FREQUENCY OF EXTENDED SPECTRUM BETA LACTAMASE PRODUCING GRAM NEGATIVE BACILLI AMONG CLINICAL ISOLATES

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ABSTRACT
Extended-spectrum β-lactamase (ESBL)–producing bacteria are emerging pathogens. They are descended by genetic mutation from native β-lactamases found in gram negative bacteria, especially infectious strains of Escherichia coli and Klebsiella species. Clinicians, microbiologists, infection control practitioners, and hospital epidemiologists are concerned about ESBL-producing bacteria because of the increasing incidence of such infections, the limitations of effective antimicrobial drug therapy, and adverse patient outcomes. The present study was undertaken to determine the frequency of ESBL producing gram negative bacilli recovered from clinical specimens in our setup. A total of 3099 gram negative isolates recovered from various clinical samples during the period of January 2007 to December 2008 were processed for the detection of ESBL production. Among them 35.5% bacterial strains were found to be ESBL producers. The commonest ESBL producing organism isolated was Eschericia coli (44.8%), followed by Klebsiella pneumoniae (38.6%). Production of ESBLs by gram negative bacteria is emerging as a widespread problem in our setup. Appropriate infection control and antibiotic management strategies are needed to stem the spread of this emerging form of resistance.

Key words: ESBL, frequency, Escherecia coli, Klebsiella pneumoniae, gram negative bacilli.

INTRODUCTION
Microbial drug resistance is an inevitable consequence of indiscriminate utilization of antimicrobial therapy. Since we live in an era when dire predictions about the lack of effective antimicrobial drugs occur with increasing frequency, it is unclear whether we will be able to keep pace with bacterial resistance patterns and effectively treat infections in the future.

Beginning with the introduction of penicillin’s half a century ago, the β-lactams have remained the largest antibiotic class of clinical relevance. Emergence of resistance to these agents in the past two decades has resulted in a major clinical crisis. Gram-negative bacteria resistant to extended spectrum cephalosporins, monobactams, carbapenems, and beta lactam beta lactamase inhibitor combinations have emerged through multiple resistance mechanisms.

First described in 1980’s in Europe, extended spectrum beta lactamase (ESBL) producing Enterobacteriaceae have emerged as serious nosocomial pathogens. Recent studies have demonstrated a high prevalence of these anti-microbial resistant bacteria and also a trend of increasing resistance under continued antibiotic selective pressure. ESBL enzymes, encoded by genes that are typically plasmid borne, hydrolyze and confer resistance to aztreonam, cefotaxime, ceftazidime and related oxyimino-beta lactams as well as to older penicillins and cephalosporins. Although these enzymes occur predominantly in Klebsiella species and Eschericia coli, they have been encountered in other gram negative bacilli as well.

Infections with ESBL producing bacterial strains are encountered singly or in out-breaks, especially in critical care units in hospitals, resulting in increasing costs of treatment and prolonged hospital stay. Prevalence of ESBL producing strains also varies from one geographical region to another paralleling the misuse or overuse of beta lactam drugs. Despite the fact that ESBL producing organisms occur frequently, many clinicians are unaware of their existence. Due to variable affinity of these enzymes for different substrates and inoculum effect, some ESBL isolates may appear susceptible to a third generation cephalosporin in vitro. Failure to detect and report antibiotic resistance due to ESBL production may result in failure to treat such infections effectively.

Clinical laboratories play a pivotal role in providing accurate information and guidance in the treatment of microbial infections. Also, early identification is important in countries such as ours, where excessive use of antibiotics without antibiotic surveillance programs is the norm.
The objective of this study was to determine the frequency of ESBL producing gram negative bacilli recovered from clinical specimens in our setup.

