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In vitro activity of prulifloxacin against *Escherichia coli* isolated from urinary tract infections and the biological cost of prulifloxacin resistance

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Abstract

Minimum inhibitory concentrations (MICs) and mutant prevention concentrations (MPCs) of prulifloxacin against 30 strains of *Escherichia coli* isolated from urinary tract infections as well as the 'biological cost' related to acquisition of resistance to the same drug in 10 uropathogenic *E. coli* were assessed. In terms of MIC_{90}, prulifloxacin was more potent than ciprofloxacin and levofloxacin. Prulifloxacin produced lower or equal MPC values than the other two fluoroquinolones (93.3% and 73.3% compared with levofloxacin and ciprofloxacin, respectively). Compared with susceptible strains, prulifloxacin-resistant mutants showed a reduced rate of growth (ranging from 20.0% to 98.0% in different culture media and incubation conditions) and a decreased fitness index (ranging from 0.959 to 0.999). They were also impaired in their ability to adhere to uroepithelial cells and urinary catheters (11.7–66.4% and 16.3–78.3% reduction, respectively) and showed a lower surface hydrophobicity (51.2–76.0%). They were more susceptible to ultraviolet irradiation (30.6–93.8% excess mortality), showed increased resistance to colicins and diminished transfer of plasmids (<1–8.5×10^{-8} vs. 3.3×10^{-7}–2.4×10^{-4}). Synthesis of haemolysin and type I fimbriae and production of flagella were also adversely affected. This study demonstrates a strict relationship between acquisition of prulifloxacin resistance and loss of important virulence traits. In this transition, *E. coli* pays a severe biological cost that entails a general reduction of fitness, thus compromising competition with susceptible wild-type strains in the absence of the drug.

Keywords: *Escherichia coli*; UTI; Antimicrobial resistance; Fluoroquinolones; Fitness

1. Introduction

Uncomplicated urinary tract infections (UTIs) are among the most prevalent infectious diseases mainly affecting women. Between one-quarter and one-half of all women experience a UTI at some time [1]. The aetiology (>80% of cases are caused by *Escherichia coli*) and treatment of lower UTI has changed little in recent years. However, the management of UTI is becoming complicated by increasing rates of resistance to several drugs among the most common uropathogens [2].

In *E. coli*, ampicillin and sulfamethoxazole/trimethoprim (co-trimoxazole) resistance rates have reached 20–50% worldwide, whilst increasing percentages of ciprofloxacin-resistant mutants have been observed in Spain, Portugal and Italy [3]. Given this situation, interest in prulifloxacin, a newly introduced fluoroquinolone that can be prescribed for the treatment of UTIs, has increased. Indeed, Montanari et al. [4] have demonstrated that this antibiotic encompasses all common uropathogens within its spectrum.

The aims of the present study were to compare the in vitro activity of prulifloxacin and other fluoroquinolones in terms of minimum inhibitory concentrations (MICs) and mutant prevention concentrations (MPCs) and to evaluate the 'biological cost' related to acquisition of prulifloxacin resistance in *E. coli* strains isolated from complicated and uncomplicated UTIs.

Previous studies have shown that when a microorganism becomes fluoroquinolone resistant through one or more mutational events, it may present reduced fitness compared with the original strain [5–9].

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2. Materials and methods

2.1. Bacterial strains

Forty isolates of *E. coli* were studied, 20 isolated from patients with uncomplicated UTIs and 20 from complicated UTIs. Strains were identified using the API system (bioMérieux, Rome, Italy).

2.2. Antimicrobial susceptibility tests

MICs were determined by the microdilution method in cation-adjusted Mueller–Hinton broth according to Clinical and Laboratory Standards Institute (CLSI) guidelines [10]. *Escherichia coli* ATCC 25922 was included in each run as a control. The following antimicrobial agents were assayed: prulifloxacin (Angelini ACRAF, Aprilia, Italy); levofloxacin, ciprofloxacin, fosfomycin/trometamol and cefuroxime/axetil (obtained from their respective manufacturers); and ampicillin, amoxicillin/clavulanic acid, co-trimoxazole and nitrofurantoin (from commercial sources). Strains were defined as antibiotic susceptible or resistant in accordance with CLSI guidelines [11].

