Extended Spectrum Beta-Lactamases and Cephalosporinases in Enterobacteriaceae

September 2010
MODE OF ACTION - β-LACTAMS IN GRAM NEGATIVES

<table>
<thead>
<tr>
<th>SUSCEPTIBLE</th>
<th>RESISTANT</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-LACTAM ANTIBIOTIC</td>
<td>PORIN BLOCKS ENTRY</td>
</tr>
<tr>
<td>DIFFUSION THROUGH OUTER MEMBRANE</td>
<td>EFFLUX PUMP</td>
</tr>
<tr>
<td>DIFFUSION THROUGH PEPTIDOGLYCAN</td>
<td>BETA-LACTAMASE HYDROLYZES β-LACTAM</td>
</tr>
<tr>
<td>PENICILLIN BINDING PROTEINS</td>
<td>CHANGES IN PBP RESULTS IN FAILURE TO BIND TO β-LACTAM</td>
</tr>
<tr>
<td>CELL DEATH</td>
<td></td>
</tr>
</tbody>
</table>
Resistance to Beta-Lactam Antibiotics

Three major mechanisms:

1. Production of beta-lactamases

2. Altered PBP

3. Lack or diminished expression of outer membrane porins (OMPs)
Beta-lactamases

- Hydrolyze the beta-lactam bond

- Active site
  - Serine residue (Ambler Class A, B, C)
  - Metal ion (zinc) (Ambler Class D)
## FUNCTIONAL CLASSIFICATION

<table>
<thead>
<tr>
<th>GROUP</th>
<th>ATTRIBUTES OF GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CHROMOSOMAL; NOT INHIBITED CLAVULANATE</td>
</tr>
<tr>
<td>2</td>
<td>INHIBITED BY CLAVULANIC ACID</td>
</tr>
<tr>
<td>2a</td>
<td>PENICILLINASE EX. <em>STAPHYLOCOCCUS</em></td>
</tr>
<tr>
<td>2b</td>
<td>BROAD-SPECTRUM $\beta$-LACTAMASES EX. TEM-1</td>
</tr>
<tr>
<td>2be</td>
<td>EXTENDED SPECTRUM $\beta$-LACTAMASES</td>
</tr>
<tr>
<td>2br</td>
<td>INHIBITOR-RESISTANT TEM</td>
</tr>
<tr>
<td>2c</td>
<td>CARBENICILLIN HYDROLYZING</td>
</tr>
<tr>
<td>2d</td>
<td>OXACILLIN HYDROLYZING</td>
</tr>
<tr>
<td>2e</td>
<td>CEPHALOSPORINASE</td>
</tr>
<tr>
<td>2f</td>
<td>CARBAPENEM HYDROLYZING (SERINE ACTIVE SITE; INHIBITED BY CLAVULANIC ACID)</td>
</tr>
<tr>
<td>3</td>
<td>METALLO-$\beta$-LACTAMASES</td>
</tr>
<tr>
<td>4</td>
<td>MISCELLANEOUS GROUP</td>
</tr>
</tbody>
</table>
**β-Lactamases**

Different Substrate Profiles

- **Penicillinases:**
  - inactivate penicillins

- **Cephalosporinases:**
  - inactivate cephalosporins

- **Broad spectrum β-lactamases:**
  - inactivate both penicillins and narrow spectrum cephalosporins

- **Extended spectrum β-lactamases:**
  - also inactivate one or more newer cephalosporins or aztreonam, not carbapenems

- **Carbapenemases:**
  - resistance against all beta-lactams
Genetics of Beta-Lactamases

Genes encoding beta-lactamases (*bla*)
- Chromosome
- Plasmids
- Transposons

Genetic environment dictates if production:
- Constitutive
- Inducible
Genetics of Beta-Lactamases

*bla* genes:
- **Integrons:**
  - Genetic elements of variable length
    - Contain 5’ conserved integrase gene(*int*)
    - Gene cassette with other resistance genes
    - Integration site for the gene cassette *attI*

**Mobile elements that contain integrons:**
- important source of spread of *bla* genes
  - plasmids
  - transposons
Extended Spectrum Beta-Lactamases
Extended Spectrum Beta-Lactamase (ESBL)

