ORIGINAL ARTICLE

Study of in Vitro Activity of Fluoroquinolones in Combination with Third Generation Cephalosporins on Clinical Isolates of Pseudomonas Aeruginosa

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Abstract

Background: *Pseudomonas (P) aeruginosa is an opportunistic pathogen and is frequently responsible for nosocomial infections. The multi drug resistance of these P. aeruginosa isolates plays an important role in the colonization or infection of chronically hospitalized patients. Synergy is one of the most common reasons for using combination antimicrobial therapy.* ***Aims & Objectives:*** *To test the susceptibility of the clinical isolates of P. aeruginosa to antimicrobials like Ciprofloxacin, Ofloxacin, Ceftazidime and Cefoperazone and to investigate the possible synergy of their combinations.* ***Materials & Methods:*** *The study was conducted in Bacteriology Laboratory, Department of Microbiology, Govt. Medical College, Miraj.* ***Study Design:*** *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 fifty 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:*** *The in vitro combination of ciprofloxacin and Ceftazidime is the most effective combination against clinical isolates of P. aeruginosa.*

Keywords*: Cefoperazone, Ceftazidime, Ciprofloxacin, Ofloxacin, Pseudomonas aeruginosa*

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Introduction

Pseudomonas (P) aeruginosa is an increasingly important cause of nosocomial infections. 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. The multi drug resistance of these P. aeruginosa isolates plays an important role in the colonization or infection of chronically hospitalized patients.[2,3,4] P. aeruginosa exhibits high level of resistance to many antimicrobials and because of its ability to develop resistance during therapy, empirical treatment for serious systemic infections usually involves 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. 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. For these reasons, there is continued search for alternative combinations. One such combination is fluoroquinolones with third generation cephalosporins (β-lactams).[2]

Aims & Objectives

Present study aims to test the antimicrobial susceptibility and to investigate the possible synergy of fluoroquinolones (Ciprofloxacin and Ofloxacin) and third generation cephalosporins (Ceftazidime and Cefoperazone) on clinical isolates of P. aeruginosa.

Materials & Methods

The study was conducted in Bacteriology Laboratory, Department of Microbiology, Govt. Medical College, Miraj. Fifty (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, Govt. Medical College, Miraj and from Padmabhushan Vasantdada General Hospital, Sangli. The samples were obtained from different sources like burn wound, urine, pus, blood. 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.

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] P. aeruginosa ATCC 27853 was used as a quality control strain. Antimicrobial agent and their diameter of zone of inhibition as per National Committee for Chemical Laboratory Standards (NCCLS) guidelines. Reading of result was obtained by measuring diameter of zone of inhibition.

Combination Study

In this Disc diffusion method the standard inoculum and Mueller Hinton agar were used. The possible interaction was observed between fluoroquinolones (Ciprofloxacin and ofloxacin) and third generation cephalosporins (ceftazidime and cefoperazone) by using following combinations.

a) Ciprofloxacin & Ceftazidime
b) Ciprofloxacin & Cefoperazone
c) Ofloxacin & Cefoperazone
d) Ofloxacin & Ceftazidime

Reading of Results

With synergistic combination, enhancement or bridging was observed at or near the junction of the two zones of inhibition. Interaction was considered to be synergistic when there was a well observed change (>2mm) in the zone of inhibition. Interaction was considered as antagonistic when truncation was observed near the junction of the two zones of inhibition.

Results

The susceptibility pattern of the clinical isolates to all four antimicrobials showed that out of 50 isolates, 22 (44%) isolates were sensitive while 17 (34%) isolates were resistant to all 4 antimicrobials. 11 (22%) isolates which exhibited mixed resistant pattern were sensitive to one or a few drugs and resistant to others.

Susceptibility of 50 isolates to individual drug showed that Ceftazidime ranked first in exhibiting antimicrobial activity against P. aeruginosa. 32 (64%) of the clinical isolates were sensitive to Ceftazidime while 18 (36%) were resistant to it. Cefoperazone ranked 2nd in the order of activity 29 (58%) were sensitive while 21 (42%) were resistant. This was followed by ciprofloxacin, 27 (54%) of isolates were sensitive to ciprofloxacin and 23 (46%) were resistant. Ofloxacin was the least active among the 4 antimicrobials to which 22 (44%) isolates were sensitive and 28 (56%) were resistant (Figure- 1).

In resistant isolates, combination of ciprofloxacin and Ceftazidime demonstrated synergy in 11 isolates and combination of ciprofloxacin and Cefoperazone exhibited synergy in 9 isolates, Ofloxacin and Ceftazidime in 10 isolates and Ofloxacin and Cefoperazone in 8 clinical isolates. The most synergistic combination in present study was that of ciprofloxacin and Ceftazidime. None of the clinical isolates displayed antagonism with

the use of combination of fluoroquinolone and third generation cephalosporins (Table- 1).

