INTRODUCTION

Pseudomonas aeruginosa (Ps. aeruginosa) is termed as an opportunistic pathogen belonging to the family Pseudomonadaceae which have a large number of species and are able to cause opportunistic infections in human. The Ps. aeruginosa is aerobic rods, non-fermentative, gram-negative motile and water soluble pigments producer. In laboratory, these characteristics are used for rapid diagnosis. Ps. aeruginosa can be isolated from the feces of healthy people and also found sporadically on the human body sites which are moist i.e. ear, axilla, throat, nasal mucosa. In hospitals; mops, sinks, respiratory equipment, food mixers and disinfectant solutions are reservoir of Ps. aeruginosa. It extensively causes nosocomial infections like nosocomial pneumonia, nosocomial urinary tract infections, nosocomial bacteremia and wounds. The pattern of antibiotic resistance of Ps. aeruginosa isolates varied from hospital environment to geographical locations. Several resistance mechanisms may be developed by Ps. aeruginosa against different antibiotics, beta-lactamase production is the major mechanism of resistance to beta-lactam antibiotics. Mostly, third generation cephalosporins are hydrolyzed by ESBL/enzymes. Also the defected methyl directed mismatching repair (MMR) system in the hypermutable strains of Ps. aeruginosa is being isolated frequently from cystic fibrosis (CF) patient’s lung. In cystic fibrosis patients, it has been involved in the formation of biofilms, a unique characteristic to develop antibiotics resistance.

Cephalosporins may be classified by their clinical pharmacology, chemical structure, antimicrobial spectrum or resistance to beta-lactamase, but the classification by generation is well accepted, very useful and based on basic features of antimicrobial activity. Among all third generation cephalosporins, Cefoperazone and Ceftazidime are the most effective against Ps. aeruginosa and, they are also stable to breakdown effect of most beta-lactamases. Ceftazidime is very effective to treat pediatric patients and nosocomial infections. Ceftriaxone is unique because of its prolonged serum half-life, which permits once or twice daily dosing.

This study was designed to determine the In-vitro susceptibility of Ps. aeruginosa isolated from clinical specimens in Karachi, Pakistan for the analysis of third generation cephalosporins. Modified Kirby-Bauer disk diffusion technique was used to determine susceptibility of isolates.

Objective

The prime intention of this study was to evaluate and accumulate the epidemiological data on the resistance of Ps. aeruginosa within the community and to compare the activity of third generation cephalosporins against Ps. aeruginosa.

MATERIALS & METHODS

Three Biological culture media were used i.e. Mueller-Hinton Agar (Batch No. CM0337, IVD, Oxoid, England), Mueller-Hinton Broth (Batch No. CM0405, IVD, Oxoid, England), and Blood Agar (Oxoid, England). Antibiotics discs used in this experiment were: Cefoperazone (CFP) 75µg, Cefixime (CFM) 50µg, Ceftizidime (CAZ), Cefotaxime (ZOX), Ceftriaxone (CTX), and Cefixime (CRO) each were of 30µg. These discs were commercially purchased from Oxoid Ltd, England.

Bacterial Cultures

Isolates of Ps. aeruginosa were collected from different laboratories of public and private hospitals of Karachi, Pakistan. They were isolated on nutrient agar slant and transported under cooled condition. Sub culturing of isolates were done on Media (Mueller Hinton Broth).

Identification of Bacterial Isolates

The identification of isolates was done on the basis of cultural characteristics, Gram staining and biochemical tests.

Susceptibility Test

The test was performed using modified Kirby-Bauer disc diffusion method according to the guidance of the Clinical and Laboratory Standards Institute. Mueller-Hinton broth was used as the growth medium in disk susceptibility test while inoculum was compared with 0.5 McFarland standard turbidity to adjust inoculum density.

ABSTRACT

The prime intention of this study was the evaluation & accumulation of epidemiological data on the resistance of Pseudomonas aeruginosa, and to compare the activity of different third generation cephalosporins against Pseudomonas aeruginosa. For this purpose Modified Kirby-Bauer Method was used for the determination of sensitivity of antibacterial agents using strains of Pseudomonas aeruginosa ATCC 27853 as control. Total 250 isolates of Pseudomonas aeruginosa were collected from different public and private hospitals of Karachi, Pakistan. In-vitro qualities (i.e. sensitive, resistant and intermediate) of six members of third generation cephalosporins (Cefoperazone, Ceftazidime, Ceftizoxime, Cefotaxime, Ceftriaxone and Cefixime) were reviewed. Results showed that Cefoperazone was the most effective antibacterial agent (80% sensitive), while the second most effective antibacterial agent was Ceftazidime (70% sensitive). Cefotaxime and Ceftizoxime also showed intermediate activity. Cefixime and Ceftriaxone didn't show any supportive activity i.e. 0% sensitive at all.

KEY WORDS: Third generation cephalosporins, Pseudomonas aeruginosa, Susceptibility test.

