RESISTANCE PATTERN OF 3RD GENERATION CEPHALOSPORINS

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ABSTRACT

Objective: To determine resistance of third generation cephalosporins against different clinical isolates obtained from various clinical laboratories in Karachi, Pakistan. Methodology: Based on convenient sampling, 100 clinical isolates of E. coli, Enterococci, K. pneumoniae, P. vulgaris, P. aeruginosa and S. aureus were collected from December 2013 to May 2014 from patients coming to various clinical laboratories in Karachi, Pakistan. Mueller-Hinton agar and Mueller-Hinton broth were used for assessing the sensitivity patterns of the clinical isolates. The third generation cephalosporins tested against the clinical isolates were cefotaxime, ceftizoxime and ceftriaxone. Results: It was found that against E. coli, cefotaxime and ceftriaxone were 67% resistant whereas ceftizoxime was 79% resistant. The antibiotics were 100% resistant to Enterococcus. Against Klebsiella spp. resistance was 47% for cefotaxime and ceftriaxone and 73% for ceftizoxime. For Proteus spp., resistance for cefotaxime and ceftriaxone was 60% each and for ceftizoxime it was 80%. Ceftizoxime (91%), cefotaxime (73%) and ceftriaxone (64%) were resistant to P. aeruginosa while ceftriaxone 77%, ceftizoxime 58% and cefotaxime 50% were found resistant to S. aureus. Conclusions: The degree of third generation cephalosporins resistance was found being increased alarmingly and this should be monitored since it may give rise to drug resistant diseases amplifying the use of other drugs; treatment options for infections with multi-resistant micro organisms are limited.

KEYWORDS: Antibiotic resistance, Cephalosporins, Ceftizoxime, Cefotaxime and Ceftriaxone.
INTRODUCTION

Antibiotic resistance is threatening the accomplishment of health services; the precise public inference has not been adequately calculated. Most recently, an upgraded effort has been directed for scheming antibiotic practice and nurture public consideration towards the cautious use of antibiotics.[1,2,3] The foremost reason is environmental, in that antibiotics tempt and pick for bacterial resistance. Antibiotic resistance has been considered as a universal risk.[4,5] Antimicrobial drug efficacy cannot be taken for granted since they are progressively getting the point of non-recurring means.3 Infections due to multidrug-resistant gram-negative microbes enact a substantial concern on both patients and healthcare professionals.[2,4] It was seen that after general application of broad-spectrum cephalosporin, Enterobacteriaceae producing extended-spectrum b-lactamas (ESBLs) became common in hospitals. Infection with ESBL-producing gram-negative organisms, principally Escherichia coli or Klebsiella pneumoniae, is linked with higher death rate, a rise in the duration of hospital stay and hospitalization costs, and deferral in treatment, compared with infection due to non-ESBL pathogens.[4]

Bacteria may have resistance as a natural feature or it can be attained. The latter can result from a mutation of cellular genes, the acquirement of foreign resistance genes or a combination of these two mechanisms. Hence, antibiotic resistance can be attained: (i) via mutation in different chromosomal loci and (ii) via horizontal gene transfer (i.e. acquisition of resistance genes from other microorganisms).[6]