First described in 1983 – Germany

Mid 1980’s - France
- Klebsiella spp
  - Jarlier et al. Rev Infect Dis 1988;10:867-878
Extended Spectrum Beta-Lactamase (ESBL)

- Point mutation to parent TEM/SHV enzymes
  - Now > 130 TEM ESBLS/ > 50 SHV ESBLS
- TEM (10/26) predominant in USA
  - Preferentially hydrolyze ceftazidime
- SHV (2/3)
  - Broad resistance to all cephalosporins and aztreonam
DEVELOPMENT OF ESBL

TEM-1 238 GLYCINE → SERINE = TEM-19
(high level resistance)

SHV-1 238 GLYCINE → SERINE = SHV-2
(cefotaxime resistance)

SHV-2 240 GLUTAMATE → LYSINE = SHV-5
(ceftazidime resistance)
Extended Spectrum Beta-Lactamase

Outbreaks - clonal spread

- Hospital
- Associated resistance- aminoglycosides/TMP-SMX

Risk Factors
- Broad antimicrobial use
- Immunosuppression
- Ceftazidime use
  - Rice et al. AAC.1990;34:2193-2199
  - Rice et al. CID 1996;23:118-24
Extended Spectrum Beta-Lactamase

**CTX-M enzymes - 2004**
- significant resistance to cefotaxime /ceftriaxone
- now multiple CTX-M beta-lactamases
- worldwide spread
  - very high prevalence in some geographic locations
- plasmid acquisition of chromosomal ESBL from *Kluyvera spp*
  - Polyclonal - plasmid spread
    - Community

**Risk factors:**
- FQ use
- Long term care facility
- UTI

Now: community → hospital
- human fecal carriage

Quinolones- important in maintaining CTX-M strains
<table>
<thead>
<tr>
<th>MIC</th>
<th>FAILURE</th>
<th>DEATH</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>100% (6/6)</td>
<td>33% (2/6)</td>
</tr>
<tr>
<td>4</td>
<td>67% (2/3)</td>
<td>0% (0/3)</td>
</tr>
<tr>
<td>2</td>
<td>33% (1/3)</td>
<td>0% (0/3)</td>
</tr>
<tr>
<td>≤1</td>
<td>27% (3/11)</td>
<td>18% (2/11)</td>
</tr>
</tbody>
</table>

Cephalosporin Breakpoints

• **Clinical data:**
  – better correlated with MIC than presence of ESBL??
  – MICs of $\geq 4\mu g/mL$ predict failure
  – Phenotypic tests not always reliable in presence of multiple mechanisms of resistance
  – ESBL + AmpC ~ false negative ESBL test

• **Screening methods:**
  – need to be adjusted as new beta-lactamases found in more species
Cephalosporin Breakpoints

**CLSI:**

- **Lower breakpoints**
  - Correct clinical breakpoints could obviate need for ESBL screening for predicting clinical outcome

- **Detection and characterization of ESBL / other mechanisms of resistance**
  - Important for Infection Control and Surveillance
### MIC breakpoints (µg/ml):

<table>
<thead>
<tr>
<th>Agent</th>
<th>Old (M100-S19)</th>
<th>Revised (M100-S20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>≤8</td>
<td>16</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>≤8</td>
<td>16-32</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≤8</td>
<td>16-32</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>≤8</td>
<td>16</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>≤8</td>
<td>16</td>
</tr>
</tbody>
</table>
## Disk diffusion breakpoints (mm):

<table>
<thead>
<tr>
<th>Agent</th>
<th>Old (M100-S19)</th>
<th>Revised (M100-S20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>≥18</td>
<td>15-17</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>≥23</td>
<td>15-22</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≥21</td>
<td>14-20</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>≥18</td>
<td>15-17</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>≥22</td>
<td>16-21</td>
</tr>
</tbody>
</table>
Cefotaxime / Escherichia coli

Antimicrobial wild type distributions of microorganisms - reference database

EUCAST MIC Distribution

Epidemiological cut-off: WT ≤ 0.25 mg/L
Clinical breakpoints: S ≤ 1 mg/L, R > 2 mg/L