2.3. MPC

MPC tests were performed as described by Roveta et al. [12]. Thirty strains of *E. coli* were studied, 15 isolated from uncomplicated UTIs and 15 from complicated UTIs. For each strain, the MPC was determined in at least three independent experiments. Variability between experiments did not exceed one concentration step (doubling dilution). The MPC was determined for levofloxacin, ciprofloxacin and prulifloxacin. *Escherichia coli* isolates showing chromosomal or plasmid-mediated resistance to antimicrobial agents used for treatment of urinary infections (ampicillin, amoxicillin/clavulanic acid, cefuroxime, co-trimoxazole, nitrofurantoin and fosfomycin/trometamol) were included.

2.4. Stepwise selection of prulifloxacin-resistant mutants

Ten isolates of *E. coli* were tested, five from uncomplicated UTIs and five from complicated UTIs. Several independent cultures of the strains were grown overnight in Luria broth (LB). From each culture, ca. 10^8 colony-forming units (CFU) were spread on Luria agar (LA) plates containing, for the first step selection, prulifloxacin at 1× MIC. Plates were incubated overnight at 37 °C. Colonies were counted, picked and streaked for the selection of single colonies on the selective medium incorporating the drug [9]. Successive stepwise selections were continued until the strains were able to grow at a concentration of 1 mg/L. After that, further mutants with levels of resistance of 2, 4 and 8 mg/L (corresponding to prulifloxacin MICs of 4, 8 and 16 mg/L, respectively) were obtained through inoculation of a single colony in prulifloxacin-containing plates.

2.5. Rate of growth in rich medium, minimal medium, urine and in the presence of bile salts under aerobic or anaerobic conditions

Flasks containing 50 mL of fresh tryptic soy broth, minimal medium, artificial urine and 0.5% bile salts (a concentration that can be reached in the intestinal tract [13]) were inoculated with 0.5 mL of an overnight broth culture of the pathogen to be tested. Subcultures were incubated under aerobic and anaerobic conditions. Growth was monitored spectrophotometically at 620 nm. Every 2 h, appropriate dilutions of cultures were plated on brain–heart agar (BHA). After overnight incubation at 37 °C, colonies were counted and the generation time was calculated.

2.6. Measurement of fitness in vitro

To determine the biological cost associated with different levels of resistance to prulifloxacin in vitro, each prulifloxacin-resistant mutant was competed against the wild-type strain in artificial urine. The experiment was carried out as described by Komp Lindgren et al. [9]. Each competition was initiated from two overnight cultures grown in urine, diluted in 0.9% NaCl and mixed together at a cell density of ca. 5000 CFU/mL in 2 mL of urine. The tubes were incubated overnight to complete one cycle of competition. After each competition cycle, the mixed culture was serially diluted and plated onto LB and LB plus 100 μg/mL rifampicin. This level of rifampicin was determined empirically to inhibit the growth of prulifloxacin-susceptible isolates. Successive cycles of growth competition were performed by transferring ca. 5000 CFU on each day from the competition tube to 2 mL of urine and performing another overnight incubation. Each experiment was repeated for five cycles. The in vitro selection coefficient (S) per generation of each mutant was calculated as: [(number of mutant cells/number of wild-type cells)/(number of generations/number of cycles)] × ln 2. Relative fitness (with respect to that of the starting strain) was defined as $1 + S$.

2.7. Adhesion to uroepithelial cells

Adhesion experiments were carried out as described by Ofek et al. [14] and Li Pira et al. [15]. Briefly, ca. 5×10^8 bacteria were incubated with a suspension of uroepithelial cells (1:100–1:1000) at 37 °C for 90 min. Microorganisms that failed to adhere to epithelial cells were washed out by centrifugation in phosphate-buffered saline (PBS). After Gram staining, 20 epithelial cells from each sample were examined microscopically and the mean number of adherent bacteria per cell was determined. Student's t-test was used to calculate P-values. Differences in adherence efficiency were considered significant at $P < 0.05$.

2.8. Adhesion to urinary catheters

Adhesion experiments were carried out as described by Marchese et al. [16]. Briefly, silicone-treated latex catheters (Foley catheter) were filled with bacterial suspension (10^8 CFU/mL) for 48 h at 37 °C to allow microorganisms to adhere to the inner surface. Catheters were then washed in PBS with a peristaltic pump to eliminate bacteria that failed to attach. Adherent bacteria were obtained from the inner surface of catheters after sonication in PBS. The suspensions were serially diluted and plated onto rich solid medium (BHA) and incubated overnight at 37 °C. The number of CFU/mL was finally evaluated. Student's *t*-test was used to calculate *P*-values. Differences in adherence efficiency were considered significant at $P < 0.05$.

