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**ORIGINAL RESEARCH**

# Extended spectrum beta lactamase production, cell surface hydrophobicity and biofilm formation of uropathogenic escherichia coli isolated from patients in a tertiary care hospital, Imphal

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## ABSTRACT

**Background:** Urinary tract infections (UTIs) caused by uropathogenic *Escherichia coli* (UPEC) is a significant health concern, exacerbated by the production of extended-spectrum beta-lactamases (ESBLs), biofilm formation, and cell surface hydrophobicity. These traits contribute to antibiotic resistance and persistent infections. This study aimed to assess the prevalence of ESBL-producing UPEC isolates and their associations with biofilm formation, cell surface hydrophobicity, and antimicrobial resistance patterns in a tertiary care hospital in Imphal, India. **Methods:** This observational cross-sectional study was conducted from October 2022 to July 2024, analyzing 398 UPEC isolates. Biofilm production was evaluated using the Congo red agar and tissue culture plate methods, while cell surface hydrophobicity was assessed via the salt aggregation test. ESBL production was determined using the potentiated disc diffusion method, and antibiotic susceptibility testing was performed following CLSI 2022 guidelines. Statistical analyses were conducted using SPSS software, with significance set at p-value<0.05. **Results:** ESBL production was observed in 68.8% of isolates, and biofilm formation in 52.8%. Among ESBL producers, 58% demonstrated biofilm formation, significantly higher than non-ESBL producers (44%, p=0.009). Cell surface hydrophobicity was detected in 31.7% of isolates and was significantly associated with biofilm formation (p=0.000). However, no association was found between ESBL production and hydrophobicity (p=0.900). Biofilm-producing isolates exhibited higher resistance rates, particularly to third-generation cephalosporins and ciprofloxacin, while maintaining sensitivity to gentamicin, fosfomycin, and amikacin. **Conclusion:** This study highlights the high prevalence of ESBL-producing UPEC isolates and their enhanced biofilm-forming capacity, emphasizing the need for stringent antimicrobial stewardship and infection control measures.

**Key-words:** Uropathogenic E. coli, ESBL, biofilm formation, cell surface hydrophobicity, antimicrobial resistance



## INTRODUCTION

Pathogenic strains of *Escherichia coli* are a leading cause of extraintestinal infections, including urinary tract infections (UTIs).[1] These strains possess specific virulence factors (VFs) that enhance their ability to establish systemic infections. The pathogenicity of *E. coli* isolates is attributed to the combined effects of one or more VFs, which set them apart from non-pathogenic intestinal strains. Over the course of their lives, approximately 12% of men and 10–20% of women experience at least one episode of acute symptomatic UTI.[2,3]

Extended-spectrum beta-lactamases (ESBLs) are enzymes predominantly produced by Enterobacteriaceae, particularly uropathogenic *Escherichia coli* (UPEC) and *Klebsiella pneumoniae*. These enzymes confer resistance to a broad range of beta-lactam antibiotics, including penicillins,

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cephalosporins, and aztreonam, posing significant challenges in the treatment of infections caused by ESBL-producing strains, especially urinary tract infections (UTIs).[4,5] The increasing prevalence of ESBL-producing bacteria globally is a critical public health concern due to their association with multidrug resistance, which limits treatment options and complicates infection management.[5,6]

The phenotypic characteristics of ESBL-producing UPEC are further influenced by factors such as cell surface hydrophobicity and biofilm formation. Cell surface hydrophobicity enhances bacterial adhesion to host tissues and medical devices, facilitating the persistence of infections and evasion of the host immune response.[7] Biofilm formation, wherein bacteria aggregate into structured communities encased in an extracellular matrix, provides an additional layer of defense, significantly increasing resistance to antibiotics and host immune mechanisms. These adaptations contribute to the chronicity and recurrence of infections, leading to worse clinical outcomes such as increased morbidity, prolonged hospital stays, and higher healthcare costs.[8,9]

Evidence suggests a strong correlation between ESBL production and biofilm formation in UPEC strains. The ability to form biofilms is often linked to the presence of specific virulence genes that contribute to both pathogenicity and resistance mechanisms.[4,10] This multifactorial survival strategy complicates the management of UTIs caused by ESBL-producing strains and underscores the importance of understanding the genetic and phenotypic traits of these pathogens.

