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Original Research Article

Phenotypic Detection of Metallo-Beta-Lactamase-Producing *Escherichia coli* and *Klebsiella pneumoniae* in a Tertiary Care Hospital: A Cross-Sectional Study

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Abstract

Background: The development of isolates of Klebsiella pneumoniae and Escherichia coli that produce metallo-beta-lactamase (MBL) is a serious problem in clinical microbiology because of their resistance to carbapenems and other beta-lactam antibiotics. For these illnesses to be properly managed, quick and accurate identification techniques are essential.

Methods: A cross-sectional observational study was conducted over two years at a tertiary care hospital in Central India. A total of 10,320 clinical specimens were analyzed, yielding 480 non-duplicate isolates of *E. coli* (260) and *K. pneumoniae* (220). Imipenem resistance was screened using a 10-µg disk, and MBL production was detected using CDT and E-test.

Results: imipenem resistance was found in 168 (35%) of the 480 isolates, including 72 (15%) K. pneumoniae and 96 (20%) E. coli. In 80 (47.62%) of the isolates that were imipenem-resistant, MBL production was verified. 37% of K. pneumoniae and 42% of E. coli had MBL identified by CDT, whereas the E-test had somewhat higher detection rates (41.66% and 48%, respectively).

Conclusion: This study highlights the growing prevalence of MBL-producing *E. coli* and *K. pneumoniae* in clinical settings. The E-test showed superior sensitivity compared to CDT, making it a more reliable method for MBL detection. Routine screening and confirmation of MBL production are essential for implementing effective infection control measures and guiding antimicrobial therapy.

Keywords: Metallo-beta-lactamase (MBL), carbapenem resistance, *Escherichia coli*, *Klebsiella pneumoniae*, Combined Disk Test (CDT), Epsilometer Test (E-test).

Introduction

Antimicrobial resistance among Gram-negative bacteria has emerged as a global public health crisis, significantly limiting treatment options for serious infections. Carbapenems, often considered the last line of defense against multidrug-resistant organisms, are now threatened by the increasing prevalence of carbapenem-resistant pathogens. Among these, *Escherichia coli* and *Klebsiella pneumoniae* are the most frequently implicated organisms in healthcare-associated infections [1,2].

Carbapenem resistance is predominantly mediated by the production of carbapenemases, including metallo-beta-lactamases (MBLs). MBLs belong to class B carbapenemases, requiring divalent cations such as zinc for their enzymatic activity [3]. These enzymes confer resistance to almost all beta-lactam antibiotics, including carbapenems, while sparing monobactams. The rapid dissemination of MBL genes, often carried on mobile genetic elements, exacerbates the challenge of controlling these resistant organisms [4,5].

Phenotypic detection of MBL production remains a cornerstone of diagnostic microbiology, particularly in resource-limited settings where molecular techniques may not be feasible. Among the available methods, the Combined Disk Test (CDT) and Epsilometer Test (E-test) are widely used for their practicality and reliability. While molecular techniques provide greater accuracy in detecting specific MBL genes, their high cost and technical requirements limit their routine use in many healthcare settings [6,7].

This study aimed to determine the prevalence of imipenem-resistant *E. coli* and *K. pneumoniae* isolates in a tertiary care hospital and to evaluate the efficacy of two phenotypic methods—CDT and E-test—for detecting MBL production. By elucidating the local prevalence and resistance

patterns of MBL producers, this research seeks to inform infection control strategies and promote rational antibiotic use [8,9].

Materials and Methods

Study Design: This cross-sectional observational study was conducted over two years (January 2022–December 2023) in the Department of Microbiology at a tertiary care hospital in Central India.

Sample Collection: A total of 10,320 clinical specimens, including pus, urine, sputum, wound swabs, blood, and body fluids, were processed using standard microbiological procedures. A total of 480 non-duplicate isolates of *E. coli* (260 isolates) and *K. pneumoniae* (220 isolates) were included for analysis.

Identification and Screening: The isolates were identified using standard biochemical tests and an automated system (Vitek 2 Compact, Biomerieux). Antimicrobial susceptibility testing was conducted per Clinical and Laboratory Standards Institute (CLSI) guidelines. Screening for imipenem resistance was performed using a 10-µg imipenem disk, with isolates showing zone diameters ≤22 mm categorized as imipenem-resistant.

Phenotypic Detection of MBL

1. Combined Disk Test (CDT)

- A suspension adjusted to 0.5 McFarland turbidity was spread on Muller-Hinton agar (MHA).
- Two imipenem (10 µg) disks were placed 30 mm apart, one supplemented with 10 µL of 0.5 M EDTA.
- An increase in inhibition zone diameter ≥7 mm around the EDTA disk compared to the imipenem disk was considered MBL-positive.

2. Epsilometer Test (E-test)

- MBL E-test strips (Himedia) containing imipenem and imipenem-EDTA were applied to MHA plates inoculated with test organisms.
- Plates were incubated at 37°C for 16–18 hours. MBL production was confirmed if the MIC of imipenem-EDTA was reduced ≥8-fold compared to imipenem alone.