Antimicrobial resistant strains of bacteria are nurturing menace to animal and human health. Resistance mechanisms to evade the harmful action of antimicrobials had been identified and defined for acknowledged antimicrobials currently available for medical use in human and veterinary treatment. Certain bacteria are naturally resistant to some kinds of antibiotics. Bacteria could become resistant by a genetic mutation or by attaining resistance from another bacterium. It is established that the most common resistance mechanisms engaged by bacteria may be enzymatic degradation or modification of the antimicrobial, mutation in the antimicrobial target site, reduced cell wall permeability to antimicrobials, and active efflux of the antimicrobial across the cell membrane. The growing occurrence of antimicrobial resistant bacterial microorganisms has severe consequences for the impending treatment and prevention of infectious diseases in both animals and humans.[7] In healthcare settings the pressure of resistance is critical where benign pathogens commonly found on the skin, inthe
alimentary tract, or in the vagina—have appeared as pathogens. This may be due to the overuse and misuse of antibiotics.\(^8,9,10\) Following are the pathogens, denoted by the acronym ESKAPE, which may cause the majority of nosocomial infections in the United States, and often proved to be resistant to the antibiotics Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonia, Acinetobacter baumanii, Pseudomonas aeruginosa, Enterobacter spp.\(^{11,12}\) It has been assessed that nearly half of all antibiotics are used incorrectly to “treat” non-bacterial viral infections and other health complications, or the incorrect antibiotics are specified, or the treatment course is either too short or too long.\(^{13}\) The extreme use and misuse of antibiotics are usually recognized due to incorrect prescribing by physicians, and also the deficiency of appropriate and precise microbiological diagnostic tests. Still, the risk for resistance rises when people can simply get antibiotics without a prescription, or when they do self-medication with antibiotics left over from former courses of management.\(^{14}\) The following three key steps have been recognized to avert the development and spread of resistance to current and forthcoming antibiotics: limit their use, (discourage their misuse, and decrease the load of infectious disease through preventive sanitization and infection control practices.\(^{15,16,17}\) Physicians considering resistant cases of infectious disease try antimicrobial susceptibility tests done in hospital laboratories to advise optimal therapeutic agent.\(^{18}\)

The third-generation cephalosporins are broad-spectrum antibiotics that have been used in various clinical situations i.e. Cefotaxime and ceftizoxime have the best gram-positive coverage; Ceftriaxone has a long half-life and a superb drug for community-acquired infections. Since the third generation cephalosporins have established record of clinical value, satisfactory pharmacokinetics, and low incidence of adverse effects, it is a better choice in various clinical situations.\(^{19}\) The cephalosporin have become a key part of the antibiotic hospital formulary in prosperous countries. It is the reduced allergenic and toxicity hazards as well as a broad spectrum of activity that inspires the selection of microorganisms that are resistant to these antibiotics. The broad-spectrum ability of these drugs, however, emboldens swift overgrowth of certain microorganisms that are neither eradicated nor repressed by treatment.\(^{20}\) Organisms that are not repressed by cephalosporins therefore overgrow, with variable probability to cause infection.\(^{21}\) Some of these are straightaway identifiable as pathogens; while the others, although initially considered as commensal, consequently cause the disease.\(^{22}\) Moreover, there is a relationship between uswe of the cephalosporin and the occurrence of multiply-resistant organisms.\(^{21, 23,24,25}\) P. aeruginosa is a common isolate from
patients which is characteristically resistant to cephalosporins\textsuperscript{[21, 26]} and most cephalosporins boost its overgrowth. Patients are more vulnerable than volunteers to variations in colonization resistance and enterococcal overgrowth leads to the infection of the urinary tract, wounds, catheter sites and/or blood.\textsuperscript{[27,28]} It has been reported in a study that if the use of cephalosporin is lessened as part of general decrease in antimicrobial prescribing, there is a reduction in nosocomial infections like enterococcal and selected Gram-negative bacteraemias, and MRSA.\textsuperscript{[29]} The association of cephalosporins with staphylococci, enterococci, multiply-resistant Gram-negative bacilli, yeasts and C. difficile has been reported in various studies.\textsuperscript{[21,30,31]}

**METHODOLOGY**

One hundred clinical isolates of Escherichia coli, Enterococcus, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, and Staphylococcus aureus were collected from patients coming to different laboratories situated across the Karachi city, irrespective of gender, age, disease and duration of disease. These clinical isolates were obtained from various patients sources like ear swab, eye, high vaginal swab, intra uterine, intra uterine contraceptive, pus, sputum, stool, tracheal aspiration, urine and wound swab. Antimicrobial discs of 3 antibiotics i.e. Cefotaxime, Ceftizoxime, Ceftriaxone, with concentration of 30µg were prepared for testing. All isolated bacterial species were stored in slant nutrient agar and standardized for the antibiotic susceptibility tests. The prepared media used for sensitivity pattern were Mueller-Hinton agar and Mueller-Hinton broth.