Humza Ahmad Ullah*, Iqbal Javeid1, Khan Khalid1, Hani Muhammad2, Jamil Sahrish3
1Faculty of Pharmacy, Hamdard University, Karachi, Pakistan
2Department of Pharmacy, GC University, Faisalabad, Pakistan
3Faculty of Pharmacy, Federal Urdu University, Karachi, Pakistan

Article Received on: 08/11/12 Revised on: 11/12/12 Approved for publication: 23/12/12

*Email: incredible_313@yahoo.com
The diameter of each zone of inhibition was measured by a ruler, and results were reported as susceptible, intermediate and resistant to the agents that were tested. Data was interpreted according to CLSI (Table 2B-1) Ver. 2010\(^2\). Diameters of Zones of Inhibition for each antibiotic were also obtained for the control strains Ps. aeruginosa ATCC 27853 to ensure that the method performed correctly.

Table 1. Measured Zone of Inhibition against *Ps. aeruginosa*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Antibiotics</th>
<th>Code</th>
<th>Resistance (R)</th>
<th>Intermediate (I)</th>
<th>Sensitive (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cefoperazone</td>
<td>CFP</td>
<td>10%</td>
<td>10%</td>
<td>80%</td>
</tr>
<tr>
<td>2</td>
<td>Ceftazidime</td>
<td>CAZ</td>
<td>11%</td>
<td>19%</td>
<td>70%</td>
</tr>
<tr>
<td>3</td>
<td>Cefotaxime</td>
<td>CTX</td>
<td>3.3%</td>
<td>80%</td>
<td>16.8%</td>
</tr>
<tr>
<td>4</td>
<td>Ceftizoxime</td>
<td>ZOX</td>
<td>20%</td>
<td>60%</td>
<td>20%</td>
</tr>
<tr>
<td>5</td>
<td>Ceftriaxone</td>
<td>CRO</td>
<td>57%</td>
<td>43%</td>
<td>0%</td>
</tr>
<tr>
<td>6</td>
<td>Cefixime</td>
<td>CFM</td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

(w.r.t percentages Resistance, Intermediate & Sensitivity)

RESULTS & DISCUSSION

Zone of Inhibition was measured by disk diffusion method. Cefoperazone (CFP) was found to be the most effective, having 80% antibacterial activity against *Ps. aeruginosa*, due to highest sensitivity in 200 isolates while Cefoperazone (CFP) was also found to be 10% sensitive as shown in table 1. Similar results were also reported by Farida Anjum et al., in 2010 after studying Cefoperazone against *Ps. aeruginosa*, they reported 60% antibacterial activity. The second most effective antibacterial agent against *Ps. aeruginosa* was Ceftazidime (CAZ) which showed 70% effect in 175 isolates. Ceftazidime was also studied by Saleem Hafeez et al., in 2000 and reported 80 % sensitivity against *Ps. aeruginosa*. Similar findings were also studied by Tahira et al., in 2009 and reported 89% sensitivity while Farida et al., in 2010 reported 62% sensitivity against the same pathogen. Results revealed that Cefotaxime (CTX) was the third choice of drug and found to be effective in 42 isolates i.e., 16.8% effective against *Ps. aeruginosa* as shown in table.1. Similar antimicrobial activities was also studied by J Puri et al, in 1996 regarding CFP and CAZ. The least choice of drug was Ceftizoxime (ZOX) which showed 20% effectiveness while Hafeez et al., in 2000 reported 16% activity against *Ps. aeruginosa* (14). Cefixime (CFM) and Ceftriaxone (CRO) were not effective against all isolates but lateral was considered 21% effective by Tahira et al., in 2009. *Ps. aeruginosa* showed resistance against Cefixime and Ceftriaxone. Hence frequent measures should be taken to control the problem of resistance. Continually updated and validated antimicrobial susceptibility profiles data are necessary to ensure the provision of effective and safe empiric therapy. Antibiotic resistance is considered due to many biochemical mechanisms like presence of beta-lactamase resistance in *Ps. aeruginosa* which is generally initiated by a chromosomal class I beta lactamase or a plasmid mediated beta-lactamase. The process of resistance to the cephalosporins (third generations) usually originated through a mutation that...
results in potentially depressed instead of inducible population. Cefoperazone is a broad spectrum third generation cephalosporin having potent activity against Ps. aeruginosa. It acts upon PBPs to stop peptidoglycan synthesis in bacteria (both gram-positive and gram-negative). The responsible factors for resistance to Cefoperazone are enzymatic hydrolysis, altered PBPs affinity and permeability barrier present in the membranes of gram negative bacteria\textsuperscript{15}. Reported comparative studies of antibiotics have some limitations of short period of study designs, hence better results would be achieved by long term studies 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 parallel studies of antibiotic resistance problems like this\textsuperscript{16}.

CONCLUSION
The results of this study provide useful and effective guidelines for choosing an appropriate antibacterial agent against infections caused by Pseudomonas aeruginosa. The drug of choice is Cefoperazone (CFP) while the second choice may be Ceftazidime (CAZ) while Cefotaxime (CTX) is also considered for the third line of therapy.

ACKNOWLEDGEMENT
We (authors) are very thankful to Faculty of Pharmacy, Hammad University Karachi, Pakistan for providing us such facilities to conduct this research work.

REFERENCES

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