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A study on Carbapenemase detection in Enterobacteraceae family in a tertiary care hospital using Modified Hodge Test (MHT)

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Abstract

Introduction: Carbapenem resistance in Enterobacteriaceae is a challenging problem for clinicians. MHT is the CDC recommended screening test with a sensitivity & specificity of >90% . Carbapenems are last resort antibiotics for multidrug-resistant *Enterobacteriaceae* . However, resistance to carbapenem is increasing at an alarming rate worldwide leading to major therapeutic failures and increased mortality rate. Early and effective detection of carbapenemase producing carbapenem-resistant *Enterobacteriaceae* (CRE) is therefore key to control dissemination of carbapenem resistance in nosocomial as well as community-acquired infection. The aim of present study was to evaluate efficacy of Modified Hodge test (MHT) for early detection of carbapenemase producing *Enterobacteriaceae* (CPE).

Aim & Objectives: The aim of the present study to detect carbapenem resistant or carbapenemase production in Enterobacteraceae using MHT.

Materials and Methods: Study period was between Feb-April 2014. 100 urine & pus/wound swab samples were included in the study. Isolates of Enterobacteriaceae were identified to genus/species level. MBL detection was done for 85 samples as per CLSI guidelines. 25 were MBL producers. MHT was done for these 25 isolates. 0.5McFarland dilution of E.coli ATCC 25922 in 5ml broth or saline was prepared. Lawn culture of E.coli ATCC 25922 done. Imipenem 10µgm disc was placed in the center. Test organism was streaked from the edge of plate to the edge of the disc & incubated overnight.

Results: In the present study, Isolates included E.coli -54 (64%), Klebsiella spp..- 17 (20%) & Proteus spp..- 14 (16%). Positive MHT had a Clover leaf type indentation at the intersection of the test organism and E.coli25922 within the sensitive zone. Of these 4 (7%)

Pseudomonas, 1(6%) Klebsiella spp. & 1(7%) E.coli spp.. were found to be Carbapenemase producers.

Conclusion: Although MHT is routinely used, advanced molecular methods is to be done for confirmation which we intend to do in the future. *Enterobacteriaceae* members are among the most common and easily transferable bacterial species responsible for severe HAI. This study revealed a high percentage of *Enterobacteriaceae* clinical isolates producing carbapenemases in India. Detection of such bacteria, formulating hospital antibiogram, and monitoring the usage of antimicrobial drugs is recommended.
Keywords: Carbapenem, MHT, Enterobacteraceae, E.coli, Pseudomonas

Introduction

Carbapenem resistance in Enterobacteriaceae is a challenging problem for clinicians. MHT is the CDC recommended screening test with a sensitivity & specificity of >90% . The global spread of carbapenemase-producing gram-negative bacilli in the last decade is a serious health threat, and limited treatment options are available for such infections . Carbapenemases have increasingly been reported in enterobacteriaceae worldwide. Carbapenemases, are *β*-lactamase enzymes with a capacity to hydrolyze not only the carbapenems but also all the other beta lactam agents. [1]

Most carbapenemases are plasmid encoded hence resistance can easily spread. Carbapenem-resistant enterobacteriaceae are reported to cause mortality in up to 50% of patients who acquire bloodstream infections.

Clinically important carbapenemases include Class A – most common type isolated clinically is *Klebsiella pneumoniae* carbapenemase (KPC), Class B – metallo-β-lactamases having a Zn^{2+} ion at its core, e.g., New Delhi metallo-beta-lactamase - 1 (NDM-1), Verona integron-encoded metallo-β-lactamase (VIM) type, and Imipenemase (IMP) type, Class D – oxacillin-hydrolyzing β-lactamases or oxacillinases which have a serine molecule at its core, e.g. OXA-48, OXA-181.[1]

Discovered in 2008 in Sweden from an Indian patient hospitalized previously in New Delhi, NDM-1-positive Enterobacteriaceae has become the focus of worldwide attention.

