APPENDIX III - DOUBLE DISK TEST FOR ESBL

I. Introduction
Class A or Bush Group 1 extended spectrum beta-lactamases (ESBLs) are inhibited by clavulanic acid. This may be detected by testing the suspected organism to a 3rd generation cephalosporin alone and in combination with clavulanic acid. If the combination results in an expanded zone of inhibition compared to that of the 3rd generation cephalosporin alone, it is indicative of the presence of an ESBL.

II. Materials
Mueller-Hinton (MH) agar (150 mm)
20/10 mg amoxicillin-clavulanate disc
30 mg ceftazidime disc
30 mg ceftriaxone or cefotaxime disc
30 mg aztreonam disc
10 mg cefpodoxime disc (optional)
30 mg cefoxitin disc
Quality control strain: *E. coli* ATCC 51446

III. Procedure
1. Prepare a bacterial suspension of the organism to be tested that has a turbidity equivalent to that of a 0.5 McFarland standard.

2. Inoculate a Mueller-Hinton agar plate with this suspension in accordance with NCCLS M100-S10 (M2) guidelines for disc diffusion testing.

3. Place the amoxicillin-clavulanic acid disc towards the centre of the plate.

4. Carefully measure 15 mm out from the edge of that disc at 90° angles marking the plate.

5. Place a ceftazidime disk on the plate so that its inner edge is 15 mm (the mark) from the amoxicillin-clavulanic acid disc (See Figure 1 KB-ESBL Template).

6. Do the same with cefotaxime (or ceftriaxone), aztreonam and cefpodoxime discs so that they are spaced 90° apart and 15mm from the centre disc.
7. Place a cefoxitin disc in any available space remaining on the plate.

8. Incubate 35°C, in O₂ x 18-24 hours and record the zone diameters for the all cephalosporins as per NCCLS guidelines.

**Figure 1.** KB-ESBL Template

[Diagram of KB-ESBL Template with labels for CPD, CRO, CAZ, AMC, ATM, and FOX]
IV. Interpretation

Note: The following applies to cefpodoxime-nonsusceptible *E. coli*, *Klebsiella* species and *Proteus* species only.

1. Document zone size for all antibiotics.
2. Observe for **potentiation** of the inhibition zone (i.e. increase in the inhibition zone) of any one of cefpodoxime, ceftazidime, ceftriaxone or aztreonam when combined with clavulanic acid (enter Yes or No to the “drug” named ESBL Inhibitor in the LIS).
3. If a reduction of zone of inhibition of any one of cefpodoxime, ceftazidime, ceftriaxone or aztreonam when combined with clavulanic acid is observed, recheck the identification of the isolate and repeat testing. Notify the charge technologist if result remains unchanged.

**Class A ESBL present:**

i) Potentiation of the inhibition zone of any one of cefpodoxime, ceftazidime, ceftriaxone or aztreonam when combined with clavulanic acid (see below for examples of different patterns of potentiation that can be seen with organisms that contain Class A ESBLs)

ii) Susceptibility to cefoxitin

iii) Susceptibility or resistance to any one of ceftazidime, ceftriaxone or aztreonam

**Class A and Class C ESBL present:**

i) Potentiation of the inhibition zone of any one of cefpodoxime, ceftazidime, ceftriaxone or aztreonam when combined with clavulanic acid

ii) Resistant or Intermediate to cefoxitin.

iii) Susceptibility or resistance to any one of ceftazidime, ceftriaxone or aztreonam

**Class C-ESBL present:**

i) No potentiation with clavulanic acid

ii) Resistance or Intermediate to cefoxitin

iii) Resistance to any one of ceftazidime, ceftriaxone or aztreonam.
ESBL not Class A or Class C present:
   i) No potentiation with clavulanic acid
   ii) Susceptibility to cefoxitin
   iii) Resistance to any one of ceftazidime, ceftriaxone or aztreonam

ESBL absent:
   i) No potentiation with clavulanic acid
   ii) Susceptibility or resistance to cefoxitin
   iii) Susceptibility to all of ceftazidime, ceftriaxone or aztreonam

V. Reporting

<table>
<thead>
<tr>
<th>Reporting Comment</th>
<th>Potentiation of the inhibition zone of any one of cefpodoxime, ceftazidime, ceftriaxone or aztreonam when combined with clavulanic acid (enter Y or N to the “drug” named ESBL Inhibitor in the LIS)</th>
<th>Cefoxitin</th>
<th>Ceftazidime, ceftriaxone or aztreonam</th>
</tr>
</thead>
<tbody>
<tr>
<td>The susceptibility pattern suggests that this organism contains a class A extended spectrum beta-lactamase (ESBL).</td>
<td>Yes</td>
<td>S</td>
<td>S/R</td>
</tr>
<tr>
<td>The susceptibility pattern suggests that this organism contains class A and C extended spectrum beta-lactamases (ESBL).</td>
<td>Yes</td>
<td>I/R</td>
<td>S/R</td>
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<tr>
<td>The susceptibility pattern suggests that this organism contains a class C extended spectrum beta-lactamase (ESBL).</td>
<td>No</td>
<td>I/R</td>
<td>R</td>
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<td>The susceptibility pattern suggests that this organism contains an extended spectrum beta-lactamase (ESBL) other than class A or C.</td>
<td>No</td>
<td>S</td>
<td>R</td>
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<tr>
<td>Not ESBL – no reporting comment</td>
<td>No</td>
<td>S/R</td>
<td>S</td>
</tr>
</tbody>
</table>

VII. References


6. NCCLS. Performance standards for antimicrobial susceptibility testing; Eighth informational supplement. NCCLS document M100-SB [ISBN 1-26238-337-X]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 1998

