



Exploring the Link Between Biofilm Formation and Multidrug Resistance in *Escherichia coli* Isolated from Urinary Tract Infections in Sialkot, Pakistan

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Abstract: *Escherichia coli* strains are among the key contributors to urinary tract infections (UTIs) around the world, presenting a significant health challenge globally. **Objectives:** This cross-sectional study aims to isolate multidrug-resistant (MDR) *Escherichia coli* strains from UTI patients in Sialkot, Pakistan, and to investigate their biofilm-forming ability, exploring the possible association between biofilm formation and antibiotic resistance. **Methods:** A total of 131 *E. coli* isolates were isolated and identified. Their ability to form biofilms was confirmed using the tube method, and their antibiotic susceptibility patterns were evaluated using the disc diffusion method. Fisher's exact test was applied to investigate the association between biofilm production and antibiotic resistance. **Results:** The findings revealed that 65.6% isolates (n = 88) were biofilm producers, while 34.4% (n = 43) were non-biofilm producers. Antibiotic sensitivity testing indicated high levels of resistance among isolates to several antibiotics, notably ceftriaxone (91.6%), cefoperazone (86.3%), ciprofloxacin (85.5%), cefotaxime (82.4%), and cefixime (81.7%). A significant association was observed between biofilm formation and resistance to most antibiotics, including nitrofurantoin, amoxicillin-clavulanate, amikacin, piperacillin-tazobactam, and sulfamethoxazole-trimethoprim, among others. **Conclusion:** These findings suggest that *E. coli* isolates resistant to these certain antibiotics are more likely to form biofilms. This research highlights the critical need to explore biofilm formation, along with antibiotic susceptibility patterns of isolates, to help select appropriate antibiotics and facilitate effective treatment approaches.

Keywords: Urinary Tract Infection, Biofilm, Multidrug Resistance, Infection, Sialkot

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Introduction

Urinary tract infections (UTIs) are one of the most widespread infections affecting people of all age groups, with about 150 million cases reported globally each year. (1, 2). Uropathogenic *Escherichia coli* (UPEC) is reported to be the most common cause of UTIs (2, 3) accounting for about 75% cases (1). Antibiotics are being used as the primary treatment for UTIs (4). However, the growing issue of antibiotic resistance poses a significant challenge to healthcare settings worldwide. (3). The overuse of antibiotics for treating UTIs, even over brief durations, has contributed to the extensive rise of antimicrobial resistance among pathogens (2). Recent findings indicate that pathogenic *E. coli* strains are becoming increasingly resistant to broad-spectrum antibiotics, such as tetracyclines, aminoglycosides, β -lactams, and fluoroquinolones (5, 6). As resistance patterns of isolates differ across regions, understanding the local antimicrobial resistance profile is critical (2), as it is leading to the emergence of multidrug-resistant (MDR) strains, making the treatment of UTIs challenging, particularly in patients with recurrent infections (4). Biofilms are complex 3D assemblies of microorganisms embedded within a self-produced extracellular polymeric matrix (EPS), attached to either biological or inanimate surfaces (7). It is crucial in UTI pathogenesis, particularly in catheter-associated UTIs (CAUTIs), which account for 40% of hospital-acquired infections. Additionally, its development is a key factor in high recurrence resistance rates frequently associated with UTIs (8). The ability of UPEC cells to form biofilms extends to the surfaces of catheters, within bladder epithelial cells, and along the bladder walls (2) aiding in providing protection and enhancing bacterial resistance (9).

Several recent studies have focused on evaluating the relationship between antibiotic resistance and biofilm formation. However, the findings have often been inconclusive. Therefore, the current study aimed to explore the possible correlation between antibiotic resistance and biofilm production in UPEC isolates at a tertiary care hospital in Sialkot, Pakistan.

Methodology

Study Design and Population

This cross-sectional study included 131 clinical *E. coli* isolates from patients diagnosed with UTIs of all age groups admitted to Allama Iqbal Memorial Hospital, Sialkot, Pakistan, between July and December 2023.