The Infectious Diseases Society of America brought major attention to this problem and to the need for novel therapeutics with the call-to-action “Bad Bugs No Drugs” campaign, and with the acronym “ESKAPE” pathogens (*Enterococcus faecium, S. aureus, Klebsiella pneumoniae, Acinetobacter baumannii* species, *Pseudomonas aeruginosa,* and *Enterobacter* species) ; 4 of the 6 “ESKAPE” bacteria are gram-negative bacilli, and 2 are Enterobacteriaceae. [2]

Multidrug resistance within Enterobacteriaceae is not a new phenomenon. The rise of extended-spectrum β-lactamase (ESBL)–producing Enterobacteriaceae (resistant to penicillins, cephalosporins, and monobactams) has reached high levels, and the number of unique ESBL protein sequences exceeded 1000 in 2011 . For serious infections, carbapenems have been the preferred, and at times only, treatment. [3]

Most recently, the emergence of carbapenemases carried on mobile genetic elements, such as transposons or plasmids that can harbor additional resistance genes affecting multiple classes of antibiotics, has led to high level antibiotic-resistant bacteria, and the mobile resistance elements often have transferred into strains capable of efficient person-to-person spread. Rapid and accurate detection of resistance mechanisms is essential for determining appropriate antimicrobial therapy and infection control measures.

The most common carbapenemases include veronica integron metallo-beta-lactamases types (VIM), imipenemase (IMP) types, *Klebsiella pneumoniae* carbapenemase (KPC), oxacillinase-48 (OXA-48), and New Delhi metallo-beta-lactamase-1 (NDM-1), encoded by carbapenem resistance determining genes *bla*VIM, *bla*IMP, *bla*KPC, *bla*OXA-48, and *bla*NDM, respectively. Phenotypic assays are used to identify carbapenemase activity while molecular assays have been developed to identify carbapenemase encoding genes . [4,5]

Carbapenem resistance in *Enterobacteriaceae* may be due to various reasons that include hyper production of the Amp C beta lactamase, loss of porins, production of metallo-beta-lactamases (MBL) and production of *K pneumonia* carbapenemases. The most important carbapenemase determinants responsible for resistance or reduced susceptibility to carbapenem group of antibiotics in *Enterobacteriaceae* members include class A KPC 1-3, Class D (OXA-48, OXA-181), IMP, VIM, NDM, NMC-A, SME1-3, IMI-1, GES-2, SHV, and SFC. [4] Class A and D enzymes have a serine-based hydrolytic mechanism for cleaving the β-lactam ring in antibiotics. MBLs, by contrast, are class B carbapenemases containing zinc at the active site. [6] Carbapenemase-producing pathogens are considered a serious nuisance as they have the ability to hydrolyze penicillins, cephalosporins, and monobactams as well as carbapenems . [7] Under such circumstances of extreme resistance towards antibiotics, the pathogens remain susceptible only to colistin and tigecycline combination therapy . [8]The actual prevalence of carbapenemase producers among Gram-negative bacteria is still unknown because many countries that are likely to be their main reservoirs have not established any search protocol for their detection.

In the present study, 16.2% (16/138) of Gram-negative uropathogens showed the occurrence of carbapenemase encoding genes, which is almost four times higher than other data reported in Bangladesh, where only 4.8% isolates were found to be carbapenemase producers. However, with its increased use, we may actually trigger resistant mechanisms against these combinations leading to the end of the current era of pharmacopoeia . [9] Multidrug resistant gram negative bacteria due to production of beta lactamase, metallo-beta-lactamases, and carbapenemases are difficult to treat. In view of the alarming increase in the appearance of Carbapenemase-producing bacteria in the clinical isolates a standard testing method should

be followed for detection of carbapenemase producing bacteria. The carbapenemase detection methods include the modified Hodge test (MHT), the double disk test (DDST), blood agar combined disk (BA-CD) assay, PCR amplification, and DNA sequencing. Several tests have been developed for the phenotypic detection of carbapenemases .The modified Hodge test (MHT) is inexpensive and feasible for practically all clinical laboratories. The MHT is a CLSI-recommended phenotypic method for carbapenemase detection.

Modified Hodge Test : All CRE isolates detected by disc diffusion and MIC were also tested by Modified Hodge test. Modified hodge test (MHT) is recommended by CLSI as isolates of Enterobacteriaceae producing KPC-type carbapenemase have a high level of sensitivity (>90%) and specificity. [10] This recommended method detects carbapenemase in *Enterobacteriaceae* isolates but not in *Pseudomonas* spp. Although the MHT often has high sensitivity , its interpretation is often difficult and subjective . Moreover, many studies have demonstrated false-positive results in the presence of extended-spectrum β-lactamases (ESBLs) and AmpC β-lactamases.

