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Impact of Low-Level Resistance to Fluoroquinolones Due to *qnrA1* and *qnrS1* Genes or a *gyrA* Mutation on Ciprofloxacin Bactericidal Activity in a Murine Model of *Escherichia coli* Urinary Tract Infection▽

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We investigated the impact of low-level resistance to fluoroquinolones on the bactericidal activity of ciprofloxacin in a murine model of urinary tract infection. The susceptible *Escherichia coli* strain CFT073 (ciprofloxacin MIC [CIP MIC] of 0.008 μg/ml) was compared to its transconjugants harboring *qnrA1* or *qnrS1* and to an S83L *gyrA* mutant. The three derivatives showed similar low-level resistance to fluoroquinolones (CIP MICs, 0.25 to 0.5 μg/ml). Bactericidal activity measured in vitro after 1, 3, and 6 h of exposure to 0.5 μg/ml of ciprofloxacin was significantly lower for the derivative strains ($P < 0.01$). In the murine model of urinary tract infection (at least 45 mice inoculated per strain), mice were treated with a ciprofloxacin regimen of 2.5 mg/kg, given subcutaneously twice daily for 2 days. In mice infected with the susceptible strain, ciprofloxacin significantly decreased viable bacterial counts ($\log_{10}$ CFU/g of tissue) in the bladder (4.2 ± 0.5 versus 5.5 ± 1.3; $P = 0.001$) and in the kidney (3.6 ± 0.8 versus 5.0 ± 1.1; $P = 0.003$) compared with those of untreated mice. In contrast, no significant decrease in viable bacterial counts was observed with any of the three derivative strains. The area under the concentration-time curve from 0 to 24 h/MIC and the maximum concentration of drug in serum/MIC ratios measured in plasma were indeed equal to 827 and 147, respectively, for the parental strain, and only 12.4 to 24.8 and 2.2 to 4.4, respectively, for the derivative strains. In conclusion, low-level resistance to fluoroquinolones conferred by a *qnr* gene is associated with decreased bactericidal activity of ciprofloxacin, similar to that obtained with a *gyrA* mutation.

Urinary tract infection (UTI) due to *Escherichia coli* is the most common bacterial infection. Fluoroquinolones are commonly used for the treatment of UTI because isolated microorganisms are frequently resistant to aminopenicillins and trimethoprim-sulfamethoxazole (22), and fluoroquinolones are given orally. However, resistance to fluoroquinolones in *E. coli* has increased due to their large use (13, 23). Classical mechanisms of quinolone resistance are due to chromosomal mutations in the genes encoding their targets (quinolone resistance-determining regions of the type II topoisomerases) or in regulatory genes affecting permeability or efflux (15, 29). More recently, plasmid-mediated mechanisms were reported, such as those due to *qnr* genes (16, 25) encoding pentapeptide repeat proteins, *aac(6′)-Ib-cr* encoding a modified acetyltransferase (32), and *qepA* encoding an active efflux pump (27). The Qnr proteins protect DNA gyrase from quinolone inhibition.

Enterobacteriaceae with plasmid-mediated resistance to fluoroquinolones due to *qnrA*, *qnrB*, or *qnrS* have been described worldwide (31) and particularly among *E. coli* from UTI (5, 39). Acquisition of *qnr* genes increases fluoroquinolone MICs by between 8- and 64-fold; however, the final MICs remain below the susceptibility breakpoints, according to CLSI (1 μg/ml) (6) and to the European Committee on Antimicrobial Susceptibility Testing (0.5 μg/ml) (19). Although it has been suggested that the Qnr protein favors the selection of mutant resistance (25, 28), the therapeutic relevance of the acquisition of the *qnr* gene on the bactericidal activity of fluoroquinolones remains unclear. Therefore, the aim of our study was to evaluate the impact of low-level fluoroquinolone resistance conferred by *qnr* genes on ciprofloxacin bactericidal activity in vitro and in vivo. We measured ciprofloxacin bactericidal activity in vitro at the concentrations reached in humans and in vivo in a murine model of UTI. Results obtained with the susceptible strain of *E. coli* used for the experimental model were compared to those obtained with two of its transconjugants harboring a *qnr* gene, also taking into account the results obtained with its *gyrA* mutant that exhibits low-level fluoroquinolone resistance similar to that of the *qnr* transconjugants.

