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# Evaluation of Biofilm Formation in Molecular Identification *E. coli* Strains that Cause Urinary Tract Infection in Children and Antibiotic Resistance

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## Abstract

**Background:** *Escherichia coli* (*E. coli*) is the primary causative agent of urinary tract infections (UTIs), one of the most common human illnesses, which frequently occurs in children.

**Objective:** This study aimed to identify *E. coli* strains that cause UTIs in children and determine the correlation between biofilm formation and antibiotic resistance.

**Patients and Methods:** A total of 290 cases of UTI patients were collected from Al-Batoul Teaching Hospital in Diyala, Iraq. The ages of these patients ranged from 1 day to 12 years, from February 2023 to January 2024. The strains of *E. coli* that cause UTIs were identified using polymerase chain reaction (PCR) and sequencing methods. Antimicrobial susceptibility was evaluated, and a microtiter plate assay was used to assess biofilm production.

**Results:** The predominant bacteria responsible for UTI in children were *E. coli* (40%), and it was noted that the lowest percentage of bacteria causing UTI in this study were *Klebsiella oxytoca* and *Pseudomonas aeruginosa*, as they appeared in 5% of cases. The strains of *E. coli* that cause UTIs in the current study are *E. coli* Y8-2 (14.8%), *E. coli* 106K88 (19.3%), *E. coli* UA32 (11.4%), *E. coli* RM11911 (20.5%), and *E. coli* EC1704-1 (34%). *E. coli* EC1704-1 showed multidrug resistance (MDR) to ciprofloxacin (100%), sulfamethoxazole-trimethoprim (100%), cephalosporins and penicillin (100%), and aminoglycosides (93.3%). *E. coli* Y8-2, *E. coli* 106K88, and *E. coli* UA32 appeared less resistant to antibiotics than *E. coli* EC1704-1 and *E. coli* EC1704-1. Additionally, it was demonstrated that biofilm formation and antimicrobial resistance were negatively correlated among the isolates.

**Conclusion:** This study demonstrated a clear link between biofilm formation and antibiotic resistance, suggesting that this bacterium with reduced resistance may depend on biofilms to enhance its survival.

**Keywords:** *16SrRNA*, *Escherichia coli* strains, Antibiotic resistance, Urinary tract infection, Biofilm formation.

## Introduction

Urinary tract infections (UTIs) are the most common bacterial infections, affecting approximately 150 million people worldwide each year, and are often seen in children. Understanding the underlying

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infections and their antibiotic resistance patterns in certain regions are crucial for providing the best possible care (1, 2). UTIs might cause short-term complications, including fever, painful urination, lower abdominal discomfort, and may lead to irreversible kidney scarring (3). Urinary tract infection is more prevalent in women than in men due to the close physical proximity of the urethra to the end of the gastrointestinal tract (4). Screening for bacterial susceptibility in each city is critical for generating essential data on antibiotic resistance (5, 6). UTI problems mostly involve kidney failure caused by severe renal damage and sepsis, which results from the infection spreading beyond the lower urinary tract to other areas of the body. These infections are often treated with antibiotics. Antibiotic susceptibility testing is crucial for doctors to select appropriate medications for patients with urinary tract infections (5). The frequency of antibiotic resistance in *E. coli* causing UTIs in children in primary care is significant. Therefore, it is essential to examine the treatment usage and antibiotic resistance patterns of bacteria in patients with UTIs. Various risk factors for UTIs in children were identified, such as sex, ethnicity, vesicoureteral reflux, neurogenic bladder, phimosis, structural anomalies of the lower urinary tract, constipation, and delaying voiding have been linked to UTIs (7). In newborns, urinary tract infections might present with nonspecific symptoms such as poor feeding, diarrhea, failure to grow, vomiting, moderate jaundice, lethargy, fever, and hypothermia. In certain instances, UTI can progress to neonatal sepsis. Infants under 2-years old with UTIs may have non-specific symptoms, including fever, gastrointestinal issues such as vomiting, diarrhea, abdominal discomfort, or urine with a strong odor. In children older than 2-years, cystitis or pyelonephritis may present with typical symptoms. (8). *Escherichia coli* is the predominant bacterium responsible for causing urinary tract infections in children. *E. coli* strains possess several genes encoding various virulence factors that significantly contribute to the bacteria's pathogenicity. The severity of urinary tract infection depends on the virulence gene characterization of the invading *E. coli* strain (9, 10). In addition, antimicrobial resistance (AMR) has become a major threat to world health. It is expected that AMR will cause 10 million deaths by the year 2050 (6, 7). The broad distribution of antibiotics to urinary tract pathogens with resistance, such as *E. coli*, is driven by the widespread use of antibiotics to treat UTIs (8). Afterward, the course of treatment of UTIs has been increasingly complex as a result of the multidrug-resistant (MDR), particularly in those suffering from repeated UTIs (9-11). One characteristic that hinders bacterial removal during antibiotic therapy is the development of biofilms on biological surfaces. The 3-dimensional multicellular biofilms are arranged in groups that have the ability to adhere to both abiotic and biological surfaces and are coated with an extracellular polymeric substance (12, 13). *E. coli* cells may produce biofilms inside and on the surface of catheters (3, 14,15). An in-depth investigation into the connection between biofilm and antibiotic resistance reveals incongruous results regarding its forming capability. Biofilm generation has been shown to improve the resistance of bacteria by a number of methods, including decreased expansion and reduced spreading rate of antimicrobials (14, 16). Biofilms might work independently of the process, mostly used for bacterial resistance by sensitive isolates as a method of surviving (17-20). A significant distinction in the sensitivity between implanted biofilm cells and planktonic cells that are part of the reports of the same strain exists (21, 22). When collectively, these Observations emphasise how crucial it is to consider biofilm formation capacity as an essential bacterial factor in the treatment strategy for UTIs (23, 24). Hence, this

