Extended Spectrum β-Lactamase (ESBL) Producing Enterobacteriaceae

Shelby Reimer
The \( \beta\)-lactams are an important class of antimicrobials used for the treatment of serious hospital and community-associated infections.
Mechanism of Bacterial Resistance to $\beta$-Lactam Antibiotics

- (I) $\beta$-lactamase production
- (II) Expressing altered and mutated PBPs
- (III) Absence or reduced expression of outer membrane proteins (OMPs)
- (IV) Overexpression of efflux pumps

**β-LACTAMASES**

β-lactamases are enzymes that mediate resistance by hydrolyzing the β-lactam ring of the β-lactam antibiotic, inactivating it (TEM-1, TEM-2, SHV)

![Diagram of β-lactamase action on antibiotic](http://spot.pcc.edu/~jvolpe/bi234/lec/6_antimicrobials/images/fig10-16_Beta-lactamase_L.jpg)
**ESBLs**

- **Extended-spectrum β-lactamases (ESBLs)** = enzymes that mediate resistance to extended-spectrum (third generation) cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone) and monobactams (e.g., aztreonam) but do not affect cephemycins (e.g., cefoxitin and cefotetan) or carbapenems (e.g., meropenem or imipenem).

- Changes in 1-5 amino acids near the serine active site of TEM-1 (or SHV-1) determine the ESBL type.
ESBLs

ESBLs

- >500 β-lactamases
  - >50% ESBLs

Most ESBLs are seen in *Enterobacteriaceae*, predominantly *E. coli* and *K. pneumoniae*

ESBLs located on transferable plasmids that may carry additional resistance genes

<table>
<thead>
<tr>
<th>Family of β-lactamase of clinical importance</th>
<th>Representative enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. SHV type</td>
<td>SHV-1, SHV-111, SHV-89,</td>
</tr>
<tr>
<td>2. TEM type</td>
<td>TEM-1, TEM-2, TEM-3</td>
</tr>
<tr>
<td>3. CTX type</td>
<td>CTX-M-1, CTX-M-44</td>
</tr>
<tr>
<td>4. OXA type</td>
<td>OXA-1, OXA-2, OXA-10</td>
</tr>
<tr>
<td>5. PER type</td>
<td>PER-1</td>
</tr>
<tr>
<td>6. GES type</td>
<td>GES-1</td>
</tr>
<tr>
<td>7. VEB-1, BES-1 and other ESBL</td>
<td>BES-1, CME-1, VE-B-1, PI</td>
</tr>
</tbody>
</table>
### ESBL TYPES

<table>
<thead>
<tr>
<th>Class</th>
<th>Type</th>
<th>Nr of subtypes</th>
<th>Most common subtypes</th>
<th>Predominant organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>TEM</td>
<td>183</td>
<td>TEM-3; 4; 24; 26; 52</td>
<td><em>Enterobacteriaceae</em></td>
</tr>
<tr>
<td></td>
<td>SHV</td>
<td>134</td>
<td>SHV-2; 4; 5; 12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CTX-M</td>
<td>102</td>
<td>CTX-M-3; 9; 14; 15</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>OXA</td>
<td>194 (18 ESBLs)</td>
<td>OXA-2; 10</td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
</tbody>
</table>

**ESBL-producing *Enterobacteriaceae***

<table>
<thead>
<tr>
<th>Period</th>
<th>Most prevalent types</th>
<th>Predominant species</th>
</tr>
</thead>
<tbody>
<tr>
<td>20th century</td>
<td>SHV + TEM</td>
<td><em>Klebsiella</em> spp. (Enterobacter spp)</td>
</tr>
<tr>
<td>21st century</td>
<td>CTX-M</td>
<td></td>
</tr>
</tbody>
</table>
The number of new and novel variants of each type of ESBL is rising rapidly, with 223 TEM, 193 SHV, and 172 CTX-M-type ESBLs reported as of January 6, 2019 (http://www.lahey.org/Studies/).

