Screening and detection of plasmid mediated AmpC betalactamases

The capability to detect AmpC is important to improve the clinical management of infections and provide sound epidemiological data.

Reduced susceptibility to cefoxitin in the Enterobacteriaceae may be an indicator of AmpC activity, but it should be confirmed by other tests.

Laboratories should be able to recognize AmpC derepressed strains and those with plasmid AmpC.

Guidelines from the CLSI are not yet available for detection of bacteria with AmpC beta-lactamases.
### TYPES OF AmpC BETA-LACTAMASES

<table>
<thead>
<tr>
<th></th>
<th>Cefepime + Clav.</th>
<th>Boronic acid</th>
<th>Cloxacillin 500µg</th>
<th>Cefoxitin or Imipenem</th>
<th>Cefepime</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosomally mediated Amp C (partially derepressed)</td>
<td>No synergism</td>
<td>Synergy with Ceftazidime+Clav. and/or Cefotaxime+Clav.</td>
<td>Synergy with Ceftazidime and/or Cefoxitin</td>
<td>Antagonism with 3rd generation cephalosporins</td>
<td>S zone &gt;26 mm</td>
</tr>
<tr>
<td>Plasmid mediated AmpC (or totally derepressed)</td>
<td>No synergism</td>
<td>Synergy As above</td>
<td>Synergy As above</td>
<td>No antagonism with 3rd generation cephalosporins</td>
<td>S zone &gt;26 mm</td>
</tr>
<tr>
<td>Inducible plasmid mediated AmpC ACT-1, DHA-1, DHA-2, CFE-1, CMY-13</td>
<td>No synergism</td>
<td>Synergy As above</td>
<td>Synergy As above</td>
<td>Antagonism with 3rd generation cephalosporins</td>
<td>S zone &gt;26 mm</td>
</tr>
<tr>
<td>Chromosomal *ESAC (E. coli) (not inducible)</td>
<td>No synergism</td>
<td>Synergy As above</td>
<td>Synergy As above</td>
<td>No antagonism with 3rd generation cephalosporins</td>
<td>S zone ≤26 mm MIC 1-8 µg/ml</td>
</tr>
</tbody>
</table>

*ESAC = Extended spectrum Amp C

### SCREENING
Derepressed/plasmid AmpC should be suspected when we see:

- Resistance to 3rd generation cephalosporins – NOT Cefepime.
- Resistance to Cefoxitin (inhibition zone < 16 mm).
- No cephalosporin / Clav. synergism.
- I / R to Amoxycillin + Clav.
- AmpC derepressed *Serratia* are S to ceftazidime.
- *Providencia, Morganella* and *Serratia* inducible & derepressed may appear S /I to cefoxitin.
- Strains producing AAC-1 beta-lactamase are susceptible to cefoxitin.

### CONFIRMATION
Apply one Cefotaxime + Clavulanate (CTAX+CL) and one Ceftazidime + Clavulanate (CAZ+CL) Neo-Sensitabs on an inoculated MH agar plate. In between apply one Boronic Acid Diatabs (BOR) at a distance of approx. 10 mm (edge to edge).

If the strain is totally resistant to the Cefalosporins+Clavulanate combination, the distance should be reduced to 5 mm.

Apply one Ceftazidime (CAZ) and one Cefoxitin (FOX) Neo-Sensitabs on an inoculated MH agar plate. In between at a distance of 5-10 mm edge to edge, apply one Cloxacillin (CLOX) 500 µg Neo-Sensitabs. Imipenem (IMP) is used to detect the inducible phenotype.
INTERPRETATION
A keyhole or ghost zone (synergism) between Boronic Acid and any of Cefotaxime + Clav. or Ceftazidime + Clav. indicates the presence of an AmpC beta-lactamase.

A keyhole or ghost zone between Cloxacillin 500 µg and Ceftazidime and/or Cefoxitin indicates the presence of an AmpC beta-lactamase.

Plasmid mediated AmpC differ from chromosomal AmpC in being uninducible (few exceptions). Strains producing inducible plasmid AmpC beta-lactamases (ACT-1, DHA-1, DHA-2, CFE-1, CMY-13) will show antagonism (distorted zone) between Cefoxitin or Imipenem and 3rd generation cephalosporins.

Strains of Klebsiella spp, Salmonella spp and P. mirabilis showing synergism with Boronic Acid and/or Cloxacillin 500 µg possess presumptively plasmid mediated AmpC beta-lactamases.

The method cannot distinguish between chromosomal and plasmid mediated AmpC beta-lactamases in E. coli, but the test is useful to select strains for further analysis. Plasmid mediated are often multiresistant and may show scattered colonies near the edge of the zone of third gen. cephalosporins and aztreonam disks.

INDUCIBLE PHENOTYPE
The inducible phenotype is identified by a tablet approximation test, using Imipenem or Cefoxitin against 3rd generation cephalosporins (distance 15 mm from edge to edge).

Distorted zones indicate the presence of an inducible AmpC beta-lactamase.

Treatment with 3rd generation cephalosporins should be avoided in severe Enterobacter, C. freundii, Serratia and Morganella infections except in UTI, because of risk for selection of cephalosporin-resistance during therapy.

AmpC + ESBL
Screening criterion for ESBL presence among AmpC-producing Enterobacter, C. freundii and Serratia marcescens is Cefepime MIC > 1 ug/ml (inhibition zone< 26 mm).

High level expression of AmpC may prevent recognition of an ESBL. Use of Cefepime is more reliable to detect these strains because high AmpC production has little effect on cefepime activity.
PRODUCTS MENTIONED IN THE BROCHURE INCLUDING REF NO:

DIATABS

<table>
<thead>
<tr>
<th>Product code</th>
<th>Diatabs</th>
</tr>
</thead>
<tbody>
<tr>
<td>10041</td>
<td>Boronic Acid 250 µg</td>
</tr>
<tr>
<td>10031</td>
<td>Cloxacillin 500 µg</td>
</tr>
</tbody>
</table>

NEO-SENSITABS

<table>
<thead>
<tr>
<th>Product code</th>
<th>Original Neo-Sensitabs</th>
<th>Product code</th>
<th>CLSI potency Neo-Sensitabs</th>
</tr>
</thead>
<tbody>
<tr>
<td>72312</td>
<td>Ceftazidime+Clav. 30+10 µg</td>
<td>64712</td>
<td>Cefotaxime+Clav. 30 + 10 µg</td>
</tr>
<tr>
<td>72212</td>
<td>Ceftazidime 30 µg</td>
<td>64612</td>
<td>Ceftazidime+Clav. 30 + 10 µg</td>
</tr>
<tr>
<td>71712</td>
<td>Cefoxitin 60 µg</td>
<td>64012</td>
<td>Cefotaxime 30 µg</td>
</tr>
<tr>
<td>74612</td>
<td>Imipenem 15 µg</td>
<td>62912</td>
<td>Cefoxitin 30 µg</td>
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<tr>
<td>71212</td>
<td>Ceferpine 30 µg</td>
<td>61212</td>
<td>Imipenem 10 µg</td>
</tr>
<tr>
<td>79512</td>
<td>Ceferpine+Clav. 30 + 10 µg</td>
<td>63712</td>
<td>Ceferpine 30 µg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>64812</td>
<td>Ceferpine+Clav. 30 + 10 µg</td>
</tr>
</tbody>
</table>

REFERENCES

5) Merlino J: Boronic acid tests for AmpC detection in E. coli and K. pneumoniae isolates. Study at Concord Hospital,Sidney (Australia),nov. 2007.