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Research Article

Determination of minimum inhibitory concentration of third generation cephalosporins and fluoroquinolones on clinical isolates of *Pseudomonas aeruginosa*

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ABSTRACT

Background: *Pseudomonas aeruginosa* has attracted much attention because of its high incidence of nosocomial infections in recent years. The multidrug resistance of these *P. aeruginosa* isolates plays an important role in the colonization or infection of chronically hospitalized patients. There is continued search for alternative drugs, such fluoroquinolones, and the third generation cephalosporins. The objective of present study was to test the susceptibility of the clinical isolates of *P. aeruginosa* to antimicrobials like ciprofloxacin, ofloxacin, ceftazidime, and cefoperazone. To determine their minimum inhibitory concentration (MIC) is in resistant isolates.
Methods: The study was conducted in Bacteriology Laboratory, Department of Microbiology, Government Medical College, Miraj. The design of study: *in vitro* study on 50 clinical isolates of *P. aeruginosa.*
Results: Among the third generation cephalosporins, ceftazidime (64%) exhibited maximum *in vitro* activity and among the fluoroquinolones ciprofloxacin (54%) exhibited maximum activity. Out of 50 clinical isolates of *P. aeruginosa*, 17 were resistant to all four antimicrobials, 22 were susceptible to all four antimicrobials, and 11 clinical isolates of *P. aeruginosa* showed mixed susceptibility-resistant pattern.
Conclusion: MIC values for resistant strains of *P. aeruginosa* ranged from 0.03 to 32 µg/ml for fluoroquinolones and 0.5-512 µg/ml for third generation cephalosporins.

Keywords: *Pseudomonas aeruginosa,* Minimum inhibitory concentration, Ciprofloxacin, Ofloxacin, Ceftazidime and cefoperazone

INTRODUCTION

Pseudomonas aeruginosa is a common Gram-negative bacterium and an important cause of infection in patients with compromised host defense. This is an opportunistic pathogen and is frequently responsible for hospital acquired infections.[1]

P. aeruginosa has attracted much attention because of its high incidence of infection in recent years. It is an important cause of morbidity and mortality. The multidrug resistance of these *P. aeruginosa* isolates plays an important role in the colonization or infection of chronically hospitalized patients.[2-4]

P. aeruginosa exhibits high-level of resistance too many antimicrobials and because of its ability to develop resistance during therapy, empirical treatment for serious systemic infections usually involves a combination of two drugs.[5]

For the effective treatment of nosocomial infections caused by *P. aeruginosa*, clinicians often have resort to the most potent fluoroquinolones or combination of different antibiotics.[2]

Synergy is one of the most common reasons for using combination antimicrobial therapy. It is well-established that combinations that demonstrate *in vitro* synergy are clinically more effective than non-synergistic combinations.

The verification of synergistic interaction between two antimicrobial agents against *P. aeruginosa* can be evaluated by several *in vitro* techniques that measure antimicrobial activity.[6]

The most widely documented synergy is seen when β-lactams are combined with aminoglycosides. These combinations are synergistic against most species including *P. aeruginosa.* Although these combinations are clinically well proven to provide effective treatment, there are limitations such an increasing resistance of *P. aeruginosa* to β-lactam antibiotics and toxicity associated with aminoglycoside therapy.

The present study aims to test the antimicrobial susceptibility and to determine the minimum inhibitory concentration (MIC) of antimicrobials like ciprofloxacin, ofloxacin, ceftazidime, and cefoperazone on clinical isolates of *P. aeruginosa.*

Objectives

1. To test the susceptibility of the clinical isolates of *P. aeruginosa,* to the following antimicrobials:
   a. Ciprofloxacin
   b. Ofloxacin
   c. Ceftazidime
   d. Cefoperazone.
2. To determine the MIC of the following antimicrobials:
   a. Ciprofloxacin
   b. Ofloxacin
   c. Ceftazidime
   d. Cefoperazone.

METHODS

The study was conducted in Bacteriology Laboratory, Department of Microbiology, Government Medical College, Miraj.

Design of study: *In vitro* study.

