RESEARCH ARTICLE

Multidrug Resistant and Biofilm formation in Uropathogenic *E.coli*

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Ann Coll Med Mosul 2026; 48 (1):17-22.
Received: 7th Feb. 2026, Revised 1st March. 2026, Accepted: 15th April 2026.
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Doi: 10.33899/acmm.v48.i1.61355

ABSTRACT

Background: Uropathogenic *Escherichia coli* (UPEC) is the main cause of urinary tract infections (UTIs). It causes a significant impact on public health due to its wide occurrence and healthcare costs. Many strains exhibit multidrug resistance (MDR) through different mechanisms such as biofilm and β-lactamase production, which enhances antibiotic tolerance and causes recurrent infections which necessitates the study of MDR and these causes.

Aim: to detect the relation between biofilm production and MDR in UPEC in Mosul City.

Patients and Methods: The study included 45 fully identified *E. coli* strain isolated from UTIs in Mosul City. Antimicrobial susceptibility was measured using Kirby-Bauer disk diffusion test according to guidelines and MDR was established as resistance to one agent in three or more groups. Biofilm measured using Tissue Culture Plate method, classifying the isolates from non-biofilm producer to strong producers based on optical density cut-off value.

Results: the study showed varying resistance level in different drugs, Amikacin, Ceftazidime, Cephalothin, Cefotaxime and Amoxiclav with resistance rate of 62.2%, 77.8%, 80%, 84.5%, 77,.8% and 86.7% respectively. Imipenem exhibited no resistance while Norfloxacin had 11.1% resistance rate. MDR was recorded in 15 out of the 45 isolates and only 2 showed extensive drug resistance. Biofilm production found in 73.3% of the isolates, categorized as high, moderate and mild in 27.3%, 60.6% and 12.1% respectively. Among those with MDR, 93.3% produced biofilm, with 46.7% demonstrating high production with significant statistical association.

Conclusion: Uropathogenic isolates revealed significant antimicrobial resistance specially Beta-lactam drugs whereas Imipenem was fully sensitive. A significant relation was noted between MDR and biofilm production that need to address biofilm mediated tolerance in treatment of persistent MDR infections.

Keywords: Biofilm, Multidrug resistant, Uropathogenic *E coli.*

INTRODUCTION

Escherichia coli is one of the primary microorganisms that is implicated in urinary tract infection (UTI). It is the most commonly acquire bacterial infections and among the top causes of global infectious burden due it is morbidity and mortality [1]. UTI affects millions of people over the world resulting in substantial health care cost & contribute to a serious economic and public health threat. Currently, Uropathogenic Escherichia coli (UPEC) strains shows an increasing resistant to multiple antimicrobial agent's classes which results in failure of empirical therapy [2].

The term MDR is applied to any bacteria that develops resistance to three or more classes of antimicrobials, while bacteria show sensitivity to only one class of antibiotics is called extensively drug resistance (XDR) [1,3]. Thereby, understanding the causes of UPEC multidrug resistance (MDR) is curtail for effective diagnosis and management. Biofilm formation and MDR often coexist in UPEC clinical isolates, however, the frequency of biofilm association and MDR has not been fully characterized [3,4]. The UPEC bacteria that exhibit MDR carries a significant challenge for public health especially the increase of antimicrobial resistance (AMR) against the β lactams drugs, tetracycline and fluoroquinolones which necessitates regional profiling of AMR to prevent treatment failure [5,6]. The AMR in UPEC can be attributed to intrinsic factors and acquiring resistance genes. Intrinsic mechanisms include decrease membrane permeability, expression of efflux pump, enzymatic destruction of the drug and biofilm formation [1,3,7]. In UPEC infection, biofilm formation within the bladder epithelial cells will serve as a protective barrier that hinder antimicrobial efficacy and reduce drug penetration, thus, enhancing the bacterial persistence and causes recurrent infection [8,9]. Researcher highlighted that UPEC strains that forms biofilm demonstrated a higher antibiotics resistance particularly to fluoroquinolones and third-generation cephalosporins when compared to biofilm negative strains [10].

This direct correlation assumption necessitates further investigation to measure the relation between biofilm formation and MRD in UPEC. This work aimed to detect the association between biofilm formation and multidrug resistance in UPEC in Mosul City.

PATIENTS AND METHODS

Study Population

Clinical isolates were collected from all age group patients complained from signs and symptoms of UTI at hospitals and tertiary care centers.

Sample Collection

Forty-five E. coli isolates were obtained from midstream urine samples under aseptic condition following standard protocols.

Exclusion Criteria

Patients on antimicrobial therapy in the 48-72 hours prior to sample collection were excluded from the study

Bacterial isolation and identification

Culture: urine sample were inoculated on standard bacteriological media including, blood agar, MacConkey agar and Eosin Methylene Blue (EMB) agar. Media were incubated aerobically at 37°C for 18–24 hours. The bacterial growth was fully identified depending on colonial morphology, gram staining and standard biochemical tests.