Epidemiological studies of ESBL-producing UPEC have shown significant variability in prevalence rates across different regions and populations. Factors such as age, sex, underlying health conditions, and healthcare-associated exposures influence these rates. While there are numerous studies globally that explore the prevalence and characteristics of ESBL-producing UPEC, data from northeast India, particularly Manipur, remain limited. No study to date has estimated the prevalence of ESBL producers among uropathogenic *Escherichia coli* in this region or examined the association between biofilm production, cell surface hydrophobicity, and ESBL production.This study aims to address this gap by determining the prevalence of ESBL-producing *Escherichia coli* among uropathogenic isolates from patients in a tertiary care hospital in Imphal. Additionally, it will assess biofilm production and cell surface hydrophobicity in these isolates and explore their association with ESBL production. The study also seeks to analyze the antibiogram profiles of uropathogenic *E. coli* to identify resistance patterns, thereby contributing valuable data to inform treatment strategies and combat the growing challenge of antimicrobial resistance.

## MATERIAL AND METHODS

### Study design and setting

This observational cross-sectional study was conducted from October 2022 to July 2024 in the Department of Microbiology at the Regional Institute of Medical Sciences (RIMS), Imphal. This institute is a prominent teaching hospital with over 1,200 beds, catering to a substantial and diverse population.

### Study population, sample size, and sampling

The study population comprised all patients provisionally diagnosed with urinary tract infections (UTIs) upon admission to the RIMS, including those from outpatient departments (OPD), inpatient wards, and emergency services. The sample size was calculated using the formula $n=4pq/d^2$, where p=44.6%, which was taken as the prevalence of ESBL producers among the E. coli isolates,from a previous study.[11] With a 95% confidence interval and 5% precision, the minimum required sample size was 398. Non-probability sampling was done until the required sample size was achieved.

### Selection criteria

Urine specimens were included in the study if *Escherichia coli* was successfully isolated. Contaminated samples were excluded to ensure the accuracy and reliability of the findings.

### Study Procedure

Data was collected using a pretested structured questionnaire to record patients' name, age, sex, laboratory number, clinical history, and diagnosis. A total of 398 clinical samples, comprising clean voided midstream and catheterized urine, were collected. Midstream samples were obtained after periurethral cleaning, while catheterized samples were aseptically aspirated.Samples were cultured on blood and MacConkey agar using a calibrated loop technique and incubated at 37°C overnight. Significant growth ($\geq 10^5$ CFU/ml) was identified based on colony morphology, Gram staining, motility tests, and biochemical assays. Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar following CLSI 2022 guidelines. ESBL production in Gram-negative isolates resistant to third-generation cephalosporins was confirmed using the potentiated disc diffusion method.

Biofilm production was detected by the Congo red agar (CRA) and tissue culture plate methods. CRA results were interpreted based on colony appearance, while the tissue culture plate method involved staining biofilms with crystal violet and measuring optical density at 620 nm. Cut-off values determined biofilm production strength. Cell surface hydrophobicity (CSH) was assessed using the salt aggregation test (SAT) with ammonium sulfate at concentrations of 2 M, 1.4 M, and 1 M; the test was considered positive if clumping was observed at 1.4 M and 1 M, and

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negative if clumping was absent at 2 M and no aggregation was observed. Standard control strains were used to maintain quality throughout all procedures.

**Statistical analysis**

Data analysis was performed using IBM Statistical Package for Social Sciences (SPSS) software (version 21.0).Categorical variables were described using frequency and percentages. To determine differences between various parameters across different groups, inferential analysis was conducted using the chi-square or Fisher's exact test. A significance level of $p<0.05$ was used to indicate statistical significance.

**Ethical issues**

The study was conducted with a waiver of informed consent, granted by the Research Ethics Board, RIMS, Imphal, as it involved laboratory analysis of microbial isolates and posed no risk to participants while offering potential public health benefits. Privacy and confidentiality were rigorously maintained throughout the study. Ethical approval was obtained under Research Ethics Board RIMS approval number A/206/REB-Comm(SP)/RIMS/2015/836/177/2022.

