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Pediatric Urinary Tract Infection: Evaluation Antibiotic Susceptibility and Biofilm Formation Dynamics

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ABSTRACT

Background: Urinary tract infections are quite prevalent among children presented with non-specific symptoms including abdominal pain, fever, and nocturnal urine incontinence. The study aimed to determine the sensitivity of uropathogenic bacteria to antibiotics and assess their ability to form biofilms.

Methods: This study was carried out in Kirkuk City. A total of 180 children with positive urine culture participated, within the age range of 6-12 years. Bacterial identification and antibiotic susceptibility tests were performed. The ability of all bacteria isolated to produce biofilms was assessed using a quantitative microtiter plate method.

Results: The uropathogenic *Escherichia coli* was the predominant isolate (40 %) from children with urinary tract infections. The prevalence of UTI in children was highest in the age group between (9-12) years, with 120 children accounting for (67 %) of the cases. Biofilm production for isolated bacteria was; *E. coli* (66.7 %), *Klebsiella pneumonia* (33.4 %), *Pseudomonas aeruginosa* (66.6 %) *Proteus mirabilis* (100 %), *Acinetobacter baumannii* (33.6 %), *Staphylococcus haemolyticus* (85.6 %), *Staphylococcus aureus* (83.3 %), *Staphylococcus epidermidis* (100 %), *Staphylococcus lentus* (100 %), *Enterococcus faecalis* (66.6 %).

Conclusion: The majority of bacteria recovered from children with urinary tract infections were capable of forming biofilms. Infections caused by bacteria that develop biofilms are more resistant to typical antibiotic treatments, requiring higher dosages or more powerful drugs.

Key words: Children; Urinary Tract Infection; Antibiotic; Biofilm formation.

INTRODUCTION

Urinary tract infection (UTI) is a prevalent bacterial infection that frequently occurs in infancy. During the initial year of life, the occurrence of this condition is more frequent in boys (3.7%) compared to girls (2 %). However, beyond infancy, it becomes notably more widespread in girls [1]. The shorter length of the female urethra, regular colonization of the perineum by enteric organisms, high vaginal pH, and greater adhesiveness of bacteria to vaginal cells could be responsible for this occurrence [2]. A UTI can affect either the lower tract alone or both the lower and upper urinary tracts [3]. Common symptoms of UTI include painful urination (dysuria), frequent urination, urgent need to urinate, burning sensation, pain in the side (flank pain) or lower abdomen (suprapubic pain), fever, and presence of blood in the urine (haematuria) [4]. Typical bacterial pathogens that cause UTI are Gram-negative species including *Escherichia coli, Klebsiella, Proteus, Enterobacter,* and *Pseudomonas*, as well as Gram-positive organisms such as group B *streptococci, Enterococcus spp,* and *Staphylococcus spp*. Fungi may cause UTIs in children such as *Candida spp*[5].

Over the past thirty years, there has been a persistent occurrence of bacterial resistance among pathogens that cause UTIs. The existing reports and data validate that the increase in resistance to routinely used antibiotics is a consequence of the improper utilization of antimicrobials. The emergence of resistance among UTI infections is a matter of great worry and necessitates immediate study to develop effective solutions to address the problem [6]. Bacterial biofilms are clusters of cells that adhere to surfaces and are enveloped by a matrix of extracellular polymeric molecules. Structured biofilm communities are believed to contain over 99 % of bacteria [7]. Bacteria that are linked with biofilms exhibit unusual characteristics compared to their free-living planktonic counterparts. These traits include distinct physiology, a strong immune system tolerance by being resistant to phagocytosis, and greater resistance to traditional antibiotic treatments compared to their planktonic cells [8]. The occurrence of antibiotic resistance in biofilm is proposed to be due to various mechanisms, including limited diffusion of antibiotics through the matrix, horizontal transmission of resistance genes, inactivation of antibiotics due to changes in metal ion concentrations and pH values, and the metabolic inactivity of bacteria. The degree of resistance is contingent upon the stage of biofilm development, with bacteria being more vulnerable during the initial reversible phase. As a result, biofilms serve as a persistent source of chronic infections [9]. Therefore, this research was suggested to examine the ability of bacteria to produce biofilm.

