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Detection of Carbapenemase-Producing Enterobacterales: A Comparative Analysis of Available Phenotypic Methods

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Carbapenems serve as a critical last-line defence against infections from multidrug-resistant Enterobacterales. The increasing prevalence of CRE (carbapenem-resistant Enterobacterales) poses significant challenges in clinical settings. This greatly complicates the handling of gram-negative bacilli (GNB) infections and represents a significant global health threat.
Aim: This study evaluates and contrasts different phenotypic techniques used to identify carbapenemase-producing Enterobacterales isolated from various clinical samples.

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Objectives: To compare the accuracy and reliability of Combined Disc Test (CDT) and Modified Carbapenem Inactivation Method/EDTA - Carbapenem Inactivation Method (mCIM/eCIM) in identifying Metallo-beta-lactamase (MBL)-producing Enterobacterales.
Methods: All clinical specimens submitted for culture and sensitivity testing at the Department of Microbiology were analysed to isolate and identify Enterobacterales. Carbapenemase production was assessed through using the phenotypic CDT Confirmation was carried out using the mCIM and eCIM in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines.
Results: During a two-year period, 254 strains of Enterobacterales were isolated. Among those, 80/254 (31.49%) isolates exhibited resistance to the Imipenem (IMP) disc. The CDT identified 30/80 (37.5%) isolates as serine carbapenemase producers and 50/80 (62.5%) isolates as MBL producers. In comparison, the mCIM/eCIM methods detected 26/80 (32.5%) isolates as serine carbapenemase producers, 48/80 (60.0%) isolates as MBL producers, and 6/80 (7.5%) isolates were tested negative for carbapenemase production.
Conclusion: This study evaluates the precision and effectiveness of the mCIM/eCIM and CDT methods for identifying CRE. The combination of the mCIM and eCIM tests may be a more reliable phenotypic method for detecting carbapenemase compared to the CDT.

Keywords: Metallo-beta-lactamase (MBL); Modified Carbapenem Inactivation Method (mCIM); EDTA Carbapenem Inactivation Method Multidrug Resistant (MDR); Enterobacterales, Combined Disc Test (CDT).

1. INTRODUCTION

Carbapenems are frequently used to treat infections caused by multidrug-resistant Enterobacterales. However, the global spread of carbapenem-resistant GNB has become a major public health concern **(**Bakthavatchalam et al., 2022). MBL is a type of carbapenemase enzyme that breaks down carbapenems, rendering these antibiotics ineffective (Ambler,1980). Enterobacterales that produce carbapenemase enzymes are referred to as carbapenemase-producing Enterobacterales (CPE). It's important to note that not all Enterobacterales produce carbapenemases. Organisms that test resistant to at least one of the carbapenem antibiotics (imipenem, meropenem, ertapenem or doripenem) are known as carbapenem-resistant organisms.

The first clinical report of MBL-producing Enterobacterales occurred in 1994, involving a Serratia marcescens strain resistant to imipenem **(**Osano et al., 1994). The ability of MBL-producing Enterobacterales to resist carbapenems is particularly concerning because they can transfer this resistance to other bacteria through a process called conjugative transfer, which involves the exchange of genetic material via plasmids (Mori et al., 2021).

Following the discovery of new strains of carbapenemase-producing bacteria, such as the outbreak of *Klebsiella pneumoniae* in the United States in 1996 and the emergence of metallo-beta-lactamase-producing bacteria in New Delhi in 2009, the Centers for Disease Control and Prevention (CDC) issued an alert in 2013 about carbapenem-resistant Enterobacteriaceae (CRE). This alert highlighted the global rise of CRE infections **(**Yigit et al., 2001), (Yong et al., 2009), (Centers for Disease Control and Prevention, 2023).

Outbreaks of CRE in healthcare settings are associated with increased mortality, morbidity, and healthcare costs. Various diagnostic methods have been developed to detect carbapenemases in clinical laboratories, including chromogenic agar, Carba NP, MALDI-TOF MS, CIM, and PCR. (Hirsch et al., 2014), (Monteferrante et al., 2016), (Moran et al., 2011) Of these, phenotypic testing remains the simplest and most widely used in routine diagnostics. These tests utilize specific inhibitors, such as EDTA for metallo-carbapenemases and phenyl boronic acid (PBA) for serine-carbapenemases, to distinguish between different carbapenemase types (Pasteran et al., 2009), (Tsakris et al., 2008).

