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Detection of biofilm among uropathogenic *Escherichia coli* clinical isolates in Suez Canal University Hospitals

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ABSTRACT

Background: *Escherichia coli* (*E. coli*) is a Gram-negative bacterium that is facultative anaerobic in nature. *E. coli* strains causing urinary tract infection (UTI) are called uropathogenic *E. coli* (UPEC). UPEC makes up to 70-95% of UTIs. UTI is a serious health problem concerning antibiotic resistance and biofilms formation. Biofilm can restrict the diffusion of antimicrobials. **Aim:** To detect *E. coli* biofilm producers to improve prognosis and treatment and reduce morbidity and mortality rates due to UTI. **Methods:** Forty-seven clinical isolates of non-duplicated UPEC were collected from clinically suspected cases of UTI in Suez Canal University Hospitals (SCUHs). Isolates were identified as *E. coli* by colony morphology, Gram staining and biochemical reactions. Antimicrobial susceptibility testing was performed by Kirby–Bauer disc diffusion method on Muller–Hinton agar plate. The detection of biofilm was carried out by Congo red agar (CRA) method and modified tissue culture plate (MTCP) method. **Results:** The CRA method detected 63.8% of *E. coli* isolates as biofilm producers while the MTCP method detected 40.4% of them as biofilm producers. The sensitivity, specificity and accuracy of CRA method in comparison to MTCP method were 100%, 60.7% and 76.6% respectively. All biofilm producers and non-producers were resistant to amoxicillin-clavulanate and ceftazidime. The maximum sensitivity was seen for imipenem (91.49 %), followed by nitrofurantoin (78.72%) and then gentamicin (63.83%). Biofilm producers showed significant resistance (73.68%) to norfloxacin when compared to nonbiofilm producers (25%) ($P=0.003$). **Conclusion:** The prevalence of biofilm production among UPEC in SCUHs is 40.4%. Imipenem is the drug of choice for treating biofilm producing UPEC.

Introduction

Urinary tract infections (UTIs) are one of the most common bacterial infections in humans. At least one symptomatic UTI episode is thought to occur in 40% of women and 12% of men over the course of their lives, and 27 to 48% of the affected

women experience recurrent UTIs. UTIs are about 40% of all hospital-acquired infections and 50% of bacterial infections that increase the risk of morbidity and prolonged hospitalization [1].

Most UTIs are caused by *Escherichia coli* (*E. coli*) bacteria, which is a Gram-negative

bacterium. It is a facultative anaerobic non sporulating rod-shaped bacterium [2].

E. coli strains that cause UTI are termed uropathogenic *E. coli* (UPEC). UPEC is a heterogeneous group of extraintestinal pathogenic *E. coli* (ExPEC) that are able to survive in the alimentary tract without causing disease. However, their presence in other sites such as blood, the urinary tract, or the central nervous system may cause serious disease [3].

Bacterial biofilms are microbially derived sessile communities characterized by cells which are irreversibly attached to a surface or to each other. They are embedded in a matrix of extracellular polymeric substances (EPS) that they produced [4].

The medical significance of biofilms is that in comparison to their free-living (planktonic) bacteria, biofilms have increased resistance to antimicrobial agents, thus, diseases in which biofilms play a major role are more likely to be chronic and difficult to eradicate [5].

Currently, there is no data documenting the prevalence of biofilm producing strains of uropathogenic *E. coli* and their antibiotic susceptibility at Suez Canal University Hospitals (SCUHs). So, this study aimed to detect the biofilm producing *E. coli* in UTI patients at SCUHs and also determine their antibiogram profile to improve prognosis and treatment and reduce morbidity and mortality rates due to this infection.

Methodology

Study population

This descriptive cross-sectional study was conducted at SCUHs in Ismailia, Egypt, during the period from December 2022 to July 2023. Forty-seven clinical isolates of non-duplicated UPEC were collected by simple random sampling technique from patients of both genders and all age groups in different departments in SCUHs who had criteria for symptomatic urinary tract infection (SUTI) according to Center of Diseases and Control (CDC) criteria [6] and had not received antibiotics within 48 hours before specimen collection. Ethics committee of Faculty of Medicine, Suez Canal University had approved the study in July 2022. This study followed the principles of the Helsinki Declaration.