MATERIAL AND METHODS

This cross-sectional study was carried out in Kirkuk City from October 2023 to March 2024; the total number of participant were 180 children with positive urine culture, and their age range was between 6 and 12 years; those children were attending the Pediatric outpatient department of Azadi teaching hospital, Pediatric hospital of Kirkuk, Tappa health center. All participants were subjected to a questionnaire specially designed for this study, which included the patient's name, age, gender, signs and symptoms, and history of medication such as antibiotics.

The inclusion criteria include patients with signs and symptoms of UTIs such as fever, poor appetite, vomiting, malaise, abdominal and flank pain, dysuria, frequency; abnormal urinalyses such as pyuria (more than 10 leukocytes per microscopic field), Red Blood cells, bacteria, monilia, and crystals. The exclusion criteria included the presence of anatomical disorder, history of vesicoureteral reflux or nephrogenic disorders, children with diabetes, and children who received antibiotics as treatment.

The sample collection and bacterial identification were achieved by Mid-stream urine collection from all children who participated in the study in a sterile wide mouth cup, after instruction to clean the urogenital area, they were delivered to the microbiology lab. less than half an hour after collection. All urine samples were subjected to general urine examination and were inoculated on the Blood, MacConkey, and Sabouraud Dextrose agar medium, then incubated aerobically at 37°C for 18-24 hours. Samples showing significant growth, bacteria growing >10^5 colony forming units (CFU/ml) with single bacterial growth, were considered significant and processed further for identification. Isolated colonies were purified, Gram stained, identified by their colony morphology, shape, size, color, and odor, observation of fermenting lactose on MacConkey agar, hemolytic activity on blood agar, followed by identification and antibiotic susceptibility test using the fully automated Vitek 2 Compact System (BioMerieux) according to the manufacturer's instructions. Vitek 2 GN, Vitek 2 GP, and Vitek 2 YST cards were used for identification, while AST-N417 and AST-P592 cards were used for antibiotic sensitivity tests.

The ability of all bacteria isolated from this study to form biofilms was evaluated using a quantitative microtiter plate method, as described below [10]:

Bacterial cultures were first inoculated into 5 milliliters of Tryptone Soy Broth (TSB) and incubated at 37°C for 24 hours. After this incubation period, the bacterial colonies were diluted to a 1:100 ratio using TSB supplemented with 1% glucose. From each diluted culture, 200 µl was pipetted into the wells of a 96-well flat-bottom microtiter plate. The plate was covered and incubated at 37°C for 48 hours.

Following the incubation, the contents of each well were washed three times with phosphate-buffered saline (PBS, pH 7.3). Subsequently, 150 µl of 0.1% crystal violet (w/v) was added to each well, and the plate was left at room temperature for 15 minutes.

The stain was then removed by washing the wells three times with distilled water, and the excess liquid was blotted using filter paper. The plates were allowed to air dry.

To resolubilize the crystal violet dye, each well was filled with 150 µl of 95% ethanol, and the plates were left at room temperature for 10 to 15 minutes.

The optical density (OD) of each sample was measured using an ELISA reader at a wavelength of 630 nm. Each sample was tested in duplicate, and the optical density of each bacterial isolate was determined by averaging the duplicate readings. These optical density values were compared to the optical density of the negative control (ODc), which consisted of TSB without bacterial inoculum.

Based on the comparison between the average optical densities of the bacterial isolates (ODi) and the negative control (ODc), the bacteria were classified into four groups as follows:

- Non-biofilm forming: $ODi \leq ODc$
- Weak biofilm forming: $ODc < ODi \leq 2 \times ODc$
- Moderate biofilm forming: $2 \times ODc < ODi \leq 4 \times ODc$
- Strong biofilm forming: $ODi > 4 \times ODc$

Notes:

- ODc: Optical density of the negative control (TSB without bacterial inoculum).
- ODi: Optical density of the bacterial isolates.

