COMPARISON OF MODIFIED HODGE TEST (MHT) AND MODIFIED CARBAPENEM INACTIVATION METHOD (mCIM) FOR DETECTION OF CARBAPENEMASES

By: Suganthing R, Gopalakrishnan V, Vignesh R and Chandran A
INTRODUCTION

- Carbepenems - antimicrobial drugs of last resort - crucial for preventing and treating life-threatening nosocomial infections
- Carbapenem resistant Enterobacteriaceae (CRE) has become a major threat for patient management in all health care facilities
- Carbapenem susceptibility breakpoints were modified over the past decade by CLSI Committee due to emergence of Carbapenem Resistant Enterobacteriaceae (CRE)
- Health care practitioners and Infection Control Unit expect more rapid and reliable result for detection of CRE
- Currently 2 methods, Modified Hodge test (MHT) and modified Carbapenem Inactivation Method (mCIM) are used regularly and will be compared for their reliability and effectiveness in this study
- Phenotypic methods (MHT & mCIM) still play a major role in Microbiology Units in assessing CRE due lack of budget to run molecular methods
A total of 258 clinical isolates of *Klebsiella pneumoniae* were isolated from a tertiary hospital. They were recovered from December 2018 to February 2019. Of these 258 clinical isolates of *Klebsiella pneumoniae*, 25 isolates were identified as resistant to carbapenem group of antibiotics by Kirby Bauer method and Etest.
DISTRIBUTION OF SAMPLES COLLECTED n=25

No of samples

- Blood
- Rectal Swab
- Tracheal Asp
- CSF

20
2
2
1
METHODS

CRE *Klebsiella pneumoniae* isolates were collected from Microbiology Unit

Isolates were sub-cultured, screened for carbapenem resistance by Kirby-Bauer disc diffusion

Resistant strains were assessed for production of carbapenemases by MHT and mCIM method
Modified Hodge Test

- Prepare 0.5 McFarland dilution of the *E. coli* 25922 in 3 ml TSB
- Dilute 1:10 by adding 0.3 ml of *E. coli* in 2.7 ml of TSB
- Make a lawn culture of this dilution on Mueller gar
- Place 10 μg meropenem disk in the centre
- Streak test organism in a straight line, from edge of the disk to the edge of the plate
- Incubate overnight at 35°C ± 2°C in ambient air for 16-24 hours

A Amjad et al., Iran J Microbiol.2011, Clinical and Laboratory Standards Institute,2010
INTERPRETATION OF MHT

After 16-24 hours of incubation, examine plate for a clover leaf-type indentation at the intersection of the test organism and the \textit{E.coli 25922}, within the zone of inhibition of the meropenem disk.

1. Positive
2. Negative
3. Positive
MODIFIED CARBAPENEM INACTIVATION METHOD

Emulsify 1µL loopful of bacteria from an overnight nutrient agar plate in 2 ml TSB

Vortex and place a 10µg meropenem disk to the above tubes. Ensure the entire disk is immersed in the suspension and Incubate at 35± 2°C in ambient air for 4 hours

Just before completion of incubation time, prepare a 0.5 McFarland suspension of *E.coli* ATCC 25922 in saline

Inoculate MHA plate with *E.coli* ATCC 25922 and place the meropenem disk from tube on the MHA plate previously inoculated with *E.coli* ATCC 25922

Incubate the MHA plates at 35± 2°C for 18-24 hours, following incubation and measure the zone of inhibition

Kim van der Zwaluw et al.,2014, Clinical and Laboratory Standards Institute, 2018
mCIM TEST INTERPRETATION

- A Zone diameter of 6-15mm is Carbapenemase positive
- A Zone diameter of ≥ 19mm Carbapenemase negative
## RESULTS

<table>
<thead>
<tr>
<th>No. of isolates</th>
<th>MHT</th>
<th>mCIM</th>
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<tbody>
<tr>
<td></td>
<td>MHT Pos</td>
<td>MHT Neg</td>
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<tr>
<td>25</td>
<td>20</td>
<td>5</td>
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DISCUSSIONS

- MHT was considered as the gold standard technique to detect carbapenemase producing bacteria in the past years.
- Presently cCIM - recommended technique for carbapenemase detection
- Our results showed that MHT failed to detect carbapenemase production in 5 isolates of CRE Klebsiella pneumoniae collected
- mCIM was able to detect carbapenemase production in all 25 isolates
- MHT is less reliable to detect carbapenemase when compared to cCIM
- On comparison of MHT and mCIM, it is recommended to use mCIM as a more reliable method for carbapenemase detection
- mCIM provides a faster and reliable result and is more sensitive and specific results
- PCR needs to be carried out to detect the presence of carbapenemase producing gene sequence to confirm if all these strains are producing
THANK YOU!