What are Beta-lactamases?

Proteins degrading Beta-lactam’s

Henrik Hasman – National Food Institute - DTU
The Beta-lactam antibiotics

- Isolated from *Penicillium chrysogenum*
- App. 50% of the antibiotics used worldwide
- The Beta-lactam group is constantly expanding
- Is now being produced semi-synthetically
- Kills growing cells by interfering with the cell-wall synthesis
- One of the most important human antibiotics.
Penicillins

Penicillin G

Ampicillin (AMP)

Amoxicillin
Cephalosporin’s

Cephalosporin C
(1. gen. Cephalosporin)

Cefoxitin (FOX)
(2. gen. cephamycin)

Cefotaxime (CTX)
(3. gen. Cephalosporin)

Cefepime (FEB)
(4. gen. cephalosporin)
Carbapenem’s og inhibitors

Imipenem (IMP)
Carbapenem

Clavulanic acid (CLA)
Inhibitor
Bakterial cellwall

Gram positive

Gram negative

Peptidoglycan
Narrow spectrum vs. Extended spectrum Beta-lactam’s

Narrow and moderate spectrum BL’s

- Penicillin G and V (PEN)
- Methicillin (MET)
- amoxicillin (AMOX) and ampicillin (AMP)
- Cephalotin (CEP)

Broad and Extended spectrum BL’s

- Cefoxitin (FOX)
- Cefotaxime (CTX) and Ceftazidime (CAZ)
- Cefepime (FEB)
- Imipenem (IMI)
What are ESBL’s then?

- Able to degrade Broad and extended spectrum beta-lactam’s
- Divided into: \texttt{ampC’s}, “True ESBL” and \texttt{Metallo-BL’s}.
- First identified 22 years ago (SHV-2).
- Different affinities to different beta-lactam’s.
- ESBL and plasmidic ampC’s mainly in \textit{Enterobacteriaceae}.
- Metallo-BL mainly in \textit{Pseudomonas}.
- now > 200 different genes.
- Approximately 20 different groups.
- Big difference in homology.
- Seen in all environments where Extended spectrum beta-lactam’s are used.
Beta-lactamase genes so far....

TEM  CMY  FOX  VEB  KLU
SHV  MOX  DHA  CME  FEC
OXA  PSE  ACC  GES  LAT
CTX  FOX  PER  TLA  ......

Pitfalls: ■ Reduced susceptibility can be caused by up-regulated efflux-pumps or defective influx pumps.
■ *E. coli* carries a down-regulated *ampC* beta-lactamase, which can be activated (up-regulated) by two mutations.
<table>
<thead>
<tr>
<th>Plasmidic AmpC’s</th>
<th>ESBL</th>
<th>MBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMY</td>
<td>TEM</td>
<td>IMP</td>
</tr>
<tr>
<td>ACC</td>
<td>SHV</td>
<td>VIM</td>
</tr>
<tr>
<td>DHA</td>
<td>OXA</td>
<td>SPM</td>
</tr>
<tr>
<td>FOX</td>
<td>CTX-M</td>
<td>GIM</td>
</tr>
<tr>
<td>BIL</td>
<td>VEB</td>
<td></td>
</tr>
<tr>
<td>MIR</td>
<td>PER</td>
<td></td>
</tr>
<tr>
<td>ACT</td>
<td>CME</td>
<td></td>
</tr>
<tr>
<td>KLU</td>
<td>SFO</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FEC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GES</td>
<td></td>
</tr>
</tbody>
</table>

Genes in yellow indicate most prevalent types!
The three different ESBL groups

The ‘True’ ESBL’s:

- Often located on transferable plasmids/elements
- often found in bacteria lacking a chromosomal AmpC’s
- rarely resistant to inhibitors (results in the ‘synergy effect’)
- resistant to both 3. and 4. generation ceph’s
- Inducible by beta-lactams.

Synergy!

\[
\text{CTX} \\
\downarrow > 5\text{mm} \\
\text{CTX-CLA}
\]
The three different ESBL groups

**AmpC’s**

- Often located on chromosomes (*E. coli, Citrobacter, Enterobacter*)
- ...or on plasmids but originating from chromosomal versions
- confers resistance to beta-lactam inhibitors (thus no ‘synergy’)
- confers resistance to cefoxitin (FOX); a 2. gen. cephamycin)
- sensitive to 4. gen. ceph’s (like cefepime (FEB))
- Not inducible by beta-lactams.

