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Carbapenemase-Producing Enterobacterales: Distribution and Evaluation of Phenotypic Detection Methods Among Carbapenem Resistant Enterobacterales.

Chetna[1], Shaheen Bhat[2], Ayesha Nazar[3], Yusuf Imran[4], Dalip K kakroo[5], Nashra Afaq[6]
[1]Chetna[1], (MSC Medical Microbiology), SMS& R, Sharda hospital,Greater Noida, India.
[2]Associate Professor[2*], Department of Microbiology, SMS & R, Sharda hospital,Greater NoidaIndia.
[3]Assistant Professor[3], Department of Microbiology, SMS & R, Sharda hospital, Greater Noida, India.
[4]Assistant Professor[4], Department of Pediatrics, NIIMS, NIU University, Greater Noida, India.
[5]Professor & Head[5], Department of Microbiology, SMS & R, Sharda hospital, Greater Noida, India.
[6]Assistant Professor[6], Department of Microbiology and Central Research Laboratory, Rama Medical College Hospital and Research Centre, Uttar Pradesh, India.

Corresponding Author: Dr Shaheen Bhat*
Email ID: shaheen.bhat@sharda.ac.in

ABSTRACT

Background: *Carbapenem-resistant Enterobacterales (CRE) represent a critical global health challenge due to resistance to last-line antibiotics and their involvement in hospital-acquired infections. Rapid detection of carbapenemase-producing Enterobacterales (CPE) is essential for guiding therapy and preventing transmission, particularly in resource-limited settings where molecular diagnostics are not routinely available.*

Methods: *A hospital-based cross-sectional study was conducted over the period of 1 year at a tertiary care centre. A total of 227 clinical isolates of CRE were processed using standard microbiological methods.Phenotypic assays, including Modified Hodge Test (MHT), EDTA inhibition test, and Boronic acid inhibition test, were employed to detect carbapenemase production among carbapenem-resistant isolates.*

Results: *Of the 227 CRE isolates, most were recovered from urine (53.3%), followed by pus (12.3%), pus swabs (10.1%), and respiratory specimens. Escherichia coli (66.08%) was the predominant isolate, followed by Klebsiella pneumoniae (16.74%) and Enterobacter spp. (4.41%). Specialty-wise, the majority of isolates originated from ICU (25.99%) and general medicine wards (22.03%). Phenotypic detection revealed the highest positivity with MHT (88.6%), followed by EDTA inhibition test (66.9%) and Boronic acid inhibition test (15%).*

Conclusion: *The study highlights the predominance of E. coli and K. pneumoniae among CRE isolates, with urine as the most frequent specimen source. Among phenotypic methods, MHT showed the highest detection efficiency, while the EDTA test was useful for identifying metallo-β-lactamase producers. Early phenotypic detection remains vital for effective management and infection control, especially in settings with limited molecular facilities.*

Keywords: *Carbapenem-resistant Enterobacterales (CRE), Carbapenemase, Modified Hodge Test, Phenotypic detection, Antimicrobial resistance.*

INTRODUCTION

Carbapenem-resistant Enterobacteriaceae (CRE) are a group of gram-negative bacteria that either produce carbapenemase enzymes or exhibit resistance to at least one carbapenem-class antibiotic[1,2]. Several phenotypic methods—such as the Modified Hodge Test, EDTA inhibition test, and Boronic Acid Inhibition Test—are routinely utilised in clinical laboratories for their detection due to ease of implementation. [3,4,5]

Globally, the rise of CRE has become a major clinical concern, associated with high morbidity and mortality rates[2,6,7]. These pathogens are typically resistant to one or more carbapenem agents, including imipenem, meropenem, ertapenem, or doripenem, either by reduced susceptibility or confirmed presence of carbapenemase genes.[2,8]

Carbapenems, members of the β-lactam antibiotic class, offer a broad antimicrobial spectrum and are typically reserved for severe or multidrug-resistant infections—especially those caused by extended-spectrum β-lactamase (ESBL) and AmpC-producing Enterobacteriaceae.[2,7] However, the proliferation of CRE has significantly undermined their therapeutic effectiveness, posing a serious threat to infection control and public health systems worldwide.[2,6]

CRE infections are complicated to manage because conventional antibiotics are largely ineffective, necessitating early and accurate identification in microbiology laboratories.[2,3,5,8]Of greatest concern are carbapenemase-producing Enterobacteriaceae (CPE), which secrete enzymes capable of hydrolysingcarbapenems and facilitating horizontal transmission within healthcare settings, thereby complicating patient outcomes and containment efforts.[5,8]