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study aimed to identify *E. coli* strains that cause UTIs in children and study the correlation between biofilm formation and antibiotic resistance of *E coli* isolates.

## Patients and Methods

**Bacterial isolation and identification:** It was collected 290 urine samples of children for cultures and sensitivity tests from February 2023 to January 2024. Information on age and gender was recorded; the ages of these patients ranged from 1 day to 12 years. The study was conducted at Al-Batoul Teaching Hospital in Diyala Province, Iraq. The clinical urine specimens were obtained from midstream and catheter-aspirated urine samples of individuals diagnosed with urinary tract infections, prior to the initiation of antimicrobial therapy. The positive urine cultures were defined as having a bacterial count of at least 105 CFU/mL (25). The colonial morphology of the isolated bacteria on various culture media, including blood agar, MacConkey agar (Oxoid, USA), and Cysteine Lactose Electrolyte Deficient (CLED) agar (Oxoid, USA), was initially used to identify the bacteria. Gram staining was also employed in this process. Several common biochemical tests (methyl red test, KIA test, Voges-Proskauer test, indole test, citrate test, and urease test) were used further to validate the identification of *E. coli* (26). For long-term preservation, the isolates were then kept in tryptic soy broth (TSB) supplemented with 20% glycerol at -80 °C.

**Antimicrobial susceptibility testing (AST):** The susceptibility tests of bacterial isolates for the antibiotics were done by following the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) (27). It was determined that the susceptible isolates to nineteen different antimicrobial drugs across thirteen different categories. Azithromycin, nitrofurantoin, tetracycline, chloramphenicol, imipenem, meropenem, cefozolin, cefuroxime, cefotaxime, ciprofloxacin, ceftazidime, gentamicin, amikacin, trimethoprim-sulfamethoxazole, aztreonam, ampicillin, and amoxicillin-clavulanic acid were among the agents employed in the Kirby-Bauer disc diffusion method. Classified isolates as multi-drug resistant (MDR) if they resisted at least three antimicrobial classes. The method previously outlined (28) was used to calculate the multiple antibiotic resistance (MAR) indexes.

**Molecular methods for *E. coli* detection:** Using 16S-R and 16S-F primers, *16S rRNA* was amplified by polymerase chain reaction (PCR), and molecular detection was applied to all 88 *E. coli* isolates. DNA was extracted from *E. coli* isolates and then subjected to PCR analysis using the identified gene. The gene was amplified by polymerase chain reaction (PCR) using primers specific to the *16SrRNA*. F (AGAGTTTGATCCTGGCTCAG) and R (TACGGTTACCTTGTTACGACTT) were the primers used. This was followed by verifying the amplified target genes using agarose gel electrophoresis (10, 11).

