Section: Microbiology

Original Research Article

MOLECULAR DETECTION OF BLACTX-M GENES AMONG ESBL-PRODUCING PROTEUS ISOLATES IN TIRUNELVELI MEDICAL COLLEGE

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Received : 23/03/2025
Received in revised form : 07/05/2025
Accepted : 28/05/2025

Keywords:
Proteus mirabilis, Extended-Spectrum Beta-Lactamase (ESBL), blaCTX-M gene, Real-Time PCR, Antibiotic resistance.

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DOI: 10.47009/jamp.2025.7.3.113

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2025; 7 (3); 589-594

ABSTRACT

Background: Extended-spectrum β-lactamase (ESBL)-producing Proteus species, particularly Proteus mirabilis, are increasingly linked to drug-resistant illnesses. The blaCTX-M gene is one of the most common ESBL genes worldwide, imparting resistance to third-generation cephalosporins. Early detection of blaCTX-M is crucial for successful treatment and infection prevention. **Materials and Methods:** A cross-sectional study was conducted from December 2015 to August 2016 at Tirunelveli Medical College, Tamil Nadu, India. From 1124 clinical specimens, 100 Proteus isolates were identified, of which 53 were phenotypically confirmed as ESBL producers. Molecular detection of the blaCTX-M gene was performed using Real-Time PCR. Phenotypic tests—Combined Disc Test (CDT), Double Disc Synergy Test (DDST), and E-Test—were evaluated against PCR results. Demographic, clinical, and risk factor data were collected and analyzed. **Result:** Out of 53 ESBL-producing isolates, 48 (90.5%) were positive for the blaCTX-Mgene. P. mirabilis accounted for 60.4% of ESBL isolates. The highest ESBL prevalence was in the 46–60 age group (37.7%). The E-Test showed the best agreement with PCR (sensitivity 98%, specificity 100%). CDT demonstrated 96% sensitivity and 80% specificity, while DDST had lower sensitivity (69.8%). UTIs (43.3%) were the most common ESBL-associated infections. Catheterization (78.2%), prolonged hospital stay (≥15 days, 75.5%), and prior cephalosporin use (94.3%) were significantly associated with ESBL positivity ($p < 0.05$). **Conclusion:** The study confirms the high prevalence of the blaCTX-M gene among ESBL-producing Proteus isolates in a tertiary care setting. Real-Time PCR proved effective for rapid detection. The strong association of ESBL with catheterization, extended hospital stay, and cephalosporin use highlights the need for improved antibiotic stewardship and infection control measures.

INTRODUCTION

The genus Proteus, a member of the Enterobacteriaceae family, is made up of Gram-negative, motile, facultative anaerobic bacteria.[1] These creatures are found throughout nature, including soil, water, and the gastrointestinal tracts of humans and animals.[2] Proteus mirabilis is the most clinically relevant species, accounting for roughly 90% of all Proteus infections.[3,4] These infections are very frequent in the urinary tract, particularly among individuals who have indwelling catheters or urinary structural abnormalities.[5,6] Proteus species are pathogenic because they generate the enzyme urease, which hydrolyzes urea into ammonia and carbon dioxide. This process raises the alkalinity of urine, resulting in the production of struvite stones.[7,8] The ensuing calculi can clog the urinary tract, leading to persistent and recurring infections and complicating therapy.[9] Furthermore, Proteus infections are not confined to the urinary system; they can also cause wound infections,[10] bacteremia,[11] pneumonia,[12] intra-abdominal abscesses,[13] and other healthcare-associated diseases, all with serious clinical effects.[14] Antibiotic resistance in Proteus species, especially through extended-spectrum β-lactamases (ESBLs), is a developing problem.[15] These enzymes, notably those encoded by the blaCTX-M gene, hydrolyze a broad variety of β-lactam antibiotics, including third-generation cephalosporins such as cefotaxime.[16] They are frequently co-expressed with resistance to non-β-lactam antibiotics, further reducing treatment

ISSN (O): 2687-5365; ISSN (P): 2753-6556

options.[17] The blaCTX-M gene, which has become the most abundant ESBL gene globally, is typically found on plasmids that promote its rapid horizontal spread among bacterial populations, amplifying its clinical and epidemiological significance.[18,19] Rapid and precise detection of ESBL producers, particularly those containing blaCTX-M, is crucial for guiding successful antibiotic therapy and adopting infection control strategies.[15,20] The purpose of this study is to investigate the prevalence of the blaCTX-M gene among phenotypically confirmed ESBL-producing Proteus isolates in a tertiary care context using both conventional and molecular diagnostic approaches.