Materials and Methods

Study period was between Feb-April 2014. 100 urine & pus/wound swab samples were included in the study. ETP disks were placed on Mueller-Hinton agar (MHA) (Becton-Dickinson, Cockeysville, MD, USA) plates seeded with *Escherichia coli* ATCC 25922 Isolates of Enterobacteriaceae were identified to genus/species level. MBL detection was done for 85 samples as per CLSI guidelines. 25 were MBL producers. MHT was done for these 25 isolates. In Modified Hodge Test the growth was suspended in Normal saline and matched to Mcfarland standard (0.5). It was diluted 1:10 by adding 0.5 ml of the Mcfarland to 4.5 ml of saline. 0.5McFarland dilution of E.coli ATCC 25922 in 5ml broth or saline was prepared. Test organism was streaked from the edge of plate to the edge of the disc & incubated overnight. ETP disks were placed on Mueller-Hinton agar (MHA) (Becton-Dickinson, Cockeysville, MD, USA) plates seeded with *Escherichia coli* ATCC 25922. Lawn culture of E.coli ATCC 25922 done. Imipenem 10µgm disc was placed in the center. The isolates were inoculated in a straight line from the edge of the disk to the edge of the plate. The plates were incubated at 35℃ for 16-20 hr. Enhanced growth of the indicator strain was measured in mm with a ruler. The length of the straight line from the enhanced growth obtained from the isolate to the end of inhibition zone was classified as negative (<2 mm), weakly positive (2-3 mm), and positive (≥4 mm). When a clear area was observed around the streak, the MHT result was considered indeterminate.

After incubation period, the plates were examined for a clover leaf type of pattern of indentation at the intersection of growth of the test organism and the standard strain *E. coli* ATCC 25922, within the zone of inhibition of the Imipenem disc . [11]

Interpretation of Modified Hodge Test: Enhanced growth = positive for carbapenemase production. A positive test shows a clover leaf like pattern of indentation of *E. coli* ATCC

25922 which grows along the growth of test isolate within the zone of disc diffusion. A negative test shows no growth of *E. coli* ATCC 25922 along the growth of test organism within the zone of disc diffusion. [12]