Antimicrobial Susceptibility Testing (AST)

Kirby-Bauer disk diffusion method was performed on Muller Hinton agar (MHA). Bacterial inoculum was prepared by suspending 3-5 fresh colonies in a normal saline adjusting the turbidity of the suspension to be equal to 0.5% McFarland. The suspension was spread on MHA and the antibiotic disk were placed aseptically on the surface of the media. After 18-24 hours of incubation, inhibition zones were measured and the results were interpreted according the standard zone of Clinical and Laboratory Standards Institute (CLSI) guidelines [11].

Antibiotic Panels

Isolates were then tested against various classes including penicillins (Amoxiclav), cephalosporins (Cefotaxime), carbapenems (Imipenem), aminoglycosides (Amikacin), and fluoroquinolones (Ciprofloxacin). Isolates that are resistant to at least one agent in three or more antimicrobial classes were considered MDR.

Biofilm Detection

biofilm was detected using the gold standard quantitative Tissue Culture Plate (TCP) method. The colonies were suspended in Trypticase soy broth with 1 % glucose adjusting the turbidity to 0.5 McFarland. A 200 µL of suspension was add 96- well U shape bottom polystyrene plates and incubated at 37°C for 18-24 hours. The wells were washed 3-4 times with phosphate buffered saline to remove planktonic cells and stained with 200 µL of 0.1% Crystal Violet for 10 minutes. The dye was solubilized with 95% ethanol. The Optical Density (OD) was measured using an ELISA reader at wavelength of 630 nm. The results were classified as non-producers, weak, moderate or strong producers based on OD cut-off value that is defined as three standard deviations above the mean OD of negative control [12].

Statistical Analysis

Data was typically analyzed using SPSS software to determine the correlation between biofilm production intensity and multidrug resistance patterns, with a P-value < 0.05 considered significant.

Ethical approval

This study was approved by the Medical Research Ethics Committee, College of Medicine, University of Mosul (Ref.: UOM/COM/MREC/24-25/APR3). This committee followed the Declaration of Helsinki for Medical Research. Prior to their involvement, all subjects gave a written informed consent.

Limitation of the study

Small sample size

RESULTS

The antimicrobial susceptibility testing of different drugs showed various findings. Amikacin, Ceftazidime, Cephalosporin, Cefixime, Cefotaxime and Amoxiclav showed high resistant rate recorded as 62.2%, 77.8%, 80.0%, 84.5%, 77.8%, and 86.7% respectively with significant statistical difference when compared to those of intermediately sensitive and sensitive isolates. Imipenem showed no resistance, while Norfloxacin revealed a 11.1% resistance. Moreover, Ciprofloxacin and Levofloxacin showed relatively similar resistance and sensitivity percentages as shown in Table 1.

Table 1
Antimicrobial susceptibility testing results

Drug	Resistance	Intermediate Sensitive	Sensitive	P-value
	No. (%)	No. (%)	No. (%)	
Amikacin	28 (62.2)	7 (15.6)	10 (22.2)	0.000
Imipenem	0 (0.0)	7 (15.6)	38 (84.4)	0.000
Ciprofloxacin	23 (51.1)	1 (2.2)	21 (46.7)	0.000
Levofloxacin	24 (53.3)	3 (6.7)	18 (40.0)	0.000
Norfloxacin	5 (11.1)	15 (33.3)	25 (55.6)	0.000
Ceftazidime	35 (77.8)	6 (13.3)	4 (8.9)	0.000
Cephalothin	36 (80.0)	3 (6.7)	6 (13.3)	0.000
Cefixime	38 (84.5)	4 (8.8)	3 (6.7)	0.000
Cefotaxime	35 (77.8)	3 (6.7)	7 (15.6)	0.000
Amoxiclav	39 (86.7)	6 (13.3)	0 (0.0)	0.000

*Chi square test for goodness of fit

Among the sample analyzed, MDR was found in 15 out of the 45 isolates (33.3%) while only 2 isolates (4.4%) showed extensive drug resistance Fig 1.

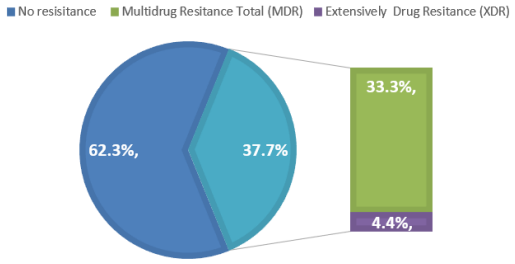

Fig 1. Distribution of Multidrug resistance in 45 UPEC isolates.