2.9. Susceptibility to ultraviolet (UV) radiation

To test the effect of UV light on susceptible and isogenic prulifloxacin-resistant mutants, adequate dilutions of bacterial suspensions in minimal medium M9 (Na_2HPO_4 40 mM, KH_2PO_4 20 mM, NaCl 9 mM, NH_4Cl 20 mM), supplemented with 20% glucose and 80 mg/L amino acids, were plated and exposed to UV light for 20 s. After incubation at 37 °C for 18–24 h, the number of survivors was determined [17].

2.10. Presence of receptors for phage T7

The presence of phage receptors was evaluated by testing the susceptibility of the strains to a phage suspension employing the plaque assay as described by Miller [17]. Suitable dilutions of ca. 10^5 phage particles were absorbed to ca. 10^8 bacteria for 2 h. Then, 2.5 mL of molten H-top agar was added to each sample and immediately poured over the surface of an H agar plate. Lytic plaques were counted after overnight incubation at 37 °C.

2.11. Susceptibility to and synthesis of colicins

Susceptibility to and synthesis of colicins by prulifloxacin-susceptible and their isogenic prulifloxacin-resistant strains were tested using the method of Debbia and Pruzzo [18]. A suspension of colicin-producing *Klebsiella pneumoniae* was spotted onto a LA plate. Then, a suspension of the strains under study mixed with molten H-top agar was poured on the plate. After overnight incubation at 37 °C, the presence or absence of an inhibition halo around the spot was interpreted as susceptibility or resistance to colicins, respectively.

2.12. Efficiency of F123 Km^R plasmid transfer by prulifloxacin-resistant E. coli strains

The efficiency of F123 Km^R plasmid transfer by prulifloxacin-susceptible *E. coli* strains and by their respective prulifloxacin-resistant mutants was assessed by the methodology described by Debbia et al. [19]. Briefly, log phase cultures (2×10^8 CFU/mL) of donor (1 mL) and recipient (1 mL) strains cultured in LB medium were mixed and incubated at 37 °C for 60 min, then washed by centrifugation and plated on selective media. After 48 h incubation at 37 °C, colonies were counted and picked.

2.13. Production of haemolysin

Haemolysis was defined as a distinct zone of clearing around or under isolated bacterial colonies after overnight incubation at 37 °C on tryptic soy agar containing 5% sheep blood [20].

2.14. Production of type 1 fimbriae

Prulifloxacin-susceptible and isogenic prulifloxacin-resistant *E. coli* strains were cultured in LB and on LA for 18 h at 37 °C. Bacteria re-suspended from the plate and from broth were adjusted in PBS to a concentration of 10^9 CFU/mL and assayed for expression of type 1 fimbriae by haemagglutination. Bacterial suspensions were serially diluted two-fold in round-bottomed, 96-well microtitre plates in duplicate. Equal volumes of 3% (v/v) horse erythrocytes were mixed with bacterial suspensions. Mannose was added to 50 mM final concentration to one row of the duplicated serial dilutions for each bacterial growth condition. Non-agglutinated horse erythrocytes formed tight buttons of cells at the bottom of the plate well, whereas agglutinated erythrocytes formed a diffuse mat of cells across the bottom of the well [21].

2.15. Motility assay

Rates of swarming were measured by inoculating tryptone agar plates with 1 microlitre of a 1/100 dilution of a fresh saturated tryptone broth culture and incubating the plates at 32 °C for 18 h, as described by Tang and Blair [22]. Each strain was subjected to three separate assays. The diameter of the circular zone of growth was measured and expressed as a mean value.

2.16. Surface hydrophobicity

Surface hydrophobicity was measured as the partition coefficient of a suspension of pathogens in water and organic solvent, as detailed by Reifsteck et al. [23]. Briefly, various amounts of an organic solvent (xylene) were added to the bacterial suspension ($2–4 \times 10^8$ CFU/mL) and then vortex mixed for 30 s. The tubes were allowed to stand for 20 min. After the xylene phase had separated from the aqueous phase, the optical density of the aqueous phase was determined spectrophotometrically at 600 nm. The increase in hydrophobicity produced a reduction in the turbidity of aqueous phase.

