bacteria. Mutations in the *gyrB* locus were identified in two strains of *Escherichia coli* K-12 (50) and in one isolate of *P. aeruginosa* (52). All three mutants have low-level resistance to quinolones with incomplete cross-resistance between fluorinated and unfluorinated compounds.

gyrA mutants typically show increased MICs of all quinolone drugs (51). However, examination of *E. coli* isolates with reduced quinolone susceptibilities by determination of the DNA sequences of the respective *gyrA* genes revealed single-step mutations to be responsible for quinolone resistance (12, 37, 51). For these mutants, the MICs of fluoroquinolones but not of unfluorinated compounds are below the breakpoint. Therefore, these mutations are not of clinical importance for *E. coli*. However, during recent years, members of the family *Enterobacteriaceae* (2, 10, 16, 28, 32) and strains of *P. aeruginosa* (20) with clinical fluoroquinolone resistance (e.g., MIC of ciprofloxacin, >4 to 256 μg/ml) were isolated from patients receiving quinolone therapy. Gyrase proteins were isolated from one strain of *E. coli* (2) and from *Serratia marcescens* (16) and characterized biochemically. For most isolates of *Enterobacteriaceae*, the genetic basis for this fluoroquinolone resistance remains obscure. In *P. aeruginosa*, which is less susceptible to fluoroquinolones, biochemical and genetic data revealed gyrase mutations or permeability mutations alone or in combination to be responsible for clinical fluoroquinolone resistance (9, 31). For *Acinetobacter calcoaceticus*, *Klebsiella pneumoniae*, and *Providencia stuartii*, no data about the genetic basis of quinolone resistance are available.

For the identification of *gyrA* mutants among quinolone-resistant strains of *E. coli* and *P. aeruginosa*, a technique based on the dominance of the *gyrA* gene of a quinolone-sensitive strain (*gyrA*$^+$) over the respective resistance allele (gyrA) (22) was developed. A plasmid carrying the *gyrA*$^+$ gene of *E. coli* K-12 was introduced into the mutants, and the resulting heterodiploid strains were examined for quinolone sensitivity. If the heterodiploid strain regained sensitivity to quinolones, this was taken as evidence that resistance in that strain was mediated by an altered *gyrA* (35, 41, 52).

The goal of our study was to develop a broad-host-range plasmid carrying the *gyrA*$^+$ gene of *E. coli* K-12, which can be efficiently transferred to fluoroquinolone-resistant isolates of *Enterobacteriaceae*, *P. aeruginosa*, and *A. calcoaceticus*. This promiscuous plasmid provides a tool for investigating the nature of quinolone resistance in many different gram-negative species.

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TABLE 1. Fluoroquinolone-resistant clinical isolates

Species and strain	MIC (μg/ml)[a]					Source or reference	Yr of isolation
	Cip	Nal	Nor	Kan	Gen		
E. coli							
4917	64	256	128	8	ND	C. Krasemann	1984
205096	128	2,048	256	2	ND	C. Krasemann	1989
HP24704-1	256	2,048	256	16	ND	28	1989
K. pneumoniae							
6623	16	2,048	32	2	ND	C. Krasemann	1987
6628	8	1,024	32	4	ND	C. Krasemann	1988
6629	8	2,048	32	2	ND	C. Krasemann	1988
HP24704-2	16	2,048	32	2	ND	28	1989
P. stuartii							
41i	64	2,048	64	1	4	C. Krasemann	1989
27091	16	2,048	16	2	8	I. Klare	1989
P. aeruginosa 014121	8	2,048	64	256	8	C. Krasemann	1990
A. calcoaceticus BK4	64	2,048	128	512	4	C. Krasemann	1989

[a] Abbreviations: Cip, ciprofloxacin; Nal, nalidixic acid; Nor, norfloxacin; Kan, kanamycin; Gen, gentamicin; ND, not determined.

MATERIALS AND METHODS

Antibiotics. All antibiotics were kindly provided by the manufacturers: ampicillin and ciprofloxacin, Bayer AG, Leverkusen, Germany; chloramphenicol, Boehringer, Mannheim, Germany; gentamicin, Merck, Darmstadt, Germany; kanamycin, Squibb, Bristol-Myers, Syracuse, N.Y.; nalidixic acid, Sterling-Winthrop, Guildford, United Kingdom; and norfloxacin, Merck Sharpe and Dohme, Rahway, N.J.

