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Microbiological rationale for the utilisation of prulifloxacin, a new fluoroquinolone, in the eradication of serious infections caused by *Pseudomonas aeruginosa*

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Abstract

Minimal inhibitory concentrations (MICs) of prulifloxacin were evaluated in comparison with ciprofloxacin, levofloxacin and moxifloxacin against a large collection ($N = 300$) of *Pseudomonas aeruginosa* strains characterised according to the CLSI/NCCLS microdilution method. Additional in vitro tests (time–kill curves and mutant prevention concentration (MPC) determinations) were carried out. Assuming a susceptibility breakpoint for prulifloxacin identical to that of ciprofloxacin, the new fluoroquinolone emerged as the most potent antibiotic (72% of susceptible strains versus 65%, 61% and 23% for ciprofloxacin, levofloxacin and moxifloxacin, respectively). Time–kill tests at 4× MIC confirmed the pronounced bactericidal potency of the drug against *P. aeruginosa*. Amongst the members of the fluoroquinolone class assessed, prulifloxacin produced the lowest MPC values ($\leq$4 mg/L). Our in vitro results indicate that prulifloxacin represents the most powerful antipseudomonal drug available today.

Keywords: Time–kill; Mutant prevention concentration; Susceptibility tests; Cystic fibrosis

1. Introduction

Pseudomonas aeruginosa is an efficient opportunistic pathogen that causes a wide range of acute and chronic infections and represents a frequent cause of morbidity and mortality in hospitalised patients [1]. The conditions produced encompass serious pneumonia in mechanically ventilated individuals and other immunocompromised hosts, bacteraemia, and urinary tract, skin and skin structure and other infections [2]. Additionally, *P. aeruginosa* is the most prevalent agent involved in pulmonary infections in cystic fibrosis (CF) [3]. Owing to its ubiquitous presence, its natural lack of susceptibility to several antimicrobial drugs and its ability to develop acquired resistance to most commonly used antibiotics, *P. aeruginosa* continues to represent a therapeutic challenge. Among the classes of drugs currently available for the eradication of *P. aeruginosa* infections, alone or in combination [4], a prominent role is played by the fluoroquinolones, whose usage has also been extended to paediatric patients with CF [5]. However, a worldwide decline in the susceptibility pattern displayed towards ciprofloxacin, the most potent among the congener drugs, has recently been reported for this pathogen [6–8]. It is therefore with considerable interest that the introduction of a new fluoroquinolone prulifloxacin [9–11], the prodrug of ulifloxacin, whose activity against *P. aeruginosa* has been assessed by Montanari et al. [12], has been met by the medical community. Since the in vitro potency of prulifloxacin was found to exceed that of ciprofloxacin on the limited number of strains tested in that study, we have expanded the aim of the present research to analyse 300 well-characterised organisms originating from pulmonary (including CF), bloodstream and urinary nosocomial infections.

Comparative determination of minimal inhibitory concentrations (MICs), time–kill kinetics and mutant prevention concentrations (MPCs) clearly establish prulifloxacin as the

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most active fluoroquinolone presently available against *P. aeruginosa*. In view of the results obtained, the place of the new drug in the treatment of serious infections caused by this pathogen is discussed and indications are proposed.

2. Materials and methods

2.1. Antimicrobials

The antibiotic powders used in this study were ulifloxacin (prulifloxacin active metabolite; Angelini, ACRAF S.p.A., Aprilia, Italy), levofloxacin (Aventis, Milan, Italy), ciprofloxacin and moxifloxacin (Bayer, Milan, Italy). Preparation of sterile stock solutions of the agents was performed in accordance with the instructions of the manufacturers.