Sample size justification

Sample size was calculated according to the following equation [7],

$$n = \left[\frac{Z_{\alpha/2}}{E} \right]^2 * P(1 - P)$$

Where: n= sample size. $Z_{\alpha/2} = 1.96$ (the critical value that divides the central 95% of the Z distribution from the 5% in the tail). P = the prevalence of *E. coli* infections = 13% [8]. E = the margin of error (= width of confidence interval) = 0.1.

So, the sample size was 43 *E. coli* clinical isolates with 10% as drop-out, total sample became 47 *E. coli* clinical isolates.

Specimen collection and processing

Forty-seven midstream urine samples were collected under complete aseptic conditions and transported rapidly after collection (within two hours after voiding) to the laboratory of the Microbiology and Immunology department, Faculty of Medicine, Suez Canal University, where they were processed for isolation and identification of *E. coli* strains. Cultures were done on blood agar (Oxoid, UK) and MacConkey's agar (Himedia, India). The cultured plates were incubated aerobically at $35 \pm 2^\circ\text{C}$ for 24 hours. Colonies suspected to be *E. coli* (being lactose fermenter on MacConkey's agar and Gram-negative bacilli by Gram stain) were confirmed by biochemical reactions including indole test, citrate test, MR test and VP test.

Antibiotic susceptibility testing

Susceptibility testing was performed according to the standard Kirby-Bauer disk diffusion method on Mueller Hinton agar (Microgen, India) and interpreted according to the Clinical and Laboratory Standard Institute (CLSI) 2023 guidelines [9]. Antibiotic discs (Bioanalyse, Turkey) include amoxicillin-clavulanate (20/10 μg), piperacillin-tazobactam (100/10 μg), cefotaxime (30 μg), ceftazidime (30 μg), aztreonam (30 μg), imipenem (10 μg), gentamicin (10 μg), amikacin (30 μg), norfloxacin (10 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg) and nitrofurantoin (300 μg).

Detection of uropathogenic *E. coli* biofilm producers

Isolates that were identified as *E. coli* were tested for biofilm production as follows:

Congo red agar method (CRA) [10]

The medium was prepared by adding 37 gm brain heart infusion broth (BHI) powder (Oxoid, UK), 50 gm sucrose and 10 gm agar to 900 ml

distilled water and autoclaved at 121°C for 15 minutes. Congo red (Techno PharmChem, India) was prepared separately by dissolving 0.8 gm of Congo red stain in 100ml distilled water as concentrated aqueous solution and autoclaved at 121°C for 15 minutes and was then added to the previous media when it cooled to 55°C. The Congo red agar was distributed in sterile plates. Plates were inoculated by the test bacteria and incubated aerobically for 24 to 48 hours at 37°C. Positive results were indicated by black colonies with a dry crystalline consistency. Non producers remain pink.

Modified tissue culture plate method (MTCP)

All isolates were screened for their ability to form biofilm by the TCP method [11] with a modification in duration of incubation, which was extended to 24 hours and the addition of glucose [12]. Few colonies suspended in physiological saline to 0.5 McFarland and vortexed for 1 min. 96 wells flat-bottomed microtiter plates were filled with 180µl Trypticase soy broth (TSB) (Oxoid, UK) + 0.5% glucose and 20µl of bacteria suspension added to each well. Three wells per strain were incubated and their mean considered as final absorbance. All plates were done in duplicate. Negative controls (Blank) were TSB + 0.5% glucose alone. Positive control - *Enterococci* - was obtained from Medical Microbiology and Immunology department laboratory, Faculty of Medicine, Suez Canal University (diagnosed before by MTCP). After stationary aerobic incubation for 24 h at 37°C, broth was carefully drawn off and the wells were washed three times with 300 µl of sterile phosphate buffered saline (PBS, room temperature). Biofilms were fixed with 150µl methanol for 20 min, flick, and air dried in an inverted position in the warm room (about 30 min). Biofilms were stained with 150µl of crystal violet solution in water (2%) for 15 min at room temperature and the wells were rinsed by placing the plate under running tap water. Microtiter plates were inverted on a paper towel and air dried. To quantify biofilm production, 150µl of 33% acetic acid was added to each well to destain the biofilms and lidded plates were placed at room temperature for 30 min without shaking. Thereafter, the optical density of the resuspended crystal violet was measured at 630nm (OD₆₃₀) by using a microtiter plate reader (Biotek Instruments ELX-800 ELISA Reader, UK).

