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BIOFILM-FORMING CAPACITY AND ANTIBIOGRAM OF UROPATHOGENIC ESCHERICHIA COLI ISOLATES FROM CATHETER-ASSOCIATED AND COMMUNITY-ACQUIRED URINARY TRACT INFECTIONS: A CROSS-SECTIONAL STUDY AT A TERTIARY CARE HOSPITAL

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ABSTRACT

Background: Urinary tract infections (UTIs) caused by uropathogenic Escherichia coli (UPEC) represent the most prevalent healthcare-associated infections globally. Biofilm formation, a key virulence attribute of UPEC, confers tolerance to antibiotics and host immune defences, contributing to treatment failure and recurrence. Understanding biofilm capacity and its correlation with antimicrobial resistance is critical for managing catheter-associated UTI (CAUTI) and complicated UTI.

Methods: This cross-sectional study included 300 non-duplicate UPEC isolates from patients attending a tertiary care hospital over 12 months. Isolates were stratified into CAUTI (n=95), complicated UTI (n=105), and uncomplicated community-acquired UTI (n=100). Biofilm detection was performed by tissue-culture plate (TCP) method (Stepanović criteria), Congo-red agar (CRA), and tube-adherence methods. Antimicrobial susceptibility was determined by Kirby-Bauer and VITEK-2 per CLSI M100-2024. ESBL and AmpC phenotypes were detected, and PCR was performed for blaCTX-M, blaTEM, blaSHV, and virulence genes (fimH, papC, hlyA).

Results: Biofilm producers constituted 62.0% of UPEC isolates (strong 24.0%, moderate 23.3%, weak 14.7%). ESBL was detected in 56.0% and MDR in 71.0% of isolates. Nitrofurantoin and fosfomycin retained highest susceptibility (84.0% and 89.0%, respectively). Fluoroquinolone susceptibility was only 28.0%. Biofilm-positive isolates showed significantly higher MDR prevalence compared to non-producers (82% vs 51%, $p<0.001$), with an adjusted OR of 2.4 (95% CI 1.5–3.9). Biofilm formation was most frequent in CAUTI isolates (79.0%).

Conclusion: Biofilm formation in UPEC is strongly associated with MDR phenotype, ESBL production, and CAUTI context. Nitrofurantoin and fosfomycin remain the most active oral agents. Empirical therapy should be guided by local antibiograms rather than historical patterns.

Keywords: Uropathogenic Escherichia coli; biofilm; ESBL; MDR; CAUTI; catheter-associated UTI; fosfomycin; nitrofurantoin

1. INTRODUCTION

Urinary tract infections (UTIs) are among the most common infectious diseases encountered

in both community and hospital settings, accounting for over 150 million cases annually worldwide [1]. Escherichia coli is responsible for approximately 80% of uncomplicated UTIs and up to 50% of healthcare-associated UTIs, with uropathogenic E. coli (UPEC) possessing a distinct arsenal of virulence factors that enable colonisation, invasion, and persistence within the urinary tract [2]. Among these, biofilm formation has emerged as a pivotal pathogenic strategy, allowing UPEC to adhere to catheter surfaces and uroepithelium, resist mechanical washout, and tolerate antibiotic concentrations well above the planktonic minimum inhibitory concentration [3].

Catheter-associated UTI (CAUTI) constitutes the most prevalent device-related healthcare-associated infection, contributing significantly to morbidity, mortality, prolonged hospitalisation, and healthcare costs [4]. Indwelling urinary catheters provide an ideal substrate for biofilm formation, creating a conduit for ascending infection and a reservoir for antibiotic-tolerant organisms. The biofilm matrix — comprising extracellular polysaccharides, proteins, and nucleic acids — reduces antibiotic penetration and shields bacterial cells from immune effectors [5].

The global escalation of antimicrobial resistance among UPEC compounds the clinical challenge. Extended-spectrum beta-lactamase (ESBL)-producing UPEC isolates, increasingly prevalent in both community and hospital settings, severely restrict the oral antibiotic treatment options available for outpatient management. In India, ESBL prevalence among E. coli UTI isolates has been reported between 40% and 72% across various tertiary care centres, depending on the patient population studied [6]. This high baseline resistance significantly impairs the utility of fluoroquinolones, which have been traditional first-line agents for UTI treatment.