2.2. Isolates

A total of 300 well-characterised *P. aeruginosa* were studied, including bacteria with multiple (more than three agents) resistance to primary antipseudomonal drugs. All strains were recent clinical isolates collected in our institution during 2004 and had known resistance phenotypes to the following antibiotics: piperacillin, piperacillin/tazobactam, ceftazidime, cefepime, aztreonam, imipenem, gentamicin, amikacin and ciprofloxacin. The microorganisms analysed included 100 mucoid and non-mucoid strains from CF patients, 50 strains from bloodstream infections (BSIs), 100 isolates from serious lower respiratory tract infections (LRTIs) and 50 pathogens from complicated nosocomial urinary tract infections (UTIs).

Pseudomonas aeruginosa was identified using commercial automated biochemical test systems (bioMérieux, Marcy l'Etoile, France).

2.3. Susceptibility tests

MICs of the antimicrobial agents were determined following the microdilution procedure suggested by the Clinical and Laboratory Standards Institute (CLSI) [13] using cation-adjusted Mueller–Hinton broth (Difco Laboratories, Milan, Italy) as test medium. Overnight cultures of bacteria were diluted to give a final concentration of ca. 5×10^5 cells/mL. Cultures were incubated for 30–45 min at 37 °C to re-establish log-phase growth. Samples were than added to equivalent volumes of the various concentrations of antibiotics distributed on a microplate and prepared from serial two-fold dilutions ranging from 0.015 μg/mL to 512 μg/mL. After 18–24 h of incubation at 37 °C, the concentrations of drugs that prevented visible growth were recorded as the MICs. *Pseudomonas aeruginosa* ATCC 27853 was included as the quality control. Susceptibility rates were calculated adopting CLSI [13] breakpoints, and the relevant value for prulifloxacin was identical to that employed for ciprofloxacin, as suggested by Montanari et al. [12,14].

2.4. Time–kill assays

Bactericidal activities of fluoroquinolones were assessed by employing the time–kill method. Time–kill studies were performed adopting standard procedures [15–17] using flasks containing 10 mL of log-phase bacterial cultures diluted to 10^6–10^7 cells/mL and previously grown at 37 °C in the test medium used in MIC assays. The drugs were added to the bacterial cultures at concentrations corresponding to 4× MIC. Drug-free flasks were included as controls and the cultures were incubated at 37 °C with shaking. Bacterial counts were carried out in triplicate just before the compounds were added (zero time) and at 2, 6 and 24 h by spreading aliquots of 0.1 mL of the suitable dilutions onto Mueller–Hinton agar plates and incubating for 24 h at 37 °C. Colony counts were performed and killing curves were plotted using the mean colony counts at each time point.

2.5. MPC determination

MPC tests were performed by plating 10^{10} cells [18,19] onto agar plates containing the selected drugs added in two-fold dilution concentration increments. A range of four drug concentrations was tested (1×, 2×, 4× and 8× MIC), and three additional drug concentrations were used (16×, 32× and 64× MIC) when necessary to define the MPC endpoint value. Following inoculation, plates were incubated at 37 °C and screened for growth at 24 and 48 h. The lowest drug concentration that allowed no growth was recorded as the MPC. For each strain, MPC was determined in at least three independent experiments. The variation between experiments did not exceed one concentration step.

3. Results

The susceptibility of this particular collection of 300 clinical isolates to primary antipseudomonal drugs is reported in Table 1. *Pseudomonas aeruginosa* with multiple resistance traits exceeded 45% of the organisms analysed.

Comparative activities of prulifloxacin, ciprofloxacin, levofloxacin and moxifloxacin are detailed in Table 2, whilst MIC distributions are presented in Fig. 1. Irrespective of the origin of the pathogen, prulifloxacin in general displayed significantly lower MIC values than those observed with all the comparator drugs studied. The incidence of susceptible strains was also correspondingly higher for prulifloxacin.

