Invitro sensitivity pattern of ESBL Amp-C producing Enterobacteriaceae members to newer antimicrobials

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Abstract:

INTRODUCTION
In the last two decades, drug resistance has emerged as a serious threat for clinicians in the treatment of the Enterobacteriaceae infections. Extended Spectrum Beta Lactamase producing Enterobacteriaceae are resistant to almost all generations of Cephalosporins except the fifth generation drugs. Carbapenems are the drug of choice for betalactamase producing (Extended Spectrum Beta Lactamase and Amp C) Enterobacteriaceae isolates. Aim of the present study is to identify the effectiveness of newer antimicrobials against Extended Spectrum Beta Lactamase Producers.

MATERIALS AND METHODS
Total of 100 samples obtained from both in-patients and out-patients of PSG Hospitals were studied. RESULTS For all the ESBLs AmpC producing organisms tested in this study Doripenem has the highest sensitivity(93) followed by Tigecycline (90), Piperacillin-tazobactum(78), Cefepime-tazobactum (68) and Cefaperazone-sulbactum(61).

DISCUSSION
In the mid-1980s, when ESBLs were discovered, the treatment of Extended Spectrum Beta Lactamase producers became difficult, which was overcome by use of betalactam and betalactam inhibitor combinations. Inappropriate therapeutic management of ESBLs is a well recognized risk factor for development of ESBLs resulting in significant morbidity and mortality. CONCLUSION Extended Spectrum Beta Lactamase producing Enterobacteriaceae warrants appropriate use of antibacterial agents. Increase in emergence of carbapenamase producing Enterobacteriaceae in recent times, necessitates the need for beta lactam and beta lactamase inhibitor combinations as an option for the treatment of infections due to ESBL-producing organisms.

Keyword: ESBL - AmpC - Enterobacteriaceae - Beta lactamase inhibitor - Susceptibility
INTRODUCTION:
In the last two decades, drug resistance is an emerging threat in the infections caused by Enterobacteriaceae. The members of this family are associated with urinary tract infections, bacteremia, pneumonia, meningitis, and diarrheal diseases. In this family, *Escherichia coli* remains the leading pathogen causing infections. Traditional treatment includes the use of -lactam antibiotics (penicillins, cephalosporins) with the main concern being the development of resistance towards these agents with production of extended-spectrum -lactamases (ESBLs) and/or AmpC -lactamases by these organisms. Most ESBLs belong to the Ambler class A of -lactamases and are inhibited by -lactamase inhibitors (clavulanate, sulbactam and tazobactam) (1,2). AmpC-type cephalosporinases are Ambler class C -lactamases. They hydrolyze penicillins, cephalosporins (including the third-generation but usually not the fourth-generation compounds) and monobactams. In general, AmpC type enzymes are poorly inhibited by -lactamase inhibitors, especially clavulanic acid(3,4).

REVIEW OF LITERATURE:
ESBLs are enzymes hydrolyzing most penicillins and cephalosporins, including oxyimino-lactam compounds (cefuroxime, third- and fourth-generation cephalosporins and aztreonam) but not cephemycins and carbapenems. Most ESBLs belong to the Ambler class A of -lactamases and are inhibited by -lactamase inhibitors (clavulanate, sulbactam and tazobactam) (1,2). AmpC-type cephalosporinases are Ambler class C -lactamases. They hydrolyze penicillins, cephalosporins (including the third-generation but usually not the fourth-generation compounds) and monobactams. In general, AmpC type enzymes are poorly inhibited by -lactamase inhibitors, especially clavulanic acid(3,4).

Antibiotic resistance in pathogens belonging to Enterobacteriaceae family is increasing worldwide in both outpatients as well as hospitalized patients. The various mechanisms of drug resistance in Gram-negative bacilli include extended spectrum beta lactamase (ESBL) production (3), AmpC lactamase production (4), efflux mechanisms(5) and porin deficiency(6). Amongst the mechanisms of resistance to third generation cephalosporins, production of mechanisms (AmpC & OXA by cefepime, ESBLs and AmpC b-lactamases are the most common (4). In a prospective study conducted at Jhalawar, Rajasthan, to know the prevalence of ESBL producing gram negative bacilli in various clinical isolates reported a prevalence of 61.6% of ESBL producers.

