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Effect of Ciprofloxacin and Trimethoprim/Sulfamethoxazole on Biofilm Formation of Multi-Drug Resistant Uropathogenic *Escherichia coli*

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ABSTRACT

Multi-drug-resistant uropathogenic *Escherichia coli* (UPEC) is considered a significant challenge due to its ability to resist antibiotics and form biofilms. UPEC biofilm formers are well protected and largely inaccessible to antibiotics, which leads to persistent infections and evasion of the host immune system. Understanding how ciprofloxacin and trimethoprim/sulfamethoxazole affect biofilm formation is essential for improving treatment strategies for urinary tract infections (UTIs). A total of 76 UPEC isolates were obtained from Iraqi patients and identified using morphological and biochemical characteristics, as well as the Vitek®-2 Compact system. Minimum inhibitory concentrations (MICs) were determined using the Vitek®-2 system, which is based on CLSI standards, followed by agar diffusion assays to determine MIC, sub-MIC (SMIC), and sub-sub-MIC (SSMIC). A 96-well microtiter plate assay was used to quantify the biofilm-forming ability of UPEC isolates and to evaluate the effects of ciprofloxacin and trimethoprim/sulfamethoxazole on UPEC biofilms. The MICs of ciprofloxacin were ≥ 4 µg/mL for resistant isolates and ≤ 0.25 µg/mL for sensitive ones. For trimethoprim/sulfamethoxazole, MICs were ≥ 320 µg/mL for resistant isolates and ≤ 20 µg/mL for sensitive isolates. Ciprofloxacin inhibited biofilm formation at SSMIC (1 µg/mL) and SMIC (2 µg/mL). Trimethoprim/sulfamethoxazole also showed inhibitory effects, although to a lesser extent than ciprofloxacin. In pre-formed biofilms, ciprofloxacin influenced biofilm integrity at MIC (4 µg/mL), SMIC (2 µg/mL), and SSMIC (1 µg/mL), while trimethoprim/sulfamethoxazole showed variable effects. Both ciprofloxacin and trimethoprim/sulfamethoxazole were capable of inhibiting biofilm formation; however, their efficacy varied. Despite their ability to inhibit initial biofilm formation, ciprofloxacin and trimethoprim/sulfamethoxazole appeared to promote the persistence of already formed UPEC biofilms. Determining the precise concentrations of these antibiotics is essential for effectively managing UTIs caused by UPEC biofilm-forming strains.

1. INTRODUCTION

Urinary tract infections (UTIs) are considered a major global health concern. Uropathogenic *Escherichia coli* (UPEC) are the primary pathogens responsible for many community-acquired and healthcare-associated illnesses [1, 2]. *E. coli*, a member of the Enterobacteriaceae family [3], is the most common cause of urinary tract infections [4]. *E. coli* possesses various virulence factors that contribute to its pathogenicity [5]. Nearly 80% of UTIs are caused by UPEC [6, 7], which can manifest as cystitis, sepsis, and pyelonephritis [8], with a particularly high incidence observed in women [9]. UPEC is considered a significant concern due to the high rates of multidrug resistance (MDR), which make infections difficult to treat [10]. The persistence and recurrence of UPEC infections are largely attributed to biofilm formation, which enables the bacteria to survive for extended periods within the urinary tract and exacerbates the severity of UTIs [11]. A biofilm is defined as a structured community of bacteria that adheres to surfaces within the urinary tract, forming a protective barrier against environmental stressors and antibiotics [12]. The bacterial cells are embedded within the biofilm's extracellular matrix, which makes them resistant to both host immune responses and antibiotics [13]. Biofilms provide a physical barrier that makes biofilm-associated UPEC infections difficult to eliminate using antibiotics. This is primarily due to the limited drug penetration and increased bacterial resistance [12, 14]. Ciprofloxacin, a fluoroquinolone, and trimethoprim/sulfamethoxazole, a folate synthesis inhibitor, are both among the most commonly prescribed antibiotics for UTIs. Ciprofloxacin acts as an inhibitor of bacterial DNA replication by targeting DNA gyrase and topoisomerase IV [15]. Trimethoprim/sulfamethoxazole disrupts folic acid metabolism, which is essential for bacterial

growth [16]. MDR UPEC infections have a high incidence in Iraq, particularly in Baghdad, highlighting the urgent need for solutions to combat antibiotic resistance [17, 18]. Both MIC and SMIC values are critical for evaluating the efficacy of antibiotics against biofilms. Whelan et al. [2] showed that biofilm-producing bacteria require higher antibiotic concentrations than planktonic cells, rendering traditional doses ineffective. Also, study [2] found that the SMIC of ciprofloxacin and trimethoprim can influence biofilm formation in some UPEC strains, and these findings highlighted the necessity of using the appropriate dosage of antibiotics because an inappropriate dose may lead to resistance and chronic infection. In Iraq, the situation is particularly disturbing due to the high prevalence of MDR UPEC. According to studies [19, 20], UPEC strains exhibit resistance to first-line treatments such as ciprofloxacin and trimethoprim/sulfamethoxazole and are capable of forming strong biofilms, complicating treatment outcomes. Therefore, the selection of effective therapeutic agents that prevent UTIs related to UPEC biofilm formers is required. It has become difficult to manage clinical UTIs, and it requires the use of appropriate doses of antibiotics or combinations, but they are sometimes not effective against infections associated with biofilms. This study aimed to evaluate the efficacy of MICs, SMICs, and Sub-SMICs (SSMIC) of ciprofloxacin and trimethoprim/sulfamethoxazole to inhibit biofilm formation and already formed Biofilms by UPEC isolated from Iraqi patients in Baghdad province.

2. METHODOLOGY

2.1 Bacterial isolation and morphological diagnosis

A total of 76 UPEC isolates were collected from patients with urinary tract infections (UTIs) in various healthcare facilities across Baghdad province between December 2023 and February 2024. Patients were males (24) and females (52) at different ages ranging from 15 days to 85 years, and different-hospitalized status (Outpatients and Hospitalized care). Ethical approval for the study was obtained from the Iraqi Ministry of Health's Ethical Review Board. Metadata collected includes the patient's gender and age. The isolates are suspected to be sourced from hospital-acquired and community-acquired UTI cases, according to the opinion of the specialist physician. Primary identification performed, considering morphological parameters and biochemical characteristics, including oxidase, catalase, indole, methyl-red, Voges-Proskauer, and citrate utilization [21]. Further confirmation was carried out with the Vitek®-2 compact system using Gram-negative identification (GNID) cards (BioMérieux, France).

2.2 MIC determination by Vitek-2 compact system

Antibiotic suitability test measured by using the Vitek®-2 compact system technique. Gram-negative antimicrobial susceptibility cards (AST) are intended to be used in the Vitek-2 system for determining susceptibility of aerobic gram-negative bacilli and to determine MICs for each isolate. Vitek®-2 compact system MIC levels depend on CLSI (2023). All *E. coli* isolates were cultured on MacConkey agar and incubated for 24 hours at 37℃. Following incubation, a single colony is taken to form a bacterial suspension with a turbidity of 0.5 cells/ml inside special tubes called Kan tubes. The bacterial suspension was placed inside the filling door of the Vitek®-2 compact system so that the suspension could be transferred from the Kan tubes to the susceptibility test kit.

