



## COMPARITIVE STUDY ON PHENOTYPIC CARBAPENEMASE ENZYME DETECTION METHODS AMONG ENTEROBACTERIACEAE CLINICAL ISOLATES IN A TERTIARY CARE HOSPITAL

### Microbiology

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### ABSTRACT

The emergence of carbapenem resistance in Enterobacteriaceae is increasingly reported all over the world. Early detection of these carbapenem resistant Enterobacteriaceae (CRE) is mandatory to contain the spread of these drug resistant organisms. This study is aimed at identifying the efficacy of phenotypic methods in detecting carbapenemase production and to compare it with genotypic method. 50 Gram Negative Enterobacteriaceae isolates showing resistance to meropenem, were tested for carbapenemase production by Modified Hodge test (MHT), Combined Imipenem-EDTA disk test and Modified carbapenemase inactivation method (mCIM) and also tested for KPC, NDM, OXA-48 genes by PCR method. Among the 50 isolates, 41 isolates demonstrated drug resistance genes by genotypic method. Remaining 9 isolates were negative for carbapenemase production by both phenotypic and genotypic method. Among the 41 isolates, 20(48.78%) were MHT positive, 21 (51.21%) were Combined Imipenem-EDTA disk test positive, 3 isolates were positive for both MHT and Combined Imipenem-EDTA disk test and 40isolates (97.56%) were positive for mCIM. This study shows that the mCIM is specific, reliable and inexpensive phenotypic method to detect carbapenemase producing Enterobacteriaceae

### KEYWORDS

Enterobacteriaceae, Carbapenemase, Modified carbapenemase inactivation method.

### INTRODUCTION

The emergence of carbapenem resistance in Enterobacteriaceae is an important threat to global health. In the Family Enterobacteriaceae, the carbapenemase producers are increasingly reported all over the world. Most commonly three types of carbapenemases are identified namely Ambler class A of the KPC type, class B of the NDM-1, IMP, and VIM types and class D of the OXA-48 type<sup>1,2</sup>. Carbapenems are frequently used in the treatment of multidrug resistant Enterobacteriaceae infections. Over the last few decades there was alarming increase in the rate of carbapenemase producing Enterobacteriaceae clinical isolates which lead to pan drug resistant bacteria with limited therapeutic options<sup>3</sup>. Reported outcomes of infections with carbapenem resistant Enterobacteriaceae (CRE) are poor. Antibiotics which are currently in use to treat CRE infections include polymyxins, tigecycline and fosfomycin which have significant adverse effects. Very few options are available for the treatment of these virulent organisms. The early and efficient detection of these CRE is a mandatory step in infection control and contain the spread of these drug resistant organisms. So this study is aimed at identifying the effective diagnostic method for detecting carbapenemase producing Enterobacteriaceae which will influence and aid in the treatment of various infections caused by carbapenemase resistant Enterobacteriaceae (CRE) organisms.

### OBJECTIVES

- To evaluate the efficacy of Combined Imipenem-EDTA disk test, Modified Hodge test (MHT) and Modified Carbapenemase Inactivation Method (mCIM) in detecting carbapenemase producing Enterobacteriaceae.
- To compare the phenotypic methods with confirmatory genotypic method.

### MATERIALS AND METHODS

This prospective cross sectional study was done for a period of 6 months during which 50 Gram negative Enterobacteriaceae isolates form various clinical samples, showing resistance to meropenem by disc diffusion technique as per CLSI guidelines were included in the study. These study isolates were further tested for carbapenemase production by phenotypic methods such as Modified Hodge test, Combined Imipenem-EDTA disk test and Modified carbapenemase inactivation method.

### MODIFIED HODGE TEST

The Modified Hodge test was performed for all the study isolates as per CLSI guidelines<sup>4</sup>. In this method, a lawn culture of *Escherichia coli* ATCC 25922 in 1:10 dilution was done on Muller Hinton agar plate and 10µg meropenem disc was placed in the center of the plate and the test organism was streaked from the edge of the disc to edge of the

plate. The plate was incubated overnight at 35°C ± 2°C in ambient air for 16 to 24 hours. After incubation the test was interpreted. The Modified Hodge test positive isolates showed clover leaf like indentation of *Escherichia coli* 25922 growing along the test organism growth streak within the disk diffusion zone which indicates carbapenemase production by the test organism. The negative test organism did not show growth of *Escherichia coli* ATCC 25922 along the test organism growth streak within the disc diffusion.