The surge in antimicrobial resistance over recent decades—exacerbated by antibiotic misuse, inadequate stewardship, and weak regulatory frameworks—has contributed to the emergence of multidrug-resistant Gram-negative pathogens.[6,9,10]Notably, members of the Enterobacteriaceae family have demonstrated high-level resistance to multiple antibiotic classes, with CRE now representing one of the most pressing threats in modern clinical microbiology and infectious disease management.[2,7]

MATERIALS AND METHODS

A hospital-based cross-sectional observational study was conducted over the period of 1 year, in the Department of Microbiology at Sharda Hospital, Greater Noida. Clinical specimens received in the bacteriology laboratory during this period— urine, blood, sputum, endotracheal tube aspirates (ETT), tracheal secretions, pus (frank and swab), tissue samples, high vaginal swabs, and bronchoalveolar lavage (BAL)—were included in the study. All samples were processed according to standard microbiological procedures.[11]

Primary bacterial isolation and identification were performed using conventional techniques such as Gram staining and a battery of biochemical tests (e.g., indole, citrate, urease, triple sugar iron test, etc.). Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, per the Clinical and Laboratory Standards Institute (CLSI) guidelines.[12]

To assess carbapenemase production among the Enterobacteriaceae isolates, three phenotypic assays were employed:

1.Modified Hodge Test (MHT) – used as a general screen for carbapenemase activity.
2.EDTA Inhibition Test – employed to detect metallo-β-lactamase (MBL) production.
3.Boronic Acid Inhibition Test – used to identify the presence of class A serine carbapenemases, such as KPC.[12]

All phenotypic tests were performed on isolates resistant or showing reduced susceptibility to carbapenems (imipenem, meropenem, or ertapenem) based on initial disk diffusion screening. The results were interpreted according to CLSI recommendations.[11]

RESULTS

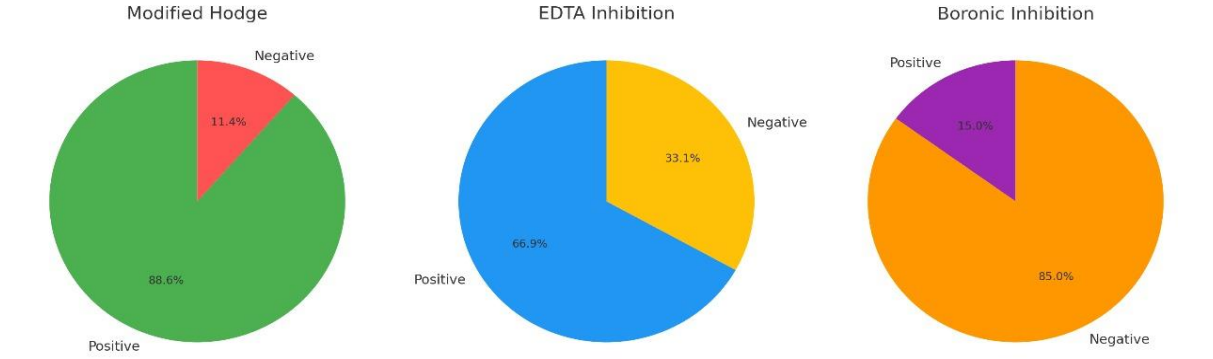
A total of 227 CRE isolates were collected from various clinical samples comprising urine (53.3%), frank pus (12.3%), pus swabs (10.1%), endotracheal tube (ETT) aspirates (6.2%), tracheal secretions (4.8%), blood (4.4%), sputum (4.4%), high vaginal swabs (3.1%), tissue samples (0.9%) and bronchoalveolar lavage (BAL) specimens (0.4%). The predominant bacterial isolate was Escherichia coli (66.08%), followed by Klebsiella pneumoniae (16.74%), Klebsiella spp. (5.29%), Enterobacter spp. (4.41%), Citrobacter spp. (3.08%), Klebsiellaoxytoca (1.76%), Citrobacterkoseri (1.32%), Citrobacterfreundii (0.88%) and Proteus mirabilis (0.44%).

