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# Comparison of Modified Carbapenem Inactivation Method (mCIM) with Novel Rapid Carbapenemase Detection Method (rCDM) for Detection of Carbapenemase-Producing Organisms from Clinical Isolates

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## ABSTRACT

***Objective:*** To determine the diagnostic accuracy of rapid Carbapenemase detection method (rCDM) for the detection of Carbapenemase-producing organisms, keeping the modified carbapenem inactivation method (mCIM) as the reference method

***Study Design:*** Cross-sectional study

***Place and Duration of Study:*** Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from Jun 21 to May 22.

***Methodology:*** A total of 180 carbapenem-resistant isolates from clinical specimens, including blood samples, respiratory samples (sputum, pleural fluid, endobronchial washings, bronchoalveolar lavage), pus, stool samples, and urine samples of patients of all ages irrespective of gender, were included. All carbapenem-resistant isolates detected using the disc diffusion method were further tested for Carbapenemase production using the novel rapid Carbapenemase detection method (rCDM), and the Clinical Laboratory Standard Institute (CLSI 2021 guidelines) recommended mCIM method.

***Results:*** The specificity, sensitivity, negative predictive value, positive predictive value, and diagnostic accuracy of the rCDM for identifying Carbapenemase-producing organisms were 97.30%, 98.60%, 94.74%, 99.30%, and 99.33%, respectively, using the modified carbapenem inactivation method (mCIM) as the reference method.

***Conclusions:*** The diagnostic accuracy for the detection of Carbapenemase-producing Gram-negative bacteria using rCDM among clinical samples is very high. It is a rapid, reliable, and cost-effective method for the detection and timely reporting of Carbapenemase-producing organisms in healthcare settings.

**Keywords:** Carbapenemase, Enterobacterales, Rapid Carbapenemase detection method (rCDM).

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## INTRODUCTION

Carbapenem-resistant bacteria have emerged worldwide in recent years.[1] Carbapenemase-producing organisms have become a major threat to public health due to their extensive resistance patterns as a result of inhibiting critical antibiotics responsible for bacterial destruction. Infections with these organisms are frequently associated with longer hospital stay, high patient morbidity, and mortality. Timely institution of specific antibiotic therapy against such resistant organisms is pivotal in reducing patient morbidity and mortality.[2] Carbapenem-resistant organisms are also at the top of the WHO threat list of drug-resistant bacteria.[3] The novel rCDM can accurately detect Carbapenemase-producing organisms with a sensitivity of 100% and specificity of 99.6% in 5 to 6 hours.[2]

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Globally, carbapenem-resistant Gram-negative bacteria pose a serious threat to hospitalized patients.[4]. Carbapenems are beta-lactam antibiotics. They are increasingly used in clinical practice as empirical treatment. However, their widespread use in hospitals has led to the rapid emergence of resistance to these agents.[5,6] Production of Carbapenemase is the main mechanism conferring resistance to these antibiotics. According to Ambler classification, they are included in class A, C, D (serine dependent), and class B (metallo-beta lactamase).[7]

Carbapenem-resistant Gram-negative bacteria constitute a significant portion of all clinical isolates. Among these resistant isolates, Carbapenemase production is a major mechanism for resistance to carbapenems, but the detection of Carbapenemase production is tedious and time-consuming. In Gram-negative bacteria, the primary categories of Carbapenemase are as follows: Ambler Class A, which can break down all β-lactams except Cephamycin;

Ambler Class B, which are zinc-dependent Metallo β-lactamases capable of hydrolyzing all β-lactams except Aztreonam; and Ambler Class D, which can hydrolyze carbapenems and broad-spectrum cephalosporins.[8]

CLSI M100 has recommended several phenotypic detection methods that include mCIM, carba NP, EDTA carbapenem inactivation method.[9] Genotypic methods include loop-mediated isothermal amplification (LAMP) and microarray techniques. FDA also recommends MALDI-TOF MS (matrix-assisted laser desorption ionization time of flight mass spectrometry). The molecular identification of Carbapenemase-producing gene based on PCR assays can also be done; these procedures are time-consuming, difficult to perform, and not cost-effective.[10]

This study was undertaken to evaluate a rapid, convenient, and economical approach for identifying Carbapenemase in resource-constraint settings. The conventional mCIM used for Carbapenemase detection requires overnight incubation (20-24 hours) in comparison to the novel rapid Carbapenemase detection method (rCDM), which takes 4-6 hours, thus facilitating the clinician in the treatment of carbapenem-resistant infections well in time.

