E-ISSN 2250-0944
ISSN 2250-1150
doi: 10.31838/ijprt/15.02.302

Research Article

Phenotypic Detection of Carbapenemases in Klebsiella Pneumoniae from Clinical Isolates in a Tertiary Care Hospital

Dr. S. Rajeswari[1], Dr. M. Umamaheswari[2], Dr. R.Vasanthi[3]
[1,2,3]Assistant Professors, Institute of Microbiology, Madurai Medical College, Madurai, Tamil Nadu, India.
Corresponding Author:
Dr. R. Vasanthi
Assistant Professor, Institute of Microbiology, Madurai Medical College, Madurai, Tamil Nadu, India.
Received: 18.08.25, Revised: 21.09.25, Accepted: 01.10.25

ABSTRACT
Introduction: The rise in carbapenem-resistant Klebsiella pneumoniae (CRKP) poses a serious therapeutic challenge, especially in resource-limited settings. Phenotypic tests serve as essential diagnostic tools for detecting carbapenemase production.
Aim: To evaluate and compare the efficacy of three phenotypic methods—Modified Hodge Test (MHT), Combined Disc Test (CDT), and Rapidec Carba NP (RCNP)—in detecting carbapenemase production among K. pneumoniae isolates.
Methods: In a prospective study from September 2016 to August 2017, a total of 196 non-repetitive clinical isolates of K. pneumoniae were collected from a tertiary care hospital. Isolates were tested for meropenem resistance using E-test, followed by phenotypic detection using MHT, CDT, and RCNP.
Results: Out of 196 isolates, 26 (13.27%) were resistant to meropenem (MIC ≥4 µg/ml). Among these 26, 16 (61.5%) were positive by MHT, 22 (84.6%) were MBL-positive by CDT, and 21 (80.8%) were positive by RCNP.
Conclusion: Among the three methods, the Combined Disc Test showed the highest detection rate, followed by RCNP. The MHT demonstrated relatively lower sensitivity. Phenotypic methods continue to be valuable diagnostic tools in settings where molecular diagnostics are unavailable.

Keywords: Klebsiella pneumoniae, Carbapenemase, Phenotypic detection, MHT, CDT, Rapidec Carba NP.

INTRODUCTION

Klebsiella pneumoniae is a Gram-negative, encapsulated, facultative anaerobic bacillus belonging to the Enterobacteriaceae family [1]. It is a major cause of healthcare-associated infections, especially in immunocompromised individuals, patients in intensive care units (ICUs), and those with prolonged hospital stays [2]. Clinically, K. pneumoniae is frequently isolated from respiratory tract infections, urinary tract infections, bloodstream infections, wound infections, and device-associated infections such as ventilator-associated pneumonia and catheter-associated infections [3, 4]. The organism's ability to acquire and disseminate antibiotic resistance genes has elevated it to a pathogen of global concern [5, 6]. Over the past decade, K. pneumoniae has shown an alarming rise in resistance to multiple antibiotics, including β-lactams, fluoroquinolones, and aminoglycosides [7, 8]. Among these, carbapenem resistance is of particular concern due to the role of carbapenems as last-line agents in treating infections caused by multidrug-resistant Gram-negative bacteria [9, 10]. Carbapenem-resistant K. pneumoniae (CRKP) poses a significant challenge to clinicians and microbiologists because of limited therapeutic options, poor patient outcomes, and increased healthcare costs [11, 12]. The World Health Organization (WHO) has recognized CRKP as a "critical priority pathogen" in the global effort to combat antimicrobial resistance [13, 14]. The primary mechanism underlying carbapenem resistance in K. pneumoniae is the production of carbapenemases—β-lactamases that hydrolyze not only carbapenems but also a wide range of other β-lactam antibiotics [15, 16]. These enzymes include KPC (Klebsiella pneumoniae carbapenemase), NDM (New Delhi metallo-β-lactamase), OXA-48, VIM (Verona integron-encoded metallo-β-lactamase), and IMP (Imipenemase) [17, 18]. The genes encoding

these enzymes are often located on plasmids, which facilitate horizontal gene transfer between bacterial strains and species, accelerating the spread of resistance [19].

