IN-VITRO COMPARATIVE ANTIBACTERIAL SUSCEPTIBILITY PATTERNS OF THIRD GENERATION CEPHALOSPORINS AGAINST PSEUDOMONAS AERUGINOSA BY USING BROTH DILUTION METHOD.

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ABSTRACT

The leading purpose of our study was the assessment of epidemiological data on the resistance of Pseudomonas aeruginosa, and to compare the activity of Cefoperazone, Ceftazidime, Ceftizoxime, Cefotaxime, Ceftriaxone and Cefixime against Pseudomonas aeruginosa. For this purpose Broth Dilution Method was used for determination of Minimum Inhibitory Concentration (MIC) of antibacterial agents using strains of Pseudomonas aeruginosa ATCC 27853 as control; according to criteria developed by Clinical and Laboratory Standards Institute (CLSI). A total of 200 clinical isolates of Pseudomonas aeruginosa were collected from different hospitals of Karachi. In-vitro susceptibility patterns (i.e. sensitive, resistant and intermediate) of third generation cephalosporins were reviewed. The study revealed that the most effective antibacterial agent was Cefoperazone (80% sensitive) while the second most effective antibacterial agent was Ceftazidime (70% sensitive). An intermediate activity was shown by Cefotaxime and Ceftizoxime. Pseudomonas aeruginosa was shown to be resistant to Cefixime and Ceftriaxone (0% sensitive).

Key Words: *Third generation cephalosporins, Pseudomonas aeruginosa, Minimum Inhibitory Concentration, Antibacterial susceptibility test.*

INTRODUCTION

Pseudomonas aeruginosa (Ps. aeruginosa) is termed as an opportunistic pathogen belongs to the family Pseudomonadaceae. Majority of Pseudomonads species are known to cause opportunistic infections in human [1]. The *Ps. aeruginosa* is an oxidase positive, aerobic rod, non-fermentative, gram-negative with unipolar motility and pigments producer. These characteristics are used in laboratory for prompt diagnosis [2]. *Ps. aeruginosa* is found in moist envoirments especially on human body sites which are moist. It extensively causes nosocomial infections in hospitalized and immunocompromised hosts and patients with cystic fibrosis [3], [4]. The antibacterial resistance in isolates of *Ps. aeruginosa* are diverse from hospital environment and geographical locations [5]. Mechanisms of antimicrobial resistance developed by *Ps. aeruginosa* against various antibacterial agents include, beta-lactamase production and multidrug efflux pumps [6]. The hypermutable strains of *Ps. aeruginosa* (in patients of cystic fibrosis) can be isolated from defected methyl directed mismatching repair (MMR) system [1]. With prolong therapy, *Ps. aeruginosa* has its unique characteristic of developing resistance to all antibiotics due to biofilm formation [2].

Cephalosporins are bacterial cell wall inhibitors consist of beta-lactam ring. These antibacterial agents are widely used in daily prescribing practices [7]. The antimicrobial activity and spectra of cephalosporins are depends upon R-side chain attachment on cephalosporin nucleus i.e. 7-amino cephalosporonic acid (7-ACA) [8]. Cephalosporins act as a bactericidal agent by blocking the transpeptidation of peptidoglycan, thus inhibit the synthesis of bacterial cell

wall [7]. The third generation cephalosporins have a broad spectrum of activity against gram-negative organisms especially *Ps. aeruginosa*, found in community and hospital acquire infections [9].

This study was designed to identify the In-vitro susceptibility profile of different clinical isolates of *Ps. aeruginosa* in Karachi against Cefoperazone, Ceftazidime, Ceftizoxime, Cefotaxime, Ceftriaxone and Cefixime. For this purpose, Minimum Inhibitory Concentration of each antibacterial agent was determined by Broth (Tube) Dilution Method [10].

MATERIALS & METHODS

Three biological culture media were used in this laboratory based study i.e. Nutrient Broth (Batch No. CM001, IVD, Oxoid, England), Mueller-Hinton Broth (Batch No. CM0405, IVD, Oxoid, England), and 5% Sheep Blood Agar (Oxoid, England).

For MIC test: Cefoperazone (Cefobid); Pfizer Pharmaceuticals, Ceftazidime (Fortazim); Bosch Pharmaceuticals Pakistan, Ceftizoxime (Cefizox); Barrett Hodgson Pakistan, Cefixime (Micronized); Pharmagen Pakistan, Cefotaxime (Exef), and Ceftriaxone (Maxef); Indus Pharma Pakistan were purchased commercially and used as antibacterial agents.

Bacterial Cultures:

Various clinical specimens of *Ps. aeruginosa* were collected from central laboratories of different hospitals of Karachi. They were isolated on nutrient agar slants and transported under cooled conditions. Sub culturing of isolates were done on Media (Mueller Hinton Broth).

Identification of Bacterial Isolates:

The identification and confirmation of isolates of *Ps. aeruginosa* were done on the basis of cultural characteristics, Gram staining and biochemical tests [11].