### COMBINED IMIPENEM-EDTA DISK TEST

The combined Imipenem-EDTA disk test was performed on all the study isolates as per Yong et al 2009<sup>5</sup>. In this method lawn culture of the test organism was made on the Muller Hinton agar plate and 10µg imipenem disk and imipenem-EDTA (750µg) disk were placed 25 mm apart and incubated at 35°C for 16 to 18 hours. After incubation the inhibition zone size of imipenem and imipenem EDTA disks were measured. A 7 mm increase in zone size of imipenem EDTA disk than imipenem disk alone was considered as Metallo-β-Lactamase producers<sup>6</sup>.

### MODIFIED CARBAPENEMASE INACTIVATION METHOD

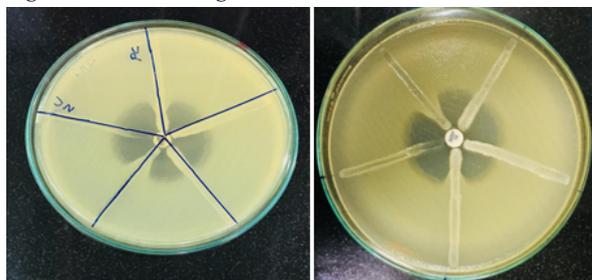
All the study isolates were subjected to Modified carbapenemase inactivation method as per CLSI 2017 guidelines<sup>7</sup>. In this method 1 µl loopful of test organism from the overnight incubated agar plate is taken and transferred to the tube containing 2 ml of trypticase soy broth then the suspension is vortexed and 10µg meropenem disk is added to the suspension and incubated at 35°C in ambient air for 4 hours. Then just prior to the completion of 4 hours incubation, 0.5 McFarland suspension of *Escherichia coli* ATCC 25922 was prepared and inoculated on to a Muller Hinton agar plate as per CLSI M02 for routine disk diffusion, then the meropenem disk was removed from the trypticase soy broth using a 10 µL loop and immediately placed in the Muller Hinton agar plate which has been inoculated with ATCC *Escherichia coli* 25922. Then the plate is incubated overnight at 35°C in ambient air and the zone of inhibition around the disc was measured. The zone size of 6-15 mm or colonies within a 16-18 mm zone were considered carbapenemase positive and zone size of ≥19 mm is considered negative and the zone size of 16-18 mm is considered indeterminate that is, unable to confirm whether the isolate is a carbapenemase producer or not<sup>7</sup>.

All the study isolates were also tested for the presence of KPC, NDM, OXA-48 genes by PCR method to confirm carbapenemase production. This genotypic method was considered as standard confirmatory method for carbapenemase production and was used to compare the other three phenotypic methods described earlier. The results were analysed and compared statistically.

## RESULTS

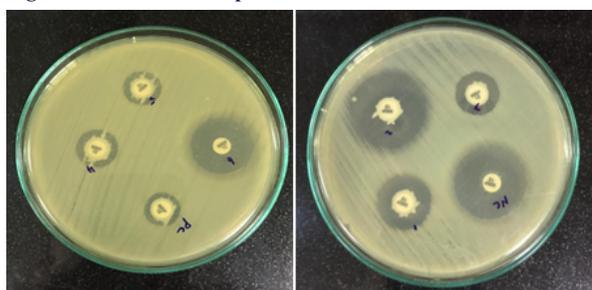
50 isolates of the Family Enterobacteriaceae showing resistance to meropenem by disk diffusion method were included in the study. All the 50 isolates were tested for carbapenemase production by both genotypic and phenotypic methods such as Modified Hodge test, Combined Imipenem-EDTA disk test and Modified carbapenemase inactivation method. Among the 50 isolates, 41 isolates demonstrated drug resistance genes by standard confirmatory genotypic method. Remaining 9 isolates were negative for carbapenemase production by both phenotypic and confirmatory genotypic method.

**Figure 1: Modified Hodge Test**



**Positive control and Negative control Test samples**

**Figure 2: Modified Carbapenemase Inactivation Method**



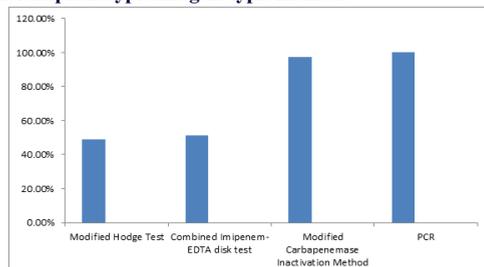
**Positive control with test samples Negative control with test samples**

**Table 1: Number of isolates showing positivity for carbapenemases by different phenotypic and genotypic methods**

TEST	NUMBER OF ISOLATES(n=41)
Modified Hodge Test	20
Combined Imipenem-EDTA disk test	21
Modified Carbapenemase Inactivation method	40
Detection of KPC,NDM & OXA-48 by PCR	41

Among the 41 isolates 20(48.78%) were Modified Hodge test positive, 21 (51.21%) were Combined disk diffusion method positive, 3 isolates were positive for both Modified Hodge test and Combined disk diffusion method and 40(97.56%) of isolates were positive for Modified Carbapenemase inactivation method.

