

CARBAPENEMASE PRODUCING BUGS: A THREAT TO ANTIMICROBIAL THERAPYAnuniti Mathias¹, Aroma Oberoi², Vipin Sam Alexander³**HOW TO CITE THIS ARTICLE:**

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ABSTRACT: CONTEXT: Carbapenemases are a group of β -lactamases capable of conferring resistance to carbapenems. Carbapenemase producing gram negative bacilli are becoming increasingly prevalent not only in nosocomial infections but in community acquired isolates also posing a threat to antimicrobial therapy of life threatening infections. **AIMS & OBJECTIVES:** This study was done to identify carbapenemase producing organisms among multidrug resistant clinical isolates and to detect MBL producers among the carbapenemase producing isolates. **MATERIALS & METHODS:** Gram negative organisms isolated from clinical samples in November-December 2014 were included. The antibiotic susceptibility of isolates was determined by Kirby Bauer method. Multi-drug resistant isolates showing reduced susceptibility or resistance to carbapenems were further tested for carbapenemase production by Modified Hodge test (MHT) and MHT positive isolates were further tested for MBL production by combined disc synergy test (CDST). **RESULTS:** A total of 32 isolates out of 62 carbapenem resistant MDR isolates were positive for carbapenemase production by MHT. Most common isolated carbapenemase producing organisms are Klebsiella species, Escherichia coli, Pseudomonas species and Acinetobacter species. Out of the 32 MHT positive isolates, 4 were positive for MBL production by CDST. MBL producers were 2 Klebsiella isolates, 1 Pseudomonas and 1 Acinetobacter isolate. **CONCLUSION:** Evaluation of effective antibiotic options and rigorous infection control measures are needed for controlling spread of carbapenemase producing multi-drug resistant organisms, especially in hospital setting.

KEYWORDS: Multi drug resistance, Carbapenemase production, MBL.

INTRODUCTION: Carbapenems are potent β -lactam antibiotics used to treat serious infections in hospital settings. They have broad antimicrobial spectrum against both Gram-positive and Gram-negative organisms as compared to penicillins, cephalosporins and β -lactam/ β -lactamase inhibitor. Carbapenem resistance in Gram-negative bacteria can be result of production of β -lactamase enzyme known as carbapenemase, expression of efflux pumps, porin loss, and alterations in PBPs.^[1] Recently, organisms belonging to the family Enterobacteriaceae, mainly Klebsiella pneumoniae, and the nonfermenter group, mainly Pseudomonas aeruginosa and Acinetobacter species have increased their potential to become extensively drug resistant by acquiring resistance to carbapenems, due mainly to carbapenemases and metallo beta lactamase (MBL) production,^[2,3] respectively.

Clinically important carbapenemases include: Class A – most common type isolated clinically is Klebsiella pneumoniae carbapenemase (KPC), Class B – Metallo- β -lactamases e.g.,-NDM -1, VIM type and IMP type, Class D–Oxacillin hydrolysing β -lactamases or Oxacillinases e.g.,- OXA-48, OXA-181.^[4] This study was done to identify carbapenemase producing organisms among multidrug resistant clinical isolates and to detect MBL producers among the carbapenemase producing isolates.

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MATERIALS & METHODS: The study was conducted in the Department of Microbiology in a tertiary care hospital in north India over a period of two months (November to December 2014). Samples received from admitted patients only were included in the study. Samples received in the microbiology laboratory were processed by standard microbiological techniques. The antimicrobial susceptibility was performed using Kirby Bauer disc diffusion method and the results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines.^[4] Multi-drug resistant (MDR) isolates showing reduced susceptibility or resistance to carbapenems were further tested for carbapenemase production by Modified Hodge test (MHT) and MHT positive isolates were further tested for MBL production by combined disc synergy test (CDST).