Against isolates from CF, which were the most susceptible among the organisms tested, the MIC of prulifloxacin ranged from 0.015 to 64 μg/mL, the MIC_{50} was 0.5 μg/mL and the MIC_{90} was 4.0 μg/mL. All other fluoroquinolones exhibited higher values, with ciprofloxacin displaying a MIC range of 0.03–128 μg/mL, levofloxacin 0.125–256 μg/mL and moxifloxacin 0.25–512 μg/mL. MIC_{50} and MIC_{90} were, respectively 1 μg/mL and 8 μg/mL for ciprofloxacin, 2 μg/mL and 16 μg/mL for levofloxacin, and 4 μg/mL and 32 μg/mL for

Table 1
Antibiotic susceptibility of the collection of 300 *Pseudomonas aeruginosa* isolates

Strain origin	PIP	TZP	CAZ	FEP	ATM	IPM	GEN	AMK	CIP	Multiresistant (%)
CF (n = 100)	53	70	70	71	49	74	45	32	62	58
LRTI (n = 100)	51	90	78	81	50	84	65	37	69	39
BSI (n = 50)	52	82	80	82	50	86	60	32	62	36
UTI (n = 50)	30	80	82	86	48	80	44	22	66	46
Total (N = 300)	48	80	76	78	49	80	54	38	65	46

CF, cystic fibrosis; LRTI, lower respiratory tract infection; BSI, bloodstream infection; UTI, urinary tract infection.
PIP, piperacillin; TZP, piperacillin/tazobactam; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; IPM, imipenem; GEN, gentamicin; AMK, amikacin; CIP, ciprofloxacin.

moxifloxacin. Susceptibility of this collection of strains to prulifloxacin was also higher (72%) than that observed with the other fluoroquinolones (ciprofloxacin 62%, levofloxacin 56%, moxifloxacin 10%). The potency patterns were similar when *P. aeruginosa* from UTIs were studied, although MIC_{90} values were higher (16 mg/L versus 32 mg/L, respectively, when only prulifloxacin and ciprofloxacin are considered). Pathogens from LRTIs and BSIs required higher fluoroquinolone levels to inhibit 90% of the strains, and the activities of prulifloxacin and ciprofloxacin, whist identical, were always superior to those shown by levofloxacin and moxifloxacin. Prulifloxacin activity was independent of the pattern of resistance displayed by *P. aeruginosa* to other antibiotics with different mechanisms of action.

Fig. 1 clearly indicates that the distribution of MIC values are consistently in favour of prulifloxacin, whose values are situated to the left of the lines produced by the comparative agents irrespective of the origin of the pathogen.

The results for prulifloxacin, ciprofloxacin and levofloxacin killing kinetic curves assessed against 15 organisms (3 mucoid and 3 non-mucoid isolates from CF; 3 from BSIs; 3 from LRTIs; and 3 from UTIs) are depicted in Fig. 2. As expected, the fluoroquinolones tested (excluding moxifloxacin) displayed a profound bactericidal effect on all *P. aeruginosa* strains, although the extent and speed of killing was highly variable depending on the origin of the isolate. CF strains were the most susceptible organisms. The effect of prulifloxacin on all strains studied was superior to that produced by the comparative agents.

Finally, MPC determinations were performed on the same 15 strains previously selected for killing kinetic studies (Fig. 3). Amongst the members of the fluoroquinolone class assessed, prulifloxacin produced the lowest MPC values, with 14 of the 15 pathogens analysed showing MPC $\leq$ 4 mg/L. At the same drug concentration, ciprofloxacin included 12 strains and levofloxacin only 1 strain.

Table 2
Comparison of minimal inhibitory concentration (MIC) values of prulifloxacin and three other fluoroquinolones on 300 *Pseudomonas aeruginosa* strains