Another phenotypic method involves incubating a carbapenem disk with a CPE isolate, which reduces the antimicrobial activity of the disk and can be assessed for carbapenemase production. This is known as the mCIM. The Clinical and Laboratory Standards Institute (CLSI) recommends using mCIM alongside the eCIM test to differentiate between metallo-carbapenemases and serine-carbapenemases

within 20 to 22 hours (Pierce et al.,2017), (CLSI, 2022),(Anand and Neha,2023).

The Enterobacterales isolates were confirmed phenotypically based on colony morphology on appropriate culture media and identified using manual biochemical tests. Antimicrobial susceptibility testing for all isolates was performed using the Kirby-Bauer disk diffusion method, following the 2022 CLSI guidelines (CLSI, 2022), (Thokar et al., 2022). Carbapenemase production was assessed using phenotypic methods, including the CDT, mCIM, and eCIM.

2. MATERIALS AND METHODS

This study was conducted at IIMSR, Integral University, Lucknow. Out of a total of 357 Enterobacterales species, 254 were identified from clinical samples collected in the microbiology laboratory between January 2022 and December 2023.

Inclusion Criteria: The study included clinical specimens such as wound swabs, urine, sputum, tracheal aspirates, ear swabs, tissue, and high vaginal swabs that yielded Enterobacterales growth. Strains identified as Enterobacterales and resistant to any of the following antibiotics, imipenem, meropenem, or ertapenem were included.

Exclusion Criteria: Samples that yielded gram-positive bacteria, *Pseudomonas* and *Acinetobacter* were excluded from the study. Additionally, strains susceptible to all three carbapenem antibiotics—meropenem, imipenem, and ertapenem were also excluded.

Phenotypic Diagnostic Methods: To detect MBL production phenotypically, bacterial suspensions were prepared from colonies grown on MacConkey agar and CLED agar. The bacterial suspension was adjusted to match a 0.5 McFarland standard and then cultured on Mueller-Hinton agar (MHA) plates using a sterile cotton swab. *Escherichia coli* ATCC 25922 was used as the quality control (QC) strain because CLSI recommends it for QC, and its well-documented antibiotic response ensures reliable results.

Combined Disc Test: In this study, CDT was performed to detect the presence of MBL enzymes in bacterial isolates. A bacterial suspension was adjusted to the 0.5 McFarland standard, ensuring consistent turbidity. The suspension was incubated at 37°C for 15 minutes. The surface of an MHA plate was uniformly swabbed with the bacterial suspension using a sterile swab for even distribution. After allowing the plate to dry, two IMP discs were placed on the MHA plate. Additionally, an IMP-Ethylenediamine Tetraacetic Acid (EDTA) disc was placed 10 mm edge-to-edge from one of the IMP discs. The plates were incubated for 16–18 hours. After incubation, the zones of inhibition around the discs were measured.

A significant increase in the inhibition zone around the IMP-EDTA disc compared to the IMP disc alone indicated the presence of MBL activity. This method helps determine the inhibitory effect of EDTA on MBL activity when combined with imipenem, providing essential information for the identification of MBL-producing bacteria, which can aid in appropriate treatment decisions (Sachdeva et al., 2017).

mCIM and eCIM Methods: To detect CPE, both the mCIM and eCIM were performed following CLSI guidelines. For each bacterial isolate, two test tubes containing 2 mL of Trypticase Soy Broth were prepared. One tube was supplemented with 20 µL of 0.5 M EDTA, while the other tube, serving as a control, had no EDTA. A fresh bacterial colony was inoculated into each tube using a 1 µL inoculation loop. A 10 µg meropenem disc was then placed into each bacterial suspension, and the tubes were incubated for 4 hours at 35°C.

After incubation, the meropenem discs were placed on an MHA plate that had been pre-inoculated with *Escherichia coli* ATCC 25922, serving as the control strain. The mCIM was considered positive if the inhibition zone surrounding the meropenem disc measured between 6–15 mm or 16–18 mm with the presence of small colonies.

The eCIM results were analysed only if the mCIM results indicated carbapenemase activity. A positive eCIM result, suggesting metallo-carbapenemase production, was confirmed if the inhibition zone around the EDTA-treated disc was at least 5 mm larger than the mCIM zone. This method provided precise differentiation of metallo-carbapenemase producers, crucial for guiding appropriate antimicrobial therapies (CLSI, 2022).

