Antibiotic resistance profile of clinical gram negative bacteria.

Aman Ullah¹, Rabia Durrani²*, Inam Ullah³, Muhammad Rafiq³

¹Department of Microbiology, University of Karachi, Karachi, Pakistan.
²Department of Microbiology, Quaid-I-Azam University Islamabad, Pakistan.
³Department of Microbiology, Federal Urdu University of Science and Technology, Karachi, Pakistan.

INTRODUCTION

Gram negative bacteria are generally resistant to antibiotics and are paramount pathogens [1-3]. Recently multidrug resistant gram negative bacteria have become more prevalent [4-6] and are causing great problems in treatment of infections.

For the treatment of bacterial infections antibiotics are utmost agent but the growing resistance of bacteria to currently used antibiotics is a major concern for clinicians, public health officials, and researchers [7,8]. Development of antibiotic resistance is canvassed by dint of wanton use of antibiotics in clinical practices and its application in animal and poultry feed at sub therapeutic doses for growth promotion. Therefore, resistant bacteria could be disseminated through the human food chain, by poor hygienic conditions, and overcrowded living conditions [7,9]. Antibiotic resistance can be native quality of an organism [10] or can be acquired as a result of mutation or acquisition of resistant genes via horizontal gene transfer [11].

The purpose of the present study was to evaluate the antibiotic resistance profile of four important clinical gram negative bacteria in local hospital of Karachi, Pakistan.

MATERIALS AND METHODS

The bacterial strains used in the given study were obtained from different clinical specimens at Dr. Essa laboratory and Dr. Ziauddin hospital in 2007, in Karachi, Pakistan. The bacterial strains had already been identified at their source. We further purified all the isolates twice on Mac Conkey’s agar and reconfirmed by using conventional microbiological methods. The susceptibilities of all the strains were determined against six antibiotics (amikacin, cefatxime, cefoperazone/sulbactam, fosfomycin, piperacillin/tazobactam, and streptomycin) by using Replica plate technique as previously used by Ahmed et al [12]. Twenty five isolated colonies were picked onto master plate of Mac Conkey’s agar and grown over night at 37ºC. Next day, with the help of sterile velvet, colonies from the master plate were replicated on Mac Conkey’s agar containing individual antibiotics to be tested and on two plate of control (Mac Conkey’s agar without antibiotic) and again incubated at 37ºC for 24 hours. After 24 hours the replicated plates of test and control were observed for growth. The culture which showed growth at the test plates (Mac Conkey’s agar containing antibiotics) was considered to be resistant and the culture which did not grow was considered to be be sensitive to that particular antibiotic at that specific

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level.

RESULTS

A total of 100 isolates including 47 strains of E.coli, 32 of Klebsiella pneumoniae, 11 of Pseudomonas aeruginosa, and 10 of Enterobacter spp. were tested. Bacterial strains were isolated from different specimen including urine, blood, pus, wound, tracheal aspirates, and other sterile body fluids.

Antibiotic susceptibilities are shown in table 1. Out of the total 100 isolates, 94% were resistant to one or more antibiotics and 6% were completely sensitive to all the six antibiotics at the level of 10µg/ml, whilst at the level of 30µg/ml 25% isolates were completely sensitive to all the six antibiotics and 75% bacteria were resistant to one or more antibiotics. Out of 94% resistant isolates at the level of 10µg/ml 11 bacteria were resistant to all the six antibiotics and at the levels of 30µg/ml, 4 were resistant to all the six antibiotics, out of total 75% resistant isolates.

Frequency of resistance of the gram negative bacteria to individual antibiotic was found to be 60% for amikacin, 59% for cefatoxime, 41% for cefoperazone/sulbactam, 38% for fosfomycin, 70% for streptomycin, and 50% for piperacillin/tazobactam at the level of 10 µg/ml. Whereas at the level of 30µg/ml the frequencies of resistance were: 28% for amikacin, 43% for cefatoxime, 11% for cefoperazone/sulbactam, 25% for fosfomycin, 50% for streptomycin, and 15% for piperacillin/tazobactam.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Level</th>
<th>E. coli</th>
<th>K. pneumoniae</th>
<th>P. aeruginosa</th>
<th>Enterobacter spp.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total No. of isolates</td>
<td></td>
<td>47</td>
<td>32</td>
<td>11</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Amikacin</td>
<td>10µg/ml</td>
<td>34</td>
<td>16</td>
<td>3</td>
<td>7</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>30µg/ml</td>
<td>16</td>
<td>6</td>
<td>1</td>
<td>5</td>
<td>28</td>
</tr>
<tr>
<td>Cefatoxime</td>
<td>10µg/ml</td>
<td>30</td>
<td>16</td>
<td>5</td>
<td>8</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>30µg/ml</td>
<td>25</td>
<td>8</td>
<td>2</td>
<td>8</td>
<td>43</td>
</tr>
<tr>
<td>Cefoperazone/sulbactam</td>
<td>10µg/ml</td>
<td>18</td>
<td>13</td>
<td>4</td>
<td>6</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>30µg/ml</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>10µg/ml</td>
<td>35</td>
<td>22</td>
<td>5</td>
<td>8</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>30µg/ml</td>
<td>27</td>
<td>13</td>
<td>3</td>
<td>7</td>
<td>50</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>10µg/ml</td>
<td>10</td>
<td>17</td>
<td>4</td>
<td>7</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>30µg/ml</td>
<td>4</td>
<td>15</td>
<td>0</td>
<td>6</td>
<td>25</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>10µg/ml</td>
<td>23</td>
<td>16</td>
<td>5</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>30µg/ml</td>
<td>2</td>
<td>7</td>
<td>2</td>
<td>4</td>
<td>15</td>
</tr>
</tbody>
</table>

Table1. Antibiotic resistance pattern of clinical isolates.

level 10µg/ml and 30µg/ml respectively. This is an alarming proportion of the antibiotic resistance and predate the clinical use of antibiotics. Noor et al in 2004 also show similar results for enteric bacilli associated with urinary tract infection [13]. Multidrug resistant gram negative bacteria are a serious problem in clinical settings, and accelerate morbidity, mortality and health cost. Present study showed high frequency of multidrug resistant which is in close agreement with the work of Tayyab et al [14]. Multidrug resistance in gram negative bacteria results from the acquisition of multiple resistance genes on mobile DNA elements such as plasmids or transposons, often as a part of an integron [15].

It is clear that more need to be done with regards to limiting resistance development and spread of resistant isolates once they occur. While ongoing efforts at developing new antibiotics and their use for treatments will certainly enhance our ability to treat infections caused by multidrug resistant gram negative pathogen.

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