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DETECTION OF CARBAPENEMASE PRODUCING GRAM NEGATIVE BACILLI WITH SIMPLIFIED CARBAPENEM INACTIVATION METHOD (SCIM)

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ABSTRACT This study reports the Simplified carbapenem inactivation method to detect carbapenemase-producing gram negative bacilli in a simple and accurate manner. This method is based on Modified Carbapenem inactivation method with the improvement of experimental procedure. Instead of incubating the antibiotic disc in the organism culture media, the organism to be tested and smeared directly onto the antibiotic disk in the sCIM. This method is suitable for routine use in most clinical microbiology lab for detection of carbapenemase-producing gram negative bacilli.

KEYWORDS :

INTRODUCTION:

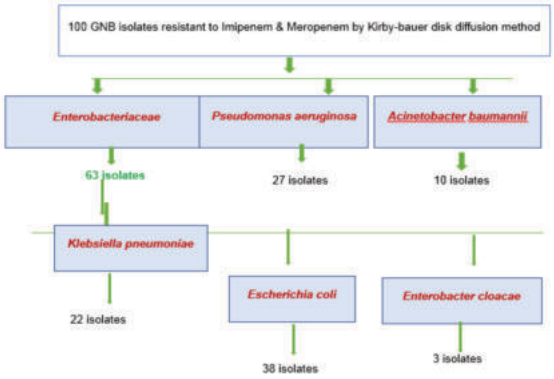
Antibiotics known as carbapenems occupy an important niche because they are able to maintain their action against chromosomal cephalosporinases and extended-spectrum beta-lactamases, which are enzymes that are present in a large number of gram-negative bacteria[1,2]. The discovery of beta-lactamases that hydrolyze carbapenems has posed a danger to the clinical utility of this class of antibiotics and brings us one step closer to the issue posed by "extreme drug resistance" in gram-negative bacteria[3].

A major cause for concern for people's health all around the world is the widespread presence of carbapenemase-producing Enterobacterales (CPE). CPE are able to hydrolyze carbapenems, which are the very last line of defence in the fight against antibiotic-resistant bacteria. In addition, CPE frequently transmitted to other strains of Enterobacterales via mobile components such as plasmids. Because of this, it is essential for clinical microbiology laboratories to be able to detect the presence of CPE. Although molecular detection methods such as polymerase chain reaction and immunochromatography are helpful tools for the detection of carbapenemase production, these sophisticated procedures are quite expensive and require specialised equipment and reagents. In addition, the results of these tests do not necessarily exhibit sensitivity and specificity levels of one hundred percent. Hence, a CPE detection approach that is both straightforward and thorough is required.[4]

The Clinical and Laboratory Standards Institute (CLSI) has recommended using the modified carbapenem inactivation method (mCIM) to detect carbapenemase producers with high sensitivity and specificity. The rapid and reliable identification of carbapenemases is of critical importance in medical care. CLSI (2010) presented the modified Hodge test as a means of carbapenemase detection; however, this technique is restricted to the accurate detection of KPC-type carbapenemases in Enterobacteriaceae and cannot be utilised for any other type of carbapenemase. The Carba NP test method was recommended by CLSI (2012) for the detection of carbapenemases in gram-negative bacilli; however, the preparation of the reagents required for this test is difficult, and the solutions cannot be stored for extended periods of time; this limits the clinical application of the test. A new detection approach known as the carbapenem inactivation method (CIM) was developed by van der Zwaluw and colleagues. This method is user-friendly and very sensitive when it comes to the identification of carbapenemases. In 2017, the CLSI recommended the modified carbapenem inactivation approach, which was based on the CIM method (mCIM). The ability of this approach to detect a wide variety of carbapenemases is demonstrated here. Unfortunately, it is a somewhat complicated technology, and the only bacteria whose carbapenemases it can detect are those belonging to the Enterobacteriaceae family and P. aeruginosa. In the present study, we followed a Simplified Carbapenem Inactivation method (sCIM) designed by Jing X. et al., 2018 which was based on the mCIM, for simple and accurate detection of carbapenemases in Gram negative bacilli and compared it with mCIM method.[5]

MATERIALAND METHODS

This is a prospective hospital-based observational study carried out in the department of microbiology that is attached to Rangaraya Medical College in Kakinada. 100 GNB isolates resistant to Imipenem & Meropenem were included. The time span under consideration for the study was from December 2022 to March 2023.

