Original Research Article

# Carbapenem Resistance in Clinical Isolates of Enterobacteriaceae : A Global Health Concern

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**Received: 26-07-2020 / Revised: 25-08-2020 / Accepted: 29-08-2020**

**Abstract**

**Introduction:** Carbapenem-resistant Enterobacteriaceae (CRE) has gradually emerged and is one of the serious public health concerns worldwide. **Aim:** To detect Carbapenem Resistance in clinical isolates of Enterobacteriaceae and Carbapenamase production by performing Modified Hodge Test (MHT) and Combined Disc Test (CDT). **Material & Methods:** Identification of Isolates was done by standard bacteriological techniques. The isolates were screened for carbapenem resistance by Kirby-bauer disc diffusion method using Ertapenem as per CLSI recommendation. Detection of carbapenemase production was done by Modified Hodge test and Combined Disc test. **Result:** A total of 931 clinical isolates of Enterobacteriaceace were obtained from various clinical samples. Out of which isolates of *Escherichia coli were* 295 (31.68%). All these isolates were screened for Carbapenem resistance..Out of 931 isolates, 710 (76.26%) isolates were carbapenem screen positive. Maximum carbapenem resistance was seen in *Klebsiella pneumoniae*, 307 (43.23 %). Out of 710 carbapenem resistant isolates, 567 (79.85%) were carbapenemase producers. **Conclusion:** Early detection, isolation and contact precaution for CRE patient will to prevent rapid dissemination of CRE infection.

**Keywords:** Carbapenem resistance, Enterobacteriacae, Modified Hodge Test.



## Introduction

The emergence of Carbapenem-Resistant Entero bacteriaceae (CRE) has become a major health concern worldwide and a new challenge in the treatment of infectious diseases.

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The Enterobacteriaceae are a large family of Gram-negative bacteria that may cause several infectious diseases such as respiratory tract infection, urinary tract infection etc. Carbapenem has been the main treatment for severe infections associated with Entero bacteriaceae.[1]

Carbapenem-resistant Enterobacteriaceae (CRE) are usually resistant to all β-lactam agents as well as most other classes of antimicrobial agents. The treatment options are very few for patients infected with CRE. Carbapenem resistance in Enterobacteriaceae occurs when an isolate acquires a carbapenemase or when an isolate produces an extended - spectrum cephalo-sporinase, such as an Amp C-type β-lactamase.[2]

Carbapenem resistance in Enterobacteriaceae is mainly mediated by the production of carbapenemases, a form of β-lactamase that cleave the β-lactam ring, an essential component of β-lactam antibiotics such as cephalosporins and carbapenems.[1]

The common mechanisms that are responsible for carbapenem resistance include changes in outer membrane proteins over expression of drug efflux pumps and carbapenem hydrolyzing enzymes.[3]

In addition, carbapenemase producers are usually associated with many other non–β-lactam resistance determinants, which give rise to multidrug- and pandrug-resistant isolates.[4]

The drug resistance genes are often carried on mobile genetic elements and hence can easily transmit from person to person often via the hands of healthcare personnel or via contaminated medical equipment. These genes confer high levels of resistance to Carbapenems and many other antimicrobials (fluoroquinolones and aminoglycosides), often leaving very limited therapeutic options.[5]

The majority of organisms in human gut flora are from Enterobactericeae family.[6] The prevention of spread of carbapenemase producers relies on early their detection ..Therefore, this study was carried out to detect carbapenem resistance in clinical isolates of Enterobacteriaceae in our hospital and to evaluate a simple, rapid cost effective method for detection of carbapenamase production**.**

## Material and Methods

The present study was carried out in the Post Graduate Department of Microbiology, Subharti Medical College and associated Chhatarpati Shivaji Subharti Hospital (CSSH), Meerut. The study included all the clinical samples received in clinical Microbiology laboratory for Culture and Sensitivity from various inpatient units (ICUs & wards) and Out Patient Departments. Isolates were identified by standard bacteriological techniques.[7] Antibiotic sensitivity was performed as per CLSI guidelines 2016.[8]

### Screening of carbapenemase producers

All the isolates were screened for carbapenem resistance using Ertapenem disc (10µg) by Kirby Bauer disc diffusion method according to CLSI guidelines.

### Detection of carbapenemase production

The MHT is a CLSI-recommended phenotypic method for carbapenemase detection.[8] MBL production was detected by performing CDT.[9]

### Ethics

Approval from Institutional Ethics Committee was obtained before conducting the study.