Early detection of carbapenemase-producing organisms (CPOs) is crucial for several reasons. Firstly, it enables clinicians to tailor antibiotic therapy based on susceptibility patterns, avoiding the use of ineffective drugs and preserving remaining treatment options. Secondly, early detection supports timely implementation of infection prevention and control (IPC) measures to contain nosocomial transmission [20-22]. Thirdly, surveillance data on carbapenem resistance can inform antibiotic stewardship programs and public health interventions [23]. While molecular techniques such as PCR, real-time PCR, and whole-genome sequencing offer rapid and highly specific identification of carbapenemase genes, their use in many developing countries remains limited due to high costs, need for skilled personnel, and lack of infrastructure [24, 25]. Consequently, there is a continued reliance on phenotypic methods in routine clinical laboratories, especially in resource-constrained settings [26]. Phenotypic detection methods are cost-effective, relatively easy to perform, and can provide valuable information on the functional expression of resistance mechanisms [27]. Among the commonly used phenotypic methods, the Modified Hodge Test (MHT) was once widely employed but is now considered less reliable due to its low specificity and high false-positive rates, particularly with certain strains of Enterobacteriaceae. Nevertheless, it still has relevance in some settings as a preliminary screening tool [28, 29]. The Combined Disc Test (CDT), which detects metallo-β-lactamase (MBL) activity based on the inhibition of carbapenemase enzymes by EDTA or other chelating agents, offers better sensitivity and specificity [30]. Another promising method is the Rapidec Carba NP (RCNP) test, a rapid colorimetric assay that detects carbapenemase activity through hydrolysis of the imipenem substrate, resulting in a pH shift and observable color change [31]. This test provides results in less than two hours and does not require specialized equipment, making it a viable option for laboratories with limited resources [32]. This study focuses on evaluating three phenotypic methods—the Modified Hodge Test (MHT), Combined Disc Test (CDT), and Rapidec Carba NP Test (RCNP)—to assess their reliability in detecting carbapenemase production in clinical isolates of K. pneumoniae from a tertiary care hospital.

MATERIALS AND METHODS

2.1 Study Design and Duration

A prospective, cross-sectional study was conducted over a period of 12 months, from September 2016 to August 2017, in the Department of Microbiology at a tertiary care hospital. The study aimed to detect carbapenemase production in K. pneumoniae isolates using three phenotypic methods and to evaluate their diagnostic performance.

2.2 Sample Collection and Identification

A total of 196 non-repetitive clinical isolates of *K. pneumoniae* were collected from various clinical specimens, including blood, urine, sputum, wound swabs, pus, tracheal aspirates, and other body fluids. Only one isolate per patient was included to avoid duplication.

All specimens were processed according to standard microbiological procedures. Isolates were cultured on appropriate media, such as MacConkey agar and blood agar, and incubated at 37°C for 18–24 hours. Colonies suggestive of *K. pneumoniae* were identified based on colony morphology, Gram staining, biochemical tests (indole negative, citrate positive, urease positive, oxidase negative), and further confirmed using an automated identification system or manual identification kits where applicable.

2.3 Antibiotic Susceptibility Testing

All isolates were subjected to antimicrobial susceptibility testing using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, following Clinical and Laboratory Standards Institute (CLSI) guidelines. Meropenem (10 µg) and imipenem (10 µg) discs were used for preliminary screening of carbapenem resistance. Isolates showing reduced susceptibility (zone diameter ≤19 mm) to meropenem or imipenem were further subjected to Minimum Inhibitory Concentration (MIC) determination by E-test (epsilometer test). An MIC of ≥4 µg/ml for meropenem was considered indicative of carbapenem resistance as per CLSI breakpoints.

2.4 Phenotypic Tests for Carbapenemase Detection

All carbapenem-resistant K. pneumoniae isolates (based on MIC) were subjected to the following phenotypic tests:

2.4.1 Modified Hodge Test (MHT)

The Modified Hodge Test was performed to detect carbapenemase activity using *E. coli* ATCC 25922 as the indicator strain. A lawn culture of the indicator strain was made on Mueller-Hinton agar, and a 10 µg meropenem disc was placed at the center. Test isolates were streaked from the edge of the disc to the periphery in straight lines. Plates were incubated at 35°C for 16–24 hours. A cloverleaf-like indentation of growth of the indicator strain towards the meropenem disc was interpreted as a positive result, indicating carbapenemase production.

2.4.2 Combined Disc Test (CDT)

The CDT was used to detect metallo-β-lactamase (MBL) production. Two imipenem discs (10 µg) were placed on a Mueller-Hinton agar plate inoculated with the test organism. To one of the discs, 10 µl of 0.5 M EDTA solution was added. After overnight incubation at 37°C, an increase in the zone of inhibition of ≥7 mm around the imipenem + EDTA disc compared to imipenem alone was considered positive for MBL production.

2.4.3 Rapidec Carba NP (RCNP) Test

The Rapidec Carba NP test (bioMérieux, France) was performed according to the manufacturer's instructions. This rapid biochemical test detects carbapenemase activity through hydrolysis of the imipenem substrate, which results in a pH change and corresponding color shift in the indicator medium. A color change from red to yellow/orange was interpreted as positive, while no color change indicated a negative result. Results were recorded within 2 hours.

2.5 Quality Control

Quality control strains used included:

- Escherichia coli ATCC 25922 – Negative control
- Klebsiella pneumoniae ATCC BAA-1705 – Positive control for KPC production
- Pseudomonas aeruginosa ATCC BAA-2108 – Positive control for MBL production

All media and reagents were quality checked before use. Tests were repeated in case of ambiguous results.

2.6 Data Analysis

The data were compiled and analyzed using Microsoft Excel. The proportion of carbapenem-resistant isolates was calculated. The sensitivity and specificity of each phenotypic method were compared using RCNP as the reference standard. Descriptive statistics (percentages, frequencies) were used to summarize the findings.