Statistical Analysis: Data were analysed using IBM SPSS Statistics (version 29.0.1.1; SPSS, Inc., Chicago, IL, USA). For categorical variables, descriptive statistics included a frequency distribution table and corresponding graphs. To assess statistical significance in differences of percentages or proportions was assessed using Pearson's chi-square test. A significance level of $P \leq 0.05$ was used to determine statistical significance.

3. RESULTS

The study included 254 (71.14%) Enterobacterales species isolated from 357 clinical specimens collected between January 2022 and December 2023. Non-Enterobacterales isolates were excluded. Antibiotic sensitivity testing of the 254 isolates revealed that 80 isolates (31.49%) were CRE.

A significant difference was observed in gender distribution, with a higher prevalence of CRE isolates in females compared to males. Among the 80 CRE isolates, 54 samples (67.5%) were from females, while 26 samples (32.5%) were from males (Fig. 1). The highest number of isolates was obtained from female patients aged 21-30 years and male patients aged 51-60 years.

The distribution of samples among the 80 CRE isolates was as follows: urine (52.5%), wound/pus (33.75%), blood (5.0%), sputum/tracheal aspirate (3.75%), vaginal swab (2.5%), tissue aspirate (1.25%), and bone (1.25%).

In terms of bacterial distribution, 47 isolates (58.75%) were identified as *Escherichia coli*, representing the highest number. This was followed by 27 isolates (33.75%) of *Klebsiella pneumoniae*, 5 isolates (6.25%) of *Citrobacter freundii*, and 1 isolate (1.25%) of *Morganella morganii.*

Table 1 explains the varying rates of MBL production across different sample types and Enterobacterales species. The highest prevalence of MBL producers was found in urine samples, with 25 out of 42 testing positive. Pus samples also showed a significant rate of MBL production, with 18 out of 26 testing positive.

Among the pathogens, *Escherichia coli* was the most prevalent MBL producer, accounting for 28 out of 47 positive samples. *Klebsiella pneumoniae* followed, with 19 out of 27 samples positive for MBL production. In contrast, *Citrobacter freundii* had the lowest rate, with only 3 out of 5 samples testing positive.

Overall, the results indicate that *Escherichia coli* is the primary MBL-producing pathogen, particularly in urine and pus samples. The CDT effectively differentiated between MBL-producing (MBL-positive) and non-MBL-producing (MBL-negative) bacterial strains. In MBL-positive strains, the addition of EDTA significantly enhanced the antibiotic's inhibitory effect, as shown by a larger inhibition zone.

Conversely, in MBL-negative strains, the inhibition zone did not increase with EDTA, confirming the absence of MBL production. This distinction is crucial for determining the appropriate treatment, as MBL production is a key mechanism of resistance to β-lactam antibiotics.