CLSI
EUCAST

0587 observations (58 data sources)
Ceftazidime / Escherichia coli

Antimicrobial wild type distributions of microorganisms - reference database

EUCAST MIC Distribution

EUCAST

CLSI

MIC

Epidemiological cut-off: WT \leq 0.5 \text{ mg/L}

Clinical breakpoints: S \leq 1 \text{ mg/L}, R > 8 \text{ mg/L}
Cefepime / Escherichia coli

Antimicrobial wild type distributions of microorganisms - reference database
EUCAST MIC Distribution

EUCAST

CLSI

MIC
Epidemiological cut-off: WT ≤ 0.125 mg/L
Clinical breakpoints: S ≤ 1 mg/L, R > 8 mg/L

3203 observations (47 data sources)
Gram-negative bacteremia and cefepime

Cefepime 1 - 2 g x 2
28 d mortality, Cart-analysis

EUCAST  S≤1, R>8 mg/L
CLSI    S≤8, R>16 mg/L

N=204 patients

FIG. 1. Twenty-eight day mortality stratified by cefepime MIC.

Cephalosporin Breakpoints

Dilemma:

• what if only one cephalosporin is tested?
  – Can you predict others??

• what if one is S and one is R??
  – Controversy to report as tested??
ESBL Detection-Laboratory

- Chromosomally encoded inducible AmpC beta-lactamase
  - *Enterobacter, Serratia, Providencia, Citrobacter* etc.
- Acquired AmpC
  - *E. coli, K. pneumoniae spp., Proteus spp*

High level expression of AmpC:
- may prevent recognition of an ESBL
- clavulanate - inducer of high level Amp
  - increase resistance to screening drugs
    → false negative ESBL confirmatory test

Option:

Cefepime +/- clavulanate
Escherichia coli

ESBL +
(control)
Enterobacter cloacae

Cefipime / Amox-clav

ESBL +
(in the presence of Amp C)
Cephalosporinases
Cephalosporinases

Amp C / Functional Group 1

- Chromosomal
  - Inducible- regulator genes
- Resistant to penicillins and 1st generation cephalosporins
- Hydrolyze all beta-lactams except carbapenems
- Not inhibited by clavulanic acid
- Vary in how they test to 2nd generation cephalosporins
  - cefoxitin and cefuroxime
Cephalosporinases

MICs to 2nd generation cephalosporins

- *E. cloacae, E. aerogenes* – cefoxitin > cefuroxime
- *Citrobacter freundii* – cefoxitin > cefuroxime
- *Serratia spp* – cefuroxime > cefoxitin
- *Morganella morganii* – cefuroxime > cefoxitin
- *Hafnia spp* – S to cefoxitin / cefuroxime
- *Providencia spp* – S to cefoxitin / cefuroxime
- *Pantoae agglomerans* – S to cefoxitin / cefuroxime
<table>
<thead>
<tr>
<th>GOOD</th>
<th>VARIABLE</th>
<th>POOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoxitin</td>
<td>Clavulanate</td>
<td>Sulbactam</td>
</tr>
<tr>
<td>Imipenem</td>
<td>Cefotaxime</td>
<td>Tazobactam</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>Cefamandole</td>
<td>Aztreonam</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>3\textsuperscript{rd} Gen Cephs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4\textsuperscript{th} Gen Cephs</td>
<td></td>
</tr>
</tbody>
</table>
Inducible Beta Lactamases

Constitutive production

- Mutant strains arise spontaneously:
  - frequencies of about $10^{-6}$ to $10^{-9}$
- Cephalosporinase produced constitutively at high levels
  - not reversible
  - antibiotics that are poor inducers:
    - good selectors of mutants
## Cephalosporinases

<table>
<thead>
<tr>
<th>Mutant Selection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GOOD SELECTORS</strong></td>
</tr>
<tr>
<td>3(^{\text{rd}}) Gen Cephs</td>
</tr>
<tr>
<td>4(^{\text{th}}) Gen Cephs</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
Cephalosporinases

Now appearing on plasmids !!!