**RESULTS**

Among the participants, the highest proportion of individuals (24.9%) falls within the 57 to 70 years category, followed by 18.8% in the 43 to 56 years category. The youngest group, aged 1 to 14 years, constitutes 20.4% of the population, while those aged 15 to 28 years and 29 to 42 years represent 7.0% and 15.6%, respectively. Individuals aged 71 to 84 years account for 12.1%, and only 1.3% are aged 85 years and above. Regarding gender distribution, females comprise the majority at 65.8%, while males make up 34.2%.In terms of admission types, 57.3% of the individuals were treated in the Outpatient Department (OPD), 37.2% were admitted to the ward, and 5.5% werefrom the Intensive Care Unit (ICU).

Among all the *E. coli* isolates, 274 (68.8%) were identified as Extended Spectrum Beta-Lactamase (ESBL) producers, while the remaining 124 (31.2%) were non-ESBL producers (Figure 1). Biofilm formation, assessed using the Tissue Culture Plate (TCP) method, was observed in 210 (52.8%)out of 398 samples (Figure 2).

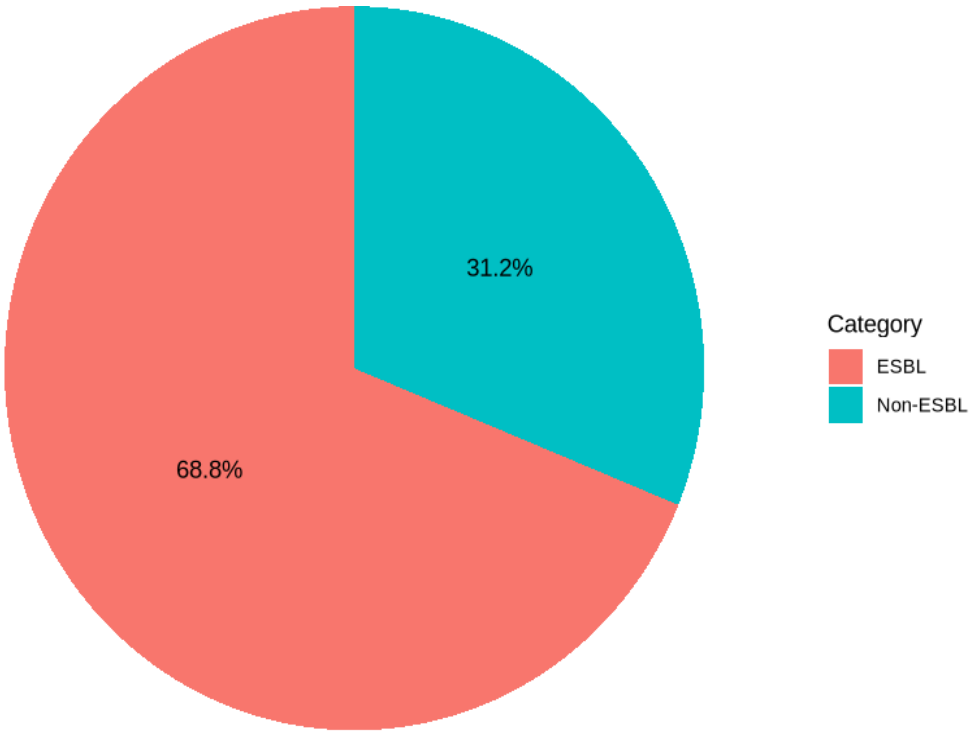


**Figure 1: Distribution according to ESBL and non-ESBL producing E. coli**

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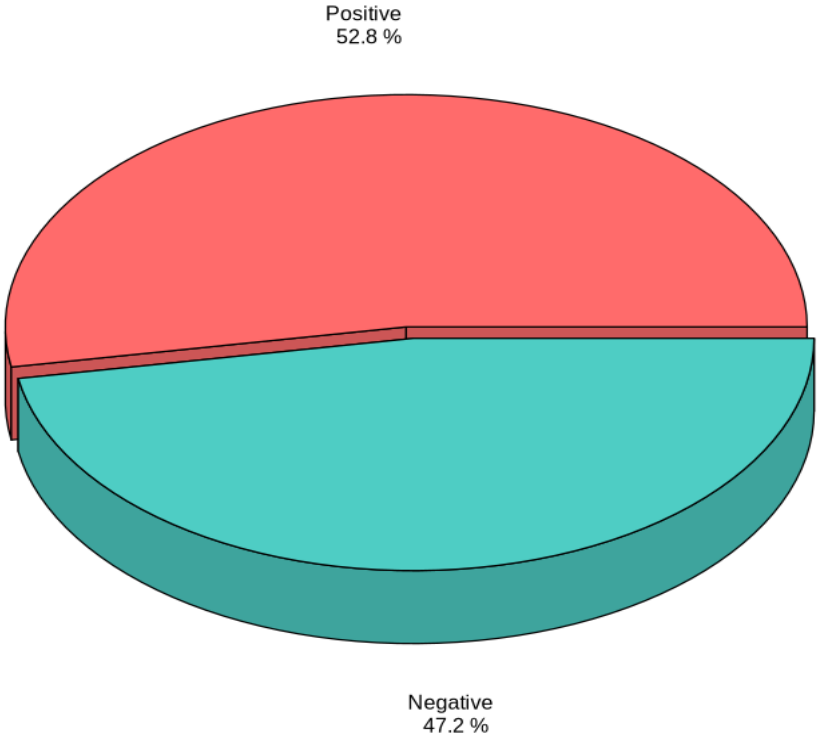