**RESULTS**

The results of susceptibility of various micro organisms are presented as follows according to the performance standards for antimicrobial disk susceptibility tests.\textsuperscript{[32]} A hundred clinical isolates of different micro organisms were collected for the study (Table 1). The susceptibility against the pathogens was mostly observed by cefotaxime and ceftriaxone whereas intermediate activity around 20% was established by all antibiotics against K. pneumoniae and P. vulgaris (Table 2). The Enterococci were 100% resistant to the antibiotics and the overall results have revealed that on an average the pathogens showed great resistance against the antibiotics employed.
Table: 1 Clinical isolates of various micro-organisms

<table>
<thead>
<tr>
<th>S.No</th>
<th>Micro-organism</th>
<th>Total clinical isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E. coli</td>
<td>42</td>
</tr>
<tr>
<td>2</td>
<td>Enterococci</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>K. pneumonia</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>P. vulgaris</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>P. aeruginosa</td>
<td>11</td>
</tr>
<tr>
<td>6</td>
<td>S. aureus</td>
<td>26</td>
</tr>
</tbody>
</table>

Table: 2 Susceptibility patterns of micro-organisms against cephalosporins according to CLSI [32]

<table>
<thead>
<tr>
<th>S.No</th>
<th>Micro-organism</th>
<th>Antibiotic</th>
<th>Susceptible %</th>
<th>Intermediate %</th>
<th>Resistant %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cefotaxime</td>
<td>17</td>
<td>17</td>
<td>67</td>
</tr>
<tr>
<td>1</td>
<td>E. coli</td>
<td>Ceftizoxime</td>
<td>12</td>
<td>10</td>
<td>79</td>
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<tr>
<td></td>
<td></td>
<td>Ceftriaxone</td>
<td>7</td>
<td>26</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cefotaxime</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Enterococci</td>
<td>Ceftizoxime</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ceftriaxone</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cefotaxime</td>
<td>27</td>
<td>27</td>
<td>47</td>
</tr>
<tr>
<td>3</td>
<td>K. pneumonia</td>
<td>Ceftizoxime</td>
<td>7</td>
<td>20</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ceftriaxone</td>
<td>27</td>
<td>27</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cefotaxime</td>
<td>20</td>
<td>20</td>
<td>60</td>
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<tr>
<td>4</td>
<td>P. vulgaris</td>
<td>Ceftizoxime</td>
<td>0</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ceftriaxone</td>
<td>20</td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cefotaxime</td>
<td>9</td>
<td>18</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>P. aeruginosa</td>
<td>Ceftizoxime</td>
<td>0</td>
<td>9</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ceftriaxone</td>
<td>36</td>
<td>0</td>
<td>64</td>
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<tr>
<td>6</td>
<td>S. aureus</td>
<td>Cefotaxime</td>
<td>15</td>
<td>35</td>
<td>50</td>
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<tr>
<td></td>
<td></td>
<td>Ceftizoxime</td>
<td>19</td>
<td>23</td>
<td>58</td>
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<tr>
<td></td>
<td></td>
<td>Ceftriaxone</td>
<td>12</td>
<td>12</td>
<td>77</td>
</tr>
</tbody>
</table>

