INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

IN VITRO COMPARATIVE STUDIES ON SUSCEPTIBILITY PATTERNS OF *PSEUDOMONAS AERUGINOSA* AND *STAPHYLOCOCCUS AUREUS* TO CEFEPIME AND CEFPIROME

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Article Received on: 18/03/13 Revised on: 03/04/13 Approved for publication: 11/05/13

DOI: 10.7897/2230-8407.04528
IRJP is an official publication of Moksha Publishing House. Website: www.mokshaph.com

ABSTRACT
The aim of this study was to gather the epidemiological data on the susceptibility patterns of *Pseudomonas aeruginosa* and *staphylococcus aureus* against fourth generation cephalosporins (Cefpirome and Cefepime). Total 100 isolates of each bacterial species were collected from central laboratories of different private hospitals of Karachi, Pakistan between September 2012 and December 2012. Modified Kirby-Bauer disc diffusion method was used for the determination of sensitivity of Cefpirome and Cefepime using strains of *Pseudomonas aeruginosa* (ATCC 27853) and *staphylococcus aureus* (ATCC 6538) as control. In-vitro comparative susceptibility patterns of Cefpirome and Cefepime were studied. Isolates of *Pseudomonas aeruginosa* were found to be resistant, 15% against Cefepime and 18% against Cefpirome while that of *staphylococcus aureus* were 3% against Cefepime and 5% against Cefpirome. Results clarify that *Pseudomonas aeruginosa* is more resistant as compare to *staphylococcus aureus* against both of the fourth generation cephalosporins. It is concluded from this study that Cefepime and Cefpirome are highly effective against *Pseudomonas aeruginosa* and *Staphylococcus aureus* and can be used safely in the treatment of infections caused by these organisms.
Keywords: Cefepime, Cefpirome, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, Susceptibility test.

INTRODUCTION

Pseudomonas aeruginosa *(Ps. aeruginosa)* is an opportunistic pathogen, one of the leading causes of nosocomial infections (pneumonia) and community acquired infections. *Ps. aeruginosa* is gram negative bacillus, non-sporing, non-fermentative, aerobic and generally non-capsulated actively motile unipolar nosocomial pathogen belonging to the family Pseudomonadaceae. This pathogen was first isolated by Gessard in 1882 from the wound in pure culture. He determined that appearance of blue green strain on surgical dressing was due to production of pigments by this pathogenic organism.[1]

The pattern of antibiotic resistance of *Ps. aeruginosa* may be developed by several resistance mechanisms against different antibacterial agents like beta-lactamase production[2] formation of biofilms, a unique characteristic to develop antibiotics resistance.[3]

Staphylococcus aureus *(S.aureus)* is one of the most successful and adaptable human pathogen. Its ability to acquire mechanism of antibiotic resistance and advantageous pathogenic determinants are the factor which contributes to it is emergence in both community setting and nosocomial infections.[4] In 1961, among nosocomial isolates of *S.aureus,* the resistance to methicillin was first appeared and since that methicillin resistant staphylococcus aureus (MRSA) has become very common in ICU's and hospitals throughout the world. MRSA can also cause aggressive infection in healthy peoples. Necrotizing pneumonias and suppurative skin infections are very common syndromes in these new strains. substantial high morbidity and mortality rates due to *S.aureus* infection suggest that it is convivially developing in to a challenging public health problem.[4] The incidence of infections caused by MRSA has increased, the strains involved in these infections are mostly multi drug resistant.[5]

Cephalosporins are classified by their chemical structure, clinical pharmacology antimicrobial spectrum or resistance to beta-lactamase.[6] In 1993, the most active fourth generation cephalosporin, Cefepime was introduced.[3] It is one of the few agents that have good activity against *Ps. aeruginosa*.[7] As compared to the third generation cephalosporins it has rapid penetration into periplasmic space. It shows remarkable activity against *Ps. aeruginosa* MDR isolate, Citrobacter species, Proteus mirabilis, Klebsiella pneumonia, Serratia species but it is less active against Bacillus fragillis.[3] It can resist the hydrolysis by the common chromosomally and plasmid mediated β-lactamases. It is used against critical pneumonia, infections of soft tissues and bones, febrile neutropenia and urinary tract infections. Cefepime monotherapy gives both an excellent microbiological clearance and a good clinical response. Irrational use of Cefepime is warranted in order to preserve its antibacterial potency.[8] Cefepime has a well-tolerated safety profile and administered twice daily. It is reflected from clinical data that Cefepime is comparable to Ceftazidime and Cefotaxime; therefore it is an effective alternative agent for susceptible pathogens.[9]