**Figure 2: Distribution according to biofilm production**

Isolates were assessed for cell surface hydrophobicity using the salt aggregation test (SAT) with ammonium sulfate (2 M, 1.4 M, and 1 M). Clumping at 1.4 M and 1 M indicated positivity, with 126 (31.7%) isolates testing positive (Table 1).

**Table 1: Distribution according to Cell surface hydrophobicity**

| Cell surface hydrophobicity | Frequency | Percentage |
|---|---|---|
| **Negative** | 272 | 68.3% |
| **Positive** | 126 | 31.7% |
| **Total** | 398 | 100% |

Upon further analysis of the isolates for biofilm production, it was observed that 158 (58%) of the ESBL-producing isolates demonstrated biofilm formation, while 116 (42%) did not. Conversely, among the non-ESBL-producing isolates, 54 (44%) were biofilm formers, whereas 70 (56%) were non-biofilm formers. Statistical analysis using the Chi-square test revealed a significant association between ESBL production and biofilm formation (Table 2).

**Table2: Association of ESBL and Biofilm production**

| ESBL | Biofilm | | p-value |
|---|---|---|---|
| | Producers | Non-Producers | |
| **Producers** | 158(58%) | 116(42%) | 0.009 |
| **Non-producers** | 54(44%) | 70(56%) | |

Among biofilm-producing isolates, 126 demonstrated positive cell surface hydrophobicity, whereas no such positivity was observed among non-biofilm-producing isolates. This association was statistically significant (Table 3).

**Table3: Association of Biofilm production and cell surface hydrophobicity**

| Biofilm | Cell surface hydrophobicity | | p-value |
|---|---|---|---|
| | No | Yes | |
| **Positive** | 86 | 126 | 0.000 |
| **Negative** | 186 | 0 | |

There was no association between ESBL production and CHS positivity (Table 4).

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**Table 4:Association of ESBL production and cell surface hydrophobicity**

| ESBL | Cell surface hydrophobicity | | p-value |
|---|---|---|---|
| | Negative | Positive | |
| Positive | 187 | 87 | 0.900 |
| Negative | 85 | 39 | |

The antibiotic susceptibility pattern among biofilm-producing *E. coli* isolates (N=212) revealed varying levels of sensitivity, intermediate susceptibility, and resistance to the tested antibiotics. High sensitivity rates were observed for Gentamicin (88.2%), Fosfomycin (88.2%), Amikacin (83.4%), and Ceftazidime-Clavulanate (82.5%). Moderate sensitivity was noted for Nitrofurantoin (75.1%), Minocycline (63.3%), and Meropenem (57.5%). Sensitivity was lower for Piperacillin-Tazobactam (37.7%), Ertapenem (41.2%), and Imipenem (41.5%). Ciprofloxacin, Ceftriaxone, Ceftazidime, and Cefotaxime showed notably low sensitivity rates of 9.5%, 19.6%, 16.6%, and 10.8%, respectively.Resistance rates were highest for Cefotaxime (71.4%), Ceftazidime (74.4%), Ceftriaxone (65.8%), and Ciprofloxacin (66.4%). In contrast, resistance was relatively low for Amikacin (11.1%), Fosfomycin (7.0%), and Gentamicin (4.3%).