**DNA sequencing:** The amplified gene product was purified using a Sanger sequencing ABI 3730XL for DNA sequencing before being sent to Macrogen Corporation in Korea for further study. After receiving the data via email, powerful software was used to analyse it and identify the different strains of *E. coli* (12, 13).

**Biofilm formation assay:** As previously mentioned, the 96-well microtiter plate assay assessed the isolates' ability to produce biofilms (29). Luria Bertani (LB) broth was used as the medium, and bacterial suspensions were shaken and cultured at 37 °C for 18 to 24 hours. Subsequently, the suspensions were diluted 1: 100 to a final level of 200 µL in M63 medium containing 0.25% glucose. The inoculated microtiter plates were then incubated for 48 hours at 30 °C without shaking. After the culture was removed, sterile phosphate-buffered saline (PBS)

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was used to wash the wells. Biofilms were dried at 65 °C for ten minutes, then cleaned with PBS and dyed with 2% crystal violet (CV). Thereafter, 33% glacial acetic acid was used to dissolve the adhering CV, and to calculate the biofilm production capacity, the optical density at 580 nm (OD580) was measured using a microplate reader (SunriseTM, TECAN, Switzerland). The medium that was not inoculated acted as a negative control. The experiment was run in duplicate. The cutoff value (ODc) was determined as three standard deviation units over the average absorbance of the sterile media, as previously mentioned (30). The isolates were then divided into four categories: moderate biofilm-producing isolates ($2ODc \geq OD \leq 4ODc$), strong biofilm-producing isolates ($OD \geq 4ODc$), weak biofilm-producing isolates ($ODc \geq OD \leq 2ODc$), and non-biofilm-producing isolates ($OD \leq ODc$) (31).

## Statistical analysis

In order to do statistical analysis, the Graphpad Prism program (Graphpad, California, United States) was utilized. Analysis of variance (ANOVA) was performed in either a one-way or two-way fashion in order to compare the groups for the investigations.

## Results

**Isolation and identification of *E. coli*:** The results of this study showed male and female patients were suspected to the urinary tract infection, 93 (32.1 %) and 197 (67.9 %), respectively. According to the current study's findings of infections were significantly greater in female patients than in male patients (Figure 1).

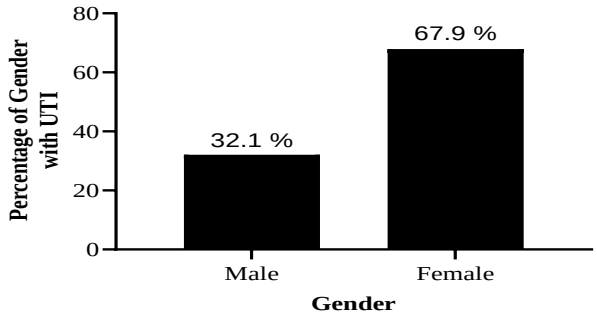


***Figure 1.*** *Distribution of UTI patients according to their gender.*

In addition, the patients were divided into four age groups, and the results showed that the percentage of infection increased in the age group of 8 to 12 (49.7%) and decreased within the first year of age. It was shown that both the gender and age of the patients had a significant impact on the prevalence of the condition (Figure 2).

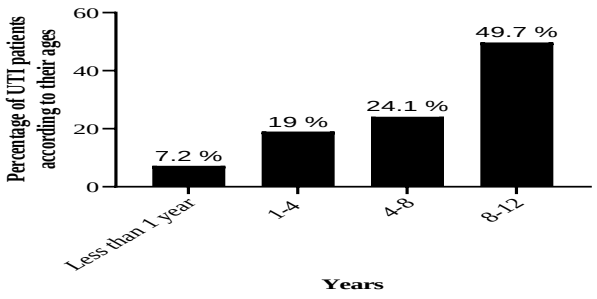


**Figure 2**. Distribution of UTI patients according to their ages.

The results of this study showed that there are six different types of bacteria were caused UTI in children and the most common bacteria that cause this infection are *E coli* 88 (40 %), *Klebsiella pneumoniae* 60 (27.3 %) and *Proteus* spp 38 (17.3 %). It appeared that *Staphylococcus saprophyticus*, *Kebsiella oxytoka* and *Psuedomonas aureginosa* have the lowest percentage of bacteria caused UTI infections among children as appeared in 12 (5.4 %), 11 (5 %) and 11 (5 %), respectively of cases (Figure 3).