- plasmid mediated Amp C
- transmission to *E. coli / Klebsiella spp*
- derived from chromosomal Amp C from;
  - *Citrobacter freundii* - CMY-2A, LAT-1, BIL-1
  - *Enterobacter cloacae* - MIR-1
  - *Pseudomonas aeruginosa* - MOX-1, FOX-1
Plasmid mediated cephalosporinases

Problems:
- Major threat to ICU/hospital setting
- Some are regulated and inducible
- Combination of porin loss plus AmpC
  → carbapenem resistance
- Potential problem with *Klebsiella spp*
Cephalosporinases

Laboratories:

- not easily detected by current laboratory methods
  - agar tends to minimize effect of β-lactamases
    *Pitout et al AAC 1997;41:35-39*
  - broth microdilution tests use small inocula
    *may not be detectable by rapid methods*
Amp C detection

• Boronic acid inhibits Amp C enzyme
  – 120mg phenylboronic acid in 3ml DMSO
    • Add 3ml sterile distilled water
  – Add 20µl to 30µg cefotetan disc

→ compare cefotetan +/- boronic acid

• Cloxacillin/Cefotetan E test
Cephalosporinase

P. vulgaris, P. penneri

Cephalosporinase

- cefuroximase
- Class A – inducible
- R : penicillins, 1st generation cephalosporins
- S : cefoxitin and amox/clav
Enterobacter cloacae

Cefotetan +/- boronic acid

Amp C + (control)
Enterobacter absuriae

Cefotetan +/- boronic acid

Ertapenem R, Amp C +
**Detection of Beta Lactam Resistance Based on Carbapenem Results**

ETP ≥ 2 mg/mL and/or MEM ≥ 1 mg/mL and/or IPM ≥ 2 mg/mL

- **No**
  - **Chart 1A**

- **Yes**
  - CTX ≥ 2 µg/mL
    - **No**
      - **Probable mechanism(s):**
        - Altered PBP2* (*P. mirabilis* only)
        - Chromosomal carbapenemase**
        - OXA enzymes***
      - **Consult Microbiologist**
    - **Yes**
      - **Chart 2**

---

* *P. mirabilis* will sometimes test resistant to imipenem. This is due to an altered PBP2 which does not affect other beta-lactam antibiotics, including ertapenem and meropenem. The *in vitro* imipenem resistance is not thought to be clinically relevant *in vivo*. If 3rd generation cephalosporins, ertapenem and meropenem test susceptible, no further testing is required.

** Chromosomal class A carbapenemases (SME, NMC, or IMI) have been found in Enterobacter spp. and Serratia spp. Although third generation cephalosporins, esp. ceftazidime, often test susceptible in-vitro (as does cefoxitin), carbapenemases should be considered resistant.

*** Certain OXA enzymes hydrolyze carbapenems, but not cephalosporins. To date, most of these enzymes have been found in non-fermentative Gram negative bacteria (Pseudomonas and Acinetobacter). However, spread to Enterobacteriaceae has been documented. Suspected isolates with OXA enzymes should be referred to a reference laboratory.
ANTIMICROBIAL SUSCEPTIBILITY TESTING MANUAL
ENTEROBACTERIACEAE Beta-Lactam Resistance Detection Charts

Chart 2B

CTX or CRO or CAZ ≥ 2µg/mL

FOX

≤ 8 µg/mL

Probable mechanism(s):
ESBL
Carbapenemase (KPC)

≥ 16 µg/mL

Probable mechanism(s):
ESBL + impermeability
Amp C (plasmid)
ESBL + Amp C
Carbapenemase