RESULTS

3.1 Isolation and Sample Distribution

A total of 196 non-repetitive clinical isolates of K. pneumoniae were collected from various clinical specimens of patients admitted to Government Rajaji Hospital, Madurai, over one year. The isolates were obtained from urine, pus, blood, sputum, wound swabs, and tracheal aspirates. The distribution of isolates by specimen type is shown in Figure 1. The highest number of isolates were recovered from urine samples (37.76%), followed by pus (30.10%), and blood (16.84%). The least number of isolates were from tracheal aspirates (2.55%).

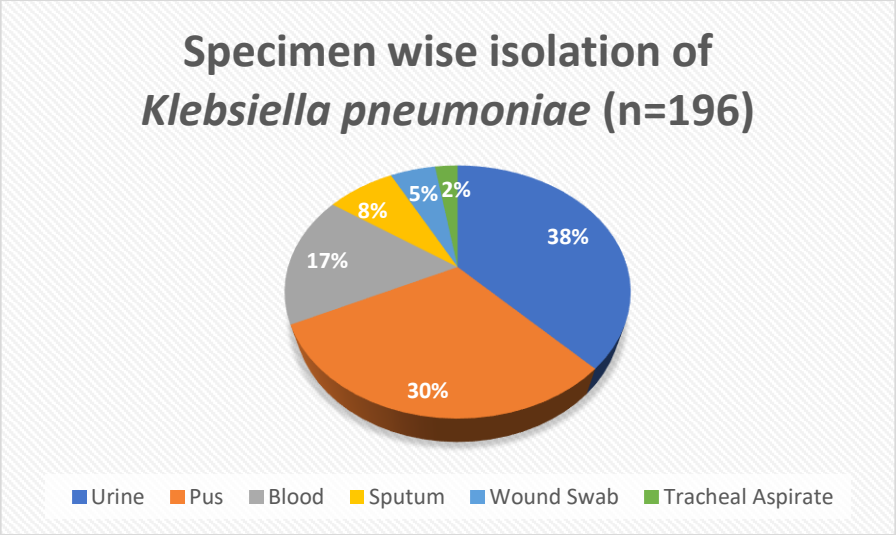

Fig: 1 Distribution of K. pneumoniae isolates by specimen type (n=196)

E-ISSN 2250-0944
ISSN 2250-1150
doi: 10.31838/ijprt/15.02.302

3.2 Age-Wise Distribution of *K. Pneumoniae* Infections among Patients

The age-wise distribution of *K. pneumoniae* infections among 196 patients shows that the highest prevalence was observed in individuals aged above 60 years, accounting for 23% of the cases. This was closely followed by the 46–60 years age group, with 21.9%, and the 31–45 years group, comprising 19.4% of the total cases. Notably, a significant number of infections were also seen in infants under 1 year, representing 21% of the patients. In contrast, younger age groups had a lower incidence, with the 1–15 years group making up 7.7%, and the 16–30 years group comprising only 7% of the cases (Figure 2). This distribution suggests that both the elderly and infants are more vulnerable to *K. pneumoniae* infections, possibly due to weaker immune defences or underlying health conditions.

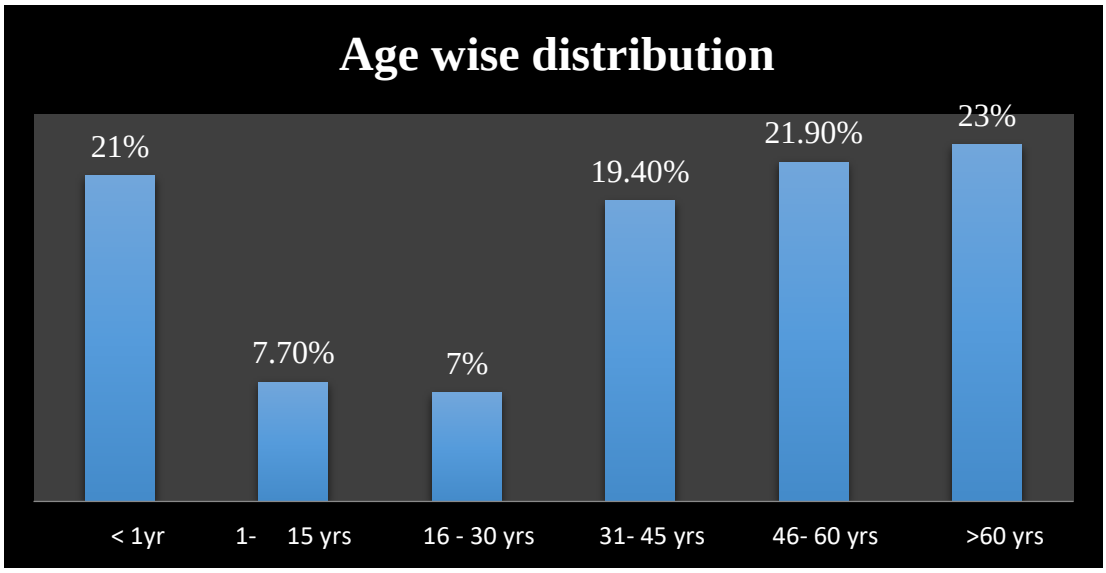

Fig: 2 Age Wise Distribution of K. Pneumoniae

3.3 Sex-Wise Distribution of K. Pneumoniae Isolates among Patients

Out of the 196 *K. pneumoniae* isolates analyzed, a higher prevalence was observed in male patients, who accounted for 125 cases (63.78%). In comparison, female patients made up 71 cases (36.22%) (Figure 3). This data indicates a male predominance in the incidence of *K. pneumoniae* infections, suggesting that males may be at a higher risk of infection in the studied population.