## METHODOLOGY

This cross-sectional study was performed at the Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from Jun 21 to May 22 after taking approval from the Institutional Review Board (IRB), vide reference number FC-MIC20-1/READ-IRB/23/2123. After a thorough literature search, we calculated the sample size using the sensitivity-specificity calculator. We calculated the sample size using the rCDM prevalence of 78.21 %, confidence level at 95%, and margin of error at 5%.[2] The estimated sample size was 180. Sampling was done using non-probability consecutive sampling. Informed consent was obtained from all patients before including them in the study. All the patient particulars, like name, age, gender, and other relevant information of the patients were noted.

**Inclusion Criteria:** The study included all clinical specimens, such as blood, respiratory samples, pus, stool, and urine samples, that yielded growth of isolates resistant to carbapenems from patients.

**Exclusion Criteria:** Repeat samples from the same patient were excluded.

All samples were processed as per standard microbiological guidelines, which are recommended for each specific clinical sample.[11] Carbapenem-resistant isolates were identified using the modified Kirby-Bauer disc diffusion method using imipenem and/or meropenem disk (10 µg, Oxoid, UK) as per CLSI guidelines.[12]

Both mCIM and rCDM were performed simultaneously on carbapenem-resistant isolates detected by the routine disc diffusion method.

All carbapenem-resistant organisms were tested for Carbapenemase production by mCIM method using Clinical Laboratory Standard Institute (CLSI) 2021 guidelines.[12]

To perform rapid carbapenem detection method (rCDM), thin Mueller-Hinton Agar OxoidTM (tMHA) plates of 3mm thickness were prepared. The plates were kept in cold storage at a temperature of 4°C. In the standard disk diffusion method, a plate containing Mueller-Hinton agar (tMHA) was evenly lawned with a 3.0 McFarland standard bacterial suspension of E. coli ATCC 25922. Colonies of test organisms, grown on blood agar (Oxoid, UK) overnight under ambient air at 37oC, were uniformly distributed on an imipenem disk (10 µg, Oxoid, UK). The surface of the disk containing the bacteria was applied to the tMHA plate that had been previously lawned with E. coli ATCC 25922. The imipenem disk without bacteria was also placed on tMHA plate as the control. All plates were then incubated in the ambient air at a temperature of 35 °C for 4-6 hours. If Carbapenemase-producing organisms were present, imipenem would be broken down, permitting susceptible strains to grow. A positive result was indicated by a zone of inhibition around the test organisms, decreasing by 5 mm or more compared to the control. A negative result occurred when the zone of inhibition decreased by 3 mm or less for the test organisms compared to the control. An indeterminate result was indicated by a decrease in the zone of inhibition ranging from 3 to 5 mm, for the test organisms compared to the control.

The data collected was entered into Statistical Package for the Social Sciences, SPSS software (version 26), for analyzing the statistical information. Descriptive statistics were used to calculate both qualitative and quantitative variables. For quantitative variables such as age, the mean and standard deviation (SD) were determined, while for the qualitative variables, percentage and frequency were computed. A 2x2 table was created to assess the

specificity, sensitivity, negative predictive value (NPV), positive predictive value (PPV), and diagnostic accuracy of rCDM in identifying Carbapenemase in clinical isolates compared to mCIM. A *p*-value of less than 0.05 was considered statistically significant.

