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Detection the phenotypic of biofilm formation and its correlation with antibiotic resistant among uropathogenic bacteria isolated from Iraqi children

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SUMMARY

AUTHORS' CONTRIBUTION: (A) Study Design · **(B)** Data Collection . **(C)** Statistical Analysis · **(D)** Data Interpretation · **(E)** Manuscript Preparation · **(F)** Literature Search · **(G)** No Fund Collection

Background: Urinary Tract Infections (UTIs) are a serious public health concern, especially in children, which can be caused by a variety of organisms, especially gram-negative bacteria. The biomarker procalcitonin has become more effective in diagnosing bacterial infections in the last decade.

Aim: This study was done to found out the ability of the bacterial isolates to form biofilm and to determine the antibiotic resistance for these biofilm-forming isolates in UTI patients.

Methodology: A case-control study included 60 cases from patients who suffer from Urinary Tract Infection (UTI) at Al-Imamain Al-Jawadain Medical City in Baghdad.

Result: Among total 60 isolates from UTI patients, The result of our study showed that 30.0% of the isolates have the ability to form strong biofilm, 33.3% were moderate, the result also showed the biofilm formation percentage of *S. haemolyticus, E. faecalis, Proteus* spp., *K. pneumonia, S. aureus, E. coli*, and *S. epidermidis* were (100%, 100%, 85.68%, 72.72%, 75.0%, 51.18% and 50% respectively). The biofilm forming isolates were highly resistance to Amoxicillin, Ampicillin, Gentamicin and Trimethoprim.

Conclusion: The majority of the bacterial isolates was biofilm- forming and showed a wide resistance to range of antibiotics.

Keywords: Urinary tract infection; UTI in children; Biofilm production; Biofilm-forming; Antibiotic resistance

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INTRODUCTION

Urinary tract infections are among the most common bacterial infections, impacting around 150 million people globally. In microbiology, a UTI is described as the urothelium's inflammatory reaction to microbial intruders [1]. The most common bacteria resbonsible for UtI is *Escherichia coli (E. coli)* [2]. Other bacteria involved are *Staphylococcus, Proteus mirabilis, Enterococcus spp, Klebsiella pneumoniae and Pseudomonas aeruginosa* [3].

According to research, pathogenic strains of certain uropathogenic bacteria exhibit many virulence phenomena, the most notable of which is known as Biofilm, which refers to the bacterium's propensity to colonize on the mucosal surfaces of the afflicted host. The severity of a urinary tract infection is determined by the virulence of the bacteria as well as the host's susceptibility [4].

Biofilms are surface-attached microbial populations encased in a self-produced extracellular polymeric matrix. They are the product of complicated intra- and intercellular signaling and communication mechanisms that are regulated by a sophisticated Quorum Sensing (QS) regulatory system that is found all across the microbial world [5].

Biofilms are responsible for up to 60% of infections in human and are difficult to eliminate with antibiotics. *In vitro* susceptibility experiments have revealed a significant increase in biofilm cell resistance to killing [6]. Biofilms may be thought of as a universal strategy for the survival of bacterial, which allows them to efeectivaly use of the available resources. They are mostly polysaccharides, which inhibit antibacterial agents, antibodies, and white blood cells from entering inside the cluster [7].

METHOD

Bacterial isolates

The study was conducted with a total of 60 consecutive isolates from children that suffer from UTI, which collected from at Al-Imamain Al-Jawadain Medical City in Baghdad. These isolates were collected over a period of 2 months from September 2022 to November 2022. The bacterial isolates were identified using Gram's stain and other biochemical tests include Oxidase, catalase, Methyl Red, Voges - Proskauer and INDOL tests.

Antimicrobial susceptibility test

The disk-diffusion method by Kirby-Bauer was used to determine the antibiotic susceptibility test, and the Clinical and Laboratory Standards Institute (CLSI) criteria were followed in interpreting the findings [8]. The antimicrobial agents that were tested in this study includes: Amoxicillin (5 mcg), amikacin (30 mcg), ampicillin (2 mcg), cefoxitin (5 mcg), CefIxime (30 mcg), ceftriaxone (30 mcg), ciprofloxacin (5 mcg), cefotaxime (5 mcg), gentamicin (10 mcg), levofloxacin (5 mcg), imipenem/cilastin (10/10 mcg), meropenem (10 mcg), and trimethoprim (25μg).

Identification of biofilms

The microliter plate test, which was detailed by Lotfi [9], was used to detect biofilm. This technique is regarded as the industry standard for detecting biofilms.