Naas et al., 2008, CMI, Suppl 1:42-52
Ambler Classification = based on AA sequence similarity

- Class A: TEM, SHV, CTX-M, ESBLs, KPC
- Class B: Metallo-enzymes (NDM)
- Class C: AmpC
- Class D: OXA

[Points to Serine active site and Zinc active site]
Bush Classification (Groups 1-2, Subgroups a-f)

- Group 1: AmpC
- Group 2: TEM, SHV, CTX-M, ESBLs, KPC
  - ESBLs designated as group 2be
- Group 3: Metallo-enzymes (NDM)
- Group 4: OXA, other
<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1928</td>
<td>Discovery of penicillin</td>
</tr>
<tr>
<td>1940</td>
<td>Clinical applications of penicillin</td>
</tr>
<tr>
<td>1960</td>
<td>Introduction of first-generation cephems (cephalosporins/β-lactamases)</td>
</tr>
<tr>
<td>1974</td>
<td>Introduction of second generation (cephems/cephalosporins)</td>
</tr>
<tr>
<td>1980</td>
<td>Development of third generation (cephems/cephalosporins)</td>
</tr>
<tr>
<td>1984</td>
<td>Development of carbapenems and monobactams</td>
</tr>
<tr>
<td>1990-to date</td>
<td>Increased use of oral cephems, monobactams, and second- and third-generation cephalosporins and carbapenems</td>
</tr>
<tr>
<td></td>
<td><strong>Emergence of resistance</strong></td>
</tr>
</tbody>
</table>

- **1950**: Emergence of penicillinase producing *Staphylococcus aureus*
- **1965**: Plasmid mediated TEM-1 emergence in *E. coli* isolated from a Greece, patient TEM-1 hydrolyzed penicillin and its derivatives including 1st-generation cephalosporins
- **1967-1970**: Penicillin intermediately resistant *Salmonella* from South America were reported. This was followed by explosive increasing report on multidrug resistant ESBL and carbapenemase producing Gram-negative bacilli all over the world
- **1974–1977**: Penicillinase producing *Haemophilus influenza* emergence of penicillinase producing *Haemophilus influenza*
- **1974–1977**: Appearance of penicillin resistant *S. pneumoniae*
- **1974–1977**: Emergence of β-lactamase nonproducing ampicillin resistant *H. influenzae* (BLNAR)
- **1983**: Appearance of ESBL producing Gram-negative bacilli
- **1986**: First report on TEM and non-SHV ESBL from Japan
- **1989**: First report of CTXM producing *E. coli* from Germany
- **1989**: In the following two years, endemics of CTXM producing *Salmonella* from South America were reported. This was followed by explosive increasing report on multidrug resistant ESBL and carbapenemase producing Gram-negative bacilli all over the world

**Figure 2**: Trend of development of antibiotics and emergence of resistance with particular emphasis on ESBL.
**TRANSMISSION**

- **bla** genes mobilized from chromosomes to plasmids
  - **bla**CTX-M mobilizes 10x more frequently than **bla**SHV & **bla**TEM

- Horizontal & vertical transmission of plasmids:
  - Horizontal transmission: plasmid-mediated conjugation
  - Vertical transmission: clonal transmission by normal cell division

EPIDEMIOLOGY

The widespread distribution of ESBLs is the result of:

- Clonal expansion of strains that produce $\beta$-lactamases
- Horizontal transfer of ESBL genes on plasmids (blaESBL genes)
- The emergence of genes de novo (as in CTX-M types)

The most successful ESBLs in *Enterobacteriaceae* in terms of dissemination and promiscuity are the CTX-M, TEM, and SHV types

- Changing pattern of predominant $\beta$-lactamases over time

Regional Prevalence of ESBL-Producing E. coli in Canada: CANWARD 2016

Denisuiik et al. ASM Microbe 2017.
% OF E. COLI BLOOD ISOLATES THAT ARE ESBL-PRODUCERS

Increasing prevalence of ESBL-producing *Enterobacteriaceae* globally
Increasing prevalence of MDR/XDR ESBL-producing *E. coli* in CANWARD study (2007-2011) from 77.4% in 2007 to 82.6% in 2011

Community-associated emergence of ESBL-producing *Enterobacteriaceae* is becoming a problem as well

