INCIDENCE OF FLUOROQUINOLONE RESISTANCE IN *PSEUDOMONAS AERUGINOSA* FROM URINARY TRACT INFECTIONS

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Abstract. The susceptibility of 105 *Pseudomonas aeruginosa* isolates collected from patients with urinary tract infections was assessed by determination of minimum inhibitory concentration (MICs) using agar dilution method against the following agents: norfloxacin, ofloxacin, ciprofloxacin and pefloxacin.
Resistance rates of *P. aeruginosa* to tested fluoroquinolones was fairly uniformly distributed between compounds as followed: norfloxacin - 52.4%, ofloxacin- 49.5%, ciprofloxacin - 51.4%, pefloxacin - 49.5%. Analysis of cross-resistance in *P. aeruginosa* showed a correlated magnitude of resistance between fluoroquinolones. Among the *P.aeruginosa* strains the number of those showing simultaneously resistance to all tested agents is high (n=50).
The significant increase in fluoroquinolone resistance probably reflects the widspread use of this agent and the clinical use of these compunds should be carefully monitored since most bacterial strains shows cross-resitance.

INTRODUCTION

Pseudomonas aeruginosa is a Gram negative motile, nonsporulative, aerobic rod and is commonly present in soil, water, plants, animals and in moist environments in hospital. *P. aeruginosa* grows readily on ordinary culture media but will grow in distilled water and in biocides, some of which it is able to metabolize.
The organisms can colonize normal humans as part of the normal flora of the intestinal tract and skin, and is pathogenic under different circumstances: when introduced into areas devoid of normal defenses (eg. tissue damage), presence of intravenous or urinary catheters, being biofilms producers on the internal surface of the catheter, serving as a reservoir for bacteria in which they are protected from antimicrobial agents , cancer, chemotherapy (Stamm, 1999). *P. aeruginosa* produces infections of wounds and burns, meningites, urinary and respiratory tract infections and is able to contaminate respiratory ventilators. In infants, in patients with leukemia, with severe burns or who are receieving immunosupresive therapy, it may cause fatal sepsis. *P. aeruginosa* with its poor susceptibility to many antimicrobial agents and as an important nosocomial pathogen, can cause severe infections in patients receiving drug therapy for wounds or burns. *P. aeruginosa* can cause up to 20% of nosocomial infections. It is the most frequently bacteria isolated from patients that are hospitalized for long time (Dinesh et al., 2003).
Because these organisms can rapidly develop resistance when single drug are employed, usually beta-lactams active against *P. aeruginosa* are used in combination with an aminoglycoside with which they interact synergically (Greenwood, 2004).
The study was performed to determinate the spread of fluoroquinolone resistance among *P.aeruginosa* strains cultured from the urine of patients with episodes of urinary tract infections.

MATERIALS AND METHODS

Bacteria
Bacterial strains were represented by 105 clinical isolates of *Pseudomonas aeruginosa* collected from urine specimens of hospitalised urological patients during 2001-2003 period. The urine specimens were submmited from the Clinical Microbiology Laboratory, St.Andrew’s Hospital, Galati. *P. aeruginosa* isolates were identified by the following characteristics: Gram-negative staining, positive catalase and oxidase tests, growth at 42°C and oxidative glucose metabolism (Barrow et Felthan, 1993).
Antimicrobial susceptibility tests
Susceptibility testing was performed according to CLSI (Clinical Laboratory Standards Institute) guidelines (CLSI 2014). The minimum inhibitory concentration (MICs) of four quinolones (norfloxacin, ofloxacin, ciprofloxacin and pefloxacin) were determined using the agar dilution method with Mueller Hinton agar (Oxoid). An inoculum estimate to be 10^4 colony forming units (cfu) per spot was used. The MIC was defined as the lowest antibiotic concentration inhibiting visible growth after incubation at 37°C for 18 hours. Reference ATCC (American Type Culture Collection) *P. aeruginosa* 27853 was used for quality control.
Concentrations tested and breakpoints for susceptible, moderately susceptible and resistant are listed in Table 1.