Cefepime, a 4th generation cephalosporin, is stable against AmpC & OXA but it lacks activity against ESBL producing organisms. The combination of Cefepime-tazobactam effectively cover all three major resistance eration cephalosporins, production of mechanisms (AmpC & OXA by cefepime, ESBLs and AmpC b-lactamases are the most common (4). In a prospective study conducted at Jhalawar, Rajasthan, to know the prevalence of ESBL producing gram negative bacilli in various clinical isolates reported a prevalence of 61.6% of ESBL producers.

Cefepime-tazobactam can be used for treatment of ESBL producing Enterobacteriaceae. This will help to reduce the usage of carbapenems in ESBL positive Enterobacteriaceae strains and prevent development of carbapenem resistance. However more invitro and clinical trials are required to assess the usefulness of cefipime-tazobactum in clinical practice.
Many clinical laboratories currently test *Escherichia coli* and *Klebsiella spp.* for production of ESBLs but do not attempt to detect plasmid mediated AmpC b-lactamases (also known as imported, transmissible, foreign, or mobile AmpC b-lactamases). These enzymes are typically associated with multiple antibiotic resistances, leaving a few therapeutic options (7).

**OBJECTIVES:**

To know the antibiotic sensitivity pattern of ESBL &amp;C producing Enterobacteriaceae members to newer antimicrobials.

**MATERIALS AND METHODS:**

Total of 100 samples obtained from both in-patients and out-patients of a tertiary care hospital were studied. **TABLE 1: DISTRIBUTION OF BACTERIAL ISOLATES IN VARIOUS SAMPLES**

<table>
<thead>
<tr>
<th>S.NO</th>
<th>SAMPLE</th>
<th>ISOLATES</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Urine</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Respiratory Tract Samples</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>Wound swabs</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>Blood</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>Body Fluids</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td><strong>TOTAL</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

For susceptibility testing, the Kirby-Bauer disk diffusion method was used. Antibiotic disks were purchased from Becton Dickinson (Franklin Lakes, NJ), and results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) 2009 guidelines (8). ESBLs were detected by the confirmatory method of Clinical and Laboratory Standards Institute (CLSI) using cefotaxime (30 mg) and ceftazidime (30 mg) and a disc of cefotaxime plus clavulanic acid (30 and 10 mg) (9).

AmpCs were detected by the confirmatory method of Clinical and Laboratory Standards Institute (CLSI) using cefoxitin (30 g/disk) screening cut off of 18 mm, is used (i.e., the CLSI susceptible breakpoint)(9,10) Antibiotic susceptibility to newer antibiotics like cefepime-tazobactum (30/10), cefaperazone-sulbactam (30/10), piperacillin-tazobactum (100/10) were performed by the CLSI method (8). Tigecycline has a breakpoint of MIC 2 mg/L for Enterobacteriaceae except proteeae (11,12).Doripenem has a breakpoint of MIC 0.12 mg/L for Enterobacteriaceae (13) which was performed by automated drug susceptibility testing by vitek2 (Biomerieux).

**RESULTS:** In the total of 100 samples which were tested positive for ESBLs the highest positivity was found to be in *Escherichia coli* (56%), followed by *Klebsiella pneumoniae* (32%), *Citrobacter spp* (10%) and *Enterobacter spp* (2%).
In the total of 100 samples which were tested positive for AmpC the highest positivity was found to be in *Klebsiella pneumoniae* (28%), followed by *Escherichia coli* (15%), *Citrobacter spp* (6%) and *Enterobacter spp* (4%).

Out of 100 isolates the drug resistance pattern was observed as follows: 39% to cefaperazone-sulbactum, 32% to cefepime-tazobactum, 22% to piperacillin-tazobactum, 10% to Tigecycline and 7% to Doripenem.

