

Original Research Article

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Antibiogram of Clinical Isolates of *Pseudomonas aeruginosa* against Selected Quinolones

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ABSTRACT

One important class of anti-pseudomonal drug is the Fluoroquinolones (FQ) which is also available in oral form, in most countries. However, *P. aeruginosa* easily becomes resistant to FQs, which severely limits its usefulness. We carried out an antibiogram analysis of five FQs viz. Ciprofloxacin, Ofloxacin, Levofloxacin, Prulifloxacin and Moxifloxacin on 80 *P. aeruginosa* clinical isolates. This prospective study was carried out in the Department of Microbiology of a tertiary care teaching hospital in southern Rajasthan. For the same, 80 non-repetitive *P. aeruginosa* isolates, obtained from various clinical samples were assayed for resistance to five Fluoroquinolones (FQs) by using Kirby-Bauer disc diffusion method. The resistance patterns were noted and statically analyzed to find any significance. A significant resistance ($p < 0.05$) was noted towards Ciprofloxacin, Ofloxacin, Levofloxacin in the *P. aeruginosa* isolates obtained from out-patients (OPD) when compared to intensive care unit (ICUs). On the other hand, significant resistance ($p < 0.05$) for Ofloxacin, Prulifloxacin and Moxifloxacin was obtained for the isolates from wards when compared to the ICU. The overall resistance rates of Ciprofloxacin, Ofloxacin and Levofloxacin were significantly higher than those to Prulifloxacin ($p = 0.000$). Also Moxifloxacin was significantly resistant when compared to all other FQs tested ($p = 0.000$). The resistance to FQs was more in the OPDs when compared to the ICUs, while Moxifloxacin had the highest resistance rates. The probable causes for this may relate to previous exposure to FQs.

Keywords

Pseudomonas aeruginosa,
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Introduction

Fluoroquinolone (FQs) are a novel class of antimicrobial agents; which have a broader acceptance in treating hospitalized and community patients. Introduced in the year 1986, fluoroquinolones are modified form of its precursors-Quinolones. The quinolones act by inhibiting topoisomerase II and DNA gyrase in Gram positive and Gram negative bacteria respectively; thereby interfering

with DNA synthesis in the bacteria. Fluoroquinolone are effective for wide range of pathogenic bacteria; as in particular; Ciprofloxacin in infections caused by *Pseudomonas aeruginosa*. As we approached the fourth decade of FQs usage; its persistence injudicious usage had led to the emergence of FQs resistance; especially in gram negative bacteria such as in

Pseudomonas aeruginosa (Tanya Strateva *et al.*, 2009; Hideki Kobayashi, 2013)

P. aeruginosa is a Gram negative pathogen with efficient environment adaptability. It is also responsible for a wide range of acute and chronic infections. Its persistence in hospital environment, resistance to several classes of antibiotics and continuous association with serious hospital acquired infections such as sepsis, UTI, etc. presents a therapeutic challenge to the clinicians. *P.aeruginosa* is also increasingly being acquired in the community. All these factors contribute for *P. aeruginosa* as a common cause of morbidity and mortality. Among the few antimicrobial classes currently available for eradication of *P. aeruginosa* infection which can be used alone or in combination, involves the use of aminoglycosides, β -lactams, and prominent role is played by FQs. The availability of orally administrable form of FQs and its minimum toxic effect had increased its convenience of use. (Tanya Strateva *et al.*, 2009; Aljun *et al.*, 2004)

FQs have been widely used for the treatment of *P. aeruginosa* infection; however *P.aeruginosa* is capable of acquiring resistance during antibiotic therapy which is a significant risk factor for the selection of resistant *P. aeruginosa* strains in an individual patient and / or in the populations. Studies have reported a worldwide decline in susceptibility pattern of ciprofloxacin, a most potent congener of FQs. Although some members of the class (Temafloxacin, Trovafloxacin, Grepafloxacin and Gatifloxacin) have been withdrawn or restricted because of adverse events, new members continue to be developed and approved (Prulifloxacin and Moxifloxacin) (Luna Adhikari *et al.*, 2010; Liam Redgrave *et al.*, 2014; Axel Dalhoff, 2012). The present study was designed to evaluate the

resistance pattern in *P. aeruginosa* isolates from various clinical samples among different patient population (both in-door and out-door) from our hospital setup against the five FQs (Ciprofloxacin, Ofloxacin, Levofloxacin, Prulifloxacin and Moxifloxacin).

Materials and Methods

The present prospective study was carried out for one year from 2013-2014 in the department of microbiology of a tertiary care teaching hospital in south Rajasthan. Institutional ethical committee clearance was obtained for the study.