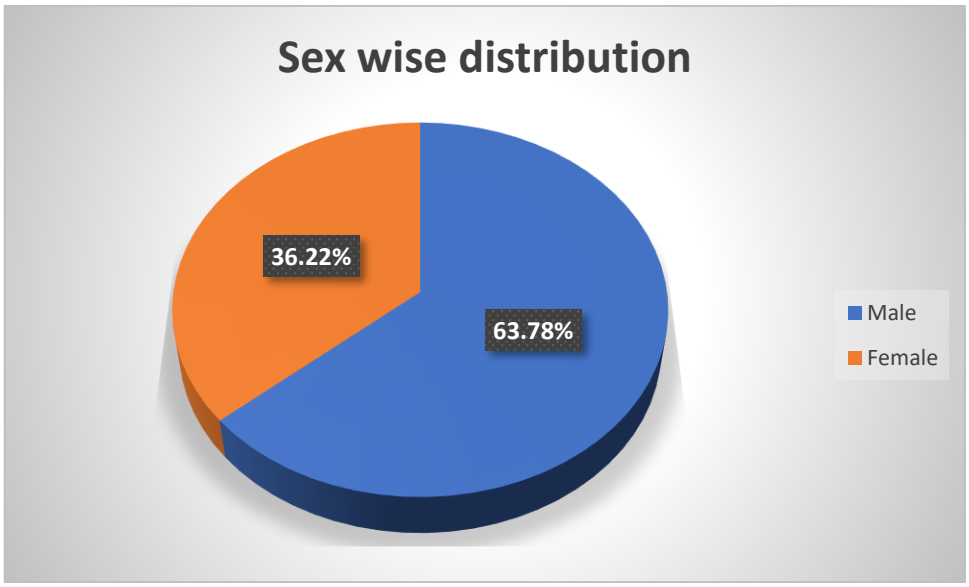

Fig: 3 Sex wise distribution of K. pneumoniae isolates

E-ISSN 2250-0944
ISSN 2250-1150
doi: 10.31838/ijprt/15.02.302

3.4 Ward-Wise Distribution of K. Pneumoniae Isolates

Among the 196 *K. pneumoniae* isolates, the highest number were obtained from Intensive Care Units (ICUs), including IMCU, IRCU, and PICU, accounting for 78 isolates (39.8%). This was followed by the Surgery department with 35 isolates (17.86%), and the Obstetrics & Gynaecology (OG) ward with 25 isolates (12.76%). The Orthopaedics and Medicine departments contributed 22 (11.22%) and 20 (10.2%) isolates, respectively. The lowest number of isolates was reported from the Paediatrics ward, with 16 cases (8.16%) (Figure 4). These findings highlight the significant burden of *K. pneumoniae* infections in critical care settings, particularly in ICUs, where patients are often more vulnerable due to underlying illnesses and invasive procedures.