**Figure1: Percentage of isolates showing positivity for carbapenemases by different phenotypic and genotypic methods**



## DISCUSSION

Over the decades, the rate of carbapenemase production in Gram negative Enterobacteriaceae isolates reported by different studies in India varies from 5.75% to 51%.<sup>6,11,12</sup> Detection of these carbapenemase producing isolates is important for the treatment of infections caused by these organisms. So we need an efficient, simple and cost effective phenotypic method to detect these carbapenemase producers. This

study is aimed at identifying one of the effective phenotypic carbapenemase detection method<sup>8,9,10</sup>.

In this study among the 50 carbapenem resistant isolates identified by disc diffusion method, 9 isolates showed negative results for phenotypic carbapenemase detection methods as well as by standard confirmatory genotypic method. This could be due to excessive Amp-C beta-lactamase or ESBL producers combined with altered membrane permeability as stated in the study done by Lutgring and Limbage et al, Vasoo et al, Peleg AY et al and Sathya Pandurangan et al<sup>6,13,14,15</sup>. The organisms that are resistant to carbapenem antibiotic due to the production of carbapenemase enzymes, also frequently produce more than one  $\beta$ -lactamase enzyme<sup>16,17</sup>.

In this study 41 isolates showed positive test results with various phenotypic carbapenemase detection methods. Among 41 carbapenem resistant isolates 20 (48.78%) showed positive Modified Hodge Test which correlates with the study done by Sathya Pandurangan et al<sup>6</sup>. The Modified Hodge test has excellent sensitivity in detecting Amblers class A(KPC) and Class D (OXA-48) exhibiting Enterobacteriaceae isolates than the class B (NDM) producing strains<sup>18</sup>. The modified Hodge test has the advantage of detecting different classes of carbapenemase, but it cannot differentiate between various classes of carbapenemase and will also give false positive results with ESBL and Amp C isolates<sup>19</sup>.

The routine antibiotic susceptibility testing indicates only resistance to carbapenem antibiotics, but it cannot differentiate Metallo- $\beta$ -lactamase (MBL) producers<sup>5</sup>. To detect the MBL producer, Combined Imipenem-EDTA disk test was performed and 21 (51.21%) isolates showed positive Combined Imipenem-EDTA disk test indicating MBL producers. In this study among the carbapenemase producers, more isolates showed positive Combined Imipenem-EDTA disk test when compared to Modified Hodge test indicating slight increase in MBL producers than the KPC and OXA-48 producers. The above results correlate with the study done by Sathya Pandurangan et al<sup>6</sup>. There are multiple mechanisms which mediate MBL resistance. Screening of multi drug resistant strains for MBL production is very important to contain the spread, implement strict infection control practices and as well as to provide therapeutic guidance for confirmed infection<sup>20</sup>.

In the late 70s itself the idea of hydrolysis of beta-lactam antibiotics by incubating them with bacterial suspension was demonstrated<sup>21</sup>. But however, the carbapenemase inactivation method is the first to use antibiotic susceptibility testing disk which is universally available at low cost and with longer shelf life. In our study, out of the 41 genotypically confirmed isolates 40(97.5%) isolates showed positive for carbapenemase production by modified carbapenemase inactivation method. mCIM shows high concordance with genotypic method(PCR) in detecting carbapenemase resistance and it demonstrates higher percentage of carbapenemase detection than Modified Hodge test and Combined Imipenem-EDTA disk test. Our study correlates with the results of the studies conducted by Zwaluw K et al<sup>22</sup>, Pierce et al<sup>23</sup> and Yamada et al<sup>24</sup>. From our study results we inferred that the mCIM can detect organisms exhibiting carbapenem resistance by different mechanisms, better than the other phenotypic methods.

## CONCLUSION

It is important to reliably detect carbapenemase producing Enterobacteriaceae isolates by the laboratory to contain the spread of these multi drug resistant pathogens. The existing phenotypic methods have limitations. This study shows that the Modified Carbapenemase inactivation method is the simple, specific, reliable, inexpensive and less labor intensive phenotypic method to detect carbapenemase producing Enterobacteriaceae and hence it can be implemented as a routine carbapenemase detection method in clinical microbiology laboratory.

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