MATERIALS AND METHODS

Study Design and Setting: This cross-sectional study was conducted in the Department of Microbiology, Tirunelveli Medical College, Tamil Nadu, India, over a period of nine months (December 2015 – August 2016).

Sample Collection and Identification: From a total of 1124 clinical isolates, 100 non-duplicate Proteus spp. isolates were chosen. Standard biochemical assays were used to identify the samples. All procedures were carried out with biosafety level measures. Prior to starting the trial, we received ethical clearance and informed consent.

Genotypic Detection by Real-Time PCR

DNA Extraction

Genomic DNA was extracted using a silica-based spin column method.[21] DNA quality was confirmed and stored appropriately.

PCR Amplification

Real-time PCR experiment targeting the blaCTX-M gene was performed using the Helini Biomolecules' kit. Fluorescent probes (FAM channel) were utilized to detect targets, while internal controls (HEX channel) ensured reaction validity. PCR conditions included:

- Taq activation: 95°C for 15 min
- 40 cycles of:
  - Denaturation: 95°C for 20 sec
  - Annealing: 58°C for 30 sec
  - Extension: 72°C for 30 sec

Positive, negative, and internal controls were included in each run. A sample was considered positive if fluorescence crossed the threshold before cycle 36 and internal control was valid.

Statistical Analysis: All data collected during the study were entered and analyzed using SPSS version 20.0. Descriptive statistics were used to summarize the data, including frequencies and percentages for categorical variables (e.g., age group, specimen type, risk factors, antibiotic exposure). For comparison between groups (e.g., ESBL vs. non-ESBL isolates), Chi-square (χ^2) test or Fisher's exact test was used wherever applicable. A p-value < 0.05 was considered statistically significant.

RESULTS

A total of 53 ESBL-producing Proteus isolates were identified phenotypically and tested molecularly. [Table 1] shows that Real-Time PCR identified the presence of the blaCTX-M gene in 48 isolates (90.5%), while only 5 isolates (9.5%) tested negative.

Table 1: Detection of CTX-M gene by Real-Time PCR

ESBL Confirmed Isolates	CTX-M Amplified	CTX-M Not Amplified
(N=53)	48 (90.5%)	5 (9.5%)

Species and Age Distribution: Among ESBL producers, P. mirabilis was more prevalent (60.4%) compared to P. vulgaris (39.6%) [Table 2]. Age-wise, the highest number of ESBL isolates was found in the 46–60 years group (37.7%), followed by the 61–75 years group (32%). This suggests higher prevalence in older adults [Table 3].

Table 2: Species-wise Distribution of ESBL-producing Proteus Isolates

Species	ESBL (n=53)	%	Non-ESBL (n=47)	%
P. mirabilis	32	60.4	31	66
P. vulgaris	21	39.6	16	34

Table 3: Age-wise Distribution of ESBL-producing Proteus Isolates

Age (years)	ESBL (n=53)	%	Non-ESBL (n=47)	%
≤15	6	11.3	9	19.1
16–30	3	5.7	4	8.5
31–45	5	9.4	9	19.1
46–60	20	37.7	11	23.4
61–75	17	32	11	23.4
≥76	2	3.8	3	6.4

Comparison of Phenotypic Tests with PCR: The E-test showed excellent agreement with PCR, with a sensitivity of 98%, specificity of 100%, PPV of 100%, and NPV of 83%. It detected 47 true positives with no false positives [Table 4]. The Combined Disc Test (CDT) had a sensitivity of 96%, specificity of 80%, PPV of 98%, and NPV of 67% [Table 5]. The Double Disc Synergy Test

ISSN (O): 2687-5365; ISSN (P): 2753-6556

(DDST) had the lowest sensitivity at 69.8%, though its specificity remained 80%, indicating reduced reliability in detecting CTX-M producers [Table 6].

Table 4: Comparison of E-Test and PCR for CTX-M Gene Detection

E-Test	PCR Positive	PCR Negative	Total
Positive	47	0	47
Negative	1	5	6
Total	48	5	53

Table 5: Comparison of CDT and PCR for CTX-M Gene Detection

CDT	PCR Positive	PCR Negative	Total
Positive	46	1	47
Negative	2	4	6
Total	48	5	53

Table 6: Comparison of DDST and PCR for CTX-M Gene Detection

DDST	PCR Positive	PCR Negative	Total
Positive	37	1	38
Negative	11	4	15
Total	48	5	53