2.3 MIC determination by the well diffusion method

Four UPEC isolates, K16, G33 (resistance), K42, and G27 (sensitive) used to determine the MICs, SMICs, and SSMICs of Ciprofloxacin and Trimethoprim/Sulfamethoxazole. Ciprofloxacin was diluted in sterilized distal water to make a series of dilutions ranging from (0.625-1024) µg/ml, while Trimethoprim/Sulfamethoxazole concentration ranged from (0.5-2024) µg/ml. By using the well diffusion method as mentioned in study [22], fresh cultures of each isolate were inoculated within 5 ml plastic tubes containing 3 ml of 0.85% normal saline and shaken using vortex. The bacterial suspensions corresponded to 0.5 McFarland (1.5×10^8) CFU/ml, and a cotton swab was flooded at the bottom of each suspension and spread on Muller-Hinton agar. After leaving the plates to dry, a 5 mm sterilized cork borer was used to make four wells in each plate, and 100µL of each antibiotic concentration was transferred to each well. Plates were incubated overnight at 37℃.

2.4 Biofilm formation assay

The Crystal Violate (CV) biofilm assay is considered the gold standard to quantify biofilm formation in UPEC. Kowalska et al. [23] procedure carried out with modification included manipulating in type of media and supplements. Brain Heart Infusion broth (BHI) and Tryptic Soy Broth (TSB) provided by Himedia were used. BHI supplemented with 2% sucrose. TSB supplemented with 1% glucose. Briefly, 200 µl of each fresh bacterial suspension adjusted to 0.5 McFarland (1.5×10^8) cfu/ml was inoculated in each well of a sterilized 96-well flat-bottom microtiter plate. Three-folds from each isolate were made, and three-folds of BHI and TSB were taken as Negative controls. The plates were incubated overnight at 37℃. After incubation, the contents of the plates were emptied and washed with three-fold of 200 µl of 0.98% Phosphate Buffer saline (PBS) and left to dry for 40 minutes to dry at room temperature. The attached cells in each well were stained with 200 µl of 0.1% CV for 10 minutes. The plates were then again emptied of the remaining stain and 200 µl of 95% Ethanol added to each well to solubilize the contents. Uninoculated broth of both BHI and TSB was taken as a Negative control. The optical density (OD) was measured at 620 nm using an ELISA plate reader in the Biotechnology Research Center/Al-Nahrain University.

2.5 Effect of antibiotics on biofilm formation

Four of the UPEC isolates were used to estimate the efficiency of ciprofloxacin and Trimethoprim/Sulfamethoxazole on biofilm formation. The selected isolates were K16 and G33 (resistant), K42 and G27 (sensitive). To dilute ciprofloxacin for the level of MICs, SMICs, and SSMICs, Rafaque et al. [24] proposed that antibiotics be diluted in Mueller-Hinton broth (MHB). Simply, stock solutions of each antibiotic are prepared, and then the concentrations are diluted in MHB. MICs of Ciprofloxacin were 4 µg/ml for both K16 and G33, while it was 8 µg/ml and 1 µg/ml for K42 and G27, respectively. The same previous

dilution was carried out for preparing Trimethoprim/Sulfamethoxazole dilutions. MICs of Trimethoprim/Sulfamethoxazole were 256 µg/ml for both K16 and G33, while it was 64 µg/ml for K42 and G27. Two microtiter plates were used, one for the K16 and G33 (Resistance-Plate), the other for K42 and G27 (Sensitive-Plate). 100µ of K16 and G33 grown MHB added to wells and left to settle. Each isolate was taken as two two-fold replicates. Then, 100µL of each ciprofloxacin concentration (4 µg/ml, 2 µg/ml, and 1 µg/ml) was added to each well containing fresh bacterial isolates. The same procedure was followed in the Resistance plate for Trimethoprim/Sulfamethoxazole at concentrations: 256 µg/ml, 128 µg/ml, and 64 µg/ml. Sensitive plate carried out in the same way using K42 and G27. Ciprofloxacin was (8 µg/ml, 4 µg/ml, and 2 µg/ml) for K42 and (1 µg/ml, 0.5 µg/ml, and 0.125 µg/ml) for G27, while Trimethoprim/Sulfamethoxazole was (64 µg/ml, 32 µg/ml, and 16 µg/ml) for each isolate. All plates incubated overnight at 37℃, emptied and washed with 200µl (two-fold) and stained with 200µl of CV for 10 minutes, followed by 200µl of Ethanol 95% and O.D. measured at 620 nm. Free antibiotics, bacterial broths taken as positive control, and bacteria-free antibiotics dilutions taken as negative control. Each reading was compared to the tested wells analyzed to detect the efficiency of Ciprofloxacin and Trimethoprim/Sulfamethoxazole on biofilm formation by each isolate.

2.6 Effect of antibiotics on UPEC already formed biofilm

The effect of antibiotics ciprofloxacin and Trimethoprim/Sulfamethoxazole was tested on already formed UPEC biofilms. Four isolates of UPEC (K16, G33, K42, and G27) were selected. The same method described in study [23], which aimed to detect the effect of ciprofloxacin on biofilm formation by optimizing some steps (adding antibiotics after biofilm formation, amount of bacteria and antibiotic inoculation, and timing of using PBS and 0.1% CV). Briefly, each isolate was grown in MHB overnight at 37℃. Once again, two 96-well microtiter plates were used, one for resistance isolates and the other for sensitive isolates. 200 µl of each isolate was inoculated into the wells. Six replicates were taken for each isolate. The plates were incubated overnight at 37℃ to allow the isolates to form biofilms. After incubation, the contents of the plates were emptied to remove unattached cells. 100 µl of ciprofloxacin and Trimethoprim/Sulfamethoxazole concentrations were added to each well as two-fold replicates for each concentration. The same concentrations of antibiotic used to affect biofilm formation were used. After adding antibiotic concentration, plates were incubated for 24 hours at 37℃, emptied of unattached content with three-fold of 200µl of PBS, and left to dry for 40 minutes. Then, the plates were stained with 200µl of 0.1% CV for 10 minutes, followed by 200µl of 95% Ethanol. O.D. measured at 620 nm. Free antibiotics, bacterial broths taken as positive control, and bacteria-free antibiotics dilutions taken as negative control. Each reading was compared to the tested wells analyzed to detect the efficiency of Ciprofloxacin and Trimethoprim/Sulfamethoxazole on biofilm formation by each isolate.

2.7 Statistical analyses

The data was tabulated in a datasheet of IBM SPSS version 25.0, which was utilized to do the statistical analysis. The mean and standard errors of continuous variables were reported, and significant differences were tested using the analysis of variance (ANOVA) test, followed by the least significant difference (LSD) test. The Pearson's correlation coefficient was utilized to determine the correlation between different parameters under study. The data were represented as (Mean±S.E.), and statistical significance was defined as a probability value ($p \leq 0.05$) [25].

3. RESULTS

3.1 Bacterial sampling and morphological diagnosis

All of the 76 UPEC isolates were lactose fermenters and produced dry, mucoid colonies on MacConkey agar. Biochemical testing revealed that all were negative for both the Oxidase and Catalase tests. Specifically, no purple discoloration was noticed in the oxidase test after reagent addition, and no bubbles formed in the catalase test, which indicates the absence of hydrogen peroxide breakdown into H_2O and O_2. Most of the isolates tested positive for the indole test, as evidenced by the red indole ring after adding Kovacs reagent, except for four isolates that showed variable results. All isolates tested positive for the Methyl test and negative for the Voges-Proskauer test and citrate utilization test. Based on phenotypic characteristics, it confirmed that all were *E. coli*. Further using Vitek®-2 compact system, which includes 64 biochemical tests, confirmed the diagnosis with accuracy ranging from 93% to 99%. After this additional verification, it was determined that 70 of 76 isolates were indeed *E. coli*. Among these 49 (70%) were females, and 21 (39%) were males.