Interpretation of the results [13]

OD values < 0.120 → Non/weak biofilm producer.

OD values 0.120 - 0.240 → Moderate biofilm producer.

OD values > 0.240 → Strong biofilm producer.

Statistical analysis

Statistical analysis was done with SPSS-25 software (SPSS Inc., Chicago, IL, USA). Data was analyzed and presented as numbers and percentages using graphs and tables with the confidence interval (CI) at 95%. *P* value of 0.05 was used as the statistical significance limit.

Results

The study population included 47 clinical isolates of non-duplicated UPEC that were collected from 17 (36.2%) males and 30 (63.8%) females. Their age ranged from 20 to 78 years with a mean ± SD of 50.8 ± 16.9 years. The highest percentage was detected among the group of (50-65) years.

Nineteen (40.43%) *E. coli* strains were isolated from the intensive care unit (ICU) patients followed by the inpatients' departments (16 isolates, 34.04 %) then the outpatients' clinics (12 isolates, 25.53 %).

Thirty (63.83 %) *E. coli* strains were isolated from catheterized patients while the remaining (17 isolates, 36.17 %) *E. coli* strains were isolated from non-catheterized patients.

Using the Kirby-Bauer disk diffusion method on Mueller Hinton agar, antibiotic susceptibility testing of the *E. coli* isolates showed that the maximum sensitivity was seen for imipenem (91.49 %), followed by nitrofurantoin (78.72 %) and then gentamicin (63.83 %).

All the isolates were resistant to amoxicillin-clavulanate and ceftazidime. Resistance to piperacillin-tazobactam, cefotaxime and ciprofloxacin was 87.23 %, 80.85 % and 87.23%, respectively as shown in **table (1)**.

Using the CRA method (**Figure 1**), out of the 47 non duplicate *E. coli* isolates, 30 (63.8 %) isolates were biofilm producers and 17 (36.2 %) isolates were non biofilm producers.

Using the MTCP method (**Figure 2**), 19 (40.4 %) out of 47 *E. coli* isolates were biofilm producers while the remaining 28 (59.6 %) isolates were non biofilm producers. Out of the 19 biofilm producers' isolates, only one isolate was a strong biofilm producer and 18 isolates were moderate biofilm producers. So, the prevalence of biofilm production among UPEC in Suez Canal University

Hospitals is 40.4% as the MTCP is the gold standard method of biofilm detection.

The difference between the two methods was statistically significant (p value < 0.05) as shown in **table (2)**.

All 19 strains that were biofilm producers by MTCP method were also producers by CRA method. However out of 28 non producers by MTCP method there were 11 producers and 17 non producers by CRA.

Table 3 shows the diagnostic accuracy of CRA in comparison to MTCP method as a gold standard method, the sensitivity, specificity and accuracy of CRA method were 100%, 60.7% and 76.6%, respectively.

As shown in **table (4)**, 13 (43.3%) out of 30 catheterized patients were biofilm producers by MTCP method, and 6 (35.3%) out of 17 non catheterized patients were biofilm producers. So, there is no statistically significant difference

between catheterized and non-catheterized patients in biofilm production.