During the study period a total of 80 *P. aeruginosa* strains were isolated from various clinical samples and were included in the study. No duplicate strain from the same patient was included in the study. For the culture, clinical samples received were cultured on Nutrient agar, Blood agar and MacConkey agar. Media plates were incubated at a temperature of 37 degrees C for 18-24 hours. The culture plates were processed using standard microbiological procedure. Characterization and identification was carried out using a combination of colony morphology, Gram stain characteristics, motility tests, pigmentation, oxidation-fermentation test, catalase test, oxidase test and pyocyanin production (Monica Cheesbrough, second edition, part 2).

All the 80 isolates thus obtained; were subjected to antibiotic susceptibility testing on Mueller-Hinton agar plates by the Kirby-Bauer disc diffusion method as defined by CLSI 2014 guidelines. The panel of FQs to which susceptibility was evaluated consisted of five Fluoroquinolones viz. Ciprofloxacin (5 μ g), Ofloxacin (5 μ g), Levofloxacin (5 μ g), Prulifloxacin (5 μ g) and Moxifloxacin (5 μ g).

The control strain used in this study was *P.aeruginosa* ATCC 27853. Zone size interpretation was done according to the criteria depicted in table no. I.

The obtained resistance pattern for the above mentioned FQs was subjected to statistical analysis by the ANOVA test and the post hoc Tukey HSD test.

Result and Discussion

Out of 80 clinical isolates of *P. aeruginosa*; highest contribution of 40 % and 33.75% were from respiratory secretions and urinary isolates, respectively. Following this, Pus, blood and pleural/biliary fluid contributed 26.25%.

On the basis of patient admission; 76.25 % of the *P. aeruginosa* isolates were from samples obtained from in-patients and 23.75 % were from out-patient. Of the 61 *P. aeruginosa* isolates from in-patients, 62.30% were from the wards, while 37.70% were from ICUs. The overall resistance pattern of 80 isolates of *P.aeruginosa* against each of the FQs (Ciprofloxacin, Ofloxacin, Levofloxacin, Prulifloxacin, Moxifloxacin) is depicted in table no.II. The distribution of each FQ resistance in the in-patients (ICUs and wards) along with out-patients is represented in table no. III.

Inter comparison of overall resistance rates to Ciprofloxacin and Ofloxacin was found significantly higher than those to Levofloxacin and Prulifloxacin ($p=0.000$). The overall resistance rates to Moxifloxacin were significantly higher when compared to all other drugs ($p=0.000$). A significant resistance ($p < 0.05$) was observed for Ciprofloxacin, Ofloxacin, and Levofloxacin in the *P. aeruginosa* isolates from the out-patient samples when compared to isolates from ICU samples. Similarly; significant resistance ($p < 0.05$) for Ofloxacin,

Prulifloxacin and Moxifloxacin was observed for *P. aeruginosa* isolates from wards in comparison to the ICUs.

Resistance to antimicrobial agents is an increasing clinical problem and a recognized public health threat. The magnitude of the problem increases several folds when organisms such as *P. aeruginosa* is involved which shows multifaceted resistance mechanisms to the available antibiotics. To tackle the grave situation of antibiotic resistance some of antimicrobial class such as FQs have been obtained by the slight modification of previous generations (Shivani saxena *et al.*, 2014).

FQs are bactericidal and widely used class of antibiotics. Their effectiveness against *P. aeruginosa* is one of their most important features; but prevalence of FQs resistance is related to its intensity of use. Several authors across the globe have reported FQ resistance rate ranging from 12.5% (Algun *et al.*, 2004) to as high as 73.2% (Deepak Arora *et al.*, 2011). In the present study the FQs resistance rate ranges from 43.75% (Moxifloxacin) to 27.5% (Levofloxacin & Prulifloxacin).

The overall resistance rate for each of tested FQs in the present study is as follows. Ciprofloxacin the most active FQ against *P. aeruginosa* was found to be overall resistant in 28.75% of the isolates. Similar to our finding Kunimoto DY *et al.*, (1999) had reported resistance rate of 30.7%. In various studies investigating the ciprofloxacin resistance in *P. aeruginosa* reported from 0% to 89%. In current study, ciprofloxacin is resistant in 47.36% of isolates obtained from out-door patients followed by resistance of 26.31% in wards and 17.33% in ICUs. U algun *et al.*, had reported a lower overall resistance rate of 12.5%; while 23.4% in ICU and 1.9% in OPD. For Ofloxacin overall 28.75% resistance was

observed in this study. Other authors have reported Ofloxacin resistance to be in between 19% and 62.5%. In the present study, Ofloxacin resistance of 21.7% in ICUs and 31.57% in wards was noticed. U algun *et al.*, had reported an overall resistance rate of 19.9% for Ofloxacin; while 32.8% in ICU and 7.5% in OPD.