**DISCUSSION:**

In the mid-1980’s, when ESBLs were discovered, the treatment of Extended Spectrum Beta Lactamase producers became difficult, which was overcome by use of betalactam and betalactam inhibitor combinations. Inappropriate therapeutic management of ESBLs is a well recognized risk factor for development of ESBLs resulting in significant morbidity and mortality. Extended spectrum beta lactamases (ESBLs) and AmpC beta lactamases are the most common mechanisms of antimicrobial resistance in Gram negative bacilli. The prevalence of ESBLs in India has now reached epidemic proportions, ranging from 62% to 100% in *Escherichia coli* and *Klebsiella spp.*, isolated from skin and soft tissue infections, blood stream infections and respiratory infections as observed in the 10 Indian medical centre SENTRY study.(14). ESBLs are usually inhibited by -lactamase inhibitors, such as clavulanic acid, sulbactam or tazobactam. ESBL producers are highly sensitive to carbapenems. According to the study conducted by Neelam Taneja et al, carbapenems are more effective than cefepime in treating serious infections that involve large numbers of AmpC producing organisms(16). Increase in emergence of carbapenemase producing Enterobacteriaceae results in prolonged stay and increased expenditure to the patient. Rationale behind the use of -lactam/-lactamase inhibitor combinations in the treatment of ESBL producing Enterobacteriaceae should be clearly understood to reduce the risk of spread.

According to IDSA(Infectious Disease Society of America) guidelines the recommendations for treating ESBL infections are
In this study Doripenem has the maximum sensitivity of 93% and Doripenem is potent against most of the Enterobacteriaceae isolates tested, including those resistant to “advanced-generation” cephalosporins and screen-positive ESBL and Amp C isolates as suggested by a study of Cefepime versus imipenem-cilastatin for treatment of nosocomial pneumonia in intensive care unit patients (15). Tigecycline has 90% sensitivity in our study and had good activity against most ESBL-producing and AmpC-hyperproducing Enterobacteriaceae. It may be a therapeutic alternative to carbapenems in some infections caused by ESBL- and AmpC-producing isolates, many of which are also multiresistant to quinolones, aminoglycosides and classical tetracyclines. (12).

Among the beta-lactam-inhibitor combinations tested in other studies, Cefoperazone-sulbactam revealed the highest activity against ESBL-producing E.coli and K.pneumoniae and may be used for empirical therapy of nosocomial infections caused by ESBLs(21).

CONCLUSION:
Extended Spectrum Beta Lactamase producing Enterobacteriaceae warrants appropriate use of antibacterial agents. Increase in emergence of carbapenemase producing Enterobacteriaceae in recent times, necessitates the need for beta lactam and beta lactamase inhibitor combinations as an option for the treatment of infections due to ESBL-producing organisms. For all the ESBLs & Amp C producing organisms tested in this study Doripenem has the highest sensitivity(93%) followed by Tigecycline (90%), Piperacillin-tazobactam(78%), Cefepime-tazobactum(68%) and Cefoperazone-sulbactum(61%).

Cefepime-tazobactum is effective against 68% of isolates. Cefepime, a 4th generation cephalosporin, is stable against Amp C & OXA, but it lacks activity against ESBL producing organism. A novel combination of Cefepime-tazobactum is expected to increase susceptibility of Enterobacteriaceae otherwise resistant to cefepime if used alone. The combination of Cefepime-tazobactum may effectively cover all three major resistance mechanisms (Amp C & OXA by cefepime, ESBLs by tazobactam)(16).

Cefoperazone-sulbactam is effective against 61% of our isolates.

In our study 78% of isolates are susceptible to piperacillin-tazobactum. Piperacillin-tazobactum contains a semisynthetic penicillin in combination with the beta-lactamase inhibitor tazobactam. Piperacillin-tazobactum was the second most active antibacterial, after imipenem, against the ESBL & Amp C producing pathogens, inhibiting 84.4% of the isolates (18).
REFERENCES:


