Original Article

# Coexistence Of Biofilm Formation And Antibiotic Resistance Profile Of Uropathogenic E. Coli (Upec)

**Ahmed Dhahir Abed[1]*, Thamer Yousif Mutter[2]**

*[1] Biology Department, College of science, University of Anbar, Iraq, E-mail:- ahm20s1002@uoanbar.edu.iq
[2] Biology Department, College of science, University of Anbar, Iraq

***Corresponding Author:** - Ahmed Dhahir Abed

*Biology Department, College of science, University of Anbar, Iraq, E-mail:- ahm20s1002@uoanbar.edu.iq
**Doi:** 10.47750/pnr.2022.13.S05.31

## Abstract

The bacteria known as Uropathogenic *Escherichia coli* (UPECs) play a major role in triggering UTIs (UTIs). Antibiotic resistance among UPEC strains may progress faster if UTIs in Anbar continue to be treated with beta-lactam antibiotics without antibiotic susceptibility testing. In a study evaluating cephalosporin-resistance among 141 *E. coli* clinical isolates, 100 were found in urine samples. Antibiotic resistance was analyzed using the VITEK-2 system. The results indicated that resistant to most β-lactamases used in this study was associated with biofilm formation. 88.5% of UPEC isolates that showed biofilm positive were resist to 3rd generation cephalosporins with a statistically highly significance (P=<0.0001).

**Keywords: -** *E. coli*, biofilm, β-lactamases, UPECs.

## INTRODUCTION

One of the leading causes of morbidity, UTIs affect an estimated 150 million people annually around the world (Flores-Mireles et al., 2015). It is estimated that 80–90% of UTIs acquired in the community are caused by *E. coli*, while only 30–50% of UTIs acquired in hospitals are caused by E. coli. (Ejrnæs, 2011). Uropathogenic E. coli (UPEC) is able to invade, grow, ascend, and persist in the uroepithelium because of its ability to form biofilms and utilize a variety of virulence factors. This ability is dependent on the ability of UPEC to form biofilms. (Terlizzi et al., 2017). The term "biofilm" refers to an accumulation of microbial cells that are permanently attached to a surface and encased in a matrix composed primarily of polysaccharide material. Biofilms can't be removed . (Speancer et al., 2014) . Biofilms offer bacteria a means of survival by putting them in a position where they can make efficient use of the nutrients that are present while also blocking the bacteria's access to antimicrobial agents, antibodies, and white blood cells. (Nandakumar et al., 2013) . They have also been found to harbor a large number of antibiotic inactivating enzymes such as beta-lactamases, which contributes to the formation of an antibiotic-resistant island. (Davies & Davies, 2010) . Researchers have identified biofilms of Escherichia coli as the most common cause of UTIs. Four main processes contribute to biofilm development: There are four stages of biofilm development: I the initial adhesion or attachment (reversible); (ii) the earliest stages of biofilm structure development; (iii) biofilm maturation; and (iv) the dispersion of cells from the biofilm to return to a planktonic state.(Sharma et al., 2016).As shown in figure 1.

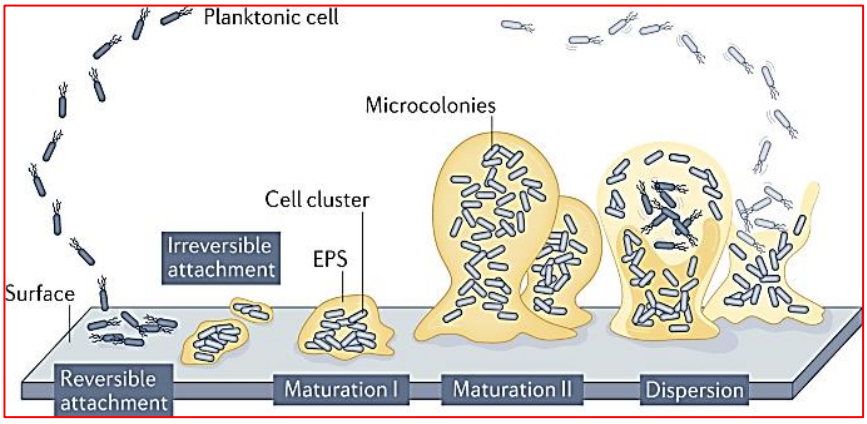


**Figure 1.** Biofilm formation steps.

In the current study, we wanted to determine how well UPEC clinical isolates in Anbar form biofilms and whether or not this capability is correlated with the antimicrobial susceptibility pattern of those isolates.