Overall resistance rate of 27.5% was noted each for Levofloxacin and Prulifloxacin in this study. Different studies have reported Levofloxacin resistance in between 16.9% to 36%, while susceptibility reports were higher for Prulifloxacin (72%) than for Ciprofloxacin (65%) and Levofloxacin (61%). In the current study, resistance of 21.7% and 26.08% was in ICUs alongwith 23.6% and 28.94% in wards; for

levofloxacin and prulifloxacin respectively. But in out-door patients levofloxacin was resistant in 42.10% and Prulifloxacin in 26.31%. U algun *et al.*, had reported a resistance rate of 16.9%; while 29.7% in ICU and 3.8 % in OPD, for levofloxacin.

In the current study, Moxifloxacin had the highest resistance rate of 43.75%, amongst all the FQs. In the study by Arpana *et al.*, had also reported lower activity of moxifloxacin in gram negative bacteria in comparison to gram positive. This variation in resistance rates of different FQs may be due to the disparity of antibiotic policies with geographical variation, prior mono-therapy with FQs, study population and type of sample included in the study.

Table.1 Zone size interpretative table of the FQs used in the study.

Sl. No	Antibiotic	Drug content (µgm)	Zone Of Inhibition		
			Resistant	Intermediate	Sensitive
1	Ciprofloxacin [#]	5	≤15	16-20	≥21
2	Levofloxacin [#]	5	≤15	16-18	≥19
3	Ofloxacin [#]	5	12	13-14	15
4	Prulifloxacin [*]	5	≤12	13-15	≥16
5	Moxifloxacin ^{**}	5	≤17	18-24	≥25

[#]The zone diameter is interpreted in accordance to CLSI 2014 guidelines (CLSI antimicrobial susceptibility testing ;vol 34 NO.1;Page :1-230).

^{*}The zone diameter is interpreted for Prulifloxacin is as per Eiken chemical, Japan.

^{**}Zone diameter is interpreted for moxifloxacin as per BSAC recommendation.

Table.2 Overall resistance to each FQs against the *P. aeruginosa* isolates (n=80)

Fluoroquinolones	Percentage of overall resistance
Ciprofloxacin (5µgm)	28.75% (23)
Ofloxacin(5µgm)	28.75% (23)
Levofloxacin (5 µgm)	27.5% (22)
Prulifloxacin(5 µgm)	27.5% (22)
Moxifloxacin (5 µgm)	43.75% (35)

Table.3 Distribution of resistance pattern for the various FQs among in-patients (ICUs & wards) and out-patients

Fluoroquinolones	Overall resistance in IPD (ICUs + wards; n=61)	Resistance in wards (n=38)	Resistance in ICU(n=23)	Resistance in OPD(n=19)
Ciprofloxacin	22.95% (14)	26.31% (10)	17.33% (04)	47.36% (09)
Ofloxacin	27.86% (17)	31.57% (12)	21.7% (05)	31.57% (06)
Levofloxacin	22.95% (14)	23.6% (09)	21.7% (05)	42.10% (08)
Prulifloxacin	27.86% (17)	28.94% (11)	26.08% (06)	26.31% (05)
Moxifloxacin	45.90% (28)	52.63% (20)	34.78% (08)	36.84% (07)

The present study has surprisingly shown that the percentage of FQs resistance in samples obtained from ICU patients was significantly lower than the patients from the OPD and Wards. The only plausible explanation to this may be had from the possible rampant and irrational use of FQs antibiotics in population. Various studies have documented that the single most important determinant of FQ resistance is prior exposure to these drugs. Because of the increasing FQs resistance empirical usage should either abandoned or restricted in order to take the developing resistance rates under control. Also a detailed history of antibiotic usage with special emphasis on prior FQ consumption might give a clue to the origin of resistance in our OPD patients. At the same time, the relative lower rates of resistance in ICU patient, points towards a scientific antibiotic use practice and meticulous attention to the prevention of nosocomial infection.

The limitations of the present study were small sample size and due to financial constraint we did not use E strips for the determination of MIC values of FQs. A follow up study therefore needs to be conducted which incorporate determination of MIC with appropriate sample size along

with details of prior antibiotic use in the study design. The findings also warrant further probe into possible genetic factors in the microbe that may contribute to FQs resistance.

To conclude, although in the present study we observed moderate resistance to FQs, but recent years have witnessed an emergence in FQs resistance. To overcome the antibiotic resistance we need a combined approach consisting of rational use of FQs, avoidance of FQs as monotherapy, improved hospital infection control measures, along with continuous monitoring of local FQs resistance pattern with an aim that the results should be made readily available to the clinician so as to maximize the possibility of administering appropriate effective antibiotic for better patient management. Thus selection of appropriate antibiotics and its use on the pharmacodynamics concept remains the best way in treating *P. aeruginosa* infections; otherwise we may be nearing to an end of antibiotics era.

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