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MATERIALS AND METHODS

Bacterial strains, conjugation assays, and plasmid stability. Experiments were performed with four isogenic strains derived from the quinolone-susceptible *E. coli* CFT073 strain used previously to set the murine model of pyelonephritis (20). A rifampin (rifampicin)-resistant mutant strain, *E. coli* CFT073-RR, was selected in vitro from *E. coli* CFT073 by plating 10^9 bacteria onto Mueller-Hinton (MH) agar containing a rifampin concentration of 100 μg/ml. *E. coli* CFT073-RR served as the quinolone-susceptible control strain. Two

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transconjugants were obtained after conjugation between the following *E. coli* CFT073-RR and clinical strains harboring *qnr* genes: *E. coli* Hm13 harboring *qnrA1* and *E. coli* PS105 harboring *qnrS1* (both resistant to amoxicillin [amoxicilline]). Plasmids of the clinical strains were negative for the presence of a second *qnr* gene, *aac(6′)-Ib-cr* or *qepA*. The transconjugants *E. coli* CFT073-RR Tc (pQnrA1) and *E. coli* CFT073 Tc (pQnrS1) were obtained after 40 min of mating in MH broth, as previously described (3). After incubation, transconjugants were selected by plating the conjugation mixture on MH agar supplemented with amoxicillin (100 μg/ml)-rifampin (100 μg/ml). The PCR experiments confirmed the presence of the transconjugants. In order to obtain a strain with low-level fluoroquinolone resistance from a different mechanism, we selected a strain with a single *gyrA* mutation from *E. coli* CFT073-RR by plating 10^9 bacteria onto MH agar containing nalidixic acid at a concentration of 40 μg/ml. PCR and DNA sequencing of the quinolone resistance-determining regions in the DNA gyrase and topoisomerase IV genes (4) confirmed a single *gyrA* S83L mutation in the clone, studied further as the *E. coli* CFT073-RR-*gyrA* mutant.

Plasmid stability in the transconjugants *E. coli* CFT073-RR Tc (pQnrA1) and *E. coli* CFT073 Tc (pQnrS1) was measured in vitro by daily subculturing for 4 weeks in antibiotic-free MH agar and by plating onto MH agar alone or containing 100 μg/ml of amoxicillin. After 4 weeks, the mean plasmid losses were 0.8% for *E. coli* CFT073-RR Tc (pQnrA1) and 0.3% for *E. coli* CFT073-RR Tc (pQnrS1).

Fluoroquinolone activities in vitro. MICs were determined by the agar dilution method in accordance with CLSI guidelines (6). Time-kill curve kinetics were performed for each strain in 10 ml MH broth, with a ciprofloxacin concentration of 0.5 μg/ml and with a ciprofloxacin concentration equal to four times the MIC of the strain tested. Antimicrobial agent-free broth was evaluated in parallel as a control. Cultures were incubated at 37°C. Viable counts were determined by serial dilution after 0, 1, 3, 6, and 24 h of incubation and by plating 100 μl of the control, test cultures, or the dilution at the indicated times onto MH agar plates. Colony counts were determined after 24 h of incubation.

Selection of resistant mutants was performed at four times the MIC of ciprofloxacin against each strain, as described previously (4). Briefly, strains were grown at 37°C overnight in antibiotic-free MH broth and centrifuged, and the pellet was suspended in 5 ml of sterile broth, giving an inoculum of $>10^{10}$ CFU/ml. Agar plates containing ciprofloxacin were inoculated with 100 μl of cell suspension and incubated at 37°C. The proportion of resistant mutants was calculated by dividing the number of CFU growing on MH agar plates with ciprofloxacin by the number of CFU growing on antibiotic-free MH agar.