Table 1
Susceptibility to fluoroquinolones of 30 *Escherichia coli* strains isolated from uncomplicated and complicated urinary tract infections (UTIs)

Strains	MIC (mg/L)								
	Ciprofloxacin			Levofloxacin			Prulifloxacin		
	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Uncomplicated UTI ($n = 15$)	0.002–0.06	0.015	0.06	0.015–0.06	0.03	0.06	0.008–0.03	0.015	0.015
Complicated UTI ($n = 15$)	0.008–0.06	0.015	0.06	0.015–0.06	0.03	0.06	0.008–0.015	0.015	0.015

MIC, minimal inhibitory concentration.

3. Results

3.1. Antimicrobial susceptibility tests

Prulifloxacin showed greater activity against all *E. coli* isolates studied in terms of MIC values compared with ciprofloxacin and levofloxacin. MICs against *E. coli* isolated from uncomplicated UTIs were 0.002–0.06 mg/L for ciprofloxacin, 0.015–0.06 mg/L for levofloxacin and 0.008–0.03 mg/L for prulifloxacin. Both ciprofloxacin and prulifloxacin showed the same MIC_{50} value (0.015 mg/L), but prulifloxacin had a lower MIC_{90} (0.015 mg/L) than ciprofloxacin (0.06 mg/L). MIC_{50} and MIC_{90} values of levofloxacin (0.03 mg/L and 0.06 mg/L, respectively) were higher than those of prulifloxacin. *Escherichia coli* isolated from complicated UTI gave similar results (Table 1).

3.2. MPC

Among the members of the fluoroquinolone class assessed, prulifloxacin showed lower or equal MPC values compared with the other two fluoroquinolones (93.3% and 73.3% compared with levofloxacin and ciprofloxacin, respectively). Fig. 1 shows the different MPC distributions

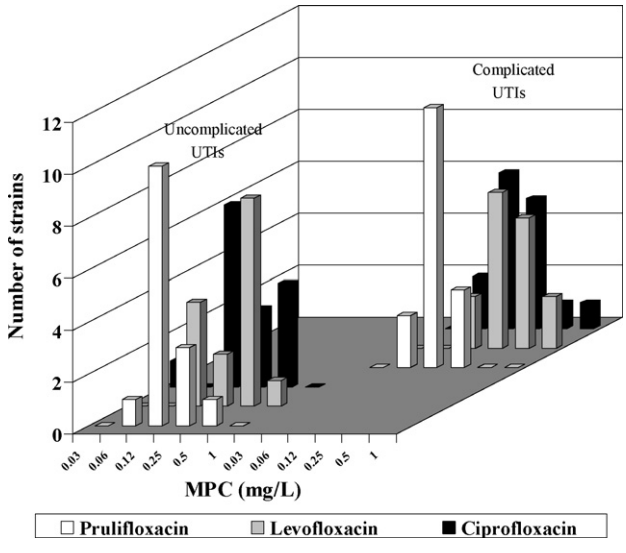

Fig. 1. Mutant prevention concentration (MPC) of 30 *Escherichia coli* strains isolated from uncomplicated and complicated urinary tract infections (UTIs).

obtained for prulifloxacin, levofloxacin and ciprofloxacin in *E. coli* derived from uncomplicated and complicated UTIs. Most isolates studied showed an MPC/MIC ratio more favourable to prulifloxacin irrespective of their origin (uncomplicated or complicated UTIs). Moreover, 70.0% (21/30) had a prulifloxacin MPC/MIC ratio higher than or equal to that of levofloxacin and ciprofloxacin. A similar situation occurred for six *E. coli* isolates showing resistance to several antimicrobial agents commonly used in the treatment of UTIs (data not shown).

3.3. Rate of growth under aerobic or anaerobic conditions in rich medium, minimal medium, urine and in the presence of bile salts

All resistant strains tested showed a significant reduction in their rate of growth compared with the susceptible parental strains, independent of culture media and incubation conditions (Fig. 2).

Under the different experimental conditions studied, the rate of growth was reduced by 20.6–95.0%, 20.6–98.0% and 20.0–95.0% for *E. coli* resistant to 2, 4 and 8 mg/L of prulifloxacin, respectively. The generation time varied from 24–120 min (susceptible strains) to 25.7–180 min (strains resistant to 2 mg/L), 27.7–180 min (resistant to 4 mg/L) and 30–180 min (resistant to 8 mg/L) (data not shown). Anaerobiosis, presence of bile salts and artificial urine were the environmental conditions that most affected the rate of growth.