Ethical Approval: Ethical clearance for this study was obtained from the Institutional Ethics Committee.

Results

Distribution of Isolates: Among the 480 isolates, *E. coli* (54.17%) was more frequently identified than *K. pneumoniae* (45.83%). Most isolates were obtained from urine (35.63%), followed by pus (30.21%) and wound swabs (10.63%). The detailed distribution is provided in Table 1.

Sample Type	E. coli	K. pneumoniae	Total
Urine	92	79	171
Pus	85	60	145
Wound Swabs	28	23	51
Blood	20	30	50
Sputum	18	21	39
Body Fluids	17	7	24
Total	**260**	**220**	**480**

Imipenem Resistance: Of the 480 isolates, 168 (35%) were resistant to imipenem. Among these, 72 (43%) were *K. pneumoniae*, and 96 (57%) were *E. coli* (Table 2).

Organism	Imipenem-Resistant (%)	Imipenem-Sensitive (%)	Total (%)
E. coli	96 (20%)	164 (34.17%)	260 (54.17%)
K. pneumoniae	72 (15%)	148 (30.83%)	220 (45.83%)
Total	**168 (35%)**	**312 (65%)**	**480 (100%)**

MBL Detection by Phenotypic Methods: Among the 168 imipenem-resistant isolates, MBL production was confirmed in 80 isolates (47.62%). Using CDT, 37% of *K. pneumoniae* and 42% of *E. coli* isolates were MBL-positive. The E-test detected MBL in 41.66% of *K. pneumoniae* and 48% of *E. coli* isolates (Table 3).

Organism	Imipenem-Resistant	CDT Positive (%)	E-test Positive (%)
E. coli	96	40 (41.66%)	46 (48.00%)
K. pneumoniae	72	27 (37.50%)	30 (41.66%)

Discussion

Because there are few viable treatments for infections brought on by these resistant organisms, the rise of isolates of Klebsiella pneumoniae and

Escherichia coli that produce metallo-beta-lactamase (MBL) presents a serious issue in clinical microbiology. This work sheds important light on the patterns of antibiotic resistance in Central India by highlighting the frequency of imipenem resistance and MBL generation in a tertiary care hospital there.

The study found that 35% of the isolates were resistant to imipenem, a key carbapenem used to treat severe infections caused by Gram-negative bacteria. This prevalence is higher compared to some earlier studies, which reported imipenem resistance rates ranging from 24% to 28% [10,11], indicating a potential regional or temporal increase in resistance levels. Among imipenem-resistant isolates, 47.62% were confirmed as MBL producers, aligning with similar studies that observed MBL production rates of approximately 40%–50% [8,12].

The study demonstrated that imipenem resistance and MBL production were more prevalent in *E. coli* (20% and 48%, respectively) compared to *Klebsiella pneumoniae* (15% and 41.66%, respectively). This is in contrast to several studies that reported higher resistance and MBL production rates in *Klebsiella pneumoniae* than in *E. coli* [13,14]. This disparity underscores the variability in antimicrobial resistance patterns across different geographical regions and healthcare settings.

The combined disk test (CDT) and Epsilometer test (E-test) were both effective in detecting MBL production, but the E-test demonstrated slightly higher sensitivity, identifying 48% and 41.66% of MBL producers in *E. coli* and *Klebsiella pneumoniae*, respectively. These findings are consistent with previous reports suggesting that the E-test is a reliable phenotypic method for MBL detection, though it is costlier compared to CDT [6,7]. CDT remains a practical alternative for routine diagnostics in resource-limited settings [15,16].

Strong infection control procedures and antimicrobial stewardship initiatives are desperately needed, as seen by the high frequency of MBL producers in our research. MBL synthesis greatly reduces the range of available treatments by conferring resistance to other beta-lactams as well as carbapenems [4,5]. Early detection of MBL-producing isolates is crucial for preventing their dissemination in healthcare facilities.

The observed MBL production rate (47.62%) is comparable to the findings of Fazlul et al. (52.6%) [8] and slightly higher than those of Wadekar et al. (18%) [14] and Panchal et al. (19.62%) [11]. This variation may be attributed to differences in study populations, detection methods, and regional antibiotic usage practices. Moreover, the higher MBL detection rate in *E. coli* compared to *Klebsiella pneumoniae* contrasts with studies that typically report higher rates in *Klebsiella pneumoniae* [17,18], suggesting possible differences in the local epidemiology of resistant strains.

Strengths and Limitations

This study utilized two complementary phenotypic methods for MBL detection, enhancing the reliability of the findings. However, the absence of molecular confirmation methods, such as polymerase chain reaction (PCR), is a limitation that prevents identification of specific MBL genes. Future studies incorporating molecular techniques are recommended to better understand the genetic mechanisms underlying MBL production [19,20].