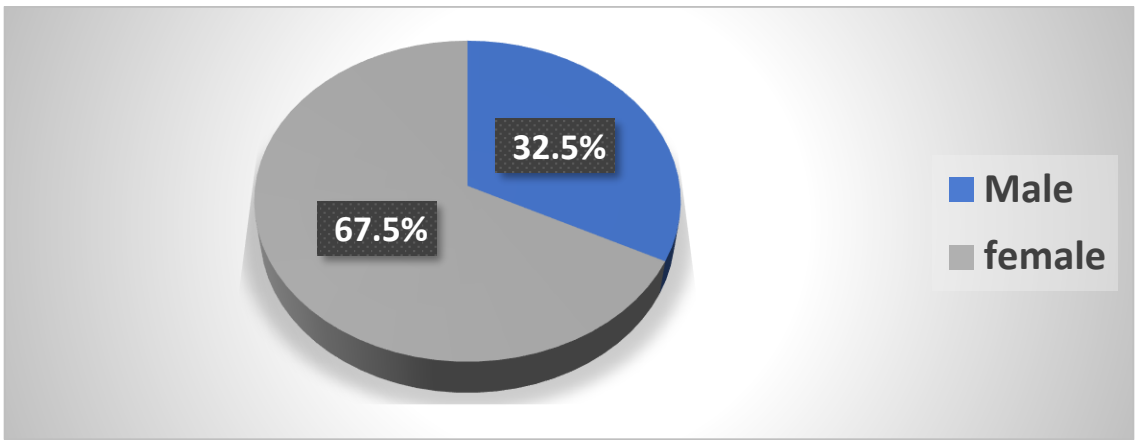

Fig. 1. Gender distribution among CRE isolates

Table 1. Distribution of MBL production across sample types and isolated species

Sample Types	*Escherichia coli (n=47)*		*Klebsiella pneumonia* (n=27)		*Citrobacter freundii (n=5)*		*Morganella morganii* (n=1)		Total MBL Producers (Sample Types)	
	MBL (%)	Total	MBL (%)	Total	MBL (%)	Total	MBL(%)	Total	MBL(%)	Total
Urine	58.62%	29	63.63%	11	100%	1	0%	1	59.52%	42
Pus	69.23%	13	81.81%	11	0%	2	0%	0	69.23%	26
Bactec	100%	1	100%	1	100%	2	0%	0	100%	4
Sputum	0%	0	66.67%	3	0%	0	0%	0	66.67%	3
Bone	0%	1	0%	0	0%	0	0%	0	0%	1
HVS	50%	2	0%	0	0%	0	0%	0	50%	2
Tissue	0%	0	0%	1	0%	0	0%	0	0%	1
Bile + Pus	0%	1	0%	0	0%	0	0%	0	0%	1
Total MBL Producers (Organisms)	59.57%	47	70.37%	27	60%	5	0%	1	62.5%	80

Fig. 2 (a). Illustrates the addition of EDTA to Imipenem, significantly increasing the inhibition zone, indicating MBL production. EDTA inhibits MBL activity by chelating zinc ions, confirming the strain as MBL-positive

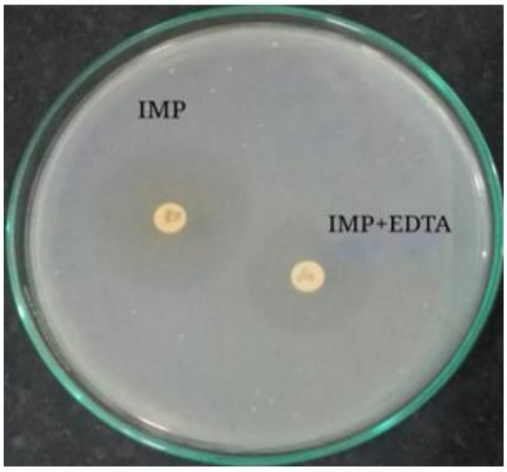

Fig. 2 (b). illustrates a decrease in the inhibition zone when EDTA was added to Imipenem, indicating the absence of MBL production, confirming the strain as MBL-negative

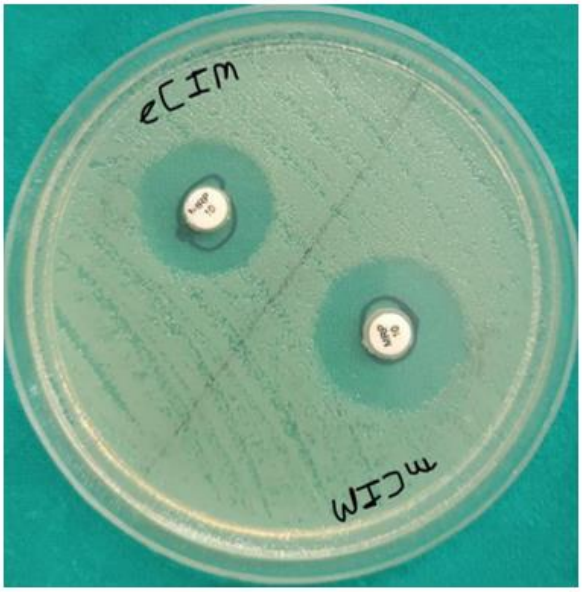

Fig. 3 (a) The zones of inhibition around both the mCIM and eCIM discs were similar, suggesting the presence of a serine carbapenemase rather than an MBL