All statistical analysis were carried out using SPSS for Windows version 27. Students' T-tests were used to compare the continuous variables. A p-value <0.05 was considered statistically significant.

The study was done in alignment with the ethical principles derived from the Declaration of Helsinki. The study protocol, subject information, and permission form underwent assessment and approval by a local ethics committee in Kirkuk Health Directorate, as per document number 594, dated 18/9/2023.

RESULTS AND DISCUSSION

Distribution UTIs according to age and gender of patients:

The prevalence of UTI in children was highest in the age group (9-12) years, with 120 children accounting for (67 %) of the cases. While for the age group of (6-8) years, there were 60 children with UTIs, representing (33%) of the cases (Table 1). This result was in agreement with the study by Bajpai S. et al. who found that (9–12) was the age group most susceptible to UTIs compared to other age groups accounting for 74 % of the total 100 children with UTIs [11]. This frequency in that age group may have resulted from their infrequent bathroom breaks or incomplete bladder emptying. Additionally, some kids with recurrent UTIs experience dysfunctional elimination syndrome, or difficulty controlling their bladder during the day [12].

Table 1. Distribution of UTI in children according to age and gender.

Age group	Children with urinary tract infection			P-value
	Male No.(%)	Female No.(%)	Total No.(%)	
6-8 years	21 (12 %)	39 (21 %)	60 (33 %)	
9-12 years	21 (12 %)	99 (55 %)	120 (67 %)	**0.1**
Total	42 (24 %)	138 (76 %)	180 (100 %)	

Regarding gender distribution, female children were more susceptible to developing UTIs, accounting for 76% of cases, compared to 24% in male patients (Table 1). This finding aligns with the study by Joya M. et al [13]. The increased susceptibility in females may be attributed to anatomical differences in the urogenital system, such as the shorter urethra in females compared to males [14].

Etiological bacterial agents:

Gram staining revealed that 111 (61.6%) of the bacteria isolated from children with UTIs were Gram-negative, 57 (31.7%) were Gram-positive, and 12 (6.7%) were yeast. The types and percentages of isolated bacteria and yeast are shown in (Table 2). These findings are in line with the study by Al-Baqer T.M. et al., where Gram-negative bacteria were the most commonly isolated, accounting for 170 isolates (84.15%), while Gram-positive bacteria represented 30 isolates (14.85%) [15]. Similarly, the prevalence of Candida in this study aligns with the findings of Seifi Z. et al., who reported a 5.2% occurrence of Candida among participants [16]. Additionally, our results are consistent with those of Salman H.A. et al., who isolated 267 (89.29%) Gram-negative and 32 (10.7%) Gram-positive bacteria from children with UTIs [17]

Table 2. Frequencies and percentage of isolated micro-organisms from children with UTI.

Type of micro-organism	Frequency	Percentage %
E. coli	72	40 %
Klebsiella pneumoniae	18	10 %
Acinetobacter baumannii	9	5 %
Proteus mirabilis	6	3.3 %
Pseudomonas aeruginosa	6	3.3 %
Staphylococcus haemolyticus	21	11.7 %
Staphylococcus aureus	18	10 %
Staphylococcus epidermidis	9	5 %
Staphylococcus lentus	6	3.3 %
Enterococcus faecalis	3	1.7 %
Candida albicans	12	6.7 %
Total	**180**	**100%**

Antibiotic resistance to Gram-negative bacteria:

