International Journal of Research in Medical Sciences
Agarwal N et al. Int J Res Med Sci. 2019 Jun;7(6):2373-2376
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20192530

Original Research Article

Phenotypic detection of carbapenem resistance and metallo-beta-lactamase among Enterobacteriaceae from clinical samples in tertiary care hospital

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Received: 05 April 2019
Accepted: 04 May 2019

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ABSTRACT

Background: Carbapenems have the broadest activity spectra of any β-lactam antibiotic and are often the most appropriate agents for use in the treatment of infections caused by multi resistant gram negative bacteria. The recent worldwide emergence and dissemination of carbapenemase-producing Gram negative rods that are resistant to carbapenems is a significant concern with respect to patient care and infection control strategies. Hence this study was undertaken to study the magnitude of carbapenem resistance among routine clinical isolates of family Enterobacteriaceae so as to guide the clinicians in selection of appropriate antimicrobial chemotherapies and infection control measures.

Methods: The present study was conducted in the Department of Microbiology over a period 18 months from January 2017 to July 2018. All the clinical isolates of Enterobacteriaceae were screened for carbapenem resistance as per CLSI guidelines. Such strains were then subjected to phenotypic confirmation of carbapenemase production by the Modified Hodge test. All isolates that gave a positive screening test were further evaluated for metallo-β-lactamase production. The technique used was the Combined Disk Test using a combination of Imipenem and Imipenem-EDTA.

Results: Out of the 400 total clinical isolates of Enterobacteriaceae isolated in the laboratory,57 were found to be Meropenem resistant (14.25%) and were labelled 'Carbapenem resistant Enterobacteriaceae" or CRE. Modified Hodge test (MHT) performed on the 57 carbapenem resistant isolates showed 41 (71.93%) isolates to be carbapenemase enzyme producers. Combined disc test (CDT) conducted on the 57 isolates of CRE detected Metallo-β-lactamase (MBL) enzyme production in 39 isolates (68.42%).

Conclusions: Since there is a high prevalence of carbapenemase resistance in our setting hence we need to be cautious with the indiscriminate use of broad spectrum antimicrobials, more so, the carbapenems.

Keywords: Carbapenem, Enterobacteriaceae, MBL, Modified Hodge test, Resistance

INTRODUCTION

Carbapenems have the broadest activity spectra of any β-lactam antibiotic and are often the most appropriate agents for use in the treatment of infections caused by multi resistant gram negative bacteria. Metallo-β-lactamase is an enzyme that can break down and destroy multiple classes of antibiotics including the carbapenems.[1] Their presence can convert an antibiotic susceptible gram negative bacterium into a multi drug resistant "superbug"! The detection of even a single MBL harboring bacterium signifies an imminent outbreak.[2] The spread of this resistance may incapacitate the health care system and limit the ability to provide invasive

procedures and immunosuppressive therapy in a safe medical environment. Because of its potential for logarithmic escalation in case of an outbreak, it is more cost-effective to combat the problem before it becomes established. Many factors, including the ease of international travel for medical tourism and migration, and the importation of food products, have been responsible for the spread of these microorganisms to several countries far beyond their place of origin. These microorganisms complicate therapy and limit treatment options. Equally worrisome is their gradual dissemination into the community. It is time to hold ourselves accountable for the careless use of antibiotics-a fragile and imperiled global health resource. We are already facing bacteria that are resistant to all available antibiotics, and the situation will only get worse without able stewardship and active measures to prevent transmission. Strict infection control measures including active surveillance for timely detection of colonized patients, separation of carriers from non-carriers, and contact precautions are of utmost importance and may be the only effective way of preventing the introduction and transmission of these bacteria in healthcare settings. Hence this study was undertaken to study the magnitude of carbapenem resistance among routine clinical isolates of family Enterobacteriaceae so as to guide the clinicians in selection of appropriate antimicrobial chemotherapies and infection control measures.

METHODS

The present study was conducted in the Department of Microbiology at Muzaffarnagar, Medical College Muzaffarnagar over a period 18 months from January 2017 to July 2018.

All 400 isolates of family Enterobacteriaceae obtained from various clinical samples: pus, blood, urine, CSF and various body fluids received in microbiology laboratory from IPD and OPD were included in the study. The isolates were identified as per the standard microbiological procedures.[3] Antimicrobial sensitivity testing was performed on Muller-Hinton agar plates with commercially available disks (Himedia) by Kirby Bauer disk diffusion method and interpreted as per CLSI guidelines.