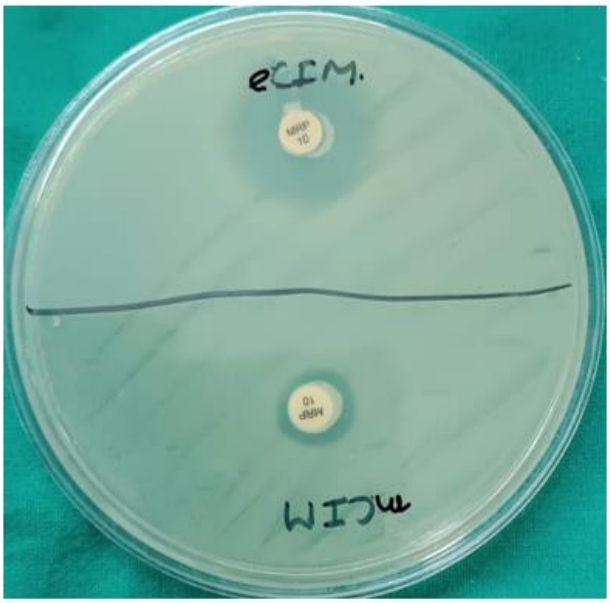

Fig. 3 (b). A significantly larger zone of inhibition around the eCIM disc compared to the mCIM disc, indicating the presence of a MBL, as EDTA effectively inhibited the MBL enzyme

For each isolate, the CDT was performed and the results were analysed and compared with those from the mCIM and eCIM tests. The mCIM and eCIM assays involved two incubation steps. First, the bacterial suspension was incubated with the antibiotic in a tube, followed by the application of the antibiotic-treated suspension onto a culture plate, with both steps requiring an incubation time of 22 hours.

4. DISCUSSION

The increasing prevalence of CPE poses a significant public health challenge due to their resistance to a wide range of antibiotics, including carbapenems—often regarded as the last line of defense against severe infections. This study emphasizes the importance of detecting carbapenemase production in clinical isolates of Enterobacterales using phenotypic

methods, which are crucial for guiding appropriate treatment strategies.

4.1 Prevalence and Distribution of CPE

Several studies have documented a rising prevalence of CRE across India. Datta et al., (2012) reported a CRE prevalence of 7.87% in North India, while Nair PK et al., (2013) observed a prevalence of 12.26% in Western India. In Southern India, Jan R et al., (2016) reported a prevalence of 8%, and Rao A and Indumathi et al., (2016) documented 13.95% in the same region.

Higher rates were observed by Khare V et al. (2017), who reported a prevalence of 37.9% in Northern India, and Pawar SK et al., (2018), who noted 31.77% in Western India. Srivastava P (2022) also found a prevalence of 29.35% in rural Uttar Pradesh, indicating the spread of resistant strains in less urbanized regions. In our study, a prevalence of 31.5% was recorded, demonstrating a troubling rise in CRE rates, consistent with both national and global trends in healthcare settings with high antibiotic usage.

4.2 Phenotypic Testing Using CDT Methods

Among the pathogens identified in our study, *Escherichia coli* accounted for 58.75% of isolates, followed by *Klebsiella pneumoniae* 33.75%. This distribution aligns with findings from Thomas et al., who reported that *E. coli* comprised 63.75% of isolates in their study.

The predominance of CRE in urine samples (52.5%) in our study mirrors results from Srivastava et al., (56.86%), Verma G et al., (40.50%), Nair et al.[19] (42.0%), Singh et al., (39.4%), Verma G et al., (35.0%) and Pawar et al., (31.76%), underscoring the association of CRE with urinary tract infections (UTIs).

The horizontal transmission of plasmid-encoded carbapenemase genes, typically spread via the fecal-oral route, may explain the high prevalence of CRE in urine specimens, particularly in hospital settings where community-acquired and nosocomial infections overlap.

4.3 Gender Disparity in CRE Infections

A notable finding in our study is the higher prevalence of CRE infections in female patients, accounting for 67.5% of resistant isolates. This observation is consistent with previous studies by Srivastava et al.,2019, May S et al.,2016, and Niranjan BP et al.,2018, which also reported higher incidences of *E. coli* and *K. pneumoniae* infections among females.

The increased vulnerability of women to UTIs is often attributed to anatomical factors, such as the shorter female urethra, which facilitates bacterial entry into the bladder, especially during sexual activity. These findings underscore the need for targeted infection prevention measures in female patients to reduce the risk of CRE infections.

4.4 Age Distribution of CRE Isolates

In this study, the highest prevalence of CRE was observed in patients aged 21-40 years (36.25%), followed by those aged 41-60 years (27.5%), 61-80 years (18.75%), and 1-20 years (17.5%). These results align with Patidar et al. (2021), who also reported the highest prevalence in the 21-40 age group (36.0%). However, our findings also highlight an increase in resistance among older individuals, suggesting that the rise in antibiotic resistance may not be confined to younger populations, as previously thought.