Cefpirome is a fourth generation cephalosporin having a wide range of antibacterial activity. Cefpirome shows greater activity against gram-negative organisms as compared to third generation cephalosporins. It is highly active against abroad range of gram negative and gram positive organisms including methicillin resistant staphylococcus aureus (MRSA) and *Ps. aeruginosa.* It also shows great activity against haemophillus influenza and many member of family Enterobacteriaceae.[10] Cefpirome is stable against most of the chromosome and plasmid mediated β-lactamases. Its tolerability is similar to that of Ceftazidime and other third generation cephalosporins. Diarrhea is commonly observed

event thus Cefpirome is usually a valuable extended spectrum agent for severe injection treatment.[11]

This study was designed to determine the susceptibility patterns of *Ps. aeruginosa* and *S.aureus* isolated from different hospitals in Karachi, Pakistan for the analysis of fourth generation cephalosporins (Cefpirome and Cefepime). Modified Kirby-Bauer disk diffusion method was used to determine susceptibility pattern of isolates.[12]

Objective

The aims of our study were:

- To evaluate and gather the epidemiological data on the resistance of *Ps. aeruginosa* and *S.aureus.*
- To compare the susceptibility of fourth generation cephalosporins against *Ps. aeruginosa* and *S.aureus.*

MATERIALS AND METHODS

Four different types of biological culture media were used for isolation, biochemical and sensitivity testing of *Ps. aeruginosa* and *S.aureus.* These were Mueller-Hinton Agar (Oxoid, England), Mueller-Hinton Broth (Oxoid, England), 5% Sheep Blood Agar (Oxoid, England), and MacConkey Agar (Oxoid, England). Antibiotics discs (with commercially available concentrations) used in this experiment were: Cefepime (FEP) 30µg, and Cefpirome (CPO) 30µg. These discs were commercially purchased from Oxoid Ltd, England.

Bacterial Isolates

Isolates of *Ps. aeruginosa* and *S.aureus* were collected from central laboratories of different private hospitals in Karachi, Pakistan including Liaqat National Hospital (LNH), Faiz Rehman Hospital, and Khyber Hospital. Sub culturing of isolates were done on Media (Mueller Hinton Agar).

Identification of Bacterial Isolates

The identification of isolates were done on the basis of cultural characteristics, gram staining and biochemical tests including positive reaction to oxidase, growth at 42°C and pyocyanin production.[13]

Antimicrobial Susceptibility Testing

The test was performed using modified Kirby-Bauer disc diffusion method according to the guidance of the Clinical and Laboratory Standards Institute (CLSI).[14], [12] Mueller-Hinton agar was used as the growth medium in disk susceptibility test while the broth culture was incubated at 37°C until it achieved the turbidity of the 0.5 McFarland standard in order to adjust inoculum density.[15]

Reading and Interpretation

After incubation period, the diameter of each zone of inhibition was measured by using Varnier caliper, and results were compared as susceptible, intermediate and resistant to the agents that with known standards i.e. according to CLSI Ver. 2010.[14] Measurements were also taken for control strains of *Ps. aeruginosa* (ATCC 27853) and *S.aureus* (ATCC 6538) to ensure that the method performed correctly.

RESULTS

Our study was designed to evaluate the in-vitro susceptibility profile of *Ps. aeruginosa* and *S.aureus* isolates. The percentage of profiles (susceptible, intermediate and resistant) was shown in (Table 1). Out of 100 isolates of *Ps. aeruginosa*, 15 isolates were to be resistant against Cefepime and 18 isolates were resistant against Cefpirome. Only 3 out of 100 isolates of *S.aureus* were shown resistant against Cefepime and 5 out of 100 isolates were resistant against Cefpirome (Table 1).The comparative susceptibility patterns of *Ps. aeruginosa* against both antibacterial agents are given in Figure 1. Similarly, the susceptibility patterns of *S.aureus* against both antibacterial agents are given in Fig (2). Overall, *Ps. aeruginosa* shows more resistant to Cefepime (15%) and Cefpirome (18%) as compare to *S.aureus* which shows resistant to Cefepime and Cefpirome 3% and 5% respectively, graphically represented in Figure 1 and 2. Measured zone of inhibition against both isolates were shown in images (I to VI).