- After being cultivated in stationary conditions for one night at 37 degrees Celsius in TSB, the bacterial isolates were diluted 1:100 with fresh TSB and TSB supplemented with 1% glucose.
- 0.2 ml of the diluent was put in each well of a sterile, 60 -well micro culture plate. After that, the culture plate was incubated for 24 hours at 37 °C.
- Then the contents in each well of the plate were removed by lightly tapping the plate.
- After three PBS washes (to eliminate any floating germs), the plates were stained with 200 micro liter of crystal violet 1% for 30 minutes after drying, then washed again with PBS and kept to dry.
- The Optical Density (OD) of biofilm was measured by using ELISA reader at a 570 nm wavelength. This experiment was performed in triplicate and repeated three times.
- The biofilm isolates were classified using the categories (**Tab. 1.**).

RESULTS

Identification of the isolates

In this study, we investigated 60 isolates from children that suffer from UTI and the findings of our investigation indicated that the proportion of isolates of Gram-negative bacteria exceeded that of Gram-positive bacteria. The most prevalent bacteria were *E. coli*, which was detected in 31 children (51.66%). Other common bacteria included *K. pneumoniae* (18.33%), *P. mirabilis* (11.68%), *S. aureus, S. epidermidis,* and each other (6.66%), followed by *Enterococcus faecalis* (3.35%) and *Staphylococcus haemolyticus* (1.66%) [10].

Detection of biofilm formation by microtiter plates method

The result of our study showed that the isolates of *S. haemolyticus and E. faecalis* were one hundred percent biofilm forming, while the isolates of *Proteus spp*. were 85.68% biofilm forming, isolates of *S. aureus, K. pneumoniae, and S. epidermidis* were 75.0%, 72.72%, and 50.0%, respectively, biofilm forming, and isolates of *E. coli* were 51.18% biofilm forming as showing in **Tab. 2.**

Drug resistance of biofilm forming isolates

The result of our study showed the Relationship between the antimicrobial resistance and the biofilm formation as mention in **Tab. 3**.

The result showed that all the biofilm forming isolates were highly resistance to Amoxicillin, Ampicillin, Gentamicin and Trimethoprim. While the most effective antibiotic against biofilm forming isolates were Meropenem, Levofloxacin and Imipenem /Cilastin.

Tab. 1. Classification of biofilm isolates by microliter plate method [10].

OD values	Adherence	Biofilm formation
<0.120	Non	Non /Weak
0.120-0.240	Moderately	Moderate
>0.240	Strong	High

Tab. 2. Distribution of bacteria according to biofilm formation and non-biofilm formation.

Bacteria spp.	No. of isolates	Biofilm formation		Non biofilm formation		P-value
		No.	%	No.	%	
Escherichia coli	31	16	51.18%	15	48.30%	0.875 NS
Klebsiella pneumonia	11	8	72.72%	3	27.27%	0.131 NS
Proteus spp.	7	6	85.68%	1	14.28%	0.050 *
Staphylococcus aureus	4	3	75%	1	25%	0.317 NS
Staphylococcus epidermidis	4	2	50%	2	50%	1.00 NS
Staphylococcus haemolyticus	1	1	100%	0	0	0.894 NS
Enterococcus faecalis	2	2	100%	0	0	0.711 NS
P-value	---	---	0.0002 **	---	0.0002 **	---

* ($P \leq 0.05$), ** ($P \leq 0.01$).

Tab. 3. The relationship between biofilm formation and antimicrobial resistance.

Antibiotic	Types of isolates							Total
	S. aureus (3)	*S.haemolyticus* (1)	*S. epidermidis* (2)	*E. faecalis (2)*	*E.coli (16)*	*K. Pneumonia*(8)	*Proteus spp.* (6)	
Amoxicillin	3(100%)	1(100%)	2(100%)	2(100%)	16(100%)	8(100%)	6(100%)	38
Amikacine	2(66.6%)	1(100%)	0	0	11(68.75%)	2(25%)	3(50%)	19
Ampicillin	2(66.6%)	0	1(50%)	2(100%)	16(100%)	8(100%)	5(83.3%)	34
Cefoxitin	3(100%)	0	2(100%)	1(50%)	13(81.25%)	3(37.5%)	4(66.6%)	26
Ceflxime	2(66.6%)	1(100%)	0	1(50%)	7(43.75%)	5(62.5%)	5(83.3%)	21
Ceftriaxone	3(100%)	0	1(50%)	0	(62.5%) 10	5(62.5%)	2(33.3%)	21
Ciprofloxacin	2(66.6%)	1(100%)	2(100%)	0	8(50%)	5(62.5%)	1(16.6%)	19
Cefotaxime	2(66.6%)	0	2(100%)	0	6(37.5%)	2(25%)	0	12
Gentamicin	3(100%)	1(100%)	2(100%)	2(100%)	16(100%)	7(87.5%)	6(100%)	37
Levofloxacin	1(33.3%)	0	0	0	4(25%)	2(25%)	0	7
Imipenem / Cilastin	1(33.3%)	0	0	0	3(18.75%)	2(25%)	0	6
Meropenem	0	0	0	0	2(12.5%)	0	0	2
Trimethoprim	3(100%)	1(100%)	2(100%)	2(100%)	15(93.75%)	8(100%)	6(100%)	37

DISCUSSION

Distribution of bacteria causing urinary tract infection

The current investigation found that the proportion of Gram-negative bacteria was much greater than the percentage of Gram-positive bacteria (81.55% *vs.* 18.45%), which is consistent with the findings of Flores-Mireles AL, et al. [11]. The results of culture and biochemical testing revealed that the most prevalent isolated bacterium was *E. coli* (51.6%), and the current study's findings are comparable to those of Sharma [12]. In 51.1% to 72.8% of cases of *E. coli* was isolated.