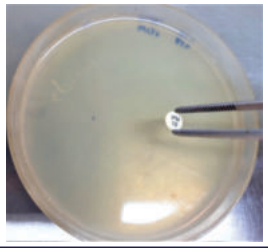


Modified Carbapenem Inactivation method (mCIM):

1 µL loopfuls of Enterobacteriaceae, 10 µL loopfuls of P.aeruginosa, A.baumannii from blood agar plates were emulsified in 2mL trypticase soy broth (TSB). A meropenem disk was then immersed in the suspension and incubated for a minimum of 4hrs at 35°C. A 0.5 McFarland suspension of E.coli ATCC 25922 was prepared in saline using the direct colony suspension method. A Mueller–Hinton agar (MHA) plate was inoculated with E.coli ATCC 25922 using the routine disk diffusion procedure. The meropenem disk was removed from the TSB and placed on an MHA plate previously inoculated with the E.coli ATCC 25922. Plates were incubated at 37°C for 18−24hrs.

Simplified Carbapenem Inactivation Method (sCIM):

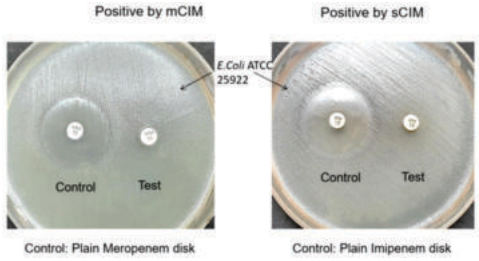
To perform the sCIM, for Enterobacteriaceae, a 0.5 McFarland standard suspension of E.coli ATCC 25922 was inoculated onto the MHA plate. For A. baumannii and P. aeruginosa, a 0.5 McFarland standard suspension of E.coli ATCC 25922 was diluted 1:10 in saline and inoculated onto the MHA plate, following the routine disk diffusion procedure. The side of the disk having bacteria was placed on the MHA plate previously inoculated with E.coli ATCC 25922.



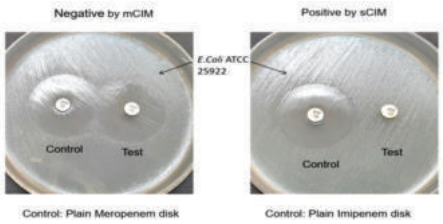
Lawn culture done on the MHA plate with E.coli ATCC 25922 The sCIM is based on the mCIM with improvement of experimental procedures. Instead of incubating the antibiotic disk in the organism culture media for 4hrs as in the mCIM, the organism to be tested was smeared directly onto an antibiotic disk in the sCIM.

Control: Plain Imipenem disk was placed on the MHA plate inoculated with E.coli ATCC 25922.

Carbapenemase producing Enterobacteriaceae



Carbapenemase producing Acinetobacter baumannii



RESULTS & OBSERVATIONS

Table 1: Detection of Carbapenemase producing Gram negative bacilli with Simplified Carbapenem Inactivation Method

Strains	Number of isolates tested	Positive by mCIM	Positive by sCIM
Enterobacteriaceae	63	42	42
Pseudomonas aeruginosa	27	11	11
Acinetobacter baumannii	10	0	9

These results showed that the sCIM and the mCIM has similar rates of detection for CPE and P.aeruginosa. sCIM was found to be superior to the mCIM for the detection of carbapenemase producing A.baumannii.

DISCUSSION:

Whereas the mCIM requires for TSB and MHA plates, the sCIM simply requires MHA plates. With the mCIM, the antibiotic disc is placed into the TSB that contains the test organisms for a period of four hours. On the other hand, in the sCIM, the test organism is immediately spread onto the antibiotic disc, which allows for a significant reduction in the amount of time required. When compared to meropenem, imipenem disc was more readily hydrolyzed by carbapenemases, which led to its selection in the sCIM. When carbapenemase-producing bacteria are smeared onto an imipenem disc, the enzyme spreads out and hydrolyzes the antibiotic on the paper. This causes the zone of inhibition to become smaller in size.

Table 2: Strains and mCIM, sCIM diameters

Strain	mCIM zone diameter(mm)	sCIM zone diameter(mm)
Enterobacteriaceace	<10mm	<10mm
Pseudomonas aeruginosa	10-12mm	<10mm
Acinetobacter baumannii	21-24mm	<10mm

Comparision of zone of inhibition between mCIM and sCIM

The rate at which carbapenem is hydrolyzed by non-fermenting bacteria that produce carbapenemase is slower than the rate at which it is hydrolyzed by enterobacteriaceae that produce carbapenemase. If the concentration of the 0.5 McFarland standard suspension of E. coli ATCC 25922 had been diluted by 10 times, the sensitivity of the sCIM as it relates to the detection of non-fermenting bacteria, such as P. aeruginosa and A. baumannii, could have been enhanced.