Notably, the relationship between biofilm formation and antimicrobial resistance is bidirectional and biologically intricate. While biofilm growth itself confers phenotypic tolerance (distinct from genotypic resistance) by reducing antibiotic penetration and inducing dormancy, biofilm-forming isolates are also independently more likely to harbour resistance genes — perhaps reflecting co-selection of fitness traits on mobile genetic elements [7]. Understanding this relationship has direct clinical implications: biofilm-forming isolates may fail treatment even when susceptibility testing on planktonic cells suggests sensitivity.

Despite the clinical importance of UPEC biofilm in the era of ESBL and MDR prevalence, systematic data characterising biofilm capacity, virulence gene profiles, and antibiogram correlations across UTI categories (CAUTI, complicated, and community-acquired) are limited from tertiary care hospitals in South Asia. This study was designed to address this gap.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

This observational cross-sectional study was conducted over a 12-month period in the Microbiology Department of a tertiary care hospital. Institutional Ethics Committee approval was obtained. Written informed consent was obtained from all patients. STROBE guidelines were followed throughout.

2.2 Study Population

All non-duplicate UPEC isolates from patients with symptomatic UTI (significant pyuria ≥10 white blood cells/high-power field and colony count $\geq 10^5$ CFU/mL) were included. Duplicate isolates (same species, same susceptibility pattern from the same patient within 30 days) were excluded. Isolates were stratified into three groups: CAUTI (from catheterised patients with

catheter in situ >48 hours, CDC/NHSN 2022 criteria), complicated UTI (structural/functional urinary-tract abnormality, pregnancy, immunosuppression, or male sex, with symptomatic UTI not fulfilling CAUTI criteria), and uncomplicated community-acquired UTI (non-catheterised, non-pregnant adult women without urological abnormality). Sample size: based on expected ESBL prevalence of 50%, with 5% absolute precision at 95% confidence (n=246 minimum), target set at 300 to allow stratified subgroup analysis.

2.3 Biofilm Detection

Biofilm formation was quantified by the tissue-culture plate (TCP) method on 96-well flat-bottomed polystyrene plates (Stepanović et al., 2007 criteria): Strong producer (OD ≥ 2×ODc), moderate (OD ≥ ODc and < 2×ODc), weak (OD < ODc and > 0.1+ODc), non-producer (OD ≤ ODc). The Congo-red agar method on Brain-Heart Infusion agar supplemented with 0.8% Congo red dye and 1% sucrose was used in parallel (black colonies with dry crystalline consistency = biofilm positive). Tube-adherence method was used as supplementary confirmation. Biofilm results are reported as concordance of TCP (reference method) with CRA and tube method.

2.4 Antimicrobial Susceptibility and ESBL Detection

AST was performed by Kirby-Bauer disc diffusion and VITEK-2 per CLSI M100-2024. Agents tested: ampicillin, amoxicillin-clavulanate, piperacillin-tazobactam, cefuroxime, ceftriaxone, cefepime, ertapenem, meropenem, nitrofurantoin, fosfomycin (disc elution method), cotrimoxazole, ciprofloxacin, levofloxacin, amikacin, gentamicin, tigecycline. ESBL phenotypic detection: combined disc diffusion (ceftazidime vs ceftazidime-clavulanate; cefotaxime vs cefotaxime-clavulanate, CLSI 2024). AmpC phenotypic detection: AmpC disc test. PCR for blaCTX-M, blaTEM, blaSHV, and virulence genes (fimH, papC, hlyA). MDR/XDR: Magiorakos et al. (2012) criteria.

2.5 Statistical Analysis

Data analysed using SPSS v26. Categorical variables expressed as frequencies/percentages. Comparisons by chi-square or Fisher's exact test; continuous variables by Student's t-test or Mann-Whitney U. Multivariable binary logistic regression for predictors of MDR status and CAUTI; results as adjusted OR (95% CI). Significance: $p<0.05$.