ESBL discs
Cefepime ESBL discs
Amp C testing

ESBL

ETP

≥ 2 µg/mL

≤ 1 µg/mL

Comment 4
ESBL + Amp C

Comment 5
ESBL + Impermeability

Chart 6

ETP

≥ 2 µg/mL

≤ 1 µg/mL

Chart 6

Comment 7
Undetermined mechanism*

Chart 6

ETP

≥ 2 µg/mL

≤ 1 µg/mL

Chart 6

Comment 7
Undetermined mechanism*

Chart 6

Chart 6

Cefepime**

Chart 6

Chart 6

Chart 6

Chart 6

Comment 7
Undetermined mechanism*

Chart 6

Chart 6

Comment 1
ESBL

Chart 6

Chart 6

Chart 6

Chart 6

Comment 7
Undetermined mechanism*

Chart 6

Chart 6

Chart 6

Chart 6

* Undetermined mechanism – Suggest repeat testing to rule out laboratory error. If cefoxitin S - possibility of ACC-1 (rare Amp C cephalosporinase that does not affect cefoxitin)

** Cefepime should be susceptible in the presence of Amp C Cephalosporinase. If resistant, this implies an ESBL or extended spectrum Amp C cephalosporinase (ESAC).
ANTIMICROBIAL SUSCEPTIBILITY TESTING MANUAL
ENTEROBACTERIACAE BETA-LACTAM RESISTANCE DETECTION CHARTS

Chart 2F

Cedecea spp
Citrobacter freundii complex
Enterobacter aerogenes
Enterobacter asburiae
Enterobacter cloacae
Enterobacter taylorae
Hafnia spp
Morganella morganii
Providencia spp
Serratia spp

CTX or CRO or CAZ ≥ 2µg/mL

Probable mechanism(s):
Chromosomal Amp C (induced/derepressed)
ESBL + Amp C (classical/induced/derepressed)
Carbapenemase

ESBL discs
Cefepime ESBL discs

ESBL

ETP

≤ 1 µg/mL

≥ 2 µg/mL

Comment 4
ESBL + Amp C (Chromosomal)

Chart 6

Cefepime

S

I/R

ETP

≤ 1 µg/mL

≥ 2 µg/mL

Comment 3
Amp C (induced/derepressed)

Chart 6

Chart 6

ESAC*

These organisms produce an inducible chromosomal Amp C cephalosporinase that typically results in resistance to penicillins and 1<sup>st</sup>/2<sup>nd</sup>-generation cephalosporins, including cefotixin (exceptions: Serratia spp, cefuroxime may test more resistant than cefotixin; Hafnia and Providencia species, both may still test susceptible to cefotixin and cefuroxime). Induction and/or derepression of the Amp C enzyme results in broad resistance including 3<sup>rd</sup> generation cephalosporins, especially cefotaxime and ceftriaxone. Cefepime, a 4<sup>th</sup> generation cephalosporin, is poorly hydrolyzed by the Amp C enzyme (typical MICs ≤ 1µg/mL).

Resistance to cefepime implies either acquisition of an extended spectrum beta lactamase (ESBL) or an extended spectrum Amp C cephalosporinases (ESAC) resulting from further derepression of the chromosomal enzyme. Acquisition of an ESBL is common but difficult to detect by standard ESBL confirmatory tests due to interference from the chromosomal Amp C enzyme that is not inhibited by clavulanate. Cefepime ESBL discs may be useful in detecting ESBL production in the presence of an Amp C cephalosporinase.

NOTE: It is not possible to detect an ESBL in the presence of an ESAC as the higher level of Amp C cephalosporinase interferes with the cefepime ESBL test. Nosocomial outbreaks of cefepime resistant organisms warrant molecular confirmation of resistance mechanism(s) (ESBL or ESAC).
Interior Health

- Detection of both ESBL and AmpC
  - Phenotypic
  - MAST discs
    - Simplify detection
    - Avoid Delays
  - Molecular Diagnosis
- Infection Control Implications
  - *Klebsiella* spp/ *Proteus* spp
  - Isolation Precautions
  - Screening
    - Prevalence study
    - Active screening??
Molecular Detection at BCCDC

Gene targets:

• **Amp C cephalosporinase:**
  - MOX 1-2, CMY 1-11, LAT 1-4, BIL 1, DHA 1-2, ACC, MIR 1T, ACT 1, FOX 1-5b

• **ESBL:**
  - SHV, TEM, CTX-M, OXA-1 and CMY-2

• **Carbapenemase:**
  - KPC, IMP and VIM