ISSN- O: 2458 - 868X, ISSN–P: 2458 – 8687
National Library of Medicine - ID: 101731606

International Journal of Medical Science and Innovative Research (IJMSIR)
IJMSIR : A Medical Publication Hub
Available Online at: www.ijmsir.com
Volume – 7, Issue – 5, September – 2022 , Page No. : 193 – 203

Phenotypic detection of Carbapenemase-producing Enterobacterales from Modified Carbapenem Inactivation Method amongst gram-negative blood culture clinical isolates

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Citation this Article: Dr. Kirti Nirmal, Vikas Saini, L. Jothisri, Narendra Pal Singh, Shukla Das, "Phenotypic detection of Carbapenemase-producing Enterobacterales from Modified Carbapenem Inactivation Method amongst gram-negative blood culture clinical isolates", IJMSIR- September - 2022, Vol – 7, Issue - 5, P. No. 193 – 203.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Introduction: The amount of members of the order Enterobacterales resistant to multiple antimicrobial classes has developed, and clinicians increasingly turn to agents from the broad-spectrum carbapenem class as options of last resort for the effective treatment of severe infections caused by these microrganisms. The worldwide emergence and extend of antimicrobial resistance in Gram-negative bacteria are severely limiting therapeutic options, posing a major threat to the public.

Materials and Methods: This prospective study was conducted in the Department of Microbiology, tertiary care hospital Delhi from January 2022 to April 2022. 30 clinical blood culture isolates of Enterobacterales which showed resistance to tested antibiotic meropenem according to the recommended latest CLSI guidline. The carbapenemase-producing Enterobacterales (CPE) microorganism was detected by the modified carbapenem inactivation (mCIM) method and interpreated according to standard guidelines.

Statistical analysis: All data were entered in MS Excel work sheet. Clinical details were taken in patient record profroma. We performed statistical analysis using SPSS 21.

Result: Out of 30 phenotypic suspected CRE isolates males were predominated over females. 46% of isolates belong from newborn babies'≤28 days of birth. 86% isolates were showed CP-CRE by mCIM. Although, 14%

isolates were showing non Carbapenemase producing-carbapenem resistant Enterobacterales (non CP-CRE) with mCIM. Klebsiella pneumoniae (50%) was the most common organism was isolated in the Enterobacterales family

Conclusion: The mCIM is a test that has high sensitivity and specificity as compared to others CRE detection methods. However, to get an accurate and consistent result, mCIM being a phenotypic test, is to be done under standardized conditions as described in CLSI 2022.

Keywords: Carbapenemase-producing Enterobacterales, Blood culture isolates, Disk diffusion method, Modified carbapenem inactivation method.

Introduction

The worldwide emergence and extend of antimicrobial resistance in Gram-negative bacteria are severely limiting therapeutic options, posing a major threat to the public.[1] The amount of members of the order Enterobacterales resistant to multiple antimicrobial classes has developed, and clinicians increasingly turn to agents from the broad-spectrum carbapenem class as options of last resort for the effective treatment of severe infections caused by these microrganisms.[2] Carbapenems are a class of β-lactam antibiotics with a broad spectrum of antibacterial activity and are generally stable against most beta-lactamase enzymes produced by bacteria. Therefore, carbapenems have been considered as the last line of defense against bacterial infection caused by multiple drug-resistant microorganisms. However, the widespread emergence and spread of carbapenem-resistant Enterobacterales (CRE) worldwide have posed problems in the appropriate treatment of infections caused by CRE and have also had implications for unproductive infection control interventions.[3] Adding to the above problems, the resistant mechanisms of CRE some how vary and are dynamic, leading to a certain degree of difficulty on detection. The prevalence of CRE, according to published literatures in the epidemic area, varies between 24.7% and 29.8% [4, 5]. In India, the prevalence of carbapenem resistance in Enterobacteriales varies widely from 12% to 97% across the country. [6-9]