3. RESULTS

3.1 Biofilm Formation, ESBL, and MDR Profile

Among 300 UPEC isolates, 186 (62.0%) were biofilm producers: strong 72 (24.0%), moderate 70 (23.3%), weak 44 (14.7%), and non-producers 114 (38.0%). TCP-CRA concordance was 82.3%; TCP-tube method concordance was 78.1%. ESBL was detected in 168 (56.0%) isolates; AmpC in 42 (14.0%); blaCTX-M in 74.4% of ESBL producers (predominantly CTX-M-15), blaTEM in 22.1%, blaSHV in 9.5%. fimH was present in 87.3% of all UPEC, papC in 38.7%, hlyA in 18.3%. Demographic and clinical characteristics are summarised in Table 1.

Table 1: Demographic and Clinical Profile by UTI Category (n = 300)

Variable	CAUTI (n=95)	Complicated UTI (n=105)	Uncomplicated UTI (n=100)
Mean age, years (±SD)	58.4 ± 14.3	52.1 ± 16.7	38.2 ± 12.4
Female sex, n (%)	49 (51.6%)	68 (64.8%)	100 (100%)
Diabetes mellitus, n (%)	48 (50.5%)	38 (36.2%)	12 (12.0%)

Catheter duration, median days (IQR)	7 (4–14)	—	—
Recurrent UTI (>2/year), n (%)	37 (38.9%)	42 (40.0%)	38 (38.0%)
Biofilm producers, n (%)	75 (78.9%)	66 (62.9%)	45 (45.0%)
ESBL positive, n (%)	62 (65.3%)	57 (54.3%)	49 (49.0%)
MDR, n (%)	79 (83.2%)	72 (68.6%)	62 (62.0%)

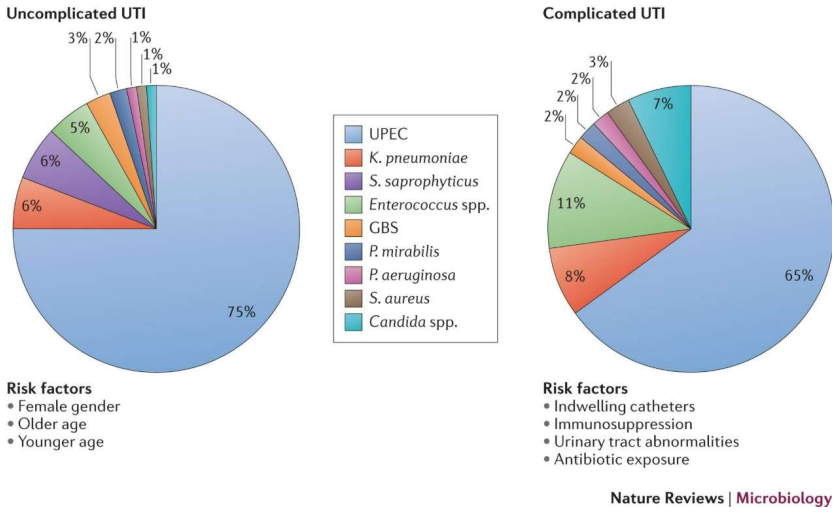

Figure 1: Comparative Clinical and Microbiological Characteristics of UTI Categories

3.2 Antibiogram and Biofilm-Resistance Association

Nitrofurantoin retained highest susceptibility (84.0%) followed by fosfomycin (89.0%), amikacin (71.3%), and piperacillin-tazobactam (54.7%). Fluoroquinolone (ciprofloxacin) susceptibility was only 28.0%. Biofilm-positive isolates showed significantly higher MDR prevalence (82.3% vs 51.4%, p<0.001), higher ESBL rates (69.4% vs 36.0%, p<0.001), and lower nitrofurantoin susceptibility (78.0% vs 92.1%, p=0.002). Full antibiogram stratified by biofilm status is presented in Table 2.