Statistical Analysis

Susceptibility data were compared by using chi-square tests through SPSS version 19.0.

Cefepime against *Ps. aeruginosa* & *S.aureus*			
	Value	**df**	**Asymp. Sig. (2-sided)**
Pearson Chi-Square	1.979[a]	4	.740
Likelihood Ratio	3.014	4	.555
Linear-by-Linear Association	.289	1	.591
N of Valid Cases	100		
a. 6 cells (66.7%) have expected count less than 5. The minimum expected count is .15.			

Cefpirome against *Ps. aeruginosa* & *S.aureus*			
	Value	**df**	**Asymp. Sig. (2-sided)**
Pearson Chi-Square	2.013[a]	4	.733
Likelihood Ratio	2.702	4	.609
Linear-by-Linear Association	.987	1	.320
N of Valid Cases	100		
a. 6 cells (66.7%) have expected count less than 5. The minimum expected count is .12.			

DISCUSSION

This study was conducted to evaluate the susceptibility patterns of *Ps. aeruginosa* and *S.aureus*. Treatment of *Ps. aeruginosa* is a challenge because the therapeutic options are limited due to resistance [16]. Our study showed that 81% were sensitive, 15% were resistant and 4% were intermediate to Cefepime (FEP) as shown in (Table 1). Approximately similar results for sensitivity i.e. 77.6% were reported by (Christenson JC et al., 2000) [17]. Another study which was conducted by (Ehimare Akhabue et al., 2011) reported 8.4% resistant to Cefepime.[7] However, (Luqman Satti et al., 2011) from Pakistan reported 71% resistant isolates of *Ps. aeruginosa* to Cefepime This variation in resistance was may be due to demographic changes accordingly.[3]

A second antibacterial agent used in this study was Cefpirome (CPO) which has been introduced for the treatment of serious infections including lower respiratory tract infections and septicemias.[10]

Table 1: Comparison of Sensitivity Pattern of Cefepime & Cefpirome (percentagewise) against *Ps. aeruginosa* & *S.aureus* (n=100)

Antibiotics	Code	Potency	Zone of Inhibition (mm) against *Ps.aeruginosa*.			Zone of Inhibition (mm) against *S.aureus*.		
			S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Cefepime	FEP	30 µg	81	04	15	85	12	03
Cefpirome	CPO	30 µg	79	03	18	86	09	05

S = Sensitivity, I = Intermediate, R = Resistant

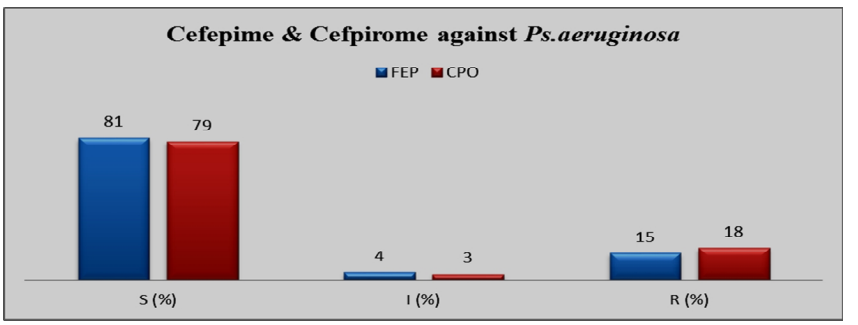

Figure 1: Comparative susceptibility patterns of *Ps. aeruginosa* to Cefepime and Cefpirome

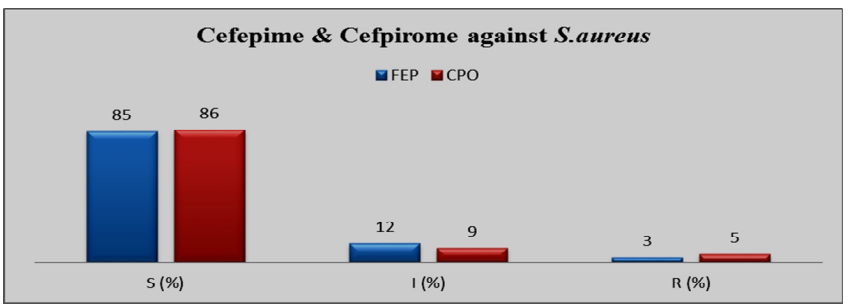

Figure 2: Comparative susceptibility patterns of *S.aureus* to Cefepime and Cefpirome
FEP = Cefepime; CPO = Cefpirome. S = Sensitivity, I = Intermediate, R = Resistant.