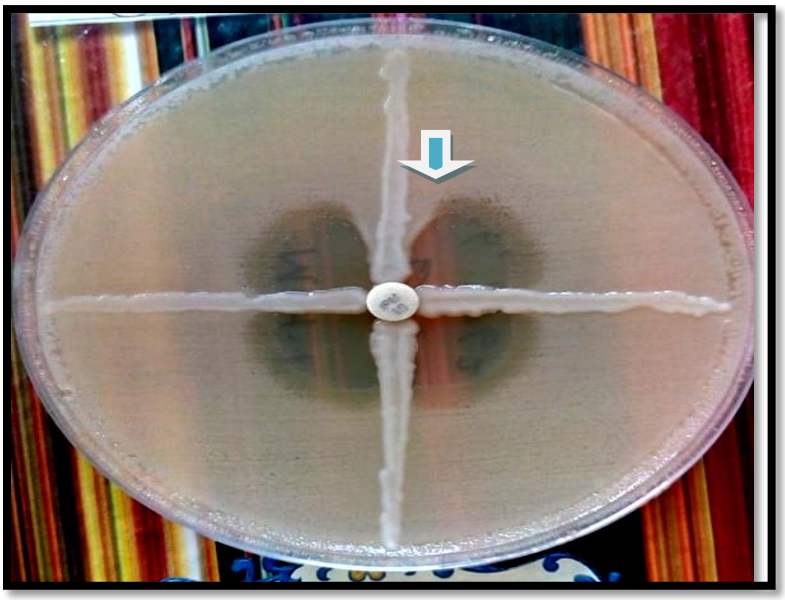

**Figure 1 : Modified Hodge Test positive: isolate showing a clover leaf like indentation (arrow) of the Escherichia coli 25922 growing along the test organism growth streak with the disc diffusion zone**

**Figure 2: Combined Disc Test positive: isolate showing an increase zone of diameter of > 7 mm around the IPM-EDTA disc as compared o to that of IPM disc alone, indicating MBL producers**

**Results**

A total of 931 clinical isolates of Enterobacteriaceace were obtained from various clinical samples. Out of which isolates of *Escherichia coli were* 295 (31.68%), *Klebsiella pneumoniae* ,279 (29.96%), *Klebsiella oxytoca* ,196 (21.05%), *Citrobacter spp.* ,89 (9.55%), *Proteus spp.* ,65 (6.98%) and *Morganella morganii*, 07 (0.75%). All these isolates were screened for Carbapenem resistance.

Out of 931 isolates, 710 (76.26%) isolates were carbapenem screen positive and 221 (23.7%) were carbapenem screen negative. Looking at the Distribution of carbapenem resistant isolates in IPD & OPD, it was found that majority were isolated from IPD patients 510/710 (71.83%) .

Maximum carbapenem resistance was seen in *Klebsiella pneumoniae*, 307 (43.23 %) followed by *Escherichia coli, 211* (29.71%), *Klebsiella oxytoca, 103* (14.50%), *Citrobacter* species, 53 (7.46%), Proteus spp. 30 (4.22%) and *Morganella morganii* 06 (0.84%).

Looking at the sample wise distribution of Carbapenem resistant isolates, it was found that urine, 220 (30.98 %) was the predominant sample followed by pus ,157 (22.11 %), sputum, 140 (19.71%), ET aspirate ,83 (11.69%), blood ,54(7.60%), Stitch line swab ,19 (2.67%), Central line tip, 14 (1.97%), Ascitic fluid, 10(1.40%), ICD fluid, 08(1.12%) and tissue 05 (0.70 %).

Out of 710 carbapenem resistant isolates, 567 (79.85%) were carbapenemase producers. Only MHT was positive in 225/710 (31.69%) isolates. Only CDT was positive in 196/710 (27.61%), both MHT & CDT were positive in 146/710 (20.56%), while both MHT & CDT were negative in 143/710 (20.14 %). (Table 1)