Isolation and Identification of *E. coli* Strains

The urine samples were processed within 1 hour of collection, as per the standard guidelines, to prevent contamination. Subsequently, *E. coli* isolates were identified employing the standard microbiological methods and biochemical testing. All urine samples were inoculated on Cystine Lactose-Electrolyte-Deficient Agar (CLED agar) medium and then incubated at 37 °C for 18-24 hours. The identified isolates of *E. coli* were then subjected to identification through colony characteristics, Gram staining, and subsequent standard biochemical testing, including Catalase, Indole, Urease, Oxidase, and Citrate tests, for further confirmation. (10).

Antimicrobial susceptibility testing for *E. coli* Isolates

Antibiotic susceptibility of *E. coli* strains were tested against a panel of antibiotics using the Kirby-Bauer disk diffusion technique on Mueller-Hinton Agar medium, as per the Clinical and Laboratory Standards Institute (CLSI) protocol. The antibiotic discs of cefoperazone-sulbactam

(105 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), cefoperazone (30 µg), Augmentin (30 µg), gentamicin (10 µg), amoxicillin (10 µg), fosfomycin (50 µg), cefixime (5 µg), cefotaxime (30 µg), nitrofurantoin (300 µg) piperacillin–tazobactam (110 µg), amikacin (30 µg), Septran (25 µg), imipenem (10 µg) and meropenem (10 µg), were used in disc diffusion test. The plates were later incubated for 16 to 18 hours at 37 °C. Following this, the zones of inhibition around the antibiotic discs were eventually measured using a scale and recorded in accordance with CLSI guidelines (11).

Biofilm Formation assay

Biofilm formation was assessed by inoculating 10 mL of Trypticase Soy Broth containing 1% glucose with a loopful of bacterial colonies from overnight culture plates, followed by incubation at 37°C for 24 hours. The tubes were then emptied and rinsed with phosphate-buffered saline (PBS) at pH 7.3 and allowed to dry. Following this, the tubes were stained with crystal violet (0.1%) and then rinsed with deionized water to remove any excess stain. The tubes were left to dry in an upside-down position.

Biofilm presence was indicated by a visible film covering the wall and bottom of the tube. (12).

Statistical Analysis

All statistical analyses were conducted employing the R software (version 4.3.2). Fisher's exact test was applied to evaluate the possible relationship between antibiotic resistance of isolates and biofilm formation, considering *P*-values below 0.01 indicating statistical significance.

Results

A total of 131 *Escherichia coli* isolates were assessed in the present study from June 2023 to December 2023 at Allama Iqbal Memorial Hospital, Sialkot, Pakistan. The patient population for the collected isolates was composed of 52 males (39.7%) and 79 females (60.3%). Among females, the most affected age group was 21-30 years, followed by 41-50 years, while in males, the highest prevalence was found in the 51–60 age range (Fig. 1). Among these isolates, 88 (65.6%) were capable of biofilm formation, whereas 43 (34.4%) were non-biofilm formers.