The second most frequent bacterium recovered was *K. pneumoniae*, which was discovered in 11 out of 60 children (18.3%), and this result agrees with the study done by Lehrasab W, et al. [13].

Detection of biofilm formation by using microtiter plates method

Our research's findings, which indicated that 51.18% of *E. coli* isolates were biofilm-forming, were in line with those of Monther A [14] study conducted in Iraq, which found that 50.0% of *E. coli* isolates were positive for biofilm formation. While the investigation of Ahmed revealed that 83.3% of *E. coli* isolates were biofilm-forming, which is a significantly high percentage when compared with our results, Ahmad's study from 2021 in Iraq was discordant with our findings.

The results of our study revealed that 72.72% of *K. pneumonia* isolates were biofilm forming, which is consistent with the findings of Karimi K, et al. [15] discovered that only 20% of *K. pneumonia* isolates were strongly biofilm formation. However, our findings contradict those of Monther A [14] who discovered that all isolates of *K. pneumonia* had the potential to form the biofilm.

Our investigation found that 85.68% of *Proteus* spp. isolates formed biofilms, which agrees with a study done in Poland by Kwiecinska-Piróg J, et al. [16] which found that all of the tested isolates formed biofilms.

Our findings demonstrated that 50% of *S. epidermidis* isolates formed biofilms, which agrees with the findings of an Iranian research conducted by Borooni S. which revealed that 45% [17] *S. epidermidis* isolates formed biofilms.

The result showed that 75% of *S. aureus* isolates seemed to produce biofilms. This finding was similar to the findings of While Wu, et al. and Piechota, who discovered that 86% - 99.2% of *S. aureus* isolates could form a biofilm [18,19]. While these findings contradict the findings of Monther A. which found that about 50% of *S. aureus* isolates formed biofilms [14].

Our data reveal that the isolates of *E. faecalis* & *S. haemolyticus* isolates were 100% biofilms, which disagree with the findings of Fallah F, et al. [20], who found that 26.5% of *E. faecalis* isolates and 75% of *E. faecium* isolates form biofilms. According to recent studies, *E. faecalis* may penetrate and bind to urinary epithelial cells, causing intracellular bacterial populations to proliferate in the bladder.

Drug resistance of biofilm formation by bacterial isolates

The results of our investigation revealed a considerable rise in antibiotic resistance for biofilm-forming isolates, with all *E. coli* biofilm-forming isolates being 100% resistant to Amoxicillin, Ampicillin, and Gentamicin, and 93.75% and 81.75% resistant to Trimethoprim and Cefoxitin, respectively. The corresponding figures for Amikacine and Ceftriaxone, were 68.75%, 62.5%, respectively. All of these findings are agree with the findings of Tajbakhsh who discovered that all *E. coli* biofilm-forming isolates were resistant to ampicillin (87.5%), tetracycline (75%), Nalidixic acid (72.5%), and Co.Trimoxazole (62.5%) [21].

According to our findings, all *K. pneumonia* biofilm-forming isolates were 100% resistant to Amoxicillin, Ampicillin, and Trimethoprim, 87.5% resistant to Gentamicin, and 62.5% resistant to Ceflxime, Ceftriaxone, and Ciprofloxacin. These findings were similar with previous research by Nirwati H, et al. [22] and Folliero

[23] which found a significant proportion of resistance to aminoglycosides, fluoroquinolones, and cephalosporin.

The results of our investigation revealed that all *Proteus* spp. biofilm-forming isolates were 100% resistant to Amoxicillin, Gentamicin, and Trimethoprim, 83.3% resistant to Ampicillin and Ceflxime, 66.6% resistant to Cefoxitin.

Our findings revealed that all *S. aureus* biofilm-forming isolates were 100% resistant to Amoxicillin, Ceftriaxone, Gentamicin, and Trimethoprim, and 66.6% resistant to Ampicillin, Cefotaxime, Amikacine, Ciprofloxacin and Cefixime. These findings are consistent with those of Monther A [14].

CONCLUSION

The majority of the bacterial isolates that responsible for urinary tract infections were capable of creating biofilms and showed resistance to a wide range of antibiotics. So more studied should be done to prevent the development of multi-drug resistant bacteria.

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