In the present study antibiotic susceptibility test was conducted for all Gram-negative bacteria isolated from children with UTIs as shown in (Table 3). The findings indicated that *E. Coli* is resistant to Trimethoprim/Sulfamethoxazole (45.8 %), Cefuroxime (50 %), Ceftazidime (33.3 %), Cefazolin (33.3 %), Ceftriaxone (25 %), Cefuroxime/Axetile (29.1 %), Cefepime (16.6 %), Ampicillin (33.3 %), Amoxicillin/ClavulanicAcid (8.3 %), Ciprofloxacin (23.8 %), Gentamicin (8.3 %),Nitrofurantoin (4.1 %), and they were sensitive to Pipracilin, Amikacin, Meropenem, Imipenem, Fosfomycin (100 %). Is disagrees with Hasan M.T et al. whose results of antibiotic sensitivity were resistance to Trimethoprim/Sulfamethoxazole (10 %), Ceftazidim (82.5 %), Ceftriaxone (60 %), while agreement with Ciprofloxacin resistance was (27 %) [18].

The result from this study is in agreement with Al-Dahhan H.A. et al. in which*E. coli* was resistant to Ampicillin (21.7 %), Amoxicillin/clavulanic acid (2.2 %), Gentamicin (13.5 %), and nitrofurantoin (1.7 %) [19], as well as comparable to the study that done by Mohammed S. et al. in which *E.coli* was sensitive to Imipenem (100 %), Amikacin (76 %) [20].

While for*Klebsiella pneumonia* isolates, their resistance to Amoxicillin/Clavulanic Acid, Cephazolin, Cefuroxime/Axetile, Ceftazidim, Ceftriaxone, Cefepem, Ciprofloxacin, Trimethoprime/Sulfamethoxzole, were (16.6 %), while there resistance to Cefuroxime and Fosfomycin were (33.3 %), forNitrofurantoin was (50 %). In a study conducted by Duicu C. et al., the resistance pattern of *Klebsiella pneumonia* were, Amoxicillin (6.04 %), Ceftazidim (3.32 %), Ceftriaxone (1.25 %), Cefepem (4.53 %), which is less than this study [21]. While in a study conducted by Majumder M. et al there was resistance to Cefuroxime (54.17 %), Ciprofloxacin (27 %), Nitrofurantoin (79.41 %), which is in agreement with this study [22].

Acinetobacter baumannii only has extremely limited antibiotic resistance; they were resistant to Piperacillin/Tazobactam, Cefazolin, Ciprofloxacin, (33.3 %, 66.6 %, 33.3 %) respectively. In a study conducted by Salman H.A. et al the rate of resistance to Piperacillin/Tazobactam and Ceftriaxone were (50 %, and 75 %) respectively [17]. While Hasan M.T. et al antibiotic susceptibility test for *Acinetobacter baumannii* were resistance to Ampicillin (100 %), Gentamicin (75 %), Meropenem (50%), Imipenem (75 %), Ciprofloxacin (50 %), Trimethoprim/Sulfamethoxazole (75 %) which disagree with current study [18]. While *Proteus mirabilis* were resistance to Cefazolin (100 %), Cefuroxime (100 %), Cefuroxime/Axetile (100 %), Ceftriaxone (100 %), Imipenem (100 %),Nitrofurantoin (100 %), which disagrees with Joya M. et al. in which *Proteus mirabilis* were resistance to Ceftriaxone (20 %), Nitrofurantion (40 %), and it was susceptible to Imipenem (100 %) [13]. For *Pseudomonas aeruginosa* they were resistant only for Cefazolin and Imipenem (100 %, 50 %) respectively. Is disagrees with Habibi A. and Honarmand R. whose isolates of *Pseudomonas aeruginosa* were highly resistant to antibiotics Ampicillin (87.5 %), Amikacin (962.5 %), Gentamicin (62.5 %), Imipenem (12.5 %), Aztreonam (62.5 %) [23].