No synergy!
The three different ESBL groups

**Metallo beta-lactamases:**

- Can be inhibited by metal chelators (like EDTA)
- mainly found in Pseudomonas
- confers resistance to all generations of ceph’s
- confers resistance to carbapenems like Imipenem
- rarely found in Enterobacteriaceae.
## Phenotypic differences of the groups

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>ampC</th>
<th>ESBL</th>
<th>MBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibited by Clavulanic acid?</td>
<td>NO</td>
<td>YES¹</td>
<td>NO</td>
</tr>
<tr>
<td>Inhibited by EDTA?</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>Resistance to cephamycins (cefoxitin)?</td>
<td>YES</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>Resistance to 4. Gen. Ceph’s?</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Resistance to carbaphenem’s?</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
</tr>
</tbody>
</table>

¹Some (especially TEM’s) are inhibitor resistant!
How to detect ESBL I?

- Phenotypically:
  - Combination disk method
  - Double disk method
  - MIC test
  - E-test
## How to detect ESBL II?

<table>
<thead>
<tr>
<th>Method (CLSI 2007)</th>
<th>Initial Screen Test</th>
<th>Phenotypic Confirmatory Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimicrobial concentration</td>
<td>Cefpodoxime 4 µg/ml or</td>
<td>Ceftazidime 0.25-128 µg/ml</td>
</tr>
<tr>
<td></td>
<td>Ceftazidime 1 µg/ml or</td>
<td>Ceftazidime+clav. 0.25/4-128/4 µg/ml</td>
</tr>
<tr>
<td></td>
<td>Aztreonam 1 µg/ml or</td>
<td><strong>And</strong></td>
</tr>
<tr>
<td></td>
<td>Cefotaxime 1 µg/ml or</td>
<td>Cefotaxime 0.25-64 µg/ml</td>
</tr>
<tr>
<td></td>
<td>Ceftriaxone 1 µg/ml</td>
<td>Cefotaxime+clav. 0.25/4-64/4 µg/ml</td>
</tr>
<tr>
<td></td>
<td><em>(The use of more than one antimicrobial agent for screening will improve the sensitivity of detection).</em></td>
<td><em>(Confirmatory testing requires use of both cefotaxime and ceftazidime alone and in combination with clavulanic acid).</em></td>
</tr>
</tbody>
</table>

**Breakpoints (CLSI):**
- **Ceftazidime:** S: ≤ 8; I: 32; R: ≥ 32
- **Cefotaxime:** S: ≤ 8; I: 16-32; R: ≥ 64

**Breakpoints (EFSA):**
- **Cefotaxime:** R: ≥ 0.5

**Synergy (CLSI):** A ≥ 3 two-fold reduction in MIC (e.g. from 8 to 1 µg/ml)
Screening for ESBL at the CRL

**Primary screening:** Ampicillin
- Amoxicillin + clavulanic acid
- Cephalothin (1. generation cephalosporin)
- Cefpodoxime (3. generation cephalosporin)
- Ceftiofur (3. generation cephalosporin)

**Secondary screening (Disc’s):**
- Ceftazidime (CAZ - 3. gen.)
- CAZ + CLA (CAZ-CLA + inhibitor)
- Cefotaxime (CTX - 3. gen.)
- CTX + CLA (CTX-CLA + inhibitor)
- Cefoxitin (FOX – 2. gen. cephamycin)
- Cefepime (PER - 4. gen.)
ESBL-tablet assay

- FEB
- CTX
- FOX
- CAZ
- CTX-CLA
- CAZ+CLA
ESBL

Synergy!

ampC

No synergy!
BUT BUT BUT ……

Different beta-lactamases can have different affinity towards different Beta-lactams!

