INCIDENCE OF β-LACTAMASE PRODUCING ORGANISMS CAUSING VARIOUS INFECTIONS AND COMPARISON OF THEIR ANTIMICROBIAL SUSCEPTIBILITY TESTING

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ABSTRACT: Higher generation cephalosporins, resistant to β-lactamases were designed to combat bacterial resistance to first generation β-lactam antibiotics. This led to the emergence of extended spectrum β-lactamases (ESBLs), which conferred bacterial resistance to these drugs. Now, combination drugs employing a β-lactam drug + a β-lactamase inhibitor (Clavulanate, Sulbactam, Tazobactam) are used against ESBL-producing pathogens. This study aimed to evaluate the efficacy of these combination drugs against ESBL-producers and also screening for the presence of inducible AmpC β-lactamase producers. Enterobacteriaceae family culture isolates exhibiting resistance to the 2nd / 3rd generation cephalosporins by the Disk Agar Diffusion test were tested for ESBL production by the Double Disk Synergy Test. Staphylococcal species culture isolates were tested for β-lactamase production by the Nitrocefin spot test. The Disk Antagonism test was used to screen for the presence of inducible Amp C β-lactamase producers. The combination drugs included in this study were Cefepime/Tazobactam, Cefoperazone/Sulbactam, Cefotaxime/Sulbactam, Ceftriaxone/Sulbactam & Ceftriaxone/ Tazobactam. 128 clinical isolates were tested for β-lactamase activity. Of the 26 S.aureus isolates, 88.4% (23) were β-lactamase positive and 57.1 % coagulase negative Staphylococci were positive. Amongst Enterobacteriaceae family, 67.8% of E.coli; 53.1% of K.pneumoniae and 53.8% of Proteus species were confirmed ESBL producers. For E.coli, the best drug was Cefepime/ Tazobactam. All drugs were effective against Proteus spp. K.pneumoniae and S.aureus isolates were resistant to these drugs due to the production of AmpC β-lactamases. 3.4% of E.coli, 4.5% of S.aureus and 14.2% of Proteus spp were confirmed inducible AmpC β-lactamase producers.

Key words: β-Lactamase, Antimicrobial, Organisms

INTRODUCTION:

The introduction of β-lactam antibiotics led to the emergence of β-lactamases and some of these resulted from simple point mutations in existing β-lactamase genes that led to a changed substrate profile. These are the extended spectrum β-lactamases (ESBLs) and the development and spread of ESBLs have most likely been caused by the overuse of expanded spectrum cephalosporins in the hospital setting. There are limited therapeutic options left for ESBL producers and thus therapeutic strategies have now been developed employing cephalosporin/β-lactamase inhibitor combinations e.g.: Ceftriaxone/Tazobactam, Cefotaxime/Clavulanate. This study was conducted to evaluate the efficacy of these combinations against ESBL producing clinical isolates. However, with this new strategy, newer types of β-lactamases emerged which conferred resistance to these inhibitor combinations also. These are AmpC β-lactamases and they pose a serious threat to therapy. This study was also undertaken to screen for the presence of inducible AmpC β-lactamase producers using standard methods presently available for their detection.

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Methodology:

a. **Bacterial strains:** Routine clinical isolates of *Enterobacteriaceae*, *Staphylococcal* species, *B. catarrhalis*, *Pseudomonas*, *Acinetobacter* and *S. pneumoniae* from Dec’06 to July’07 were included in this study. The antibiotic susceptibility patterns of the β-lactamase producing strains were determined by the disk agar diffusion method in Mueller-Hinton Agar. The two CLSI quality control organisms, *E.coli* ATCC 25922 and *K.pneumoniae* ATCC 700603 were included in all experiments.

b. **Antibiotics:**
   - **Himedia:** Cefdinir (Cdn), Cefepime (Cpm), Cefixime (Cfx), Cefoperazone (Cs), Cefpodoxime (Cep), Cefuroxime (Cu), Cefoxitin (Cn), Imipenum (I), Piperacillin/Tazobactam (Pt), Ticarcillin/Clavulanate (Tc).
   - **EOS Labs:** Cefotaxime (CTX), Ceftazidime (CAZ), Cefipime/Tazobactam (30 µg/5 µg), Cefixime/Sulbactam (75µg/30µg), Cefixime/Sulbactam (20µg/10µg), Ceftriaxone/Sulbactam (30µg/10µg), Ceftriaxone/Tazobactam (30µg/5µg), Cefotaxime/Clavulanate (30µg/10µg), Cefotaxime/Sulbactam (30µg/10µg), Cefazidime/Clavulanate (30µg/10µg).