Sample size: 50 clinical isolates of *P. aeruginosa.*

A. 50 clinical isolates of *P. aeruginosa* were used in this *in vitro* study
   Isolates were obtained from samples coming to the Bacteriology Laboratory of Department of Microbiology, Government Medical College, Miraj and from Padmabhushan Vasantdada General Hospital, Sangli. The samples were obtained from different sources like burn wound, urine, pus, blood.
B. Media
   Muller Hinton agar was used for susceptibility testing and determination of MIC.
C. Antimicrobial agents
   In the present study, total four antimicrobials including two fluoroquinolones (ciprofloxacin and ofloxacin) and two antimicrobials from third generation cephalosporins (ceftazidime and cefoperazone) were used.

The antimicrobial susceptibility testing

The antimicrobial susceptibility of all the clinical isolates of *P. aeruginosa* was done on Mueller-Hinton agar plates by Kirby-Bauer disc diffusion method.[7]

Antimicrobial agent and their diameter of the zone of inhibition as per Clinical and Laboratory Standards Institute (CLSI) guidelines.

Antimicrobials	Diameter of zone of inhibition (mm)
Ciprofloxacin	25-33
Ofloxacin	17-21
Ceftazidime	22-29
Cefoperazone	23-29

P. aeruginosa ATCC 27853 was used as a quality control strain.

Reading of results

Reading of result was obtained by measuring the diameter of zone of inhibition.

Antimicrobials	Resistant	Sensitive
Ciprofloxacin	15 mm or less	18 mm or more
Ofloxacin	12 mm or less	16 mm or more
Ceftazidime	14 mm or less	18 mm or more
Cefoperazone	15 mm or less	21 mm or more

Susceptibility and resistance were measured as per CLSI guideline.

Determination of MIC

Method: Agar dilution method.

Preparation of plates

The medium used for MIC determination was Mueller-Hinton agar. An appropriate quantity of each antimicrobial agent under study was mixed with 1 ml of distilled water, and this was placed in sterile Petri plate of diameter 9 cm, 25 ml of molten agar, cooled to 55°C was added to each plate and mixed. After the plates had set, they were dried at 37°C with their lids tipped for 30 mins in an incubator.[8]

Antimicrobials

The antimicrobials used were ciprofloxacin, ofloxacin, ceftazidime, and cefoperazone.

The drug concentration used for MIC determination was:
1. Ceftazidime (μg/ml)
   0.5. 1, 2, 4, 8, 16, 32, 64, 128, 256, 512

2. Cefoperazone (µg/ml)
   0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512
3. Ciprofloxacin (µg/ml)
   0.03, 0.06, 0.12, 0.25, 0.5, 1, 2, 4, 8, 16, 32
4. Ofloxacin (µg/ml).
   0.03, 0.06, 0.12, 0.25, 0.5, 1, 2, 4, 8, 16, 32.

Inoculum

This was prepared by adding standard loopful (4 mm diameter) of an overnight broth culture to 5 ml of nutrient broth. This was standardized with McFarland's No. 1 tube (0.5%) tuibidimeter standard.[8]

The standard broth cultures were inoculated on Mueller-Hinton plates prepared with the addition of appropriate concentration of antimicrobials.

Incubation

The plates were incubated at 35-37°C for 16-18 hrs.

Reading of the results

MIC was taken as the lowest concentration of the antimicrobial inhibiting visible growth after 18-24 hrs. Quality control of the method was assured using ATCC 27853 *P. aeruginosa* with known MIC as a control strain as recommended by standard literature.[9]

RESULTS

The susceptibility pattern of the clinical isolates to all four antimicrobials showed that out of 50 isolates, 22 isolates were sensitive while 17 isolates were resistant to all 4 antimicrobials. 11 isolates which exhibited mixed resistant pattern were sensitive to one or a few drugs and resistant to others (Table 2).

Susceptibility of 50 isolates to individual drug showed that ceftazidime ranked the first in exhibiting antimicrobial activity against *P. aeruginosa*. 64% of the clinical isolates were sensitive to ceftazidime while 36% were resistant to it. Cefoperazone ranked 2nd in the order of activity 58% were sensitive while 42% were resistant. This was followed by ciprofloxacin, 54% of isolates were sensitive to ciprofloxacin, and 46% were resistant. Ofloxacin was the least active among the 4 antimicrobials to which 44% isolates were sensitive and 56% were resistant (Table 3).