3.4. Measurement of fitness in vitro

All mutants showed a reduction of fitness (Table 2) directly related to the levels of resistance: 0.987–0.999, 0.974–0.998 and 0.959–0.998 for mutants able to grow at 2, 4 and 8 mg/L of prulifloxacin, respectively.

Table 2
Relative fitness of prulifloxacin-resistant (Pru^R) *Escherichia coli* strains in comparison with their prulifloxacin-susceptible (Pru^S) parental strains

Strain (level of resistance)	Relative fitness (range)
Pru^S	1.000
Pru^R (2 mg/L)	0.987–0.999
Pru^R (4 mg/L)	0.974–0.998
Pru^R (8 mg/L)	0.959–0.998

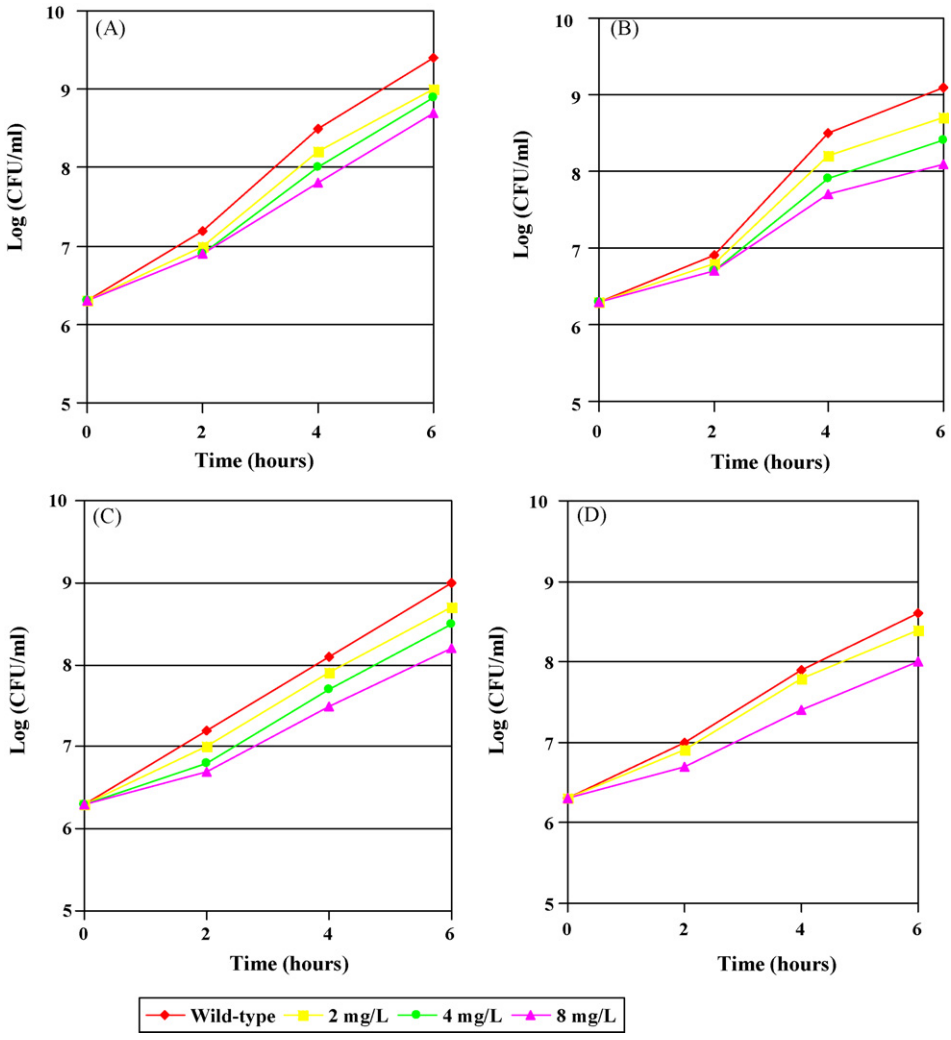

Fig. 2. Rate of growth of a prulifloxacin-susceptible *Escherichia coli* strain compared with its isogenic prulifloxacin-resistant strains (minimal inhibitory concentrations of 4, 8 and 16 mg/L) in different growth media: (A) rich medium; (B) minimal medium; (C) 0.5% bile salts; and (D) artificial urine. CFU, colony-forming units.