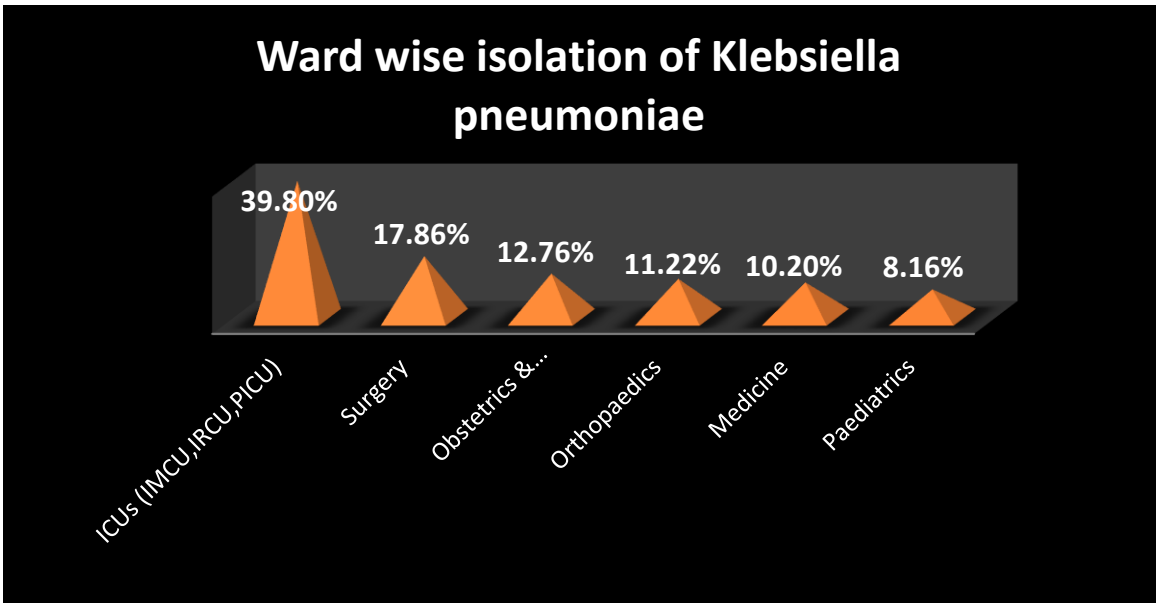

Fig: 4 Ward-Wise Distributions of K. Pneumoniae Isolates

3.5 Antimicrobial Susceptibility Testing

All isolates underwent antibiotic susceptibility testing using the Kirby-Bauer disc diffusion method for multiple antibiotics, including meropenem. The susceptibility results revealed that the isolates exhibited the highest sensitivity to tigecycline (100%), followed by amikacin and netilmicin (both 85.71%), and meropenem (83.67%). Ampicillin showed the lowest sensitivity, with only 5.10% of isolates susceptible. The susceptibility pattern for meropenem specifically is detailed in Table 1. Meropenem resistance was observed in 30 isolates (15.31%), while 164 isolates (83.67%) were susceptible. Two isolates (1.02%) displayed intermediate susceptibility.

Table 1: Meropenem Susceptibility Pattern among *K. Pneumoniae* Isolates (N=196)

CLSI Interpretation	Number of isolates	Percentage (%)
Susceptible (≥ 23 mm zone)	164	83.67
Intermediate (20-22 mm zone)	2	1.02
Resistant (≤ 19 mm zone)	30	15.31
Total	**196**	**100**

3.5.1 Confirmation of Carbapenem Resistance by MIC

The 32 isolates that showed resistant or intermediate susceptibility to meropenem by disc diffusion were further analyzed by E-test to determine the Minimum Inhibitory Concentrations (MIC). Out of these, 26 isolates (81.25%) had MIC values ≥ 4 µg/mL,

confirming carbapenem resistance, while 6 isolates (18.75%) had MICs within the susceptible range (≤ 1 µg/mL). No isolates had intermediate MIC values. This confirmed that 13.27% (26/196) of the total isolates were carbapenem-resistant *K. pneumoniae* (Table 2).

Table 2: Meropenem MIC Distribution Among Resistant And Intermediate Isolates (N=32)

MIC Range (µg/mL)	Number of Isolates	Percentage (%)
Susceptible (≤ 1)	6	18.75
Intermediate (2)	0	0
Resistant (≥ 4)	26	81.25
Total	**32**	**100**

3.6 Phenotypic Detection of Carbapenemase Enzymes

The 26 confirmed carbapenem-resistant isolates were tested for carbapenemase production using three phenotypic assays: Modified Hodge Test (MHT), Combined Disc Test (CDT) with EDTA, and Rapidec Carba NP (RCNP) test (Table 3).

- Modified Hodge Test (MHT) was positive in 16 isolates (61.54%), indicating carbapenemase production.
- Combined Disc Test (CDT) detected carbapenemase production in 22 isolates (84.6%), showing the highest sensitivity among the phenotypic tests.
- Rapidec Carba NP test showed positive results in 21 isolates (80.77%), providing rapid and reliable results.

Table 3: Phenotypic Detection of Carbapenemase Production

Phenotypic Test	Positive isolates	Percentage (%)	Negative isolates	Percentage (%)
Modified Hodge Test (MHT)	16	61.54	10	38.46
Combined Disc Test (CDT)	22	84.6	4	15.4
Rapidec Carba NP Test (RCNP)	21	80.77	5	19.23

3.7 Comparative Analysis of Phenotypic Tests

The Combined Disc Test demonstrated the highest detection rate of carbapenemase producers, with a sensitivity of 84.6%, closely followed by the Rapidec Carba NP test (80.77%). The Modified Hodge Test detected carbapenemase production in only 61.54% of resistant isolates, highlighting its limitations, especially in detecting certain carbapenemase types like NDM (Table 4).

Table 4: Comparative Performance of Phenotypic Tests for Carbapenemase Detection (n = 26)

Phenotypic Test	Positive Isolates (n)	Detection Rate (%)
Modified Hodge Test (MHT)	16	61.54%
Combined Disc Test (CDT)	22	84.60%
Rapidec Carba NP Test (RCNP)	21	80.77%

DISCUSSION

The emergence of carbapenem-resistant Klebsiella pneumoniae (CRKP) is a growing public health concern worldwide due to its association with high morbidity, mortality, and limited therapeutic options. This study evaluated the efficacy of three phenotypic tests—Modified Hodge Test (MHT), Combined Disc Test (CDT), and Rapidec Carba NP (RCNP)—for detecting carbapenemase production in clinical isolates of *K. pneumoniae* in a tertiary care hospital setting. In our study, 13.27% (26/196) of *K. pneumoniae* isolates were confirmed to be carbapenem-resistant based on MIC determination (MIC ≥ 4 µg/ml for meropenem). This prevalence is consistent with other studies conducted in Indian tertiary care centers, where the prevalence of CRKP

has ranged from 10% to 20% depending on geographical region, hospital practices, and patient population [33, 34].