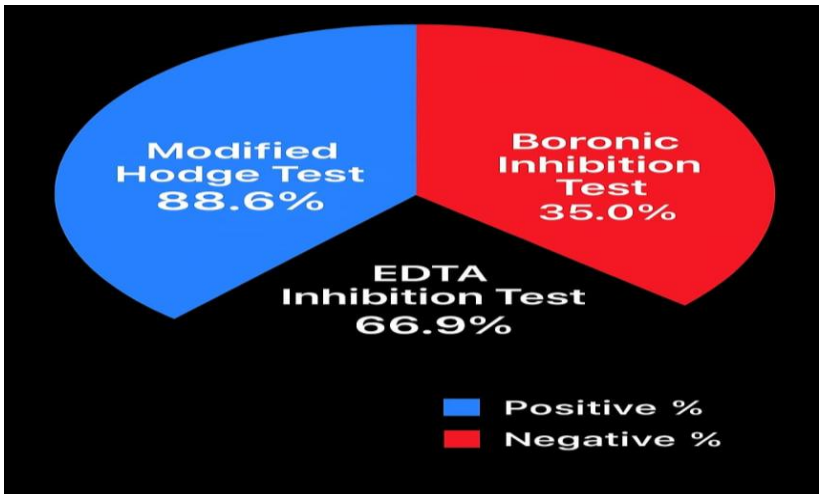
Carbapenemase production was evaluated using three different phenotypic assays. The Modified Hodge Test (MHT) yielded the highest detection rate, identifying 201 (88.6%) isolates as carbapenemase producers, while 26 (11.4%) were negative. The EDTA Inhibition Test, indicative of metallo-β-lactamase activity, detected carbapenemase production in 152 isolates (66.9%), with 75 (33.1%) testing negative. In contrast, the Boronic Acid Inhibition Test, which targets class A carbapenemases, showed comparatively lower sensitivity, detecting carbapenemase activity in only 34 isolates (15.0%), while 193 (85.0%) remained negative.

PHENOTYPICTEST RESULTS OF CARBAPENEM RESISTANT ENTEROBACTERALES

TEST	RESULT	PERCENTAGE (%)
MODIFIED HODGE TEST	POSITIVE	88.6
MODIFIED HODGE TEST	NEGATIVE	11.4
EDTA INHIBITION TEST	POSITIVE	66.9

EDTA INHIBITION TEST	NEGATIVE	33.1
BORONIC INHIBITION TEST	POSITIVE	15.0
BORONIC INHIBITION TEST	NEGATIVE	85.0





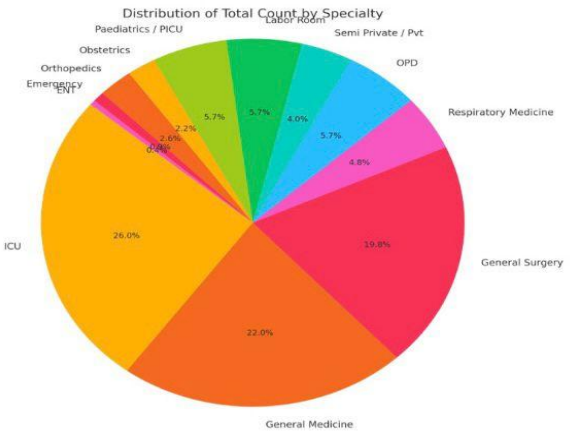
SPECIALITY WISE DISTRIBUTION OF CARBAPENEM RESISTANYT ENTEROBACTERALES

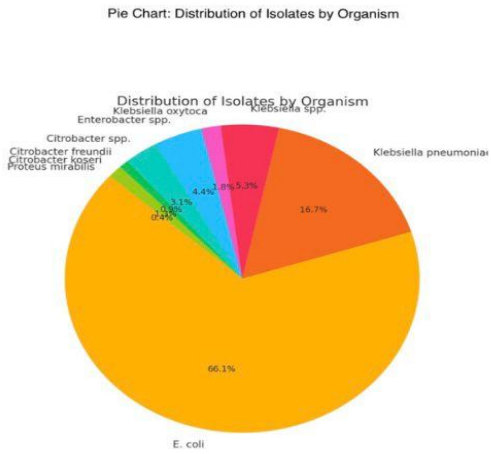
SPECIALITY	TOTAL COUNT	RATIO (%)
ICU	59	25.99%
GENERAL MEDICINE	50	22.03%
GENERAL SURGERY	45	19.82%
RESPIRATORY MEDICINE	11	4.85%
OPD	13	5.73%
SEMI PRIVATE/ PRIVATE	9	3.96%
LABOR ROOM	13	5.73%
PAEDIATRICS/ PICU	13	5.73%
OBSTETRICS	5	2.2%
ORTHOPEDICS	6	2.64%
EMERGENCY	2	0.88%
ENT	1	0.44%

