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Original Research Article

Improving carbapenem resistance identification: A comprehensive evaluation of bc-CIM in positive blood cultures

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Abstract

Background: Effective therapeutic therapy of blood culture positives requires early identification of medication resistance. By lowering mortality and morbidity, it improves patient outcomes, permits prompt and focused antibiotic therapy, and slows the spread of resistant infections. Early detection can also aid in the prevention of inefficient therapies, which can result in more economical healthcare costs and more effective use of resources. For all intents and purposes, it is essential in mitigating the impact of drug-resistant illnesses.

Materials and Methods: To determine whether 100 positive blood cultures produced carbapenemase, the bcCIM test was used. All Gram-negative bacilli (GNB) were subjected to the mCIM test and subcultures of positive blood cultures. The outcomes were contrasted with each isolate's VITEK MIC results.

Results: *Klebsiella pneumoniae* was the most common isolate (55/100, 55%), followed by *Escherichia coli* (41/100, 41%), *Enterobacter cloacae* (3/100, 3%), and *Proteus mirabilis* (1/100, 1%). 47 isolates have carbapenemase production identified by mCIM. In comparison with mCIM, bcCIM demonstrated 92% overall accuracy, 100% specificity (53/53; 95% CI 93.3–100), and sensitivity 82.98% (39/47; 95% CI 69.2–91.6). Strong consistency was indicated by the bcCIM and mCIM's 0.838 kappa agreement.

Conclusion: Rapid pathogen identification with susceptibility profiling is necessary for sepsis caused by multidrug-resistant gram-negative pathogens. In order to improve infection management and targeted medication, this study suggests standardizing the blood culture carbapenem inactivation method (bc-CIM) for faster and more economical carbapenemase detection. When positive blood cultures are subjected to Direct Susceptibility Testing (DST), turnaround times are considerably reduced, which improves results by enabling quicker, more appropriate treatment.

Keywords: Drug resistance, Blood cultures, Carbapenem inactivation method, bc-CIM.

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1. Introduction

Blood cultures are crucial for diagnosing bloodstream infections (BSIs), especially in patients with sepsis. They enable the quick identification of pathogens and determination of their antibiotic susceptibility, which is vital for starting appropriate antimicrobial treatment. Timely and accurate therapy significantly influences patient outcomes.[1] The rise of multidrug-resistant (MDR) organisms, especially Gram-negative bacteria, has made it increasingly important to quickly identify and thoroughly characterize these pathogens.[2] Carbapenemase-producing gram-negative bacteria are among these multidrug-resistant pathogens. Their resistance to last-resort antibiotics makes them a serious threat.[3]

Standard techniques for identifying carbapenemase production, including the Modified Hodge Test (MHT) and molecular assays, generally need isolated bacterial colonies. This process can take 24 to 72 hours, leading to delays in initiating effective treatment and implementing infection control measures.[4,5] Increase in trend of carbapenem-resistant organisms highlights the need for faster diagnostic techniques.[6]

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Detecting carbapenemase production directly from positively flagged blood culture bottles offers significant advantages over traditional methods performed on isolated colonies, reducing turnaround time by 24 to 36 hours and enabling earlier administration of targeted therapy.[7,8] This early intervention is crucial in managing sepsis and preventing the spread of resistant bacteria within healthcare settings.[9]

To avoid false positives or negatives, that could result in treatment decisions that are not appropriate, direct testing techniques such as the blood culture carbapenem inactivation method (bc-CIM) need to be sensitive and specific.[10] Due of the more pronounced growth and response patterns seen on pure isolates, conventional methods—despite considerably slower—are frequently regarded as reliable.[11] Consequently, even though direct detection techniques offer quicker outcomes, they need to undergo extensive verification to ensure that diagnostic accuracy is not compromised.[12]

The bc-CIM test provides an affordable and efficient method for detecting carbapenemase-producing bacteria directly from blood cultures, significantly reducing diagnostic time compared to enzyme detection assays.[13] Considering the availability of commercial carbapenemase detection kits, their high cost limits broad use, particularly in settings with limited resources.[14] The bc-CIM provides a useful substitute that may be easily incorporated into current laboratory procedures with little further help.[15]

Despite these benefits, the accuracy of the approach in directly detecting carbapenemase from blood cultures remains an important issue. The need for additional validation and standardization is made clear by the potential that medicines that interact with the blood culture media or unfavourable growth circumstances could affect the test's sensitivity and specificity.[16]

2. Materials and Methods

2.1. Study design and study setting

This is a laboratory- based prospective study, this was conducted over one year (May 2023–April 2024) at JSS Medical College and Hospital, Mysuru. Total 100 positive blood culture samples obtained from patients those who suspected bloodstream infections, were included using a convenient technique.

