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Prevalence of AmpC β-Lactamase Production and Antimicrobial Resistance Patterns among Proteus spp. at a Tertiary Care Hospital in Lahore

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ABSTRACT

BACKGROUND & OBJECTIVE: AmpC beta-lactamase production among Gram-negative bacteria is an emerging therapeutic challenge due to resistance to several beta-lactam antibiotics. Limited local data are available on AmpC production among Proteus species. The main objective of this study was to determine AmpC beta-lactamase production in Proteus species using phenotypic methods and assess their antibiotic resistance patterns.

METHODOLOGY: This cross-sectional study was conducted at the Microbiology Laboratory, Department of Pathology, King Edward Medical University/Mayo Hospital, Lahore, Pakistan, during 01-02-2025 to 31-10-2025. Sixty non-duplicate clinical isolates of Proteus spp. were included. Identification was based on Gram staining, colony morphology, swarming motility, and biochemical testing. Antimicrobial susceptibility was determined by Kirby–Bauer disc diffusion according to CLSI guidelines (M100, 35th edition, 2025). Isolates with a cefoxitin zone <18 mm were screened for AmpC production and further evaluated using the AmpC disc test and Modified Hodge Test. Data were analyzed using SPSS version 27.

RESULTS: Of 60 isolates, 41 (68.3%) were from males and 19 (31.7%) from females. AmpC production was detected in 7 (11.7%) isolates by the AmpC disc test and 6 (10.0%) by the Modified Hodge Test. Highest resistance was observed to amoxicillin-clavulanate (93.3%), cefotaxime (83.3%), ceftriaxone (85%), and ciprofloxacin (68.3%). Resistance to meropenem and piperacillin-tazobactam was 6.7% and 10.0%, respectively.

CONCLUSION: A significant number of AmpC beta lactamase proteus was found, which also showed MDR. Regular diagnostic labs should have no trouble incorporating these phenotypic detection techniques.

KEY WORDS: AmpC Beta- lactamase, Antibiotic resistance, Cefoxitin Hodge test, Enterobacteriaceae, MDR, Proteus species.

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INTRODUCTION

AmpC β-lactamases are clinically important cephalosporinases that can compromise the activity of several β-lactam antibiotics and contribute to therapeutic failure. Local data on phenotypically detected AmpC production among Proteus spp. remain limited. Previous studies from Pakistan have demonstrated a substantial burden of AmpC-mediated resistance among Gram-negative clinical isolates, highlighting the importance of institution-specific surveillance.[1-3]

Antimicrobial resistance is a major challenge to the effective treatment of bacterial infections worldwide. Although β-lactam antibiotics remain among the most frequently prescribed antimicrobial agents, their clinical effectiveness is increasingly threatened by β-lactamase production, one of the principal resistance mechanisms in Gram-negative bacteria.[4,5] These enzymes hydrolyse the β-lactam ring and render susceptible antibiotics ineffective.

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AmpC enzymes are Ambler class C β-lactamases and are distinct from class B metallo-β-lactamases, which require zinc ions for catalytic activity.[6] AmpC β-lactamases may be chromosomally encoded or acquired through transferable plasmids. They hydrolyse many penicillins, cephamycins and broad-spectrum cephalosporins and are generally poorly inhibited by clavulanate, sulbactam and tazobactam.[7] Their detection may be complicated by coexistence with extended-spectrum β-lactamases or other resistance mechanisms, while routine susceptibility testing may not reliably distinguish AmpC-producing isolates. Consequently, laboratory detection and appropriate antimicrobial reporting are important for guiding therapy and limiting the spread of resistant organisms.[7]

Proteus spp., particularly Proteus mirabilis, are clinically important Gram-negative bacilli associated with urinary tract, wound and other healthcare-associated infections. These organisms possess several virulence characteristics—including motility, urease production, biofilm formation and catheter colonization—that facilitate persistent infection. Their ability to acquire and disseminate antimicrobial-resistance determinants further emphasizes the need for continuous susceptibility surveillance.[8,9]