Modified Hodge Test: MHT was performed according to CLSI guidelines for phenotypic detection of carbapenemase production by the isolate (Figure 1). Inoculum of E.coli ATCC 25922 was uniformly swabbed onto Mueller Hinton Agar and 10µg Imipenem disk placed at the centre of the plate. The test isolate was streaked as a straight line of at-least 20-25mm length from the edge of the disk to the edge of the plate and the plate incubated at 37°C in ambient air for 16-20 hours and thereafter examined for enhanced growth around the test organism streak at the intersection of the streak and the zone of inhibition. Enhancement of growth was considered as positive result and no enhancement of growth as negative result for carbapenemase production, respectively.^[5,6]

Combined Disk Synergy Test: CDST was performed for testing MBL production using two 10µg Imipenem disks with one disk containing 292µg EDTA, placed 25mm apart (figure 2). An increase in zone diameter of ≥4mm around the Imipenem-EDTA disk as compared to that of Imipenem disk alone was considered positive for MBL production.^[2,7]

RESULTS: A total of 62 clinical isolates that were resistant either to imipenem or meropenem or both were tested by MHT. These included 20 *Escherichia coli* (Urine=14, blood=1, pus=3, ET tip=1, peritoneal fluid=1), 5 *Klebsiella pneumoniae* (Urine=5), 16 *Pseudomonas* spp. (Urine=1, blood=1, pus=2, ET tip=1, wound swab=11), 13 *Acinetobacter* spp. (urine, pus, BAL, sputum, suction tip=1 each, ET tip=4, wound swab=4), 6 *Enterobacter* species (Urine=3, pus 2, ET tip=1), 1 *Citrobacter koseri* (Blood=1) and 1 *Proteus vulgaris* (Tissue=1) isolates. Of these, 32 (51.6%) isolates were positive for carbapenemase production by MHT which included 10 (50%) *E.coli* (Urine=7, pus=3), 5 (100%) *Klebsiella pneumoniae* (Urine=5), 6 (37.5%) *Pseudomonas* spp. (Pus=6), 8 (61.5%) *Acinetobacter* spp. (Pus=8), 1(16.6%) *Enterobacter* spp. (pus=1), 1(100%) *Citrobacter koseri* (Blood=1) and 1(100%) *Proteus vulgaris* (Pus=1).

Out of the 32 MHT positive isolates, 4 (12.5%) were positive for MBL production by CDST. MBL producers were 2 *Klebsiella pneumoniae* isolated from urine, 1 *Pseudomonas* spp. isolated from wound swab and 1 *Acinetobacter* spp. isolated from endotracheal secretions (Table 1).

DISCUSSION: Carbapenems like Imipenem, Meropenem and Ertapenem are mainly used as reserve drugs in treatment of life threatening infections in severely ill, immune compromised, patients hospitalised for prolonged duration, etc who are more prone to acquire multidrug resistant infections. They are clinically important for treating extended spectrum β-lactamase (ESBL) producing enterobacteriaceae which along with *Pseudomonas* species and *Acinetobacter* species are predominantly isolated pathogens from clinical samples.

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Production of carbapenem hydrolysing enzymes, also known as carbapenemases, by the enterobacteriaceae has led to emergence of carbapenem resistant enterobacteriaceae (CRE). Carbapenemase production in these organisms has reduced the clinical utility of carbapenem group of drugs posing a major challenge in treatment of severe infections.^[8] Therefore a rapid, simple and reliable method for detection of carbapenemase production is needed.

Among carbapenemase producers, metallo- β -lactamase production was 12.5% in this study which is less than that reported in other studies. Shenoy et al reported phenotypic MBL production in 93.24% MDR isolates.^[6] MBL production among carbapenem resistant *K. pneumoniae* isolates has been detected as 88.33%^[7] and 75%.^[9] Another study in south India has reported carbapenemase production and MBL production as 14.3% and 6.5% in *Acinetobacter baumannii*, 28.1% and 50% in *Pseudomonas aeruginosa* and 14.3% and 28.6% in *Pseudomonas* species,^[10] respectively. A comparatively lesser occurrence of carbapenemase production among carbapenem resistant isolates in this study can be due to presence of other mechanisms like Amp C co-production, loss of porin channels, expression of efflux pumps and presence of altered PBPs.

