Screening and detection of carbapenemases

For many isolates with carbapenemases the MICs of carbapenems are around the susceptible breakpoint making resistance difficult to detect - particularly with automated systems.

Therefore special zone breakpoints are needed in first line screening

Strains with reduced susceptibility to Imipenem, Meropenem (both inhibition zone < 22 mm) or Ertapenem (zone < 21mm) on Mueller-Hinton Agar with McFarland 0.5 inoculum, should be suspected of possessing carbapenemases.

Most isolates with KPC and GES enzymes are highly resistant to Ceftazidime.

Ertapenem Neo-Sensitabs is the most sensitive indicator for possible carbapenemase, but in approximately 20% of cases other resistance mechanisms are involved (confirmation of carbapenemase with Modified Hodge Test is necessary).

It is important to recognize small resistant colonies growing inside the Ertapenem disk zone.
**A) PROCEDURE FOR METALLO-BETA-LACTAMASE (MBL) DETECTION.**

**ENTEROBACTERIACEAE**
Apply one Dipicolinic Acid Diatabs (DPA) on an inoculated Mueller Hinton (MH) plate. Apply one Meropenem Neo-Sensitabs and one Ertapenem Neo-Sensitabs onto the plate on either side of the DPA, 5mm from the DPA (edge to edge).

Apply Imipenem + EDTA (IM+ED) on an inoculated MH plate. Apply one Meropenem Neo-Sensitabs and one Ertapenem Neo-Sensitabs at either side of the Imipenem + EDTA, 10 mm from the Imipenem + EDTA (edge to edge).

**NON-FERMENTERS**
Apply one DPA Diatabs on the MH plate. Apply one Imipenem Neo-Sensitabs and one Meropenem Neo-Sensitabs at either side of the DPA, 5 mm from the DPA (edge to edge).

Apply Imipenem + EDTA (IM+ED) on the inoculated MH plate. Apply one Meropenem Neo-Sensitabs and one Ertapenem Neo-Sensitabs at either side of the Imipenem + EDTA, 10 mm from the Imipenem + EDTA (edge to edge).

**INTERPRETATION**
The use of two chelating agents EDTA and DPA will enhance the detection of metallo-β-lactamases (MBL) in the clinical laboratory. A key hole or ghost zone between carbapenems (one or more) and Dipicolinic Acid indicates the presence of an MBL. A key hole or ghost zone between IM+ED and the carbapenems (one or more) indicates the presence of an MBL.

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*A key hole or ghost zone between carbapenems (one or more) and Dipicolinic Acid indicates the presence of a MBL.*

**Please note:**
Most MH agar brands contain physiological levels of Zn²⁺ ions and should be used for carbapenem testing. Iso-Sensitest agar has low levels of zinc ions and may give false susceptibility results for carbapenems in the presence of MBL. Some MH agar brands should also be supplemented with zinc ions (70 mg zinc sulphate.6 H₂O per liter medium). This applies also to the Modified Hodge Test (MHT). Strains of *Acinetobacter baumannii* producing certain oxacillinases may give a false positive metallo-β-lactamase test result.
Isolates giving negative metallo-beta-lactamase tests, may produce other carbapenemases.

The most current are KPC enzymes isolated from Enterobacteriaceae (K. pneumoniae, E. coli, Enterobacter spp., P. mirabilis) particularly K. pneumoniae.

To detect these strains in rectal swab screening samples, direct plating on McConkey agar in the presence of Ertapenem Neo-Sensitabs and Imipenem Neo-Sensitabs may be used.

Place one Amoxycillin + Clavulanate Neo-Sensitabs between one Ertapenem and one Imipenem Neo-Sensitabs (distance 10 mm from edge to edge).

Place one Boronic Acid Diatabs between one Ertapenem and one Imipenem Neo-Sensitabs (distance 6 mm from edge to edge).

Perform Modified Hodge Test (MHT) with Ertapenem and Meropenem Neo-Sensitabs.