Strain origin	Antimicrobial agent	MIC (μg/mL)			Susceptibility (%)
		Range	50%	90%	
CF (n = 100)	Prulifloxacin	0.015 to 64	0.5	4	72
	Ciprofloxacin	0.03 to 128	1	8	62
	Levofloxacin	0.125 to 256	2	16	56
	Moxifloxacin	0.25 to 512	4	32	10
LRTI (n = 100)	Prulifloxacin	0.03 to 128	0.25	16	74
	Ciprofloxacin	0.06 to 256	0.5	16	69
	Levofloxacin	0.125 to 256	1	32	65
	Moxifloxacin	0.5 to >512	2	64	29
BSI (n = 50)	Prulifloxacin	0.03 to 64	0.25	32	70
	Ciprofloxacin	0.03 to 128	0.5	32	62
	Levofloxacin	0.125 to 128	2	64	56
	Moxifloxacin	0.5 to 256	2	64	42
UTI (n = 50)	Prulifloxacin	0.03 to 32	0.125	16	70
	Ciprofloxacin	0.06 to 64	0.25	32	66
	Levofloxacin	0.25 to 256	1	64	66
	Moxifloxacin	1 to >512	4	128	20
Total (N = 300)	Prulifloxacin	0.015 to 128	0.25	16	72
	Ciprofloxacin	0.03 to 256	0.5	16	65
	Levofloxacin	0.125 to 256	2	32	61
	Moxifloxacin	0.25 to >512	4	64	23

CF, cystic fibrosis; LRTI, lower respiratory tract infection; BSI, bloodstream infection; UTI, urinary tract infection.

