

## Original Research Article

# Phenotypic detection of carbapenem resistance in gram negative bacilli from various clinical specimens of a tertiary care hospital in Western Uttar Pradesh

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### ABSTRACT

**Background:** Carbapenemase producing multidrug-resistant organisms (i.e., MDROs) is a critical medical and public health issue globally. These bacteria are often resistant to all beta-lactam agents and are also co-resistant to other multiple classes of antimicrobial agents, leaving very few antimicrobial options.

**Methods:** This study was carried out at UP University of medical sciences Saifai, Etawah, Uttar Pradesh, India, from January 2015 to June 2016. 110 isolates were found resistant by the Kirby Bauer's disc diffusion method according to the CLSI guidelines. Modified Hodge test and combined disk test were performed for resistant isolates.

**Results:** A total of 800-gram negative isolate were included in the study. 110 isolates were found resistant to imipenem by disk diffusion method. Out of these 90 (81.81%) were positive for carbapenemase production by modified Hodge test.

**Conclusions:** We conclude that the modified Hodge test is a useful method for detection of carbapenemase production. Combined disc method is useful to detect metallo beta lactamase production.

**Keywords:** Carbapenemases, Gram negative bacteria, Modified Hodge test, Metallo beta lactamase (MBL)

### INTRODUCTION

Carbapenems are  $\beta$ -lactam antibiotics, presently considered as the most potent agents of treatment for multidrug resistant gram negative bacterial infections because of the stability of these agents against majority of  $\beta$ -lactamases and their high rate of permeation through bacterial outer membranes.<sup>1</sup>

Prevalence of carbapenem resistance in Gram-negative bacteria is increasingly encountered in healthcare-associated infections in India. Bacteremic episodes due to these organisms carry a high mortality as shown by numerous studies from other countries also.<sup>2</sup> Carbapenems antibiotic bind to PBP 1 and PBP 2 of

gram-negative and gram-positive bacteria, causing cell elongation and lysis.<sup>3</sup> Bacterial resistance arises from production of carbapenemases, which hydrolyse the carbapenem nucleus and alteration of the porin channels in the bacterial cell wall, reducing the permeability of the drugs.<sup>4</sup>

The vast majority of acquired carbapenems in gram negative bacilli belong to 3 ambler classes of  $\beta$ -lactamases, namely class A carbapenems (KPC) which hydrolyse all  $\beta$ -lactams, and are inhibited by clavulanic acid and tazobactam, zinc dependent class B carbapenems (NDM, VIM, and IMP) that hydrolyse all  $\beta$ -lactams except aztreonam, and are inhibited by metal chelators like EDTA, and ambler class D (OXA-48-like)

that hydrolyse carbapenems and weakly hydrolyse (or do not hydrolyse) broad-spectrum cephalosporins and are poorly inhibited by clavulanic acid and EDTA.<sup>5</sup>

The modified Hodge test (MHT) has been extensively used as a phenotypic method for detection of carbapenems activity and the only method of carbapenems detection so far recommended by the CLSI. The test is sensitive for the detection of a carbapenems mediated mechanism of resistance to carbapenems, but does not provide information on the type of carbapenems involved.<sup>6</sup> Detection of carbapenems is a crucial infection control issue because they are often associated with extensive antibiotic resistance, treatment failures and infection-associated mortality.

## METHODS

This study was carried out at UP University of medical sciences Saifai, Etawah, Uttar Pradesh, India from January 2015 to June 2016. In this study 810 gram-negative bacilli randomly selected, non-duplicate strain from all clinical samples like urine, pus, vaginal swab, blood, sputum and other body fluids received routinely in microbiology laboratory of tertiary care hospital.

Gram negative bacilli isolated from the various samples; which were having less sensitivity zone size of Imipenem on modified Kirby Bauer disk diffusion method were suspected for carbapenem resistance and further tested. Antimicrobial susceptibility of all the isolates was performed by the Kirby Bauer's disc diffusion method according to the CLSI guidelines.

