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RESEARCH ARTICLE

A SINGLE STEP CARBAPENEM INACTIVATION METHOD FOR THE DETECTION OF CARBAPENEMASES IN CARBAPENEM RESISTANT *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE.*

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ABSTRACT

The carbapenem resistance is gradually increasing particularly among Enterobacteriales isolated from patients admitted to the ICUs. Therefore a simple, convenient and accurate method for early detection of resistant isolates is essential to curtail rapid dissemination among the patients. The present study aimed to perform the newer single step carbapenem inactivation method (ssCIM) for the detection of carbapenemase production in carbepenem resistant *Escherichia coli* and *Klebsiella pneumoniae* and compared with modified CIM (mCIM) and polymerase chain reaction. The ssCIM test was performed according to Jing et al. The mCIM was performed according to CLSI- 2021 guidelines. A total of 160 carbapenem resistant *E.coli* and *K.pneumoniae* were tested for carbapenemase production by ssCIM and mCIM. The MIC of imipenem ranges between 4 mg/L and16 mg/L. Out of 160 CREK 86 were *E.coli* and 74 were *K.pneumoniae*. Among *E.coli* 84/86 (97%) and 69/74(93%) *K.pneumoniae* isolates tested positive for carbapenemase production by mCIM and ssCIM. In total 153/160(95.6%) CREK isolates were positive by phenotypic tests. A total of 152/160 (95%)isolates were positive for the presence of carpenemase genes. The results of ssCIM are in concordance with mCIM including one false positive result. The overall sensitivity and specificity of ssCIM with PCR was 100% and 87.5% respectively. The ssCIM is a less laborious, more convenient, and reliable method for detection of carbapenamase production among carbapenem resistant bacteria.

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INTRODUCTION

Antimicrobials are the most powerful weapons in the continuous combat between bacteria and human beings. The armamentarium of antimicrobials is becoming depleted with the development of resistance by bacteria to carbapenems, the 'last-resort' drug to treat severe infections caused by Enterobacterales. According to Antimicrobial Resistance Research & Surveillance Network (AMRSN) data, Imipenem susceptibility of *E. coli* was 86% in 2016 and dropped to 63% in 2019, and recovered to 72% in 2020 but the susceptibility of *Klebsiella pneumoniae has* dropped steadily from 65% in 2016 to 45% in 2020. CP-CRE are placed first among difficult -to -treat drug resistant pathogens in the Indian hospitals group by AMRSN. The carbapenem resistance is mainly by plasmid- mediated carbapenem hydrolyzing enzymes. The cross-border transmission of carbapenemase producing carbapenem resistant Enterobacteriales [CP-CRE] has become an important public health concern world wide.[1,2&3] The carbapenem resistance is gradually increasing particularly among Enterobacteriales isolated from patients admitted to the ICUs. The Presence of mobile genetic elements facilitates faster dissemination of CP-CRE between patients. Therefore a simple, convenient ,rapid, and accurate method for early detection of CP-CRE is essential to curtail the spread.[4] Even though the molecular detection of carbapenemases by polymerase chain reaction is considered as a gold standard, it is not suitable for routine use in developing countries. PCR may not detect novel genes coding for newer or unknown carbapenemases other than the target or known genes. Therefore phenotypic tests are still indispensable for the detection of carbapenemase producers in diagnostic laboratories.[3] Clinical Laboratory Standard Institute [CLSI]introduced the Modified Hodge test for the detection of carbapenemases in 2010. Later in the year 2012, CLSI recommended Carba NP test for detection of carpenemase production in gram negative bacilli. Owing to the limitations of carba NP test in clinical application, CLSI recomeneded the modified carbapenem inactivation method (mCIM) in 2017, based on the CIM method designed by van der Zwaluw et al.

(2015). Based on the principle of mCIM, Jing et al. designed simplified CIM [sCIM] for easy and accurate detection of carbapenemase producing Gram negative bacilli[5]. Although CLSI recommended these tests only for epidemiological and infection prevention purposes and not for routine use, understanding the mechanism of carbapenem resistance has treatment implications in optimizing antibiotic therapy, avoiding inappropriate prescription of antimicrobials and prevention of dissemination of these multi drug resistant bacteria to other patients in the hospital[6]. Hence the present study aimed to perform the newer single step ssCIM test for the detection of carbapenemase production in carbepenem resistant *Escherichia coli* [*E.coli*] and *Klebsiella pneumonia* [*K.pneumoniae*] and compared with mCIM and polymerase chain reaction (PCR).