## DISCUSSION

Among the study participants majority were females (262; 65%) compared to males (136; 35%), highlighting a clear female predominance in the prevalence of urinary tract infections (UTIs). These findings align with the literature, where researchers reported that females are more prone to UTIs due to anatomical and physiological factors, including a shorter urethra, its proximity to the anus, urethral dilation, and urinary stasis during pregnancy.[12–14]

In this study, uropathogenic *Escherichia coli* (UPEC) was predominantly isolated from individuals aged 40–70 years, consistent with the findings of Poovendran et al., who reported a peak prevalence among those aged 21–60 years.[15] Within this age range, a higher prevalence was observed among the elderly, likely due to associated comorbidities and increased susceptibility to complications during late reproductive or post-reproductive periods.

The observed high antibiotic resistance rates among *E. coli* isolates causing UTIs are alarming, though not unexpected. Of the 398 UPEC isolates, 274 (69%) were Extended Spectrum Beta-Lactamase (ESBL) producers, a proportion higher than the 50% reported by Nigudgi et al. but lower than the 80% documented by Shakya et al. in Nepal.[3,16] Resistance rates in this study were particularly high for cefotaxime (71%), ceftazidime (75%), ceftriaxone (67%), and ciprofloxacin (72%). Conversely, higher sensitivity rates were observed for gentamicin (85.4%), fosfomycin (88.7%), amikacin (82.4%), and nitrofurantoin (71.6%). These findings align with those of Pardeshi et al., who reported resistance rates of 70.2% for fluoroquinolones, 85.8% for amoxicillin, and 70.2% for third-generation cephalosporins, alongside sensitivity rates of 90.76% for gentamicin, 96.8% for meropenem, and 79.62% for nitrofurantoin.[17] Sensitivity to fosfomycin (88.7%) in the current study is comparable to the 79% reported by Bakhatet al.[18] Additionally, resistance to piperacillin-tazobactam (32.7%) observed here is consistent with findings by Prasada et al., who noted a rising trend in resistance to this agent from 9.4% to 23% over five years in their study conducted in Mangalore, India.[19]

Cell surface hydrophobicity, assessed using the salt aggregation test, was observed in 126 (31%) of the 398 isolates, with clumping occurring at salt concentrations ≤1.4M. These findings are similar to those of Raksha et al., who reported hydrophobicity in 26.36% of isolates, and Sharma et al., who documented hydrophobicity in 27.6% of isolates along with multiple virulence factors.[20,21]

Among the 274 ESBL-positive isolates, 158 (57.6%) demonstrated biofilm formation compared to only 54 (43.2%) of the non-ESBL isolates. These findings are consistent with the study by Nigudgi et al., where 68% of ESBL-positive isolates formed biofilms, compared to only 24% of non-ESBL isolates.[3] This study highlights the greater biofilm-forming capacity of ESBL-producing isolates compared to non-ESBL producers. Furthermore, biofilm development was significantly associated with increased cell surface hydrophobicity compared to non-biofilm-producing isolates. However, no statistically significant difference in cell surface hydrophobicity was observed between ESBL and non-ESBL isolates, similar to the findings of Hashemizadeh et al.[22]

This study was limited by its single-center design and the lack of molecular identification of resistance genes or other mutations due to financial and time constraints. Despite these limitations, the findings emphasize the need for further research, particularly focusing on gene expression studies, to better understand the pathogenicity of ESBL and non-ESBL isolates. A multicentric approach with larger sample sizes and advanced molecular analyses is crucial to comprehensively evaluate these associations and to develop effective strategies for combating antibiotic resistance and biofilm-related infections.

## CONCLUSION

This study underscores the high prevalence of Extended Spectrum Beta-Lactamase (ESBL)-producing *Escherichia coli* among uropathogenic isolates in a tertiary care hospital in Imphal, India. The findings reveal that ESBL-producing isolates exhibit a significantly enhanced capacity for biofilm

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formation compared to their non-ESBL counterparts, with a notable association between biofilm production and cell surface hydrophobicity. The alarming levels of antibiotic resistance, particularly among biofilm-forming isolates, highlight the critical need for robust antimicrobial stewardship programs and stringent infection control measures. Despite inherent limitations, including the single-center design and the absence of molecular characterization, this study provides valuable insights into the interplay of virulence factors and resistance mechanisms in uropathogenic *E. coli*. These findings highlight the importance of further molecular investigations to explain the genetic determinants of pathogenicity and resistance. A multicenter approach incorporating larger sample sizes and advanced molecular techniques is strongly recommended to guide clinical practices and combat antimicrobial resistance effectively.

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