There are various risk factors for carbapenem producing Enterobacterales (CPE) in bloodstream infections (BSI). A study published in a teaching hospital in Shanghai, China suggested that skin and soft tissue infection (odds ratio [OR] 26.63 and ICU-acquired infection (OR 5.82) was a risk factor for CPE BSI. [10] Multisite colonization (hazard ratio [HR] 13.73), ICU stay (HR 3.14) & previous BSI (HR 6.62) was associated with the development of CPE BSI in colonized patients. [11] Primary liver disease and hepatitis C virus infection or hepatocellular cancer were significantly associated with the development of CPE in intensive care unit (ICU) patients after orthotopic liver transplantation .[12]

It is generally accepted that carbapenem-resistant BSIs are associated with high morbidity and mortality, mostly because of the paucity of antimicrobials active against CRE and the multiple comorbidities of patients.[13] Severe infection causes organ dysfunction and/or failure via complex mechanisms, including pathogenic microorganisms, an excessive inflammatory response, and immune dysfunction. Although two groups of resistant mechanisms, non-carbapenemase (such as a combination of other beta-lactamase enzymes, including AmpC or extended-spectrum b-lactamase (ESBL) and loss of porins) and carbapenemase-producing are reported, the latter group is responsible for greater impact on the spreading of resistant strains. At present, more than a hundred carbapenemase genes are reported. [14] Several carbapenem-resistant (CR) genes such as

blaKPC, blaNDM, and blaOXA-48-like are commonly found worldwide. However, the distribution of some CR genes, that is, blaIMP, blaVIM, blaSPM, blaSIM, and blaGIM is reported predominantly in certain regions. Accurate, rapid, and uncomplicated methods for the detection of carbapenemase are required to identify the resistant mechanism of the suspect organisms. Several phenotypic detection methods of carbapenemase-producing isolates have been developed and used routinely in clinical microbiology laboratories. [15-17]

At present, phenotypic tests modified carbapenem inactivation method (mCIM), is recommended by the Clinical Laboratory Standard Institute (CLSI) for the production of carbapenemases since 2015.[18] The initial description of the CIM reported very promising results, including high sensitivity for the detection of a variety of carbapenemases and excellent specificity. [19] The test was straightforward to perform and interpret and involved low-cost materials already readily available in clinical laboratories. [19, 20] This method mCIM has shown to be an excellent candidate for the detection of carbapenemases.[20-22] The present study aimed to studied the phenotypic detection of Carbapenemase producing Enterobacterales from Modified Carbapenem Inactivation Method amongst gram negative blood culture clinical isolates.

Materials and Methods

This prospective study includes 30 clinical blood culture isolates of Enterobacterales which showed resistance to tested carbapenems antibiotic (meropenem) according to the recommended latest clinical laboratory standard institute (CLSI M100-Ed 31st) guidelines by disk diffusion method. This study was conducted in the Department of Microbiology, University College of medical sciences, and associated GTB hospital, Delhi from January 2022 to April 2022[18] Antimicrobial susceptibility testing (AST) of these isolates was performed using the Kirby-Bauer disk diffusion method. The carbapenemase-producing Enterobacterales (CPE) microorganism was detected by the modified carbapenem inactivation (mCIM) method and interpreated according to CLSI M100-Ed 31st guidelines. [21]