4.5 Comparison of Phenotypic Detection Methods

The prevalence of carbapenem-resistant Enterobacteriaceae (CRE) has been steadily rising across different regions of India. For example, Datta et al., (2012) reported a CRE prevalence of 7.87% in North India. Nair PK et al., (2013) observed a higher prevalence of 12.26% in Western India. In Southern India, Jan R et al., (2016) reported a CRE prevalence of 8.0%, and Rao A and Indumathi et al., (2016) noted an increase to 13.95% in the same region. Khare V et al., (2017) documented a significantly higher prevalence of 37.9% in Northern India, while Pawar SK et al., (2018) reported a prevalence of 31.77% in Western India.

Our study compared two phenotypic methods for detecting carbapenemase production: the CDT and mCIM/eCIM. CDT identified MBL production in 62.5% of the isolates, while mCIM/eCIM detected MBL production in 60% of the isolates. Both methods demonstrated high specificity, which is crucial in minimizing false positives and ensuring accurate diagnosis and treatment decisions.

Our results align with previous studies, though variations in prevalence rates were observed.

Verma G et al. reported a combined prevalence of 97.5% using mCIM and eCIM, indicating a higher detection rate. In contrast, Koul et al. found combined prevalences of 58.5% and 58.4 % for mCIM and eCIM, respectively, which are more consistent with our findings.

The CDT method, which employs EDTA to inhibit MBL activity, was found to be particularly effective in identifying MBL-producing strains. This effectiveness is demonstrated by the significant increase in the inhibition zone observed in positive cases. The CDT method's advantages include its simplicity, cost-effectiveness, and quick turnaround time, making it a practical choice for routine clinical use. On the other hand, while the mCIM method also provides reliable results, it requires more technical skill and has a longer incubation period. Therefore, CDT is generally preferred due to its straightforward and efficient approach (Kumudunie et al., 2021).

5. CONCLUSION

The CDT and mCIM tests showed high specificity but moderate sensitivity for detecting MBL-producing isolates, highlighting their utility in clinical settings even in the absence of molecular confirmation. Our study underscores the effectiveness of phenotypic assays, particularly the combined disk test and the modified carbapenem inactivation method, in identifying carbapenemase-producing Enterobacterales.

The CDT test proved advantageous due to its simplicity, requiring minimal technical expertise and delivering results within 16-18 hours. In contrast, while the mCIM method is effective, it requires greater proficiency and a longer turnaround time of 20-22 hours.

Despite the lack of confirmatory MIC testing, the high specificity of both assays supports their reliability in detecting MBL production, which is crucial for guiding appropriate antibiotic therapy. These findings advocate for the practical application of CDT in routine diagnostic workflows, emphasizing its role as a cost-effective and efficient tool for managing multidrug-resistant pathogens in clinical settings.

In clinical practice, the use of these phenotypic assays can significantly improve the management of multidrug-resistant infections, ensuring timely intervention and reducing the spread of resistant pathogens. Future research should focus on enhancing the sensitivity of these methods and integrating them with molecular approaches for more comprehensive detection of resistance mechanisms. Additionally, expanding their application across diverse healthcare settings can further strengthen antimicrobial stewardship efforts.

6. CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS

Accurate detection of CPE is crucial for guiding effective antimicrobial therapy and preventing the spread of these resistant organisms. Both the CDT and mCIM methods demonstrated high specificity, making them valuable tools in clinical practice, particularly where molecular testing is not readily available. However, the moderate sensitivity of these phenotypic tests indicates a need for further refinement to enhance their ability to detect all CPE cases comprehensively.

Given the rising prevalence of CPE, there is an urgent need for improved surveillance and the development of more sensitive diagnostic methods. Implementing stringent infection control measures is also essential. Future research should aim to enhance phenotypic detection techniques and integrate molecular methods for more accurate and thorough identification of CPE. Additionally, investigating the factors behind the gender disparity in CRE infections could lead to more effective prevention and treatment strategies.

7. LIMITATIONS

This study did not include other phenotypic methods, such as the Modified Hodge Test and Carba NP test.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

We hereby declare that generative AI technology (MLA Style: OpenAI. ChatGPT. Version 4, 2023, https://chatgpt.com) was used during the editing process.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Ambler, R. P. (1980). The structure of beta-lactamases. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, *289*, 321–331. Available:https://doi.org/10.1098/rstb.1980.0049

Anand, K. P., & Neha, T. (2023). Detection of methicillin-resistant *Staphylococcus aureus* and determination of minimum inhibitory concentration of vancomycin for *Staphylococcus aureus*. *ISSN: 2320-8708*, *11*(1).