DISCUSSION

Studies to determine the resistance pattern of micro organisms are directed widely to observe the movement of microbial resistance. In this study, 100 clinical isolates were obtained from different sources from patients coming to various clinical laboratories in Karachi, Pakistan (Table 1). Among all the micro organisms found in the clinical isolates, the dominant pathogens were E. coli (n=42), S. aureus (n=26), Klebsiella (n=15) and P. aeruginosa (n=11) while P. vulgaris and Enterococci were (n=5) and (n=1) respectively. Several similar studies had been conducted in this connection.[33-38] The sources of clinical isolates for this study included urine (n=65), pus (n=14), ear swab (n=5), high vaginal swab (n=4), sputum (n=5) and stool (n=3). Urine had been used as the major source of specimen in other such studies also.[33,39] Several S. aureus clinical isolates had been collected from the sources like pus,
urine, blood, high vaginal swabs and other secretions to determine resistance patterns.\textsuperscript{[40]} In Karachi, third generation cephalosporins are prescribed widely by the physicians. Mostly these antibiotics are prescribed empirically without culture and sensitivity testing due to which the resistance might develop against this important class of antibiotics. Sometimes, the bacteria might convert from susceptible to resistant forms to the antibiotics during therapy.\textsuperscript{[41]} The results of susceptibility and resistance pattern of third generation cephalosporins of this study are shown in Table 2. Out of 42 isolates of E.coli, 67\% were found resistant to cefotaxime and also to ceftriaxone. In 2011, it was observed that E. coli isolates from UTI of patients were 46\% resistant to ceftriaxone.\textsuperscript{[36]} It was found that 79\% isolates were susceptible to ceftizoxime. There was only 1 isolate of Enterococcus faecalis which showed resistance to all the tested cephalosporins; about 90\% vancomycin resistance was also observed in a study in 2009.\textsuperscript{[42]} Out of 15 isolates of K. pneumoniae, 47\% were resistant to cefotaxime and ceftriaxone. Ola Ibrahim Ahmed (2013) studied K. pneumoniae isolates in patients susceptible to nosocomial infections and found that the pattern of susceptibility showed about 65\% resistance to cefotaxime and ceftazidime.\textsuperscript{[43]} Almost 40\% resistance of Klebsiella to cefotaxime and ceftizoxime was found in a study in 2012;\textsuperscript{[44]} 73\% isolates of Klebsiella were found resistant to ceftizoxime in our study. Such studies were also conducted by Zhong et al in 2012.\textsuperscript{[45]} The results of this study have shown 60\% resistance to ceftriaxone and ceftizoxime against Proteus spp. whereas 80\% resistance to cefotaxime while in 2007, Cao and Haiyan reported the resistance to 3\textsuperscript{rd} generation cephalosporins.\textsuperscript{[46]}

In this study 91\% resistance against ceftizoxime, 73\% resistance against cefotaxime and 64\% resistance against ceftriaxone were observed for isolates of P. aeruginosa. In an in vitro comparative study performed for these cephalosporins by Ullah hamza in 2013, it was found that cefotaxime and ceftizoxime showed intermediate activity against P. aeruginosa while 0\% activity was shown by ceftriaxone.\textsuperscript{[47]} In another study by Xu et al. (2010) the resistance rates of cephalosporins were found below 20\%.\textsuperscript{[48]} The present study showed that 50\% isolates of S. Aureus were resistant to cefotaxime; 58\% were resistant to ceftizoxime and 77\% were resistant to ceftriaxone. In various studies, resistance was found to around 50\% for cefotaxime and ceftizoxime whereas for ceftriaxone study results differed from each other i.e. 22\% and 75\% resistance.\textsuperscript{[49,50,51]} Overall results reveal that ceftizoxime was most resistant cephalosporin against various clinical isolates used in the present study.
CONCLUSION

The increase in number of resistant organisms would result in therapy failure, economic burden on patients and poor infection control thus increasing morbidity rates. In Karachi, resistance against 3rd generation cephalosporins is increasing alarmingly which may be due to irrational use of the antibiotics. It is necessary to select antibiotics on basis of susceptibility tests so as to prevent prevalence of drug resistant bacteria. Empirical use of antibiotics focused on antibiotic policy guidelines is highly recommended.

REFERENCES


32. Performance standards for antimicrobial susceptibility testing; 22nd informational supplement., M100-S22: 32(3), replaces M100-S21: 31(1).