Table 2. Antibiogram by Biofilm Formation Status (n = 300 UPEC isolates)

Antibiotic	**Biofilm Positive (n=186) Susceptible %**	**Biofilm Negative (n=114) Susceptible %**	**p-value**
Nitrofurantoin	78.0%	92.1%	0.002
Fosfomycin	84.9%	95.6%	0.008
Amikacin	61.3%	87.7%	<0.001
Piperacillin-tazobactam	46.2%	68.4%	<0.001
Cotrimoxazole	22.0%	41.2%	<0.001
Ciprofloxacin	20.4%	39.5%	<0.001
Ceftriaxone	28.5%	52.6%	<0.001
Ertapenem	92.5%	97.4%	0.10
ESBL positive	69.4%	36.0%	<0.001
MDR (≥3 classes)	82.3%	51.4%	<0.001

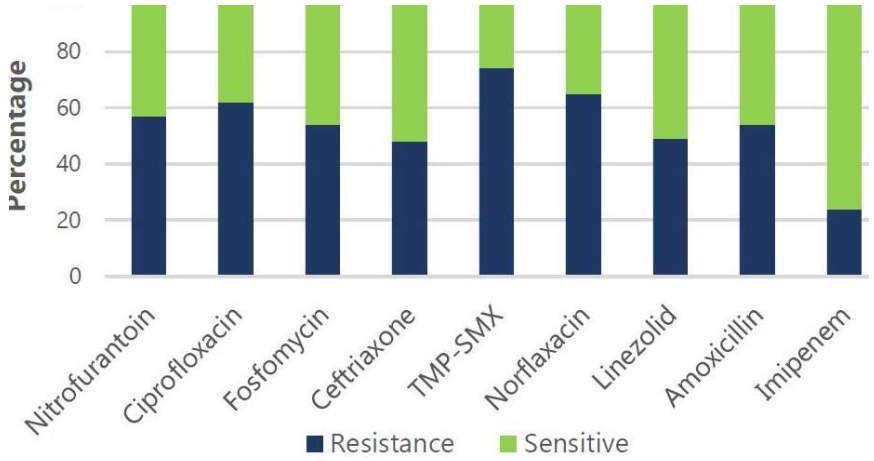

Figure 2: Comparison of Antibiotic Susceptibility Between Biofilm-Positive and Biofilm-Negative Isolates

3.3 Multivariable Predictors of MDR

Multivariable logistic regression identified four independent predictors of MDR phenotype: strong/moderate biofilm formation (aOR 2.4; 95% CI 1.5–3.9; p<0.001), CAUTI origin (aOR 2.7; 95% CI 1.6–4.6; p<0.001), prior antibiotic exposure in 3 months (aOR 2.1; 95% CI 1.3–3.4; p=0.002), and presence of papC virulence gene (aOR 1.7; 95% CI 1.1–2.8; p=0.03). Results are in Table 3.

Table 3. Multivariable Logistic Regression: Predictors of MDR Phenotype (n = 300)

Variable	**Unadj. OR (95% CI)**	**p-value**	**Adj. OR (95% CI)**	**p-value**
Strong/moderate biofilm	2.8 (1.8–4.4)	<0.001	2.4 (1.5–3.9)	<0.001
CAUTI origin	3.1 (1.9–5.1)	<0.001	2.7 (1.6–4.6)	<0.001
Prior antibiotic use (3 mo)	2.4 (1.5–3.8)	<0.001	2.1 (1.3–3.4)	0.002
papC gene positive	1.9 (1.2–3.0)	0.005	1.7 (1.1–2.8)	0.030
Diabetes mellitus	1.5 (0.9–2.5)	0.09	1.3 (0.8–2.2)	0.30
Recurrent UTI	1.6 (1.0–2.6)	0.05	1.4 (0.8–2.4)	0.20

