International Journal of Research in Medical Sciences
Singh VP et al. Int J Res Med Sci. 2026 Oct;14(10):4694-4699
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20263543

Original Research Article

Phenotypic detection of presumptive AmpC β-lactamase production among cefoxitin screen-positive gram-negative bacilli in a tertiary care teaching hospital

Vijay Prakash Singh[1]*, Anju Rani[2], Varuna Gupta[2]

[1]Department of Microbiology, Autonomous State Medical College (ASMC), Gonda, Uttar Pradesh, India
[2]Department of Microbiology, K.D. Medical College, Mathura, Uttar Pradesh, India

Received: 18 August 2026
Accepted: 19 September 2026

***Correspondence:**
Dr. Vijay Prakash Singh,
E-mail: vpsingh82@yahoo.com

ABSTRACT

Background: AmpC β-lactamase-mediated resistance among Gram-negative bacilli is an increasingly important therapeutic challenge because it reduces the effectiveness of several β-lactam antibiotics and limits treatment options. In resource-limited laboratories, reliable phenotypic methods remain essential for the routine evaluation of cefoxitin screen-positive isolates for presumptive AmpC β-lactamase production.
Methods: This prospective descriptive observational study was conducted at a tertiary care teaching hospital in Western Uttar Pradesh, India, from January to December 2025. A total of 2,860 clinical specimens yielded 1,486 clinically significant, non-duplicate Gram-negative isolates. Cefoxitin screen-positive isolates (inhibition zone diameter ≤18 mm) were evaluated using the Cefoxitin–Cloxacillin Double Disk Synergy Test (CC-DDST), Phenylboronic Acid Disc Test (PBA), and Disk Approximation Test (DAT) in accordance with CLSI M100, 35th edition (2025). Frequencies, percentages, and 95% confidence intervals were calculated.
Results: Among the 1,486 Gram-negative isolates, 626 (42.1%; 95% CI: 39.6–44.6%) were cefoxitin screen-positive. *Escherichia coli* (40.6%) and *Klebsiella pneumoniae* (28.6%) were the predominant cefoxitin screen-positive isolates. CC-DDST identified the highest proportion of presumptive AmpC β-lactamase-positive isolates (303/626; 48.4%; 95% CI: 44.5–52.3%), followed by PBA (285/626; 45.5%; 95% CI: 41.6–49.4%) and DAT (206/626; 32.9%; 95% CI: 29.3–36.7%).
Conclusions: A considerable proportion of Gram-negative isolates were cefoxitin screen-positive. Among the evaluated phenotypic methods, CC-DDST showed the highest positivity rate for presumptive AmpC β-lactamase production and appears to be a practical and cost-effective method for routine evaluation of cefoxitin screen-positive Gram-negative isolates in laboratories where molecular diagnostic facilities are unavailable.

Keywords: AmpC β-lactamase, Cefoxitin screening, Gram-negative bacilli, Phenotypic detection, Antimicrobial resistance, CC-DDST

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as a major global public health challenge that compromises the effectiveness of antimicrobial therapy and contributes to increased morbidity, mortality, prolonged hospitalization, and healthcare costs. Gram-negative bacilli are among the most important multidrug-resistant pathogens because of their ability to acquire and disseminate multiple resistance mechanisms, particularly through the production of β-lactamases. Among these enzymes, AmpC β-lactamases are of particular clinical importance because they hydrolyse penicillins, cephalosporins, cephamycins, and monobactams and are poorly inhibited by conventional β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam. Consequently, infections caused by

AmpC-producing organisms are associated with limited therapeutic options and an increased risk of treatment failure.[1-3]

AmpC β-lactamases may be either chromosomally encoded or plasmid-mediated. The widespread dissemination of plasmid-mediated AmpC enzymes among clinically significant Gram-negative pathogens, particularly *Escherichia coli* and *Klebsiella pneumoniae*, is a major concern because these resistance determinants are frequently associated with multidrug-resistant phenotypes. Their presence may compromise the activity of several commonly used β-lactam agents and complicate empirical antimicrobial therapy.[2,3]