**INTERPRETATION**

The following results will presumably indicate the presence of a KPC beta-lactamase:

a) Negative metallo-beta-lactamas tests.

b) Positive synergy test between Boronic Acid and the carbapenems (one or both).

c) Negative synergy test between Cloxacillin 500 μg and the carbapenems (11).

d) Positive synergy test between clavulanate (AMC) and carbapenems (one or both). Not always easy to see. Although isolates with ESBL + impermeability will give false positive results.

e) Positive Modified Hodge Test.

**Please notice:**

Test only ertapenem-resistant strains. Ertapenem susceptible strains may provide a false positive result with Boronic Acid.

**Conclusion:**

Reduced susceptibility to ertapenem, synergy between Boronic Acid and the carbapenems, and no synergy between Cloxacillin 500 g and the carbapenems is clearly indicative of KPC enzyme being present.

Isolates producing high level AmpC + impermeability can be detected by synergy between Cloxacillin 500 μg and the carbapenems (11).
C) PROCEDURE FOR OXACILLINASE DETECTION  
(CLASS D ENZYMES)

Strains producing oxacillinases will currently show zones of inhibition < 21 mm with Ertapenem and/or <23 mm with Meropenem Neo-Sensitabs. Most are resistant to Aztreonam.

These enzymes are mainly found in *Acinetobacter baumannii* but also in *Enterobacteriaceae* (*K. pneumoniae, Enterobacter*) and *P. aeruginosa* although these are rare.

**INTERPRETATION**

The following results will presumably indicate the presence of oxacillinases:

a) Negative metallo-beta-lactamase tests.

b) Negative (or weak positive) synergy test between clavulanate (AMC) and carbapenems (one or both).

c) Positive Modified Hodge Test. (Addition of zinc ions: 70 mg zinc sulphate.6H2O /liter medium may be necessary to detect oxacillinases).

An isolate is counted positive when it fulfils the 3 criteria.

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**Screening of carbapenemases**

IM+ED = Imipenem + EDTA Neo-S, MER = Meropenem Neo-S, ERT = Ertapenem Neo-S, DPA = Dipicolinic Acid Ditabs, AMC = Amoxycillin+Clavulanate Neo-S.

CLOX = Cloxacin 500 μg Ditabs

With non-fermenters use Imipenem Neo-S instead of Ertapenem Neo-S.
MODIFIED HODGE TEST (MHT)

Is used to determine if resistance to carbapenems is caused by a carbapenemase.

A MH agar plate (or a McConkey plate) is inoculated with the susceptible strain *E. coli* ATCC 25922 (Mc Farland 0.5, diluted 1/10) as for disk diffusion.

When testing *Enterobacteriaceae*, one Ertapenem Neo-Sensitabs and one Meropenem Neo-Sensitabs are applied onto the plate approx. 30 mm apart from each other.

For non-fermenters one Imipenem Neo-Sensitabs and one Meropenem Neo-Sensitabs are applied.

A suspension of the microorganism to be tested for carbapenemase is adjusted to Mc Farland 0.5 standard and a loop is used to make a streak passing through the two carbapenem disks.

Two more streaks are placed perpendicularly making a cross.

Thereafter incubation for 18-24 hours at 35-37 C.

Alteration in the shape (indentation) of the zones of inhibition around the test organism is considered indicative of the presence of a carbapenemase (figure).

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*K. pneumoniae* KPC positive

Modified Hodge Test (MHT)

With non-fermenters use Imipenem Neo-S instead of Ertapenem.
PRODUCTS MENTIONED IN THE BROCHURE INCLUDING REF NO:

**DIATABS**

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<td>10051</td>
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<td>Cloxacillin 500 μg</td>
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**NEO-SENSITABS**

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<td>60112</td>
<td>Amoxycillin + Clavulanate (20 + 10 µg)</td>
</tr>
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**REFERENCES**

11) Giske Chr.: Personal communication, 2009