All biofilm producers and non-biofilm producers were resistant to amoxicillin-clavulanate and ceftazidime. Comparing the resistance to antibiotics, it was found that biofilm producing isolates showed more resistance than non-biofilm producing isolates to the following antibiotics; imipenem (10.53% versus 3.57%), gentamicin (15.79% versus 14.29%), norfloxacin (73.68% versus 25%), trimethoprim-sulfamethoxazole (73.68% versus 71.43%), nitrofurantoin (26.32% versus 3.57%), levofloxacin (57.9% versus 32.15%) and ciprofloxacin (94.74% versus 82.14%) as shown in **table (5)**. Norfloxacin was the only antibiotic that showed significant difference in resistance between biofilm and non-biofilm producing isolates ($p = 0.003$)

Table 1. Antibiotic susceptibility patterns of the isolated *E. coli* strains.

Antibiotic	Resistant	Intermediate	Sensitive
	No. (%)	No. (%)	No. (%)
Amoxicillin-clavulanate	47 (100 %)	0 (0 %)	0 (0 %)
Piperacillin-tazobactam	41 (87.23 %)	5 (10.64 %)	1 (2.13 %)
Cefotaxime	38 (80.85 %)	5 (10.64 %)	4 (8.51 %)
Ceftazidime	47 (100 %)	0 (0 %)	0 (0 %)
Aztreonam	32 (68.09 %)	0 (0%)	15 (31.91 %)
Imipenem	3 (6.38 %)	1 (2.13 %)	43 (91.49 %)
Gentamicin	7 (14.89 %)	10 (21.28 %)	30 (63.83 %)
Amikacin	15 (31.92 %)	12 (25.53 %)	20 (42.55 %)
Norfloxacin	21 (44.68 %)	4 (8.51 %)	22 (46.81 %)
Trimethoprim-sulfamethoxazole	34 (72.34 %)	0 (0 %)	13 (27.66 %)
Nitrofurantoin	6 (12.77 %)	4 (8.51 %)	37 (78.72 %)
Levofloxacin	20 (42.55 %)	4 (8.51 %)	23 (48.94 %)
Ciprofloxacin	41 (87.23 %)	0 (0%)	6 (12.77 %)

Table 2. Results of MTCP and CRA methods of biofilm detection.

Biofilm production	Biofilm detection methods				P value
	MTCP		CRA		
	No.	%	No.	%	
Biofilm producers	19	40.4%	30	63.8%	.000*
Non producers	28	59.6%	17	36.2%	
Total	47	100%	47	100%	

P value ≤ 0.05 is considered statistically significant, comparison between groups done by pearson Chi-Square test, *= significant.

Table 3. Diagnostic accuracy of CRA in comparison to MTCP method.

Method	CRA
Sensitivity	100%
Specificity	60.7%
Positive predictive value (PPV)	63.3%
Negative predictive value (NPV)	100%
Accuracy	76.6%

Table 4. Prevalence of biofilm production among catheterized and non-catheterized patients

Catheterization		Biofilm production by MTCP		Total	P value
		Producers	Non producers		
Catheterized	No (%)	13 (43.3%)	17 (56.7%)	30 (100%)	.589 (NS)*
Non catheterized	No (%)	6 (35.3%)	11 (64.7%)	17 (100%)	

-P value ≤.05 is considered statistically significant, comparison between groups done by pearson Chi-Square test. * Not significant.

Table 5. Antibiotic susceptibility among biofilm and non-biofilm producing isolates.