Strains and plasmids. Clinical isolates with high-level fluoroquinolone resistance are listed in Table 1. *E. coli* K-12 strains used in this study were C600S (26), which is a *recA* derivative of C600 (*thr leu thi lacY tonA supE44 hsdR rpsL* [3]) and its *gyrA* derivative, C600SN (48a); JM83 (47); KL16nal-31 (*gyrB* [24]), obtained from J. T. Smith; and JF703 (*ompF* [8]), obtained from B. Bachman, *E. coli* Genetic Stock Center. The plasmids are listed in Table 2.

Chemicals, biochemicals, and media. All chemicals, unless otherwise stated, were purchased from Merck (Germany). Restriction enzymes, T4 DNA ligase, T4 DNA polymerase, DNA polymerase Klenow fragment, deoxynucleotide triphosphates, and incubation buffers were supplied by Boehringer (Germany) or by New England BioLabs, Schwalbach, Germany. The enzymes were used according to the manufacturers' instructions. Cellulose-acetate filters (0.45-μm pore size) were from Sartorius, Goettingen, Germany.

Media. Standard agar no. I (NI agar), standard broth no. I, and Simons citrate agar were purchased from Merck (Germany). Mueller-Hinton broth was from Difco, Detroit, Mich. M9 minimal medium was prepared as described by Maniatis et al. (30) without added thiamine.

TABLE 2. List of plasmids

Plasmid	Size (kb)	Resistance marker(s)	Source or reference
pRSFK	8.4	Sul^r Kan^r	E. Scherzinger
pBP84	10	Amp^r Spc^r Gen^r	49
pMK90	18	Amp^r	33
pRP1	61	Amp^r Tet^r Kan^r	29
pUC12	2.7	Amp^r	47
pBP500	7.4	Kan^r	This study
pBP513	10.7	Kan^r $gyrA^+$	This study
pBP515	11.6	Kan^r Gen^r $gyrA^+$	This study
pRP1H	61	Amp^r Tet^r	This study

Susceptibility testing. MICs were determined by the microbroth dilution method with unsupplemented Mueller-Hinton broth according to the methods of the National Committee for Clinical Laboratory Standards (36).

The susceptibilities of the transconjugants towards quinolones were determined by the single-cell MIC (scMIC) method of Richmond and Wotton (40). Briefly, overnight cultures of the quinolone-resistant strain and the respective transconjugant, which was grown in the presence of either kanamycin (50 μg/ml) or gentamicin (25 μg/ml for *A. calcoaceticus*, 50 μg/ml for *P. aeruginosa*) to maintain selective pressure against the loss of pBP515, were serially diluted 10-fold. Twenty-five microliters of the 10^{-7}, 10^{-6}, and 10^{-5} dilutions were spotted onto NI-agar plates containing twofold serial dilutions of either ciprofloxacin (256 to 0.004 μg/ml) or nalidixic acid (2,048 to 1 μg/ml) to yield single CFU after incubation for 16 to 18 h at 37°C. The scMIC was determined and defined as the minimum drug concentration that reduces the number of viable cells by at least 10-fold compared with a drug-free control. To compare scMICs of ciprofloxacin and nalidixic acid for the transconjugants with those for the wild type, five strains of each species with MICs identical to the respective MICs for 50% of the strains tested were chosen for scMIC determinations. Median values were calculated and taken as wild-type scMICs. MICs for 50% of the strains testing were assessed by determining the MIC for a population of about 100 randomly collected clinical isolates of each species.

DNA transfer techniques. Transformation of competent *E. coli* K-12 cells was performed as described by Maniatis et al. (30).