Although molecular methods are considered the reference standard for detecting AmpC β-lactamase genes, their routine use remains limited in many clinical microbiology laboratories because of financial, technical, and infrastructural constraints. Consequently, cefoxitin screening followed by phenotypic evaluation using methods such as the Cefoxitin-Cloxacillin Double Disk Synergy Test (CC-DDST), Phenylboronic Acid Disc Test (PBA), and Disk Approximation Test (DAT) continues to be widely employed for the presumptive detection of AmpC β-lactamase production.[3-7]

Several phenotypic methods have been described for the evaluation of presumptive AmpC β-lactamase production; however, the proportion of cefoxitin screen-positive isolates identified by these methods may vary across laboratories and bacterial populations. In resource-constrained settings, information on the positivity rates of commonly used phenotypic methods under routine laboratory conditions is important for guiding laboratory practice and supporting antimicrobial stewardship programmes.

Despite the increasing burden of antimicrobial resistance, data on cefoxitin screen-positive Gram-negative bacilli and presumptive AmpC β-lactamase production from Western Uttar Pradesh remain limited. Regional epidemiological data are important for guiding empirical antimicrobial therapy, strengthening antimicrobial stewardship programmes, formulating institutional antibiotic policies, and supporting infection prevention and control strategies.[2,7,8]

The present study was undertaken to determine the prevalence of cefoxitin screen-positive Gram-negative bacilli and to describe the positivity rates of CC-DDST, PBA, and DAT for the phenotypic detection of presumptive AmpC β-lactamase production in a tertiary care teaching hospital.

METHODS

Study design and setting

This prospective descriptive observational study was conducted in the Department of Microbiology, K.D. Medical College Hospital & Research Centre, Mathura, Uttar Pradesh, India, from January to December 2025 after obtaining approval from the Institutional Ethics Committee.[9,10]

Study population

During the study period, 2,860 clinical specimens received from both outpatient and inpatient departments were processed for bacterial culture. Clinically significant, non-duplicate Gram-negative bacilli isolated from these specimens were included in the study. Only the first isolate obtained from each patient was analysed. Duplicate isolates, mixed bacterial growth, contaminants, and repeat isolates with identical bacterial identification and antimicrobial susceptibility profiles were excluded.

Isolation and identification of bacterial isolates

Clinical specimens were collected using standard aseptic precautions and processed according to established microbiological procedures. Gram-negative bacilli were isolated on appropriate culture media and identified on the basis of colony morphology, Gram staining, and conventional biochemical tests following standard microbiological procedures.[11]

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar using a standardized 0.5 McFarland bacterial suspension. Inhibition zone diameters were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) M100, 35th edition (2025) guidelines.[12]

Cefoxitin screening for presumptive AmpC β-lactamase production

All Gram-negative isolates were screened for presumptive AmpC β-lactamase production using a cefoxitin (30 µg) disk. Isolates with an inhibition zone diameter of ≤18 mm was considered cefoxitin screen-positive and were subjected to further phenotypic evaluation. Cefoxitin resistance was used as a screening criterion for presumptive AmpC β-lactamase production and not as a definitive diagnostic marker, because resistance to cefoxitin may also occur through mechanisms other than AmpC β-lactamase production.[5]

Phenotypic detection of presumptive AmpC β-lactamase production

Cefoxitin screen-positive isolates were evaluated using the following phenotypic methods.