4.1 Performance of Phenotypic Tests

Among the phenotypic tests evaluated, the Combined Disc Test (CDT) demonstrated the highest detection rate (84.6%), followed by the Rapidec Carba NP test (80.77%), and the Modified Hodge Test (61.54%). The high sensitivity of CDT in our study supports its effectiveness in detecting metallo-β-lactamase (MBL) producers, which are common in Indian isolates, particularly those harboring NDM genes [35]. The Rapidec Carba NP test, a colorimetric assay based on imipenem hydrolysis, showed a detection rate of over 80%, consistent with findings from other studies that have reported sensitivity ranging from 70–95%, depending on the prevalence of specific carbapenemase genes and bacterial species [36, 37]. Its rapid turnaround time (under 2 hours) makes it a practical option for routine diagnostics, especially where molecular methods are unavailable. The Modified Hodge Test, while once considered a reference method, showed the lowest sensitivity (61.54%) in our study. This is in line with published data highlighting its poor performance in detecting certain carbapenemases, particularly NDM and OXA-48-like enzymes, and its tendency to yield false-positive results due to AmpC production or porin loss [38, 39]. Consequently, MHT is no longer recommended by the CLSI (Clinical and Laboratory Standards Institute) as a confirmatory test for carbapenemase detection [40].

4.2 Clinical Implications

The predominance of CRKP isolates from ICUs (39.8%), as seen in our ward-wise distribution, highlights the vulnerability of critically ill patients to multidrug-resistant infections. ICU patients often undergo invasive procedures, have prolonged hospital stays, and are frequently exposed to broad-spectrum antibiotics—all of which contribute to the emergence and spread of resistant pathogens [41]. The higher proportion of CRKP in the elderly population (>60 years) and infants (<1 year) indicates age-related immunosuppression as a significant risk factor. Similar demographic patterns have been reported in previous epidemiological studies, suggesting that these age groups should be closely monitored for multidrug-resistant infections [42].

4.3 Utility of Phenotypic Methods in Resource-Limited Settings

In many low- and middle-income countries, phenotypic methods remain the mainstay for detecting carbapenemase-producing organisms due to the cost and infrastructure limitations associated with molecular techniques. While molecular assays (e.g., PCR, multiplex PCR) provide accurate identification of carbapenemase genes (e.g., *bla_KPC*, *bla_NDM*, *bla_OXA-48*), their high cost and technical requirements limit their accessibility in routine hospital laboratories [43]. Hence, CDT and RCNP offer valuable alternatives for early detection and surveillance. However, phenotypic methods have limitations—they may fail to detect low-level enzyme producers or rare carbapenemases and cannot differentiate between different carbapenemase types. As such, a combination of phenotypic and genotypic approaches, where feasible, is ideal for comprehensive surveillance and infection control.

5. Limitations

This study has a few limitations. First, molecular confirmation of the carbapenemase genes was not performed due to resource constraints, which would have validated the phenotypic test results and provided genotype-phenotype correlation. Second, sample size of carbapenem-resistant isolates (n=26) was relatively small, though reflective of the actual prevalence in the hospital during the study period. Further multicenter studies with larger sample sizes and molecular confirmation are recommended.

CONCLUSION

Carbapenem-resistant *K. pneumoniae* poses a major threat in healthcare settings due to limited treatment options and high mortality. In this study, 13.27% of isolates were carbapenem-resistant. Among the phenotypic methods evaluated, the Combined Disc Test showed the highest sensitivity (84.6%), followed by Rapidec Carba NP (80.77%), while the Modified Hodge Test (61.54%) was least effective. Phenotypic tests remain valuable diagnostic tools, especially in resource-limited settings lacking molecular facilities. Early detection of carbapenemase producers is critical for effective patient management and infection control. Strengthening laboratory