Antibiotic resistance of Gram-positive bacteria:

Antibiotic susceptibility test was conducted for All Gram-positive bacteria in this study, as indicated in (Table 4). The findings demonstrate that the Gram-positive bacteria that were isolated from children who had UTIs were extremely resistant to the antibiotics utilized in this investigation. *Staphylococcus haemolyticus* isolates show high resistance to Cefoxitin, Benzylpenicillin, Oxacillin, Erythromycin, and Fusidic acid as (100%) for each antibiotic, while (85.75%) for Clindamycin, Tetracycline (71.4%), Ciprofloxacin (42.8%), both Gentamicin and Vancomycin was (28.5%), while Teicoplanin, Moxifloxacin, Rifampicin, Trimethoprime-sulfamethoxazole were (14.2%), all isolates were sensitive for Tigecycline and Linezolid (100%). These results in agreement with Luty R.S. et al. in resistance to, Benzylpenicillin (100%), Erythromycin (89%), Ciprofloxacin(46%), Teicoplanin (11%), Tetracycline (54%) , Fusidic acid (95%), and agreement with sensitivity to Tigecycline (100%) [24]. And in agreement with Afik A. et al. study in resistance to Cefoxitin, Benzylpenicillin both (100%), Gentamicin (22%), Trimethoprime-sulfamethoxazole (27.8%) and sensitive (100%) to Linezolid [25].

Out of all the coagulase negative staphylococci that have been examined, *Staphylococcus haemolyticus* is recognized for its resistance to a wide range of antibiotics, such as cephalosporins, penicillins, macrolides, tetracyclines, quinolones, and amino-

Table 3. Antimicrobial resistance rate number and percentage of Gram-negative uropathogen.

Drugs classes	Antibiotics	Strain (Number of isolates)				
		E.coli (72)	*K. pneumonia* (18)	*A. baumanii* (9)	*P. mirabilis* (6)	*P. aeruginosa* (6)
Penicillins	Ampicillin	24 (33.3%)	0	0	0	0
	Amoxicillin/ Clavulanic Acid	6 (8.3%)	3(16.6%)	0	0	0
	Piperacillin/ Tazobactam	0	0	3 (33.3%)	0	0
Cephalosporins	Cefazolin	24 (33.3%)	3(16.6%)	6 (66.6%)	6 (100%)	6 (100%)
	Cefuroxime	36 (50%)	6 (33.3%)	0	6 (100%)	0
	Cefuroxime/ Axetile	21 (29.1%)	3 (16.6%)	0	6 (100%)	0
	Ceftazidime	24 (33.3%)	3 (16.6%)	0	0	0
	Ceftriaxone	18 (25%)	3 (16.6%)	0	6 (100%)	0
	Cefepime	12 (16.6%)	3 (16.6%)	0	0	0
Aminoglycosides	Amikacin	0	0	0	0	0
	Gentamicin	6 (8.3%)	0	0	0	0
Carbapenems	Meropenem	0	0	0	0	0
	Imipenem	0	0	0	6 (100%)	3 (50%)
	Ertapenem	0	0	0	0	0
Quinolones	Ciprofloxacin	30 (23.8%)	3(16.6%)	3 (33.3%)	0	0
Trimethoprim	Trimethoprime/ Sulfamethoxzole	33 (45.8%)	3 (16.6%)	0	0	0
Others	Nitrofurantoin	3 (4.1%)	9 (50%)	0	6 (100%)	0
	Fosfomycin	0	6 (33.3%)	0	0	0

Table 4. Antimicrobial resistance rate number and percentage of Gram-positive uropathogen.