MATERIAL AND METHODS

The study was conducted in the Microbiology laboratory of a tertiary care hospital attached to a teaching institute in Karnataka, India. A total of 160 Carbapenem resistant *E.coli* and *K.pneumoniae* [CREK] previously isolated from rectal swab cultures of patients admitted in the ICU were retrospectively tested for carbapenemase production by carbapenem inactivation methods. Identification of the isolates and susceptibility to carbapenem was tested by using Vitek 2 compact automated culture system. Isolates resistant to any one of the carbapenems [Imepeem, meropenem, ertapeenem] were further tested for carbapenemase production by modified carbapenem inactivation method [mCIM], single step carbapenem inactivation method [ssCIM] and multiplex polymerase chain reaction [PCR]. The mCIM was performed according to CLSI- 2021 guidelines. Briefly, a loopful of overnight grown test isolate on blood agar was inoculated into 2 mL Tryptic Soy Broth (TSB). Subsequently, a meropenem (10 µg) disc was placed in bacterial suspension and incubated at 37˚C. After 4 hours of incubation, meropenem disk from the bacterial suspension was removed carefully and placed on to Muller Hinton Agar[MHA] plates, freshly lawn cultured with the 0.5 McFarland dilution of *E. coli* indicator strain (ATCC 25922). The plates were incubated at 37˚C for 24 hrs. After incubation, the zone diameters were measured. An inhibition zone diameter of 6-15 mm was considered positive test.[7] The ssCIM test was performed according to Jing et al. In brief, the test isolates were freshly sub cultured on to blood agar plates and incubated at 37^0C for 18-24 hrs. Then a 0.5 McFarland dilution of *E. coli* indicator strain (ATCC 25922) was lawn cultured on MHA plates and allowed to dry for 3-5 mimutes. After that, 1-3 colonies of the overnight grown test isolates were smeared onto an imipenem disc(10 µg), then the side of the disc with the test isolate was placed onto MHA plate that was previously lawn cultured with indicator strain. An imepenem disc without any bacterial colonies was placed as a control. All plates were incubated at 37 ^{0} C for 16–18 hrs. Eventhough ssCIM is based on carbapenem inactivation similler to mCIM, the step of 4 hrs incubation of the test isolate along with antibiotic disc was skipped in ssCIM. The test isolate was considered positive for carbapenemase production, if the diameter of zone of inhibition around the disc was within 6-20 mm.[5]

Polymerase chain reaction (PCR): Multiplex PCR was performed to detect the blaVIM, blaIMP, blaNDM, blaKPC, and blaOXA-48 like genes by using previously published primers and cycling conditions.DNA was isolated from bacterial colonies using the boiling lyses method. PCR was performed in 20 µL reaction mixture containing 10 µL Quick-load Taq 2X PCR Master Mix, 2 µL primer mixture, and 2 µL DNA template and 6µL water with the following cycling conditions: 95°C for10 min; 95°C for 1min, 59°C for 30 sec, and 72°C for 1 min for 30 cycles, final extension at 72°C for 10 min. PCR products were visualized on a 1.8% agarose gel[8].

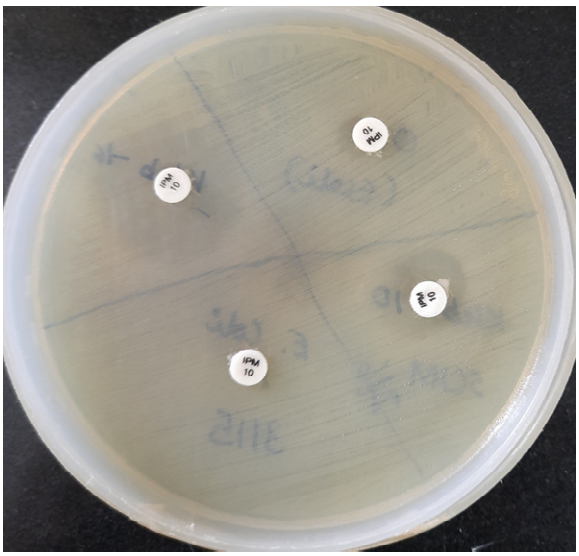

Figure 1. Results of ssCIM- positive test result with 6-10 mm zone of inhibition by 03 isolates and one isolate was negative for carbapenemase production showing 22mm zone of inhibition