In an observational study at five U.S. medical centers between 2009-2010, 56% of all ESBL-producing *Escherichia coli* isolates stemmed from community-associated episodes; 37% of these episodes had no healthcare association

PREVALENCE OF *E. coli* O25:H4 ST131

**bla**CTX-M-15 – ESBL plasmid with multi drug co-resistance:
- **bla**OXA-1
- **bla**TEM-1
- **aac**6'-Ib-cr (aminoglycosideacetyltransferase)
- **tet**(A) (tetracycline efflux protein)

**MLST clone ST131**
- Disseminated by a widespread, successful clone: Serotype O25:H4 & MLST profile ST131
- O25:H4-ST131 not exclusively associated with **bla**CTX-M-15

Denisuk et al. JAC 2013; Denisuk et al. ICAAC/ICC 2015.
Since 2003, multidrug-resistant CTX-M-15-producing *E. coli* has emerged worldwide as an important pathogen causing community- and hospital-associated infections.

PREVALENCE OF *E. coli* O25:H4 ST131


### CANWARD Study Year

<table>
<thead>
<tr>
<th>Year</th>
<th>Total ESBL-<em>E. coli</em></th>
<th>Total ST131 ESBL-<em>E. coli</em></th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>53</td>
<td>26</td>
<td>49.1</td>
</tr>
<tr>
<td>2008</td>
<td>55</td>
<td>27</td>
<td>49.1</td>
</tr>
<tr>
<td>2009</td>
<td>47</td>
<td>25</td>
<td>53.2</td>
</tr>
<tr>
<td>2010</td>
<td>30</td>
<td>18</td>
<td>60.0</td>
</tr>
<tr>
<td>2011</td>
<td>46</td>
<td>33</td>
<td>71.7</td>
</tr>
</tbody>
</table>

P-value\(^a\) 0.0368 (0.0179)

\(^a\)P-value comparing ST131 ESBL-EC vs. non-ST131 ESBL-EC: chi-square (one-tailed Fisher’s exact test)
ESBL-producing *Enterobacteriaceae* can cause several types of bacterial infection:

- Urinary tract infections (UTIs)
- Kidney infections
- Intra-abdominal infections
- Hospital-acquired/ventilator associated pneumonia
- Wound infections
- Bacteremia (bloodstream infections)
TREATMENTS

- Carbapenems
- Nitrofurantoin and fosfomycin
- β-lactam / β-lactamase inhibitors
- Aminoglycosides
- Colistin
Carbapenems are widely regarded as the drugs of choice for the treatment of severe infections due to ESBL-producing *Enterobacteriaceae*.

Stewart et al. 2015. Biochem. 54:588-597.
**β-LACTAMASE INHIBITORS**

**β-lactamase inhibitors** = molecules that irreversibly bind to and inhibit β-lactamases (Clavulanic acid, Avibactam, Sulbactam, Tazobactam)

**β-lactam/β-lactamase inhibitor** combination treatments:

- ampicillin/sulbactam (Unasyn™)
- amoxicillin/clavulanate (Augmentin™/Amoclantm)
- piperacillin/tazobactam (Zosyn™)
- ticarcillin/clavulanate (Timentin™)

Nitrofurantoin and fosfomycin are alternative options for the treatment of uncomplicated UTIs.

Aminoglycosides are sometimes effective against ESBL infections, but adverse effects include nephrotoxicity and ototoxicity.

Colistin, a last-resort drug, also has a high risk for adverse effects (nephrotoxicity and neurotoxicity).
CONCLUSION

- Worldwide dissemination of ESBLs has presented a concern for treatment of hospital and community-associated infections

- Limited treatment options
  - Need to avoid overuse of carbapenems to delay wide-spread appearance of drug resistant CRE superbugs
  - β-lactam/β-lactamase inhibitors offer a potential solution to the CRE problem, however studies on combination treatments show they are not as effective at treating ESBL infections

- Antibiotic stewardship (avoiding unnecessary antibiotic use) is crucial to preserve the activity of β-lactam antibiotics


QUESTIONS?