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Modified Hodge Test For Detection Of Carbapenemase In Clinical Isolates Of *Pseudomonas Aeruginosa*.

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Purpose:

The clover leaf test or modified Hodge test (MHT) has been extensively used as a general phenotypic method for the detection of carbapenemase activity, and it is the only method of carbapenemase detection so far recommended by the CLSI. The test is sensitive for the detection of a carbapenemase mediated mechanism of resistance to carbapenems, but does not provide information on the type of carbapenemase involved.

Principle: The cloverleaf technique, or modified Hodge test (MHT), is a phenotypic technique for detecting carbapenemase activity. It is based on the inactivation of a carbapenem by carbapenemase-producing strains that enable a carbapenem-susceptible indicator strain to extend growth towards a carbapenem-containing disk, along the streak of inoculum of the tested strain.

Reagents

1. 5 ml Mueller Hinton broth (MHB) or 0.85% physiological saline
2. Mueller Hinton agar (MHA)
3. 10 µg meropenem or ertapenem susceptibility disk
4. *E. coli* ATCC 25922: 18–24hr subculture

Equipment

1. Turbidity meter
2. $35^0C \pm 2^0C$ ambient air incubator

Supplies

1. Sterile cotton-tipped swabs
2. 1 ml sterile pipette
3. Sterile loop

Specimen

Test organisms: 18–24 hr subculture

Special safety precautions

Biosaftey Level 2

Quality control

Perform quality control of the carbapenem disks according to CLSI guidelines.

Perform quality control with each run.

. MHT Positive *Klebsiella pneumoniae* ATCC BAA-1705

. MHT Negative *Klebsiella pneumoniae* ATCC BAA-1706

Procedure

Step 1	Prepare a 0.5 McFarland dilution of the *E.coli* ATCC 25922 in 5 ml of broth or saline.
Step 2	Dilute 1:10 by adding 0.5 ml of the 0.5 McFarland to 4.5 ml of MHB or saline.
Step 3	Streak a lawn of the 1:10 dilution of *E.coli* ATCC 25922 to a Mueller Hinton agar plate and allow to dry 3–5 minutes.
Step 4	Place a 10 µg meropenem or susceptibility disk in the center of the test area.
Step 5	In a straight line, streak test organism from the edge of the disk to the edge of the plate. Up to four organisms can be tested on the same plate with one drug.
Step 6	Incubate overnight at $35^0C \pm 2^0C$ in ambient air for 16–24 hours

Interpretation/Result

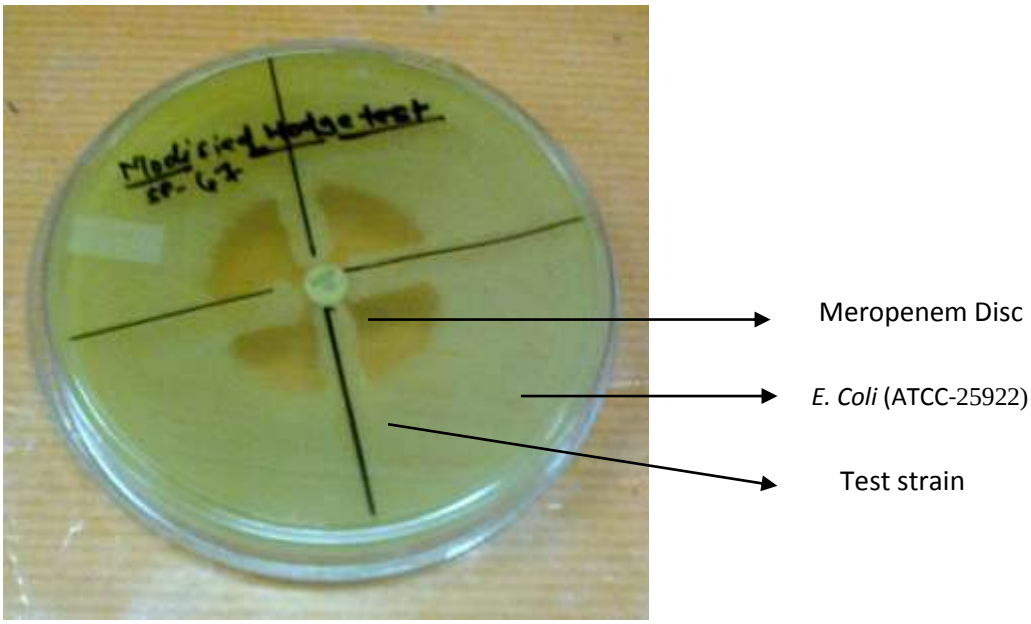

Fig - The clover leaf test or modified Hodge test (MHT) Positive .

- After 16-24 hours of incubation, examine the plate for a clover leaf-type indentation at the intersection of the test organism and the *E. coli* 25922, within the zone of inhibition of the carbapenem susceptibility disk.
- MHT Positive test has a clover leaf-like indentation of the *E.coli* 25922 growing along the test organism growth streak within the disk diffusion zone.
- MHT Negative test has no growth of the *E.coli* 25922 along the test organism growth streak within the disc diffusion.

See the CLSI guidelines (Ml00) for recommendations on detection of carbapenemase production in *P.aeruginosa* that test susceptible to carbapenem.

Expected values

A positive MHT indicates that this isolate is producing a carbapenemase. A negative MHT indicates that this isolate is not producing a carbapenemase.

Method limitations

The class of carbapenemase are not be determined by the results of the MHT. Some isolates show a slight indentation but do not produce carbapenmase.

Procedure notes

Up to four organisms can be tested on the same MHA plate with one drug. Two drugs with up to 4 organisms can be tested on a 150 mm Mueller Hinton agar plate.

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