Methodology of mCIM method: The 10-µg meropenem (MEM) disk (HiMedia Laboratories Pvt. Ltd) was added to 2 ml of tryptic soy broth (TSB) (HiMedia Laboratories Pvt. Ltd) inoculated with a 1-µl loopful of bacterial isolate (about one colony), and the tube was incubated for 4 h at 37°C in the incubator. Simultaneously, a Mueller-Hinton agar (MHA) plate was streaked with the carbapenem-susceptible E. coli ATCC 25922 reference strain from a 0.5 McFarland standard inoculum and incubated in ambient air for 35˚C± 2°C. This early incubation promoted the start of E. coli growth. After 4 hours of incubation, the meropenem disks (10µg) were removed from the TSB bacterial suspension and placed on the meropenem susceptible E.coli ATCC 25922 indicator strain streaked MHA plates. The plates were incubated in ambient air at 35˚C± 2°C for 18–24 h. Following incubation, the zone diameters were measured and interpreted according to the standard CLSI guidelines. The inhibition zone was measured after overnight incubation for the detection of carbapenemase activity. Therefore, a zone diameter of 6 to 15 mm or the presence of pinpoint colonies within a 16-18 mm zone and if the tested isolates produce a carbapenemase, the meropenem in the disk were be hydrolyzed and there was no inhibition or limited growth inhibition of the meropenem susceptible E.coli ATCC 25922 considered a positive result. The zone diameter of 16 to 18 mm or zone diameter of ≥ 19mm and the presence of pinpoint

colonies within the zone was considered an indeterminate result (requiring further testing to establish the presence or absence of carbapenemase production). A zone diameter of ≥19 mm and if the tested isolates do not produce carbapenemase, the meropenem in the disk were not hydrolyzed and there was inhibit the growth of the meropenem susceptible E.coli ATCC 25922 was considered a negative result.[18]

Statistical analysis

All data were entered in MS Excel work sheet.Clinical details were taken in patient record profroma. We performed statistical analysis using SPSS 21. Fisher's exact test was used to find significant association. P value <0.05 was considered statistically significant.

Results

In this study, 30 phenotypic suspected carbapenem-resistant Enterobacterales (CRE) blood culture clinical isolates from the Kirby Bauer disk diffusion method were enrolled for differentiation of carbapenemase-producing carbapenem-resistant Enterobacterales (CP-CRE) from modified carbapenem inactivation method (mCIM). The clinical parameters and blood investigation of all the suspected CRE isolates were taken in the case record proforma.

Out of 30 phenotypic suspected CRE isolates males were predominated over females (M: F= 2.5:1). 46% of isolates belong from newborn babies (≤28 days of birth) while, 64% of patients belong to more than 28 days of birth up to 60 years.

Out of 30 suspected CRE blood culture isolates from the Kirby Bauer disk diffusion method, 26 (86%) isolates were showed CP-CRE by mCIM. Although, 4 (14%) isolates were showing non Carbapenemase producing-carbapenem resistant Enterobacterales (non CP-CRE) with mCIM. (Table 1) So, there could be possibility that it's could be non-CP CRE may be result of amplification of non- carbapenmase beta lactamase gene with concurrent outer memberane protein porin disruption.

Out of 30 suspected CRE blood culture isolates, 85 % concordance and 15% disconcordant results of antibiotic inhibition zone size were found between mCIM and Kirby Bauer disk diffusion methods. Four Enterobacterales isolates showed CRE by Kirby Bauer disk diffusion method while these isolates were resistant (≥15mm) inhibition zone size by mCIM. It predicted non-carbapenemase producing carbapenem-resistant Enterobacterales. (Table 2)

Amongst CRE blood culture isolates, Klebsiella pneumoniae (50%) was the most common organism was isolated in the Enterobacterales family. (Figure 1)

Out of 30 CRE isolates, 4 isolates/ patients were non-carbapenememase producing Enterobacterales. These 4 patients were sick clinically and died later as a final outcome. These 4 patients were resistant to multiple groups of antimicrobial classes of drugs. The clinical risk factors and outcomes of phenotypic detection of carbapenem resistant Enterobacterales in blood culture isolates were predicting in Table no 3.