Inoculum Adjustment:

The density of inoculum was adjusted by comparing with 0.5 McFarland standard after incubation to achieve turbidity [10].

MIC Determination:

The minimum inhibitory concentrations (MICs) for this organisms was determined by using Serial Two-fold dilution method [10].

Reading and Interpretation:

The MICs were read as the minimum concentrations of antibiotics at which no visible growth present. Data was interpreted according to CLSI (Table 2B-1) Ver. 2010 [12]. The MIC of each antibiotic was also obtained for the control strains *Ps. aeruginosa* ATCC 27853 to ensure the method was performed correctly.

RESULTS & DISCUSSION

Our study was conducted with 200 clinical isolates of *Ps. aeruginosa.* Antibacterial susceptibility of six selected third generation cephalosporins was determined. Results revealed that the most effective antibacterial agent is Cefoperazone (CFP) against *Ps. aeruginosa,* as it shown the highest sensitivity i.e. (160)80% (Table.1) measured by broth dilution method (MIC method), while (20)10% isolates are resistant to Cefoperazone (CFP) (Table.1).

Another study conducted by Farida et al,. [6] Also revealed the highest effectiveness of Cefoperazone against isolates of *Ps. aeruginosa*.

Ceftazidime (CAZ) was the second most effective antibacterial agent against isolates of *Ps. aeruginosa* showing (140)70% (Table.1). Almost similar results for Ceftazidime were shown in the study for [13] i.e. 80 % sensitivity and also in the experiment of [14] Ceftazidime showed 89% sensitivity. In the study conducted by [6] *Ps. aeruginosa* showed 62% sensitivity.

Cefotaxime (CTX) was shown (60)30% (Table.1) sensitivity against *Ps. aeruginosa* and (120)60% isolates shown intermediate effect. These results confirmed the outcome of J Puri et al, (1996) [15]. Ceftizoxime (ZOX) was shown the least (20)10% (Table.1) sensitivity against the isolates of *Ps. aeruginosa*. Almost similar results i.e. 16% were shown in the experiment conducted by Saleem et al,. [13]. Cefixime (CFM) and Ceftriaxone (CRO) did not show any satisfactory activity against isolates of *Ps. aeruginosa* but study conducted by Tahira Mansoor et al,. shown positive results i.e. 21% of Ceftriaxone [14]. *Ps. aeruginosa* is a prominent gram-negative pathogen that causes nosocomial infections and its treatment is a challenge because resistance reduces the optimal therapeutic options. Therefore repeated measures should be taken to control the delinquent of resistance. Frequently updated and validated susceptibility profiles data are required to ensure the provision of effective therapy.

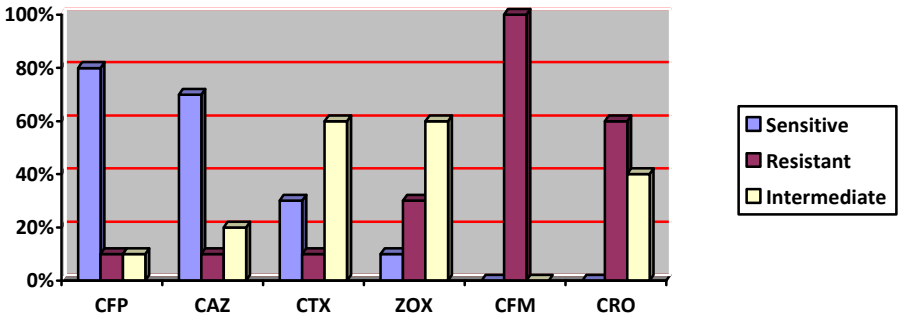
In order to make a control over infections caused by *Ps. aeruginosa*, efforts should be raised to control limitations like insufficient published data conducted in Pakistan and designing randomized clinical trials in collaboration of governmental and non-governmental organizations. In third world countries like Pakistan where the total health budget is less than 1% of GDP, cannot afford the antibiotic resistance problems alone [16].

TABLE AND FIGURES

Table: 1. MICs of Third Generation Cephalosporins (Percentages) against *Ps. aeruginosa*

S.No.	Antibiotics	Code	Resistance (R)	Intermediate (I)	Sensitive (S)
1	Cefoperazone	CFP	10 %	10 %	**80 %**
2	Ceftazidime	CAZ	10 %	20 %	**70 %**
3	Cefotaxime	CTX	10 %	60 %	30 %
4	Ceftizoxime	ZOX	30 %	60 %	10 %
5	Cefixime	CFM	100 %	0 %	0 %
6	Ceftriaxone	CRO	60 %	40 %	0 %

Figure: 1. Graphical Representation of Susceptibility patterns of Third Generation Cephalosporins (Percentages)



CONCLUSION

The results of our study provide useful strategies for selecting suitable antibacterial agent against infections caused by *Pseudomonas aeruginosa*. Cefoperazone (CFP) is found to be the most effective antibacterial agent against *Ps. aeruginosa*. While the second choice is Ceftazidime (CAZ) and Cefotaxime (CTX) may also considered for the treatment of infection caused by *Ps. aeruginosa*.

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