EXTENDED SPECTRUM β-LACTAMASES (ESBL) – AN EMERGING THREAT TO CLINICAL THERAPEUTICS

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Abstract

Extended spectrum β-lactamases (ESBLs) are plasmid mediated, TEM and SHV derived enzymes, first isolated in Western Europe in mid 1980s, most commonly in Klebsiella spp., followed by Escherichia coli. These 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. Several risk factors have been suggested. A variety of classification schemes have been developed. Recently, Bush-Jacoby-Medeiros scheme integrated functional and molecular characteristics. ESBLs have serine at their active site and attack the amide bond in the β-lactam ring of antibiotics causing their hydrolysis. Because of inoculum effect and substrate specificity their detection is a major challenge. Two indicators of ESBLs are eight fold reduction in MIC and potentiation of the inhibitor zone of third generation cephalosporin in the presence of clavulanic acid. Incidence of these organisms is being continuously increasing throughout the world with limited treatment alternatives. It becomes necessary to know the prevalence of these organisms and to formulate treatment policy. Moreover, restricted use of the third generation cephalosporins lead to withdrawal of selective pressure and use of β-lactam and β-lactamase inhibitor combinations may exert reverse mutation on these enzymes.

Key words: Broad spectrum cephalosporins, Klebsiella pneumoniae, β-lactams, clavulanic acid

Resistant bacteria are emerging world wide as a threat to the favourable outcome of common infections in community and hospital settings. β-lactamase production by several gram negative and gram positive organisms is perhaps the most important single mechanism of resistance to penicillins and cephalosporins. In the past it was believed that cephalosporins were relatively immune to attack by β-lactamases. It was surprising to find cephalosporin resistant Klebsiella spp. among the clinical isolates. The mechanism of this resistance was production of extended spectrum β-lactamases (ESBLs).1

The ESBL enzymes are plasmid mediated enzymes capable of hydrolyzing and inactivating a wide variety of β-lactams, including third generation cephalosporins, penicillins and aztreonam. These enzymes are the result of mutations of TEM-1 and TEM-2 and SHV-1. All of these β-lactamase enzymes are commonly found in the Enterobacteriaceae family. Normally, TEM-1, TEM-2 and SHV-1 enzymes confer high level resistance to early penicillins and low level resistance to first generation cephalosporins. Widespread use of third generation cephalosporins and aztreonam is believed to be the major cause of the mutations in these enzymes that has led to the emergence of the ESBLs.2 These enzymes mediate resistance to cefotaxime, cefazidime and other broad spectrum cephalosporins and to monobactams such as aztreonam, but have no detectable activity against cephamycins and imipenem. Because, of their greatly extended substrate range these enzymes were called extended spectrum β-lactamases.3

The first ESBL isolates were discovered in Western Europe in mid 1980s and subsequently in the US in the late 1980s.2 The resistant organisms are now a worldwide problem. They can be found in a variety of Enterobacteriaceae species, however, the majority of ESBL producing strains are K. pneumoniae, K. oxytoica and E.coli. Other organisms reported to harbour ESBLs include Enterobacter spp., Salmonella spp., Morganella morganii, Proteus mirabilis, Serratia marcescens and Pseudomonas aeruginosa. However, the frequency of ESBL production in these organisms is low.2

Major risk factors for colonization or infection with ESBL producing organisms are long term antibiotic exposure, prolonged ICU stay, nursing home residency, severe illness, residence in an institution with high rates of cefazidime and other third generation cephalosporin use and instrumentation or catheterisation.2

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**Evolution and Dissemination of β-Lactamases**

Fifty years ago the antibiotic era began with the discovery of penicillin. With in a few years of introduction of penicillin into clinical use, penicillinase producing *Staphylococcus aureus* started to proliferate in hospitals. To overcome this problem, penicillinase resistant penicillins came into picture. Shortly afterward, the broad spectrum penicillins and first generation cephalosporins were introduced. They remained a first line of defense against microbes for over 20 years, before resistance due to β-lactamases produced by gram negative bacilli became a serious problem.4

To counter this threat, the pharmaceutical industry marketed six novel classes of β-lactam antibiotics (cephamycins, oxyimino cephalosporins, carbapenems, monobactams and clavam and penicillanic acid sulfone inhibitors) within a relatively short span of 7-8 years. Although, novel β-lactamases had emerged gradually after the introduction of new β-lactam agents, their number and variety accelerated at an alarming rate (Table 1).4,5 More than 170 β-lactamases have been recognised.4 Their growth spurt shows no signs of slowing down.