CARBAPENEM RESISTANT ENTEROBACTERALES DISTRIBUTION

ORGANISM	COUNT	PERCENTAGE (%)
E.coli	150	66.08%
Klebsiella pneumoniae	38	16.74%
Klebsiella spp.	12	5.29%
Klebsiellaoxytoca	4	1.76%
Enterobacter spp.	10	4.41%
Citrobacter spp.	7	3.08%

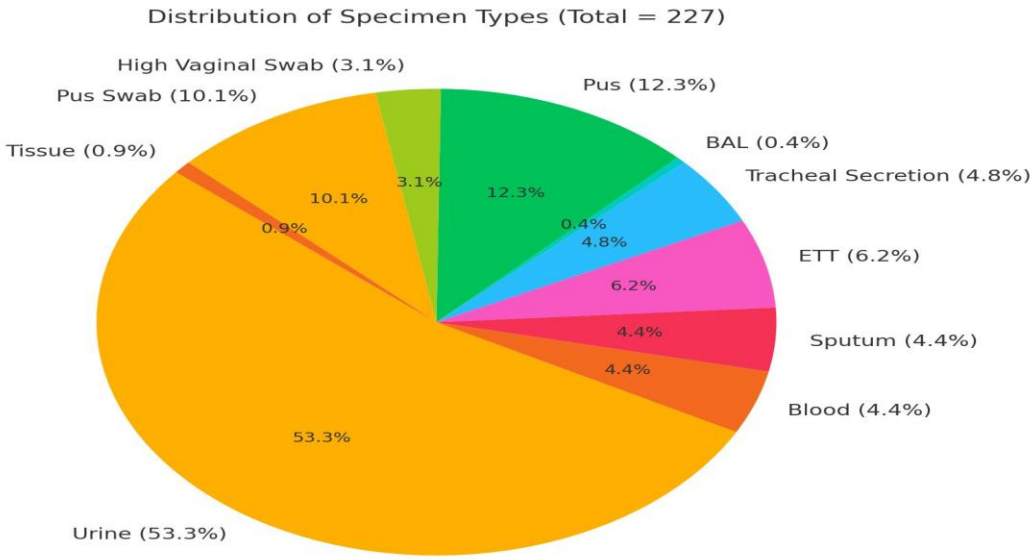
Citrobacterfreundii	2	0.88%
Citrobacterkoseri	3	1.32%
Proteus mirabilis	1	0.44%





SPECIMEN TYPE DISTRIBUTION OF CARBAPENEM RESISTANT ENTEROBACTERALES

SPECIMEN TYPE	COUNT	RATIO (%)
URINE	121	53.3%
BLOOD	10	4.4%
SPUTUM	10	4.4%
ETT	14	6.2%
TRACHEAL SECRETION	11	4.8%
BAL	1	0.4%
PUS	28	12.3%
HIGH VAGINAL SWAB	7	3.1%
PUS SWAB	23	10.1%
TISSUE	2	0.9%



GENDER WISE DISTRIBUTION OF CARBAPENEM RESISTANT ENTEROBACTERALES

CATEGORY	COUNT	RATIO (OUT OF 227)	PERCENTAGE (%)
FEMALE	105	105:227	46.26%
MALE	122	122:227	53.74%

DISCUSSION

Our study observed that Escherichia coli was the predominant carbapenem-resistant Enterobacterales (CRE) isolate (66.08%), followed by Klebsiella pneumoniae (16.74%). This is consistent with several previous studies, including those by Thomas et al.[13] and Shree RS et al.[14], who reported E. coli as the leading organism in 52.1% and 54% of cases, respectively. However, other reports indicate a reversed trend. Sharma K et al. [15] documented K. pneumoniae in 67.6% of isolates, while Pawar SK et al. [16] found 63% K. pneumoniae and only 19% E. coli. Wattal C et al. [17] also highlighted high carbapenem resistance rates in both E. coli and Klebsiella spp., emphasising that the prevalence can vary according to institutional epidemiology and patient population.