Drugs classes	Antibiotics	Strain (Number of isolates)				
		Staph. haemolyticus (21)	*Staph. aureus* (18)	*Staph. epidermidis* (9)	*Staph. lentus* (6)	*E. faecalis* (3)
Cephalosporins	Cefoxitine	21 (100%)	18 (100%)	9 (100%)	6 (100%)	0
Penicillins	Benzylpenicillin	21 (100%)	18 (100%)	9 (100%)	6 (100%)	0
	Oxacillin	21 (100%)	18 (100%)	9 (100%)	6 (100%)	0
Aminoglycosides	Gentamicin	6 (28.5%)	18 (66.6%)	9 (100%)	3 (50%)	3(100%)
Quinolones	Ciprofloxacin	9 (42.8%)	15 (83.3%)	0	0	0
	Moxifloxacin	3 (14.2%)	3 (16.6%)	0	0	0
Macrolides	Erythromycin	21 (100%)	15 (83.3%)	6 (66.6%)	6 (100%)	2(66.6%)
Lincosamide	Clindamycin	18(85.7%)	3 (16.6%)	9 (100%)	3 (50%)	0
Glycopeptide	Vancomycin	6 (28.5%)	0	3 (33.3%)	3 (50%)	0
	Teicoplanin	3 (14.2%)	3 (16.6%)	6 (66.6%)	0	0
Tetracycline	Tetracycline	15(71.4%)	12 (66.6%)	9 (100%)	3 (50%)	2(66.6%)
	Tigecycline	0	0	0	0	0
Rifamycins	Rifampicin	3 (14.2%)	0	0	0	0
Trimethoprim	Trimethoprim-sulfamethoxazole	3 (14.2%)	6 (33.3%)	6 (66.6%)	3 (50%)	0
Others	Linezolid	0	0	0	0	0
	Fusidic acid	21 (100%)	18 (100%)	6 (100%)	6 (100%)	0

glycosides. The role of *Staphylococcus haemolyticus* as a reservoir for antibiotic resistance and virulence traits is a significant but overlooked issue that demands further scientific investigation. This bacterium may possess numerous enzymes that contribute to antibiotic resistance and pathogenicity. Additionally, horizontal gene transfer plays a crucial role in the acquisition of antibiotic resistance genes in staphylococci [26]. *Staphylococcus aureus* antibiotic susceptibility test was as follows, resistance to Cefoxitin, Benzylpenicillin, Oxacillin, and Fusidic acid as (100%) for each one, while both Gentamicin, Tetracycline were (66.6%), Erythromycin (83.3%), Ciprofloxacin (83.3%), Trimethoprime-sulfamethoxazole (33.3%), Moxifloxacin, Clindamycin, Teicoplanin were resisted in rate (16.6%), on the other hand, all isolates were susceptible to Vancomycin, Tigecycline, Rifampicin, Linezolid (100%). This study is in agreement with study conducted by Gurung R.R. et al. there isolated *Staphylococcus aureus* was resistant to Cefoxitin, Benzylpenicillin (100%) for both antibiotics, Erythromycin (74%), Tetracycline (51.3%), Ciprofloxacin (53.9%), Clindamycin (25.6%), and they were sensitive to Vancomycin (100%) [27]. There was agreement in resistance to Gentamicin in rats (85%) with Garba G.I.B.I. et al. [28]. And in agreement with Alman H.A. et al. study in resistance to Teicoplanin (33%) [17].

The uroepithelium employs local defensive mechanisms, such as the formation of mucous and secretion of antimicro-