Cefoxitin-Cloxacillin Double Disk Synergy Test (CC-DDST): An increase in the inhibition zone around the cefoxitin–cloxacillin disk compared with that around the

cefoxitin disk alone was interpreted as a positive result for presumptive AmpC β-lactamase production.[7,8]

Phenylboronic Acid Disc Test (PBA): An increase in the inhibition zone around the phenylboronic acid-containing disk compared with the cefoxitin disk alone was considered indicative of presumptive AmpC β-lactamase production.[4,13]

Disk Approximation Test (DAT): Distortion or blunting of the inhibition zone towards the inducing antibiotic disk was interpreted as a positive result for presumptive AmpC β-lactamase production.[5,7]

Quality control

Quality control for antimicrobial susceptibility testing was performed in accordance with CLSI M100 (35th edition, 2025) recommendations using *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Klebsiella pneumoniae* ATCC 700603 as reference strains.[12]

Statistical analysis

Data were entered into Microsoft Excel and analysed using IBM SPSS Statistics version 26.0. Categorical variables were summarized as frequencies and percentages. The prevalence of cefoxitin screen-positive isolates and the positivity rates of the evaluated phenotypic methods were calculated with 95% confidence intervals (95% CI). Because this was a descriptive observational study, no inferential statistical tests were performed.

RESULTS

Study population

During the study period, 2,860 clinical specimens were processed for bacterial culture, yielding 1,486 (51.9%) clinically significant, non-duplicate Gram-negative bacilli. All isolates were screened for presumptive AmpC β-lactamase production using a cefoxitin (30 µg) disk.

Cefoxitin screening

Among the 1,486 Gram-negative isolates, 626 (42.1%; 95% CI: 39.6–44.6%) were cefoxitin screen-positive (inhibition zone diameter ≤18 mm), whereas 860 (57.9%; 95% CI: 55.4–60.4%) were cefoxitin screen-negative.

Distribution of cefoxitin screen-positive isolates by organism

Escherichia coli was the predominant cefoxitin screen-positive isolate, accounting for 254 (40.6%) of the 626 isolates, followed by *Klebsiella pneumoniae* [179 (28.6%)]. *Acinetobacter spp*., *Citrobacter spp*., *Pseudomonas spp*., and *Proteus spp*. together constituted the remaining 193 (30.8%) isolates (Table 1 and Figure 1).

Table 1: Distribution of cefoxitin screen-positive isolates by organism (n=626).

Organism	Number (%)
Escherichia coli	254 (40.6)
Klebsiella pneumoniae	179 (28.6)
***Acinetobacter* spp.**	78 (12.5)
***Citrobacter* spp.**	67 (10.7)
***Pseudomonas* spp.**	28 (4.5)
***Proteus* spp.**	20 (3.2)
Total	626 (100)

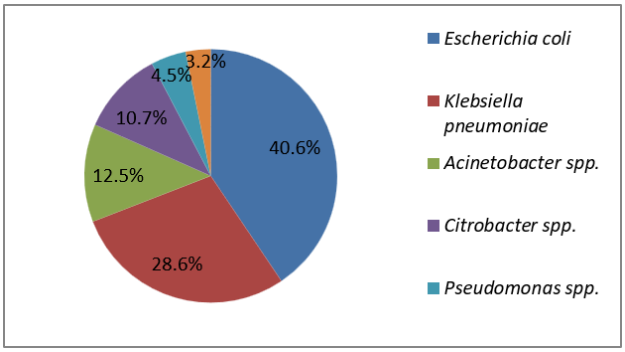

Figure 1: Distribution of cefoxitin screen-positive isolates by organism.

Positivity rates of phenotypic methods

Among the 626 cefoxitin screen-positive isolates, CC-DDST identified the highest proportion of presumptive AmpC β-lactamase-positive isolates [303/626 (48.4%; 95% CI: 44.5–52.3%)], followed by PBA [285/626 (45.5%; 95% CI: 41.6–49.4%)] and DAT [206/626 (32.9%; 95% CI: 29.3–36.7%)] (Table 2).

Table 2: Positivity rates of phenotypic methods for presumptive AmpC β-lactamase production (n=626).