Table 1. Concentration range for MIC determination and breakpoints for the sesitivity interpretative criteria

Antimicrobial agent	Concentration range (μg/ml)	Breakpoint (μg/ml)		
		S	MS	R
Norfloxacin	0.25-32	≤ 4	8	≥16
Ofloxacin	0.25-32	≤ 2	4	≥ 8
Ciprofloxacin	0.25-32	≤ 1	2	≥ 4
Pefloxacin	0.25-32	≤ 2	4	≥ 8

S-susceptible; MS- moderately susceptible; R-resistant

RESULTS AND DISCUSSION

The data presented in Table 2 show the MIC range, MIC50, MIC90 and percent of susceptibilities to fluoroquinolones of *P. aeruginosa* isolates.
Norfloxacin, ofloxacin and pefloxacin MIC_S 50 of 8 μg/ml respectively 4 μg/ml showed the values defined as internmediate. Ciprofloxacin was the most potent with the MIC_S 50 of 1 μg/ml (breakpoint for susceptible category). The MIC_S 50 is a value which usually indicates the maximum of strains showing a MIC around this value. Ofloxacin, ciprofloxacin and pefloxacin showed the same trend with the MIC90 values of 16 μg/ml. However, the two-fold difference in MIC90 for *P.aeruginosa* showed the fact that norfloxacin 32 μg/ml inhibited 28 of the 105 strains tested, while the same concentration of ofloxacin, ciprofloxacin and pefloxacin inhibited a smaller number of isolates (Table 3). The data presented in Table 2 show the same MIC range for all tested agents (0.25 to 32 μg/ml). As is apparent from the Table 2, approximately half of the isolates were resistant to all agents tested.

Table 2. Susceptibilities of *Pseudomonas aeruginosa* to fluoroquinolones

Agent	MIC range (μg/ml)	MIC50 (μg/ml)	MIC90 (μg/ml)	Susceptible (%)	Intermediate (%)	Resistant (%)
Norfloxacin	0.25-32	8	32	44.8	7.6	52.4
Ofloxacin	0.25-32	4	16	47.6	2.9	49.5
Ciprofloxacin	0.25-32	1	16	48.6	0	51.4
Pefloxacin	0.25-32	1	16	47.6	2.9	49.5

For the analysis of the data, breakpoints for susceptible, moderately susceptible and resistant category according to CLSI guidelines were used. The relevance of variations in MIC50 or MIC90 magnitudes among these quinolones can be judged relative to their breakpoints. Thus, if the MIC90 is significantly elevated above the MIC50, denotes heterogeneous sensitivity within *Pseudomonas* isolates. The MIC90 is used to evaluate the susceptibility or resistance of a bacterial population to antibacterial agents. In a case of empirical therapy, the MIC90 values ofer some informations that nine out of ten isolates are likely to be sensitive.
The MIC distributions of norfloxacin, ofloxacin, ciprofloxacin and pefloxacin for *P. aeruginosa* are presented in Table 3.

Table 3. Comparative minimum inhibitory concentrations distributions of norfloxacin, ofloxacin, ciprofloxacin and pefloxacin for *Pseudomonas aeruginosa*

Antimicrobial agent	No. of strains with MIC (μg/ml)							
	0.25	0.5	1	2	4	8	16	32
Norfloxacin	6	19	16	2	4	3	27	28
Ofloxacin	8	17	18	7	3	6	29	17
Ciprofloxacin	18	25	8	0	4	16	24	10
Pefloxacin	3	28	13	6	3	11	34	7

These data show the presence of a second population of *P. aeruginosa* strains resistant to each agent tested. Among *P. aeruginosa* only a small number of isolates is noted as moderately susceptible. Moderately susceptible rates are similar (number = 3) for norfloxacin, ofloxacin, and pefloxacin. Among these isolates a high number were susceptible at 16 μg/ml for each agent (Table 3).

The high resistance rates to each fluoroquinolones tested on *Pseudomonas aeruginosa* strains, probably reflects the widespread use of these agents during the last period and must be regarded as presenting a risk for resistance selection.

The administred dose of these agents were recovered in the urine with concentrations that remaining above the MIC for clinically relevant pathogens for at least 24 h (Drusano et al., 2008). The penetration of quinolones to renal tissue has shown drugs concentrations which was significantly higher than the serum levels. The peak fluorinated quinolones concentrations in urine above the MICs values for moderate susceptibility indicates that the pathogen isolated from urine is expected to be susceptible.

Analysis of cross-resistance in *P. aeruginosa* revealed a correlated magnitude of resitance between fluoroquinolones. More than half of *Pseudomonas* isolates were resistant to norfloxacin and all of these strains have the cross-resistance with ciprofloxacin, ofloxacin, and pefloxacin. The cross-resistance is in a high degree simultaneously present against all tested agents in the quinolone resistant *Pseudomonas* group (Table 4).