bial peptides, to prevent bacterial adhesion to uroepithelial cells, any breakdown in these defense mechanisms will allow the entry of normal flora that have colonized the urogenital area into the urinary tract [29]. *Staphylococcus epidermidis* was resistant to Cefoxitin, Benzylpenicillin, Oxacillin, Gentamicin, Clindamycin, Tetracycline, Fusidic acid in rate of (100%) for all isolates, while it was resist to Erythromycin, Teicoplanin, Trimethoprime-sulfamethoxazole (66.6%), Vancomycin (33.3%) while it was sensitive to Ciprofloxacin, Moxifloxacin, Tigecycline, Rifampicin, Linezolid (100%) for each one. This was in agreement with Luty R.S. et al. in which there results were resistant to Benzylpenicillin, Clindamycin, and Fusidic acid (100%), while Trimethoprime-sulfamethoxazole was (100%), and all of them were sensitive to Tigecycline, Linezolid (100%) [24]. While *Staphylococcus lentus* were resistant to Cefoxitin, Benzylpenicillin, Oxacillin, Erythromycin, and Fusidic (100%) for all isolates, and resist at a rate of (50%) for Gentamicin, Clindamycin, Vancomycin, Tetracycline, Trimethoprime-sulfamethoxazole, while it was sensitive (100%) to Ciprofloxacin, Moxifloxacin, Teicoplanin, Tigecycline, Rifampicin, Linezolid (100%). This in agreement with a study conducted by Al-Salamy M.H. in which *Staphylococcus lentus* was resist to Benzylpenicillin (100%), Cefoxitin (94%), Erythromycin (96%), while disagree in resistance to Clindamycin (96%), Tetracycline (28%), *Trimethoprime-sulfamethoxazole* (20%), while there isolates was sensitive to Vancomycin (100%) [30]. *Enterococcus faecalis* isolates was resistance to Gentamicin (100%), Erythromycin and Tetracycline (66.6%). It was sensitive to the rest of the antibiotics used in the study. The overall number of MDR bacteria isolated in the present study was 57, accounting for 34% of the total bacterial isolates.

Biofilm formation of Gram-negative bacteria:

Biofilm production is one of the primary strategies employed by bacteria to survive in harsh environments. Closed bacterial communities provide safety and protection, enabling bacteria to elude human defenses and conceal themselves from pathogens. Bacteria exhibit a higher rate of horizontal gene transfer, resistance gene exchange, and virulence gene exchange among biofilm communities [31]. In the current study, *E.coli* produced biofilm at a rate of 66.7 %, this result was in agreement with Karigoudar R.M. et al. study as they obtained 69 % biofilm producer *E.coli* [32]. While *Klebsiella pneumonia* produced biofilm at a rate of (33.4 %), which disagreed with the study conducted by Ramadan R. et al. they recorded (86.8%) of biofilm former [33]. These differences may have contributed to the sampling method in which they obtained their samples from catheterized patients, while in this study the samples were midstream urine. For *Pseudomonas aeruginosa* biofilm formation was in rate of (66.6%) while *Proteus mirabilis* biofilm producing were in rate of (100%), which were in agreement with study conducted by Kwiecinska-Pirog J. et al. where *Proteus mirabilis* form biofilm (100%) [34], for *Acinetobacter baumannii* biofilm formation was (33.6%) (Table 5).

Biofilm formation of Gram-positive bacteria:

In Gram-positive bacteria (Table 6), *Staphylococcus haemolyticus* was biofilm former at the rate of (85.6 %), this high rate of biofilm forming *Staphylococcus haemolyticus* may be contributed to the fact that most of the isolates were MDR [26], the result of a study conducted by Rafik A. et al. the biofilm forming in *Staphylococcus haemolyticus* was (95 %) [25]. In *Staphylococcus aureus* isolates biofilm forming was (83.3 %), as in the study by Samadi R. et al. they obtained (81.6%) biofilm former [35]. For *Staphylococcus epidermidis* the rate was (100%) which disagreed with Borooni S. et al. they obtained 45 % biofilm forming from their isolates [36], this difference may contributed to the fact that most isolates from this study were MDR. *Staphylococcus lentus* biofilm formation was (100%), this result was in agreement with Al-Azawi I.H. study who obtained (100 %) biofilm former *Staphylococcus lentus* [37]. This capability is attributed to the fact that coagulase-negative Staphylococci have the ability to create urease, which raises the pH of urine and influences the production of calcium and magnesium phosphate crystals. These crystals, in turn, promote the creation of biofilm [37]. On the other hand, there were two biofilm forming bacteria for *Enterococcus faecalis* in a total of three isolates.