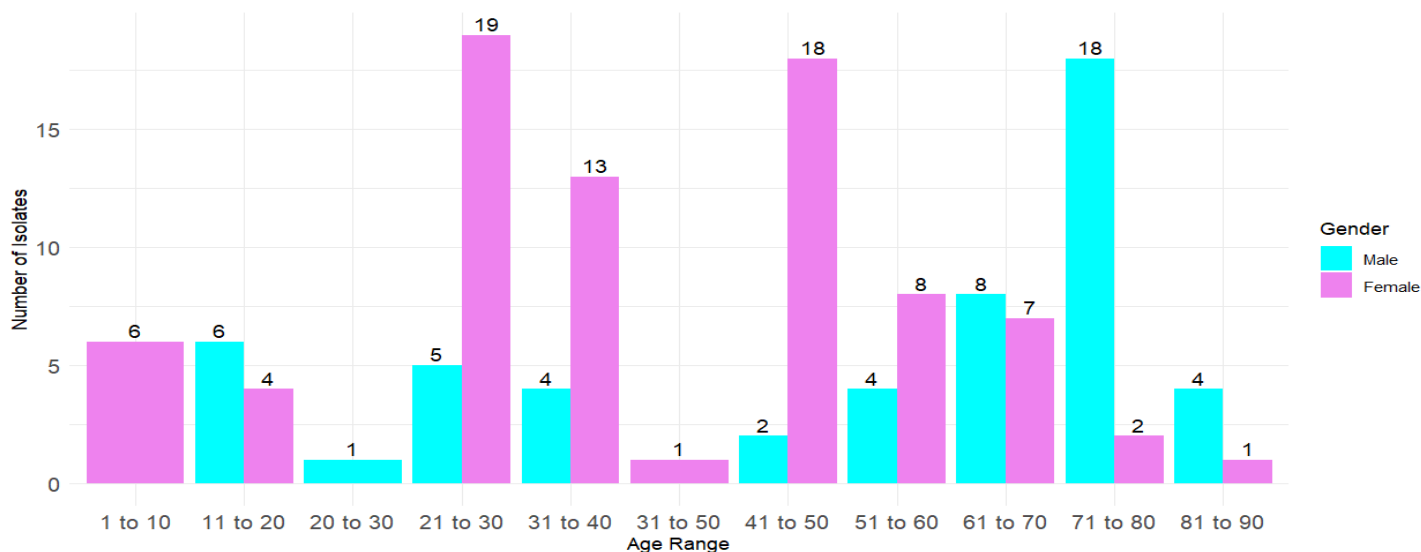


Figure 1. Age-Gender-Based Distribution of *Escherichia coli* Isolates

Antibiotic Susceptibility Testing

Figure 2 demonstrates the antibiotic susceptibility patterns of *E. coli* isolates. The testing revealed the highest levels of resistance for ceftriaxone, cefoperazone, and ciprofloxacin, with 91.6%, 86.3% and 85.5% of isolates resistant, respectively. Moreover, the isolates showed

an increasing pattern of resistance to amikacin (77.9%), amoxicillin-clavulanic acid (71.8%), gentamicin (71.8%), fosfomycin (71%), and nitrofurantoin (74.8%). However, the lowest resistance rates were observed with imipenem and meropenem, at 26.7% and 28.2%, respectively.