capacity and antimicrobial stewardship is essential to combat the spread of CRKP.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Hu Y, Anes J, Devineau S, Fanning S. Klebsiella pneumoniae: prevalence, reservoirs, antimicrobial resistance, pathogenicity, and infection: a hitherto unrecognized zoonotic bacterium. Foodborne pathogens and disease. 2021 Feb 1;18(2):63-84.
2. Liu Y, Huang L, Cai J, Zhu H, Li J, Yu Y, Xu Y, Shi G, Feng Y. Clinical characteristics of respiratory tract infection caused by Klebsiella pneumoniae in immunocompromised patients: a retrospective cohort study. Frontiers in Cellular and Infection Microbiology. 2023 Aug 16;13:1137664.
3. Dadi NC, Radochová B, Vargová J, Bujdáková H. Impact of healthcare-associated infections connected to medical devices—An update. Microorganisms. 2021 Nov 11;9(11):2332.
4. Chakraverty R, Kundu AK. Hospital-Acquired Infections in Intensive Care Unit and Their Management: The Indian Perspective. Springer Nature; 2025 Feb 10.
5. Navon-Venezia S, Kondratyeva K, Carattoli A. Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. FEMS microbiology reviews. 2017 May 1;41(3):252-75.
6. Li Y, Kumar S, Zhang L, Wu H, Wu H. Characteristics of antibiotic resistance mechanisms and genes of Klebsiella pneumoniae. Open Medicine. 2023 May 12;18(1):20230707.
7. Li Y, Kumar S, Zhang L. Mechanisms of antibiotic resistance and developments in therapeutic strategies to combat Klebsiella pneumoniae infection. Infection and Drug Resistance. 2024 Dec 31:1107-19.
8. Hou G, Ahmad S, Li Y, Yan D, Yang S, Chen S, Qiu Z, Yu X, Li N, Li Y, Liang Y. Epidemiological, virulence, and Antibiotic Resistance Analysis of Klebsiella pneumoniae, a Major Source of Threat to Livestock and Poultry in some regions of Xinjiang, China. Animals. 2024 May 10;14(10):1433.
9. Gajic I, Tomic N, Lukovic B, Jovicevic M, Kekic D, Petrovic M, Jankovic M, Trudic A, Mitic Culafic D, Milenkovic M, Opavski N. A comprehensive overview of antibacterial agents for combating Multidrug-Resistant bacteria: the current landscape, development, future opportunities, and challenges. Antibiotics. 2025 Feb 21;14(3):221.
10. Mó I, da Silva GJ. Tackling carbapenem resistance and the imperative for One Health strategies—Insights from the portuguese perspective. Antibiotics. 2024 Jun 14;13(6):557.
11. Shah AA, Alwashmi AS, Abalkhail A, Alkahtani AM. Emerging challenges in Klebsiella pneumoniae: Antimicrobial resistance and novel approach. Microbial Pathogenesis. 2025 Feb 19:107399.
12. Zhu J, Chen T, Ju Y, Dai J, Zhuge X. Transmission Dynamics and Novel treatments of high risk carbapenem-resistant Klebsiella pneumoniae: the Lens of One Health. Pharmaceuticals. 2024 Sep 12;17(9):1206.
13. Kain MJ, Reece NL, Parry CM, Rajahram GS, Paterson DL, Woolley SD. The rapid emergence of hypervirulent klebsiella species and burkholderia pseudomallei as major health threats in southeast Asia: The urgent need for recognition as neglected tropical diseases. Tropical Medicine and Infectious Disease. 2024 Apr 8;9(4):80.
14. Igweonu CF. Molecular characterization of antibiotic resistance genes in multidrug-resistant Klebsiella pneumoniae clinical isolates. Int J Eng Technol Res Manag. 2024 Aug;8(08):241.
15. Di Pilato V, Pollini S, Miriagou V, Rossolini GM, D'Andrea MM. Carbapenem-resistant Klebsiella pneumoniae: the role of plasmids in emergence, dissemination, and evolution of a major clinical challenge. Expert Review of Anti-infective Therapy. 2024 Mar 3;22(1-3):25-43.
16. Falagas ME, Asimotou CM, Zidrou M, Kontogiannis DS, Filippou C. Global Epidemiology and Antimicrobial Resistance of Klebsiella Pneumoniae Carbapenemase (KPC)-Producing Gram-Negative Clinical Isolates: A Review. Microorganisms. 2025 Jul 19;13(7):1697.