The mutant prevention concentration (MPC) of ciprofloxacin was determined as previously described (1). Briefly, each strain was grown at 37°C overnight in antibiotic-free MH broth. Cultures (100 ml) were centrifuged at 4,000 × *g* for 15 min, and the pellet was suspended in 5 ml of sterile broth, giving an inoculum of $>10^{10}$ CFU/ml. Agar plates containing ciprofloxacin concentrations ranging from 1× MIC to 16× MIC against each strain were inoculated with 100 μl of cell suspension and incubated at 37°C. The MPC was recorded as the lowest concentration of ciprofloxacin completely inhibiting bacterial growth after incubation at 37°C for 72 h.

All the in vitro experiments described above were repeated at least five times. Geometric means were used to express the results for MICs and MPCs, and the means ± standard deviations were calculated for CFU counts and rates of spontaneous mutant.

Mouse model of UTI. The ascending unobstructed mouse model of UTI was used as previously described (20). Animal experiments were performed in accordance with prevailing regulations regarding the care and use of laboratory animals by the European Commission (10). The experimental protocol was approved by the Departmental Direction of Veterinary Services in Paris, France. Eight-week-old immunocompetent female CBA mice (weight, 20 to 23 g) were used. Inocula of different strains were obtained by overnight incubation in brain heart infusion broth, washing of the cells by centrifugation at 15,000 × *g* for 15 min in saline, and resuspension in saline to a final inoculum of 5×10^{10} CFU/ml. Pyelonephritis was induced during general anesthesia (with an intraperitoneal administration of 0.3 ml of 0.66% pentobarbital solution) by injecting 50 μl (i.e., 10^8 CFU) into the bladder through a urethral catheter.

In vivo growth experiments and plasmid stability. Mice were inoculated with each of the four different strains. The growth rate of individual strains was studied in groups of at least 10 infected mice, in order to evaluate their potentials for performing stable pyelonephritis and to evaluate in vivo plasmid stability for the two transconjugants. Mice were sacrificed at 5 and 10 days after inoculation, and bladders and kidneys were aseptically taken out and homogenized in 1 ml of saline solution. One hundred microliters of the solution or its dilution was spread onto MH agar plates and incubated for 24 h. The number of CFU were counted and expressed as the number of CFU/g of tissue. Plasmid loss on day 5 and 10 was assessed for strains harboring plasmid pQnrA1 or pQnrS1 by comparing the number of CFU growing on MH plates alone or containing amoxicillin (100 μg/ml).

TABLE 1. Ciprofloxacin MICs, MPCs, and proportion of resistant mutants against the four *E. coli* strains used in the study

E. coli strain (plasmid)	MIC (μg/ml)	MPC (μg/ml)	MPC/MIC ratio (μg/ml)	PRM[a]
CFT703-RR	0.0075	0.06	8	$0.8 \times 10^{-8} \pm 0.4 \times 10^{-8}$[b]
CFT073-RR Tc (pQnrA1)	0.5	4	8	$0.6 \times 10^{-8} \pm 0.7 \times 10^{-8}$[b]
CFT073-RR Tc (pQnrS1)	0.25	2	8	$1.1 \times 10^{-8} \pm 1.5 \times 10^{-8}$[b]
CT073-RR-*gyr*A	0.25	2	8	$0.3 \times 10^{-8} \pm 0.4 \times 10^{-8}$[c]

[a] PRM, proportion of resistant mutants (selection by ciprofloxacin at four times the MIC). Each value is the mean ± standard deviation from five independent experiments.
[b] *P* was 0.4 compared with *E. coli* strain CFT073-RR.
[c] *P* was 0.2 compared with *E. coli* strain CFT073-RR.