3.2 Antimicrobial susceptibility test

According to Vitek®-2 compact system, which depends on CLSI (2023), it seems that and as shown in Figure 1, the results of AST of UPEC isolates, showed that Amoxicillin and Ampicillin were resistant by 63 isolates (90%), 7 (10%) were sensitive and no isolates showed moderate resistance. The results also showed that the number of isolates resistant to Amoxicillin/Clavulanic acid was 11 (16%), and the number of sensitive isolates was only seven at a rate of 10%, while the rest of the isolates did not show any response to the antibiotic. As for the Ticarcillin and Piperacillin, the results showed that 59 (84%) were resistant and 7 (10%) were sensitive, and no isolate showed moderate resistance. While the number of isolates resistant to the Piperacillin/Tazobactam was 17 (24%), and 50 (71%) were sensitive, the number of isolates with moderate sensitivity was only two isolates at a rate of 3%. The results showed that the highest percentage of antibiotic resistance was recorded for both Amoxicillin and Ampicillin, as the resistance rate was 90%, and the resistance rate for Amoxicillin/Clavulanic acid, Ticarcillin, and Piperacillin was 16%, 84%, and 84%, respectively. Our results showed that 59 (84%) and 51 (73%) were resistant, while there were 11 (16%) sensitive for Cefazolin and Cefuroxime, respectively, with no evidence of any intermediate sensitive isolates for both antibiotics. Cefoxitin and Cefixime were resistant to 22 (31%) and 7 (10%) isolates, respectively, while there were 45 (64%) and 12 (17%) sensitive to these antibiotics, and only 3 (4%) isolates were moderately sensitive to Cefoxitin. For

Ceftazidime, 43 (61%) showed resistance and 25 (36%) were sensitive, and only 2 (3%) were moderate. Ceftizoxime was effective against 11 (16%) isolates, and it did not show any activity against the rest of the isolates. Ceftriaxone was resistant by 51 (73%), and the remaining isolates were sensitive, while Cefepime was resistant by 26 (37%), and 44 (63%) were sensitive to Cefepime. Carbapenems (Ertapenem, Imipenem, and Meropenem) give the highest percentage of sensitivity. Out of 70 isolates only 6 (9%) were resist to Ertapenem and 54 (77%) were sensitive and 7 (10%) resist Imipenem and 53 (76%) were sensitive, no evidence of resistant or intermediate sensitivity was noticed for Meropenem but it was effective against 53 (76%) of the isolates. Resistance to Amikacin was very decreased, with only 3 (4%) being resistant, while the remaining isolates, 22 (31%) and 45 (64%) were intermediate and sensitive, respectively. For Gentamicin 21 (30%) resist it, 48 (69%) were sensitive, and only one isolate (1%) was intermediate in sensitivity. Out of the tested isolates, it seems that 44 isolates (66%) resist ciprofloxacin and levofloxacin, while 48 (34%) and 24 (33%) were sensitive for these antibiotics, respectively and there was no isolate with intermediate sensitivity observed for ciprofloxacin and only one isolate (1%) for levofloxacin. Regarding Nitrofurantoin, 52 (74%) exhibited sensitivity, while 11 isolates (16%) were resistant and 7 (10%) were moderately sensitive. For the combination of Trimethoprim/Sulfamethoxazole, it seems that 20 isolates (29%) resist it, while 51 (71%) were sensitive, and there was no isolate with intermediate sensitivity.

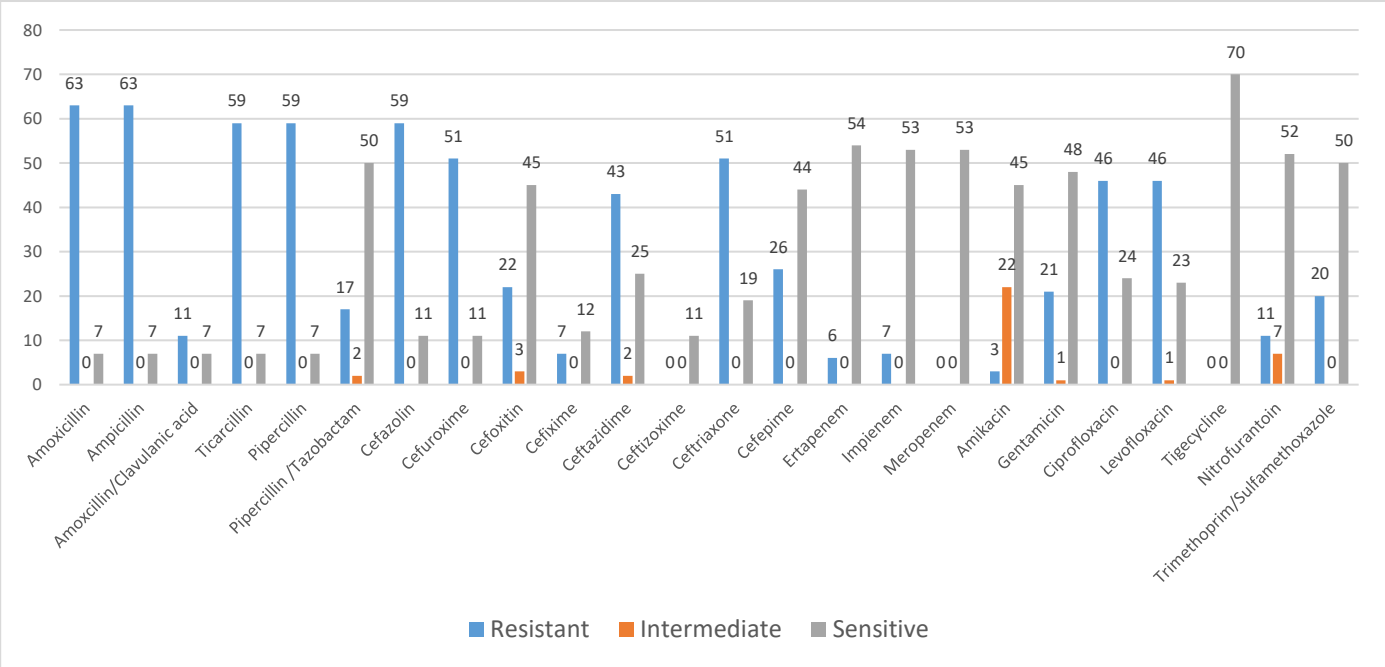

Figure 1. Antimicrobial susceptibility tests profile of UPEC

Table 1. MICs of ciprofloxacin and trimethoprim/ sulfamethoxazole by Vitek-2 compact system and well diffusion method

Isolate	Antibiotic	Vitek®-2 MICs (µg/ml)	Well Diffusion MBCs (µg/ml)	Well Diffusion MICs (µg/ml)	Well Diffusion SMICs (µg/ml)	Well Diffusion SSMICs (µg/ml)
K16	CIP	≥ 4	8	4	2	1
G33		≥ 4	8	4	2	1
K42		≤ 0.25	4	2	1	0.5
G27		≤ 0.25	1	0.5	0.25	0.125
K16	TRM/SMX	≥ 320	512	256	128	64
G33		≥ 320	512	256	128	64
K42		≤ 20	128	64	32	16
G27		≤ 20	128	64	32	16

*Ciprofloxacin (CIP), Trimethoprim/Sulfamethoxazole (TRM/SMX)

3.3 MICs of ciprofloxacin and trimethoprim/sulfamethoxazole

Using Vitek®-2 compact system with AST cards, the MICs of ciprofloxacin and Trimethoprim/Sulfamethoxazole were determined for each UPEC isolate in this study. Four isolates were selected to compare MICs obtained by Vitek®-2 compact system with those from the well diffusion method. The results of Vitek®-2 compact system showed that MICs of ciprofloxacin measured by Vitek®-2 compact system were≥ 4 µg/ml for both K16 and G33, ≤ 0.25 µg/ml for K42 and G27. For Trimethoprim/Sulfamethoxazole, the Vitek®-2 compact system revealed that MICs of ≤ 20 µg/ml for G27 and K42 and ≤ 320 for K16 and G33. According to the well diffusion method used to determine MICs, SMICs, and SSMIC of ciprofloxacin and Trimethoprim/Sulfamethoxazole, revealed that the MIC of ciprofloxacin was 4 µg/ml for K16 and G33. While the SMIC of ciprofloxacin for K16 and G33 was 2 µg/ml and the SSMIC was 1 µg/ml. MIC of ciprofloxacin for K42 was 2 µg/ml and 0.5 µg/ml for G27. The SMIC and

SSMIC for K42 were 1 µg/ml and 0.5 µg/ml, respectively, and 0.25 µg/ml and 0.125 µg/ml for G27. For Trimethoprim/Sulfamethoxazole, well diffusion method results revealed that, MIC of Trimethoprim/Sulfamethoxazole was 256 µg/ml for K16 and G33, while it was 64 µg/ml for K42 and G27. The SMIC of Trimethoprim/Sulfamethoxazole was 128 µg/ml for K16 and G33, while the SSMIC was 64 µg/ml. The SMIC of Trimethoprim/Sulfamethoxazole was 32 µg/ml for K42 and G27, while the SSMIC was 16 µg/ml, as listed in Table 1.

3.4 Ability of biofilm formation by UPEC

This study investigates the ability of all UPEC isolates to form biofilm using a 96-well microtiter plate, assessing their growth in different broths to determine their capacity for biofilm formation. In this experimental approach, each isolate was cultured in a 96-well plate with three replicates to ensure the accuracy and consistency in results. By using an ELISA plate reader at 620 nm, the OD. was measured to achieve quantification of biofilm formation. The results listed in Tables 2 and 3 demonstrated that all of the tested UPEC isolates were capable of biofilm formation in TSB 1% glucose and BHI 2% sucrose. As a baseline, the control group showed optical density values of 0.160 in TSB 1% glucose and 0.300 in BHI 2% sucrose. After comparing these baseline measurements with those obtained from each isolate, it was observed a remarkable elevation in the optical density values in which was consistently high. For the UPEC isolate freshly cultured in TSB 1% glucose, the OD. values ranged from 0.182 to 0.458. This range indicates that all UPECs have the ability to form biofilm, but the extent of this was variable. The OD. measurements for UPEC freshly cultured in BHI 2% sucrose were somewhat high, ranging from 0.376 to 1.124.

The results listed in Table 4 showed that, all *E. coli* isolates were able to form biofilm when using TSB 1% glucose, as 37 (52.85)% were weak formers, as the optical density ranged between 0.182-0.319, and 33 (47.15)% were moderate formers of biofilms, as the optical density ranged between 0.312-0.458 after comparing them with the control optical density 0.160. In addition, all of the tested isolates were able to form biofilm using the BHI 2% sucrose. It was found that 38 (54.28)% were weak formers, as the optical density rate ranged between 0.375-0.597, and 32 (45.71)% were moderate formers, as the optical density rate ranged between 0.605-1.124 after comparing it with the control density rate of 0.300, as shown in the Table 4.

3.5 Effect of antibiotics on UPEC Biofilm formation

Ciprofloxacin and Trimethoprim/sulfamethoxazole on biofilm formation were evaluated using a 96-well microtiter plate. This was conducted after demonstration of MICs and SMICs through the previous experiments. As mentioned previously, four isolates were selected; among these isolates, K16 and G33 were resistant to all tested antibiotics, while the other two remaining isolates, K42 and G27, were sensitive. The concentrations 1 and 2 µg/ml of ciprofloxacin and 64 and 128 µg/ml of Trimethoprim/Sulfamethoxazole were applied on K16 and G33 isolates. While ciprofloxacin was tested at 2 and 4 µg/ml and Trimethoprim/Sulfamethoxazole at 16 and 32 µg/ml for the K42 isolate. G27 isolate was tested at 0.25 and 0.5 µg/ml for ciprofloxacin and 16 and 32 µg/ml for Trimethoprim/Sulfamethoxazole.

Table 2. Optical density values of UPEC strains in TSB 1% glucose

Isolate	O.D	Isolate	O.D	Isolate	O.D	Isolate	O.D	Isolate	O.D	Isolate	O.D	Isolate	O.D	Control
K3	0.252	K33	0.275	K54	0.279	K68	0.322	K105	0.339	K125	0.353	G23	0.242	0.160
K7	0.284	K34	0.325	K55	0.276	K79	0.329	K106	0.341	K129	0.277	G26	0.341	
K8	0.182	K35	0.282	K58	0.328	K82	0.458	K108	0.422	G2	0.276	G27	0.267	
K16	0.258	K42	0.241	K59	0.233	K83	0.374	K113	0.342	G3	0.345	G28	0.205	
K18	0.332	K44	0.263	K60	0.299	K86	0.311	K115	0.377	G5	0.31	G29	0.431	
K19	0.217	K45	0.331	K61	0.317	K87	0.370	K117	0.379	G6	0.259	G33	0.310	
K20	0.238	K46	0.375	K62	0.358	K89	0.393	K118	0.371	G10	0.391	G34	0.209	
K24	0.321	K47	0.276	K64	0.319	K96	0.366	K120	0.386	G14	0.241	G35	0.193	
K26	0.239	K49	0.285	K65	0.394	K100	0.393	K121	0.272	G16	0.269	G36	0.355	
K28	0.223	K53	0.314	K67	0.264	K102	0.368	K122	0.261	G22	0.356	A6	0.365	

Table 3. Optical density values of UPEC strains in BHI 2% sucrose

Isolate	O.D	Isolate	O.D	Isolate	O.D	Isolate	O.D	Isolate	O.D	Isolate	O.D	Isolate	O.D	Control
K3	0.84	K33	0.559	K54	0.739	K68	0.547	K105	0.595	K125	0.488	G23	0.612	0.300
K7	0.565	K34	0.564	K55	0.597	K79	0.620	K106	0.712	K129	1.124	G26	0.445	
K8	0.76	K35	0.657	K58	0.605	K82	0.533	K108	0.687	G2	0.665	G27	0.411	
K16	0.491	K42	0.782	K59	0.505	K83	0.543	K113	0.566	G3	0.487	G28	0.673	
K18	0.775	K44	0.535	K60	0.48	K86	0.557	K115	0.621	G5	0.386	G29	0.631	
K19	0.619	K45	0.493	K61	0.483	K87	0.703	K117	0.582	G6	0.73	G33	0.480	
K20	0.627	K46	0.825	K62	0.513	K89	0.572	K118	0.663	G10	0.663	G34	0.423	
K24	0.766	K47	0.76	K64	0.502	K96	0.661	K120	0.753	G14	0.449	G35	0.594	
K26	0.653	K49	0.756	K65	0.484	K100	0.570	K121	0.571	G16	0.375	G36	0.548	
K28	0.813	K53	0.511	K67	0.5663	K102	0.720	K122	0.576	G22	0.734	A6	0.452	