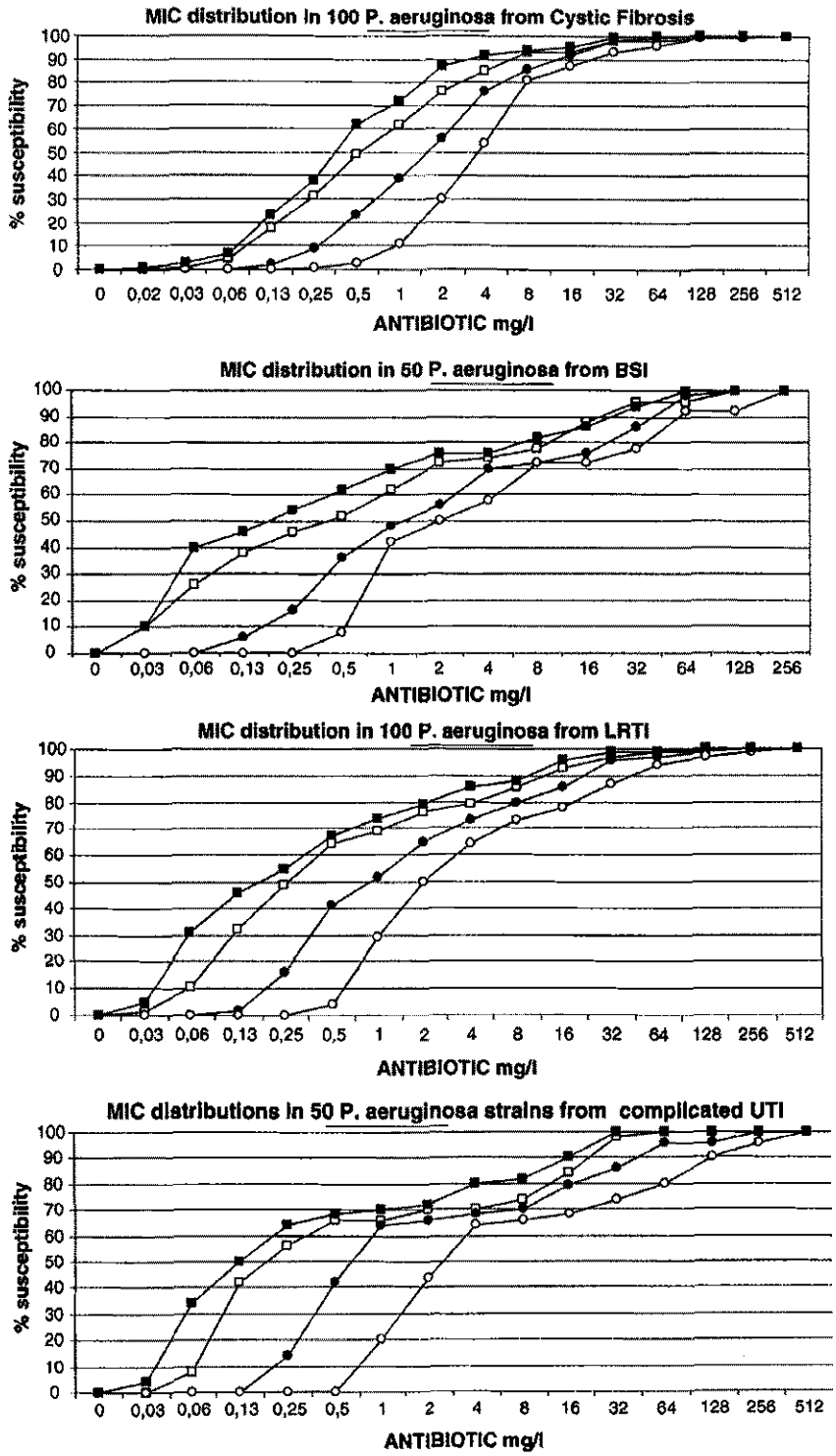

Fig. 1. Minimal inhibitory concentration (MIC) distribution of *Pseudomonas aeruginosa* strains isolated from different infections. (■) Prulifloxacin; (□) ciprofloxacin; (●) levofloxacin; (○) moxifloxacin. BSI, bloodstream infection; LRTI, lower respiratory tract infection; UTI, urinary tract infection.