Table 3: Test Strains and isolates

	Jing X. et al study[5]			Present study		
Test Strains	No. of isolates	mCIM positives	sCIM positives	No. of isolates	mCIM positives	sCIM positives
Enterobacteriaceae	196	148 (75.5%)	148 (75.5%)	63	42 (66.6%)	42 (66.6%)
Pseudomonas aeruginosa	158	(15.8%) 25	25 (15.8%)	27	11 (40.7%)	11 (40.7%)
Acinetobacter baumannii	73	0	53 (72.6%)	10	0	9 (90%)

Our results in the present study were correlating with the results reported by the sCIM method done by Jing X. et al.[5] sCIM has picked up more carbapenemase producing *A.baumannii* than mCIM.

According Wan D et al.,[6] the spread of carbapenemase-producing Enterobacteriaceae (CPE) is a major threat to public health. A disc containing meropenem and imipenem was used in conjunction with the simplified carbapenem inactivation method (sCIM) in order to identify gram-negative bacteria that produce carbapenemase. The samples that were put to the test included 106 Enterobacteriaceae, of which 74 were CPE, 17 Pseudomonas aeruginosa, of which 10 were carbapenemase-producing isolates, and 36 Acinetobacter baumannii, of which 20 were carbapenem-resistant isolates that had been stored at our laboratory. The test microorganisms were examined using the sCIM method, and each of the antibiotic disks—meropenem and imipenem—was used to examine them. There was a high level of concordance (99.1%) between the utilisation of meropenem disc and imipenem disc in the Enterobacteriaceae family. Due to the limited carbapenem hydrolytic capacity of the carbapenemase produced by these organisms, the Meropenem disc is unable to identify positive isolates among the 10 P. aeruginosa and 20 A. baumannii isolates. Hence, it was discovered that meropenem disc is comparable to imipenem disc, displaying good specificity and sensitivity in the detection of carbapenemase in Enterobacteriaceae; nevertheless, it is unable to be utilised for the detection of carbapenemase in P. aeruginosa and A. baumannii.

According to van der Zwaluw K et al.,[7] Within eight hours, the Carbapenem Inactivation Technique (CIM) was designed to identify carbapenemase activity in Gram-negative rods. This approach demonstrated a high degree of concordance with the data obtained by PCR when it was used to detect genes coding for carbapenemases KPC, NDM, OXA-48, VIM, IMP, and OXA-23. It enables the accurate detection of carbapenemase activity that is encoded by various genes in species of Enterobacteriaceae (such as Klebsiella pneumoniae, Escherichia coli, and Enterobacter cloacae), but it also enables detection of carbapenemase activity in non-fermenters such as Pseudomonas aeruginosa and Acinetobacter baumannii. The CIM has been demonstrated to be an efficient, highly reliable, and cost-effective phenotypic screening approach that is capable of accurately detecting carbapenemase activity.

Authors from various parts of India have reported varying resistance rates of carbapenem in Enterobacteriaceae ranging from 5.75 % to 51% in various gram negative isolates[8,9].In Chandola et al.[10] raised MIC for carbapenems was detected in 27.4% (171/624) of *E.coli*, 58.72%(138/235) of *Klebsiella* and 36.5% (81/222) of *Pseudomonas*. These findings have also been reported by Sathya Pandurangan et al. where in they have reported resistance in *E.coli* at 31%, and *Klebsiella* at 51%.[9]

In Chandola et al.[10] total of 58.55% isolates tested mCIM test positive of which the highest percentage (71.4%) were Pseudomonas spp, while 17.2% isolates were not found to be carbapenemase producers i.e mCIM negative. Of all the methods recommended for carbapenemases detection mCIM is the easiest and cheapest to perform and its interpretation is also very objective[11,12]

CONCLUSION:

The capacity of clinical microbiology laboratories to detect CPE with a

high degree of accuracy is an essential component in the fight against the propagation of these infections. The genotypic test is still considered to be the gold standard for carbapenemase identification; nevertheless, it is not possible to actually perform this test in the majority of clinical laboratories. This article presents the simplified carbapenem inactivation method, also known as sCIM, which was developed to detect carbapenemase-producing Gram-negative bacilli in a straightforward, precise, and speedy manner.

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