Table 4. Degree of biofilm formation by *E. coli* isolates isolated from urinary tract infections

Type of Culture Media	Number of Isolates	Average of Optical Density (OD nm) of Biofilm		
		Weak	Moderate	Strong
TSB 1% Glucose	70	37 (52.85)%	33 (47.15)%	-
BHI 2% Sucrose	70	38 (54.28)%	32 (45.71)%	-

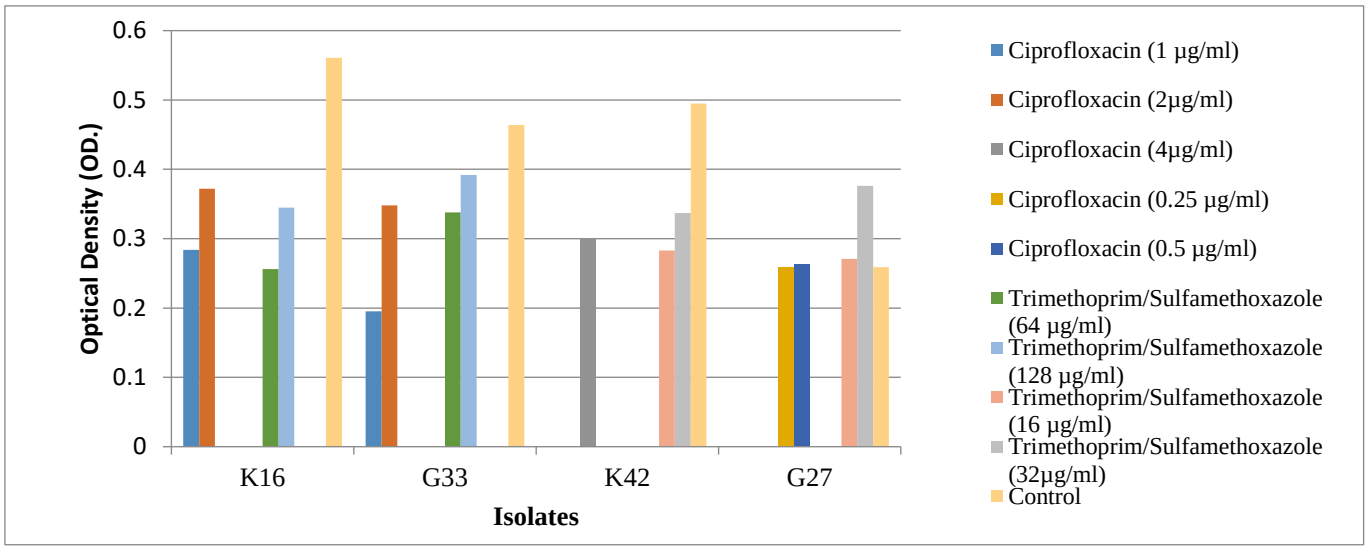

Figure 2. Effect of antibiotics before the biofilm formation of UPEC

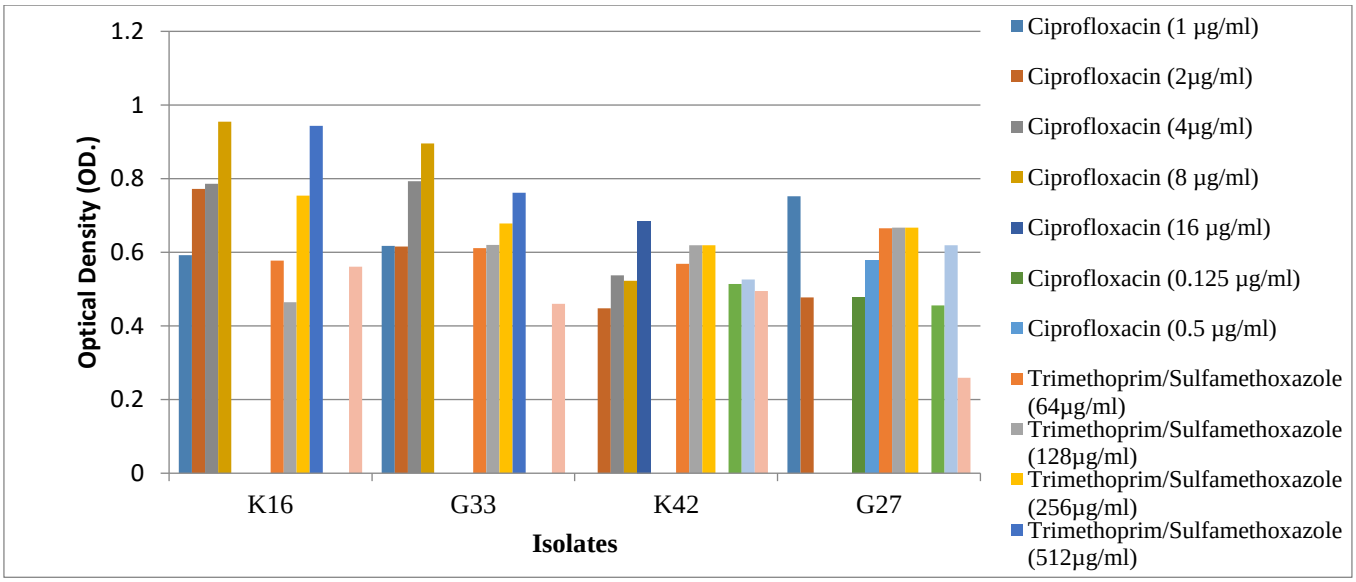

Figure 3. Effect of antibiotics after biofilm formation of UPEC

As shown in Figure 2, the results showed that ciprofloxacin has the ability to inhibit the biofilm formation of K16 and G33 at concentrations of 1 and 2 µg/ml, as a gradual decline in the optical density rates was observed at a series of different concentrations. The optical densities at concentrations of 1 and 2 micrograms/ml were 0.284 and 0.372 for K16, compared to the control group, which was 0.561. As for isolate G33, the optical densities were 0.195 and 0.348, compared to the control group (0.464). Additionally, Trimethoprim/Sulfamethoxazole had the ability to inhibit biofilm formation for K16 and G33 at all concentrations, as shown in Figure 2, as a gradual decrease in the rates of optical density was observed as the concentration of the antibiotic decreased. The optical density at the concentration of 64 and 128 µg/ml was 0.256 and 0.345 for K16 and 0.338 and 0.392 for the G33, compared to the control group, which reached 0.561 and 0.464, respectively. It was found that the concentrations SMIC and SSMIC led to inhibition of biofilm formation through a decrease in the rates of optical density. After evaluating the ability of Ciprofloxacin to inhibit biofilm formation of the sensitive isolates K42 and G27 at concentrations of 2, 4, and 0.25, 0.5 µg/ml, respectively. The results shown in Figure 2 showed that the optical density was (0.300, 0.300) for K42 compared to the control group (0.495), while the optical density was 0.259, 0.263 for G27 compared to the control group (0.259). In addition, Trimethoprim/Sulfamethoxazole was tested on K42 and G27 to determine its impact on inhibiting biofilm at a concentration of 16 and 32 µg/ml. The optical densities were 0.283 and 0.337 for K42 compared to the control group (0.495), and were 0.271 and 0.376 for G27 compared to the control group (0.259) as shown in Figure 2. It was found that all concentrations of Ciprofloxacin and Trimethoprim/Sulfamethoxazole tested on the sensitive isolate K42 showed an inhibitory effect on biofilm formation represented by a significant decrease in optical density, while all concentrations were not effective to inhibit biofilm formation of the sensitive isolate G27, but on the contrary, they showed an activating effect of biofilm formation.