The modified Hodge test was performed according to the standard clinical and laboratory standards institute (CLSI) guidelines for the detection of carbapenems in *Enterobacteriaceae*. 1:10 dilution of 0.5 McFarland of negative control *E. coli* ATCC 25922 was uniformly swabbed onto Muller Hinton agar (MHA) and test isolate

was streaked as a straight line from the edge of the meropenem (mrp) disk (10 µg), to the edge of the plate. An indentation in the growth towards the imipenem disk on either side of the test isolate was considered as positive to produce carbapenems by the test isolate. *Klebsiella pneumoniae* BAA 1705 was used as positive control.<sup>6</sup>

The combined disk test Imipenem-EDTA- the IMP-EDTA combined disk test performed as the test organisms inoculated on to plates with Muller Hinton agar as recommended by the CLSI. Two 10 µg imipenem disks (Hi-Media) placed on the plate, and appropriate amounts of 10 µl of EDTA solution added to one of them to obtain the desired concentration (750 µg). The inhibition zones with the Imipenem and EDTA disc was ≥ 7 mm than the Imipenem disc alone, will be considered as MBL positive.

E-test MBL- the E-Test strip (Hi-Media) containing a double sided seven-dilution range of MRP (4 to 256 µg/mL) and MRP (1 to 64 µg/mL) in combination with a fixed concentration of EDTA has been reported to be the most sensitive format for MBL detection. MIC ratio of MRP (meropenem)/MRP-E (meropenem-EDTA) of >8 or >3 log 2 dilutions indicates MBL production.<sup>7</sup>

## RESULTS

By disk diffusion method, from 800 strains, 110 (43,4%) were resistant to imipenem. Isolates susceptible to imipenem were excluded. Out of 110 resistant strains 30 was *Klebsiella pneumonia* strains, 26 *Escherichia coli* strains, 15 *Citrobacter freundii*, 13 *Pseudomonas aeruginosa* strains, 13 *Acinetobacter Baumanni* strains, 5 *Citrobacter koseri* strains, 4 *Enterobacter spp.* Strains, 3 strains of *Proteus mirabilis* and 2 strains of *Proteus vulgaris* isolated. Maximum number of sample was urine 29 (26.36%) followed by pus 27 (24.54%), blood 22 (20%) and 32 (29.08%) from other infections.

**Table 1: Phenotypic characterization and distribution of carbapenems producer from clinical isolates.**

Bacterial strains isolated	Total no. of isolates	Carbapenems production by					
		Modified Hodge test		E-test MBL		Combined disk test	
		Positive	Negative	Positive	Negative	Positive	Negative
<i>K. pneumoniae</i>	30	25 (83%)	5 (17%)	15 (50%)	15 (50%)	15 (50%)	15 (50%)
<i>E. coli</i>	25	21 (84%)	4 (16%)	12 (48%)	13 (52%)	12 (48%)	13 (52%)
<i>C. freundii</i>	15	12 (80%)	3 (20%)	7 (46%)	8 (53%)	7 (46%)	8 (53%)
<i>P. aeruginosa</i>	13	11 (85%)	2 (15%)	6 (46%)	7 (54%)	6 (46%)	7 (54%)
<i>A. baumani</i>	13	12 (92%)	1 (8%)	7 (54%)	6 (46%)	7 (54%)	6 (46%)
<i>C. koseri</i>	5	4 (80%)	1 (20%)	2 (40%)	3 (60%)	2 (40%)	3 (60%)
<i>Enterobacter spp.</i>	4	2 (50%)	2 (50%)	2 (50%)	2 (50%)	2 (50%)	2 (50%)
<i>P. mirabilis</i>	3	2 (67%)	1 (33%)	1 (34%)	2 (67%)	1 (34%)	2 (67%)
<i>P. vulgaris</i>	2	1 (50%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)
Total	110	90 (82%)	20 (18%)	52 (47%)	58 (53%)	52 (47%)	58 (53%)

In present study, it is found that out of 110 carbapenem resistant isolates, 90 (81.81%) were positive for carbapenems production by modified Hodge Test. Total 52 cases were MBL reported out of 110 CRGNB by Meropenem with and without EDTA Ezy MIC™ Strips (Hi-Media) and combined disc test (Table 1).