c. **Disk Agar Diffusion method (DAD):**
   The antibiotic susceptibility patterns of all strains were determined by the Kirby Bauer method in Mueller Hinton Agar with the above mentioned antibiotic disks and the results were interpreted according to the CLSI criteria.

d. **Double Disk Synergy Test (DDST):**
   This test consists of an initial screen test and a confirmatory test. The initial screen test is a presumptive identification for ESBL producers and is based on the sensitivity profile against Cefpodoxime, Ceftazidime, Aztreonam, Cefotaxime and Ceftriaxone. If the zone size is ≤ 17mm, 22mm, 27mm, 27mm and 25mm for the respective antibiotics it may indicate ESBL production. Strains showing resistance to the above antibiotics were tested for ESBL production in duplicate. The confirmatory test required the use of Cefotaxime and Ceftazidime disks alone and in combination with Clavulanate, which were placed on a MH Agar plate inoculated with a suspension of the test organism equal to a 0.5 Mc Farland turbidity standard. A >5mm increase in the zone diameter of either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was regarded as a confirmation for ESBL production.

e. **Nitrocefin Spot Test:**
   Cefinase™ discs (BD BBL™) was used to test bacterial strains of *Staphylococcus*, *B.catarrhalis* and *S.pneumoniae* for the presence of β-lactamase. The disc (being impregnated with a chromogenic cephalosporin) changes from yellow to red if the organism being tested produces β-lactam.

f. **Metallo β-lactamase detection:**
   The test strain was inoculated on a plate of MH Agar. An Imipenum (10µg) was placed 10mm apart from a disc containing 10 µl of 0.5M EDTA solution. After overnight incubation, the presence of an enlarged zone of inhibition was interpreted as metallo- β- lactamase positive.

g. **Disk Antagonism Test (DAT):**
   In this test, an inducing agent, Cefoxitin, was placed adjacent (15mm apart) to other cephalosporin disks (Cpm, Ci, Cep, CTX) on the surface of MH Agar plate inoculated with the test organism. If blunting of the cephalosporin disk adjacent to the cefoxitin disk occurred, the inducibility of AmpC β- lactamase was confirmed.
RESULTS:
A total of 251 isolates from various clinical sites was included in this study. Among these 141 were β-lactamase positive (~55%) of which *S.aureus* was the highest (79.4%) and the second highest being *E.coli* (68.3%) in case of ESBLs. The site-wise characterization of the clinical isolates (Table 1) and the percentage positivity for beta-lactamase/ESBL is tabulated below (Table 2).

Among the β-lactam antibiotics the maximum degree of resistance was seen against Cefexime. The percentage sensitivity profiles of the various clinical isolates have been graphically demonstrated. (Graphs 1-10)

Inducible Amp C beta-lactamase detection was performed by the Disk Antagonism Test (DAT), which demonstrated blunting of specific cephalosporin disk adjacent to the cefoxitin disks.

Amp C production was found in 4 isolates; *Klebsiella pneumoniae* (4.5%), Proteus spp. (14.2%) and *Staphylococcus aureus* (3.2%)

### Table 1: Site wise characterization of clinical isolates

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Urine</th>
<th>Blood</th>
<th>Body fluids</th>
<th>Pus</th>
<th>Wound swab</th>
<th>Throat swab</th>
<th>Tracheal Secretion</th>
<th>Sputum</th>
<th>Invasive devices</th>
<th>Misc</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Proteus</em> species</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>3</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>25</td>
</tr>
<tr>
<td><em>K.pneumoniae</em></td>
<td>19</td>
<td>2</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>36</td>
</tr>
<tr>
<td><em>E.coli</em></td>
<td>38</td>
<td>-</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>61</td>
</tr>
<tr>
<td><em>S.aureus</em></td>
<td>-</td>
<td>6</td>
<td>-</td>
<td>22</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>5</td>
<td>39</td>
</tr>
<tr>
<td><em>CoNS</em></td>
<td>13</td>
<td>3</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td><em>P.aeruginosa</em></td>
<td>10</td>
<td>-</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>-</td>
<td>3</td>
<td>5</td>
<td>-</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td><em>Acinetobacter</em> species</td>
<td>8</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>18</td>
</tr>
<tr>
<td><em>S.pneumoniae</em></td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td><em>B.catarrhalis</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>8</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td><em>Enterobacter</em> species</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
</tbody>
</table>