Antibiotic	Biofilm producers (n=19)						Non biofilm producers (n=28)						P value
	sensitive		intermediate		Resistant		sensitive		intermediate		Resistant		
	No	%	No	%	No	%	No	%	No	%	No	%	
Amoxicillin-clavulanate	0	0%	0	0%	19	100%	0	0%	0	0%	28	100%	-
Piperacillin-tazobactam	1	5.26%	2	10.53%	16	84.21%	0	0%	3	10.71%	25	89.29%	.471
Cefotaxime	2	10.53%	2	10.53%	15	78.94%	2	7.15%	3	10.71%	23	82.14%	.920
Ceftazidime	0	0%	0	0%	19	100%	0	0%	0	0%	28	100%	-
Aztreonam	7	36.84%	0	0%	12	63.16%	8	28.57%	0	0%	20	71.43%	.551
Imipenem	17	89.47%	0	0%	2	10.53%	26	92.86%	1	3.57%	1	3.57%	.461
Gentamicin	14	73.68%	2	10.53%	3	15.79%	16	57.14%	8	28.57%	4	14.29%	.327
Amikacin	8	42.10%	6	31.58%	5	26.32%	12	42.86%	6	21.43%	10	35.71%	.680
Norfloxacin	5	26.32%	0	0%	14	73.68%	17	60.71%	4	14.29%	7	25%	.003*
Trimethoprim-sulfamethoxazole	5	26.32%	0	0%	14	73.68%	8	28.57%	0	0%	20	71.43%	.865
Nitrofurantoin	13	68.42%	1	5.26%	5	26.32%	24	85.72%	3	10.71%	1	3.57%	.067
Levofloxacin	7	36.84%	1	5.26%	11	57.9%	16	57.14%	3	10.71%	9	32.15%	.211
Ciprofloxacin	1	5.26%	0	0%	18	94.74%	5	17.86%	0	0%	23	82.14%	.204

-P value ≤.05 is considered statistically significant, comparison between groups done by Pearson Chi-Square test. *-significant

Figure 1. Congo red method (A: non-biofilm producer, B: biofilm producer).

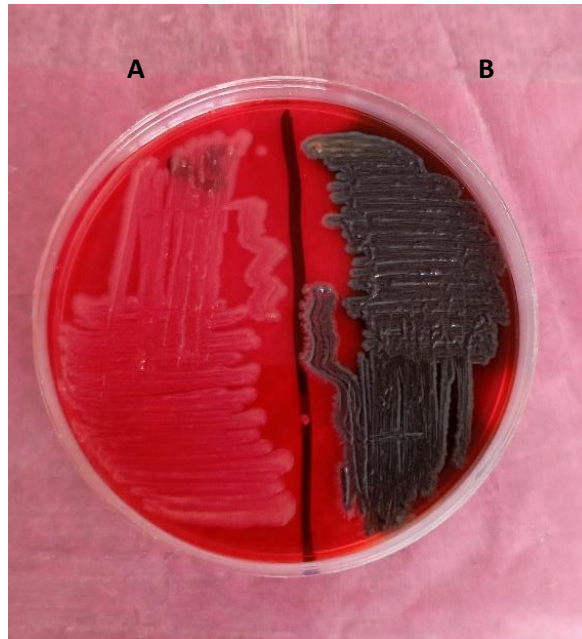
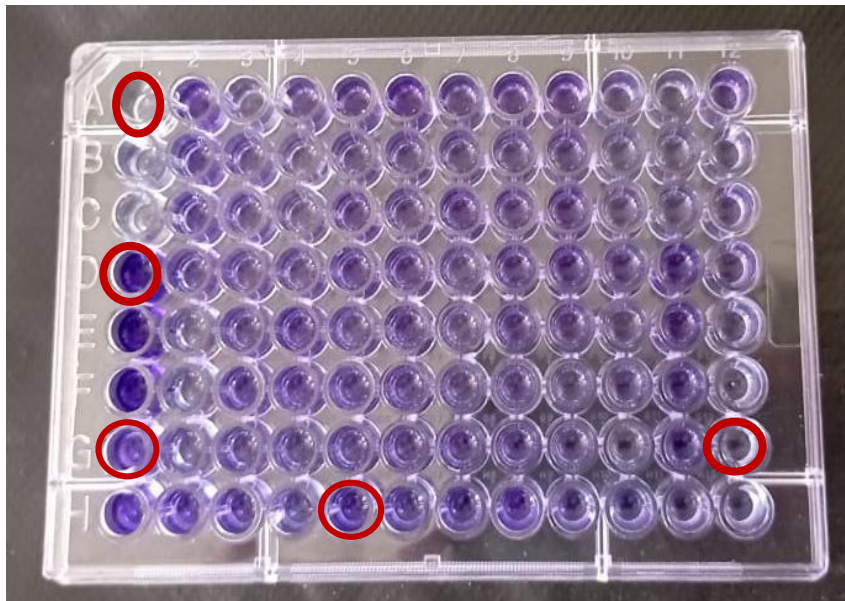


Figure 2. Modified tissue culture plate (A1: -ve control, D1: +ve control, G1: strong producer, H5: moderate producer, G12: non producer).