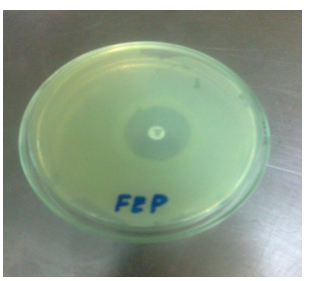

Image I. FEP against *Ps.aeruginosa*

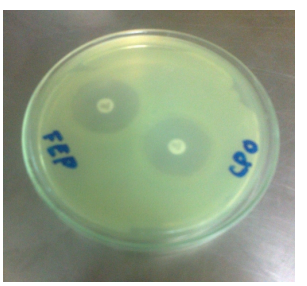

Image II. FEP & CPO against *Ps.aeruginosa*

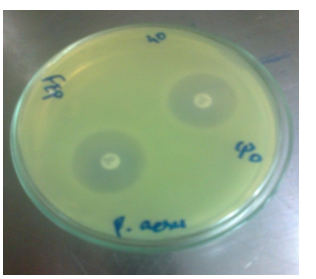

Image III. FEP & CPO against *Ps.aeruginosa*

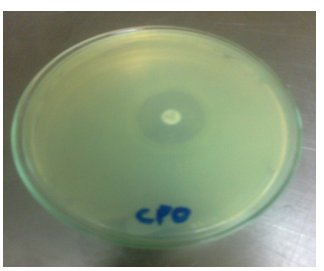

Image IV. CPO against *Ps.aeruginosa*

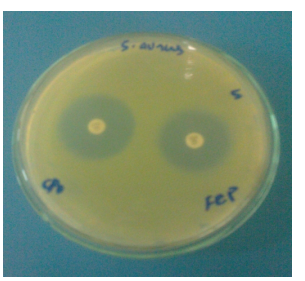

Image V. FEP & CPO against *S.aureus*

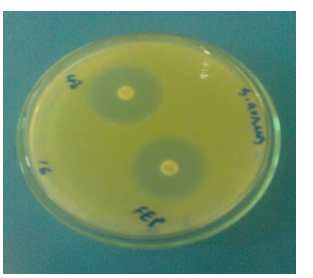

Image VI. FEP & CPO against *S.aureus*

FEP= Cefepime; CPO=Cefpirome

When isolates of *Ps. aeruginosa* were tested against Cefpirome, it showed marked susceptibility to Cefpirome; 79% sensitive, 18% resistant and 3% intermediate. Similar study was carried out in Pakistan by (Saleem Hafeez et al., 2000) which revealed that Cefpirome was 74% sensitive against *Ps. aeruginosa* while pathogen was 15% resistant to Cefpirome.[18]

In present study, Cefepime and Cefpirome showed approximately similar activity against isolates of *S.aureus* i.e. 85% and 86% respectively. A study carried out in Pakistan by (Saleem Hafeez et al., 2000) revealed same results regarding activity of Cefpirome against isolates of *S.aureus*.[18]

Based on our data, isolates of *Ps. aeruginosa* were more resistant to Cefepime and Cefpirome (Fig 1.) as compare to isolates of *S.aureus* against Cefepime and Cefpirome (Fig 2.)

In vitro comparative studies of antibiotics have certain limitations such as; short period of study designs, therefore in order to achieve better results especially in third world countries like Pakistan, we need long term studies in association of governmental and non-governmental organizations.[19]

It is highly recommended that, there should be an appropriate guideline for the use of these antibacterial agents to avoid any bitter experience of developing resistance against above organisms and also to other organisms susceptible to it.

CONCLUSION

It is concluded from this study that Cefepime and Cefpirome are highly effective against *Ps. aeruginosa* and *S.aureus* and can be used safely in the treatment of infections caused by these organisms.

ACKNOWLEDGEMENT

Authors are very thankful to Faculty of Pharmacy, Hamdard University Karachi, Pakistan for providing us such facilities to conduct this project.

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Cite this article as:

Humza, Ahmad Ullah, Khan Khalid, Jamil Sahrish, Taj Ayaz, Anwer Atif. In vitro comparative studies on susceptibility patterns of Pseudomonas aeruginosa and Staphylococcus aureus to Cefepime and Cefpirome. Int. Res. J. Pharm. 2013; 4(5):137-140

Source of support: Nil, Conflict of interest: None Declared