Antimicrobial treatment. In order to determine the bactericidal activity of ciprofloxacin, at least 45 mice per strain were inoculated with strain CFT073-RR, CFT073-RR Tc (pQnrA1), CFT073-RR Tc (pQnrS1), or CFT073-RR-*gyrA*. Two days after inoculation, at least 15 mice in each group were treated with 2.5 mg/kg of ciprofloxacin injected subcutaneously twice a day for 2 days (four injections). This regimen was chosen because it provided peak levels of plasma that were in the range of those achieved in humans after an oral administration of 500 mg of ciprofloxacin (2) and because it provided peak concentration/MIC and area under the concentration-time curve from 0 to 24 h (AUC_{0-24})/MIC ratios against the susceptible parental strain that were above those required to achieve efficacy in humans (11, 30).

Mice were sacrificed 18 h after the last dose of ciprofloxacin. Bladders and kidneys were aseptically taken out and homogenized in 1 ml of saline solution. One hundred microliters of the solution or its dilution was spread onto MH agar plates and incubated for 24 h. The number of CFU were counted and expressed as the number of CFU/g of tissue. Kidney and bladder homogenates (0.1 ml of each) were also spread onto MH agar plates containing ciprofloxacin at concentrations of 1× MIC to 4× MIC and incubated at 37°C for 72 h in order to detect spontaneous, resistant mutants. Control mice were killed for organism quantification just before the start of ciprofloxacin therapy (15 to 19 mice, start-of-therapy controls) and 18 h after the end of therapy (15 to 17 mice, end-of-therapy controls).

Ciprofloxacin pharmacokinetics. Single-dose pharmacokinetic studies were performed after an injection of 2.5 mg/kg of ciprofloxacin subcutaneously in infected mice. Blood samples of 200 μl were obtained by intracardiac puncturing from three to six anesthetized mice at 15, 30, 45, 60, 120, 240, 360, and 480 min after ciprofloxacin injection. Blood was centrifuged, and plasma samples were treated with a methanolic solution containing ofloxacin as an internal standard in order to precipitate proteins. Plasma ultrafiltrate was prepared from 200-μl plasma samples by centrifugation in a Centrifree micropartition unit (Amicon, Beverly, MA) at 2,000 × g for 30 min at 25°C. Ciprofloxacin concentrations were determined by liquid chromatography, with fluorimetric detection after deproteinization, as described previously (36). The method was linear over the concentration range from 0.1 to 40 μg/ml. Intra- and interday coefficients of variation obtained were less than 10%. The limit of quantitation used was 0.05 μg/ml. The peak concentration (maximum concentration of drug in serum [C_{max}]) and the AUC_{0-24} were calculated using the WinNonlin pharmacokinetic program (Scientific Consulting).

Statistical analysis. Results were expressed as means ± standard deviations for continuous variables. Continuous variables were compared by nonparametric testing (Mann-Whitney U test). A *P* value of less than 0.05 was considered significant. Analysis was performed using SAS statistical software (version 8.2; Cary, NC).

RESULTS

MICs, MPCs, and in vitro selection of ciprofloxacin-resistant mutants. Ciprofloxacin MIC and MPC values against the four study strains are presented in Table 1. Acquisition of the plasmids pQnrA1 or pQnrS1 increased the ciprofloxacin MIC

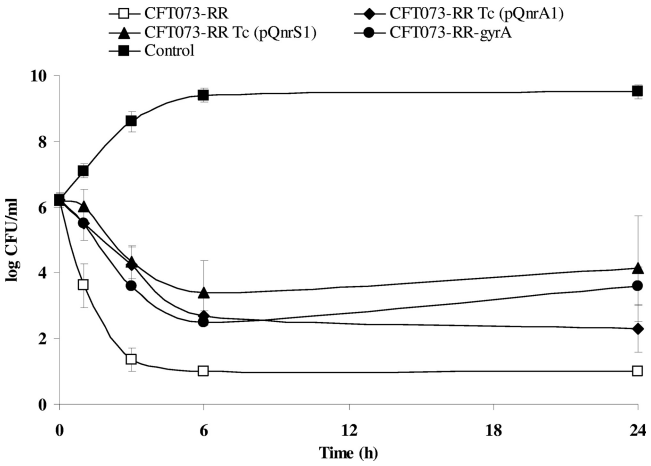

FIG. 1. Bactericidal activity of ciprofloxacin at a concentration of 0.5 μg/ml against the four different *E. coli* strains. *P* was <0.01 after 1, 3, and 6 h of incubation for any of the three strains with low-level of resistance compared with the susceptible *E. coli* CFT073-RR strain. *P* was <0.05 for *E. coli* CFT073-RR Tc (pQnrS1) and *E. coli* CFT073-RR-*gyrA* and 0.07 for *E. coli* CFT073-RR Tc (pQnrA1) compared with *E. coli* CFT073-RR after 24 h of incubation.

