**Extended Spectrum β-lactamase (ESBL) detection: Phenotypic Confirmatory Method**

**Purpose:**
ESBLs are enzymes that mediate resistance to extended-spectrum (third generation) cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone), penicillins and monobactams (e.g., aztreonam). The presence of an ESBL-producing organism in a clinical infection can result in treatment failure if one of the above classes of drugs is used. ESBL-producing organisms are not affected by cephemycins (e.g., cefoxitin and cefotetan) or carbapenems (e.g., meropenem or imipenem); and show variable susceptibility to aminoglycosides, fluoroquinolones, and trimethoprim-sulfamethoxazole. The phenotypic confirmatory test for ESBL detection as recommended by CLSI is described here.

**Principle:** ESBLs are inhibited by β-lactamase inhibitors like clavulanic acid. Hence when tested by disk diffusion method using cefotaxime(30µg), cefotaxime-clavulanic acid(30/10 µg) and ceftazidime(30 µg) ceftazidime-clavulanic acid(30/10 µg) there is an increase of ≥ 5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone.

**Procedure:**

*Preparation of inoculum*
- Using a sterile straight wire, 2-3 colonies of overnight growth of the test organism are inoculated into 3.0ml Mueller-Hinton broth and incubated at 35±2°C for 4-6h. The turbidity of the growth is adjusted to 0.5 McFarland standards using fresh broth.

*Inoculation and incubation:*
- A sterile cotton swab is dipped into the organism suspension. Any excess fluid is expressed by pressing the swab against the side of the tube. The swab is then swabbed over the dry surface of a Mueller-Hinton agar plate so as to obtain a lawn culture.
- The plates are allowed to dry for 3-10 minutes.
- Discs containing cefotaxime(30 µg), cefotaxime-clavulanic acid(30/10 µg) and ceftazidime(30 µg) ceftazidime-clavulanic acid(30/10 µg) are arranged so that the distance between them is approximately twice the radius of the inhibition zone produced by the cephalosporin.
- The same is repeated with the QC strain.
- The plates are incubated overnight at 35°C.

**Observation:**
Examine bacterial lawn for acceptable growth (confluent or almost confluent). Measure zones of inhibition as for the standard disc diffusion test (in mm).

**Interpretation:**
A ≥ 5 mm increase in the zone diameter around either cephalosporin disc in combination with clavulanic acid as compared to the zone diameter for the cephalosporin when tested alone indicates ESBL activity.
Report:
Routine ESBL testing of clinical isolates is no longer necessary if the interpretive criteria mentioned in CLSI 2012 guidelines are being used to report susceptibility results by disc diffusion; i.e., it is not necessary to edit results for cephalosporins, aztreonam and penicillins to resistant. But if 2010 guidelines are being used, then ESBL testing should be performed as described above; and if the result is positive, then the isolate should be reported as being resistant to all penicillins, cephalosporins and aztreonam. At present, CLSI 2012 guidelines, recommend ESBL testing for epidemiological and infection control purposes only.

Quality control: Positive and negative QC organisms should be tested on each day of testing.

<table>
<thead>
<tr>
<th>Control</th>
<th>Bacterial strain</th>
<th>ATCC number</th>
<th>Criterion</th>
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<tbody>
<tr>
<td>ESBL Positive</td>
<td><em>K. pneumoniae</em></td>
<td>ATCC 700603</td>
<td>≥ 5 mm increase in zone diameter of ceftazidime-clavulanic acid vs ceftazidime alone; AND ≥ 3 mm increase in zone diameter of cefotaxime-clavulanic acid vs cefotaxime alone</td>
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<tr>
<td>ESBL Negative</td>
<td><em>E. coli</em></td>
<td>ATCC 25922</td>
<td>≤ 2 mm increase in zone diameter for antimicrobial agent tested in combination with clavulanic acid vs the zone diameter when tested alone.</td>
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References :-

Figure: 1- Positive ESBL test