Modified hodge method

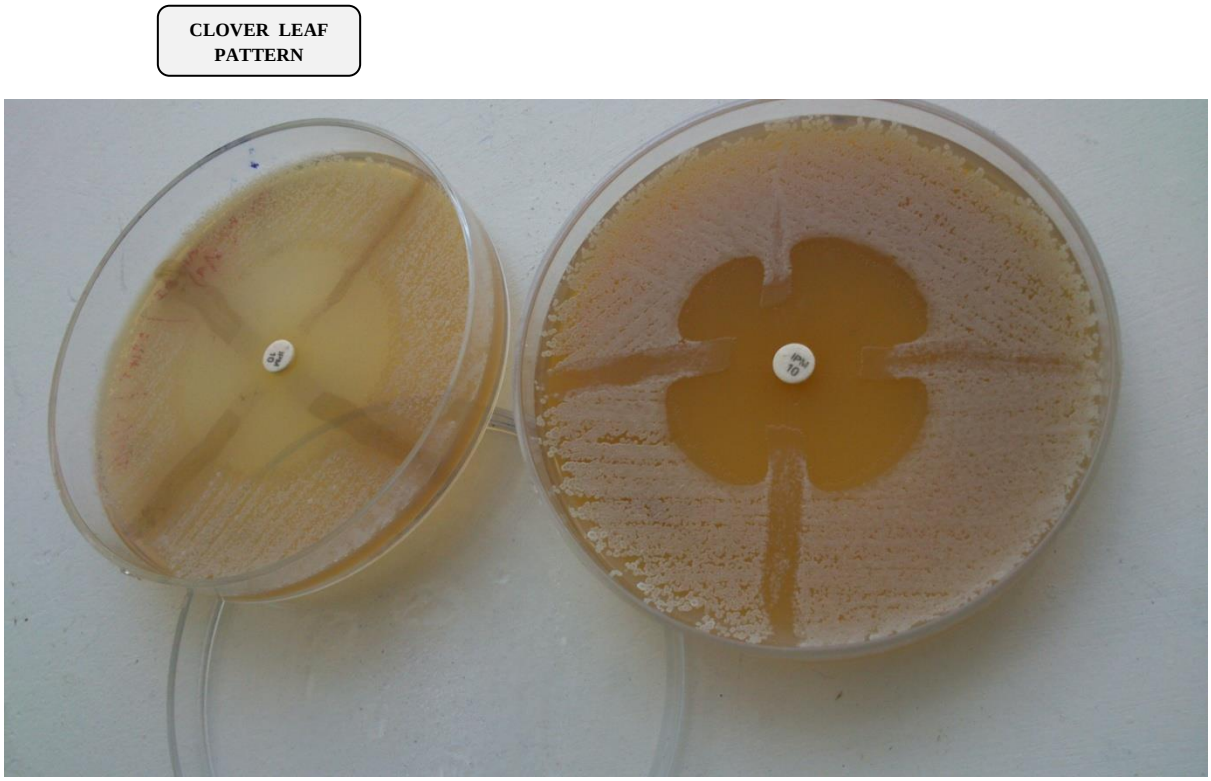


Fig: 1

Fig ; 2

Results

Isolates included E.coli -54 (64%), Klebsiella spp..- 17 (20%) & Proteus spp..- 14 (16%). Positive MHT had a Clover leaf type indentation at the intersection of the test organism and E.coli25922 within the sensitive zone. Of these 4 (7%) Pseudomonas, 1(6%) Klebsiella spp. & 1(7%) E.coli spp.. were found to be Carbapenemase producers.