And one strain can easily have more than one beta-lactamase!
Table 3

<table>
<thead>
<tr>
<th>Cephalosporins / Strains</th>
<th>Strain S 1.3</th>
<th>Strain S 1.4</th>
<th>Strain S 1.6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strain #3</td>
<td>Strain #4</td>
<td>Strain #6</td>
</tr>
<tr>
<td></td>
<td>ESBL not detected</td>
<td>ESBL detected</td>
<td>ESBL not detected</td>
</tr>
<tr>
<td></td>
<td>Number, n: Percentages, %</td>
<td>Number, n: Percentages, %</td>
<td>Number, n: Percentages, %</td>
</tr>
<tr>
<td>CTX, CAZ, XNL</td>
<td>1 17%</td>
<td>5 83%</td>
<td>0 0%</td>
</tr>
<tr>
<td>CTX, CAZ</td>
<td>4 50%</td>
<td>4 50%</td>
<td>2 25%</td>
</tr>
<tr>
<td>CTX, XNL</td>
<td>2 33%</td>
<td>4 67%</td>
<td>0 0%</td>
</tr>
<tr>
<td>CTX</td>
<td>1 33%</td>
<td>2 67%</td>
<td>0 0%</td>
</tr>
<tr>
<td>XNL</td>
<td>0 0%</td>
<td>4 100%</td>
<td>0 0%</td>
</tr>
<tr>
<td>CTX/Cl:CTX</td>
<td>2 33%</td>
<td>4 67%</td>
<td>1 17%</td>
</tr>
<tr>
<td>CAZ/Cl:CAZ</td>
<td>0 0%</td>
<td>0 0%</td>
<td>2 33%</td>
</tr>
</tbody>
</table>

**CTX-M-9** | **CTX-M-14** | **CTX-M-1**
Future project on phenotypic detection and characterization of ESBL

- ≈250 well-characterized *E. coli* and *Salmonella* isolates
  - 50 AMP<sup>S</sup> *E. coli* and 50 AMP<sup>S</sup> *Salmonella* isolates
  - 25 AMP<sup>R</sup> *E. coli* and 25 AMP<sup>R</sup> *Salmonella* isolates
  - 25 *ampC* up-regulated *E. coli*
  - 75 geno-typed ESBL resistant *E. coli* and *Salmonella*

- 8 relevant veterinary and human cephalosporin’s
- Long-rang MIC testing as well as disc diffusion testing
- Two different laboratories (FOOD-DTU and CIDC-Lelystad)
Future project on phenotypic detection and characterization of ESBL

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (TREK)</th>
<th>Disc’s (BD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoperazone</td>
<td>[0.06-128]</td>
<td>75 µg</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>[0.015-32]</td>
<td>30 µg</td>
</tr>
<tr>
<td>Ceftiofur</td>
<td>[0.06-128]</td>
<td>30 µg</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>[0.015-32]</td>
<td>30 µg</td>
</tr>
<tr>
<td>Cefquinome</td>
<td>[0.015-32]</td>
<td>30 µg</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>[0.12-128]</td>
<td>30 µg</td>
</tr>
<tr>
<td>Cefpodoxime</td>
<td>[0.06-64]</td>
<td>10 µg</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>[0.03-32]</td>
<td>30 µg</td>
</tr>
</tbody>
</table>

REQUEST: Please inform us, in case you have ESBL resistant E. Coli or Salmonella isolates with KNOWN resistance genes!
Genotypic detection of the groups

- PCR
- Southern blotting (RLB)
- Microarray
- Cloning and sequencing
### E. Coli from Danish veterinary submissions in 2006

<table>
<thead>
<tr>
<th></th>
<th>Cefpodoxime (µg/ml)</th>
<th>Ceftiofur (µg/ml)</th>
<th>Amox / clav. (µg/ml)</th>
<th>Cefotaxime (Disc’s)</th>
<th>Ceftazidime (Disc’s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=11)</td>
<td>&gt;4</td>
<td>8 / ≥8</td>
<td>4/2 → 16/8</td>
<td>R</td>
<td>S / I / R</td>
</tr>
<tr>
<td><strong>Type 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=9)</td>
<td>&gt;4</td>
<td>1 / 2</td>
<td>≥ 32/16</td>
<td>S</td>
<td>S / I / R</td>
</tr>
</tbody>
</table>

**Type 1:** ”True ESBL’s” (CTX-M1, CTX-M-2 or CTX-M-9)

**Type 2:** Chromosomal *ampC* up-regulators.
Cefotaxime / Salmonella spp
Antimicrobial wild type distributions of microorganisms - reference database
EUCAST

MIC
Epidemiological cut-off: WT ≤ 0.5 mg/L

Clinical breakpoints: S ≤ - mg/L, R > - mg/L

4147 observations (3 data sources)
Ceftiofur / Salmonella spp

Antimicrobial wild type distributions of microorganisms - reference database

MIC
Epidemiological cut-off: WT $\leq 2$ mg/L

Clinical breakpoints: $S \leq -$ mg/L, $R > -$ mg/L

1992 observations (5 data sources)