## DISCUSSION

Carbapenem resistance in gram-negative bacteria is increasingly encountered in healthcare-associated infections in India. Bacteremic episodes due to these organisms carry a high mortality as shown by previous studies from other countries.<sup>2</sup>

According to CLSI document,<sup>6</sup> carbapenemase-producing isolates usually test intermediate or resistant to one or more carbapenems on susceptibility is the most sensitive indicator of carbapenems production. In this study, out 110 carbapenem resistant gram-negative bacilli isolated. In the present study, the overall resistance to carbapenems was 13.75% which is in comparison with the study of Kaur M and Gupte S et al.<sup>2</sup> who reported 17% resistance to carbapenems in Gram negative bacilli. Also, Manoharan et al, Priyadutta et al, Wattal C et al, and Gupta E et al, showed 17%, 7.87%, 13-57% and 17-22% resistance to carbapenems respectively.<sup>8-11</sup>

From India, numerous studies have found different rates of carbapenem resistance. A study was conducted in Aligarh showed overall Imipenem resistance was 12% for *Klebsiella* species.<sup>8</sup> In July 2011 to January 2013 a study was conducted in Meerut which showed 5-6% carbapenem resistant in *Enterobacteriaceae*.<sup>12</sup> Aswaniet al, found 7% carbapenem resistance in *E.coli* and 5% carbapenem resistant in *Klebsiella species*.<sup>13</sup> In other developing countries from African continent, the prevalence of carbapenems producing bacteria ranged from 2.3% to 6.7% in North Africa and from 9% to 60% in Sub-Saharan Africa.<sup>14</sup>

Maximum number of sample was urine 29 (26.36%) followed by pus 27 (24.54%), blood 22 (20%) and 32 (29.08%) from other infections obtained in this study. A comparable study in north India, most of the carbapenem resistant organism was isolated from urine 47.1% (n=20) followed by pus 27.1% (n=13).<sup>15</sup> In another study Mohamudha RP et al, also found that the distributions of the sources of the isolates were: urine 37% (n=39), blood 22.3% (n=23), wound discharge 11.7% (n=12), peritoneal fluid 5.8% (n=6), ascitic fluid 10.7% (n=11), tracheal aspirate 6.8% (n=7), and sputum 4.9% (n=5).<sup>16</sup> Nagaraj S et al, had comparable findings where they observed that the carbapenem-resistant organisms were isolated mainly from urine samples up to 42% (n = 21), followed by wound discharge 18% and respiratory secretions 16%.<sup>17</sup>

The MHT screening test for carbapenems is currently proposed by the clinical and laboratory standards institute (CLSI) for phenotypic screening of carbapenemases

producers. The MHT method is easy to perform, but diverse specificity values have been reported by authors, so should be aware of false-positive results.<sup>6</sup> In this study 90/110 (81.81%) CRGNB isolates were positive by modified Hodge test. Carbapenems production by MHT was highest with *Acinetobacter spp.* with 12/13 (92.3%), followed by *Pseudomonas spp.* 11/13 (84.61%) and *E. coli spp.* 21/25 (84%). Similar study done by Sahin et al, found concordant results with 85% sensitivity of MHT.<sup>18</sup> Another study such as Delphine G et al and Sanjeev K et al, found positive results 68.57% and 34% respectively by MHT.<sup>19,20</sup> Study by Delphine G et al, showed that the MHT technique is highly sensitive for detecting class A, B, and D carbapenems after addition of zinc in the culture medium.<sup>19</sup>

In current study, we screened only carbapenem resistant isolates with MBL E -Test, in which 52 isolates found to be MBL positive by combined disc and E-test MBL. The E test MBL strip can detect metallo-β-lactamases, both chromosomally and plasmid mediated.<sup>21</sup> These MBL positive strains are usually resistant to β-lactams, aminoglycosides and fluoroquinolones.

## CONCLUSION

Although molecular techniques are regarded as the most appropriate method for the detection of carbapenem resistance, it becomes impractical in a routine diagnostic laboratory setup up due to cost factors and availability of molecular set up. The MHT is one of the simplest techniques used to indicate carbapenems activity. In addition to phenotypic tests, genotypic investigations should be commonly used to decrease mortality and morbidity due to infections with CRGNB.

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