The limitations in this study are relatively small sample size, short duration of the study and its restriction to phenotypic detection of carbapenemase production and MBL identification. Genotypic characterisation which confirms presence carbapenemase producing genes was not done.

Carbapenemase producing pathogens cause infections that are difficult to treat and have high mortality rates, due to their appearance in multidrug-resistant pathogens like *K. pneumoniae*, *P. aeruginosa*, and *Acinetobacter* spp. Their occurrence in outbreak settings is being reported increasingly. Careful detection is required, because high carbapenem MICs are not usually evident.

Various factors like over the counter use of antibiotics, irrational use of antibiotics, easily accessible higher antibiotics, lack of adequate health measures and improper sanitation and living conditions are considered crucial for their development and spread.^[7] Evaluation of effective antibiotic options and rigorous infection control measures will help in controlling spread of carbapenemase producing MDR organisms in hospitals. Combination antibiotic therapy and avoidance of irrational carbapenem use are effective measures to prevent evolution of MDR & XDR organisms.

Phenotypic tests for carbapenemase detection like MHT and CDST are simple, cost effective and easy to perform and hence can be used in any microbiology laboratory to detect carbapenemase production and applied clinically to guide the antimicrobial therapy, especially in severe and life threatening infections.

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Isolate (Total)	Specimen (MHT* +ve)			Total MHT +ve	Total CDST† +ve
	Urine	Blood	Pus/Body fluids		
<i>E.coli</i> (20)	14 (07)	01	05 (03)	10	-
<i>K.pneumoniae</i> (05)	05 (05)	-	-	05	02
<i>Pseudomonas</i> (16)	01	01	14 (06)	06	01
<i>Acinetobacter</i> (13)	01	-	12 (08)	08	01
<i>Enterobacter</i> (06)	03	-	03 (01)	01	-
<i>Citrobacter koseri</i> (01)	-	01 (01)	-	01	-
<i>Proteus vulgaris</i> (01)	-	-	01 (01)	01	-
Total : 62				32	04

Table 1: Summary of Carbapenemase production by multidrug resistant gram negative bacilli

* Modified Hodge Test

† Combined Disc Synergy Test

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Fig. 1: Positive Modified Hodge Test - Clover leaf shaped enhancement of growth around the test streak at the intersection of streak and the zone of inhibition.

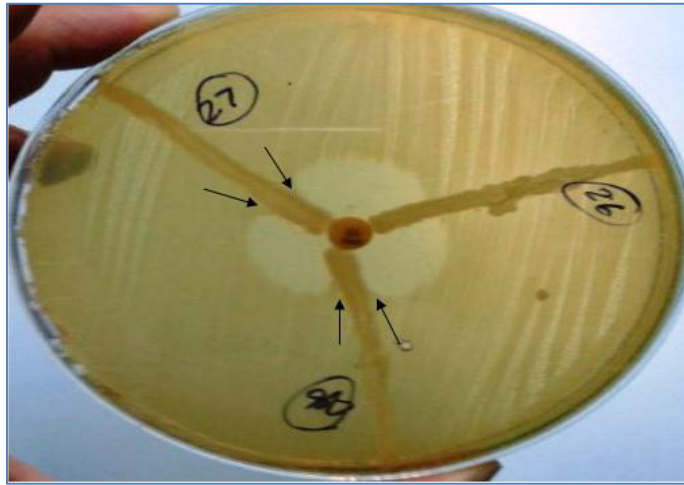


Fig. 1: Modified Hodge Test

Fig. 2: Positive CDST[‡] - Difference of ≥ 4 mm in the diameter of zone of inhibition of Imipenem and Imipenem + EDTA^{**}.



Fig. 2: Combined Disc Synergy Test

[‡]Combined Disc Synergy Test.

^{**}Ethylenediaminetetraacetic acid.

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