Antibiogram of Extended Spectrum Beta-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from Hospital Samples

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Abstract

Extended spectrum beta-lactamase (ESBL) producing organisms create a major problem for clinical therapeutics. The frequency of ESBL producing strains among clinical isolates has been steadily increasing over the past few years resulting in limitation of the therapeutic options. These resistant bacteria are emerging world wide as a threat to human health in both the community and hospital settings. -lactamase production by several organisms is the most important mechanism of resistance to beta-lactam antibiotics, such as penicillins and cephalosporins. This study was done to determine the susceptibility of different antimicrobials to ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from wound swabs, blood, urine, fluid, tracheal aspirates and sputum in Shahid Bahonar Hospital of Tehran from July, 2007 to June, 2008. A total of 115 ESBL-producing isolates were obtained from outdoor and indoor patients. Out of 115 isolates, 60% were *E. coli* and 40% were *K. pneumoniae*. All ESBL-producing isolates were confirmed using the Clinical and Laboratory Standards Institute (CLSI)-approved double-disk diffusion method. 29.6% of these isolates were collected from medical wards and 24.3% were collected from outdoor. Urine (70.4%) was the main source of ESBL-producing isolates from all patients, followed by blood (16.5%). All isolates were susceptible to both imipenem and meropenem. Of all isolates, 93.9% were susceptible to amikacin. The cephalosporins (1-4 generations) were almost 100% resistant. For Nitrofurantoin, 57.4% were sensitive. High rate resistance (74.8%) was observed to all quinolones tested. Aztreonam, Ampicillin, Co-amoxyclav and Ampicillin/Sulbactam were 100% resistant. This study shows that the frequency of ESBL producing strains of *E. coli* and *K. pneumoniae* is high in both hospital and community levels and it has a significant implication for patients’ management. Advance drug resistance surveillance and molecular characteristics of ESBL isolates is necessary to guide the appropriate and judicious antibiotic use.

Key words: Extended spectrum beta-lactamase (ESBL), Drug sensitivity, *Escherichia coli*, *Klebsiella pneumoniae*.

Introduction

Extended spectrum beta-lactamase (ESBL) are plasmid mediated, TEM-1, TEM-2 and SHV-1 derived enzymes conferring broad resistance to penicillin, cephalosporin and monobactam but not to carbapenem.1 These enzymes are produced by Enterobacteriaceae mainly by *Escherichia coli*, *Klebsiella pneumoniae* and *oxytoca*. They have been detected in other gram-negative bacilli such as Proteus species, Salmonella species, *Pseudomonas aeruginosa* and other Enteobacteriaceae.3-6 The first ESBL-producing organism was isolated in Germany in 1983. Thereafter, such organisms were reported in the USA following outbreaks of infections caused by these pathogens.7-9 The ESBL enzymes are capable of hydrolyzing broad spectrum cephalosporins and monobactams but inactive against cephamycins and imipenem. In addition, ESBL producing organisms exhibit co-resistance to many other classes of antibiotics resulting in limitation of therapeutic option. For this reason, the
significance of such ESBL–mediated infections has been increasingly reported worldwide.\textsuperscript{10-13} The ESBL have serine at their active site and attack the amide bond in the lactam ring of antibiotics causing their hydrolysis. Because of incoherent effect and substrate specificity, their detection is a major challenge. Two indicators of ESBL are eight-fold reductions in MIC and potentiation of the inhibitor zone of third generation cephalosporin in the presence of clavulanic acid.\textsuperscript{14} For this reason, detection of ESBL, using conventional antimicrobial susceptibility methods and delay in the recognition and reporting of ESBL production by Gram-negative bacilli is associated with prolonged hospital stay, increase morbidity, motility and health care expenses.\textsuperscript{1} So, it becomes necessary to know the prevalence of these organisms and to formulate the treatment policy. This study was mainly done to determine the susceptibility of different antimicrobials to ESBL producing \textit{E. coli} and \textit{K. pneumoniae} isolated from various samples in Shahid Bahonar Hospital of Tehran, Iran.