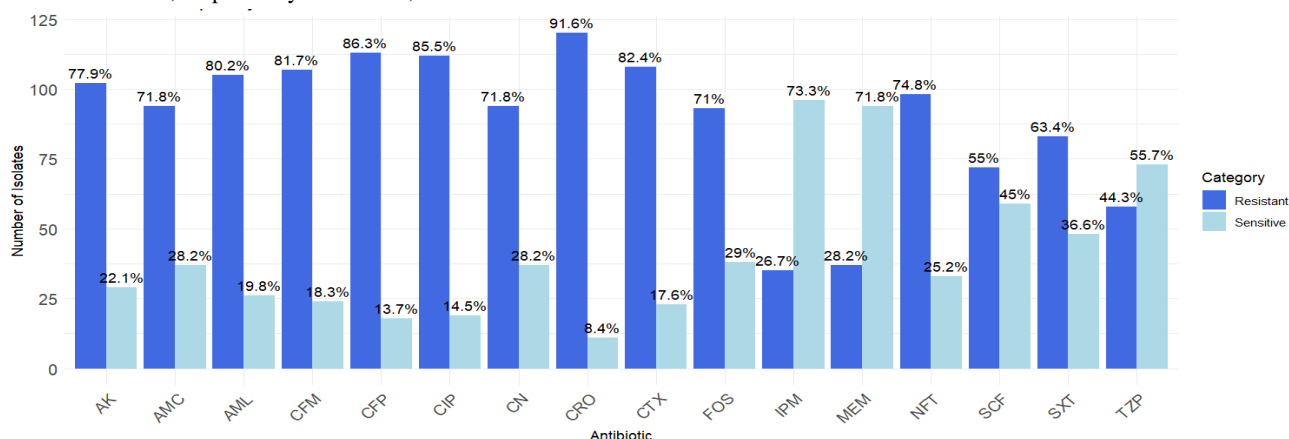


Figure 2. Antibiotic Susceptibility Pattern of UPEC Isolates (%age) AMC: amoxicillin-clavulanic acid. AML: Amoxicillin, CTX: Cefotaxime, CIP: Ciprofloxacin, SXT: sulfamethoxazole-trimethoprim, NFT: Nitrofurantoin, AK: Amikacin, CN: Gentamicin, FOS: Fosfomycin, TZP: piperacillin-tazobactam MEM: Meropenem, IPM: Imipenem, CFM: Cefixime, CFP: Cefoperazone, SCF: cefoperazone-sulbactam, CRO: Ceftriaxone

A heatmap was generated to illustrate further the resistance patterns of *E. coli* isolates across the different age groups of UTI patients, as displayed in Figure 3. It revealed the high prevalence of antibiotic resistance among the isolates. The majority of the isolates exhibited a high percentage of resistance (indicated by shades of purple) against antibiotics, including cefixime, amoxicillin-clavulanic Acid, ampicillin, amikacin, and ciprofloxacin, suggesting the limited effectiveness of these antibiotics. Conversely, lower resistance (shades of pink) to imipenem and

meropenem was observed in some age groups, highlighting their efficacy. Moreover, the heatmap shows a consistent resistance pattern exhibited by the antibiotics for most age groups, suggesting that age does not significantly determine the resistance patterns in our study. These outcomes underscore the importance of continuous surveillance for emerging resistant patterns and the judicious use of antibiotics to optimize treatment strategies.