Discussion

The mCIM is a new detection method announced by CLSI with further improvements such as that for incubation conditions. [23] The present study was showing the superior performance of mCIM compared to the disk diffusion method for the detection of carbapenemase-producing Enterobacterales. The CIM is a method for detecting carbapenemase reported by vander Zwaluw et al., in 2015 that not only exhibits high sensitivity and specificity but has also been evaluated as a cost-effective method with high practicality. [24] In this study mCIM showed excellent results with a sensitivity and specificity

of 100% for the detection of CRE. This excellent result was equal to that in the evaluation report of Yamada et al. and CLSI guidelines. [23, 25] This mCIM is considered to be superior as cmpared to the conventional Modified Hodge test (MHT) carba NP test and carbapenem inactivation method (CIM). The MHT and carba NP test have been replaced because of subjective interpretation, false positives with some Enterobacter spp. possessing AmpC enzymes and porin alterations and false negatives with NDM-1 carbapenemase. [26]

In this study patients' ages ranged from newborn to 60 yrs old. This wide range was because the study included all blood samples which were CRE positive by a conventional method, and the place of study was a tertiary care government hospital where all age group population reports for the treatment. It was observed that the measures of central tendency for age belong to newborn babies (≤28 days of birth), which may be because newborn babies group are frequently admitted and also have decreased immune response, which makes them prone to infections, increase use of antibiotics, subjecting to increased risk of harboring resistant organisms. [27] This finding was in contrast to other published studies where the elderly age group people were more prevalent. [28] Males were more predominated than females (M: F= 2.5:1) in present study. Our gender distribution findings corroborate with other studies. [29] The higher male prevalence in our study was because this study was conducted in a tertiary care hospital in east Delhi where predominant population are males.

In this study prevalence of CRE was estimated to be 41.66 % which coincides with the various studies conducted in various parts of India which showed CRE prevalence ranging from 3 % to 60 % depending on the place of study and the study population, being higher in hospitalized ICU patients. [30] Whereas a study from Philadelphia, USA showed a high prevalence of CRE(60.22 %).[31] Present study showed the high prevalence of CRE in blood culture isolates because the study was framed in a government tertiary care setting, where critically ill patient got referred who have already been received treatment so not responding to the treatment. The latest standard CLSI guidelines 2022, with revised zone diameter for resistance and sensitive criteria, do not mandate conducting mCIM on a routine basis on a patient's sample.[18] As mCIM detects only CP-CRE, however, there are other mechanisms of resistance other than carbapenemase production like excessive efflux pump system, decrease in drug penetration by modification of diffusion barriers, and altered metabolic activity. [32, 33] In this study there are four isolates that were mCIM negative which probably may be due to the presence of non-CP-CRE which needs to be further validated through genetic study.

The most common CRE microorganisms were Klebsiella pneumonia (50%) followed by Citrobacter species (40%) and E.coli (10%) which correlates with different previous studies. The published studies also showed that Klebsiella pneumonia is the most common organism among Enterobacterales. [34-36] The mCIM and Kirby Bauer disk diffusion tests are phenotypic tests used to detect carbapenem resistance in CREs which is dependent on many variables like media components and thickness, incubation period and temperature, reading method, subjective variation in interpreting the result depending on the person's reading the result, etc. Though most of the parameters are standardized few are difficult to standardize consistently which affects the reproducibility of phenotypic tests like mCIM and Kirby Bauer disk diffusion tests. However, mCIM is shown to

have excellent reproducibility. In one of the studies, a validation study on mCIM was conducted to see its reproducibility, where the mCIM test which was carried out by a lab was repeated by nine other labs. The result showed excellent reproducibility across laboratories.[37]

The limitation of this study is that the sample size was limited and the result of CRE was not compared with the genetic study to see the presence of molecular resistant genes in the isolates. These tests were carried out in a laboratory setting where the environment subjected to the organisms is not similar to in vivo, hence, the induction of certain genes may vary which may affect the results of the phenotypic study.[38]

Acknowledgment

I would like to acknowledge to blood seat senior technical staff Mr. Narender pal Singh for processing of blood samples.