Table 1. Organisams isolated

ORGANISM ISOLATED	NO.	%
E.COLI	54	64%
KLEBSIELLA SPP.	17	20%
PROTEUS SPP	14	16%

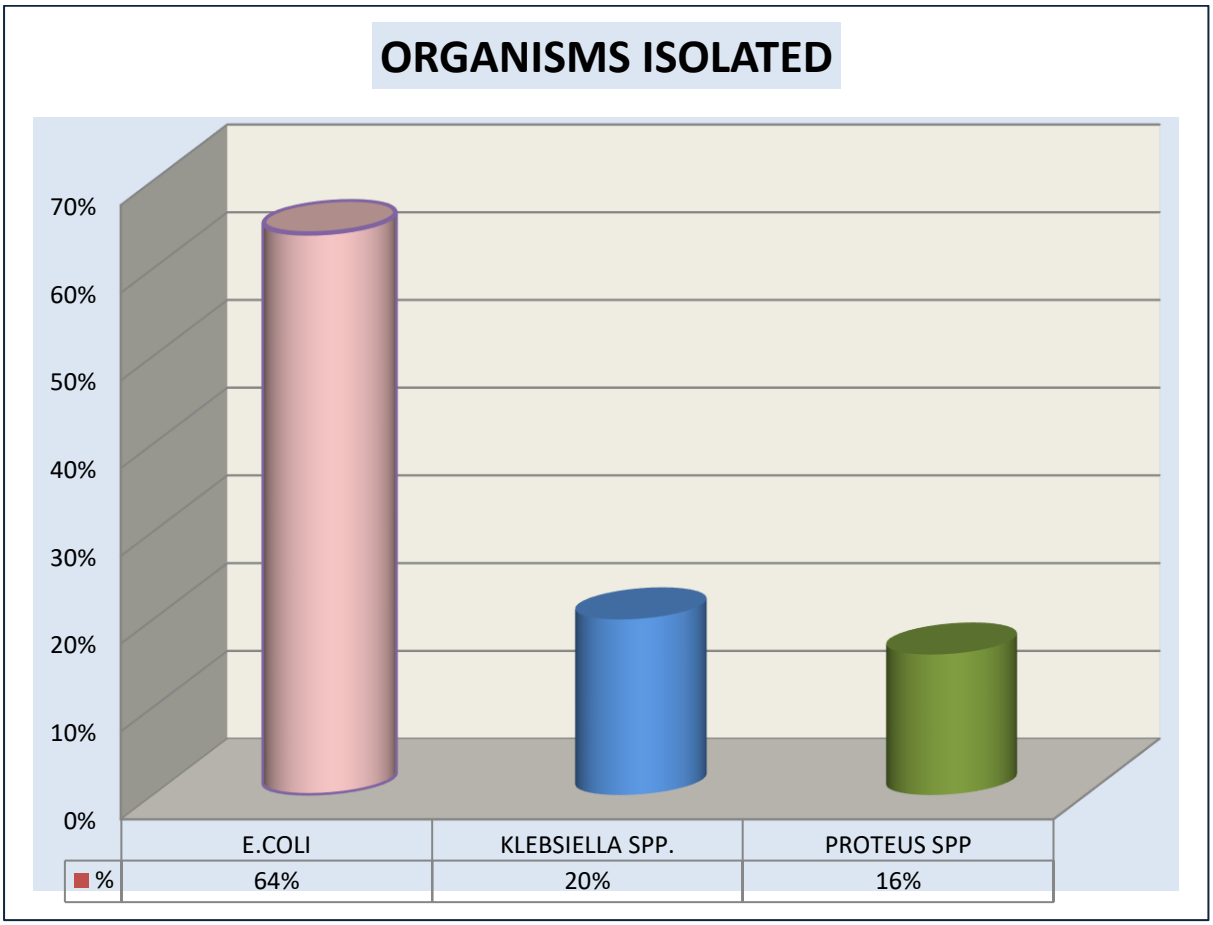
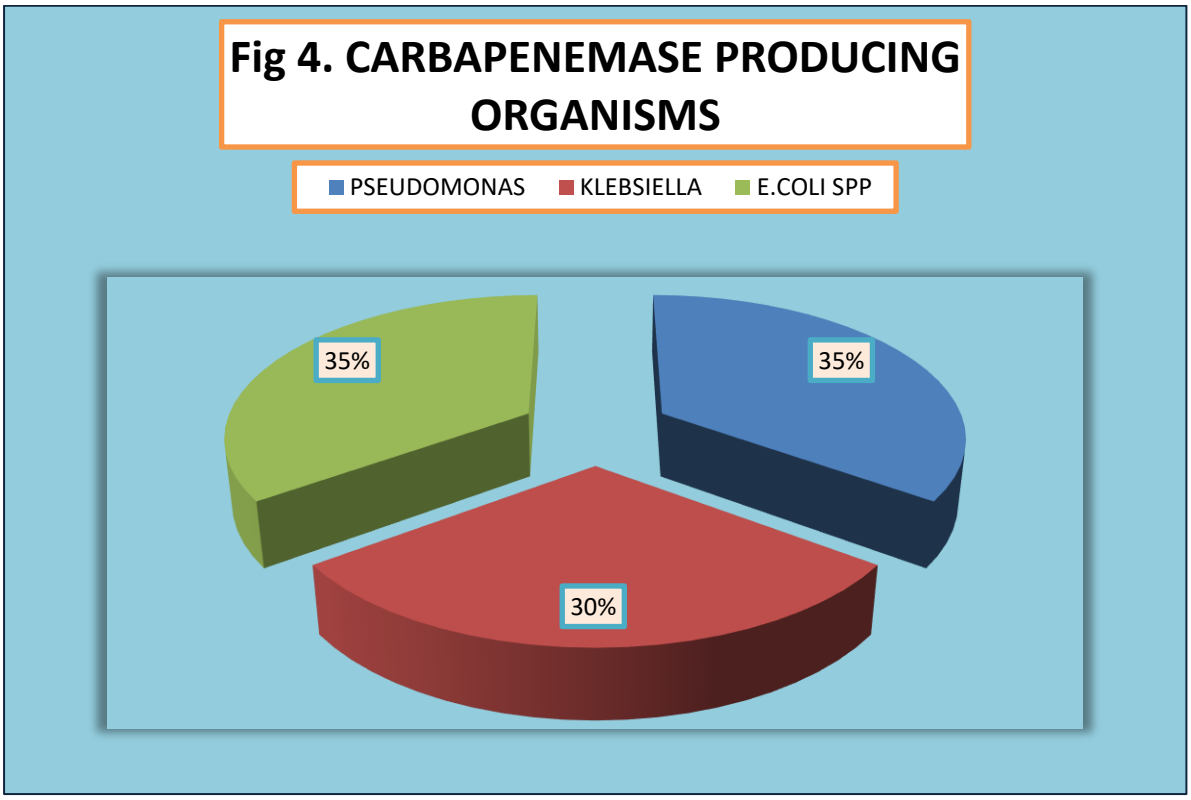