For the transfer of plasmid pBP515 by mobilization, equal volumes of donor [C600SN(pRP1H)(pBP515)] and recipient cells grown to mid-log phase were mixed, concentrated 10-fold by centrifugation, and mated on a 0.45-μm-pore-size cellulose-acetate filter placed on a prewarmed NI-agar plate. After 3 h at 37°C, the filters were washed in 5 ml of a 0.9% NaCl solution. Tenfold dilutions of the resulting suspension (10^0 dilution) were plated on M9 minimal agar containing kanamycin (50 μg/ml) for selecting transconjugants of *E. coli* and *K. pneumoniae* or gentamicin (50 μg/ml) for selecting transconjugants of *P. aeruginosa*. Transconjugants of *A. calcoaceticus* were selected on NI-agar plates containing chloramphenicol (10 μg/ml) in addition to gentamicin (25 μg/ml), and transconjugants of *P. stuartii* were selected on Simons citrate agar containing kanamycin (50 μg/ml). As controls, donor and recipient cells were individually plated and incubated under identical conditions. The viable cell counts of the recipients in the mating mixture were determined on the respective agar plates without antibiotics, and the viable cell count of the donor strain was determined on NI agar containing ampicillin (100 μg/ml), tetracycline (10 μg/ml), and kanamycin (50 μg/ml). Colonies were counted after 24 to 48 h of incubation at 37°C.

Identification of isolates. Strains of *Enterobacteriaceae* were identified by the API 20E system (Biomerieux), while strains of *P. aeruginosa* and *A. calcoaceticus* were identified by the API 20NE system (Biomerieux).

DNA isolation. Small-scale and large-scale isolations of plasmid DNA followed standard protocols (30).

TABLE 3. scMICs of ciprofloxacin and nalidixic acid for *E. coli* K-12 strains and their transconjugants

E. coli K-12 strain[a]	Plasmid(s)	scMIC (μg/ml)[b]	
		Nal	Cip
C600S[a]		2	0.008
C600S	pRP1H	2	0.008
C600S	pBP500	2	0.008
C600S	pBP513	2	0.008
C600S	pBP515	2	0.008
C600S	pRP1H, pBP500	2	0.008
C600S	pRP1H, pBP513	2	0.008
C600S	pRP1H, pBP515	2	0.008
C600SN		512	0.25
C600SN	pRP1H	512	0.25
C600SN	pBP500	512	0.25
C600SN	pBP513	2	0.008
C600SN	pBP515	2	0.008
C600SN	pRP1H, pBP500	512	0.25
C600SN	pRP1H, pBP513	2	0.008
C600SN	pRP1H, pBP515	2	0.008
KL16nal-31		128	0.015
KL16nal-31	pBP500	128	0.015
KL16nal-31	pBP515	128	0.015
JF703		16	0.06
JF703	pBP500	16	0.06
JF703	pBP515	16	0.06

[a] Strains were C600S(*gyrA*$^+$), C600SN(*gyrA*), KL16nal-31(*gyrB*), and JF703 (ompF).
[b] Abbreviations: Nal, nalidixic acid; Cip, ciprofloxacin.

RESULTS

Construction of broad-host-range vector pBP515 for dominance studies. IncQ plasmids, like the prototype pRSF1010 (21), are small, well-characterized, broad-host-range plasmids (4). Even though they are not self-transmissible, they can be mobilized by IncP plasmids like pRP1 (13), effecting transfer to several gram-negative bacteria (6). For cloning the *gyrA*$^+$ gene of *E. coli* K-12 plasmid pRSFK, a derivative of pRSF1010 was chosen. It is an 8.4-kb plasmid that encodes kanamycin and sulfonamide resistance (42a). To introduce unique restriction endonuclease cleavage sites suitable for insertion of the *gyrA*$^+$ gene, the 35-bp *Eco*RI-*Pst*I polylinker fragment of pUC12 was ligated to the 7.4-kb *Eco*RI-*Pst*I fragment of pRSFK, which contains the origin of replication, the site for mobilization, all genes essential for autonomous replication, and the *aphA* gene mediating kanamycin resistance. This step deletes a 1-kb fragment of pRSFK encoding sulfonamide resistance. The resulting plasmid, pBP500, contains unique restriction sites for *Eco*RI, *Bam*HI, *Xba*I, *Sal*I, *Pst*I, and *Sac*I and can be selected for on kanamycin.