2.2. Ethical considerations

The JSS Medical College Institutional Ethics Committee gave its approval to the study protocol. (Ref: JSS/MC/PG/91/2022-23).

All clinical samples in the study were first tested with the bc-CIM method. Subsequently, isolates from blood culture bottles that tested positive were analyzed using the m-CIM and e-CIM methods. In addition the drug susceptibility profile of the isolates was also studied. A brief methodology of bc-CIM, m-CIM and e-CIM is as follows:

2.3. Blood culture carbapenem inactivation method (bc-CIM)

Approximately 2 mL of blood from positive culture bottles was incubated with a meropenem disk in the presence of $ZnSO_4$ for 2 h at 37 °C. After incubation, the disk was placed on E. coli ATCC 25922-seeded Mueller–Hinton agar, and zone diameters were interpreted according to CLSI guidelines.

2.4. Modified carbapenem inactivation method (m-CIM)

Test isolates were inoculated into tryptic soy broth containing a meropenem disk and incubated at 37 °C for 4 h. The disk was then transferred to E. coli ATCC 25922-seeded Mueller–Hinton agar plates, and inhibition zones were recorded. Interpretation criteria followed CLSI standards.

2.5. EDTA-modified carbapenem inactivation method (e-CIM)

The procedure was identical to m-CIM, except that EDTA was added to the broth to detect metallo-β-lactamase activity. An increase of ≥5 mm in inhibition zone compared with m-CIM was considered positive for MBL production.[17]

2.6. Statistical analysis

Data analysis was performed using SPSS software, version 22.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation, while categorical data were summarized as frequencies and percentages. Associations between qualitative variables were tested using the Chi-square test, with a P-value <0.05 considered statistically significant. Agreement between bc-CIM and m-CIM was assessed using Cohen's kappa statistic, interpreted as: ≤0 (no agreement), 0.01–0.20 (slight), 0.21–0.40 (fair), 0.41–0.60 (moderate), 0.61–0.80 (substantial), and 0.81–1.00 (almost perfect).[18]

3. Results

Of 100 positively flagged blood culture bottles yielding Gram-negative bacteria

Table 1: Distribution of gram-negative isolates

Organism	Number of Isolates(n)	Percentage (%)
Klebsiella pneumoniae	55	55
Escherichia coli	41	41
Enterobacter cloacae	3	3
Proteus mirabilis	1	1
Total	100	100

According to the data collected from the VITEK-2 automated system, among the hundred GNB isolates highest amount of resistance was recorded for Ceftriaxone, while the

lowest resistance was seen against amikacin. Antimicrobial susceptibility profile of the isolates were depicted in **Figure 1**.

Out of 100 GNB isolates obtained from positively flagged blood culture bottles, 47 (47%) were carbapenemase positive by mCIM and 53 (53%) were negative. Among the positive cases, patients were aged 40-50 years, including 23 males (55%) and 22 females (45%).

Carbapenem-resistant isolates were mainly *Klebsiella pneumoniae* (33, 70%), *Escherichia coli* (11, 23%), *Enterobacter cloacae* (2, 4%), and *Proteus mirabilis* (1, 2%).

Using bcCIM, 39/100 samples (39%) were positive, 60/100 (60%) were negative, and 1 (1%) gave an intermediate result, which was considered positive for analysis.

There was a substantial agreement between mCIM and bcCIM for GNB, *K. pneumoniae*, *E. coli*, *E. cloacae*, and *P. mirabilis* which is statistically not significant (P-value is 1.0) (**Table 2**).