In Lahore, a recent analysis of 422 culture reports identified Proteus spp. in 30 samples, representing 7.1% of all isolates and demonstrating their continuing clinical relevance in local healthcare settings.[10] Another study from Lahore identified Proteus spp. among commonly recovered Gram-negative organisms and documented a substantial burden of extended-spectrum β-lactamase production.[11] Earlier research from Lahore also demonstrated AmpC production among Gram-negative isolates, while a study conducted in Rawalpindi reported phenotypic AmpC production among resistant Gram-negative clinical isolates.[2,3]

Despite these findings, published local evidence specifically addressing phenotypic AmpC production among Proteus spp. remains scarce. Information regarding the frequency of AmpC production and the associated antimicrobial-resistance patterns is necessary to support empirical treatment decisions, antimicrobial-stewardship interventions and infection-prevention strategies. Therefore, this study aimed to determine the proportion of clinical Proteus isolates demonstrating phenotypic evidence of AmpC production and to describe their antimicrobial-resistance profile at a tertiary-care hospital in Lahore.

The objectives were to determine phenotypic AmpC β-lactamase production among Proteus spp. isolates using the AmpC disc test and Modified Hodge Test; and to describe the antimicrobial resistance pattern of the isolates.

Study aims to determine the AmpC beta lactamase production in Proteus species by using phenotypic detection methods and to study the patterns of antibiotic resistance of Proteus species.

METHODOLOGY

A hospital-based cross-sectional study was conducted in the Microbiology Laboratory, Department of Pathology, King Edward Medical University/Mayo Hospital, Lahore, Pakistan, from 1 February to 31 October 2025. Ethical approval was obtained from the Institutional Review Board of King Edward Medical University (IRB No. 787/RC/KEMU; dated 20 November 2024).

Clinical specimens submitted for bacterial culture were processed using standard microbiological procedures. Specimens were inoculated onto appropriate conventional culture media, including blood agar and MacConkey agar, according to specimen type, and incubated aerobically at 35–37°C for 18–24 hours. Cultures with suspected Proteus growth were examined for colony morphology, characteristic swarming on blood agar and non-lactose-fermenting colonies on MacConkey agar.

Identification was based on Gram staining and conventional biochemical tests. Isolates were identified as Proteus spp. by characteristic reactions, including oxidase negativity, urease production, phenylalanine deaminase activity, hydrogen sulphide production and motility. Indole testing was used, where required, to differentiate Proteus mirabilis from Proteus vulgaris. A total of 60 non-duplicate clinical isolates of Proteus spp. were included. Only the first isolate recovered from each patient during the study period was analysed to avoid duplication.

Antimicrobial susceptibility was determined using the Kirby–Bauer disc-diffusion method on Mueller–Hinton agar. A suspension equivalent to a 0.5 McFarland turbidity standard was prepared from isolated colonies and uniformly inoculated onto the agar surface. Antimicrobial discs were applied under aseptic conditions, and the plates were incubated aerobically at 35±2°C for 16–18 hours.

The antimicrobial agents tested were imipenem, meropenem, co-trimoxazole, cefoperazone–sulbactam, amoxicillin–clavulanate, cefotaxime, ceftriaxone, gentamicin, amikacin, cefepime, ciprofloxacin, levofloxacin and piperacillin–tazobactam. Inhibition-zone diameters were measured in millimetres and interpreted as susceptible, intermediate or resistant according to the Clinical and Laboratory Standards Institute M100 guidelines applicable during the study period the 35th edition published in 2025.

The recovered Proteus isolates were screened for AmpC β-lactamase production using a cefoxitin (30 μg) inhibition zone of <18 mm by disc diffusion. Screen-positive isolates were subsequently confirmed using confirmatory method, AmpC disc test and modified Hodge test. AmpC production was interpreted according to the predefined difference in inhibition-zone diameter or indentation criterion recommended for the selected phenotypic method.