The *gyrA*$^+$ gene of *E. coli* K-12 was isolated on a 3.3-kb *Bsm*I fragment from plasmid pMK90. The sticky ends of this fragment were made blunt ended by treatment with T4 DNA polymerase. The modified fragment was inserted into the *Bam*HI site of pBP500, which was made compatible by filling in overlapping 5′ ends by treatment with DNA polymerase I large fragment (Klenow fragment). The resulting plasmid, pBP513, carries an additional unique *Kpn*I site within the *gyrA*$^+$ gene. After transfer of plasmid pBP513 into C600SN, a quinolone-resistant *gyrA* derivative of strain C600S, plasmid-bearing cells became quinolone susceptible (dominance test) (Table 3). In order to detect transfer of a plasmid encoding *gyrA*$^+$ in strains naturally resistant to kanamycin (like *P. aeruginosa*), the *aadB* gene mediating gentamicin resistance was introduced in plasmid pBP513. The *aadB* gene was isolated on an 885-bp *Mse*I-*Sty*I fragment from plasmid pBP84. The fragment was made blunt ended and inserted into the *Xba*I site of pBP513, which was made compatible by treatment with Klenow fragment. The resulting plasmid was named pBP515. Transfer of plasmid pBP515 into C600SN resulted in cells resistant to kanamycin and gentamicin but sensitive to quinolones (Table 3). Since pBP515 contains a functional mobilization site but no autonomous transfer system, transfer functions have to be provided in *trans*. Plasmid pRP1H was chosen as helper plasmid, since it meets all requirements for conjugational transfer of pBP515 via mobilization. Plasmid pRP1H is a derivative of pRP1, whose *aphA* gene encoding kanamycin resistance is inactivated by insertion of a 125-bp *Hin*dIII fragment of bacteriophage lambda DNA into the unique *Hin*dIII site. To confirm that the *gyrA*$^+$ gene alone and no additional sequences on pBP515 or pRP1H are responsible for the dominant phenotype, the scMICs of ciprofloxacin and nalidixic acid were determined for strains C600S and C600SN either alone or carrying plasmids pRP1H, pBP500, pBP513, and pBP515. The results listed in Table 3 demonstrate that dominance is completely based on the presence of the *gyrA*$^+$ gene and is uninfluenced by vector sequences of pBP500. Additionally, to exclude any nonspecific dominance effect of pBP515, either pBP500 or pBP515 was introduced by transformation into *E. coli* K-12 strains KL16nal-31 and JF703, which are low-level quinolone resistant because of mutations in *gyrB* and *ompF*, respectively. The results included in Table 3 demonstrate that the dominance effect mediated by pBP515 is specific for *gyrA* mutants rather than for *gyrB* or *ompF* mutants.

Isolation and characterization of transconjugants. Under the conditions used for selection of transconjugants, no growth of recipient or donor cells alone was detectable. Transfer of plasmid pBP515 from donor strain C600SN(pRP1H)(pBP515) was most efficient for isolates of *E. coli*, *K. pneumoniae*, and *A. calcoaceticus* and yielded more than 10^5 transconjugants per ml in a standard mating procedure (Materials and Methods). Transfer to *P. aeruginosa* and *P. stuartii* resulted in about 10^3 and 10^2 transconjugants per ml, respectively. Single colonies of all transconjugants were purified on selective agar plates. To verify plasmid transfer into different gram-negative species, plasmid pBP515 was isolated from one transconjugant strain each of *E. coli*, *P. aeruginosa*, and *A. calcoaceticus* and digested with restriction endonuclease *Acc*I. In all cases, a DNA fragment pattern identical to that of pBP515 was obtained (data not shown).

The effect of the presence of the plasmid-encoded *gyrA*$^+$ gene in the different strains was examined by comparing the susceptibilities of the transconjugants and the recipient strains to ciprofloxacin and nalidixic acid. Results obtained with a conventional MIC determination technique (36) showed that the MICs for the transconjugants were identical within 1 dilution step to the MICs for the corresponding resistant isolates. With the scMIC technique, the values of both quinolones were 4- to >8,000-fold lower. The reason for this discrepancy is a loss of plasmid pBP515 in a few transconjugant cells, which are selected in conventional MIC tests. However, if the scMIC is determined, only a few single colonies grew on drug concentrations above the scMIC (≤1% of the inoculum). These colonies were identified as plasmidless cells by replica plating on agar containing either nalidixic acid (growth) or kanamycin or gentamicin (no growth). These observations do not reflect an impaired

stability of plasmid pBP515 in the different species, since incubation for at least 20 generations without selective pressure yields over 95% plasmid-carrying cells as measured by comparing viable cell counts on NI-agar plates in the absence and presence of either kanamycin or gentamicin (data not shown).