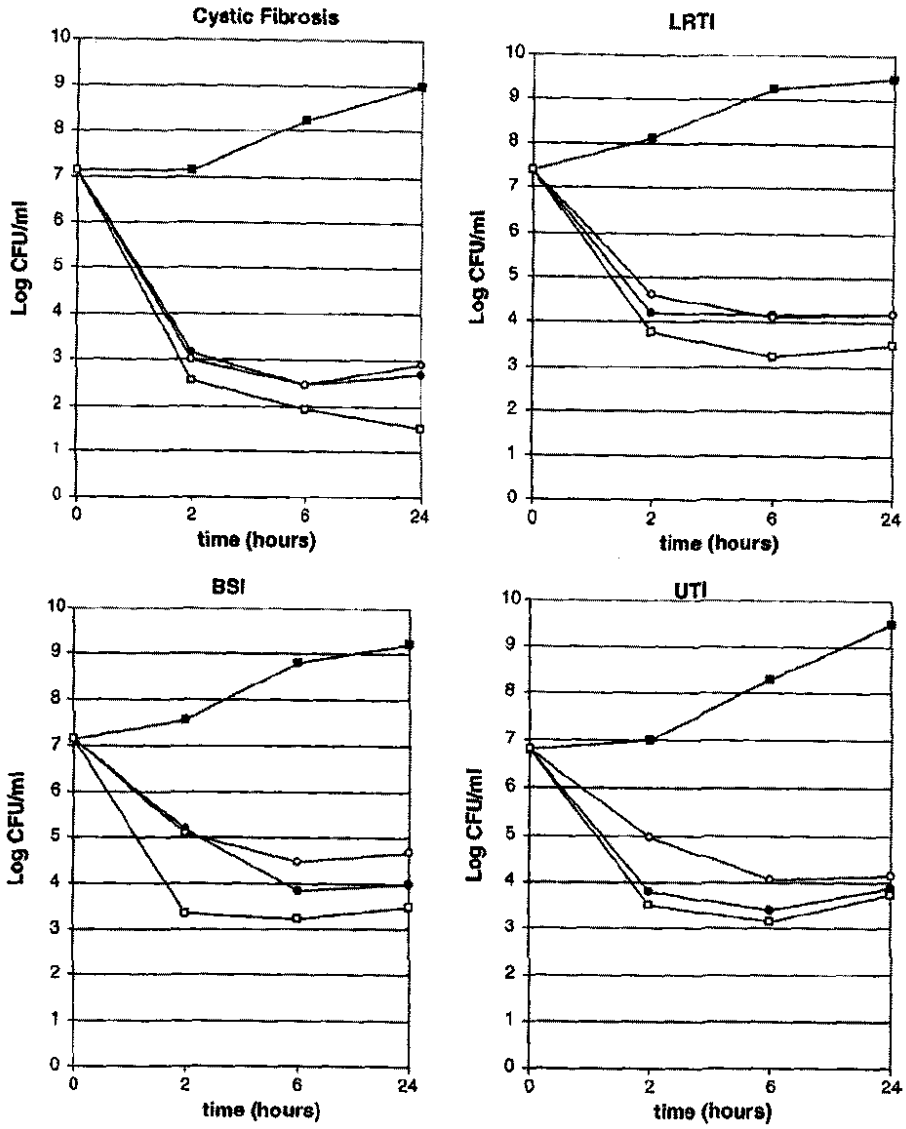

Fig. 2. Time–kill kinetic curves of prulifloxacin (□), ciprofloxacin (●) and levofloxacin (○) against four representative clinical isolates of *Pseudomonas aeruginosa* from different infections. (■) Control. CFU, colony-forming units; LRTI, lower respiratory tract infection; BSI, bloodstream infection; UTI, urinary tract infection.

4. Discussion

In assembling the present collection of *P. aeruginosa* strains originating from a variety of serious infections, care was taken to include pathogens displaying the maximum incidence of resistance traits to commonly used antimicrobials. Therefore, the picture must not be assumed to reflect the actual patterns of susceptibility to be expected in this country for this pathogen [6], although the relative distribution of resistance in organisms arising from different clinical conditions may be of some relevance, with multiple resistance being more common in isolates from CF.

On a weight-by-weight basis, prulifloxacin, the new fluoroquinolone recently introduced in Italy, displayed an in vitro potency superior by a factor of two to that shown by the comparative congeners tested on strains originating from CF and complicated UTIs, whilst the activity was similar for the remaining organisms. Ciprofloxacin performed better than levofloxacin and, as expected, moxifloxacin was the least potent compound. Assuming a susceptibility breakpoint for prulifloxacin identical to that proposed for ciprofloxacin by the CLSI [12,13], the results obtained, whilst clearly showing the phenomenon of cross resistance in the fluoroquinolone class of drugs, translate into better performance (5–7 percentage points) of the new agent compared with the values shown by ciprofloxacin and of ca. 10 percentage points when confronted with levofloxacin. Whether this feature will hold true and provide clinical advantages depends on a clearer understanding of the pharmacokinetic and pharmacodynamic parameters of the new antibiotic.

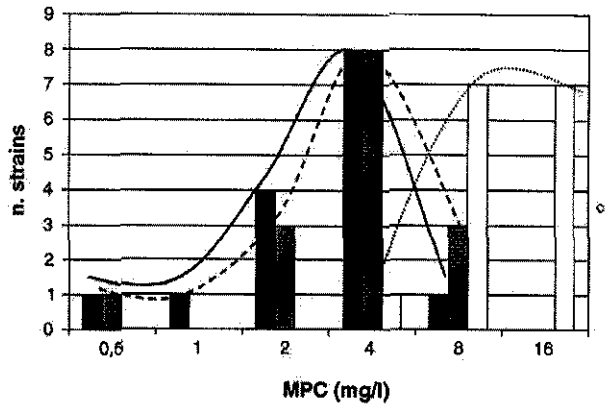

Fig. 3. Mutant prevention concentration (MPC) of 15 *Pseudomonas aeruginosa* strains: prulifloxacin (black); ciprofloxacin (grey); levofloxacin (white).

Time–kill kinetic studies confirmed that all antipseudomonal fluoroquinolones behaved as bactericidal drugs, reducing the viable population by at least three $\log_{10}$ figures within 6 h. Isolates from CF patients were the most susceptible organisms. Prulifloxacin was more rapidly bactericidal than ciprofloxacin and levofloxacin. This feature was particularly prominent with strains originating from BSIs. Achieving a swift and profound bactericidal effect by drugs administered alone or in combination is instrumental in the attempt to eradicate the pathogen in difficult-to-treat infections such as those caused by *P. aeruginosa* [4]. At present, this condition is best satisfied within the fluoroquinolone class by prulifloxacin and it therefore seems mandatory in the near future to assess the identity, among the possible companion drugs, of those molecules capable of providing valuable synergistic killing interactions.