Phenotypic method	Presumptive AmpC-positive isolates N (%)	95% CI
CC-DDST	303 (48.4)	44.5–52.3
Phenylboronic Acid Disc Test (PBA)	285 (45.5)	41.6–49.4
Disk Approximation Test (DAT)	206 (32.9)	29.3–36.7

Distribution according to clinical specimen

Pus specimens accounted for the majority of cefoxitin screen-positive isolates [361 (57.6%)], followed by urine [222 (35.4%)] and sputum [43 (7.0%)] (Table 3).

Department-wise distribution

Most cefoxitin screen-positive isolates were recovered from the Surgery department [266 (42.5%)], followed by

Orthopaedics [179 (28.6%)], Obstetrics and Gynaecology [115 (18.4%)], and Medicine [66 (10.5%)] (Table 4).

Table 3: Distribution of cefoxitin screen-positive isolates according to clinical specimen (n=626).

Clinical specimen	Number (%)
Pus	361 (57.6)
Urine	222 (35.4)
Sputum	43 (7.0)
Total	626 (100)

Table 4: Department-wise distribution of cefoxitin screen-positive isolates (n=626).

Department	Number (%)
Surgery	266 (42.5)
Orthopaedics	179 (28.6)
Obstetrics and Gynaecology	115 (18.4)
Medicine	66 (10.5)
Total	626 (100)

Patient characteristics

Male patients accounted for 361 (57.7%) of the cefoxitin screen-positive isolates, while 265 (42.3%) isolates were obtained from female patients. The majority of isolates were recovered from inpatients [476 (76.0%)], whereas 150 (24.0%) were obtained from outpatients.

Summary of findings

Overall, cefoxitin screening identified a substantial proportion of Gram-negative isolates requiring further phenotypic evaluation for presumptive AmpC β-lactamase production. Among the evaluated phenotypic methods, CC-DDST showed the highest positivity rate for presumptive AmpC β-lactamase production.

DISCUSSION

This study demonstrated that 42.1% of Gram-negative clinical isolates were cefoxitin screen-positive, indicating a substantial burden of isolates requiring further evaluation for presumptive AmpC β-lactamase production in our tertiary care teaching hospital. The high proportion of cefoxitin screen-positive isolates highlights the continued clinical relevance of AmpC-mediated resistance and emphasizes the importance of routine laboratory screening, particularly in resource-limited settings where molecular diagnostic facilities are not readily available. The prevalence observed in the present study is broadly comparable with reports from other tertiary care centres in India, although variations across studies are expected because of differences in patient populations, bacterial species distribution, antimicrobial prescribing practices, and laboratory methodologies.[1–8]

Escherichia coli and *Klebsiella pneumoniae* were the predominant cefoxitin screen-positive isolates, together accounting for nearly 70% of all screened-positive isolates. This finding is consistent with previous studies identifying these organisms as the principal Gram-negative pathogens associated with both community-acquired and healthcare-associated infections. The predominance of these species is clinically important because plasmid-mediated AmpC β-lactamases are increasingly reported among Enterobacterales and are frequently associated with multidrug-resistant phenotypes, thereby limiting the effectiveness of several commonly used β-lactam antibiotics.[2,3]

The present study described the positivity rates of three phenotypic methods for the evaluation of presumptive AmpC β-lactamase production among cefoxitin screen-positive isolates. CC-DDST showed the highest positivity rate (48.4%), followed by PBA (45.5%) and DAT (32.9%). These findings indicate that the evaluated phenotypic methods identified different proportions of presumptive AmpC β-lactamase-positive isolates among cefoxitin screen-positive Gram-negative bacilli.