When evaluated by sample type, urine emerged as the most frequent specimen in our study (53.3%), followed by pus, respiratory, blood, and vaginal samples. This mirrors the findings of Thomas et al. [13] (52.1% urine, 20% pus) and Shree RS et al. [14] (42% urine, 34% pus). Such a pattern likely reflects the high burden of urinary tract infections in hospitalised patients and the efficiency of urinary screening in detecting CRE.

Regarding gender distribution, our data demonstrated a slight male predominance (53.7%), similar to observations by Thomas et al. [13] and Pawar SK et al. [16], indicating potential demographic or exposure-related factors contributing to the distribution. Specialty-wise, our highest CRE isolation rate was from ICU patients (25.99%), followed by general medicine wards (22.03%). This aligns with the ICU-associated high resistance rates reported by Wattal C et al. [17], though Thomas et al. [13] found a peak in surgical units (36.23%), underscoring variability in patient population risk.

The comparison of phenotypic methods across multiple studies shows that the Modified Hodge Test (MHT) remains a widely used screening tool, with our study recording the highest positivity (88.6%) compared to EDTA Inhibition Test (66.9%) and Boronic Acid Inhibition Test (15%). Our findings align with Josa M et al. [19], who reported 93.2% positivity with MHT, and Bartolini A et al. [20], who documented ~94% sensitivity with 100% specificity. Kamel et al. [21] and Elsherif R et al. [24] also confirmed high MHT specificity. However, other authors have questioned MHT's diagnostic precision: Datta et al. [18], Seah C et al. [23], and Falahat S et al. [22] noted limitations, including false-negative results and reduced specificity, especially for non-KPC carbapenemases.

Boronic acid-based tests demonstrated variable sensitivity—ranging from 15% in our work to >80% in Josa M et al. [19], Falahat S et al. [22],and Elsherif R et al. [24]—likely reflecting differences in enzyme type prevalence (e.g., KPC vs. OXA-48) and methodology. EDTA-based assays, while useful for detecting metallo-β-lactamases, exhibited lower sensitivity in several reports, including Bartolini A et al. [20] (~54.5%) and Kamel et al. [21] (~62%), consistent with our own moderate detection rate. Interestingly, some studies (e.g., Seah C et al. [23] highlight the complementary value of combining boronic acid and EDTA assays to detect a broader range of carbapenemases.

Recent studies from 2024 and 2025 further reinforce the growing concern of carbapenem-resistant Enterobacterales (CRE) as a global health challenge. A multicenter Canadian surveillance report in 2024 demonstrated increasing prevalence of E. coli and K. pneumoniae isolates harboring carbapenemase genes, particularly in intensive care units, with notable therapeutic failures despite newer β-lactam–β-lactamase inhibitor combinations . Similarly, an international study in 2025 highlighted that phenotypic methods such as Modified Hodge Test continue to serve as valuable frontline tools in low-resource laboratories, although molecular assays are rapidly becoming standard in high-resource settings . Moreover, a 2025 review emphasized that regional epidemiology significantly influences the dominant carbapenemase types detected, with KPC and NDM remaining most frequent in Asia and rising OXA-48 reports in Europe . These findings align with our results, underscoring the importance of strengthening local surveillance, integrating phenotypic with molecular detection strategies, and implementing rigorous infection control measures to mitigate the spread of CRE[25-28]..

Overall, organism distribution patterns indicate that both E. coli and K. pneumoniae remain key CRE pathogens, with urine samples and ICU patients being frequent sources. Phenotypic test performance varies considerably across settings, and while MHT offers consistent specificity, integrating multiple phenotypic assays may enhance detection, particularly in resource-limited laboratories.

CONCLUSION

The present study underscores the growing prevalence of carbapenem-resistant Enterobacterales (CRE) in clinical settings, highlighting a critical public health concern. Among the tested isolates, Escherichia coli and Klebsiella pneumoniae were the most frequently identified, with urine being the most common specimen source. The Modified Hodge Test (MHT) demonstrated the highest detection rate for carbapenemase production, indicating its strong potential as a primary screening method in resource-limited laboratories. While the EDTA inhibition test proved useful for identifying metallo-β-lactamase activity, the Boronic acid inhibition test showed comparatively low sensitivity, suggesting limited utility in routine diagnostics. Overall, the findings emphasize the importance of early detection through phenotypic assays to guide appropriate therapeutic strategies and containment efforts, especially in regions with limited access to molecular diagnostics.