3.6 Effect of antibiotics on already formed biofilms of UPEC

Using a 96-well microtiter plate, an average of optical

densities measured using ELISA at 620 nm was used to test the effect of Ciprofloxacin and Trimethoprim/Sulfamethoxazole on already formed biofilms of *E. coli.* Minimum Bactericidal Concentration (MBC), Minimum Inhibitory Concentration (MIC), Sub-Minimum Inhibitory Concentration (SMIC), and Sub-Sub Minimum Inhibitory Concentration (SSMIC) were tested. Four isolates were selected, two of which (K16, G33) were resistant to all antibiotics and two (K42, G27) were sensitive to all antibiotics. The Concentrations 1, 2, 4 and 8 µg/ml of Ciprofloxacin and 64, 128, 256 and 512 µg/ml of Trimethoprim/Sulfamethoxazole were tested on isolates K16 and G33, while concentrations of Ciprofloxacin were 2, 4, 8 and 16 µg/ml and 16, 32, 64 and 128 µg/ml of Trimethoprim/Sulfamethoxazole tested for K42. As for G27, the effect of Ciprofloxacin was tested at 0.25, 0.5, 1, and 2 µg/ml, while the Trimethoprim/Sulfamethoxazole concentration was 16, 32, 64, and 128 µg/ml. The results revealed after testing the effect of Ciprofloxacin on the already formed biofilms of UPEC, that Ciprofloxacin has an activating effect on the already formed biofilms of the resistance isolates K16 and G33. The results showed that the averages of optical density at a concentration of 1, 2, 4 and 8 µg/ml were 0.592, 0.772, 0.786, and 0.955 for K16 and were 0.617, 616, 0.793, and 0.896 for G33 compared to the control group (0.561, 0.464) respectively as listed in Figure 3. Also, all concentrations of Trimethoprim/Sulfamethoxazole had the ability to activate the already formed biofilms of the two UPEC isolates K16 and G33 at the concentrations of 64, 128, 256, and 512 µg/ml. The averages of optical density were 0.577, 0.646, 0.754, 0.943, respectively, for K16, and were 0.611, 0.620, 0.678, 0.762, respectively, for G33 as presented in Figure 3. After testing the ability of the antibiotic Ciprofloxacin to inhibit the already formed biofilms of the sensitive isolate K42, as shown in Figure 3, it was found that Ciprofloxacin showed an effect similar to its effect on the resistant isolates. As its effect was somewhat stimulating for the biofilm at 2, 4, 8, and 16 µg /ml, as the optical density averages were 0.448, 0.537, 0.523, 0.685, respectively, for K42 compared to the control group, which was 0.495. Trimethoprim/Sulfamethoxazole, at concentrations 16, 32, 64, and 128 µg/ml, the optical density averages were (0.514, 0.526, 0.569, 0.619) respectively for K42 and for four concentrations compared to the control group (0.495). At concentrations 0.25, 0.5, 1, and 2 µg/ml, the isolate G27 showed averages of optical density 0.447, 0.578, 0.752, and 0.755, respectively, for the four concentrations compared to the control group of 0.259. While Trimethoprim/Sulfamethoxazole at 16, 32, 64, and 128 µg/ml, the averages of optical density were 0.456, 0.619, 0.665, and 0.667, respectively, and for the four concentrations compared to the control group of 0.259, as shown in Figure 3.

4. DISCUSSION

According to our result, all UPEC were lactose fermenters, these isolates grew a dry to mucoid colonies on MacConkey agar. As for biochemical tests, the oxidase test was negative (no purple discoloration) and the catalase test positive (no formation of bubbles), both of which are criteria for the *E. coli* profile. Most UPEC isolates tested positive in the indole test, which is a classical characteristic of *E. coli*, which produces indole from tryptophan. Methyl red tested positive, while Voges-Proskauer tested negative. Typically, *E. coli* produces a mixture of acids during fermentation, leading to a positive methyl red result, while *E. coli* lac the ability to produce acetone, which results in a negative result in the Voges-Proskauer test as mentioned in study [21]. Vitek®- 2 compact system provides a high accuracy confirmation ranging between 93% - 99%. This system uses biochemical tests a vast array for the purpose of bacterial identification, and its accuracy supports rigor in *E. coli* identification. From the 76 isolates, it seems that only 70 were confirmed to be *E. coli*. The gender distribution in our study was 49 (70%) females and 21 (30%) males. This deviation to females agrees well with the epidemiology of Uropathogenic infections, which are most commonly seen in females. Al-Awkally et al. [26] demonstrate that females are more susceptible than men to urinary tract infection, which leads to many differences, like anatomical, physiological, hormonal, and the short urethra of females. AST performed by Vitek®-2 compact system provides us with valuable insights about the antibiotic resistance profiles of UPEC tested isolates. Our findings show that, highly significant resistance to Amoxicillin and Ampicillin. The high resistance reflects the truth of wildly spread of UPEC capable of producing β-lactamase, which hydrolyzes the β-lactam ring and makes these antibiotics ineffective. The absence of intermediate resistance within UPEC isolates is attributed to the complete efficacy of resistance mechanisms or full susceptibility either by Extended Spectrum β-lactamases (ESBLs) or variants of β-lactamase [2]. Low resistance demonstrated for Amoxicillin/Clavulanic acid. This indicates that the addition of an inhibitor of β-lactamase, such as Clavulanic acid, restores the efficacy [27]. High resistance rates were observed for each Ticarcillin and Piperacillin compared to Piperacillin/Tazobactam. The activity of Tazobactam, a β-lactamase inhibitor, underscores its effectiveness among UPEC isolates. Tazobactam was able to neutralize a wide range of β-lactamases, especially within infections caused by organisms that produce ESBLs [28]. For Cephalosporin, Cefazolin and Cefuroxime show a high resistance among UPEC isolates, while low resistance is noted for Cefoxitin and Cefixime. The variability in resistance rates is attributed to different resistance mechanisms that may be used by the UPEC, and these mechanisms may be due to β-lactamases, mutation in the target site, or efflux pumps [2, 29, 30]. High sensitivity observed for Carbapenems. All of Ertapenem, Imipenem, and Meropenem revealed high efficacy against UPEC isolates. Carbapenems are considered as the best option to treat ESBL UPEC [31], still, Carbapenems are considered the last choice for treating MDR UPEC infections because resistance to them is relatively rare [10]. The absence of Meropenem resistance suggests that it is considered a potent choice for infections caused by MDR UPEC. Very reduced resistance was noticed for Amikacin and Gentamicin. The reduced resistance reflects the utility of using aminoglycosides as treatment for UPEC infection. Aminoglycosides are effective against infections caused by gram-negative bacteria, and as a result of Amikacin's low resistance, it's still a viable option for the severe cases of MDR UPEC [32]. Resistance for ciprofloxacin and levofloxacin as fluoroquinolones was high. These came in line with the global trends in resistance of UPEC. This resistance is due to mutations in quinolone resistance-determining regions present in DNA gyrase and Topoisomerase IV [33, 34]. The absence of moderate sensitivity among UPEC isolates may reflect complete resistance or susceptibility. Nitrofurantoin exhibited low

resistance by UPEC isolates. Nitrofurantoin effectiveness should be considered as the first line of choice for uncomplicated UTIs because it targets many pathways within bacterial cells after breaking down to its metabolites. These metabolites start to act as bacteriostatic agents by binding to bacterial ribosomes, leading to inhibition of protein synthesis as well as the citric cycle, and finally prevent DNA and RNA synthesis, thus making the resistance development very low [2, 35, 36].