<table>
<thead>
<tr>
<th>Year</th>
<th>Enzyme</th>
<th>Organism</th>
<th>Place</th>
</tr>
</thead>
<tbody>
<tr>
<td>1944</td>
<td>Penicillinase</td>
<td><em>S. aureus</em></td>
<td>-</td>
</tr>
<tr>
<td>1963</td>
<td>TEM-1</td>
<td><em>E. coli</em></td>
<td>Athens</td>
</tr>
<tr>
<td>1974</td>
<td>SHV-1</td>
<td><em>E. coli</em></td>
<td>Switzerland</td>
</tr>
<tr>
<td>1978</td>
<td>OXA-10</td>
<td><em>P. aeruginosa</em></td>
<td>-</td>
</tr>
<tr>
<td>1982</td>
<td>SME-1</td>
<td><em>S. marcescens</em></td>
<td>London</td>
</tr>
<tr>
<td>1984</td>
<td>IMI-1</td>
<td><em>E. cloaceae</em></td>
<td>California</td>
</tr>
<tr>
<td>1988</td>
<td>Metallo β-lactamase</td>
<td><em>P. aeruginosa</em></td>
<td>Japan</td>
</tr>
<tr>
<td>1989</td>
<td>Inhibitor-resistant penicillin</td>
<td><em>E. coli, K. pneumoniae</em></td>
<td>France, Spain, Greece</td>
</tr>
<tr>
<td>1990</td>
<td>NMe A</td>
<td><em>E. cloaus</em></td>
<td>Paris</td>
</tr>
<tr>
<td>1991</td>
<td>OXA-11</td>
<td><em>P. aeruginosa</em></td>
<td>Turkey</td>
</tr>
<tr>
<td>1991</td>
<td>OXA-14</td>
<td><em>P. aeruginosa, S. typhimurium</em></td>
<td>Turkey</td>
</tr>
<tr>
<td>1992</td>
<td>PER-1</td>
<td><em>E. coli, K. pneumoniae</em></td>
<td>France</td>
</tr>
<tr>
<td>1994</td>
<td>TOHO-1</td>
<td><em>E. coli</em></td>
<td>Japan</td>
</tr>
<tr>
<td>1996</td>
<td>PER-2</td>
<td><em>E. coli, K. pneumoniae, S. typhimurium, P. mirabilis</em></td>
<td>Germany</td>
</tr>
<tr>
<td>1997</td>
<td>VEB-1</td>
<td><em>E. coli</em></td>
<td>Germany</td>
</tr>
</tbody>
</table>

**Classification Schemes**

Various classification schemes have been proposed by many researchers.6 Classification of Sawai *et al* in 1968 was based on response to antisera. Richmond and Sykes scheme in 1973 was on the basis of substrate profile. Extension of the Richmond and Sykes scheme by Sykes and Mathew in 1976 was based on differentiation by isoelectric focussing. In the scheme proposed by Mitsuhashi and Inoue in 1981, the category “cefuroxime hydrolyzing β-lactamases” was added to “penicillinate and cephalosporinase” classification. The groupings proposed by Bush in 1989 was based on correlation of substrate and inhibitory properties with molecular structure.6

However, the number and variety of enzymes have proliferated beyond the boundaries of the scheme. A more modern scheme based on molecular structure classification was proposed by Ambler includes, of necessity, only those enzymes that have been characterized.7

Recently a new classification scheme has been developed to integrate functional and molecular characteristics.7 The Bush-Jacoby-Medeiros scheme puts 178 β-lactamases from naturally occurring bacterial isolates into four groups based on substrate and inhibitor profiles (Table 2).4
Table 2: The Bush Jacoby – Medeiros functional classification scheme for β-lactamases