Infection Type and Risk Factor Analysis: [Table 7] presents the distribution of ESBL and non-ESBL Proteus isolates across various infection types. Urinary tract infections (UTIs) were the most common among ESBL cases, accounting for 43.3% (23/53), followed by wound infections (26.4%), cellulitis (9.4%), abscesses (7.5%), and surgical site infections (SSIs) and diabetic foot infections (both 5.7%). In contrast, non-ESBL isolates were more frequently associated with wound infections (38.2%) and diabetic foot infections (14.8%). Notably, SSIs were only found among ESBL isolates. [Table 8] highlights the association between catheterization and the presence of ESBL-producing Proteus isolates in urinary tract infections. Among 23 ESBL-positive cases, 78.2% (18/23) were from catheterized patients, while only 21.7% (5/23) were from non-catheterized individuals. In contrast, ESBL-negative isolates were equally distributed between catheterized and non-catheterized patients (50% each). Analysis of hospital stay duration revealed a strong association between extended hospitalization and ESBL production. Among the 53 ESBL-positive isolates, 40 isolates (75.5%) were recovered from patients who had been hospitalized for 15 days or more, while only 13 isolates (24.5%) were from those with stays shorter than 15 days. In contrast, the majority of non-ESBL cases (76%) were associated with shorter hospital stays (<15 days). This difference was statistically significant ($P < 0.05$), indicating that prolonged hospitalization is a key risk factor for acquiring ESBL-producing Proteus infections [Table 9].

[Table 10] illustrates the pattern of prior antibiotic exposure among patients infected with ESBL and non-ESBL producing Proteus isolates. A statistically significant association was observed with cephalosporin use, where 50 out of 53 ESBL-positive cases (94.3%) had prior exposure compared to only 8 out of 47 non-ESBL cases (17%), indicating cephalosporin use as a major risk factor for the emergence of ESBL-producing strains ($p < 0.05$). In contrast, prior exposure to other antibiotic classes—including aminoglycosides, fluoroquinolones, β-lactam/β-lactamase inhibitor (BL+BLI) combinations, and carbapenems—showed no statistically significant difference between ESBL and non-ESBL groups. This finding underscores the importance of judicious cephalosporin use in clinical settings to mitigate the selection pressure driving ESBL emergence.

Table 7: Infection-wise Categorization of ESBL Isolates

Infection Type	ESBL (n=53)	%	Non-ESBL (n=47)	%
UTI	23	43.3	14	29.8
Wound infections	14	26.4	18	38.2
Cellulitis	5	9.4	3	6.3
Abscess	4	7.5	2	4.3
Diabetic foot	3	5.7	7	14.8
Respiratory infections	1	1.9	2	4.3
SSI	3	5.7	0	0

Table 8: ESBL in Catheterized vs Non-Catheterized Patients

Risk Factor	ESBL (n=23)	%	Non-ESBL (n=14)	%
Catheterized	18	78.2	7	50
Non-catheterized	5	21.7	7	50

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Table 9: Distribution of ESBL Isolates by Duration of Hospital Stay

Duration of Hospital Stay	ESBL (n = 53)	(%)	Non-ESBL (n = 47)	(%)	Total
< 15 days	13	24.50%	34	76.00%	47
≥ 15 days	40	75.50%	13	24.00%	53
Total	53	100%	47	100%	100

Table 10: Antibiotic Exposure in Patients with ESBL and Non-ESBL Isolates

Antibiotic Class	ESBL (n = 53)	Non-ESBL (n = 47)	Total	Significance
Cephalosporins	50	8	58	Significant
Aminoglycosides	3	21	24	Non-Significant
Fluoroquinolones	1	10	11	Non-Significant
BL+BLI	2	5	7	Non-Significant
Carbapenems	3	2	5	Non-Significant

DISCUSSION

The study found that 90.5% of confirmed ESBL-producing Proteus isolates had the blaCTX-M gene. This is consistent with the findings of Wang et al. in China (2011),[22] who observed a comparable prevalence. Mashwal et al, 2017,[23] found an even higher rate (97.4%) in Saudi Arabia, whereas Hassan et al 2013,[24] found 82%, indicating that CTX-M is currently the dominant ESBL gene in many parts of the world. These data demonstrate the continuous proliferation and dominance of plasmid-borne CTX-M enzymes, which have displaced previous ESBLs such as TEM and SHV. However, Maninder Kaur et al. in Amritsar discovered just the TEM gene among Proteus ESBLs, suggesting spatial variation in gene distribution. Similarly, Ho P et al., 2005 reported a 70.2% CTX-M prevalence, lower than in this study.[25] Among the 53 ESBL-positive isolates, five (9.5%) were PCR-negative for blaCTX-M, suggesting the presence of other ESBL genes like blaTEM or blaSHV, emphasizing the need for multiplex PCR in future surveillance.