The combination of Trimethoprim/Sulfamethoxazole shows moderate resistance by UPEC isolates, and vast majority were sensitive. The resistance to this combination is linked to alterations in folic acid metabolism pathways [37]. All UPEC isolates were capable of biofilm formation. Biofilm formation was observed regardless of the media supplementation with sucrose or glucose, which both enhance the ability to form biofilm. Although the degree of biofilm formation was variable, it indicates the formation of it. According to studies [38, 39], *E. coli* isolated from urine samples are able to form biofilm at different rates. Also, study [40] indicated that 96.7% of the isolates were able to form biofilms, and 50% of them were moderate, while 46.7% of them were strong in forming biofilms. Tajbakhsh et al. [41] indicated that all *E. coli* isolates isolated from urinary tract infections were 100% biofilm formers, while the study [42] indicated that the biofilm production rate of multidrug resistance isolates was 80%, 29% of which were strong, 34% moderate, 17% weak, and 20% non-biofilm-forming. Also, study [43] indicated that 87.8% of *E. coli* isolated from patients of urinary tract infections from Ramadi city. The study [44], which was conducted to determine the effect of adding sugars on the process of biofilm formation showed that the process of adding both glucose at a concentration of 10% and sucrose at a concentration of 15% gave the same result, which is the formation of biofilm, as no differences were observed in the intensity of biofilm formation after adding sugars at different concentrations. The study [23], which was conducted to detect the ability of *E. coli* to form biofilm using brain heart infusion, supplemented with 2% sucrose, and Tryptone soy broth supplemented with 2% sucrose, showed that all bacterial isolates formed biofilm. Variability in biofilm formation was documented [45], that UPEC is able to form biofilm and is influenced by many genetic and environmental factors such as availability of nutrients and cell-to-cell communications, thus leading to differences in capacities of biofilm formation [46, 47]. In contrast, the use of sucrose as a supplement leads to the enhancement of biofilm formation. Enhancement of biofilm formation by sucrose is attributed to the use of sucrose as a carbon source. Using sucrose influences the production of extracellular polymeric substance, which is considered part of the biofilm matrix [48-50]. In another study, Katongole et al. [51] highlighted sucrose impact on UPEC biofilm formation and suggested that sucrose improves adherence and aggregation of bacterial cells, which makes the biofilm more robust. The use of different media leads to demonstrating that biofilm formation can be varied in its degree according to growth conditions and bacterial isolates, which reflects limitations. Biofilm formation can be varied, and it's influenced by many factors such as bacterial strain and growth conditions, and this variation reflects limitations in biofilm formation under optimum and suboptimum conditions [52-54]. In addition, biofilm formation could be affected by many factors such as availability of nutrients, temperature, and pH [49, 55]. Differences in the values of the series of inhibitory concentrations for bacterial growth after determining them using the Vitek®-2 compact system and comparing them with the well diffusion method. As a difference was observed in the minimum inhibitory concentrations for the growth of *E. coli* using ciprofloxacin and Trimethoprim/Sulfamethoxazole, except for isolates K16 and G33, the minimum inhibitory concentration for bacterial growth was the same using Vitek®-2 system and well diffusion method, as the difference is due to the measurement method. This is what was indicated by study [56], as the minimum inhibitory concentration using the Broth Culture method differs from the minimum inhibitory concentration using the Disc diffusion method, and these differences are due to the difference in the state of bacteria in being planktonic cells and biofilm-forming cells. The cells in the biofilm state showed higher resistance to antibiotics compared to their state in planktonic cells. As for the antibiotic Trimethoprim/Sulfamethoxazole, no difference was observed in the minimum inhibitory concentrations for bacterial growth.

The results of the current study provide a valuable vision on the efficacy of ciprofloxacin and trimethoprim/sulfamethoxazole against biofilm formation of variant isolates (antibiotic-resistant and antibiotic-sensitive) UPEC. Our findings reveal that there is complexities about inhibition of biofilm formation and how it varied according to antibiotic type, concentration type of bacterial isolate it was either resistant or sensitive and isolate source, which may be isolated from an adult or a child, as well as the variance may be attributed to the difference in the gender of the affected person, whether male or female, as it was noted that the effect was inhibitory through observing the decrease in light density rates. For the resistance isolates, ciprofloxacin demonstrated an effective activity to inhibit biofilm at low concentrations (SSMIC and SMIC) with significantly low optical density, despite their resistance to ciprofloxacin. These findings suggest that at higher concentrations of ciprofloxacin, it might lose its activity in inhibiting biofilm formation, and this may be due to stress responses of bacterial cells to survive. Ciprofloxacin on the sensitive isolates had robust activity. At the SSMIC and SIMC levels, the biofilm formation of K42 was significantly inhibited, while for G27, the SSMIC and SMIC were not effective in inhibiting biofilm formation, but rather stimulated it. These findings suggest that, although the G27 isolate is classified as sensitive according to MIC tests but it responds differently to ciprofloxacin, which this possibly due to strain-specific factors that enhance biofilm formation as a response to the antibiotic. Trimethoprim/Sulfamethoxazole was also effective in inhibiting biofilm formation of K42, with significantly reduced optical density. This activity mirrors ciprofloxacin activity. In contrast, its influence on biofilm formation of the G27 isolate, and this finding highlights the response to antibiotics by this isolate. The results of testing the effect of the antibiotic Ciprofloxacin and Trimethoprim/Sulfamethoxazole on the process of biofilm formation by antibiotic-sensitive and antibiotic-resistant isolates showed that the effect of the antibiotic varies depending on the source of the isolation, which may be isolated from an adult or a child. In addition, the variation may be attributed to the difference in the sex of the infected person, whether male or female, as it was noted that the effect was inhibitory, through observing the decrease in optical density rates. The study by Wojnicz and Tichaczek-Goska [57] aimed to determine the effect of sub-inhibitory concentrations (sub-MICs) of Ciprofloxacin, Amikacin, and Colistin on biofilm

formation, motility, and villus formation of *E. coli* isolated from the urine of patients with various urinary tract infections. The results of the study indicated that all antibiotics used at sub-inhibitory concentrations reduced biofilm formation and decreased bacterial survival. This study suggests that Ciprofloxacin, Amikacin, and Colistin may be useful in the treatment of biofilm-associated infections caused by *E. coli* strains, as indicated by the study [58]. The possibility of inhibiting the biofilm of *E. coli* bacteria using Ampicilin, Cephalothin, Ceftriaxone, Ceftazidime, Amikacin, and Ciprofloxacin antibiotics at different concentrations after 48 hours of biofilm formation, as the study showed the ability of all antibiotics to inhibit the biofilm of *E. coli* bacteria except for the antibiotic Ampicilin, as these antibiotics work to reduce the mass of biofilms. According to study [59], the process of biofilm formation can be inhibited when using ¼ of the minimum inhibitory concentration (MIC) of the antibiotic Ciprofloxacin, as their study indicated that the antibiotic has an effect on the fim gene in addition to its effect on the gene locus pgaABC, as this site is an active part in the process of polysaccharide synthesis [60], while the fim gene encodes for the fimbriae type 1, which plays an essential role in the process of bacterial cell adhesion [51].