The National Committee for Clinical Laboratory Standards (NCCLS) recommended that Microbiology laboratories reported ESBL-producing isolates of \textit{E. coli} and \textit{Klebsiella} species are resistant to all penicillins, cephalosporins (including cefepime), and aztreonam, irrespective of their individual \textit{in vitro} test results. The presence of ESBL in some \textit{K. pneumoniae} and \textit{E. coli} strains poses an important challenge in clinical practice, since these organisms are common causes of serious infections. Imipenem and meropenem are considered the choice of therapy for patients with serious infections due to ESBL producing strains. Many ESBL-producing isolates are not always phenotypically resistant to oximino-cephalosporins. However, patients suffering from infections caused by ESBL-producing organisms are at risk of treatment failure if an extended spectrum of cephalosporins (ESC) are prescribed. Therefore, it is imperative for the clinical Microbiology laboratory to identify the isolates that possess increased MICs (2 \textmu g/mL) to oximino-cephalosporins, even though they may be equal to or below the susceptibility breakpoint (MIC 8 \textmu g/mL). Retrospective study at Shahid Bahonar Hospital of Tehran demonstrated an alarming increase in the prevalence of cefazidime-resistant \textit{K. pneumoniae}. This led us to look into our antibiotic resistance problems that may be being caused by the ESBL with our hospitalized patients as well as those attending the outpatient clinic.

**Methods**

During the period July, 2007 to June, 2008, various specimens (blood, fluid, urine, swabs, tracheal aspirates/sputum) were processed for significant bacteremia in the Microbiology laboratory of Shahid Bahonar Hospital, Tehran from clinically suspected patients. This study was done on 115 Gram-negative bacilli that were confirmed as ESBL producing isolates. Medical and demographic data of the patients were collected using patients files. Data recorded were as follows: demographic (age, sex, occupation), presence of urinary catheter/ abdominal tubes/ respiratory tubes/others, admission ward etc. Samples were collected aseptically in sterilized bottles or disposable sterile tubes and submitted to clinical Microbiology laboratory. The specimens received were inoculated on blood and MacConkey agar plates. Then all plates were incubated at 37°C for 24 hours. Significant isolates were identified as species level using conventional bacteriological methods.

**Antimicrobial susceptibility testing:** Isolates were screened initially using Kirby-Bauer method and MIC assays using microdilution method. All ESBL producing isolates were confirmed using the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS 2004)\textsuperscript{19} approved double-disk diffusion method. A positive result required an increased zone (5 mm), using combination disk technique with antibiotic disks containing ceftazidime (30\textmu g), cefpodoxime (30\textmu g) and cefotaxime (30\textmu g) either alone or in combination with clavulanic acid (10\textmu g). Susceptibility testing to other antibiotics was performed by disk diffusion methods as recommended by clinical laboratory standard institute (CLSI). The following antibiotic disks were used: gentamicin, amikacin, tobramycin, imipenem, meropenem, and four generation of cephalosporins (cefazoline, cephalothin, cefuroxime sodium, cefoxitin, ceftazidime, cefotaxime and cefepime), aztreonam, ampicillin, amoxicillin/clavulanate, ampicillin/sulbactam, piperacillin-tazobactam, trimethoprim/sulfamethoxazole, nitro-furantoin, ciprofloxacin, norfloxacin and nalidixic acid. According to the suggestion of CLSI, the results were interpreted. The quality control check for susceptibility testing was performed once in a week. All data were analyzed using Statistical Package for Social Sciences (SPSS).

**Results**

Among the patients, 68 (59.1%) were females and 47 (40.9%) were males. Out of 115 ESBL producing isolates, 60% were \textit{Escherichia coli} and 40% were \textit{Klebsiella pneumoniae}. Most of these isolates (29.6%) were from the medical wards, followed by outpatient's clinic (24.3%). Accident and Emergency and Surgical Units contributed 10.4% each, followed by Intensive Care Unit (9.6%). The least number of ESBL producing pathogens were isolated from the children wards (7%), neonatology wards (5.2%) and obstetric and gynecology wards (3.5%) (Table 1).
Table-1: Rate of isolation of ESBL producing bacteria from different wards of Shahid Bahonar Hospital, Tehran.

<table>
<thead>
<tr>
<th>Place of Sample Collection</th>
<th>No. of ESBL producing Bacteria</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medical Wards</td>
<td>34</td>
<td>29.6</td>
</tr>
<tr>
<td>Out Patient Department</td>
<td>28</td>
<td>24.3</td>
</tr>
<tr>
<td>Accident and Emergency Units</td>
<td>12</td>
<td>10.4</td>
</tr>
<tr>
<td>Surgical Units</td>
<td>12</td>
<td>10.4</td>
</tr>
<tr>
<td>Intensive Care Unit</td>
<td>11</td>
<td>9.6</td>
</tr>
<tr>
<td>Children Wards</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Neonatology Wards</td>
<td>6</td>
<td>5.2</td>
</tr>
<tr>
<td>Obstetric and Gynecology Wards</td>
<td>4</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Urine (70.4%) was the main source of ESBL producing isolates from all patients, next was the blood samples (16.5%). Tracheal aspirates/sputum contributed to 8.7%, followed by 3.5% from swabs and 0.9% from fluids (Table 2).