<table>
<thead>
<tr>
<th>Group</th>
<th>Enzyme Type</th>
<th>Inhibition by Clavulanate</th>
<th>Molecular Class</th>
<th>No. of Enzymes</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cephalosporinase</td>
<td>No</td>
<td>C</td>
<td>53</td>
<td><em>E. cloacae</em> P 99, MIR-1</td>
</tr>
<tr>
<td>2a</td>
<td>Penicillinase</td>
<td>Yes</td>
<td>A</td>
<td>20</td>
<td><em>S. aureus, S. albus</em></td>
</tr>
<tr>
<td>2b</td>
<td>Broad spectrum</td>
<td>Yes</td>
<td>A</td>
<td>16</td>
<td>TEM-1, SHV-1</td>
</tr>
<tr>
<td>2be</td>
<td>Extended spectrum</td>
<td>Yes</td>
<td>A</td>
<td>38</td>
<td>TEM-3, SHV-2, <em>K. oxytoca</em> K1</td>
</tr>
<tr>
<td>2br</td>
<td>Inhibitor resistant</td>
<td>Diminished</td>
<td>A</td>
<td>9</td>
<td>TEM-30, TRC-1</td>
</tr>
<tr>
<td>2c</td>
<td>Carbenicillinase</td>
<td>Yes</td>
<td>A</td>
<td>15</td>
<td>PSE-1, CARB-3, BRO-1</td>
</tr>
<tr>
<td>2d</td>
<td>Cloxacillinase</td>
<td>Yes</td>
<td>D or A</td>
<td>18</td>
<td>OXA-1, PSE-2, <em>Streptomyces cacaoi</em></td>
</tr>
<tr>
<td>2e</td>
<td>Cephalosporinase</td>
<td>Yes</td>
<td>A</td>
<td>19</td>
<td><em>Proteus vulgaris, Bacteroides fragilis, Cep A</em></td>
</tr>
<tr>
<td>2f</td>
<td>Carbapenemase</td>
<td>Yes</td>
<td>A</td>
<td>3</td>
<td><em>E. cloacae</em> IMI-1, NMC-A</td>
</tr>
<tr>
<td>3</td>
<td>Metalloenzyme</td>
<td>No</td>
<td>B</td>
<td>15</td>
<td><em>Xanthomonas maltophilia</em> L1</td>
</tr>
<tr>
<td>4</td>
<td>Pencillinase</td>
<td>No</td>
<td></td>
<td>7</td>
<td><em>Pseudomonas cepacia</em></td>
</tr>
</tbody>
</table>

### Structure of β-Lactamase and Mechanism of Action

All ESBLs have serine at their active sites except for a small (but rapidly growing) group of metallo β-lactamases belonging to class B. They share several highly conserved amino acid sequences with penicillin binding proteins (PBP)s.

β-lactamases attack the amide bond in the β lactam ring of penicillins and cephalosporins, with subsequent production of pencillinoic acid and cephalosporic acid, respectively, ultimately rendering the compounds antibacterially inactive.

Plasmids responsible for ESBL production tend to be large (80 Kbp or more in size) and carry resistance to several agents, an important limitation in the design of treatment alternatives. The most frequent core resistances found in ESBL producing organisms are aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol and sulfamethoxazole-trimethoprim.

Except for one brief report, none of these enzymes have been shown to be transposable. The usual transmissibility of the responsible plasmids, however, allows resistance to spread readily to other pathogens so that extended spectrum enzymes have been found in nearly all species of *Enterobacteriaceae*. Reports of ESBLs in *P. mirabilis* have been relatively rare. In this species, it may be due to low frequency of plasmid conjugation.

Since, ESBL production is usually plasmid mediated it is possible for one specimen to contain both ESBL producing and non ESBL producing cells of the same species. This suggests that for optimal detection, several colonies must be tested from a primary culture plate.

### Problems in Detection

Identifying organisms that are ESBL producers is a major challenge for the clinical microbiology laboratory. Due to the variable affinity of these enzymes for different substrates and inoculum effect, some ESBL isolates may appear susceptible to a third generation cephalosporin *in vitro*. However, treatment of infections due to an ESBL producing organism with third generation cephalosporins may result in clinical failure if infection is outside the urinary tract.

Cefpodoxime and ceftazidime have been proposed as indicators of ESBL production as compared to cefotaxime and ceftriaxone. Hence, an institution where only cefotaxime and ceftriaxone are used in the routine sensitivity testing panel may have difficulty in detecting ESBLs.

These enzymes can be induced by certain antibiotics, amino acids or body fluids. Organisms possessing genes for inducible β-lactamases show false susceptibility if tested in the uninduced state.

For ESBL producing bacteria there is a dramatic rise of MIC for extended spectrum cephalosporins as the inoculum is increased beyond that used in routine susceptibility. Same isolates test susceptible at the standard inoculum of 10^6 CFU/mL but resistant at an inoculum of 10^7 CFU/mL. Therefore, they may be reported as false sensitive if tested by routine methods.
Sensitivity breakpoints of MIC as designated in the National Committee for Clinical Laboratory Standards (NCCLS) guidelines for *Klebsiella* and *E. coli* against cefotaxime, ceftriaxone and ceftazidime are less than or equal to 8 µg/mL. Some ESBL producing organisms may have increased MIC against these agents compared with those of non-ESBL isolates. However, the increased MIC is still lower than the cut off value for susceptibility.2

Latest guidelines by NCCLS recommend screening *Klebsiella* spp. and *E. coli* isolates with a MIC greater than or equal to 2 µg/mL against cefpodoxime, ceftazidime, aztreonam, cefotaxime, or ceftriaxone as potential ESBL producers. Two indicators of ESBLs are an 8 fold reduction in MIC in the presence of clavulanic acid when using the broth dilution and the potentiation of the inhibitor zone by clavulanic acid (>5 mm increase in diameter of inhibition zone) when using disc diffusion method. These methods, though useful, may not detect those ESBLs that are poorly inhibited by β-lactamase inhibitors.2

Two recent studies evaluated the ability of clinical laboratories to detect and report the presence of ESBLs. A survey in Connecticut found that 21% of laboratories fail to detect ESBL producing isolates. A proficiency testing project for clinical laboratories participating in the National Nosocomial Infections Surveillance System indicated that as many as 58% laboratories failed to detect and report ESBL isolates correctly. These data suggest that improvements in the ability of clinical laboratories to detect ESBL are needed.2

**Methods of Detection**

Several tests have been developed to confirm the presence of ESBLs.