The MIC range of both the fluoroquinolones ranged from 0.03 to 32 µg/ml and similarly the MIC of both the third generation cephalosporins ranged from 0.5 to 512 µg/ml. Similar to the susceptibility and resistance patterns, there was a wide variation in the MIC range of the antimicrobials seen in different studies (Table 4).

Table 1: Sources of clinical isolates of *P. aeruginosa*.

Sources	Number of *P. aeruginosa* isolates	Percentage of *P. aeruginosa* isolates
Urine	9	18
Pus	15	30
Burn wound	24	48
Blood	2	4
Total	50	100

P. aeruginosa: Pseudomonas aeruginosa

Table 2: Susceptibility pattern of the clinical isolates of *P. aeruginosa*.

	Number of isolates	%
Isolates susceptible to all four antimicrobials	22	44
Isolates resistant to all four antimicrobials	17	34
Isolates exhibiting mixed susceptibility and resistance	11	22

P. aeruginosa: Pseudomonas aeruginosa

Table 3: Susceptibility of 50 isolates of *P. aeruginosa* to individual drug.

Antibiotic	Susceptible isolates (%)	Resistant isolates (%)
Ciprofloxacin	27 (54)	23 (46)
Ofloxacin	22 (44)	28 (56)
Ceftazidime	32 (64)	18 (36)
Cefoperazone	29 (58)	21 (42)

P. aeruginosa: Pseudomonas aeruginosa

Table 4: MIC of clinical isolates of *P. aeruginosa*.

Antimicrobials	The MIC ranges
Ciprofloxacin	0.03-32 µg/ml
Ofloxacin	0.03-32 µg/ml
Ceftazidime	0.5-512 µg/ml
Cefoperazonc	0.5-512 µg/ml

MIC: Minimum inhibitory concentration, *P. aeruginosa: Pseudomonas aeruginosa*

DISCUSSION

P. aeruginosa contributes to morbidity and mortality in nosocomial infections. Multidrug resistant isolates pose a major therapeutic problem for clinicians worldwide.

The application of antimicrobial combination therapy in such patients attempts to take advantage of synergistic antimicrobial interaction and to enhance the efficacy in the treatment of the infections at clinically achievable non-toxic concentration of the antimicrobials and to

decrease the risk of development of resistance during therapy.

In vitro combination of antimicrobial drugs is one of the tools used to determine the synergistic interaction between these antimicrobials on clinical isolates of *P. aeruginosa.*

Most of the clinical isolates of *P. aeruginosa* were obtained from burn wound (48%), pus (30%), urine (18%) and the lowest number of clinical isolates was from blood (4%). Wilson et al.[10] differentiated clinical isolates of *P. aeruginosa* as blood isolates and non-blood isolates. Non-blood isolates were from pus, bum wound, urine and other clinical specimens. In their study proportion of non-blood clinical isolates were greater than the clinical isolates from blood.

In a study conducted by Giamarellou and Petrikkos,[11] clinical isolates were derived from various sources like urine (15 strains). Sputum (5 strains), pus (4 strains), and blood (2 strains).

Thus, the sources of isolates of *P. aeruginosa* in the above studies were nearly similar to the present study.

The first objective of the present study was to lest the susceptibility of the clinical isolates of *P. aeruginosa* to ciprofloxacin, ofloxacin, ceftazidime, and cefoperazone. Out of 50 clinical isolates of *P. aeruginosa*, 22 isolates were susceptible to all four antimicrobials used while 17 isolates were resistant to all the four antimicrobials. 11 isolates which exhibited mixed susceptibility pattern were susceptible to a few drugs and resistant to others. The susceptibility pattern of these isolates to the four individual antimicrobials and to the combination used during the study was studied in detail later.

When the isolates of *P. aeruginosa* were exposed to susceptibility tests using individual antimicrobials, it was seen that a high proportion (64%) of these isolates were susceptible to ceftazidime, followed by cefoperazone and ciprofloxacin and only 44% of the isolates were susceptible to ofloxacin.