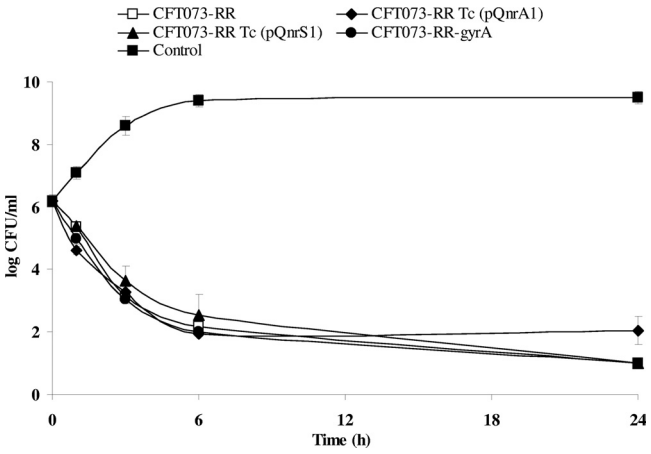

FIG. 2. Bactericidal activity of ciprofloxacin at a concentration that was four times the MIC of each of the *E. coli* study strains. *P* was >0.2 at all times for the three strains with low-level resistance compared with the susceptible *E. coli* CFT073-RR strain.

by 32 to 64 times and the MPC by 20 times. However, the strains harboring these plasmids remained susceptible to ciprofloxacin, according to currently accepted clinical breakpoints by CLSI or the European Committee on Antimicrobial Susceptibility Testing, and the MPC/MIC ratios were similar for the four strains (equaling 8). Results for the *gyrA* mutant were similar to those for the *qnr* transconjugants.

No significant differences were observed in the proportion of ciprofloxacin-resistant mutant obtained at four times the MIC of each strain between *E. coli* CFT073-RR and the three derivative strains with either the *qnr* genes or a *gyrA* mutation ($P > 0.4$) (Table 1).

Time-kill curves. The viable counts of the four strains in time-kill curves are shown in Fig. 1. At a concentration of 0.5 μg/ml, the bactericidal activity of ciprofloxacin significantly decreased after 1, 3, and 6 h of antibiotic exposure against *E. coli* strains harboring plasmid pQnrA1 or pQnrS1 and against the *E. coli* CFT073-RR-*gyrA* mutant in comparison with the susceptible *E. coli* CFT073-RR strain ($P < 0.01$). At a concentration of four times the MIC of each strain, no significant difference in the bactericidal activity of ciprofloxacin was measured among any of the three strains with low-level resistance or the susceptible *E. coli* CFT073-RR strain (*P* of >0.2 after 1, 3, 6, and 24 h of antibiotic exposure for any of the three strains with low-level resistance compared with the parental strain) (Fig. 2).

Pharmacokinetics and pharmacodynamic parameters. After a single subcutaneous injection of 2.5 mg/kg of ciprofloxacin, the mean C_{max} value obtained was 1.1 ± 0.2 μg/ml at 15 min after the injection, and the mean AUC_{0-24} obtained was 6.2 ± 1.2 μg/h/ml. Corresponding C_{max}/MIC and AUC_{0-24}/MIC ratios for the different study strains are shown in Table 2. Because the level of serum protein binding for ciprofloxacin is near zero in mice (38), the free and total serum levels for ciprofloxacin were considered to be similar.