The MPC has recently emerged as a new parameter that may help in the selection of a specific drug within selected classes of antimicrobials whose mechanisms of acquisition of resistance are well established [20–23]. The basic finding emerging from application of this principle is that a direct correlation between MIC and MPC values does not constantly exist because of the complex variables linked to the specific pharmacokinetic behaviour of each compound [21]. However, it is to be expected that the most powerful molecule (lowest MIC) will also be the least likely to evoke selection of resistant mutants (lowest MPC) following use. In the case of fluoroquinolones, history has it that the least active drugs, in terms of weight-by-weight, have been introduced first into clinical practice. Specifically, if one considers agents that include *P. aeruginosa* in their spectrum of activity, the chronological succession of events includes the sequential appearance of lomefloxacin, pefloxacin, ofloxacin (levofloxacin) and ciprofloxacin. Of all these fluoroquinolones, ciprofloxacin was by far the most efficacious. Unfortunately, because of the almost complete cross resistance to the other members that each drug was able to evocate, the situation met by the most active fluoroquinolone after introduction has not been ideal, with consistent rates of resistance broadly although unevenly represented worldwide. If the most powerful drugs had been employed since the beginning, *P. aeruginosa* would certainly have not evolved toward the levels of resistance reached today [6–8]. The fate of prulifloxacin, whose very low and certainly in vivo achievable MPCs support once more the contention that it now represents the most powerful in vitro antipseudomonal drug available, must therefore be attentively considered in this context. Adoption in the eradication of serious infections caused by *P. aeruginosa*, in conjunction with molecules inhibiting other biochemical targets when needed, instead of less active congeners, will certainly occur, has a strong rationale and has already been explored in clinical trials. Complicated and uncomplicated UTIs that have failed previous courses of therapy or that occur in environments where resistance to other recommended drugs is rampant may represent preferential targets, as suggested by international guidelines [24–26] and confirmed by recent clinical studies employing the antibiotic at 600 mg once daily for 10 days in complicated cases and single-dose prulifloxacin 600 mg in acute, uncomplicated lower UTIs [27]. The outstanding ability of other fluoroquinolones to provide a profound bactericidal activity against *P. aeruginosa* when organised in biofilms known to be present even in uncomplicated urinary tract conditions [28–30] may support the preferential usage of the more active among these compounds. Confirmation that prulifloxacin also possesses this interesting property is eagerly awaited.

LRTIs with special emphasis on serious acute exacerbations of chronic obstructive bronchitis in the elderly [31], where *P. aeruginosa* is often involved, and CF are definite indications for a new drug that displays superior in vitro antipseudomonal activity. The recently discovered immunomodulating effect of prulifloxacin on cytokine production by polymorphonuclear neutrophils [32] and its potentiation of the phagocytic and microbicidal effects of human macrophages [33] may represent added advantages. The availability of a parenteral formulation of the drug may in the future broaden its indications to bloodstream and nosocomial pulmonary infections. However, the degree of success to be expected in these conditions will largely depend on the local epidemiology of pre-existing resistance traits that prulifloxacin will face.

Replacement of less suitable fluoroquinolones through cycling represents another important option for prulifloxacin and may carry the additional benefit of fostering an increase in the incidence of susceptible strains in the population of *P. aeruginosa*. This sequence of events, attributed to the fact that levofloxacin but not ciprofloxacin selects for resistance because of widely different MPCs, has been described following substitution of levofloxacin by ciprofloxacin in some hospital settings [34–36]. Our experimental results clearly indicate that, among the fluoroquinolones available, prulifloxacin displays the lowest MPC, prompting its use for the purpose of re-establishing at least part of the original susceptibility of this primary pathogen to this class of antimicrobial agents.

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