Apart from the present study a large number of studies have evaluated the susceptibly patterns of *P. aeruginosa* to different antimicrobials. Most of the studies have included extended spectrum penicillin and aminoglycosides in their study in addition to fluoroquinolones and cephalosporins. In addition to the variations in the antimicrobials used, the patterns of susceptibility of isolates quoted by these studies also vary to a great extent. The resistance to ceftazidime ranges from as high as 82.8% in a study conducted by Suresh Chaware et al.[12] to as low as 21.08% in a study conducted by Mehta et al.,[13] similarly the resistance to ciprofloxacin ranges from 85% in a study conducted by Rastegar Lari et al.[14] to 50% in a study conducted by Sarkar et al.[15]

Although no exact correlation was found, the pattern of resistance exhibited by *P. aeruginosa* in our study falls in

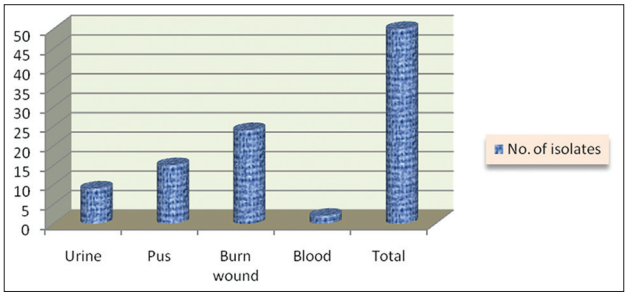
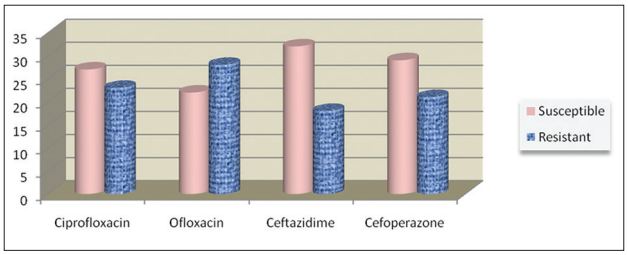


Figure 1: Sources of clinical isolates of *Pseudomonas aeruginosa*.



Figrue 2: Susceptibility of 50 isolates of *Pseudomonas aeruginosa* to individual drug.

the range of resistance pattern observed in the other studies. The wide variation in the observed resistance pattern of the isolates in different studies might be a result of different degrees of utilization of a specific drug leading to resistance by selection pressure or due to resistance transmitted by conjugation in isolates from hospital acquired infections were multidrug resistance *P. aeruginosa* is highly prevalent.

The second objective of our study was to determine the MIC of resistant isolates of *P aeruginosa* to the four antimicrobials used in the present study (Table 4).

The MIC range of both the fluoroquinolones ranged from 0.03 to 32 µg/ml and similarly the MIC of both the third generation cephalosporins ranged from 0.5 to 512 µg/ml. Similar to the susceptibility and resistance patterns, there was a wide variation in the MIC range of the antimicrobials seen in different studies.[11,16] The reason for this variation in MIC values might be because of different degrees of resistance developed by *P. aeruginosa* to antimicrobials in different geographical areas depending on the antibiotic utilization pattern and the prevalence of resistance in *P. aeruginosa* in those regions.

CONCLUSIONS

Most of the isolates (48%) of *P. aeruginosa* were obtained from bum wound. Analysis of susceptibility patterns of the isolates showed maximum susceptibility to ceftazidime (64%). This was followed by cefoperazone (58%), ciprofloxacin (54%), and ofloxacin (44%), respectively.

Among the third generation cephalosporins, ceftazidime (64%) exhibited maximum *in vitro* activity and among the

fluoroquinolones ciprofloxacin (54%) exhibited maximum activity.

Out of 50 clinical isolates of *P. aeruginosa,* 17 were resistant to all four antimicrobials, 22 were susceptible to all four antimicrobials, and 11 clinical isolates of *P. aeruginosa* showed mixed susceptibility-resistant pattern. MIC values for resistant strains of *P. aeruginosa* ranged from 0.03 to 32 µg/ml for fluoroquinolones and 0.5-512 µg/ml for third generation cephalosporins.

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