DECLARATIONS

Conflicts of interest: There is no any conflict of interest associated with this study
Consent to participate: There is consent to participate.
Consent for publication: There is consent for the publication of this paper.
Authors' contributions: Author equally contributed the work.

REFERENCES

1. ZhangZ,WangD,LiY,LiuYand Qin X (2022) Comparison of the Performance of Phenotypic Methods for the Detection of Carbapenem- Resistant Enterobacteriaceae (CRE) in Clinical Practice. Front. Cell. Infect. Microbiol. 12:849564. doi: 10.3389/fcimb.2022.849564
2. Nair, P. K., &Vaz, M. S. (2018). Prevalence of carbapenem resistant Enterobacteriaceae from a tertiary care hospital in Mumbai, India. Journal of Microbiology and Infectious Diseases, 3(04), 207-210.
3. Suwaiba M, Dadah AJ and Sanusi SB. Prevalence of carbapenem-resistant Escherichia coli and Klebsiella pneumoniae in urine samples of patients attending selected general hospitals within Kaduna Metropolis. Science World Journal Vol. 15(No 4) 2020
4. ZhangZ,WangD,LiY,LiuYand Qin X (2022) Comparison of the Performance of Phenotypic Methods for the Detection of Carbapenem- Resistant Enterobacteriaceae (CRE) in Clinical Practice. Front. Cell. Infect. Microbiol. 12:849564. doi: 10.3389/fcimb.2022.849564
5. Földes, A.; Székely, E.; Voidăzan, S.T.; Dobreanu, M. Comparison of Six Phenotypic Assays with Reference Methods for Assessing Colistin Resistance in Clinical Isolates of Carbapenemase-Producing Enterobacterales: Challenges and Opportunities. Antibiotics**2022**, 11, 377.
6. Taha R, Mowallad A, Mufti A, et al. (February 16, 2023). Prevalence of Carbapenem-Resistant Enterobacteriaceae in Western Saudi Arabia and Increasing Trends in the Antimicrobial Resistance of Enterobacteriaceae. Cureus 15(2): e35050. doi:10.7759/cureus.35050
7. Neil Gupta, Brandi M. Limbago, Jean B. Patel, Alexander J. Kallen, Carbapenem-Resistant Enterobacteriaceae: Epidemiology and Prevention, Clinical Infectious Diseases, Volume 53, Issue 1, 1 July 2011, Pages 60–67, https://doi.org/10.1093/cid/cir202
8. Dwomoh FP, Kotey FCN, Dayie NTKD, Osei MM, Amoa-Owusu F, et al. (2022) Phenotypic and genotypic detection of carbapenemase-producing Escherichia coli and Klebsiella pneumoniae in Accra, Ghana. PLOS ONE 17(12): e0279715.
9. Yusuf, I., Rabiu A.T., Haruna, M. and Abdullahi, S. A. (2015). Carbapenem-Resistant Enterobacteriaceae (CRE) in Intensive Care Units and Surgical Wards of Hospitals with No History of Carbapenem Usage in Kano, Northwest Nigeria. Nigerian Journal of Microbiology, 27(1), 2612-2618
10. Akinduti, P.A., Oluwaseun, E., Motayo, B.O. and Adeyakinu, A.F. (2012). Emerging Multidrug resistant Ampc Beta-Lactamase and Carbapenemase enteric Isolates in Abeokuta. Nature and Science, 10(7),70-74.
11. Mackie & McCartney Practical Medical Microbiology is the 14th edition.
12. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 32nd ed. CLSI supplement M100. Wayne (PA): CLSI; 2022.
13. Thomas N, Sarwat T. Prevalence of carbapenem resistant Enterobacteriaceae in a tertiary care hospital. Int J CurrMicrobiol App Sci. 2019;8(11):1418-24. doi:10.20546/ijcmas.2019.811.166.
14. Shree RS, Sumangala B. Detection of carbapenem resistant Enterobacteriaceae from various clinical samples: a record based study in a tertiary care hospital in Mandya. Indian J Microbiol Res. 2023;10(2):86-9. doi:10.18231/j.ijmr.2023.015.
15. Sharma K, Tak V, Nag VL, Bhatia PK, Kothari N. An observational study on carbapenem-resistant Enterobacterales colonisation and subsequent risk of infection in an adult intensive care unit (ICU) at a tertiary care hospital in India. Infect PrevPract. 2023;5:100312. doi:10.1016/j.infpip.2023.100312.