Fig 3. Organisms isolated

Table 2. Carbapenemase producing organisms

CARBAPENEMASE PRODUCING ORGANISMS	NO.	%
PSEUDOMONAS	4	7%
KLEBSIELLA	1	6%
E.COLI SPP	1	7%



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. From India various studies have found different rates of carbapenem resistance. In August 2004 and July 2005 a study was conducted in Aligarh. In this study overall Imipenem resistance was 12% for *Klebsiella* species. [13] In July 2011 to January 2013 a study was conducted in Meerut which showed 5-6% carbapenem resistant in *Enterobacteriaceae* . [11,14] Aswani et al. found 7% carbapenem resistance in *E.coli* and 5% carbapenem resistant in *Klebsiella* species. [15] In other developing countries from African continent, the prevalence of carbapenemase producing bacteria ranged from 2.3% to 6.7% in North Africa and from 9% to 60% in Sub - Saharan Africa. [16] In the present study, the overall resistance to carbapenems was 20 % which is in comparison with the study of Manoharan and Premalatha et al. [17]who reported 17% resistance to carbapenems in *Enterobacteriaceae.* Also, Priya dutta et al.,[18] and Gupta E et al. [19] showed 7.87%, 13-57% and 17-22% resistance to carbapenems respectively. The MHT screening test for carbapenemases is currently proposed by the Clinical and Laboratory Standards Institute (CLSI) for phenotypic screening of Carbapenemase 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.

A study by Okoche D et al, showed resistance of 22.4% among Enterobacteriaceae resistant to third generation cephalosporins. [21] Our phenotypic prevalence was very high compared to 2.8% observed in Morocco using MHT screening .[22] The difference in these findings could be

because the data from Morocco came from reported epidemic outbreaks of infection due to carbapenem-resistant *Klebsiella* spp. and *E.coli* strains in a hospital setting.

Our prevalence is also much higher than that obtained in studies from China and Germany. [22,23,24] as well as in a surveillance study in Spain which reported carbapenemase-encoding gene prevalence of 0.04% . [25] These differences may be due to restricted use of antibiotics in these countries compared to Uganda where most drugs are available over the counter without prescription by a clinician. [26] Our findings are however comparable to those observed in Nigerian, where a study reported a revalence of 33.5% in a hospital setting. [27] Khajuria *et al*., in a study in carbapenem-resistant isolates of *A. baumannii*, have reported MIC values for imipenem and meropenem ranging from 16 to 64 mg/L in 83.87% isolates and 60% isolates positive for carbapenemase production by MHT. [28] High MIC values of imipenem (MIC_{50} = 64 µg/ml and MIC_{90} = 256 µg/ml) and meropenem (MIC_{50} = 32 µg/ml and MIC_{90} = 256 µg/ml) were also reported in a study involving carbapenem-resistant isolates of *P. aeruginosa*. [29] Datta *et al*., in a study in North India, have reported phenotypic carbapenemase production in 51% *K. pneumoniae* and 15% *E. coli* bloodstream infection isolates. [30] Another study in South India has reported carbapenemase production as 14.3% in *A. baumannii* and 28.1% in *P. aeruginosa* by MHT. [31]

A study done in a tertiary care center in Italy has reported that MHT correctly identified 95/101 carbapenemase producers with sensitivity and specificity of 94% and 100%, respectively. [32]

Arnold *et al*. also have reported MHT to be 100% sensitive for detection of carbapenemases though not very specific for KPC production. [33] There is coexistence of non-β-lactam resistance determinants along with carbapenemase enzyme. Also, since this enzyme can easily be transmitted via transposon and or integron, there is possibility of widespread dissemination among susceptible gram negative bacterial isolates in the hospital. Additionally detection and surveillance of CRE has become a matter of major importance for the selection of appropriate therapeutic schemes and implementation of infection control measures. The evolution of carbapenemases in terms of host range is rapid and there is potential for emergence of new variants because the genes are associated with mobile genetic elements. Detection tests are still evolving, hindered by the heterogeneity of both enzymes and hosts, which confer different levels of carbapenem susceptibility. The need of the hour is microbiological laboratory that detects CRE accurately and timely for better patient outcomes. [34]

Conclusion

Carbapenemase production in Gram negative bacteria is not the cause of specific types of clinical infections. The role of these bacteria is related to the difficult-to-treat infections rather than to expression of specific virulence traits due to non availability of higher drugs. Carbapenem resistance in Gram-negative bacteria is increasingly encountered in healthcare-associated infections in India. But, active surveillance, hand hygiene, contact precautions, and

appropriate antibiotic usage form an effective approach in reducing the incidence of infections caused by these life threatening microorganisms.

Non molecular tests for detection of carbapenemases have variable results for Modified Hodge Test, EDTA disk synergy test, MIC by Agar Dilution Test. Out of these three tests the Modified Hodge Test (MHT) often lacks specificity (false positive results for high - level Ampicillin C producers) and sensitivity (weak screening of NDM producers). But this test works well for the detection of KPC and OXA - 48 producing Gram negative bacteria.

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Conflict of Interest

None

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Nil

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