Detection of Metallo-β-lactamase production in Gram negative bacilli by Combined Disc Test

Purpose:
Metallo β-lactamases (MBLs) are Ambler class B enzymes that are capable of hydrolysing all β-lactam antibiotics including carbapenems, with the exception of monobactams (aztreonam). In addition, there are no clinically useful inhibitors to MBLs and they have a potential for rapid and generalised dissemination since their genes are carried on mobile genetic elements. The combined disc test (CDT) to detect production of MBL enzymes in Gram negative bacilli is described here.

Principle:
The CDT is an inhibitor-based test for the specific detection of MBL producers. Metallo β-lactamases possess metal biding sites and require divalent zinc cations as cofactors for enzymic activity. These enzymes are inactivated by the action of metal ion chelators like EDTA. The test takes advantage of the metalloenzyme dependence on zinc ions, and uses the chelating agents to inhibit β-lactam hydrolysis.

Procedure:
Preparation of inoculum
Using a sterile straight wire, 2-3 colonies of test isolate is 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. 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 broth culture is then swabbed over the surface of a Mueller Hinton agar plate so as to obtain a lawn culture of the test organism.
  b. Two imipenem and two meropenem disks are placed on the plate.
  c. 10μL of 0.1M solution of EDTA is added to one of each disk of imipenem and meropenem (amounting to 292μg of anhydrous EDTA/disc).
  d. A blank disc containing 10μL of 0.1M solution of EDTA (292 μg) is also placed on the plate, to rule out inhibition of test strain by EDTA.
  e. The plate is incubated at 35° C for 18-24 hours.

Observation:
Examine bacterial lawn for adequate growth and measure the zones of inhibition around each disc as for standard disc diffusion tests (in mm). There should be no zone of inhibition around the plain disc with EDTA.

Interpretation:
A zone diameter increase of >4 mm around the carbapenem-EDTA disk compared to that of the carbapenem disk alone is considered positive for production of metallo β-lactamase enzyme. (Figure-1)
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>Criterion</th>
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<tbody>
<tr>
<td>MBL Positive control</td>
<td>known in-house MBL positive isolate of <em>Pseudomonas aeruginosa</em> or <em>Klebsiella pneumoniae</em></td>
<td>diameter increase of &gt;4 mm around the carbapenem-EDTA disk compared to that of the carbapenem disk alone</td>
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<tr>
<td>MBL Negative control</td>
<td>A known in-house MBL negative strain</td>
<td>≤ 4mm increase in zone diameter around the carbapenem-EDTA disk compared to that of the carbapenem disk alone</td>
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References:


Figure-1: Positive CDT for MBL production