All the clinical isolates of Enterobacteriaceae were screened for carbapenem resistance using Meropenem as the representative member of the carbapenem group of drugs, as per initial screening test recommendations of the CLSI guidelines, 2016.[4]

Such strains were then subjected to phenotypic confirmation of carbapenemase production by the modified Hodge test. MHT was performed on Mueller Hinton Agar plates, in accordance with CLSI guidelines. The results were read and interpreted as per CLSI recommendations, 2016.[4]

All isolates that gave a positive screening test were further evaluated for Metallo-β-lactamase production. The technique used was the combined disk test using a combination of imipenem and imipenem-EDTA on Mueller-Hinton agar plates.[5]

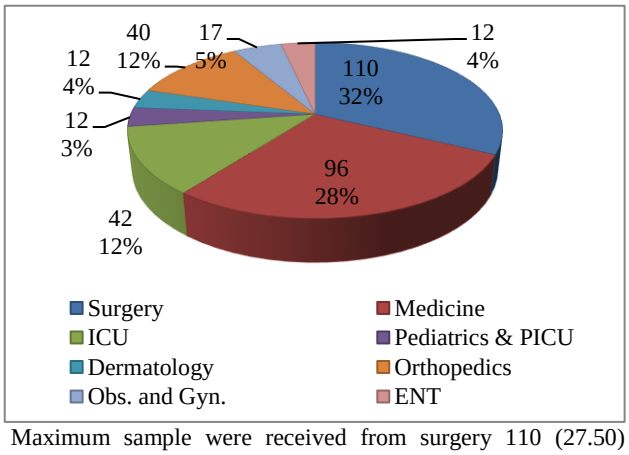
Statistical analysis was done using Chi-square test. Mean were calculated for various results.

RESULTS

The Microbiology laboratory received a total of 1800 specimens for aerobic bacterial culture and antimicrobial susceptibility during the study period. These comprised 948 consecutive clinical samples of pus and purulent fluids, 735 consecutive samples of urine, 94 samples of blood and 23 samples of CSF.

Of the total 1800 specimens, 1074 (59.67%) did not yield any growth on aerobic bacterial culture. Of the remaining 726 samples that did show growth, a total of 841 bacterial isolates were obtained, since 13.67% of specimens yielded >1 organism. Amongst these 268 [67%] were obtained from male patients and 132 [33%] from female patients. The male to female ratio was 2.03:1.

Out of 400 isolates, 104[26%] isolates were obtained from the Outpatients Department and 296[74%] were from inpatients. Among the inpatients 110 [27.50%] isolates were obtained from Surgery ward followed by 96 (24%) from Medicine ward (Figure 1). Different genus of family Enterobacteriaceae isolated from various samples were *Escherichia coli* followed by *Klebsiella* spp. (Figure 2).



Maximum sample were received from surgery 110 (27.50) followed by medicine 96 (24.00).

Figure 1: The ward wise distribution of samples.

In this study, out of the 400 total clinical isolates of Enterobacteriaceae, 57 were found to be Meropenem resistant (14.25%) and were labelled 'Carbapenem resistant Enterobacteriaceae" or CRE. It was noted that all Meropenem resistant strains were also resistant to one or more agents of cephalosporin subclass 3. Thus, all

CRE strains were tentatively considered carbapenemase producers as well.

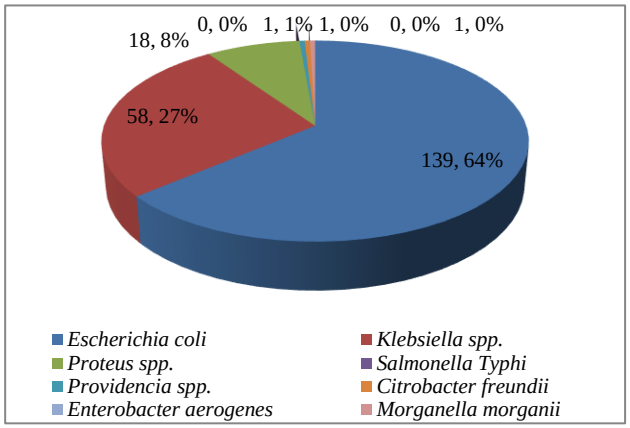


Figure 2: The genus wise distribution of various members of family Enterobacteriaceae.