DISCUSSION

Dominance of a *gyrA*$^+$ allele over a *gyrA* allele was first demonstrated by Hane and Wood in 1969 for *E. coli* K-12 (22). Since these studies, the dominance phenomenon has been exploited by several groups to confirm that strains contained plasmids encoding *gyrA*$^+$ (27, 33, 46) or to verify the nature of quinolone resistance in laboratory or clinical *E. coli* and *P. aeruginosa* strains. Investigators in these studies used species-specific narrow-host-range plasmids derived from either pBR322 (7) for E. coli (35) or pME294 (25) for *P. aeruginosa* (52) as vectors. These plasmids had to be transferred by transformation, thereby restricting the dominance test to a limited number of transformable strains. Recently, in 1990, Robillard (41) used the broad-host-range cosmid pLA2917 (1), a pRK2 derivative, as a vector for cloning the *E. coli* K-12 *gyrA*$^+$ gene. The resulting plasmid, pNRJ3-2, has a size of 21 kb and has to be transferred by mobilization with *E. coli* K-12 strain S17-1 (43), which provides the functions required for conjugation in *trans*. All investigators demonstrated dominance of the *E. coli* K-12 *gyrA*$^+$ allele in *gyrA* mutants of *E. coli* and *P. aeruginosa*, indicating both the expression of the *gyrA*$^+$ gene in *P. aeruginosa* and the formation of a functional hybrid gyrase in vivo.

The broad-host-range plasmid pBP515 developed in our study was shown to be transferable by mobilization not only to *E. coli* and *P. aeruginosa* but also to *K. pneumoniae*, *P. stuartii*, and *A. calcoaceticus*. Additionally, transfer by transformation to different *Enterobacteriaceae* (*K. pneumoniae*, *Enterobacter cloacae*, *Citrobacter freundii*, and *Salmonella typhimurium*; data not shown) was also possible. In the absence of selective pressure, plasmid pBP515 can be stably maintained in all species tested. However, for determining the susceptibilities of heterodiploid strains to quinolones, the scMIC technique is required to eliminate problems arising from the loss of plasmid pBP515 during conventional MIC determinations in some cells. Thus, pBP515 is a versatile broad-host-range plasmid which is well suited for the genetic characterization of *gyrA* mutants of several gram-negative species.

The results of the dominance test (Table 4), which show an increase in susceptibility to ciprofloxacin and nalidixic acid for transconjugants of all mutants tested, indicate that the *E. coli* K-12 *gyrA*$^+$ gene on pBP515 is dominant over the *gyrA* alleles of all five species. These are the first data providing strong indirect evidence that high-level fluoroquinolone resistance (MIC of ciprofloxacin, 8 to 256 μg/ml) in isolates of normally susceptible gram-negative species like *E. coli*, *A. calcoaceticus*, *K. pneumoniae*, and *P. stuartii* is due to *gyrA* mutations.

For estimating the influence of the *gyrA* mutations on the resistance phenotype, the scMICs for the transconjugants were compared with those for quinolone-susceptible strains of the respective species (summarized in Table 5) and for *E. coli* K-12 strain C600S (Table 3). scMICs for transconjugants of all three *E. coli* isolates decreased to the level for *E. coli* K-12, which is identical to that for wild-type *E. coli* isolates, irrespective of the scMIC of the corresponding fluoroquinolone-resistant isolate. These results demonstrate that alteration of the *gyrA* gene leads to an increase in the MICs of ciprofloxacin from 0.015 μg/ml (wild type) to 256 μg/ml (HP24704-1). Since high-level fluoroquinolone-resistant strains are rarely isolated from patients treated with these drugs and since several investigators were unable to select in vitro for such mutants in *E. coli* or *K. pneumoniae* (5, 14, 15), there is a strong possibility that multiple changes rather than a single-step mutation in the *gyrA* gene are responsible for fluoroquinolone resistance.