3.5. Adhesion to uroepithelial cells

All prulifloxacin-resistant mutants were less able to adhere to uroepithelial cells than the wild-type isogenic strains. Reduction of adhesion was directly proportional to increased levels of resistance: 11.7–58.8%, 24.5–60.0% and 36.0–66.4% for isolates inhibited by 4, 8 and 16 mg/L of prulifloxacin, respectively (Table 3).

Table 3
Adhesion to uroepithelial cells and urinary catheters of prulifloxacin-resistant (Pru^R) *Escherichia coli* isolates compared with their prulifloxacin-susceptible parental strains

Strain (level of resistance)	Adhesion reduction (%)	
	Uroepithelial cells	Urinary catheters
Pru^R (2 mg/L)	11.7–58.8	16.3–57.0
Pru^R (4 mg/L)	24.5–60.0	37.8–73.2
Pru^R (8 mg/L)	36.0–66.4	60.5–78.3

3.6. Adhesion to urinary catheters

All resistant isolates tested were impaired in their ability to adhere to urinary catheters compared with their prulifloxacin-susceptible parental strains. Reduction depended on the level of prulifloxacin resistance. The most evident effect was observed in *E. coli* mutants characterised by the highest level of resistance to prulifloxacin (MIC = 16 mg/L) (60.5–78.3%) (Table 3).

3.7. Susceptibility to UV radiation

The 10 prulifloxacin-resistant strains studied were less resistant to UV radiation than their susceptible isogenic counterparts. For the mutants displaying different levels of prulifloxacin resistance (MICs of 4, 8 and 16 mg/L) the reduction in the number of viable cells was 30.6–80.0%, 46.6–88.2% and 68.3–93.8%, respectively, compared with 32.5–78.8% for the susceptible organisms (Table 4).

Table 4
Susceptibility to ultraviolet radiation of prulifloxacin-resistant (Pru^R) *Escherichia coli* strains and their prulifloxacin-susceptible (Pru^S) parental strains expressed as percentage mortality of irradiated bacteria

Strain (level of resistance)	Mortality (%) (range)
Pru^S	32.5–78.8
Pru^R (2 mg/L)	30.6–80.0
Pru^R (4 mg/L)	46.6–88.2
Pru^R (8 mg/L)	68.3–93.8

3.8. Receptors for phage T7

No significant differences in the susceptibility to phage T7 were observed between the prulifloxacin-resistant isolates and their relative wild-type counterparts. The expression of receptors for this phage on the surface of uropathogenic *E. coli* remained the same irrespective of the level of fluoroquinolone resistance.

3.9. Susceptibility to and synthesis of colicins

All strains studied were originally susceptible to colicins. However, the 10 prulifloxacin-resistant *E. coli* studied were completely resistant to colicins, independent of their level of resistance to fluoroquinolones. In addition, no *E. coli* isolates able to synthesise colicins were found.

3.10. Efficiency of F123 Km^R plasmid transfer by prulifloxacin-resistant E. coli strains

The frequencies of recombination obtained in mating experiments showed that prulifloxacin-resistant strains, when acting as donors, transferred the F123 Km^R plasmid with a lower frequency than their isogenic prulifloxacin-susceptible isolates ($<1–8.5 \times 10^{-8}$ vs. $3.3 \times 10^{-7}–2.4 \times 10^{-4}$) (Table 5).

3.11. Production of haemolysin

Total inhibition of haemolysin production was visually observed only in prulifloxacin-resistant mutants able to grow at a concentration of 8 mg/L. No change was noticed in mutants refractory to lower concentrations of prulifloxacin (Fig. 3).

3.12. Production of type 1 fimbriae

Only 7 of 10 prulifloxacin-susceptible *E. coli* isolates studied were able to produce type I fimbriae. No prulifloxacin-resistant mutants were able to produce type I fimbriae, independent of the level of resistance to the drug.

Table 5
Efficiency of plasmid transfer in prulifloxacin-resistant (Pru^R) *Escherichia coli* isolates and in their prulifloxacin-susceptible (Pru^S) parental strains

Phenotype	No. of recombinants (CFU/mL) (range)	Efficiency of plasmid transfer (range)
Pru^S	$4 \times 10–7.8 \times 10^3$	$3.3 \times 10^{-7}–2.4 \times 10^{-4}$
Pru^R	$<10–1.5 \times 10^3$	$<1–8.5 \times 10^{-8}$

CFU, colony-forming units.