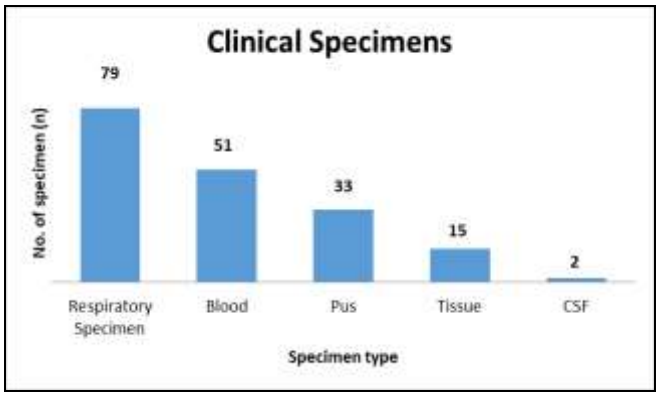


**Figure-1: Study Specimen Stratification according to Sample Type (n=180)**

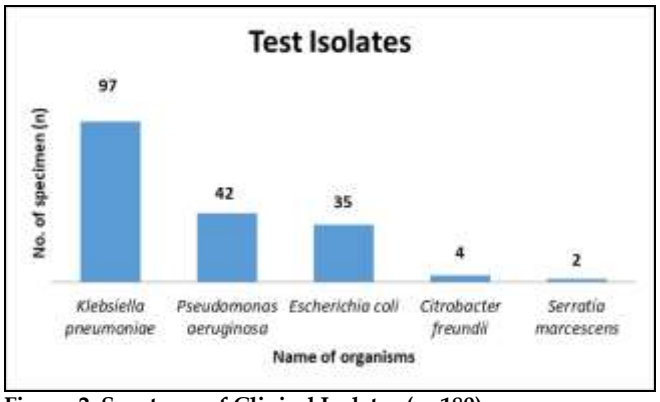


**Figure-2: Spectrum of Clinical Isolates (n=180)**

## RESULTS

A total of 180 clinical specimens were included in this study. The distribution of specimens is shown in figure-I. The average age of the patients was 41.45±14.57 years. Of the 180, 71.67% were males and 28.33% were females. The participants were categorized into two groups according to their age. Among the participants, 93 individuals, comprising 51.67% of the total sample, fell within the 18-40-year-old age group, while 87 individuals, making up 48.33% of the total, belonged to the 41-70-year-old age group. The spectrum of clinical isolates included in the study is given in Fig-2. Out of 180 isolates, 143(79.4%) were positive for Carbapenemase production using mCIM, and 142(78.9%) were positive by the rCDM method.

The results of rCDM were compared with mCIM in terms of sensitivity, specificity, positive and negative predictive values. Overall specificity, sensitivity, negative predictive value, positive predictive value, and diagnostic accuracy of rCDM for detection of Carbapenemase-producing organisms, with mCIM as the reference method, were 97.30%, 98.60%, 94.74%, 99.30%, and 98.33%, respectively. (Table-I)

**Table-I: 2 x 2 table showing the performance of the Rapid Carbapenemase Detection Method (rCDM) against the Modified Carbapenem Inactivation Method (mCIM) as the reference standard for the detection of Carbapenemase-Producing Gram-negative bacteria (n = 180)**

| | rCDM Carbapenemase detected | rCDM Carbapenemase not detected | *p*-Value |
|---|---|---|---|
| mCIM Carbapenemase detected | 141(TP) | 2 (FN) | < 0.001 |
| mCIM Carbapenemase not detected | 1(FP) | 36 (TN) | |

Sensitivity= TP/(TP+FN)= 141/(141+2)*100=98.60%
Specificity= TN/(TN+FP)= 36/(36+1)*100=97.30%
Positive Predictive Value= TP/(TP+FP)*100= 141/(141+1)= 99.30%
Negative Predictive Value= TN/(TN+FN)*100=36/(36+2)= 94.74%
Diagnostic Accuracy=(TP+TN)/All patients*100 = (141+36)/180=98.33%

**TP – True Positive, TN – True Negative, FP – False Positive, FN – False Negative, Modified Carbapenem Inactivation Method (mCIM), Rapid Carbapenemase Detection Method (rCDM)*

## DISCUSSION

The findings of this study demonstrate that the rapid Carbapenemase Detection Method (rCDM) possesses a high diagnostic accuracy for identifying Carbapenemase-producing Gram-negative bacteria in clinical isolates. The method showed excellent performance in distinguishing Carbapenemase producers, supporting its reliability as a diagnostic tool. Its rapid turnaround time and high accuracy make rCDM a valuable approach for timely detection and appropriate antimicrobial stewardship in clinical practice.

Carbapenemase-producing Gram-negative bacilli severely limit treatment options for life-threatening, complicated infections caused by these bacteria. Carbapenem-resistant Enterobacterales (CRE) and carbapenem-resistant Pseudomonas aeruginosa (CRPA) pose the greatest threat to human health.[13] In 2017, WHO acknowledged this by placing them at the top of the list of antibiotic-resistant "priority pathogens"[14]. According to the WHO's 2021 surveillance report on antimicrobial resistance, antimicrobial resistance is a major threat to human health, implying the need to explore newer treatment avenues.[15]

A novel antibiotic can prove advantageous in the fight against the ever-rising burden of antimicrobial resistance due to Carbapenemase-producing organisms. There have been various tests developed to detect different forms of Carbapenemase activity as explained by Workneh *et al*. These include tests that inhibit the activity of Carbapenemase, tests that detect carbapenem hydrolysis using MALDI-TOF MS, biochemical tests like the Carba NP test and its variations, and the carbapenem inactivation method (CIM).[16] These tests can identify the existence of Carbapenemase and occasionally distinguish between Ambler class A and Ambler class B Carbapenemase, as mentioned by Neonakis *et al*.[17] Immunochromatographic assays have also been useful in rapidly detecting OXA-48-like, IMP-like, and OXA-48/KPC CPEs from solid cultures. However, molecular methods remain the most reliable way to detect Carbapenemase producers.