Discussion

Escherichia coli (*E. coli*) is one of the most frequently isolated causative agents of UTIs, and is responsible for uncomplicated UTIs, community acquired infections, and hospital-acquired infections (80%, 95% and 50% respectively) [14]. *Escherichia coli* can cause infections that are difficult to eradicate due to formation of biofilms. Bacterial

biofilm causes chronic infections as they show increased tolerance to antibiotics and disinfectant chemicals as well as resisting phagocytosis and other components of the host immune system [15].

In this study, 47 clinical isolates of non-duplicated UPEC were collected from clinically suspected cases of UTI in different departments in SCUHs. Out of the 47 *E. coli* isolates, the CRA

method detected 30 (63.8 %) isolates as biofilm producers while the MTCP method detected 19 (40.4%) isolates as biofilm producers.

Similar results were reported by **Katongole et al.** [16] who found that 62.5% of their *E. coli* isolates were biofilm producers by CRA method. While **Dawadi et al.** [17] and **Shah et al.** [18] detected 97.2% and 87.7% biofilm producers by MTCP respectively. Another study found that biofilm producers were 74.8 % of isolates by CRA method and 69.2% by MTCP method [19].

On the other hand, Yadav and his colleagues detected 64% of *E. coli* isolates were biofilm producers by CRA method and 76% by MTCP method [20]. Another study detected 49% and 69% were biofilm producers by CRA and TCP method respectively [21].

In the present study, all 19 (40.4%) strains that were biofilm producers by MTCP method were also producers by CRA method. However, out of 28 (59.6%) non producers by MTCP method there were 11 producers and 17 non producers by CRA. The diagnostic accuracy of CRA in comparison to MTCP method as a gold standard method [4] revealed that the sensitivity, specificity and accuracy of CRA method were 100%, 60.7% and 76.6% respectively.

Tissue culture plate (TCP) method is the most suitable specific, reliable method with the advantage of both qualitative and quantitative analysis and with no subjective errors. Considering the ease of doing the test, rapidity, sensitivity and cost effectiveness, CRA method can be used as a screening test for biofilm detection.

Dhanalakshmi and his colleagues found that biofilm producers were 46.97% of isolates by CRA method and 39.77% by TCP method and reported that the sensitivity of CRA method was 80% and the specificity was 75.47% [22].

A study conducted in Egypt found sensitivity, specificity and accuracy of CRA in comparison with TCP method were 100%, 88.8% and 90.7% [23].

Furthermore, Mohammed and his colleagues in a study comparing CRA and PCR for detection of biofilm formation found that the sensitivity of CRA was 77.1% and the specificity was 33.3% [24]. Another study found that sensitivity, specificity and accuracy of CRA in comparison with MTCP method were 40%, 35% and 36% respectively [25].

On the other hand, Meena and Sharma detected that sensitivity of CRA method was 60% while specificity was 81.81%, with respect to MTCP method [26]. Also, Gogoi and Sharma reported the sensitivity and specificity of CRA method were 21.9% and 85% respectively [27].

A study done by Bose and his colleagues comparing CRA method with respect to MTCP method reported that sensitivity of CRA method was 8.25% and specificity was 96.34% [28]. Similarly, another study showed very little correlation between CRA and TCP and reported sensitivity (7.6 %), specificity (97.2 %) and accuracy (51.3 %) [13]. And a study conducted in Egypt showed that CRA sensitivity was very low (0.9%), but specificity was 97.4% [29].