17. Kimani R. A review of Carbapenems Resistance in the Current World. Journal of Medical and Biomedical Laboratory Sciences Research. 2024 May 15;4(1).
18. Dominguez CM, Oueslati S, Al Laham N, Nermont R, Volland H, Naas T. Comparison of Two Lateral Flow Immunochromatographic Assays for Rapid Detection of KPC, NDM, IMP, VIM and OXA-48 Carbapenemases in Gram-Negatives. Microorganisms. 2025 Sep 12;13(9):2140.
19. Wachino JI. Horizontal Gene Transfer Systems for Spread of Antibiotic Resistance in Gram-Negative Bacteria. Microbiology and Immunology. 2025 Jul;69(7):367-76.
20. Otu A, McCormick J, Henderson KL, Ledda A, Meunier D, Patel B, Brown CS, Singleton S, Mason EL, Islam J, Moore G. Understanding the landscape of carbapenemase-producing organisms (CPOs), and spotlighting opportunities for control in England. Infection Prevention in Practice. 2025 Aug 12:100480.
21. Cusack R, Little E, Martin-Loeches I. Practical lessons on antimicrobial therapy for critically Ill patients. Antibiotics. 2024 Feb 6;13(2):162.
22. Thakur H, Rao R. Emphasis of infection prevention and control: a review. J Popul Therap Clin Pharmacol. 2024;31:2238-49.
23. Mó I, da Silva GJ. Tackling carbapenem resistance and the imperative for One Health strategies—Insights from the portuguese perspective. Antibiotics. 2024 Jun 14;13(6):557.
24. Kao K, Alocilja EC. A Review of the Diagnostic Approaches for the Detection of Antimicrobial Resistance, Including the Role of Biosensors in Detecting Carbapenem Resistance Genes. Genes. 2025 Jun 30;16(7):794.
25. Ofori B, Twum S, Yeboah SN, Ansah F, Sarpong KA. Towards the development of cost-effective point-of-care diagnostic tools for poverty-related infectious diseases in sub-Saharan Africa. PeerJ. 2024 Jun 21;12:e17198.
26. Chakraborty S. Democratizing nucleic acid-based molecular diagnostic tests for infectious diseases at resource-limited settings–from point of care to extreme point of care. Sensors & Diagnostics. 2024;3(4):536-61.
27. Pawar L, Chouhan N, Singh A, Nag M, Reddy BS, Khan A, Singh SK, Awugo V. Pros and cons of diagnostic methods used for AMR surveillance in aquaculture. InAntimicrobial Resistance in Aquaculture and Aquatic Environments 2025 Mar 1 (pp. 137-161). Singapore: Springer Nature Singapore.
28. Chakraborty D, Bhatia M, Gupta P, Nagaraj G, Shamanna V, Srinitha P, Ashwini KV, Ravikumar KL, Srinitha P, Ashwini KV, Ravikumar KL. Assessment of the Diagnostic Accuracy of the Modified Hodge Test and Modified Carbapenem Inactivation Method for Identifying Carbapenem Resistance Mechanisms in Klebsiella pneumoniae: A Whole Genome Sequencing-Based Exploratory Study. Cureus. 2025 Mar 9;17(3).
29. Kumar N, Kaur N, Kumar H, Katyal A, Chauhan S. Selection of a Phenotypic Method for Detecting Carbapenemase-Producing Organisms: A Dilemma for Resource-Limited Settings. Bratislava Medical Journal. 2025 May 28:1-1.
30. Harsh T, Patil HV, Patil SR. Antibiotic Resistance in Metallo-ß-Lactamase-Producing Pseudomonas aeruginosa in Clinical Isolates: Challenges and Phenotypic Detection in a Tertiary Care Setting. Frontiers in Health Informatics. 2024 Apr 1;13(3).
31. Rakovitsky N, Lurie-Weinberger MN, Temkin E, Hameir A, Efrati-Epchtien R, Wulffhart L, Keren Paz A, Schwartz D, Carmeli Y. Evaluation of the CARBA PAcE test, a colorimetric imipenem hydrolysis test for rapid detection of carbapenemase activity. Microbiology Spectrum. 2024 Dec 5;12(12):e00891-24.
32. Lifshitz Z, Adler A, Carmeli Y. Comparative study of a novel biochemical assay, the Rapidec Carba NP test, for detecting carbapenemase-producing Enterobacteriaceae. Journal of Clinical Microbiology. 2016 Feb;54(2):453-6.
33. Wattal C, Goel N, Oberoi JK, et al. Surveillance of multidrug resistant organisms in a tertiary care hospital in Delhi, India. *J Assoc Physicians India*.2010;58:32–36.
34. Veeraraghavan B, Shankar C, Karunasree S, Kumari S, Ravi R, Ralph R. Carbapenem resistant Klebsiella pneumoniae isolated from bloodstream infection: Indian experience. Pathogens and global health. 2017 Jul 4;111(5):240-6.

35. Nordmann P, Gniadkowski M, Giske CG, Poirel L, Woodford N, Miriagou V, on Carbapenemases EN. Identification and screening of carbapenemase-producing Enterobacteriaceae. Clinical microbiology and infection. 2012 May 1;18(5):432-8.
36. Dortet L, Poirel L, Nordmann P. Rapid detection of carbapenemase-producing Pseudomonas spp. Journal of Clinical Microbiology. 2012 Nov;50(11):3773-6.
37. Yusuf I, Yusha'u M, Sharif AA, Getso MI, Yahaya H, Bala JA, Aliyu IA, Haruna M. Detection of metallo betalactamases among gram negative bacterial isolates from Murtala Muhammad Specialist Hospital, Kano and Almadina Hospital Kaduna, Nigeria. Bayero Journal of Pure and Applied Sciences. 2012;5(2):84-8.
38. Anderson KF, Lonsway DR, Rasheed JK, Biddle J, Jensen B, McDougal LK, Carey RB, Thompson A, Stocker S, Limbago B, Patel JB. Evaluation of methods to identify the Klebsiella pneumoniae carbapenemase in Enterobacteriaceae. Journal of clinical microbiology. 2007 Aug;45(8):2723-5.
39. Gajdács M, Ábrók M, Lázár A, Jánvári L, Tóth Á, Terhes G, Burián K. Detection of VIM, NDM and OXA-48 producing carbapenem resistant Enterobacterales among clinical isolates in Southern Hungary. Acta microbiologica et immunologica Hungarica. 2020 Dec 17;67(4):209-15.
40. Wayne P. CLSI performance standards for antimicrobial susceptibility testing. CLSI supplements M. 2020;100:20-30.
41. Falagas ME, Tansarli GS, Karageorgopoulos DE, Vardakas KZ. Deaths attributable to carbapenem-resistant Enterobacteriaceae infections. Emerging infectious diseases. 2014 Jul;20(7):1170.
42. Codjoe FS, Donkor ES. Carbapenem resistance: a review. Medical Sciences. 2017 Dec 21;6(1):1.
43. Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. The Journal of infectious diseases. 2017 Feb 15;215(suppl_1):S28-36.