RESULTS

A total of 160 carbapenem resistant *E.coli* and *K.pneumoniae*[CREK] were tested for carbapenemase production by ssCIM and mCIM. The MIC of imipenem ranges between 4 mg/L and16 mg/L. Out of 160 CREK 86 were *E.coli* and 74 were *K.pneumoniae*. Among *E.coli* 84/86(97%) and 69/74(93%) *K.pneumoniae* isolates tested positive for carbapenemase production by mCIM and ssCIM. In total 153/160 (95.6%) CREK isolates were positive by phenotypic tests. The zones of inhibition of isolates found to be carbapenemase positive by the ssCIM were 6 mm to 15mm. for CREK isolates. All isolates were subjected for multiplex PCR. A total of 152/160 (95%)isolates were positive for the presence of carpenemase genes. Among *E.coli* isolates, bla NDM gene was detected in 72/86,(83.7%) bla OXA-48 like gene in 07/86(8.1%) and both the genes in 04/86(4.6%) isolates. None of the target genes were found among 03/86 (3.4%)isolates. The carbapenamase genes detected in *K.pneumoniae* isolates were 24/74(32.4%) blaXOA-48 like and 34/74(46%) bla NDM. Both blaNDM and bla OXA -48 like genes were present in 11/74(14.8%) isolates and 05/74 (6.75%) isolates none of the target genes were found. other target genes blaVIM, bla IMP and bla KPC genes were not detected in any of the isolates. Among 08/160 PCR negative isolates one *E.coli* isolate with an MIC for imipenem of 4 mg/L was found to be carbapenemase negative by PCR and positive by both ssCIM and mCIM. The phenotypic tests showed one false positive result. Remaining 05 *K.pneumoniae* and 02 *E.coli* isolates were tested negative for carbapenemases by both mCIM and ssCIM. The results of ssCIM are in concordance with mCIM including one false positive result.

The overall sensitivity and specificity of ssCIM with PCR was 100% and 87.5% respectively.

DISCUSSION

The results of the present study showed that, 95% of the CREK were carbapenemase producers. Hence a reliable method with a shorter turnaround time is required for the early detection of carbapenemase-producing Enterobacteriales for the clinical laboratories to prevent rapid dissemination of carbapenem resistance and to implement proper infection prevention and control policies in the hospital, especially in the ICUs. Even though molecular methods are available phenotypic detection methods are essential for small laboratories in low-resource countries. Among the available phenotypic assays, mCIM is widely used in clinical laboratories because of its simplicity accuracy, and reliability. But the test is labor intensive and time-consuming. Even though the ssCIM is based on CIM, preparation of tryptic soy broth, insertion of meropenem disc into the broth, and the 4hrs incubation step of mCIM is omitted in ssCIM which makes the protocol more convenient and less time-consuming than mCIM[9] In the present study, both mCIM and ssCIM are in 100% concordance including one false positive result. The false positivity may be due to the overproduction of ESBLs and ampC enzymes by the isolate. Wan et al. also stated that, for most carbapenemases, the Km values for imipenem are much larger than meropenem which might lead to more false positive results.[9] Other carbapenem-resistant isolates that were negative by both PCR and ssCIM indicates the existence of other carbapenem resistance mechanisms or the presence of novel gene other than the target genes or variant of target genes.[10] The variations in the size of the zone of inhibition around the imipenem disc might be owing to the enzyme producing capacity of the test strain, slow hydrolytic nature of the enzyme, rate of diffusion of the enzyme into the medium, and concentration of the test isolate on the disc and the indicator strain on the MHA medium. Wan et al. stated that some hypermucoviscous and encapsulated *K. pneumoniae* cells affect the rate of release and diffusion of carbapenemases.[5,9]

CONCLUSION

The ssCIM is a less laborious, more convenient, and reliable method. It can be used as a better alternative method for the detection of carbapenemases in the clinical microbiology laboratory to curtail the rapid spread of carbapenem resistance in the hospital.

Conflict of interest: 'None declared'

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