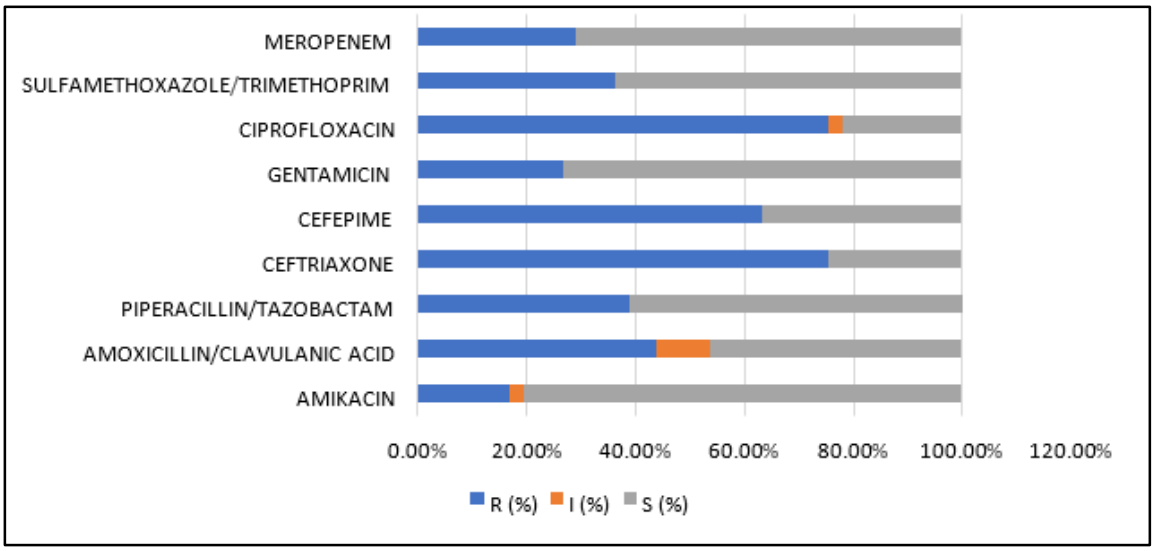

Figure 1: Showing susceptibility pattern of test isolates

Table 2: Demonstrates the results of carbapenem susceptibility by mCIM, and bcCIM, in different isolates of GNB. When comparing the results obtained through bcCIM and mCIM, intermediate results were considered positive.

	Meropenem			**Modified CIM (mCIM)results**			**Related results of Blood culture CIM (bcCIM)**		
	Resistant	**Sensitive**	**Intermdiate**	**Negative**	**Indeterminate**	**Positive**	**Negative**	**indeterminate**	**Positive**
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Klebsiella pneumoniae	28(51%)	27(49%)	0%	22(40%)	0%	33(60%)	27(49%)	0%	28(51%)
Escherich coli	12(29%)	29(71%)	0%	30(73%)	0%	11(27%)	32(78%)	1(2%)	9(22%)
Enterobacter cloacae	2(66%)	1(33%)	0%	1(33.)	0%	2(67%)	1(33%)	0%	2(67%)
Proteus mirabilis	0%	1(100%)	0%	0%	0%	1 (100%)	0%	0%	0%

Table 3: Results of carbapenem susceptibility, mCIM, and bcCIM testing for various Gram-negative bacteria.

mCIM		**bcCIM Method**		**Total**	**P-value**	**The measure of Agreement Kappa**
		Negative	**Positive**			
All GNB	Negative	53	8	61	<0.001	0.838
	Positive	0	39	39		
Total		53	47	100		
Klebsiella pneumoniae	Negative	22	5	27	<0.001	0.818
	Positive	0	28	28		
Total		22	33	55		
Escherichia coli	Negative	30	2	32		

	Positive	0	9	9	<0.001	0.868
Total		30	11	41		
Enterobacter cloacae	Negative	1	0	1	<0.001	1.0
	Positive	0	2	2		
Total		1	2	3		

***Others**: *Proteus mirabilis* which is statistically not significant due to an insufficient sample size

Intermediate results were considered as positive.[19]

Considering eCIM and mCIM as gold standard, the bc-CIM showed a sensitivity of 82.98% and 100% specificity with a diagnostic accuracy of 92% in this current study.(**Table 4**)

Table 4: Correlation between results of mCIM, and bcCIM in different GNB

Statistic	Value
Sensitivity	82.98% (39/47; 95% CI: 69.2–91.6)
Specificity	100% (53/53; 95% CI: 93.3–100)
Positive Predictive Value (PPV)	100%
Negative Predictive Value (NPV)	86.89%
Accuracy	92%
Negative Likelihood Ratio	0.17
Kappa (κ)	0.838

4. Discussion

Antimicrobial resistance (AMR) among gram negative bacteria (GNB) has become a major global health concern. Complicating the selection of effective empiric antibiotic treatments for critically ill patients.