Modified Hodge test (MHT) was performed on the 57 carbapenem resistant isolates. After 16-24 hours of incubation, the plate was examined for a clover leaf-type indentation at the intersection of the test organism and the *E. coli* ATCC 25922, within the zone of inhibition of the carbapenem disc. The presence of indentation or 'cloverleaf pattern' in the growth of *E. coli* ATCC 25922 around the test strain within the disc diffusion zone was regarded as a carbapenemase producer. 41 (71.93%) isolates came out to be carbapenemase enzyme producers while 12 (21.05 %) isolates gave a negative result (Table 1).

Table 1: The Results of Modified Hodge test for detection of carbapenemase enzyme production among carbapenem resistant enterobacteriaceae (CRE).

Result of MHT	No. of CRE isolates	Percentage %
Positive	41	71.93
Negative	12	21.05
Indeterminate	04	7.02
Total	57	100

On statistical analysis using Chi-square test, value was non-significant

Combined disc test (CDT) conducted on the 57 isolates of CRE. The technique used was the combined disk test using a combination of imipenem and imipenem-EDTA on Mueller-Hinton agar plates. CDT detected Metallo-β-lactamase (MBL) enzyme production in 39 isolates (68.42%). A notable observation was that MHT failed to detect carbapenemase activity in 9 out of 39 (23.08%) MBL positive strains.

On sample-wise distribution, the CRE isolates from urine were found to be the most frequent MBL producers (73.91%) closely followed by isolates from pus and purulent fluids (68.75%). CRE from blood were found to be the least frequent MBL producers. No out of the one CSF isolates was an MBL producer.

DISCUSSION

Enterobacteriaceae form a major part of gut flora and like other bacteria, MBL and carbapenemase Enterobacteriaceae are capable of colonizing the gut of patients. They serve as reservoirs for the spread of infection or for contamination of the environment and fomites more so in the health care setting. Foreign travel further causes worldwide dissemination of these multi drug resistant strains. The last few years have seen an alarming increase in the incidence of carbapenemase producing Enterobacteriaceae.[6]

In the present study, maximum isolation of Enterobacteriaceae was obtained from urine specimens (77.33%), followed by pus and purulent fluids (45.99%), blood (42.85%) and CSF (15.38%).

In this study, we found the overall resistance to Meropenem among Enterobacteriaceae to be 14.27%. This value is similar to those reported by Balan K et al (15%) and Behera B et al (19.50%).[7,8]

MBL gene from India by Kumarasamy KK et al in 2010 reported a carbapenem resistance rate of 24%, which is much higher than what we observed, certain other studies like those of Rai S et al also observed the same.[9,10] Saranraj P et al and Datta P et al, have reported much lower rates of resistance to carbapenems.[11,12]

On analysis of carbapenem resistance among the major genera of Enterobacteriaceae encountered among our isolates, i.e. *Escherichia coli* and *Klebsiella spp.* we observed figures of 12.9% and 18.55% respectively. Both these values are more or less similar to those reported by Behera B et al who found a slightly lower rate of resistance among *Escherichia coli* and a slightly higher rate among *Klebsiella* isolates.[8] However, most studies reported much lower overall resistance (figures ranging from 3.4% to 4%) as compared to ours. Resistance rates in *Klebsiella* reported by other workers, though slightly lower, were quite similar to our own findings.

In our study, only 71.93% carbapenem resistant strains tested positive with MHT (Clover leaf indentation) using 10µg Ertapenem discs as per CLSI guidelines.

In our study we used the Combined DiscTest (CDT) recommended by Franklin C where two 10µg imipenem discs were placed 25mm apart and 10µg of 0.1M EDTA solution was added to one of the discs.[5] A zone enlargement of >4mm around the disc containing EDTA as compared to Imipenem alone was considered a positive test. We found 31 isolates to be MBL producers. Thus, the overall prevalence of MBL among the 400 isolates was 9.75% which is in accordance with study

done by Nayar PK at a tertiary care hospital in Mumbai, the overall prevalence of MBL among Enterobacteriaceae from their study found to be 7.56 %.[13]

CONCLUSION

The presence of MBL has serious repercussions. Infections by bacterial strains are difficult to treat because of the presence of multi-drug resistance. In conclusion we would therefore caution the indiscriminate use of broad spectrum antimicrobials, more so, the carbapenems.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Agarwal N, Chauhan S, Singh P, Sharma VK. Phenotypic detection of carbapenem resistance and metallo-beta-lactamase among Enterobacteriaceae from clinical samples in tertiary care hospital. Int J Res Med Sci 2019;7:2373-6.