Biofilm production was detected in 33 samples out of the 45 total studied isolates accounting for (73.3%). This result was distributed as 27.3% high, 60.6% moderate, and only 12.1% as mild producers. These different proportions were statistically significant (p=0.002), as shown in Fig 2.

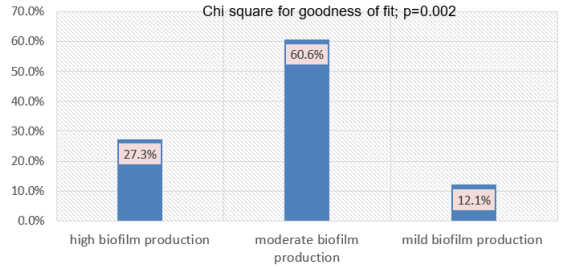

Fig 2. Distribution of biofilm production.

Among strains presented with multidrug resistance, 14 out of the 15 (93.3%) produced biofilm and only 1 (6.7%) was non biofilm producer Table 2. From the 14 bacterial biofilm producer isolates, 6 (42.8%) presented with high biofilm formation, and 8 (57.2%) had moderate biofilm formation (Fig 3). The relation between MDR and biofilm formation was statistically significant p (0.038) and OR =8.11. Table 2

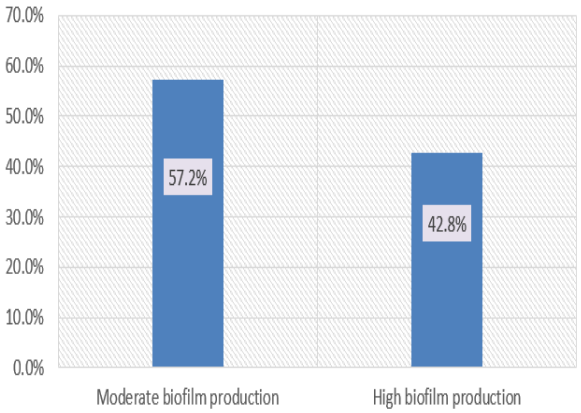

Fig 3. Distribution of biofilm production in MDR

Table 2
MDR and biofilm formation.

Biofilm	MDR No. (%)	Non-MDR No. (%)	P-value
Biofilm positive	14 (93.3)	19 (63.3)	
Biofilm negative	1 (6.7)	11 (36.7)	0.038
Total	15	30	

DISCUSSION

The present study demonstrated a high antimicrobial resistance rate in UPEC isolates obtained from patients with UTI in Mosul city, Iraq. The resistance was especially high for Amoxiclav, Cefixime and Cephalothin, whereas Imipenem retained full activity. These findings are consistent with regional trends which show an increased resistance to β-lactams drugs. However, the small size of the study limits generalization of these findings. The high resistance to Amoxiclav goes with the findings of Barbara Kot, 2019 who reported that resistance of UPEC may reach more than 80% in developing countries [13], while cephalosporins resistance was slightly higher than those recoded in Iranian study [14]. The high resistance to Amoxiclav and cephalosporins in our study is statistically significant compared to the other isolates proportions, suggesting these agents may be no longer suitable for empirical therapy. As highlighted by Goleanu et al (2025) [15], these high resistance levels are often a suggestion of a widespread production of Extended Spectrum Beta Lactamases (ESBLs) among clinical isolates. On the other hand, Imipenem showed an absence of resistance, maintaining 100% susceptibility in the tested isolates. This emphasizes the continuous role of carbapenems as highly effective treatment resort for MDR bacterial infections. Similar observation denoted in a surveillance study by Fu et al. (2024) [16], where carbapenems remained the most potent drug against gram negative pathogens. However, Amikacin showed a relatively higher resistance rate of 62.2%, which is greater than other reports but goes with recent increase of resistance to aminoglycosides in certain clinical settings [14,17]. Wu et al. (2024) [17] recently recorded that amikacin resistance is climbing reaching over 40% in some regions which explain the elevated level observed in our study.

The current study also revealed interesting patterns for fluoroquinolones. Norfloxacin resistance was proportionally low (11.1%), while Ciprofloxacin and levofloxacin showed relatively similar resistance percentages. This similarity may be explained by the close relationship between ciprofloxacin and levofloxacin resistance due to shared mutational pathways in gyrA and parC genes [18], which can lead to cross resistance between these agents. The difference in the pattern of resistance to Norfloxacin despite it belong to fluoroquinolones may be explained by the difference of usage pattern in this specific population or specific pharmacokinetics properties of this drug.