**MATERIAL AND METHODS**

Bacterial isolates included in this study were obtained from clinical samples sent for culture analysis from different hospitals of Lahore during the period of January 2007 to December 2008. A total of 3099 gram negative isolates recovered from various clinical samples of urine, blood, pus, aspirates, sputum, and bronchial secretions were processed. Study design was descriptive cross sectional and sampling technique was non probability purposive. Samples were initially inoculated on Blood and MacConkey's agar. Urine samples were cultured on Cysteine Lactose Electrolyte Deficient agar (CLED). Cultures were incubated aerobically at 37°C for 24 hours, followed by bacterial identification up to species level using API 20 E systems.

**Screening for ESBL detection**

Strains were screened using double disc diffusion technique according to the standards of NCCLS. A single, separated colony of the test organism was picked and emulsified in 0.9% normal saline to match the turbidity with 0.5% McFarland's standard. The suspension was spread on Mueller Hinton agar surface with a cotton swab soaked in the suspension. An antibiotic disc containing amoxicillin-clavulanate (20/10ug) as inhibitor of beta lactamase was placed in the center of the plate. Cefotaxime (30ug), ceftazidime (30ug), ceftriaxone (30ug) and aztreonam (30ug) discs were placed at a distance of 30 mm from the central disc as well as from each other. Zones of inhibition around the 3rd generation cephalosporin’s discs and aztreonam were observed after overnight incubation at 37°C. If the inhibition zone around one or more cephalosporin’s discs and aztreonam was extended on the side nearest to amoxicillin-clavulanate, the organism showing this synergism was identified as an ESBL producer. Escherecia coli ATCC 25922 and Klebsiella pneumoniae ATCC 700603 were used as controls.

**RESULTS**

3099 gram negative isolates were tested during the study period. These included organisms recovered from 1407 urine samples, 985 wound swabs / pus samples, 259 sputum, 219 blood, 158 body fluids and 73 bronchial washings (Table 1).

1094 (35.5%) bacterial strains were found to be ESBL producers. The commonest ESBL producing organism isolated was Escherecia coli (44.8%), followed by Klebsiella pneumoniae (38.6%), Proteus mirabilis (31.6%) and Acinetobacter baumannii (7.1%) (Table 2).

**Table 1: Frequency of gram negative bacilli among various clinical species n = 3099.**

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Urine</th>
<th>Sputum</th>
<th>B. washings</th>
<th>Wound and pus</th>
<th>Fluids</th>
<th>Blood</th>
<th>Total / % age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherecia Coli</td>
<td>859</td>
<td>68</td>
<td>28</td>
<td>413</td>
<td>34</td>
<td>68</td>
<td>1470 / 47.4</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>126</td>
<td>139</td>
<td>17</td>
<td>22</td>
<td>23</td>
<td>15</td>
<td>342 / 11.0</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>21</td>
<td>--</td>
<td>02</td>
<td>17</td>
<td>04</td>
<td>02</td>
<td>46 / 1.5</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>33</td>
<td>06</td>
<td>08</td>
<td>29</td>
<td>03</td>
<td>07</td>
<td>86 / 2.8</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>29</td>
<td>03</td>
<td>06</td>
<td>81</td>
<td>12</td>
<td>11</td>
<td>142 / 4.6</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>129</td>
<td>--</td>
<td>--</td>
<td>117</td>
<td>04</td>
<td>--</td>
<td>250 / 8.1</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>89</td>
<td>--</td>
<td>--</td>
<td>91</td>
<td>09</td>
<td>--</td>
<td>189 / 6.1</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>62</td>
<td>24</td>
<td>07</td>
<td>128</td>
<td>48</td>
<td>63</td>
<td>332 / 10.7</td>
</tr>
<tr>
<td>Acinetobacter spp.</td>
<td>59</td>
<td>19</td>
<td>05</td>
<td>87</td>
<td>21</td>
<td>51</td>
<td>242 / 7.8</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Resistant bacteria are emerging as a threat to the favorable outcome of common infections in the community and hospital settings. Beta lactamase enzymes produced by gram negative organisms confer resistance against broad spectrum beta lactam antibiotics which normally have activity against such organisms. Recent studies
Table 2: Frequency of ESBL producers amongst gram negative isolates (n = 3099).