**Double disc synergy test**12

In this test discs of third generation cephalosporins and augmentin are kept 30 mm apart from center to center on inoculated Mueller-Hinton Agar (MHA). A clear extension of the edge of the inhibition zone of cephalosporin towards augmentin disc is interpreted as positive for ESBL production.

**Three dimensional test**13

This test provides the advantage of simultaneous determination of antibiotic susceptibility and β-lactamase substrate profile. Inoculum produced in this method contains between 10⁹ and 10¹⁰ CFU/mL of cells that actively produce β-lactamase. Two types of inocula are prepared one disc diffusion test inoculum (optical density equal to that of 0.5 McFarland standard) and a three dimensional inoculum (contain between 10⁹ and 10¹⁰ CFU of cells). Plate is inoculated by disc diffusion procedure. A circular slit is cut on the agar 4mm inside the position at which the antibiotic discs are placed. Conventional (two dimensional) disc diffusion susceptibility test results are measured according to the recommendations of NCCLS. Distortion or discontinuity in the circular inhibition zone is interpreted as positive for ESBL production.

**Inhibitor potentiated disc diffusion test**14

Cephalosporin disc is placed on clavulanate containing and with out clavulanate containing MHA plates. More than10 mm increase in the zone of inhibition on the clavulanate containing MHA plate indicates ESBL production.

**Disk approximation test**10

Cefoxitin (inducer) disc is placed at a distance of 2.5 cm from cephalosporin disc. Production of inducible β-lactamase is indicated by flattening of the zone of inhibition of the cephalosporin disc towards inducer disc by ≥1 mm.

**MIC reduction test**2

An 8 fold reduction in the MIC of cephalosporin in the presence of clavulanic acid indicates production of ESBL.

**Vitek ESBL Test**15

Four wells containing cards are inoculated. A predetermined reduction in growth of cephalosporin well containing clavulanic acid; when compared with the level of growth in well with cephalosporin alone indicates presence of ESBL.

**E Test**16

The E test ESBL strip carries two gradients, on the one end, ceftazidime and on the opposite end ceftazidime plus clavulanic acid. MIC is interpreted as the point of intersection of the inhibition ellipse with the E test strip edge. Ratio of ceftazidime MIC and ceftazidime clavulanic acid MIC equal to or greater than 8 indicates the presence of ESBL.

**Treatment**

Of all the available β lactams carbapenems are the most effective and reliable as they are highly resistant to the hydrolytic activity of all ESBL enzymes, due to the trans-6 hydroxy ethyl group. Meropenem is the most active with MICs generally lower than those of imipenem.
of cephalosporin use, especially third generation cephalosporins. Several studies have shown that by limiting the use of these agents alone or in combination with infection control measures, the frequency of ESBL isolates can be reduced substantially. Educational programs for medical staff to increase awareness also should be developed.2

Conclusion

ESBL producing organisms pose a major problem for clinical therapeutics. The incidence of ESBL producing strains among clinical isolates has been steadily increasing over the past few years resulting in limitation of therapeutic options.19

Initially restricted to hospital acquired infections, they have also been isolated from infections in outpatients. Major outbreaks involving ESBL strains have been reported from all over the world, thus making them emerging pathogens.19

The routine susceptibility tests done by clinical laboratories fail to detect ESBL positive strains and can erroneously detect isolates sometimes to be sensitive to any of the broad spectrum cephalosporin20 like cefotaxime, ceftazidime, ceftriaxone.

With the spread of ESBL producing strains in hospitals all over the world, it is necessary to know the prevalence of ESBL positive strains in a hospital so as to formulate a policy of empirical therapy in high risk units where infections due to resistant organisms is much higher.20 Equally, important is the information on an isolate from a patient to avoid misuse of extended spectrum cephalosporins, which still remain an important component of antimicrobial therapy in high risk wards.20

A knowledge of resistance pattern of bacterial strains in a geographical area will help to guide the appropriate and judicious antibiotic use. There is possibility that the restricted use can lead to the withdrawal of selective pressure and resistant bacteria will no longer have survival advantage in such settings.20

References