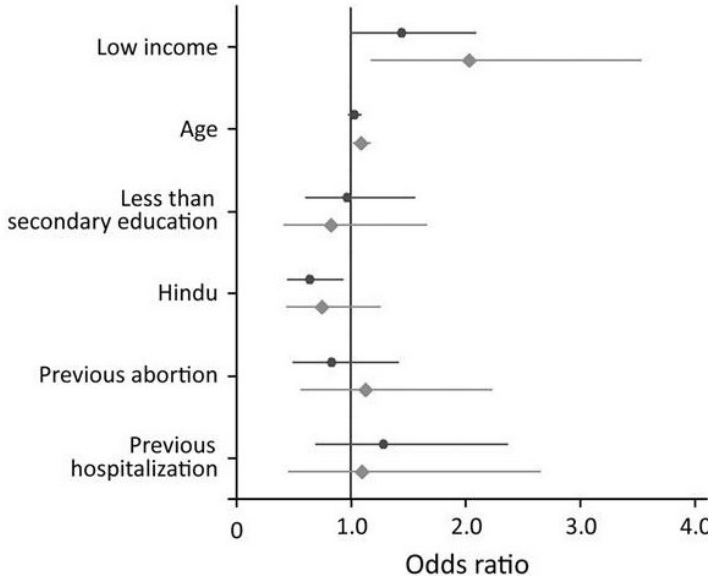

Figure 2: Forest Plot of Predictors Associated with Multidrug-Resistant UTI

4. DISCUSSION

This cross-sectional study characterised the biofilm-forming capacity and antimicrobial resistance profile of 300 UPEC isolates from a tertiary care hospital. The biofilm production rate of 62.0% — with strong producers constituting nearly a quarter of all isolates — is consistent with published Indian data reporting biofilm prevalence of 55–75% in UPEC clinical isolates [8]. The significantly higher biofilm production rate in CAUTI isolates (78.9%) compared to community-acquired UTI (45.0%) reflects the predisposing role of catheter surfaces as biofilm substrates and the selective pressure of prolonged catheterisation on adherent phenotypes.

The association between biofilm formation and MDR phenotype (aOR 2.4) observed in this study concurs with the concept of co-selection of virulence and resistance traits. Mobile genetic elements, particularly IncF plasmids prevalent in E. coli, frequently carry both biofilm-enhancing genes and antibiotic-resistance determinants, including blaCTX-M-15 — the predominant ESBL variant in our series [9]. The high prevalence of fimH (87.3%), encoding type I fimbriae essential for catheter and uroepithelial adhesion, suggests that this virulence trait is nearly universal in UPEC regardless of biofilm-forming capacity, while papC (P-fimbriae; 38.7%) and hlyA (alpha-haemolysin; 18.3%) were more selectively expressed in biofilm producers.

The antibiogram profile — characterised by fluoroquinolone susceptibility of only 28.0% and ESBL prevalence of 56.0% — mirrors the escalating resistance burden documented in national AMRSN data [5]. Encouragingly, nitrofurantoin (84.0%) and fosfomycin (89.0%) retained the highest susceptibility rates, aligning with their recommendation as first-line oral agents for uncomplicated UTI and step-down therapy for CAUTI by current IDSA and EAU guidelines [10]. The near-universal carbapenem susceptibility (ertapenem 92.5–97.4%) suggests carbapenems remain reserved for complex cases, consistent with responsible stewardship practice.

From a clinical management perspective, biofilm detection methods (TCP, CRA, tube method) are under-utilised in routine microbiology practice despite their predictive value for treatment failure. The 82.3% concordance between TCP and CRA suggests CRA could be a practical screening method in resource-limited settings where spectrophotometric measurement is

unavailable. The tube method, while simple, showed lower concordance (78.1%) and should be considered supplementary.

Limitations include the cross-sectional design, which precludes longitudinal tracking of biofilm-forming UPEC as a risk factor for recurrence; lack of time-kill or antibiofilm assays that would clarify biofilm-specific antibiotic tolerance; and single-centre design limiting generalisability. Future studies incorporating whole-genome sequencing would better characterise the interplay between virulence-gene clusters, resistance determinants, and biofilm capacity.

5. CONCLUSION

Biofilm formation was documented in 62.0% of UPEC isolates and was independently associated with MDR phenotype, ESBL production, and CAUTI context. Nitrofurantoin and fosfomycin retained the highest susceptibility and should be prioritised in empirical therapy for uncomplicated UTI. Biofilm phenotyping should be considered in isolates from CAUTI and recurrent UTI cases to guide therapeutic decisions and catheter-care strategies.

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