The higher positivity rate observed with CC-DDST may be related to the inhibitory effect of cloxacillin on AmpC enzymes, which facilitates the identification of isolates with presumptive AmpC β-lactamase production. In contrast, DAT depends on inducible enzyme expression and interpretation of zone distortion, which may influence the proportion of isolates identified under routine laboratory conditions. The lower positivity rate observed with DAT in the present study is consistent with previous reports suggesting that inhibitor-based phenotypic methods may identify a larger proportion of presumptive AmpC-positive isolates than induction-based methods.[4,6,7,13-15]

These findings should be interpreted in the context of the study design. Because molecular confirmation of AmpC genes was not performed, the positivity rates reported in this study represent the proportion of presumptive AmpC β-lactamase-positive isolates identified by the evaluated phenotypic methods rather than their diagnostic sensitivity, specificity, or overall diagnostic accuracy. Cefoxitin resistance may arise from mechanisms other than AmpC β-lactamase production, including porin loss, altered membrane permeability, efflux mechanisms, or the presence of other β-lactamases. Consequently, the evaluated phenotypic methods should be regarded as screening or presumptive diagnostic methods rather than confirmatory tests.

Pus specimens accounted for the largest proportion of cefoxitin screen-positive isolates, and most isolates were recovered from the Surgery and Orthopaedics departments. These findings probably reflect the higher burden of wound infections, postoperative infections, trauma-related infections, prolonged hospitalization, invasive procedures, and empirical broad-spectrum

antimicrobial use in these clinical settings. The predominance of isolates among inpatients further supports the role of hospital-based selective pressure in the emergence and dissemination of resistant Gram-negative bacilli.

The study has important implications for clinical microbiology laboratories and antimicrobial stewardship programmes. Failure to recognize isolates with presumptive AmpC-mediated resistance may result in inappropriate use of third-generation cephalosporins, delayed effective therapy, treatment failure, prolonged hospitalization, and increased healthcare costs. In laboratories where molecular testing is unavailable, routine cefoxitin screening followed by CC-DDST may represent a practical and affordable approach for evaluating cefoxitin screen-positive isolates for presumptive AmpC β-lactamase production. Such an approach may facilitate earlier recognition of resistant isolates, support more appropriate antimicrobial selection, and strengthen institutional antimicrobial stewardship and infection prevention and control programmes.

The principal strengths of this study include the evaluation of a large number of clinically significant, non-duplicate Gram-negative isolates, the use of standardized CLSI-recommended antimicrobial susceptibility testing, and the assessment of three commonly used phenotypic methods under routine laboratory conditions. Nevertheless, several limitations should be acknowledged. The study was conducted at a single tertiary care centre, molecular characterization of AmpC genes was not performed, and agreement among phenotypic methods could not be assessed against a molecular reference standard. In addition, cefoxitin resistance attributable to non-AmpC mechanisms may have influenced the observed positivity rates. Future multicentre studies incorporating molecular confirmation are required to validate these findings and to further evaluate the role of phenotypic methods in the routine laboratory assessment of presumptive AmpC β-lactamase production.

CONCLUSION

A substantial proportion (42.1%) of Gram-negative clinical isolates were cefoxitin screen-positive, indicating a considerable burden of isolates requiring further evaluation for presumptive AmpC β-lactamase production in this tertiary care teaching hospital. *Escherichia coli* and *Klebsiella pneumoniae* were the predominant cefoxitin screen-positive isolates. Among the evaluated phenotypic methods, CC-DDST showed the highest positivity rate for presumptive AmpC β-lactamase production, followed by PBA and DAT. The observed positivity rates indicate that the evaluated phenotypic methods identified different proportions of presumptive AmpC β-lactamase-positive isolates among cefoxitin screen-positive Gram-negative bacilli.

In the absence of molecular confirmation of AmpC genes, the findings represent the proportion of presumptive AmpC-positive isolates identified by each phenotypic method rather than their diagnostic accuracy. CC-DDST appears to be a practical and cost-effective phenotypic method for routine evaluation of cefoxitin screen-positive Gram-negative isolates, particularly in microbiology laboratories where molecular diagnostic facilities are unavailable.