<table>
<thead>
<tr>
<th>Organism isolated</th>
<th>No. of ESBL</th>
<th>% age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eschericia coli</td>
<td>659</td>
<td>44.8</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>132</td>
<td>38.6</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>01</td>
<td>2.2</td>
</tr>
<tr>
<td>Citrobaacter freundii</td>
<td>09</td>
<td>10.5</td>
</tr>
<tr>
<td>Enterobacter cloaceae</td>
<td>28</td>
<td>19.7</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>79</td>
<td>31.6</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>03</td>
<td>1.6</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>78</td>
<td>23.5</td>
</tr>
<tr>
<td>Acinetobacter spp.</td>
<td>47</td>
<td>19.4</td>
</tr>
</tbody>
</table>

on ESBL production among enterobacteriaceae isolated from clinical specimens show a variable incidence ranging from as low as 1% to as high as 74%.5,6,19-20 In our study, overall prevalence of ESBL production in gram negative isolates was found to be 35.5%. Shah et al25 and Ali et al4 have reported prevalence rates of 37.5% and 45% respectively from the northern areas of Pakistan; figures which are close to the results obtained from our area. The high percentage of ESBL producers reflects the extensive and indiscriminate use of antimicrobials in our set-up.

Klebsiella pneumoniae, Eschericia coli, Proteus mirabilis, Enterobacter spp., Citrobaacter spp., Acinetobacter spp. and Pseudomonas aeruginosa are amongst the important ESBL producing gram negative bacilli reported.25 Usually one of the three species-Klebsiella pneumoniae, Eschericia coli or Enterobacter have been reported to be as the major ESBL producers.26 The prevalence of Eschericia coli and Klebsiella pneumoniae reported around the world varies greatly although it tends to be less than 50% in most studies conducted.2,1,11,16,19 In our study, a prevalence of 44.8% and 38.6% was found for Eschericia coli and Klebsiella pneumoniae respectively which is similar to the results seen in other studies.

Therapeutic options available for the treatment of ESBL-associated infections are limited by drug resistance conferred by the ESBLs, along with frequently observed co-resistance to various antibiotic classes, including cephamsycins, fluoroquinolones, aminoglycosides, tetracyclines, and trimethoprim/sulfamethoxazole. In our study, amoxicillin-clavulanate, piperacillin-tazobactam, ampicillin-sulbactam, cefoxitin, imipenem, meropenem, amikacin, gentamicin and ciprofloxacin were the drugs included in the antibiotic susceptibility testing panel. Imipenem and meropenem were the only drugs to which all the isolates showed 100% susceptibility. This is in accordance with results seen in other studies.23-24 The second drug showing good susceptibility (85%) in ESBL producers was Amikacin. Similar results have been reported by other researchers as well.

Relevant clinical data regarding the effectiveness of different regimens for ESBL-associated infections are limited. Although certain cephalosporins may appear active in vitro, clinical outcomes are often suboptimal. Carbapenems are regarded as the agents of choice, and may be more effective than quinolones for serious infections.

The high levels of ESBL producers mainly amongst gram negative isolates is alarming and warrants special attention, both by the clinicians and the microbiologists. In our region, testing for ESBL production is not done routinely. This may allow the undetected spread of these strains within our hospitals for long periods leading to serious outbreaks particularly in intensive care units. While the clinician has to re-evaluate the antibiotic policies, the lab must be capable of readily identifying these isolates, so that proper therapy can be instituted to avoid misuse/overuse of antibiotics.

It is concluded that ESBL isolates are prevalent in our setting. Routine detection of these isolates and proper control measures are recommended so that appropriate management can be instituted and spread of these organisms curtailed.

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REFERENCES