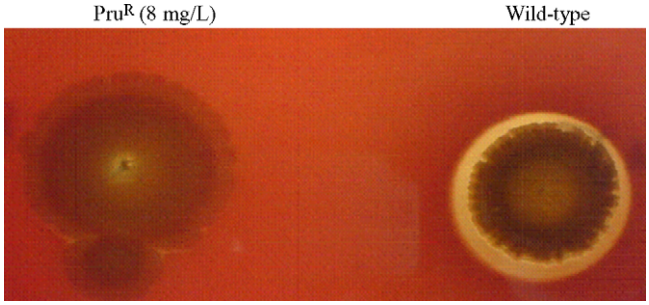

Fig. 3. Inhibition of haemolysin production in an *Escherichia coli* strain resistant to prulifloxacin (at 8 mg/L) (left) in comparison with the wild-type strain (right).

3.13. Motility assay

All strains analysed showed reduced motility. This feature was directly related to the level of resistance to prulifloxacin. The decrease was lower (21.6%) for organisms showing low-level resistance (2 mg/L), higher (40.0%) for strains with an intermediate level of resistance (4 mg/L) and maximal (68.2%) for those strains able to grow at a concentration of 8 mg/L (Table 6).

3.14. Surface hydrophobicity

All prulifloxacin-susceptible isolates studied showed a lower absorbance of the aqueous phase than their resistant counterparts. Consequently, the reduction of hydrophobicity was higher for strains resistant to prulifloxacin (51.2–76.0%, 51.3–70.0% and 55.8–75.8% for organisms refractory to concentrations of 2, 4 and 8 mg/L, respectively) compared with the values shown by the susceptible organisms (41.3–62.3%) (Fig. 4; Table 7).

Table 6
Reduction in motility of prulifloxacin-resistant (Pru^R) *Escherichia coli* strains compared with their prulifloxacin-susceptible parental isolates

Strain (level of resistance)	Mean reduction in motility (%)
Pru^R (2 mg/L)	21.6
Pru^R (4 mg/L)	40.0
Pru^R (8 mg/L)	68.2

Table 7
Hydrophobicity reduction in prulifloxacin-resistant (Pru^R) and prulifloxacin-susceptible (Pru^S) *Escherichia coli* isolates

Strain (level of resistance)	Mean reduction in hydrophobicity (%)
Pru^S	41.3–62.3
Pru^R (2 mg/L)	51.2–76.0
Pru^R (4 mg/L)	51.3–70.0
Pru^R (8 mg/L)	55.8–75.8

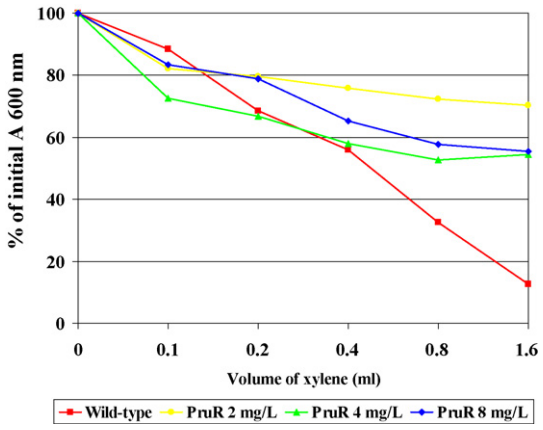

Fig. 4. Reduction of hydrophobicity in a prulifloxacin-resistant (Pru^R) *Escherichia coli* (levels of resistance of 2, 4 and 8 mg/L) compared with its wild-type prulifloxacin-susceptible parental strain.

4. Discussion

Our data indicate that prulifloxacin, a new fluoroquinolone recently introduced in Italy, displays an in vitro potency superior (in terms of MIC_{90}) by a factor of at least two to that of the other quinolones tested, thus confirming the report previously published by Montanari et al. [4].

The MPC has recently emerged as a new parameter that may help in the selection of a specific drug within a selected class of antimicrobials where the mechanism of acquisition of resistance is well established [24–27]. The basic finding emerging from application of this principle is that a direct correlation between MIC and MPC does not always exist because of the complex variables linked to the pharmacokinetic behaviour of each compound [26]. However, it is to be expected that the most potent molecule (lowest MIC) will also be the least likely to evoke selection of resistant mutants (lowest MPC and lowest MPC/MIC ratio). This study demonstrates that prulifloxacin has the lowest MIC and MPC values against *E. coli* strains both causing complicated and uncomplicated UTIs, irrespective of their resistance patterns.