Figure- 1: Antimicrobial Susceptibility

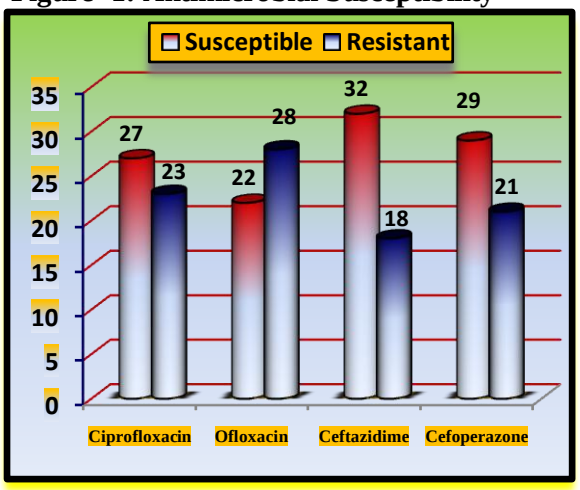

Table- 1: Antimicrobial combination on resistant isolates

Combination	Synergy	No synergy	% of synergy
Ciprofloxacin & Ceftazidime	11	6	64.71
Ciprofloxacin & Cefoperazone	9	8	52.94
Ofloxacin & Ceftazidime	10	7	58.82
Ofloxacin & Cefoperazone	8	9	47.06

In susceptible isolates of P aeruginosa, combination of Ciprofloxacin and Ceftazidime shows synergy on 20 (90.91%) isolates. Ciprofloxacin and Ccfoperazone 18 (81.82%), Ofloxacin and Ceftazidime 20 (90.91%) and Ofloxacin and Ccfoperazone on 19 (86.36) isolates. None of the clinical isolates displayed antagonism with the use of combination of fluoroquinolones and third generation cephalosporins.

In isolates showing mixed susceptibility pattern, 3 isolates were susceptible to ciprofloxacin. 5 were susceptible to Ofloxacin, 8 were susceptible to Ceftazidime and 7 were susceptible to Cefoperazone. Ceftazidime was the most active antimicrobial on the mixed susceptible isolates of P. aeruginosa. Among the clinical isolates showing mixed susceptibility pattern, 9 isolates demonstrated not only susceptibility but also synergy to the combination of Ciprofloxacin and Ceftazidime. This was most effective combination on the mixed susceptible isolates of P aeruginosa followed by Ciprofloxacin and Cefoperazone which demonstrated synergy on 8 isolates. Ofloxacin and Cefoperazone demonstrated synergy on 5 isolates and Ofloxacin and Ceftazidime demonstrated synergy on 4 isolates.

Discussion

Multi drug resistant isolates of P. aeruginosa 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.

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 [8] 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 Helen Giamarellou et al,[9] clinical isolates were derived from various sources like urine (15 strains), Sputum (5 strains), pus (4 strains) and blood (2 strains).

In the study it was observed that 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. A large number of studies have evaluated the susceptibly patterns of P. aeruginosa to different antimicrobials. Patterns of susceptibility of isolates quoted by many studies 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 [10] to as low as 21.08% in a study conducted by Mehta et al [11] similarly the resistance to ciprofloxacin ranges from 85% in a study conducted by Rustegar Laro et al [12]

to 50% in a study conducted by Bharti Sarkar et al. [13]

Although no exact correlation was found the pattern of resistance exhibited by P. aeruginosa in our study falls in 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 where multi drug resistance P. aeruginosa is highly prevalent.

Out of the 17 clinical isolates that were resistant to all four antimicrobials used in the present study, 11 isolates displayed not only susceptibility but also synergism to the combination of Ciprofloxacin and Ceftazidime, similarly 9 isolates exhibited susceptibility and synergism to the combination of Ciprofloxacin and Cefoperazone, 10 isolates exhibited susceptibility and synergism to the combination of Ofloxacin and Ceftazidime and 8 isolates exhibited susceptibility and synergism to the combination of Ofloxacin. Thus the combination of Ciprofloxacin and Ceftazidime was the most effective synergistic combination on the clinical isolates of P aeruginosa already resistant to individual antimicrobials.

The results of the present study correlated with the study conducted by Douglaus N Fish et al [14] who reported that the combination of Ciprofloxacin and Ceftazidime was active and exhibited synergy in 60-80% of tested P. aeruginosa clinical isolates. Also the results of the present study were similar to the study conducted by Mayer and E Nagy [2] who investigated the effect of fluoroquinolones in combination with third generation cephalosporins and demonstrated high synergistic activity with the combination.

Among the 22 clinical isolates susceptible to all four drugs, a very high proportion (99.91%) of isolates exhibited synergy with the use of combination of Ciprofloxacin and Ceftazidime. Synergism was very low with use of the combination of Ofloxacin and Cefoperazone. Some of the isolates although susceptible to individual antimicrobials did not display synergy with the use of combination of drugs. This might be simply due to additive effect without clear cut synergism. A similar study was conducted by Madaras-kelly K J et al [15] to demonstrate the synergistic effect between fluoroquinolones and cephalosporins on clinical isolates of P. aeruginosa, the results of which correlated with the present study.

In mixed susceptibility pattern, 9 clinical isolates demonstrated not only susceptibility but also synergism to the combination of Ciprofloxacin and Ceftazidime. This was the most effective combination on the mixed susceptible- resistant isolates of P. aeruginosa followed by the combination of Ciprofloxacin and Cefoperazone which demonstrated synergy on the 8 isolates. Combination of Ofloxacin with third generation cephalosporins was less active as compared to the above Combinations. The results of this study are similar to the study conducted by Douglas N Fish et al [14] where the combination of Ciprofloxacin and Ceftazidime was found to be the most effective combination on mixed susceptible- resistant isolates of P. aeruginosa.

Conclusion

Multi drug resistant P. aeruginosa is the single most important factor influencing the hospital environment. This in turn facilitates their transmission from one source to another resulting in variable degree of nosocomial infection in any hospital. This study was conducted in search of an alternative synergistic combination against P. aeruginosa and from present study we suggest that the combination of Ciprofloxacin and Ceftazidime can be one such effective alternative. The synergy between fluoroquinolones and third generation cephalosporins can thus be effectively utilized in the treatment of infections caused by multi drug resistance P. aeruginosa.

Conflict of Interest: *None declared*
Source of Support: *Nil*
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