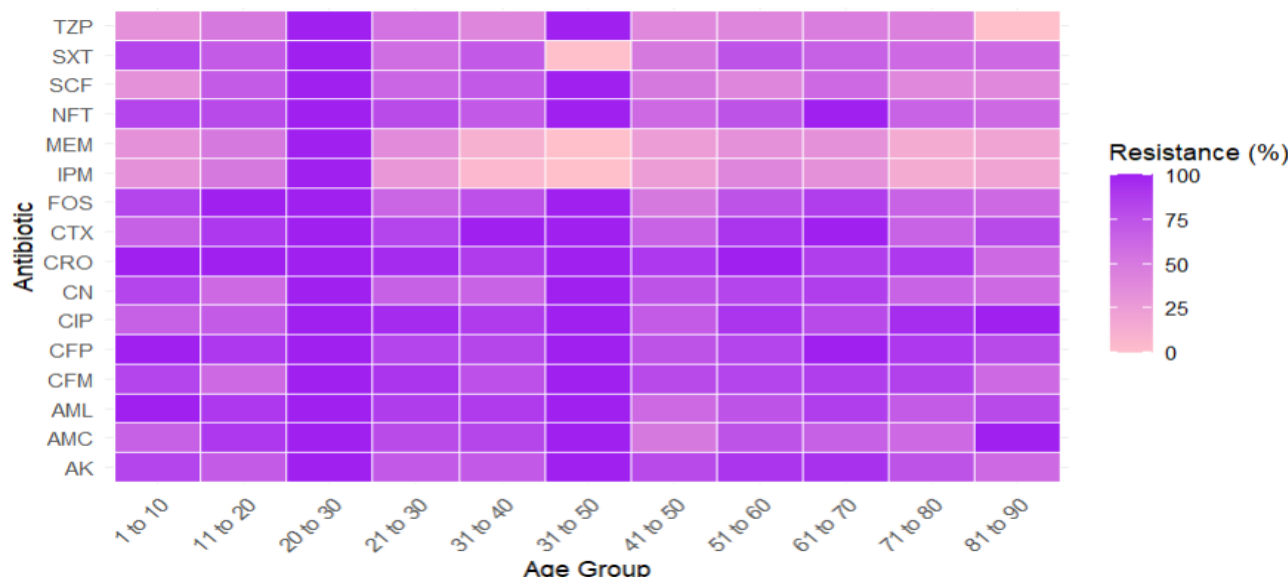


Figure 3. Heatmap displaying the patterns of antibiotic resistance across age groups of UTI patients. Purple represents resistance, and pink indicates sensitivity. *AMC*: amoxicillin-clavulanic acid, *AML*: Amoxicillin, *CTX*: Cefotaxime, *CIP*: Ciprofloxacin, *SXT*: sulfamethoxazole-trimethoprim, *NFT*: Nitrofurantoin, *AK*: Amikacin, *CN*: Gentamicin, *FOS*: Fosfomycin, *TZP*: piperacillin-tazobactam, *MEM*: Meropenem, *IPM*: Imipenem, *CFM*: Cefixime, *CFP*: Cefoperazone, *SCF*: cefoperazone-sulbactam, *CRO*: Ceftriaxone

Association between Biofilm Production and Antibiotic Resistance

The results of Fisher's exact test, used to investigate the association between antibiotic resistance and biofilm formation, revealed that several antibiotics, including nitrofurantoin, sulfamethoxazole-trimethoprim, amikacin, piperacillin-tazobactam, amoxicillin-clavulanic acid, and others, demonstrated significant associations ($P < 0.01$). In contrast, other classes of antibiotics, carbapenems and ciprofloxacin, showed non-significant results (Table 1).

Table 1. Fisher's Analysis for the association between the antibiotic resistance and Biofilm Production among UPEC isolates (n = 131)

Antibiotic	Biofilm producers Resistant (%)	Non-Biofilm Resistant (%)	P-value
AMC	74 (84.09%)	20 (46.51%)	$P < .001^{**}$
AML	82 (93.18%)	23 (53.49%)	$P < .001^{**}$
CTX	83 (94.32%)	25 (58.14%)	$P < .001^{**}$
CIP	79 (89.77%)	33 (76.74%)	$P = .06$
SXT	73 (82.95%)	10 (23.26%)	$P < .001^{**}$
NFT	79 (89.77%)	19 (44.19%)	$P < .001^{**}$
AK	81 (92.05%)	21 (48.84%)	$P < .001^{**}$
CN	76 (86.36%)	18 (41.86%)	$P < .001^{**}$
FOS	77 (87.50%)	16 (37.21%)	$P < .001^{**}$
TZP	47 (53.41%)	11 (25.58%)	$P = 0.003^{**}$
MEM	29 (32.95%)	8 (18.60%)	$P = 0.10$
IPM	26 (29.55%)	9 (20.93%)	$P = 0.40$
CFM	78 (88.64%)	29 (67.44%)	$P = 0.007^{**}$
CFP	81 (92.05%)	32 (74.42%)	$P = 0.10$
SCF	62 (70.45%)	10 (23.26%)	$P < 0.001^{**}$
CRO	84 (95.45%)	36 (83.72%)	$P = 0.40$

** = highly significant, *AMC*: amoxicillin-clavulanic acid, *AML*:

Amoxicillin, *CTX*: Cefotaxime, *CIP*: Ciprofloxacin, *SXT*: sulfamethoxazole-trimethoprim, *NFT*: Nitrofurantoin, *AK*: Amikacin, *CN*: Gentamicin, *FOS*: Fosfomycin, *TZP*: piperacillin-tazobactam, *MEM*: Meropenem, *IPM*: Imipenem, *CFM*: Cefixime, *CFP*: Cefoperazone, *SCF*: cefoperazone-sulbactam, *CRO*: Ceftriaxone

Discussion

UTIs caused by UPEC are a serious clinical concern, as a large number of patients are affected worldwide (Behzadi et al., 2020). Biofilm-forming infections majorly contribute to antimicrobial resistance (AMR) and recurrent UTIs, posing a growing concern on a global scale (13). However, research detailing the characterization of biofilm characteristics of UPEC and their antibiotic resistance patterns, specifically in Sialkot, Pakistan, is still scarce.