16. Pawar SK, Mohite ST, Shinde RV, Patil SR, Karande GS. Carbapenem-resistant Enterobacteriaceae: Prevalence and bacteriological profile in a tertiary teaching hospital from rural western India. Indian J Microbiol Res. 2018;5(3):342-7. doi:10.18231/2394-5478.2018.0072.
17. Wattal C, Goel N, Oberoi JK, Raveendran R, Datta S, Prasad KJ. Surveillance of multidrug resistant organisms in a tertiary care hospital in Delhi, India. J Assoc Physicians India 2010;58:32-6.
18. Datta P, Gupta V, Garg S, Chander J. Phenotypic method for differentiation of carbapenemases in Enterobacteriaceae: Study from north India. Indian J PatholMicrobiol. 2012;55(3):357-60. doi:10.4103/0377-4929.101744.
19. Josa M, Leal R, Rojas J, Torres M, Cortés-Muñoz F, Esparza G, et al. Comparative evaluation of phenotypic synergy tests versus RESIST-4 O.K.N.V. and NG Test Carba 5 lateral flow immunoassays for the detection and differentiation of carbapenemases in Enterobacterales and Pseudomonas aeruginosa. MicrobiolSpectr. 2022;10(1):e01080-21. doi:10.1128/spectrum.01080-21.
20. Bartolini A, Frasson I, Cavallaro A, Richter SN, Palù G. Comparison of phenotypic methods for the detection of carbapenem non-susceptible Enterobacteriaceae. Ann ClinMicrobiolAntimicrob. 2014;13:38. doi:10.1186/s12941-014-0038-3.
21. Kamel NA, Tohamy ST, Yahia IS, Aboshanab KM. Insights on the performance of phenotypic tests versus genotypic tests for the detection of carbapenemase-producing Gram-negative bacilli in resource-limited settings. BMC Microbiology. 2022 Oct 14;22(1):248. doi:10.1186/s12866-022-02660-5.
22. Falahat S, Shojapour M, Sadeghi A. Detection of KPC carbapenemase in Pseudomonas aeruginosa isolated from clinical samples using modified Hodge test and boronic acid phenotypic methods and their comparison with the polymerase chain reaction. Jundishapur J Microbiol. 2016 Mar 3;9(9):e27249. doi:10.5812/jjm.27249.
23. Seah C, Low DE, Patel SN, Melano RG. Comparative evaluation of a chromogenic agar medium, the modified Hodge test, and a battery of meropenem-inhibitor discs for detection of carbapenemase activity in Enterobacteriaceae. J ClinMicrobiol. 2011 May;49(5):1965–1969. doi:10.1128/JCM.00203-11
24. Elsherif R, Ismail D, Elawady S, Jastaniah S, Al-Masaudi S, Harakeh S, Karrouf G. Boronic acid disk diffusion for the phenotypic detection of polymerase chain reaction-confirmed, carbapenem-resistant, gram-negative bacilli isolates. BMC Microbiol. 2016 Jul 1;16(1):135. doi:10.1186/s12866-016-0754-z.
25. Public Health Agency of Canada, Canadian Nosocomial Infection Surveillance Program. Carbapenemase-producing Enterobacterales in acute-care hospitals, Canada, 2010–2023. Emerg Infect Dis. 2025;31(5):985-94. doi:10.3201/eid3105.240110.
26. van Duin D, Doi Y. The global epidemiology of carbapenemase-producing Enterobacterales. Clin Microbiol Rev. 2022;35(3):e00054-22. doi:10.1128/cmr.00054-22.
27. Messaoudi S, Power M, Belanger L, Martineau C, Doualla-Bell F. Phenotypic methods for detection of carbapenemases: strengths and limitations. Microorganisms. 2023;11(6):1491. doi:10.3390/microorganisms11061491.
28. De Vries C, Badawy D, Diwan M, Chung M, Angeles K, Bashiruddin S, et al. Frontline staff understanding of carbapenemase-producing Enterobacterales infection prevention and control measures: a cross-sectional survey in Ontario, Canada. Can J Infect Control. 2024;39(2):21-7. Available from: https://www.cjic.ca/102-fall-2024/414-frontline-staff-understanding-of-carbapenemase-producing-enterobacterales-infection-prevention-and-control-measures-a-cross-sectional-survey-conducted-in-ontario-canada