## METHODS:

### ISOLATION OF BACTERIAL ISOLATES:

The cross-sectional study was carried out in the Department of Microbiology, Ramadi teching Hospital, Anbar, Iraq, and Department of biology, science College, university of Anbar from November to December, 2022. A clinical and socio-demographic study of patients was performed. A total of 215 mid-stream urine were cultured MacConky agar and blood base agar Agar plates and incubated at 37 °C for 24 h (Shrestha et al., 2019) .

### IDENTIFICATION OF BACTERIAL ISOLATES :

All isolates were identified based on conventional methods in addition to modern methods using the Vitek 2 system, and molecular methods using PCR technique.

### ANTIBIOTIC SUSCEPTIBILITY TEST :

Antibiotic susceptibility was determined using a disk difusion technique based on the modified Kirby-Bauer method in accordance with CLSI standards (CLSI), 2018(Belley et al., 2021).

## DETECTION OF BIOFILM FORMATION IN E. COLI

### A- QUALITATIVE DETECTION OF BIOFILM USING CONGO RED AGAR TEST

1- Congo's red agar (CRA) medium consists of 37 gm / L brain heart infusion, 50 gm/L sucrose, 10 gm/L agar, and 0.8 gm/L red Congo stain.
2- Congo red stain was prepared separately as a concentrated watery solution and sterilized by autoclave at 121 C for 15 minutes. When the agar cooled to 55°C, Congo red stain solution was added.
3-Petri dishes were cultivated and incubated aerobically at 37˚C for 1-2 days.
4- Black colonies with a dry crystalline consistency indicated a positive result. Even though sporadic darkening at the middle of colonies was seen, weak biofilm producers typically stayed pink.
5-A darkening of the colonies with the nonappearance of a dry crystalline colonial morphology shown an uncertain result (Oliveira and Cunha, 2010; Catana et al., 2009).

### B- QUANTITATIVE DETECTION OF BIOFILM MICRO-TITTER PLATE METHOD:

Biofilm formation assays were conducted using a quantitative adherence assay. (Hassan et al., 2011). The production of biofilm was measured at this stage. Three independent samples were taken for each isolation, and the average was determined. The microtiter-plate reader data was then categorized into four groups: no biofilm formation, weak biofilm formation, moderate biofilm formation, and high biofilm formation. Table (1).

**Table 1.** Micro titer plate classification of biofilm formation capabilities.

| Cut-off value calculation | Biofilm production abilities |
|---|---|
| OD >(4×ODc) | Strong |
| (2×ODc)< OD≤(4×ODc) | Moderate |
| (ODc)< OD≤(2×ODc) | Weak |
| OD≤(ODc) | None |

OD: mean of samples at 630 nm. ODc: mean of control at 630 nm.

## RESULTS AND DISCUSSION

To confirm the diagnosis, the collected isolates were initially diagnosed as E.coli based on conventional methods and automated method using Vitek-2 system.

## SUSCEPTIBILITY TO ANTIBIOTICS

The Kirby-Bauer disk diffusion susceptibility test was used to determine the antibiotic susceptibility of one hundred E. coli isolates obtained from patients with urinary tract infections (Figure 2) and confirmed by automated Vitek-2 according to the recommendations of CLSI (CLSI, 2021). The high percentage of UPEC that is resistant to the third generation of -lactam antibiotics is due to excessive uptake of these antibiotics, improper usage of drugs as prescribed by a physician, and a lack of personal education demonstrated by an incomplete full course of antibiotics to destroy the pathogen in order to increase infection cure rates and avoid the formation of resistance or treatment failures . (ESBL) (Al-khikani et al., 2020).

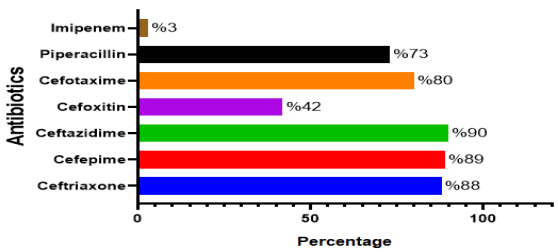


**Figure 2.** Antibiotic resistance percentage for *E. coli* based on kirby bauer disk diffusion.

## BIOFILM FORMATION IN UPEC

Bacterial biofilm formation is an ordered matrix that helps bacterial organisms adhere to the surface and to each other. Biofilm structure allows pathogens to be more resistant to the stress such as antibiotic and other environmental factors (Kumar et al., 2016).

Many recurrent infections are caused by biofilm-producing bacteria, which are difficult to eliminate. They manifests resistance to antibiotics by a variety of ways, including limited antibiotic penetration into biofilms and the expression of resistance genes (Devrari and Pai, 2018). All UPEC isolates were examined for their capability to produce a biofilm using two in vitro screening methods.