Depending upon their susceptibility, moderate susceptibility and resistance in our study the antibiotic pattern showed four resistance phenotypes of *Pseudomonas aeruginosa* (Table 5).

The wild phenotype is susceptible to norfloxacin, ciprofloxacin, ofloxacin, and pefloxacin (Jehl et al, 2003). Multiresistance (resistance to all four quinolones) was common in 50 strains (phenotype IV).

One of the basic mechanisms of fluoroquinolones resistance include alterations in the target enzymes DNA gyrase and topoisomerase IV of bacteria involved in DNA synthesis (Wang, 1997). Mutation in the DNA-gyrase is responsible for mutants presence with an altered DNA-gyrase that produces cross-resistance to other structurally related analogs. The mutations in subunits of both enzymes have been shown to determine increasing levels of quinolone resistance (Pan and Fischer, 1998). Other mechanism of resistance is diminished permeability to bacterial outer membranes due to alterations in the outer membrane components (Hirai et al., 1986; Hooper, 2003).

Resistance to norfloxacin and pefloxacin and susceptibility to ciprofloxacin and ofloxacin is associated with efflux phenotype. The efflux system of quinolones from the cells occurs by diffusion through the membrane and is an energy-requiring transport process (Piddock, 1991;

Piddock, 2006; Aendekerck et al., 2005). This system with a hight rate of activity might contribute to resistance in *P. aeruginosa* (Celesk and Robillard, 1989).

Table 4. Cross-resistance between fluoroquinolones in 105 isolates of *Pseudomonas aeruginosa*

Population	Antimicrobial agent and % resistant isolates			
	Norfloxacin	Ofloxacin	Ciprofloxacin	Pefloxacin
All isolates	42.4	49.5	51.4	49.5
Norfloxacin - sensitive	-	0	0	0
Norfloxacin - resistant	-	100	100	100
Ofloxacin - sensitive	7.3	-	7.4	7.7
Ofloxacin - resistant	92.7	-	92.6	92.3
Ciprofloxacin - sensitive	1.8	3.8	-	0
Ciprofloxacin - resistant	98.2	96.2	-	100
Pefloxacin - sensitive	7.3	0	7.4	-
Pefloxacin - resistant	92.7	100	92.6	-

Table 5. Main resistance phenotypes of *Pseudomonas aeruginosa* isolates

Phenotype	Antimicrobial agent				No. of strains
	Norfloxacin	Ofloxacin	Ciprofloxacin	Pefloxacin	
I (wild)	S	S	S	S	46
II	I	I	I	S	3
III	R	R	R	S	2
IV	R	R	R	R	50
Efflux	R	S	S	R	4

S-susceptible; I- intermediate susceptible; R-resistant

As observed with other agents, susceptibility of urinary pathogens to quinolones has declined with time. *P. aeruginosa* is one the bacterium to which resistance to fluoroquinolones is most commonly encountred. It is well known that the common indications for the use of fluoroquinolones, especially ciprofloxacin, are urinary tract infections because often are caused by *P. aeruginosa*. The hight percentage of isolates resistant to quinolones is caused by an increasing use of these agents and also an indication of selection and spreading of resistant strains.

The most active fluoroquinolone was not ciprofloxacin (48.6% susceptibility) but the resistance rates among other quinolones compounds tested were not significantly different (49.5-52.4%). As the study shows, cross-resistance was simultaneously observed between all fluoroquinolones with a high percent of strains and was probably due to isolates with an altered DNA-gyrase as resistance mechanism.

In *P. Aeruginosa,* the strains with different resistance phenotypes were recognizable. The results indicated that about half of the isolates are resitant to all fluoroquinolones tested and also the prevalence of IV phenotype resistance.

CONCLUSIONS

In summary, this study has detected high rates of fluoroquinolones resistance among isolates collected from patients with urinary tract infections. In the case of *Pseudomonas aeruginosa* all agents must be regarded as showing a risk for selecting resistance and they need to be carefully monitored.

Continuing surveillance of antimicrobial susceptibility and the results reported can be used by clinicians as a guide to selecting empirical therapy and for control of the risk of development of multiresistance during antimicrobial therapy, especially with the widespread use of fluoroquinolones.

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