Culture media, biochemical reagents and antimicrobial discs were subjected to routine quality-control procedures. Escherichia coli ATCC 25922 was used as the quality-control strain for disc-diffusion susceptibility testing. A previously characterized AmpC-producing isolate and a confirmed AmpC-negative isolate were used as positive and negative controls, respectively, where available.

Data were entered and analysed using IBM SPSS Statistics for Windows, version 27.0. Categorical variables, including demographic characteristics, specimen type, AmpC-production status and antimicrobial susceptibility results, were summarized as frequencies and percentages. The proportion of AmpC-producing isolates was calculated by dividing the number of phenotypically confirmed AmpC producers by the total number of non-duplicate Proteus isolates examined.

Isolates showing a cefoxitin (30 μg) inhibition zone of <18 mm by disc diffusion were considered potential AmpC producers. For the AmpC disc test, a lawn culture of Escherichia coli ATCC 25922 was prepared on Mueller–Hinton agar, and a cefoxitin disc was placed adjacent to a sterile disc inoculated with the test organism. Flattening or indentation of the cefoxitin inhibition zone toward the test disc was interpreted as positive. For the modified Hodge test, the test isolate was streaked radially

from the edge of a cefoxitin disc across an E. coli ATCC 25922 lawn. Cloverleaf-like indentation of growth along the streak indicated AmpC production

RESULTS

A total of 60 non-duplicate Proteus spp. isolates were included. Of these, 41 (68.3%) were obtained from male patients and 19 (31.7%) from female patients. The largest proportions of isolates were from the outpatient department (14/60, 23.3%), inpatient department (13/60, 21.7%) and surgical wards (12/60, 20.0%). Swab specimens accounted for 30 (50.0%) isolates, followed by pus and wound specimens, each contributing 14 (23.3%).Table I

Table I: Distribution of Proteus spp. isolates according to patient sex, clinical location and specimen type.

Variable	*n*	%
Gender		
Male	41	68.3
Female	19	31.7
Ward type		
Surgery	12	20
OPD	14	23.3
IPD	13	21.7
Oncology	3	5
Orthopedic	2	3.3
Plastic surgery	6	10
ICU	1	1.7
Emergency	7	11.7
Burn unit	2	3.3
Sample type		
Swab	30	50
Pus	14	23.3
Wound	14	23.3
Fluid	1	1.7
Tracheal secretion	1	1.7

Note: n =Frequency; % = Percentage.

Table II: Phenotypic detection of AmpC β-lactamase among Proteus spp. isolates.

Variable	*n*	%
Cefoxitin Hodge Test		
Positive	6	10
Negative	54	90
AmpC Disc Test		
Positive	7	11.6
Negative	53	88.3

Note: n =Frequency; % = Percentage.

Percentages are based on n=60 isolates.

Abbreviations: ICU, intensive care unit; IPD, inpatient department; OPD, outpatient department.

he Modified Hodge Test was positive in 6/60 (10.0%) isolates, whereas the AmpC disc test was positive in 7/60 (11.7%) isolates. These results indicate the presence of resistant strains (see the Table II).

Antimicrobial resistance was highest for amoxicillin-clavulanate (56/60, 93.3%), followed by ceftriaxone (51/60, 85.0%), cefotaxime (50/60, 83.3%) and ciprofloxacin (41/60, 68.3%). Resistance to meropenem, piperacillin-tazobactam and levofloxacin was comparatively lower at 6.7%, 10.0% and 11.7%, respectively.

Table III: Antimicrobial resistance pattern of Proteus spp. isolates.

Antibiotics	n resistant	% resistant
Imipenem	11	18.3
Meropenem	4	6.7
Co-trimxazole	21	35
Cefoperzone+sulbactam	15	25
Amoxicillin+Clavulanate	56	93.3
Cefotaxime	50	83.3
Ceftriaxone	51	85.0
Gentamycin	25	41.7
Amikacin	17	28.3
Cefepime	13	21.7
Ciprofloxacin	41	68.3
Levofloxacin	7	11.7
Piperacillin+tazobactam	6	10

Percentages are based on n=60 isolates.