## A- CONGO RED AGAR METHODS (CRA) (QUALITATIVE DETECTION)

UPEC were tested for biofilm formation by Congo red agar method (Table 2; Figure 3). The results showed that 70% of the isolates were biofilm producers. Biofilm producer isolates were higher in males (84.3%) compared in females (63.2%).

| Table 4-4 Biofilm production ratio between Females and Males (CRA) | | | |
|---|---|---|---|
| Biofilm producer (CRA) | Female no=68 (%) | Male: no=32 (%) | Total no=100 |
| | 43 (63.2%) | 27 (84.3%) | 70 (70%) |
| P value | 0.0366 | | |

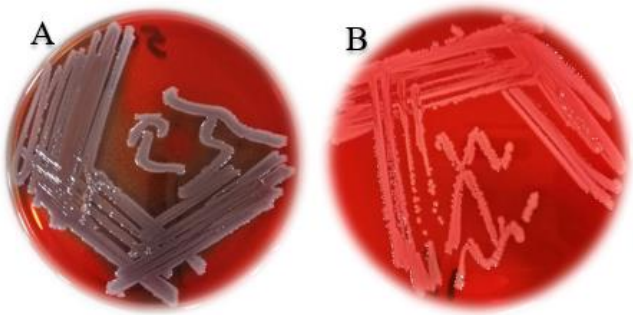


**Figure 3.** Biofilm formation using Congo-red method. (A) Positive result (black colored); (B) negative result (pink-colored).

## B- MICRO-TITER PLATE METHOD (MTP) (QUANTITATIVE DETECTION):

The ability of 100 isolates of *E. coli* to form biofilm was examined on the surface of polystyrene microtiter plate. Based on absorbance (630 nm), biofilm producers were classified into three categories: weak, moderate, and strong biofilm producers.

**Table 3.** Shows the number and percentage of biofilm producers according to above classifications. The results showed that 23 out of 100 isolates were strong biofilm producers, whereas 27/100 and 41/100 of isolates were moderate and weak producers, respectively. The remaining isolates 9/100 did not produce a biofilm.

| Table 3. Distribution of biofilm producers by microtiter plate. | | | | |
|---|---|---|---|---|
| Gender | Biofilm producer No. (%) | | | |
| | Strong | Moderate | Weak | Non producer |
| Female= 68 | 18 (26.4%) | 19 (27.9%) | 28 (41.1%) | 3 (4.4%) |
| Male= 32 | 5 (15.6%) | 8 (25%) | 13 (40.6%) | 6 (18.7%) |
| Total= 100 | 23 (23%) | 27 (29%) | 41 (41%) | 9 (9%) |
| P value | 0.0283 | | | |

MPT results showed that females were higher than males in biofilm production with a statistically highly significance (P= 0.0283). Our finding agrees with other studies who showed that the biofilm formation is twice the number in females than in males (Mittal et al., 2015; Bhatta et al., 2019).

## CORRELATION OF THE RESISTANCE PATTERN WITH THE VIRULENCE MARKERS

Table 4 shows the relationship between the resistance pattern and virulence genes expressed in UPEC. The results indicated that resistant to most β-lactamases used in this study was associated with biofilm formation. 88.5% of UPEC isolates that showed biofilm positive were resist to 3rd generation cephalosporins with a statistically highly significance (P=<0.0001). Same correlation was observed with the sulphonoamides and quinolones classes, which indicates that most biofilm former strains were MDR; high association between MDR isolates and biofilm formation. Same finding was explained by previous works that indicated the high relationship between antibiotic resistance mechanisms and biofilm formation (Naves et al., 2008; Karam et al., 2018). In a study that performed in Ugunda, the researchers showed the high association between the MDR strains and biofilm formation, which agrees with our findings (Katongole et al., 2020).

**Table 4.** Relationship between antibiotic resistance and biofilm formation.

| Virulence Marker | Antibiotic resistance profile N (%) | | | | | |
|---|---|---|---|---|---|---|
| | *COR | CX | AK | TS | CIP | IMP |
| Biofilm Positive =91 (S+M+W) | 85(88.5%) | 53(55.2%) | 29(30.2%) | 76(79.1%) | 62(64.5%) | 5(5.2%) |
| Negative=9 | 3(75%) | 2(50%) | 0 | 1(25%) | 2(50%) | 0 |
| P value | <0.0001 | 0.0743 | 0.05 | <0.0001 | 0.0099 | >0.9999 |

## CONCLUSION

In conclusion, antimicrobial susceptibilities, and biofilm formation profiles of *E. coli* isolates recovered from UTIs were determined. High rates of multi-drug resistance were reported. High correlation between β-lactam antibiotic resistance pattern and biofilm formation.

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