Bakthavatchalam, Y. D., Routray, A., Mane, A., et al. (2022). In vitro activity of Ceftazidime-Avibactam and its comparators against carbapenem-resistant *Enterobacterales* collected across India: Results from ATLAS surveillance 2018 to 2019. *Diagn Microbiol Infect Dis*, *103*(1), 115652. Available:https://doi.org/10.1016/j.diagmicrobio.2022.115652

Centers for Disease Control and Prevention. (2013). Action needed now to halt spread of deadly bacteria. Available:https://www.cdc.gov/media/releases/2013/p0305_deadly_bacteria.html

CLSI. (2022). *Performance standards for antimicrobial susceptibility testing* (32nd ed.). CLSI supplement M100.

Datta, P., Gupta, V., Garg, S., & Chander, J. (2012). Phenotypic method for differentiation of carbapenemases in *Enterobacterales*: Study from North India. *Indian J Pathol Microbiol*, *55*(3), 357-360. Available:https://doi.org/10.4103/0377-4929.101744

Hirsch, E. B., Chang, K. T., Zucchi, P. C., et al. (2014). An evaluation of multiple phenotypic screening methods for *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Enterobacterales*. *J Infect Chemother*, *20*, 224-227.

Jan, R., George, N., Mathew, M., et al. (2016). Prevalence of carbapenem-resistant *Enterobacterales* in a tertiary care referral centre: Kerala, South India. *Int J Curr Res*, *8*(12), 44353-44355.

Khare, V., Gupta, P., Haider, F., & Begum, R. (2017). Study on MICs of Tigecycline in clinical isolates of carbapenem-resistant *Enterobacterales* (CRE) at a tertiary care centre in North India. *J Clin Diagn Res*, *11*(3), DC18-DC21. Available:https://doi.org/10.7860/JCDR/2017/24594.9629

Kumudunie, W. G. M., Wijesooriya, L. I., & Wijayasinghe, Y. S. (2021). Comparison of four low-cost carbapenemase detection tests and a proposal of an algorithm for early detection of carbapenemase-producing *Enterobacterales* in resource-limited settings. *PLoS One*, *16*(1), e0245290. Available:https://doi.org/10.1371/journal.pone.0245290

May, S., Shinu, N., Samia, W., Mohamed, M., Asma, A., Kazem, B., et al. (2016). Prevalence of urinary tract infection and antimicrobial susceptibility among diabetic patients with controlled and uncontrolled glycemia in Kuwait. *J Diabetes Res*, *2016*, 1155, 7–17.

Monteferrante, C. G., Sultan, S., Ten Kate, M. T., et al. (2016). Evaluation of different pretreatment protocols to detect accurately clinical carbapenemase-producing *Enterobacterales* by MALDI-TOF. *J Antimicrob Chemother*, *71*, 2856-2867.

Moran Gilad, J., Carmeli, Y., Schwartz, D., et al. (2011). Laboratory evaluation of the CHROMagar KPC medium for identification of carbapenem-nonsusceptible *Enterobacterales*. *Diagn Microbiol Infect Dis*, *70*, 565-567.

Mori, N., Tada, T., Oshiro, S., Kuwahara-Arai, K., Kirikae, T., & Uehara, Y. (2021). A transferrable IncL/M plasmid harboring a gene encoding IMP-1 metallo-β-lactamase in clinical isolates of *Enterobacterales*. *BMC Infect. Dis.*, *21*, 1061. Available:https://doi.org/10.1186/s12879-021-06758-5

Nair, P. K., & Vaz, M. S. (2013). Prevalence of carbapenem-resistant *Enterobacterales* from a tertiary care hospital in Mumbai, India. *J Microbiol Infect Dis*, *3*(04), 207-210. Available:https://doi.org/10.5799/ahinjs.02.2013.04.0110

Niranjan, B. P., Shetye, S., Manjusha, A., Mihir, V., Zaheer, V., Parijat, G., et al. (2018). Prevalence and susceptibility analysis of gram-negative pathogens in a tertiary care transplant hospital, Mumbai. *Asian J Res Med Pharm Sci*, *4*, 1–8.