**Table 1 : Phenotypic characterization and distribution of Carbapenemase producers (n=710)**

| ORGANISM | *Klebsiella pneumoniae* | | *Escherichia coli* | | *Klebsiella oxytoca* | | *Citrobacter spp.* | | *Proteus spp.* | | *Morganella Morganii* | | TOTAL/ % | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TEST | TOTAL | % | TOTAL | % | TOTAL | % | TOTAL | % | TOTAL | % | TOTAL | % | TOTAL | % |
| Only MHT +ve | 75 | 24.42 | 80 | 37.91 | 35 | 33.98 | 19 | 35.85 | 13 | 43.33 | 03 | 50 | 225 | 31.69 |
| Only CDT +ve | 74 | 24.12 | 72 | 34.13 | 26 | 25.24 | 14 | 26.42 | 09 | 30 | 01 | 16.66 | 196 | 27.61 |
| BOTH | 64 | 20. | 48 | 22. | 20 | 19. | 10 | 18. | 04 | 13. | 00 | 00 | 146 | 20. |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MHT & CDT +ve | | 84 | | 74 | | 41 | | 86 | | 33 | | | | 56 |
| BOTH MHT & CDT –ve | 94 | 30.61 | 11 | 5.21 | 22 | 21.36 | 10 | 18.86 | 04 | 13.33 | 02 | 33.33 | 143 | 20.14 |
| TOTAL n=710 | 307 | 100 | 211 | 100 | 103 | 100 | 53 | 100 | 30 | 100 | 06 | 100 | 710 | 100 |

## Discussion

The study included a total of 931 isolates of Enterobacteriaceace from various clinical samples. Out of which, 710/931 (76.26%) isolates were carbapenem screen positive. Out of 710 carbapenem resistant isolates, 567/710 (79.85%) were carbapenemase producers ,which is much higher in comparison with the study done by Diwakar J *et al* in 2017 who reported carbapenem resistance in 43.4% isolates.[10] From India, numerous studies found different rates of carbapenem resistance, Akshaya R *et al*[5] in 2016 reported carbapenem resistance in 13.95% isolates, Chauhan K *et al* in 2014[9] reported 20.72% (183/883) isolates were carbapenem screen positive which is much lesser than our finding. These findings suggest that carbapenem resistance is on the rise year by year indicating misuse and overuse of carbapenems. So, they should be used judiciously.

In the present study MHT was positive in 225/710 (31.69%) isolates. Diwakar J *et al* in 2017 reported that 81.81% isolates were positive for carbapenemase production by modified Hodge Test.[10] In 2018 Gupta V *et al* also reported 78% isolates of *K. pneumoniae* showed positive Modified Hodge Test (MHT).[11] Hence, screening should be done routinely in the laboratories for their early detection and initiation of appropriate therapy and appropriate measures can be taken to limit the spread of CRE.

The authors found that CDT was positive in 196/710 (27.61%) isolates. In 2018, Gupta V *et al* reported Metallo Beta Lactamase (MBL) production in 64% isolate by Combined Disc Test (CDT). [11] Diwakar J *et al* in 2017 reported MBL production in 47.27% isolates by Meropenem with and without EDTA Ezy MIC™ Strips (Hi-Media) and combined disc test. [10] Chauhan K *et al* reported Carbapenemase production in 45.45% of *E. coli* and 38.67% of *Klebsiella* spp. using CDT.[9] The present study demonstrates the wide spread presence of MBLs in members of Enterobacteriaceae. Overall comparing the two phenotypic tests used for detection of carbapenamase production, it was observed that MHT could definitely detect more number of cases, 225/710 (31.69%) as compared to CDT 196/710 (27.61%).

It is clearly seen in the present study that both MHT & CDT were positive in 146/710 (20.56%) isolates showing production of serine carbapenemases and MBLs. However, both MHT & CDT were negative in 143/710 (20.14%). This negative result could be due to other important causes of carbapenem resistance among Enterobacteriaceae like porin loss etc.

Maximum carbapenem resistance was seen in *Klebsiella pneumoniae* 307 (43.23 %) followed by *Escherichia coli 211* (29.71%). Similarly, Diwakar *et al* in 2017 reported carbapenemase production predominantly in *Klebsiella pneumoniae* (27.27%) strains followed by *Escherichia coli (23.63%)*.[10] It might be due to the fact that predominant isolate in our study was *Klebsiella pneumoniae* followed by *Escherichia coli.*

On analyzing the sample wise distribution of Carbapenem resistant isolates, it was found that urine, 220 (30.98 %) was the predominant sample followed by pus and other samples. This may be attributed to the fact that urine and pus were the most predominant samples received in our laboratory. Similar finding was reported by Diwakar *et al* who also found urine, 26.36% to be the most predominant, followed by pus , *Akshaya Rao et al* 2016 also reported similar finding.[2]

## Conclusion

The phenotypic assays, MHT and CDT have been suggested as gold standard techniques to detect carbapenemases and Metallo beta lactamases producing gram negative bacteria. To conclude, spread of carbapenamase is therapeautic threat. Thus, there is a need to identify correctly class A and class B carbapenamase producers.

## Limitation

Molecular detection of genes responsible for Carbapenem resistance could not be done due to limited resources.

**Funding**
This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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**Source of Support:Nil**
**Conflict of Interest: Nil**