Comparison of Phenotypic Methods with PCR

Phenotypic detection of ESBLs remains essential for routine screening. In our study, the E-test showed the highest concordance with PCR, with sensitivity and specificity of 98% and 100%, respectively. These results are consistent with Mohmid et al., 2013 in Egypt (2009–2010), who found a sensitivity of 96.4% for CTX-M detection by E-test.[26] The added advantage of determining MIC values makes the E-test useful for guiding therapy, though its high cost limits routine use. The Combined Disc Test (CDT) also showed good performance with 96% sensitivity and 98% PPV, aligning with findings by Hisham et al., 2016 who reported 100% sensitivity.[27] The Double Disc Synergy Test (DDST) showed reduced sensitivity (69.8%) despite a high PPV (97.3%), likely due to factors like inappropriate disc spacing or degradation of clavulanate. Similar reduced sensitivity (66.6%) for DDST was reported by Hisham et al 2016.[27] The use of Modified DDST (MDDST), involving cefepime or piperacillin-tazobactam, may improve sensitivity in such cases.

Prevalence and Risk Factor Analysis: Our study found that 53% of Proteus spp. were ESBL producers, consistent with Rudresh et al., in Karnataka (57%).[28] However, Jobayeret al 2017,[29] reported a higher rate (69.4%) among urinary isolates, whereas Balan, 2017 found only 19.4% ESBL prevalence, indicating variability based on geographic and temporal factors.[30] Species-wise, ESBL production was more common in P. mirabilis (60.4%) than P. vulgaris, which aligns with the results of Jog et al 2013.[31] Interestingly, some studies observed a reverse trend, suggesting institutional or regional variations. Age-wise, the 46–60 years group had the highest ESBL burden (37.7%), consistent with Kiratisin et al., 2011 and Basavaraj et al 2011 who noted that increased hospitalization and invasive procedures among older patients contribute to this distribution.[32,33]

Specimen-wise, the highest ESBL recovery was from urine (43.3%) and pus (52.8%). This mirrors findings by Dalela et al., 2012 in Rajasthan,[34] while Sasirekha et al. reported only 42.9% ESBLs in urinary isolates, again highlighting regional differences.[35]

Catheterization, Antibiotic Exposure, and Hospital Stay

In our study, 78.2% of ESBL-positive urinary isolates were from catheterized patients. This closely parallels the findings of Khan et al., (65.9%) and De Champs et al (69%).[36,37] The role of urinary catheters as a nidus for biofilm formation and persistent infection is well-established, especially with biofilm-forming organisms like P. mirabilis. Prior cephalosporin exposure was reported in 94.3% of ESBL cases, a statistically significant risk factor. Similar findings were reported by De Champs et al., where 75.4% had cephalosporin exposure.[37] Fluoroquinolones and other antibiotic classes did not show a significant association, underlining the selective pressure imposed specifically by cephalosporins. Hospital stay ≥15 days was significantly associated with ESBL isolation (75.5%). This is consistent with studies by Rupp, 2003,[38] and De Champs et al,[37] all of whom highlighted prolonged hospitalization as a major risk factor due to increased exposure to invasive procedures and antimicrobials.

Treatment and Control: Therapeutically, Imipenem showed the highest sensitivity (98%), followed by Piperacillin/Tazobactam (94.3%) and

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Amikacin (72%), similar to the findings of Shenoy et al.[39] The resistance evasion properties of carbapenems, due to their trans-6 hydroxyethyl group, make them highly effective against ESBLs, though judicious use is vital to prevent further resistance development. Preventive strategies must emphasize hand hygiene, catheter care, antimicrobial stewardship, and infection surveillance, especially in high-risk wards like surgery and ICU. De Champs et al,[37] stressed these measures, noting their role in curbing ESBL spread in healthcare settings.

CONCLUSION

The present study highlights a high prevalence (90.5%) of the blaCTX-M gene among phenotypically confirmed ESBL-producing Proteus isolates, underscoring its dominant role in mediating resistance to third-generation cephalosporins. The majority of ESBL isolates were recovered from urinary samples and were associated with key risk factors such as prolonged hospital stay, prior cephalosporin use, and urinary catheterization. Among the diagnostic methods evaluated, the E-test showed the highest sensitivity and specificity in detecting CTX-M-mediated resistance, making it a reliable phenotypic alternative to PCR, though cost limits its routine use. The findings emphasize the importance of early detection of ESBL producers using both phenotypic and molecular methods to guide effective antimicrobial therapy. Additionally, the study reinforces the need for stringent infection control practices, antibiotic stewardship, and surveillance programs to contain the spread of resistant strains, particularly in high-risk hospital settings.

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ISSN (O): 2687-5365; ISSN (P): 2753-6556

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ISSN (O): 2687-5365; ISSN (P): 2753-6556