In the current study, 131 *E. coli* isolates from UTI patients were characterized using the standard microbiological identification procedures. UTIs caused by UPEC were found to be highly prevalent in females (60.3%) as compared to males (39.7%), which coincided with different previous studies (13-15). It is more likely that the shorter urethra in women, with its position close to the perineum, contributes to a greater vulnerability to UTIs relative to men (16). This study also showed that the majority of UPEC isolates formed biofilms (65.6%), which is in agreement with the findings reported by many previous studies (17, 18).

UPEC are becoming increasingly resistant to frequently used antibiotics, as the majority of isolates show high levels of resistance to cephalosporins, penicillins, and fluoroquinolones (ciprofloxacin), and demonstrate intermediate resistance rates to aminoglycosides (amikacin and gentamicin). However, less resistance was observed to carbapenems (imipenem and meropenem) and piperacillin-tazobactam. The high resistance rates of *E. coli* isolates to cephalosporins, penicillins, and ciprofloxacin are comparable to those reported in a previous study from

Pakistan (19). High resistance to antibiotics such as ciprofloxacin, Trimethoprim-Sulfamethoxazole (SXT), penicillins, and cephalosporins was also consistent with the findings reported in a study by AlShaikh et al. (2024), conducted in Egypt. Karigoudar et al. (2018) and Ibrahim et al. (2024) also reported similar results in their respective studies. The increased resistance might be more likely due to the overuse of broad-spectrum antibiotics during empirical therapy (20). However, lower resistance rates against these antibiotics were reported by Mlugu et al. (2023). Our study showed that the bacterial isolates were less resistant to imipenem (26.7%), meropenem (28.2%), and piperacillin-tazobactam (44.3%), indicating that carbapenems exhibited high efficacy compared to other antibiotics. This is in agreement with another study from India (21). A higher efficacy of piperacillin-tazobactam was also observed, as reported in an earlier study from Pakistan. In another study conducted in Pakistan, imipenem and meropenem demonstrated the highest efficacy in UTI patients, a finding similar to the present study (22).

This study further highlights significant associations between biofilm formation in *Escherichia coli* isolates and resistance to nearly all antibiotics ($P < 0.01$), except for three, including ciprofloxacin, imipenem, and meropenem. Our findings reveal that biofilm-producing isolates exhibit higher resistance rates to antibiotics compared to non-biofilm-producing isolates. Hence, it suggests that resistance to these antibiotics is associated with an increased likelihood of biofilm formation. Previous studies also reported significant associations between biofilm formation and antimicrobial resistance, suggesting a correlation between the two factors. (13, 18, 23), which may impact the persistence and treatment outcomes of urinary tract infections caused by *E. coli*.

It is essential to note that the present study was conducted at a single tertiary hospital with size limitations; therefore, the findings should not be generalized. Future studies are recommended to monitor the antibiotic resistance patterns of the isolates and to further explore the relationship between biofilm formation and these patterns, particularly at the molecular level, in developing regions with limited resources and research capabilities, in order to incorporate effective treatment and prevention strategies.

Conclusion

This study revealed an increasing pattern of resistance of UPEC isolates against commonly used antibiotics and their significant tendency towards biofilm formation. Thus, insights into antibiotic resistance patterns and biofilm formation are essential for effective management of biofilm-associated infections.

Declarations

Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate

Approved by the department concerned. (IRBEC-23)

Consent for publication

Approved

Funding

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Conflict of interest

The authors declared the absence of a conflict of interest.

Author Contribution

KS, RA, HAS

Data Collection, Laboratory work analysis, First Draft of Manuscript
Conception of Study, Data organization, Supervision, Revision of manuscript, Critical Input,

Conception of Study, Data organization, Supervision, Revision of manuscripts, Critical and Final Draft Review, and Approval

HE, SM, UN, SA, SI

Data analysis, Manuscript Review, Critical Input

Final draft Review, Editing, and Approval

Revision of manuscript, Critical Input, Final draft Review

Improvement of the first Draft,

All authors reviewed the results and approved the final version of the manuscript. They are also accountable for the integrity of the study.

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