The variations observed in various studies might be due to the differences in the sources from which the strains were isolated and differences in the methodology employed in the study.

In the present study, thirty (63.83 %) *E. coli* strains were isolated from catheterized patients while the remaining 17 (36.17 %) *E. coli* strains were isolated from non-catheterized patients. There was no statistically significant difference between catheterized and non-catheterized patients in biofilm production ($p= 589$) as 43.3% of catheterized patients were biofilm producers by MTCP method, and 35.3% of non-catheterized patients were biofilm producers.

In contradiction, **Karigoudar et al.** [21] found that 49% and 51% of *E. coli* strains were from catheterized and non-catheterized patients respectively. And reported a significant correlation between biofilm production and catheterization ($p=0.0001$) as 89.7% of catheterized patients were biofilm producers by TCP method and 49% of non-catheterized patients were biofilm producers. Also, SarojGolia and his colleagues detected 89.5% of catheter associated UTI were biofilm producers by all the three methods (TCP, CRA, TM) [19]

The reason that biofilm is so prevalent on urinary catheters is that it conveys a survival advantage to the microorganisms; for this same reason urinary catheter biofilm is difficult to eradicate. Organisms in a biofilm function as a community and communicate closely with one another. Survival advantages conferred by the biofilm community include resistance to being swept away by simple shear forces, resistance to phagocytosis, and resistance to antimicrobial agents [30].

The appropriate treatment for UTIs is the selection of effective, accessible, high-performance antibiotics, but in the past decade, the number of antibiotics to which bacteria have developed resistance has increased considerably. As a consequence, some agents are no longer useful for the treatment of infections, and bacteria that are resistant to antimicrobial agents, including bacteria with multidrug resistance (MDR), are an increasing problem in healthcare in both community and hospital settings [31].

In the present study, all the isolates were resistant to amoxicillin-clavulanate and ceftazidime. Resistance to piperacillin-tazobactam, ciprofloxacin and cefotaxime was 87.23 %, 87.23 % and 80.85 % respectively. Medium resistance was observed for trimethoprim-sulfamethoxazole (72.34 %), aztreonam (68.09 %), norfloxacin (44.68 %), levofloxacin (42.55 %) and amikacin (31.92 %). The maximum sensitivity was seen for imipenem (91.49 %), followed by nitrofurantoin (78.72 %) and then gentamicin (63.83 %).

A study evaluating the antibiotic susceptibility pattern of uropathogenic *E. coli*, revealed that the maximum sensitivity was seen for imipenem (100%), followed by gentamicin (79.2%) and amikacin (65.3%). A high resistance rate was seen for amoxicillin-clavulanate (91.7%) cotrimoxazole (83.3%) and norfloxacin (62.5%) [32].

Nemr and his colleagues reported that the antimicrobial susceptibility of uropathogenic *E. coli* isolates exhibited the highest susceptibility to imipenem, meropenem, amikacin, nitrofurantoin, levofloxacin and ciprofloxacin (86.3%, 80.4%, 62.7%, 62.7%, 49% and 49% respectively) [14].

Another study found that the highest numbers of *E. coli* strains were susceptible to amikacin (87.5%) followed by nitrofurantoin (72.6%), and gentamicin (60.6%), and the least numbers of the strains were susceptible to amoxicillin (10.6%) [33].

Yadav and his colleagues reported that the highest sensitivity of *E. coli* isolates was found for amikacin (93.6%), nitrofurantoin (90.8%), meropenem (88%) and gentamicin (75.2%) followed by cotrimoxazole (50%), piperacillin/tazobactam (40%) and ciprofloxacin (23.2%). Very few isolates (10%) were sensitive to ampicillin, amoxicillin-clavulanate (9.2%) and ceftazidime (5.2%) [20].

A study conducted by Dawadi and his colleagues showed that the least resistance was toward meropenem (9.8%) followed by nitrofurantoin (18.3%) and gentamicin (21.1%). The bacterial resistance was extensively high toward ampicillin (88.7%) followed by cotrimoxazole (73.2%) and ciprofloxacin (40.8%) [17].