Routine cefoxitin screening followed by appropriate phenotypic evaluation may facilitate the early recognition of presumptive AmpC-mediated resistance, support more appropriate antimicrobial therapy, and strengthen antimicrobial stewardship and infection prevention and control programmes. Further multicentre studies incorporating molecular confirmation are required to validate these findings and to further evaluate the role of phenotypic methods in the routine laboratory assessment of presumptive AmpC β-lactamase production.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Jacoby GA. AmpC β-lactamases. Clin Microbiol Rev. 2009;22(1):161-82.
2. Bush K, Jacoby GA. Updated functional classification of β-lactamases. Antimicrob Agents Chemother. 2010;54(3):969-76.
3. Tamma PD, Doi Y, Bonomo RA, Johnson JK, Simner PJ. A primer on AmpC β-lactamases: necessary knowledge for an increasingly multidrug-resistant world. Clin Infect Dis. 2019;69(8):1446-55.
4. Hemalatha V, Padma M, Sekar U, Vinodh TM, Arunkumar AS. Detection of AmpC β-lactamases production in Escherichia coli and Klebsiella by an inhibitor-based method. Indian J Med Res. 2007;126(3):220-3.
5. Coudron PE, Moland ES, Thomson KS. Occurrence and detection of AmpC β-lactamases among *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* isolates at a veterans medical center. J Clin Microbiol. 2000;38(5):1791-6.
6. Ronni Mol P, Bindayna KM, Shanthi G. Evaluation of two phenotypic methods for the detection of plasmid-mediated AmpC β-lactamases among Enterobacteriaceae isolates. J Lab Physicians. 2021;13(2):151-5.
7. Inamdar DP, Anuradha B. Phenotypic methods for detection of AmpC β-lactamases in Gram negative clinical isolates of a tertiary care hospital. Indian J Microbiol Res. 2020;7(2):125-9.
8. Mohd Khari FI, Karunakaran R, Rosli R, Tay ST. Genotypic and phenotypic detection of AmpC β-lactamases in Enterobacter spp. isolated from a

teaching hospital in Malaysia. PLoS One. 2016;11(3):e0150643.
9. Indian Council of Medical Research. National ethical guidelines for biomedical and health research involving human participants. New Delhi: Indian Council of Medical Research. 2017. Available from: https://www.icmr.gov.in/icmrobject/custom_data/pdf/resource-guidelines/ICMR_Ethical_Guidelines_2017.pdf. Accessed on 08 August 2026.
10. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191-4.
11. Tille PM. Bailey & Scott's Diagnostic Microbiology. 14th ed. St. Louis (MO): Elsevier. 2017. Available from: https://shop.elsevier.com/books/bailey-and-scotts-diagnostic-microbiology/tille/978-0-323-35482-0. Accessed on 08 August 2026.
12. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 35th ed. CLSI supplement M100. Wayne (PA): Clinical and Laboratory Standards Institute. 2025. Available from: https://clsi.org/standards/products/microbiology/documents/m100/
13. Coudron PE. Inhibitor-based methods for detection of plasmid-mediated AmpC β-lactamases in Klebsiella spp., Escherichia coli, and Proteus mirabilis. J Clin Microbiol. 2005;43(8):4163-7.
14. Black JA, Moland ES, Thomson KS. AmpC disk test for detection of plasmid-mediated AmpC β-lactamases in Enterobacteriaceae lacking chromosomal AmpC β-lactamases. J Clin Microbiol. 2005;43(7):3110-3.
15. Singhal S, Mathur T, Khan S, Upadhyay DJ, Chugh S, Gaind R, et al. Evaluation of methods for AmpC β-lactamase in Gram negative clinical isolates from tertiary care hospitals. Indian J Med Microbiol. 2005;23(2):120-4.

Cite this article as: Singh VP, Rani A, Gupta V. Phenotypic detection of presumptive AmpC β-lactamase production among cefoxitin screen-positive gram-negative bacilli in a tertiary care teaching hospital. Int J Res Med Sci 2026;14:4694-9.