Mouse model of UTI. Individual UTI experiment showed that the four derivative strains of *E. coli* CFT073 were all able to induce stable pyelonephritis in mice at least until day 10. On day 10, bacterial counts in kidneys and bladders (log_{10} CFU/g of tissue) were 5.0 ± 1.0 and 5.9 ± 1.9, respectively, for strain CFT073-RR; 3.0 ± 0.7 and 3.7 ± 0.7, respectively, for the strain harboring plasmid pQnrA1; 4.1 ± 0.5 and 5.9 ± 1.1, respectively, for the strain harboring plasmid pQnrS1; and 5.5 ± 1.0 and 5.4 ± 1.1, respectively, for the strain with a single *gyrA* mutation. The mean plasmid loss at day 10 was 4.5% for *E. coli* CFT073-RR harboring plasmid pQnrA1 and 4.2% for the strain harboring plasmid pQnrS1.

Therapeutic efficacy in experimental murine UTI. In mice infected with *E. coli* strain CFT073-RR and treated with ciprofloxacin, a significant decrease of viable bacterial counts was observed in kidneys (Table 3) and in bladders (Table 4) compared with end-of-treatment control mice. In contrast, no statistically significant decrease in viable bacterial counts in kidneys (Table 3) and in bladders (Table 4) was observed when UTI was induced with any of the three resistant derivative *E. coli* strains between treated mice and end-of-treatment control mice ($P > 0.2$) (Tables 3 and 4). Of note, ciprofloxacin did not reduce the CFU counts in kidneys and bladders even against *E.*

TABLE 2. Pharmacokinetic-pharmacodynamic parameters of the ciprofloxacin dosing regimen used in experimental UTI against the four *E. coli* strains used in the study

E. coli strain (plasmid)	Pharmacokinetic-pharmacodynamic ratios[b]	
	C_{max}/MIC[a]	AUC_{0-24}/MIC[c]
CFT703-RR	147	827
CFT703-RR Tc (pQnrA1)	2.2	12.4
CFT703-RR Tc (pQnrS1)	4.4	24.8
CT073-RR-*gyr*A	4.4	24.8

[a] The mean C_{max} for ciprofloxacin was 1.1 ± 0.2 μg/ml.
[b] The level of serum protein binding for ciprofloxacin in mice was near 0% (38). Therefore, the levels of total and unbound drug binding were considered similar.
[c] The AUC_{0-24} value used was 6.2 ± 1.2 μg/h/ml.

TABLE 3. Effect of ciprofloxacin on viable organisms in kidneys of mice infected with the four *E. coli* strains used in the study

E. coli strain (plasmid)	Results (log CFU/g of kidney ± SD [no. of mice]) for mice treated with:		
	Start-of-treatment control	End-of-treatment control	Ciprofloxacin
CFT703-RR	5.0 ± 0.5 (15)	5.0 ± 1.1 (15)	3.6 ± 0.8 (15)[a]
CFT703-RR Tc (pQnrA1)	3.4 ± 0.9 (15)	3.0 ± 0.8 (15)	2.7 ± 0.6 (16)[b]
CFT703-RR Tc (pQnrS1)	4.6 ± 1.0 (15)	4.5 ± 1.1 (15)	4.2 ± 0.7 (16)[b]
CFT703-RR-*gyrA*	3.9 ± 1.4 (15)	4.0 ± 1.3 (15)	4.1 ± 1.4 (16)[b]

[a] *P* was 0.001 compared with the end-of-treatment control group.
[b] *P* was >0.2 compared with the end-of-treatment control group.