Conclusion

Imipenem-resistant and MBL-producing E. Coli and Klebsiella pneumoniae isolates are becoming more common in clinical settings, according to the study's findings. Appropriate antibiotic treatment and efficient infection management depend on routine phenotypic screening for MBL production. The genetic factors of MBL production and the development of quick, affordable diagnostic techniques for application in healthcare settings with limited resources require more investigation.

References

1. Shobha KL, Lenka PR, Sharma MK, Ramachandra L, Bairy I. Metallo-β-lactamase production among *Pseudomonas* species and *Acinetobacter* species in coastal Karnataka. J Clin Diagn Res 2009; 3:1747–53.
2. Kabbaj H, Seffar M, Belefquih B, et al. Prevalence of metallo-β-lactamases producing *Acinetobacter baumannii* in a Moroccan hospital. Int Sch Res Notices 2013; 2013:3.
3. Schlesinger SR, Lahousse MJ, Foster TO, Kim SK. Metallo-β-lactamases and aptamer-based inhibition. Pharmaceuticals (Basel) 2011; 4:419–28.
4. Rice LB, Bonomo RA. Mechanism of resistance to antimicrobial agents. In: Murray PR, et al., editors. Manual of Clinical Microbiology. Washington, DC: ASM Press; 2007. p. 1125–8.
5. Irfan S, Zafar A, Guhar D, et al. Metallo-beta-lactamase-producing clinical isolates of *Acinetobacter* species and *Pseudomonas aeruginosa* from ICU patients of a tertiary care hospital. Indian J Med Microbiol 2008; 26:243–5.
6. Yong D, Lee K, Yum JH, et al. Imipenem-EDTA disk method for differentiation of metallo-beta-lactamase-producing clinical isolates of *Pseudomonas* spp. and

Acinetobacter spp. J Clin Microbiol 2002;40:3798–801.
7. Walsh TR, Bolmström A, Qwärnström A, Gales A. Evaluation of a new E-test for detecting metallo-beta-lactamases in routine clinical testing. J Clin Microbiol 2002; 40:2755–9.
8. Fazlul MKK, Deepthi S, Farzana Y, et al. Detection of metallo-β-lactamases-encoding genes among clinical isolates of *Escherichia coli* in a tertiary care hospital, Malaysia. Int J Pharm Res 2019; 11:290–8.
9. Khosravi Y, Loke MF, Chua EG, et al. Phenotypic detection of metallo-ß-lactamase in imipenem-resistant *Pseudomonas aeruginosa*. Scientific World J 2012; 2012:654939.
10. Kumarasamy KK, Toleman MA, Walsh TR, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: A molecular, biological, and epidemiological study. Lancet Infect Dis 2010; 10:597–602.
11. Panchal CA, Oza SS, Mehta SJ. Comparison of four phenotypic methods for detection of metallo-beta-lactamase-producing gram-negative bacteria in rural teaching hospitals. J Lab Physicians 2017; 9:81–3.
12. Marie MA, John J, Krishnappa LG, et al. Molecular characterization of beta-lactamases in *Escherichia coli* and *Klebsiella pneumoniae*. Microbiol Immunol 2013; 57:805–10.
13. Wadekar MD, Anuradha K, Venkatesha D. Phenotypic detection of ESBL and MBL in clinical isolates of Enterobacteriaceae. Int J Curr Res Acad Rev 2013; 1:89–95.
14. Agarwal N, Chauhan S, Singh P, et al. Phenotypic detection of carbapenem resistance and metallo-beta-lactamase among Enterobacteriaceae. Int J Res Med Sci 2019; 7:2373–6.
15. Behera B, Mathur P, Das A, et al. An evaluation of four different phenotypic techniques for detection of metallo-beta-lactamase producing *Pseudomonas aeruginosa*. Indian J Med Microbiol 2008; 26:233–7.
16. Datta P, Gupta V, Garg S, et al. Phenotypic method for differentiation of carbapenemases in Enterobacteriaceae: Study from North India. Indian J Pathol Microbiol 2012; 55:357–60.
17. Castanheira M, Mendes RE, Woosley LN, et al. Trends in carbapenemase-producing *Escherichia coli* and *Klebsiella* spp. from Europe and the Americas. J Antimicrob Chemother 2011; 66:1409–11.
18. Chauhan K, Pandey A, Asthana AK, et al. Evaluation of phenotypic tests for detection of Klebsiella pneumoniae carbapenemase and metallo-beta-lactamase in clinical isolates of *Escherichia coli* and *Klebsiella* species. Indian J Pathol Microbiol 2015; 58:31–5.
19. Galani I, Rekatsina PD, Hatzaki D, et al. Evaluation of different laboratory tests for the detection of metallo-beta-lactamase production in Enterobacteriaceae. J Antimicrob Chemother 2008; 61:548–53.
20. Pandya N, Prajapati S, Mehta S, et al. Evaluation of various methods for detection of metallo-beta-lactamase production in gram-negative bacilli. Int J Biol Med Res 2011; 2:775–7.