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Legend Figure and Tables

Figure 1: Distribution of phenotypic detection of carbapenem-resistant Enterobacterales organisms in blood culture isolates (n=30).

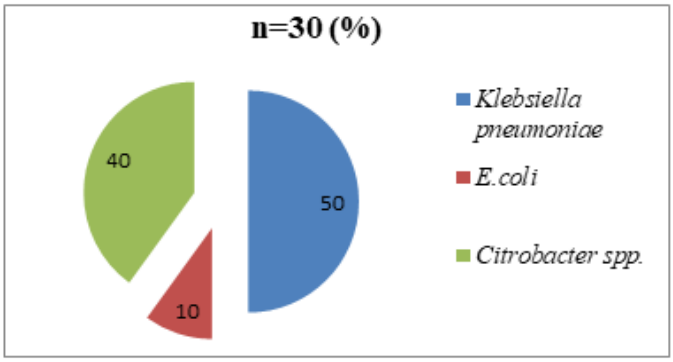

Figure 1: Distribution of phenotypic detection of carbapenem-resistant Enterobacterales organisms in blood culture isolates (n=30).

Table 1: Phenotypic detection of carbapenem producing Enterobacterales amongst blood culture isolates. (n=30)

Enterobacterales Isolates Tests	Carbapenemase detection method in Enterobacterales isolates (n=30)		Statistical analysis
	Kirby Bauer Disk Diffusion method	Modified Carbapenem Inactivation Method (mCIM)	
Carbapenemase- producing carbapenem- resistant Enterobacterales (CP-CRE)	30	26	0.112 (Fischer's

Non Carbapenemase- producing carbapenem -resistant Enterobacterales (Non CP-CRE)	0	4	exact test)

Table 2: Discordant of Modified Carbapenem Inactivation Method & disk diffusion method for carbapenememase detection in Enterobacterales amongst blood culture isolates. (n=30)

Isolates number	Organisms	Disk diffusion method Zone size (mm)	Modified Carbapenem Inactivation method (mCIM) zone size (mm)
Isolate 1	Citrobacter koseri	17 mm	8 mm
Isolate 2	Klebsiella pneumoniae	18 mm	10 mm
Isolate 3	Klebsiella pneumoniae	20 mm	12 mm
Isolate 4	Citrobacter freundii	21 mm	9 mm

Table 3: Clinical risk factors and outcomes of phenotypic detection of carbapenem resistant Enterobacterales in blood culture isolates (n=30).

Parameters	Category	N=30 (%)
Newborn (≤28 days of birth)	Low birth wieght	10 (33)
	Neonatal jaundice	5 (16.7)
	Meconium stained liquor	4 (13.4)
	Preterm/ premature baby	3 (10)
	Septiceamia (early/late)	5 (16.7)
	Congenital pneumonia	2 (6.6)
Invasive mechnanical ventilation	Yes	10 (33)
	No	20 (66.7)
ICU admission	yes	11(36.7)
	No	19 (63.3)
Steroid therapy	Yes	18 (60)
	No	12 (40)
Diabetes mellitus	Yes	10 (33)
	NO	20 (67)
Hypertension	yes	4 (13.3)
	No	26 (87)
Chronic kidney disease	Yes	5 (17)
	No	25 (83)
Chronic liver disease	Yes	2 (7)
	No	28 (93)

Blood transfusion	Yes	6 (20)
	No	24 (80)
C Reactive protein (mg/dl)	Raised	12 (40)
	Normal	18 (60)
Procalictonin (ng/ml)	Raised	8 (27)
	Normal	22 (73)
Interlukin-6 (pg/ml)	Raised	6 (20)
	Normal	24 (80)
Thrombocytopenia	Present	4 (13.3)
	Absent	26 (87)
Antibiotic therapy (at present)	Present	30 (100)
	Absent	Nil
COVID-19 status	Recovered	18 (60)
	Don't know	12 (40)
Survival outcome	Yes	26 (87)
	No	4 (13.3)