Table-2: Frequency and percentage of samples yielding ESBL producing isolates

<table>
<thead>
<tr>
<th>Source</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>81</td>
<td>70.4</td>
</tr>
<tr>
<td>Blood</td>
<td>19</td>
<td>16.5</td>
</tr>
<tr>
<td>Tracheal aspirates/Sputum</td>
<td>10</td>
<td>8.7</td>
</tr>
<tr>
<td>Swabs</td>
<td>04</td>
<td>3.5</td>
</tr>
<tr>
<td>Fluid</td>
<td>01</td>
<td>0.9</td>
</tr>
</tbody>
</table>
| Total           | 115       | 100            

All isolates were susceptible to both imipenem and meropenem. Of all isolates, 93.9% were susceptible to amikacin, whereas only 31.3% was susceptible to gentamicin and 20% to tobramycin. The Cephalosporins (1-4 generations) were almost 100% resistant except for Cefoxitin, which demonstrated a sensitivity of 77.4% against all isolates. For nitrofurantoin, 57.4% were sensitive, Tazocin showed a sensitivity of 49.6%, whereas low sensitivity was shown towards Co-trimoxazole (13%). Ciprofloxacin and Norfloxacin were 23.5% sensitive whereas only 17.4% were sensitive to Nalidixic acid (Table-3).

Discussion
During the past decade, ESBL producing Gram-negative bacilli especially *Escherichia coli* and *Klebsiella pneumoniae* have emerged as serious pathogens both in hospital and community acquired infections worldwide. Recent studies revealed that patients with infection such as septicemia with ESBL producing organisms had significantly higher fatality rate than those with non-ESBL isolates. The occurrence of ESBL among clinical isolates vary greatly world wide and geographically and are rapidly changing over time. Our study demonstrated clear differences in susceptibility patterns with our 115 ESBL producing isolates, between *Klebsiella*
producing organisms. Several studies have demonstrated that the use of third-generation cephalosporins, along with implementation of infection control measures, are the most effective means of controlling and decreasing the spread of ESBL producing isolates. The high percentage of ESBL producing isolates from outpatient clinics and accident and emergency wards should alert the physician in the primary care regarding the complication of uncontrolled prescription of oral cefalosporin, such as, cefuroxime. It should also alert them regarding the probability of ESBL producing isolates in infections not responding to the first line antibiotics, such as amoxicillin. Urine (70.4%) was the main source of ESBL producing isolates from all patients, followed by blood (16.5%). Generally, the pathogens isolated from medical wards and from blood appeared to be more sensitive than isolates from other areas and source. Respiratory and other swab samples did not demonstrate any sensitivity for all the quinolones and trimethoprim-sulfamethoxazole tested. Tobramycin demonstrated a 100% resistance towards all ESBL-producing bacteria. The ESBL-producing organisms are increasing rapidly and becoming a major problem in the area of infectious diseases. In one study from Turkey the prevalence rate of ESBLs was 12-47%. High rates of third-generation cephalosporin use have been implicated as a major cause of this problem. Problems associated with ESBL producing isolates include multidrug resistance, difficulty in detection and treatment, and increased mortality of patients. Of all available anti-microbial agents, carbapenem are the most sensitive and reliable treatment options for infections caused by ESBL producing bacteria. The ESBL-producing organisms are increasing rapidly and becoming a major problem in the area of infectious diseases. In one study from Turkey the prevalence rate of ESBLPs was 12-47%. High rates of third-generation cefalosporin use have been implicated as a major cause of this problem. Problems associated with ESBL producing isolates include multidrug resistance, difficulty in detection and treatment, and increased mortality of patients. Of all available anti-microbial agents, carbapenem are the most sensitive and reliable treatment options for infections caused by ESBL producing isolates. However, overuse of carbapenem may lead to resistance of other gram-negative organisms. Therefore, restricting the use of third-generation cefalosporins, along with implementation of infection control measures, are the most effective means of controlling and decreasing the spread of ESBL producing isolates.

References


13. Aminzadeh Z, Sadat KM, Sha’bani M. Bacteriuria by *Escherichia coli* and *Klebsiella pneumoniae* isolated from Hospital Samples Alipourfard et al

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