A study by Piere *et al*., comparatively assessed phenotypic approaches for identifying Carbapenemase. PCR was taken as the gold standard. The CarbaNP demonstrated a sensitivity of 99.6% and a specificity of 100%. The sensitivity and specificity of mCIM was 99.6% and 99%, respectively.[18] This study concluded that both CarbaNP and mCIM have excellent specificity and sensitivity for detecting Carbapenemase producing organism; however, the Modified Hodge Test is no longer recommended.

Zhou *et al*., conducted a study with total of 257 Enterobacterales and 236 Pseudomonas aeruginosa isolates were tested in this study. The study revealed that sensitivity and specificity of rCDM were 100% and 99.6 %, respectively, with mCIM as the reference method. For Enterobacterales and P. aeruginosa, the agreement rate between rCDM and mCIM was 100%.[19] In our study sensitivity and specificity of rCDM were 98.6 and 97.3, respectively, which is almost in concordance with this study. The novel rCDM can accurately detect Carbapenemase-producing organisms with a sensitivity of 100% and a specificity of 99.6% in 5 to 6 hours.

The rCDM has several advantages over mCIM.[2] The detection time of rCDM is 5-6 hours, as compared to the modified carbapenem inactivation method, which requires overnight incubation and has a detection time of 18-24 hours.3 The short detection time of rCDM is due to the following reasons. Bacterial growth is hindered when a certain amount of antibiotic is present. To inhibit a higher concentration of bacteria, a greater concentration of the drug is necessary.[6] In comparison to regular MHA plates, which are 4mm thick, tMHA plates only measure 3mm in thickness. This causes a higher concentration of antibiotic to be present at the same distance from the disk, effectively inhibiting a higher concentration of bacteria.[9] Consequently, a greater concentration of bacteria is needed when tMHA is utilized. Additionally, Wei et al., demonstrated in a study that a shorter incubation period results in less time for bacterial growth to become visible. The hydrolysis rate of the imipenem disk is particularly rapid due to a large Kcat (catalytic constant) value for most Carbapenemases. As a result, there is a notable reduction in the diameter of the zone of inhibition in a short period of time.[20]

Cost analysis of both methods of Carbapenemase detection shows a difference in per-test cost. The cost of every consumable was taken from the annual tender of the laboratory, and the per-test average cost was calculated for each Carbapenemase detection method. The rCDM was economical compared to mCIM. The average cost of rCDM was PKR 45 vs PKR 57 of mCIM. This shows rCDM is 17.6 % cheaper than mCIM. rCDM turned out to be cost-effective as it does not require tryptic soy broth incubation, and less MHA base is utilized for making thin Mueller-Hinton agar.

To enhance the clinical effectiveness of rapid testing for carbapenem-producing organisms, effective coordination is essential between the clinical microbiology laboratory, antimicrobial stewardship/infection control teams, and clinicians. Early detection and reporting of Carbapenemase-producing organisms by microbiologists would help treat physicians in clinical decision-making. The empirical use of carbapenems could be curtailed in a timely resulting in a significant decrease in treatment cost and emergence of multidrug-resistant pathogens. In addition, it is crucial to inform hospital epidemiologists and infection control personnel without delay when patients are infected or carrying CRE. This will enable the necessary infection control measures to be put into place.

## LIMITATIONS OF THE STUDY

This study has several limitations. It was a single-center study with limited geographic representation of carbapenem-resistant isolates. In addition, rCDM cannot differentiate serine from Metallo-Carbapenemases, so further testing is needed, while molecular methods, though the gold standard, may be inaccessible, costly, and time-

consuming in resource-limited settings. Finally, rCDM is not suitable for detecting Carbapenemase produced by Acinetobacter spp.

## CONCLUSIONS

This study has shown that the diagnostic accuracy for the detection of Carbapenemase-producing Gram-negative bacteria using rCDM among clinical samples is very high. It is a rapid, reliable, and cost-effective method. Based on the results of the present study, rCDM can be recommended for use as a primary diagnostic tool for the rapid and timely detection of Carbapenemase-producing organisms from clinical isolates.

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**Authors' Contribution**

Following authors have made substantial contributions to the manuscript as under:

AZ & IAM: Data acquisition, data analysis, critical review, approval of the final version to be published.

SKMN & AI: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

AG & RS: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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