The present data also confirm a strict relationship between acquisition of prulifloxacin resistance and loss of important traits conferring bacterial virulence, demonstrating that this phenomenon causes profound alterations in bacterial physiology, reducing bacterial fitness. Resistant mutants are characterised by a slower rate of growth compared with the sensitive parental strains. Impairment of the rate of multiplication has also been confirmed in different experimental (rich and minimal medium, presence of urine and bile salts) and incubation conditions (aerobiosis and anaerobiosis), mimicking situations that might be encountered naturally by bacteria in their host and in the environment.

All resistant strains studied showed increased susceptibility to UV radiation. This behaviour could be the result of alterations in some aspects of bacterial physiology of the mutants. The activity of the SOS system, dedicated to repair UV damage, could be diminished in isolates with acquired prulifloxacin resistance. The SOS response is a strictly energy-dependent process and when intracellular concentrations of ATP are low the SOS activity is impaired, causing a different degree of susceptibility to UV light [28].

A reduction of adhesion to uroepithelial cells as well as to urinary catheters was also detected in all prulifloxacin-resistant mutants. This behaviour is of clinical importance since long-term indwelling catheters usually cause recurrent infections due to uropathogens that, through a process of adhesion, form difficult to eradicate biofilms [29]. Prulifloxacin-resistant microorganisms, even if selected, cannot make full use of the biofilm environment. Reduced adhesion to uroepithelial cells may be attributed to the profound modification of the main adherence ligands present on these strains as also suggested by defective production of type I fimbriae and by concomitant reduction of surface hydrophobicity. Thus, structural modifications of the cell surface may explain the reduced adherence to urinary catheters described in this study.

The fact that prulifloxacin resistance affects some important physiological functions of *E. coli* is also confirmed by the significant reduction in swarming observed in mutants. Together with adhesiveness, motility is correlated with pathogenicity, since inhibition of motility also reduces the formation of new colonies and the spread from the portal of entry of infection [30].

Prulifloxacin-resistant strains appear to be more resistant to colicins than their isogenic isolates. This could be due to modifications of the outer cell membrane, resulting in alteration of the expression of receptors for colicins.

Isolates of reduced susceptibility to prulifloxacin showed reduced transfer of plasmids. This feature may hinder the spread of resistance elements or other genes with adaptive mutations that are known to contribute to survival of bacterial populations [31].

The decreased production of haemolysin and the sluggish motility displayed by prulifloxacin-resistant mutants represent a further reduction of virulence traits. Andreu et al. [32] reported haemolytic activity in 57% and 55% of *E. coli* isolates causing pyelonephritis and cystitis, respectively, in patients with a normal urinary tract anatomy. Haemolysin contributes to tissue injury through cytolytic effects, thus promoting bacterial invasion and persistence of the microorganism in the host. Death of cells and the ensuing damage to tissues provides the microorganisms with a source of iron and may also destroy phagocytes, sparing pathogens from ingestion.

In conclusion, the present results show that prulifloxacin resistance has a high biological cost, entailing a generally reduced fitness that might compromise competition with the normal microflora both in the human host and in the environment. Resistant bacteria are seemingly compelled to shut down or to downregulate non-essential metabolic pathways and this may in turn lead to a reduction in the expression of several genes, including determinants of pathogenicity. Prulifloxacin-resistant strains are therefore impaired bacteria,

unable to express the potential to maintain or initiate infections since they are less adherent and have a slower growth rate and are therefore more likely to be washed out, displaced and overcome once in the environment. These limitations are likely to affect their ability to thrive, spread and initiate infection.

Because of the increasing resistance rates among *E. coli* to several antibiotics commonly prescribed for UTIs observed worldwide, there is an urgent need for new active molecules.

Lack of susceptibility to ampicillin and co-trimoxazole is a common finding, irrespective of the geographic area considered, whilst resistance to other antimicrobials is gaining momentum in several countries. Complicated and uncomplicated UTIs that have failed previous courses of therapy or that occur in environments where resistance rates are high may represent preferential targets for the appropriate usage of fluoroquinolones, as suggested by international guidelines [1,33,34]. In this connection, prulifloxacin is the drug of choice within the fluoroquinolone class of agents because of its potent and wide coverage of major uropathogens, low MPC/MIC ratios that render selection of refractory strains improbable, high biological cost related to acquisition of resistance in the very rare mutants that eventually arise, and strongly diminished virulence of these mutants. Recent clinical studies [35] confirm the value of this new therapeutic option.

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