The antimicrobial susceptibility profile in the current study, as illustrated in Fig 1, revealed a significant burden of resistance, specifically MDR which was detect in 33.3% of the isolates while 4.4% reached the critical threshold of XDR. These records underscore the increasing challenge of resistance in medical practice. The observed MDR rate is consistent with recent epidemiological reports from tertiary care facilities. For instance, Almakrami et al. (2024) [19] identified a high prevalence of MDR isolates, noting that such results are often driven by selective pressure of broad-spectrum antibiotic use. Although our MDR rate is substantial, it remains within the range of 30% to 60% that frequently reported in contemporary hospital acquired infection surveillance studies [20]. The emergence of XDR isolates (4.4%), although it is low, paramount clinical concern. This emergence will extremely limit therapeutic options for clinicians. Assefa et al. [21] emphasizes that even small percentage of XDR isolates in a clinical population can serve as a reservoir for highly resistant genes which may lead to outbreaks of microorganism nearly impossible to treat with standard protocols. These finding suggesting that superbugs are already circulating in the population. The transition from MDR to XDR represents a critical point in management of bacterial infections, which may be independently associated with prolonged hospital stays as denoted by Padmaja et al. [22]. Furthermore, the categorization used in this study (MDR as resistance to ≥3 classes and XDR as resistance to all but ≤2 classes) follows the standardized international criteria proposed by Magiorakos et al. [23], ensuring that our findings are comparable with global surveillance data. The current work studied the ability of the bacteria to form biofilm, revealing that nearly three quarters of the bacteria were biofilm producers. This high prevalence points the significant role of biofilms in the survival and persistence of these pathogens in clinical environments. These findings are consistent with those of Hasnaw and Al-Hilu [24] suggesting that biofilm formation may represent a central mechanism for bacterial pathogenesis rather that an incidental trait. The categorization of biofilm positive samples revealed heterogenous distribution of production intensity. Most of the microorganism 60.6% were moderate biofilm producers followed by high producer strains by 27.3% and least common were mild producer 12.1%. This observed distribution was statistically relevant (p=0.002) which reflects that this distribution is not random and may indicates genetic or environmental adaptation of the isolates to hostile environment of antimicrobial drugs. Similar findings have been reported by Hasnaw and Al-Hilu [24], who noted that the intensity of biofilm production often skews toward moderate or strong producers especially in hospital acquired infections, which is potentially due to selective pressure of antimicrobial treatment. The dominance of moderate and high biofilm producers carries profound clinical implications. Biofilm serves as barrier that enhances AMR. Research by Vestby et al. [25] denoted that even moderate biofilm production can increase the minimum inhibitory concentration of antibiotics by up to a 1000-fold compared with non-biofilm producer. Furthermore, the statistical significance of prevalence of moderate and high biofilm producers suggest that these isolates have increased ability for chronic colonization [26]. Grading of biofilm production is crucial for managing therapeutics strategies, as strong producers are often associated with treatment failure and recurrent infections [26]. The significant p-value in our study reinforces the reliability of these phenotypic types as diagnostic markers for assessment of virulence potential of clinical strains. The significant correlation between MDR and the capacity for biofilm formation and the high percentage of biofilm production in majority of MDR (93.3%) suggest that biofilm is not a merely coincidental findings but a fundamental survival strategy for MDR bacteria. These results align with recent academic literature identifying the synergism between these two parameters. [27] The fact that 100% of biofilm positive MDR isolates in this study fell into high or moderate groups is worth to note. These findings go with that of Facchin et al. [28] who observed an association between MDR and biofilm production suggesting that the physiological stress of antibiotics resistance may select for more robust biofilm producing strains. Clinically, the dominance of strong (42.8 %) and moderate biofilm (57.2%) producer among MDR represent a great challenge for infection control. This challenge is reported by Donadu et al. [29] where the strong biofilm MDR strains were significantly more difficult to eradicate from clinical settings and medical devices leading to chronic and difficult to treat infections. To summarize, our results reinforce the necessity of screening biofilm production alongside with routine antimicrobial susceptibility testing, as the presences of biofilm production render usual MDR treatments ineffective.

CONCLUSIONS

Based on the findings of this study, it can be concluded that UPEC isolates exhibit a high rate of antimicrobial resistance and relevant capacity for biofilm formation. The data showed critical level of resistance to several first line antimicrobial agent specially beta lactam drugs, whereas Imipenem remain fully effective agent. These results underscore the role of biofilms as an important contributing to antibiotics tolerance, suggesting that therapeutic strategies must address biofilm mediated resistance to manage multidrug resistant infections.

Acknowledgment

I would like to express my gratitude to those who contributed to the completion of this work. I am also grateful to the faculty staff of Department of Microbiology in the College of Medicine /University of Mosul who provided the necessary facilities and supportive academic environment to complete this work. Finelly,

I extend my heartfelt appreciation to my family for their patience, understanding and unwavering support.

Conflict of interest

The author declares that there is no conflict of interest with respect to the research authorship.

Funding

Personal Funding

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