Globally, the prevalence of carbapenem-resistant Enterobacteriaceae (CRE) is on the rise. Cai et al. reported 4.5% of carbapenem resistance among enterobacteriaceae in the US.[20]

In our study 55% of patients were male and 45% female, similar to M. Fan et al. were also reported (56.4% males, 43.6% females). Additionally, 47% of isolates in our study produced Ambler class B carbapenemase (NDM), compared with 73.8% reported by M. Fan et al.[21]

In our study, the maximum number of CPE (carbapenem producing enterobacteriaceae) is *Klebsiella pneumoniae* consistent with reports from Metwally et al. (39%) was the major pathogen. and other international studies where Klebsiella spp. predominated in bloodstream infections.[22]

According to WHO guidelines and the study by Gopi Patel et al., Klebsiella pneumoniae isolates from ICU patients showed 63.4% resistance to third-generation cephalosporins, and Escherichia coli isolates showed 75% resistance.[23] In our study, higher resistance rates were observed: 89.1% for K. pneumoniae and 75.6% for E. coli, consistent with global trends reported by Alvarez-Uria et al.[24] Additionally, carbapenem-resistant Enterobacteriaceae (CRE) were detected in 57.1% of isolates, in line with previous surveillance data.

Klebsiella spp. (39%) and Pseudomonas spp. (21.9%) were the most common pathogens in the major bloodstream infections reported by Metwally et al., and there was a fair agreement (kappa = 0.327) between mCIM (CLSI reference) and bcCIM for identifying GNB that produces carbapenemase. The common pathogens in our investigation were Escherichia coli (41%) and Klebsiella pneumoniae (55%), with a higher agreement between mCIM and bcCIM (K. pneumoniae: kappa = 0.818; E. coli: kappa = 0.868).[22,26] In line with our results, De Lima-Morales et al. found that when the test was conducted directly on blood, only 80% of isolates found on pure culture were recognized.[22,25]

Metallo-beta-lactamases (MBLs) were produced by 47% of multidrug-resistant Enterobacterales in the current investigation, which is slightly lower than the 75.6% reported by Dalia El Nobi et al. from Assiut University, Egypt.[26] Our study's sensitivity of 82.98% and specificity of 100% for bcCIM, using mCIM as the gold standard, were in agreement with those of Sfeir et al., who reported sensitivity of 97.9–100% and specificity of 91.4–100% for comparable tests.[27]

4.1. Research gap

There is not much evidence that carbapenemase can be quickly and directly detected from positive blood cultures, even if the number of carbapenem-resistant Gram-negative bacteria is increasing. The majority of current research focuses on pure cultures, which may cause antibiotic therapy to be delayed. To aid in early detection, direct focused therapy, lower morbidity and mortality, and slow the spread of multidrug-resistant infections, validated, economical, and quick phenotypic techniques as bcCIM are required.

5. Limitations

There are various limitations to the study. First, because research was only done at one location, the results could not be as applicable to other hospitals or areas. Second, the accuracy of sensitivity and specificity estimates may be impacted by the relatively small sample size of 100 isolates. Third, intermediate bcCIM values were deemed positive for analysis, which may have an impact on the accuracy of the diagnosis. Additionally, only Gram-negative bacteria from bloodstream infections were included in the study, and

carbapenemase types were not molecularly confirmed. Despite these drawbacks, our work shows that bcCIM is a practical and dependable technique for easily and inexpensively detecting carbapenemase straight from positive blood cultures. The results support future multicenter studies with larger cohorts and molecular validation, and they offer useful preliminary data to recommend timely antibiotic treatment.

6. Conclusion

Multidrug-resistant gram-negative organisms remain a major threat in sepsis patients. It is vital for the Microbiology laboratory to devise methods to identify the pathogen with the susceptibility profile at the earliest. This study proposes standardizing the blood culture carbapenem inactivation method (bcCIM), a modification with the potential to expedite carbapenemase detection by up to 24 hours compared to conventional enzyme detection tests. While commercial kits for detecting carbapenemase are available, they can be costly to implement in routine laboratory practice. bc-CIM offers a cost-effective alternative, utilizing modified protocols that can be standardized within existing laboratory workflows. Rapid identification of carbapenemase producers through bc-CIM contributes to infection control efforts by facilitating the early implementation of infection prevention measures. This is crucial in preventing the spread of resistant bacteria within healthcare facilities and the community. The implementation of direct susceptibility testing (DST) from positively flagged blood cultures represents a significant advancement, reducing turnaround times by up to 36 hours compared to traditional methods.

7. Source of Funding

None.

8. Conflict of Interest

None.

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