Association between biofilm formation bacteria and MDR bacteria:

The result of the current study revealed that there was a significant association between biofilm formation bacteria and MDR bacteria (P-value < 0.001). All MDR bacteria that were isolated from children with UTIs were able to produce biofilm (Table 7). This result was in agreement with Maione A. et al. in which all of their MDR bacteria that were isolated from patients with UTIs were able to produce biofilm [38]. Also in agreement with Mirzahosseini H.K. et al., the majority of their bacterial isolates were biofilm producers and rendering high antimicrobial resistance [39].

Table 5. Biofilm formation among Gram-negative uropathogen bacteria.

Strain (number of isolate)	Types of biofilm				Total biofilm former No.%
	Weakly adherent No.%	Moderately adherent No.%	Strongly adherent No.%	Non biofilm former	
E.coli (72)	39 (54.1 %)	6 (8.4 %)	3 (4.2 %)	24 (33.3 %)	48 (66.7 %)
K.pneumoniae(18)	3 (16.7 %)	3 (16.7 %)	0	12 (66.6 %)	6 (33.4 %)
P.aeruginosa(6)	4 (66.6 %)	0	0	2 (33.4 %)	4 (66.6 %)
P.mirabilis(6)	6 (100 %)	0	0	0	6 (100 %)
A.baumannii(9)	3 (33.6 %)	0	0	6 (66.4 %)	3 (33.6 %)

Table 6. Biofilm formation among gram positive uropathogen.

Strain (number of isolate)	Types of biofilm				Total biofilm former No.%
	Weakly adherent No.%	Moderately adherent No.%	Strongly adherent No.%	Non biofilm former	
Staph.haemolyticus(21)	9 (42.8 %)	9 (42.8 %)	0	3 (14.4 %)	18(85.6 %)
Staph.aureus(18)	12 (66.8 %)	3 (16.6 %)	0	3 (16.6 %)	15(83.4 %)
Staph.epidermidis(9)	6 (66.7 %)	0	3 (33.3 %)	0	9 (100 %)
Staph.lentus(6)	6 (100 %)	0	0	0	6 (100 %)
E.faecalis(3)	1(33.3 %)	1(33.3 %)	0	1 (33.4 %)	2(66.6 %)

Table 7. Association between biofilm formation and MDR bacteria.

Biofilm formation	MDR		P-value
	Yes	No	
Negative	0	51	
Weak	36	54	
Moderate	18	3	< 0.001
Strong	3	3	
Total	57	111	

MDR=Multidrug-Resistant

Biofilm provides an additional protective mechanism for enclosed bacterial cells to evade the harmful effects of antimicrobial agents, as well as harsh environmental conditions [40].

Bacteria within the biofilm exhibit distinct behaviors compared to their planktonic counterparts, particularly in relation to antibiotics, resulting in constraints on standard antibiotic treatments. Studies have revealed that bacteria residing within the biofilm exhibit a resistance to antimicrobial treatments that is 10–1000 times greater than that of bacteria in the planktonic state [41].

CONCLUSION

The study identified multidrug-resistant bacteria in children with urinary tract infections, with *Escherichia coli* being the most common Gram-negative bacterium and *Staphylococcus haemolyticus* the predominant Gram-positive bacterium. Amikacin, Meropenem, and Ertapenem were the most effective antibiotics against Gram-negative isolates, while Linezolid, Tigecycline, and Rifampicin were most effective against Gram-positive bacteria. Most bacteria especially MDR strains, were capable of biofilm formation, making them more resistant to standard antibiotic treatments and necessitating stronger or higher-dose therapies.

ETHICAL DECLARATIONS

- **Acknowledgements**

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- **Ethics Approval and Consent to Participate**

  The study protocol, subject information, and permission form underwent assessment and approval by a local ethics committee in Kirkuk Health Directorate, as per document number 594, dated 18/9/2023.

- **Consent for Publication**

  Non.

- **Availability of Data and Material**

  The datasets are available from the corresponding author upon reasonable request.

- **Competing Interests**

  The authors declare that there is no conflict of interest.

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- **Authors' Contributions**

  All authors contributed significantly, directly, and intellectually to the work and consented to its publication.

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