Osano, E., Arakawa, Y., Wacharotayankun, R., Ohta, M., Horii, T., Ito, H., Yoshimura, F., & Kato, N. (1994). Molecular characterization

of an enterobacterial metallo beta-lactamase found in a clinical isolate of *Serratia marcescens* that shows imipenem resistance. *Antimicrob. Agents Chemother*, *38*, 71–78. Available:https://doi.org/10.1128/AAC.38.1.71

Pasteran, F., Mendez, T., Guerriero, L., et al. (2009). Sensitive screening tests for suspected class A carbapenemase production in species of *Enterobacterales*. *J Clin Microbiol*, *47*, 1631-1639.

Patidar, N., Vyas, N., Sharma, S., & Sharma, B. (2019). Phenotypic detection of carbapenemase production in carbapenem-resistant *Enterobacterales* by modified Hodge test and modified strip Carba NP test.

Pawar, S. K., Mohite, S. T., Shinde, R. V., Patil, S. R., & Karande, G. S. (2018). Carbapenem-resistant *Enterobacterales*: Prevalence and bacteriological profile in a tertiary care teaching hospital from rural western India. *Ind J Microbiol Res*, *5*(3), 342-347.

Available:https://doi.org/10.18231/2394-5478.2018.0072

Pierce, V. M., Simner, P. J., Lonsway, D. R., et al. (2017). Modified Carbapenem Inactivation Method for phenotypic detection of carbapenemase production among *Enterobacterales*. *J Clin Microbiol*, *55*, 2321-2333.

Rao, A., & Indumathi, V. A. (2016). Detection of carbapenem-resistant *Enterobacterales* from clinical isolates. *Int J Curr Microbiol App Sci*, *5*(5), 864-869.

Available:https://doi.org/10.20546/ijcmas.2016.505.089

Sachdeva, R., Sharma, B., & Sharma, R. (2017). Evaluation of different phenotypic tests for detection of metallo-β-lactamases in imipenem-resistant *Pseudomonas aeruginosa*. *J Lab Physicians*, *9*(4), 249-253. Available:https://doi.org/10.4103/JLP.JLP_118_16

Singh, P., Bisht, D., Kumar, A., & Tripathi, A. (2023). Prevalence of carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* in rural Uttar Pradesh.

Singh, S., Samant, S. A., Bansal, M., Talukdar, A., & Arif, D. (2016). Phenotypic detection of carbapenemase-producing gram-negative bacteria by modified Hodge test. *Int J Curr Microbiol App Sci*, *5*, 315–320.

Thokar, M. A., Tiwari, N., Hussain, A., Ahmad, A., & Mahmood, S. E. (2019). Bacteriological study of surgical site infections in a tertiary care hospital of district Lucknow. *National Journal of Medical and Allied Sciences*, *8*(2), 39-44.

Thomas, N., & Sarwat, T. (2019). Prevalence of carbapenem-resistant *Enterobacterales* in a tertiary care hospital. *Int J Curr Microbiol App Sci*, *8*(11), 1418-1424.

Available:https://doi.org/10.20546/ijcmas.2019.811.166

Tsakris, A., Kristo, I., Poulou, A., et al. (2008). First occurrence of KPC-2-possessing *Klebsiella pneumoniae* in a Greek hospital and recommendation for detection with boronic acid disc tests. *J Antimicrob Chemother*, *62*, 1257-1260.

Verma, G., Nayak, S. R., Jena, S., et al. (2023). Prevalence of carbapenem-resistant *Enterobacterales*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* in a tertiary care hospital in Eastern India: A pilot study. *J Pure Appl Microbiol*, *17*, 2243-2249.

Verma, G., Singh, N., Smriti, S., Panda, S. S., Pattnaik, D., Tripathy, S., Praharaj, A. K., & Patro, A. R. K. (2024). Modified Carbapenem Inactivation Method and Ethylenediaminetetraacetic Acid (EDTA)-Carbapenem Inactivation Method for detection of carbapenemase-producing *Enterobacterales* and *Pseudomonas aeruginosa*. *Cureus*, *16*(6), e63340. Available:https://doi.org/10.7759/cureus.63340

Yigit, H., Queenan, A. M., Anderson, G. J., Domenech-Sanchez, A., Biddle, J. W., Steward, C. D., Alberti, S., Bush, K., & Tenover, F. C. (2001). Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.*, *45*, 1151–1161. Available:https://doi.org/10.1128/AAC.45.4.1151-1161.2001

Yong, D., Toleman, M. A., Giske, C. G., Cho, H. S., Sundman, K., Lee, K., & Walsh, T. R. (2009). Characterization of a new metallo-beta-lactamase gene, bla

(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob. Agents Chemother.*, *53*, 5046–5054.

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