Another study detected that the *E. coli* isolates were most resistant to trimethoprim sulfamethoxazole and Amoxicillin (93%) followed by gentamicin (87%) and the least were imipenem (0.5 %) and nitrofurantoin (25.5%) [16].

In a study conducted by Shah *et al.* [18] who evaluated the overall resistance pattern of UPEC and confirmed that the highest resistance was toward ampicillin (96.5%) and nitrofurantoin (91.2%), followed by amoxicillin-clavulanate (82.5%). Medium resistance was observed for ceftazidime (73.7%), co-trimoxazole (66.7%), piperacillin/tazobactam (49.1%), and gentamicin (45.6%) and minimum resistance was observed for norfloxacin (17.5%), followed by amikacin (22.8%) and imipenem (33.3%).

Treatment of biofilm related infections is typically performed by prolonged and high dose of antimicrobial therapy as well as elimination of infected medical devices [34].

In the present study, all biofilm producers and non-biofilm producers were resistant to amoxicillin-clavulanate and ceftazidime. Comparing the resistance to antibiotics, it was found that biofilm producing isolates showed more resistance than non-biofilm producing isolates to the following antibiotics; imipenem (10.53% versus 3.57%), gentamicin (15.79% versus 14.29%), norfloxacin (73.68% versus 25%), trimethoprim-sulfamethoxazole (73.68% versus 71.43%), nitrofurantoin (26.32% versus 3.57%), levofloxacin (57.9% versus 32.15%) and ciprofloxacin (94.74% versus 82.14%). Norfloxacin was the only antibiotic that showed significant difference in resistance between biofilm and non-biofilm producing isolates ($p=0.003$).

Biofilm producing isolates showed more resistant due to some suggested factors: (a) the extracellular polymeric substances (EPS) matrix can restrict diffusion of antibiotics (b) interaction of antibiotics with the polymeric matrix lowers their activity, (c) enzyme-mediated resistance such as β -lactamase, (d) lower metabolic activity, growth, and division inside the biofilm, (e) genetic changes on

target cells or hiding the target sites, (f) extrusion of antibiotics using efflux pumps [35].

A study comparing antibiotic susceptibility and biofilm formation, revealed that most antibiotics showed an insignificant relationship. Against ampicillin, amoxicillin-clavulanate, ciprofloxacin, co-trimoxazole, cefepime, ceftriaxone, gentamicin, and meropenem, the p -value was $\geq .05$ which signifies biofilm-formation may not have a relation with these antibiotics used in vitro in non-biofilm forming environment. The correlation could be established between biofilm production and antibiotic resistance for cephalixin ($p = .038$) and nitrofurantoin ($p = .042$) only [17].

Karigoudar and his colleagues reported that biofilm producers showed maximum resistance to co-trimoxazole (73.9% versus 38.7%), gentamicin (94.2% versus 38.7%), and imipenem (11.6% versus 3.2%) when compared to non-biofilm producers. Significant association was seen between resistance to antibiotic and biofilm formation with a $p = 0.01$ (<0.05) [21].

On the other hand, a study showed significant association between resistance to amoxicillin-clavulanate, ceftazidime, cefepime, imipenem, and nitrofurantoin and biofilm formation ($p < 0.05$) [18].

This study had some limitations, including the relatively small sample size and absence of funds. Although phenotypic methods for detection of biofilm are easy and cheap, they may cause some difficulties in result interpretation since they can be influenced by variations in medium composition and cultivation conditions and are prone to subjective errors. So, genotypic evaluation must be considered in further studies.

Conclusion

This study demonstrated that the prevalence of biofilm production among UPEC in SCUHs is 40.4% as the MTCP is the gold standard method of biofilm detection. The CRA method can be used for the routine detection of biofilm production in *E. coli* because of its easy application, low cost and reliable results with good sensitivity. Imipenem is the drug of choice for treating biofilm producing *E. coli* strains.

Conflicts of interest

The authors declare that they have no financial or non-financial conflicts of interest.

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