DISCUSSION

This study identified phenotypic evidence of AmpC β-lactamase production in 10.0% of Proteus spp. isolates using the modified Hodge test and 11.7% using the AmpC disc test. Although the difference was small, it demonstrates that phenotypic assays may not classify all isolates identically. Because these methods have different performance characteristics and do not provide molecular confirmation, the findings should be interpreted as phenotypic evidence rather than definitive characterization of the underlying AmpC genes.

The local relevance of these findings is supported by previous Pakistani studies. A Lahore study of neonatal sepsis reported AmpC production among 22% of Gram-negative isolates, while a Rawalpindi study detected phenotypic AmpC production among resistant Gram-

negative clinical isolates.[2,3] However, these studies included broader Gram-negative bacterial populations and are therefore not directly comparable with the present Proteus-specific sample. A 2022 study from Lahore also demonstrated a substantial burden of ESBL-producing Gram-negative organisms, with Proteus spp. accounting for 18% of the isolates.[11] More recently, an antimicrobial susceptibility study from Lahore identified Proteus spp. in 7.1% of 422 culture reports, further supporting its clinical relevance in the local setting.[10]

The resistance profile observed in the present study was notable for high resistance to amoxicillin–clavulanate (93.3%), cefotaxime (85.0%), ceftriaxone (83.3%) and ciprofloxacin (68.3%). High levels of antimicrobial resistance among Proteus spp. have also been reported elsewhere, although direct comparisons are limited by differences in patient populations, specimen types, antimicrobial panels and laboratory methods. A study of Proteus vulgaris from a tertiary-care hospital in Islamabad reported high resistance to several antimicrobial agents but comparatively lower resistance to imipenem, supporting the observation that carbapenems may retain greater activity against some resistant Proteus isolates.[12-14]

The two phenotypic AmpC tests did not produce identical positivity rates. This discordance may reflect differences in their analytical principles, sensitivity and specificity. The study did not include molecular detection of blasubAmpC/sub genes; therefore, the genotype, plasmid or chromosomal location, and transferability of the resistance determinants could not be established. Future studies incorporating molecular characterization and correlation with clinical outcomes would provide more definitive evidence.

The strengths of this study include its focus on a clinically relevant organism, the use of two phenotypic methods for detecting AmpC production, and assessment of a broad antimicrobial susceptibility profile. However, the single-centre design and relatively small sample of 60 isolates limit the generalizability of the findings. Molecular confirmation was not performed, while clinical outcomes, previous antimicrobial exposure and patient-level risk factors were not evaluated. Furthermore, the total number of isolates screened before selection, antimicrobial-disc manufacturers and complete quality-control records should be documented before submission.

Future multicentre studies should include larger numbers of Proteus isolates, standardized phenotypic testing with documented quality control, molecular characterization of AmpC determinants, and assessment of relevant clinical, treatment-related and epidemiological risk factors.

CONCLUSION

Phenotypic evidence of AmpC β-lactamase production was identified among Proteus spp. isolates at a tertiary care hospital in Lahore, with positivity of 11.7% by the AmpC disc test and 10.0% by the Modified Hodge Test. The isolates also demonstrated substantial resistance to several commonly used antimicrobial agents. These findings support continued local antimicrobial-resistance surveillance and standardized laboratory detection of clinically important resistance mechanisms.

Ethical approval:

Ethical approval was taken from institutional review board of King Edward Medical University, Lahore with the IRB number 787/RC/KEMU, Dated 20-11-2024.

Conflict of Interest:

Authors declare no conflict of interest.

Financial Disclosure: None

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Authors' Contributions:

QUA & RT: Conceptualization and study design

MS & AN: Data Collection and manuscript drafting.

QUA & RT: Data Analysis and critical review.

MS, AN & RT: Supervision & Manuscript drafting & proof reading.

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