TABLE 4. Effect of ciprofloxacin on viable organisms in bladders of mice infected with the four *E. coli* strains used in the study

E. coli strain (plasmid)	Results (log CFU/g of bladder ± SD [no. of mice]) for mice treated with:		
	Start-of-treatment control	End-of-treatment control	Ciprofloxacin
CFT703-RR	6.2 ± 1.1 (19)	5.5 ± 1.3 (17)	4.2 ± 0.5 (15)[a]
CFT703-RR Tc (pQnrA1)	5.1 ± 0.8 (16)	4.7 ± 0.5 (15)	4.7 ± 0.3 (20)[b]
CFT703-RR Tc (pQnrS1)	6.6 ± 1.5 (15)	5.0 ± 0.9 (15)	5.0 ± 0.9 (16)[b]
CFT703-RR-*gyrA*	5.4 ± 0.8 (15)	5.4 ± 0.8 (15)	5.4 ± 0.7 (16)[b]

[a] *P* was 0.003 compared with the end-of-treatment control group.
[b] *P* was >0.6 compared with the end-of-treatment control group.

coli CFT073-RR harboring plasmid pQnrA1, which produced the lowest bacterial counts in start-of-treatment and end-of-treatment control mice. Therefore, treatment with ciprofloxacin did not influence, under our experimental conditions, the evolution of UTI due to strains harboring low-level resistance to fluoroquinolones due to *qnr* genes or a *gyrA* mutation.

In vivo selection of resistant mutants. No ciprofloxacin-resistant mutant was detected after antibiotic exposure when the experimental UTI was induced with any of the four *E. coli* strains.

DISCUSSION

The *qnr* gene is a novel plasmid-mediated gene used for fluoroquinolone resistance (25). The resistance level conferred is so low that *qnr*-positive strains remain susceptible to fluoroquinolones, according to international susceptibility breakpoints. Our in vitro and in vivo studies of isogenic strains of *E. coli* harboring *qnrA* or *qnrS* showed that the bactericidal activity of ciprofloxacin is markedly reduced against these strains.

Few studies have shown the therapeutic impact of low-level resistance to fluoroquinolones in *Enterobacteriaceae* either experimentally (12), in clinical reports (9, 26, 40), or in a retrospective clinical study (8). However, the *qnr* gene was not involved in any of these studies. The only report investigating the therapeutic impact of a *qnr* gene was performed in a murine model of pneumonia due to a strain of *Klebsiella pneumoniae* alone or expressing the *qnrA1* gene (34). However, in contrast with our experiments, since the *K. pneumoniae qnr*-negative parental strain harbored several mechanisms of resistance to fluoroquinolones (has a *gyrA* mutation, deficient in porins, and has an active efflux pump), the derivative strain harboring the *qnrA1* gene became resistant to fluoroquinolones in vitro, with a ciprofloxacin MIC of 4 μg/ml. Consequently, this led to an in vivo failure with ciprofloxacin.

In the present study, we showed that low-level resistance to fluoroquinolones due to a *qnr* gene is sufficient to significantly reduce the bactericidal activity of ciprofloxacin in vitro and in vivo. Mice infected with *qnr*-positive transconjugants of *E. coli* did not show any significant reduction in bacterial count when they were treated with ciprofloxacin compared with the counts of mice infected with the susceptible parental strain. We also showed that the therapeutic impact was similar to that observed with a derivative harboring a single *gyrA* mutation. However, two comments should be made concerning the initial bacterial inoculum used in our in vivo experiments. First, our experimental conditions were associated with a relatively low initial inoculum, since the largest bacterial counts we observed were $\sim 10^5$ CFU in kidneys and $\sim 10^7$ CFU in bladders. Thus, the relevance of our results with a higher inoculum deserves further investigations. Second, the CFU counts obtained before therapy in kidneys and bladders tended to be lower for *qnr*-containing strains than those for the parenteral strain or the derivative harboring a single *gyrA* mutation. To investigate that, we have performed competition studies in the same model, between the susceptible parental strain and each of the resistant derivative strains, in a 1/1 ratio. Results confirmed that the *qnr*-containing strains lost most of the competitions, while there was no difference for the *gyrA* derivative (data not shown). These data suggest a decrease in fitness for the strains harboring *qnr* but not for the strains with a *gyrA* mutation.