Transconjugants of the other four species did not become as susceptible to quinolones as wild-type *E. coli* K-12. The scMICs for transconjugants of *A. calcoaceticus*, *E. coli*, and *P. aeruginosa* were identical to those for the corresponding wild type (Table 5). For *P. aeruginosa* transconjugants, this is consistent with observations made by others (41, 52).

TABLE 4. scMICs for fluoroquinolone-resistant isolates of gram-negative species and their transconjugants

Species and strain	Plasmid	scMIC (μg/ml)[a]	
		Nal	Cip
E. coli			
4917		64	8
4917	pBP515	4 (16)	0.015 (530)
205096		1,024	64
205096	pBP515	2 (512)	0.015 (4,260)
HP24704-1		2,048	256
HP24704-1	pBP515	4 (512)	0.03 (8,530)
K. pneumoniae			
6623		2,048	8
6623	pBP515	32 (64)	0.5 (16)
6628		1,024	8
6628	pBP515	32 (32)	0.5 (16)
6629		>2,048	8
6629	pBP515	32 (>64)	0.5 (16)
HP24704-2		>2,048	32
HP24704-2	pBP515	128 (>16)	1 (32)
P. stuartii			
41i		>2,048	128
41i	pBP515	64 (>32)	4 (32)
27091		2,048	16
27091	pBP515	32 (64)	4 (4)
P. aeruginosa			
014121		>2,048	8
014121	pBP515	64 (>32)	0.125 (64)
A. calcoaceticus			
KB4		1,024	128
KB4	pBP515	8 (128)	0.5 (256)

[a] Abbreviations: Nal, nalidixic acid; Cip, ciprofloxacin. The reduction of the scMIC of each transconjugant compared with that of the respective isolate is shown in parentheses as fold change.

TABLE 5. Comparison of scMICs of ciprofloxacin and nalidixic acid for transconjugants and quinolone-susceptible wild-type strains

Species	scMIC range (μg/ml)[a]			
	tc	wt	tc	wt
E. coli	2–4	2	0.015–0.03	0.03
A. calcoaceticus	8	8	0.5	0.25
K. pneumoniae	32–128	4	0.5–1	0.125
P. aeruginosa	64	64	0.125	0.125
P. stuartii	32–64	4	4	0.06

[a] Abbreviations: Nal, nalidixic acid; Cip, ciprofloxacin; tc, transconjugant; wt, wild type.

Additionally, transfer of pBP515 to wild-type strains of *A. calcoaceticus* and *P. aeruginosa* did not result in reduction of the scMICs of ciprofloxacin and nalidixic acid (data not shown). Most likely, these species-specific differences in quinolone susceptibility are due to differences in the permeability of the outer membrane to quinolones.

Transfer of plasmid pBP515 to *K. pneumoniae* and *P. stuartii* isolates resulted in partial dominance (Table 5). For *K. pneumoniae* transconjugants, the scMIC of ciprofloxacin is 4- to 8-fold above that for wild type (0.125 μg/ml) and that of nalidixic acid is 8- to 32-fold above that for wild type (MIC for wild type, 4 μg/ml). For *P. stuartii* transconjugants, the scMIC of ciprofloxacin is 64-fold above that for the wild type (0.06 μg/ml) and that of nalidixic acid is 8- to 16-fold above that for wild type (MIC for wild type, 4 μg/ml). Both transconjugants of *P. stuartii* remain quinolone resistant (scMIC of ciprofloxacin, 4 μg/ml, and scMIC of nalidixic acid, 32 to 64 μg/ml) by clinical means.

One reason for this partial dominance in *K. pneumoniae* and *P. stuartii* might be reduced expression of the plasmid-coded *gyrA*$^+$ gene of *E. coli* K-12 in these strains. The formation of hybrid gyrase enzymes with reduced quinolone sensitivities is also consistent with partial dominance. Additionally, mutations affecting gyrase subunit B or the permeability of the outer membrane might be involved. Detailed biochemical and genetic analyses are required to fully characterize these mutants.

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