### Table 2: Percentage Beta lactamase/ESBL/Metallo Beta lactamase positive strains

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Total Number</th>
<th>Beta-lactamase / ESBL Positive</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>39</td>
<td>31</td>
<td>79.4</td>
</tr>
<tr>
<td>Coagulase Negative <em>Staphylococci</em></td>
<td>25</td>
<td>11</td>
<td>44</td>
</tr>
<tr>
<td><em>E.coli</em></td>
<td>61</td>
<td>41</td>
<td>68.3</td>
</tr>
<tr>
<td><em>K.pneumoniae</em></td>
<td>36</td>
<td>22</td>
<td>61.1</td>
</tr>
<tr>
<td><em>Acinetobacter</em> species</td>
<td>18</td>
<td>8 (Metallo Beta lactamase)</td>
<td>44.4</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>30</td>
<td>11 (Metallo Beta lactamase)</td>
<td>36.6</td>
</tr>
<tr>
<td><em>Proteus species</em></td>
<td>25</td>
<td>14</td>
<td>58.3</td>
</tr>
<tr>
<td><em>Branhamella catarrhalis</em></td>
<td>13</td>
<td>2</td>
<td>15.38</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>2</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td><em>S.pneumoniae</em></td>
<td>2</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Total:</td>
<td>251</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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Graph 1: Comparison of percentage sensitivity profiles of *Pseudomonas* isolates

Graph 2: Comparison of percentage sensitivity profiles of *S.aureus* isolates

Graph 3: Comparison of percentage sensitivity profiles of coagulase negative *Staphylococcal* isolates
Graph 4: Comparison of percentage sensitivity profiles of *E.coli* isolates

Graph 5: Comparison of percentage sensitivity profiles of *Klebsiella pneumoniae* isolates

Graph 6: Comparison of percentage sensitivity profiles of *Proteus sp.* isolates
Graph 7: Comparison of percentage sensitivity profiles of Enterobacter sp. Isolates

Graph 8: Comparison of percentage sensitivity profiles of Acinetobacter sp. Isolates

Graph 9: Comparison of percentage sensitivity profiles of Brahanmella catarrhalis isolates

Graph 10: Comparison of percentage sensitivity profiles of Streptococcus pneumoniae isolates
Discussion:

The overuse of β-lactam drugs has imposed a selective pressure on pathogens to acquire resistance genes and mutate these to confer a broader range of activity. Therefore a search for their inhibitors to protect the antibiotic activity in vivo against β-lactam resistant pathogens was initiated. This study showed most of these inhibitor combinations showed a marginal increase in susceptibility for β-lactamase/ESBL producers. In case of Enterobacteriaceae, Ceftriaxone + Sulbactum (Ci/Sbm), Ceftriaxone + Tazobactum (Ci/Tzb) and Cefoperazone + Sulbactam (Cs/Sbm) combination proved to be the most effective in containing ESBL production.

- In case of *E.coli* Ci/Sbm & Ci/Tzb brought about 98% increase in susceptibility and for *K.pneumoniae* Cs/Sbm about 86% sensitivity.
- For *Proteus* spp. isolates most combinations showed a 50% increase in susceptibility and so was the case for *Enterobacter* spp. isolates.
- Cpm/Tzb & Cs/Sbm were the most effective for the Metallo-β-lactamse producers *Pseudomonas* sp. and *Acinetobacter* sp. respectively.
- For *Staphylococcal* isolates all the combinations showed equal efficacy.
- In case of *Branhamella catarrhalis* only Cs/Sbm & Cefexime/Sulbactum (Cfx/Sbm) brought about significant change in sensitivity and Cfx/Sbm in case of *Streptococcus pneumoniae*.

According to our study, Amp-C β-lactamase production amongst the clinical isolates was comparatively less (~3%) when compared with other studies reported from Delhi, Chennai, Aligarh but more than that found in Kolkata. In 2005, 1.4% inducible Amp- C β-lactamase producers were found in Kolkata hospitals. In Chennai, Subha et al found Amp C β-lactamase production in 24.1% of *Klebsiella* sp and 37.5% of *E.coli* in 2003 [7,8,9]

If the type of β-lactamase produced by the pathogens can be identified prior to therapy, therapeutic failure can be averted.

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