The results of study [61] indicate that the use of high concentrations of the antibiotics Trimethoprim and Nitrofurantoin did not show any inhibitory effect on the biofilm formation process, while the antibiotic Ciprofloxacin showed an inhibitory effect on the formation of biofilms. The study attributed the inhibitory effect of the antibiotic Ciprofloxacin to the fact that it is responsible for inhibiting the replication of DNA. Rafaque et al. [24] indicated that the low concentration of Ciprofloxacin, lower than the minimum inhibitory concentration (SMIC), which is added or mediates the process of biofilm formation, enhance the formation of biofilms of *E. coli* isolated from cases of urinary tract infection, as five out of 6 isolates were stimulated to form biofilms at the level of the minimum inhibitory concentration (MIC) of the Ciprofloxacin. A recent study by Whelan et al. [2] refers to that biofilm formation can be inhibited by Ciprofloxacin at SMIC concentration, even if the bacterial strains are resistant, most likely due to the interference of biofilm-related pathways and the quorum-sensing system. The study also indicated that Trimethoprim/Sulfamethoxazole is known to inhibit folic acid synthesis and stop bacterial proliferation at low concentrations; however, it is similar to Ciprofloxacin in inhibiting biofilm formation but can also promote biofilm formation at higher concentrations. The effect of antibiotics on biofilm formation was variable, which may be due to the variation in the isolate strains that were resistant and susceptible and were isolated from males and females and at different age groups, as was the case in the study [2] which showed that the minimum inhibitory concentration (MIC) was effective in inhibiting *E. coli* biofilm. However, as shown in the results of the current study, it appears that the higher the concentration of the antibiotic Ciprofloxacin, the less effective it is in inhibiting biofilms. This decrease in effectiveness is attributed to the stress of bacterial cells in order to form biofilms as a survival mechanism. In addition, the effect of the minimum inhibitory concentration and sub-minimum inhibitory concentration on inhibiting biofilm formation for some isolates and activating biofilm for some isolates. There was no difference in the effect on biofilm formation between sensitive and resistant isolates, as indicated by study [24], while studies [62-64] indicated that bacterial cells communicate with each other using quorum sensing systems, which are responsible for regulating group behaviour such as biofilm formation. Sometimes, the concentration of the antibiotic at a concentration level below the SMIC may interfere with quorum sensing, leading to the promotion of biofilms rather than their inhibition. Several studies [46, 63, 65, 66] have indicated that the extracellular matrix of biofilms acts as a physical barrier, preventing the penetration of antibiotics. As a result, bacterial cells embedded within the matrix, especially in the deeper layers, are only exposed to non-lethal doses of antibiotics, which promote and maintain biofilm formation.

The results of testing Ciprofloxacin and Trimethoprim/Sulfamethoxazole on already formed biofilms by *E. coli* show alarming results. The results showed that the antibiotics at the MBC, MIC, SMIC, and SSMIC concentrations, instead of inhibiting biofilms, work to enhance biofilms, as an increase in optical density rates was observed at all concentrations. Biofilms contain multiple and different alternatives or forms of bacterial cells, which cannot be reached by antibiotics and are known as persistent cells. Persistent cells are characterized by being metabolically inactive, and they can survive for a long time because they are protected and cannot be reached by antibiotics, and thus work to renew biofilms after the antibiotic activity has disappeared [46, 63, 66]. We note from the results of the experiment that ciprofloxacin and Trimethoprim/Sulfamethoxazole at concentrations lower than the minimum inhibitory concentrations led to the inhibition of biofilm formation. This is due to its effect on the suspended cells, while it led to the activation of the biofilm formed by not affecting the cells due to their surrounding materials that prevent the access of antibiotics. The studies [67, 68] confirm that *E. coli* resistant to the antibiotic Ciprofloxacin shows significant differences in their behavior from bacteria sensitive to the antibiotic in the case of planktonic cells and biofilm cells when exposed to the antibiotic Ciprofloxacin. Planktonic cells, which are metabolically active and free-floating, show higher sensitivity to the antibiotic Ciprofloxacin because the antibiotic targets the enzymes DNA gyrase and topoisomerase IV responsible for the process of DNA replication in bacterial cells, which leads to effective killing of bacteria at lower concentrations. In contrast, *E. coli* cells forming biofilms are significantly less susceptible to the antibiotic Ciprofloxacin, as the extracellular polymeric substance (EPS) matrix surrounding the biofilm cells acts as a barrier, which hinders the penetration of antibiotics. In addition, biofilm cells are in a state of inactivity or slow growth, which reduces the effectiveness of the antibiotic Ciprofloxacin, which targets active cellular processes. The study also indicates that concentrations below the minimum inhibitory concentration promote the survival of biofilms and allow for genetic exchange, such as horizontal gene transfer, which enables the spread of resistance. This is what indicated by study [69] that the antibiotic Ciprofloxacin appears to be effective in killing planktonic cells, which effectively reduces their survival over time, while its effect on biofilm cells shows that these cells appear the opposite of what planktonic cells show, as they have a higher ability to tolerate the antibiotic Ciprofloxacin. The study [70] also indicated that the acquisition of resistance in planktonic cells occurs at a slower rate, and thus, strains appear that are inhibited at lower concentrations compared to biofilm cells, because they need long periods and high concentrations of the antibiotic Ciprofloxacin to show the resistance trait. As for biofilm cells, they showed rapid development of resistance to the antibiotic

Ciprofloxacin when exposed for long periods and to high concentrations of the antibiotic Ciprofloxacin, in addition to their need for higher minimum inhibitory concentrations compared to planktonic cells. This rapid development of resistance is attributed to the protective environment of biofilms, which facilitates the occurrence of genetic mutations and horizontal gene transfer, enhancing the ability of bacteria to withstand the pressure of antibiotics. As for the antibiotic Trimethoprim/Sulfamethoxazole, the study [71], which indicated that the antibiotic Trimethoprim/Sulfamethoxazole is effective against planktonic cells, effectively inhibiting their growth, while its effectiveness appears less in biofilm cells, which was also indicated by the study [72], that it is effective in inhibiting the growth of planktonic cells and biofilm cells at a concentration of 50 µg/ml. In the study of [73], the antibiotic Trimethoprim/Sulfamethoxazole was effective in inhibiting planktonic cells, but its effect decreased in the case of biofilm cells. The effectiveness of ciprofloxacin and trimethoprim/sulfamethoxazole depends on many factors such as timing, dosage, and adherence to the prescribed treatment strategy. Ciprofloxacin and trimethoprim/sulfamethoxazole should be administered during active infection to eliminate biofilm formation, due to these biofilms provide protection to these bacterial cells against antibiotics, forming a barrier similar to immune response to these therapeutic agents, which eventually makes the infection harder to heal. If these antibiotics (ciprofloxacin and trimethoprim/sulfamethoxazole) are used incorrectly, such as at their suboptimal doses or during insufficient durations, bacterial cells easily form biofilm, reducing drug penetration and leading to persistence of infection. Therefore, restricting adherence to the prescribed antibiotic protocol is currently essential to prevent biofilm-associated resistance.

5. CONCLUSIONS

All of the tested UPEC isolates exhibited 100% biofilm formation, with weak to moderate capacity. Ciprofloxacin at SMIC (2 and 4µg/ml) and Trimethoprim/Sulfamethoxazole at (32 and 128µg/ml) were capable of inhibiting biofilm formation of MDR UPEC, while they were unable to break down the already formed biofilms. Particularly, the resistance isolates were more sensitive to their effect. However, the efficacy of these antibiotics was variable and depended on the type of isolate, whether it was resistant or sensitive, and the concentration. According to these results, the importance of determining the precise concentration of antibiotic to manage UTIs associated with biofilm formation, which is caused by UPEC. Further studies should be performed to optimize the antibiotic doses for UTIs associated with biofilm formation.

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