Our therapeutic results were obtained with a ciprofloxacin regimen, producing C_{max} and AUC_{0-24} values in plasma that are in the lower range of those observed in humans with a ciprofloxacin dosing regimen of 500 mg twice a day given orally (14). In contrast, the proportion of active unbound ciprofloxacin was higher in mice than in humans, since the level of serum protein binding of ciprofloxacin is near 0% (38) at this range of ciprofloxacin concentrations in mice and ranges from 30% to 40% in humans (14, 37). The efficacy of ciprofloxacin treatment in mice infected with the parental quinolone-susceptible *E. coli* strain (CFT073-RR) and the reduced activity in mice infected with *E. coli qnr*-positive transconjugants can be explained by pharmacodynamic-pharmacokinetic indexes. In vivo, the parameters that best predict fluoroquinolone efficacy are the AUC_{0-24}/MIC ratio (11) and the C_{max}/MIC ratio (30). Values higher than 30 to 125 and 10 to 12, respectively, have been shown to be predictive of clinical and microbiological efficacy in different foci of infection (11, 30). In our experiments, the AUC_{0-24}/MIC ratio of ciprofloxacin was higher than 125 for the susceptible *E. coli* strain CFT073-RR, whereas this ratio was below 30 for the three derivative strains. Likewise the C_{max}/MIC ratio was widely higher than 12 for *E. coli* CFT073-RR and was below 10 for the three derivative strains.

In vitro, we showed that, at a concentration of 0.5 μg/ml, which is a concentration under the susceptibility breakpoint value for ciprofloxacin against *Enterobacteriaceae* (6) and one that is achievable in human serum during therapy with cipro-

floxacin (14), bactericidal activity of ciprofloxacin was significantly decreased during the first 6 hours of exposure against the derivative strains harboring plasmid pQnrA1 or pQnrS1 and against *E. coli* CFT073-RR-*gyrA* in comparison with the susceptible *E. coli* CFT073-RR parental strain. Jacoby (17) observed similar results with *E. coli* J53, *E. coli* J53 with a plasmid carrying a *qnrA1* gene, and *E. coli* J53 with a single mutation in *gyrA*. In contrast, at a concentration of four times the MIC of each strain, we did not find any significant difference in the bactericidal activity of ciprofloxacin against the parental and derivative strains, as shown by Mammeri et al. (24). The ciprofloxacin MPC was at least 20 times higher for the *qnr* transconjugants and for the *gyr*A mutant than for the parental strain, as previously reported (4, 35). However, MPC/MIC ratios remained similar for the four strains. This is in agreement with a similar proportion of resistant mutants for the four strains when exposed to a concentration of ciprofloxacin equal to four times the MIC. Acquisition of *qnr* genes has been reported to favor the selection of *gyrA* and *parC* mutations during a treatment with a fluoroquinolone and to select strains with higher levels of fluoroquinolone resistance (25, 28). In vivo, we have not detected ciprofloxacin-resistant mutants after antibiotic exposure. This result is probably due to the relatively low level of inoculum in our experimental model of UTI, which made the selection of resistant mutants unlikely.

The prevalence of microorganisms harboring the *qnr* gene is high, from 2% to 32% in *Enterobacteriaceae* resistant to expanded-spectrum cephalosporins (2, 7, 18, 21, 31, 33). The genes encoding Qnr proteins are usually located in multidrug-resistant, transferable plasmids. The antimicrobial treatment for the *Enterobacteriaceae* harboring these plasmids is often restricted to carbapenems and fluoroquinolones. Detection of strains with only one mechanism of resistance to quinolones, such as a single gyrase mutation, may benefit from susceptibility testing with quinolone nalidixic acid. In contrast, in strains harboring only *qnr* genes, the increase in MIC is lower for nalidixic acid than for fluoroquinolones, and fluoroquinolone MICs remain below the susceptibility breakpoints (6, 19).

In conclusion, our results suggest that low-level fluoroquinolone resistance conferred by *qnr* genes or a *gyrA* mutation is associated with a reduced bactericidal activity of ciprofloxacin in vivo. Therefore, detection of a mechanism of fluoroquinolone resistance resulting in a fluoroquinolone MIC below but close to breakpoint may become necessary for the treatment of severe infections due to *Enterobacteriaceae*.

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