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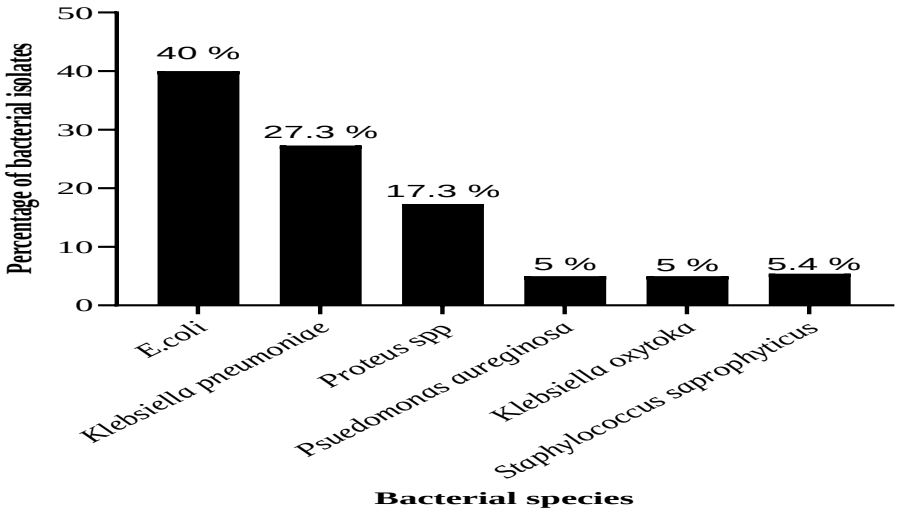


**Figure 3.** The types of bacteria that cause UTIs in children.

**Molecular detection of *E. coli*:** In this study, the molecular identification of *E. coli* was done using *16SrRNA* that was amplified via PCR by using the specific primers, and the results showed an amplified 1500 bp PCR product of the same size as the target gene, as shown in Figure 4.

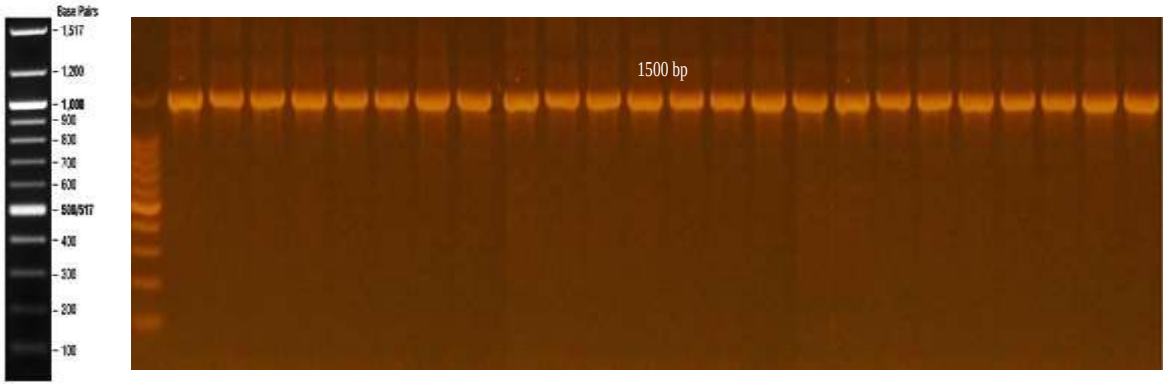


**Figure 4:** Agarose gel electrophoresis of PCR products. The results appeared to amplify 1500 bp fragments, which is the size of *16SrRNA* as compared to the molecular ladder, which is between 1500 and 100 base pairs.

**Sequencing results:** In order to determine the strains of *E. coli* that are responsible for urinary tract infections (UTIs), the PCR products were purified and then sent for sequencing. According to the analysis of DNA sequencing alignment findings, it was shown that several strains of this bacterium that cause urinary tract infections in children, which are *E. coli* Y8-2 13 (14.8 %), *E. coli* 106K88 (19.3 %), *E. coli* UA32 (11.4 %), *E. coli* RM11911 (20.5 %), and *E. coli* EC1704-1 (34 %) as shown in Figure 5.

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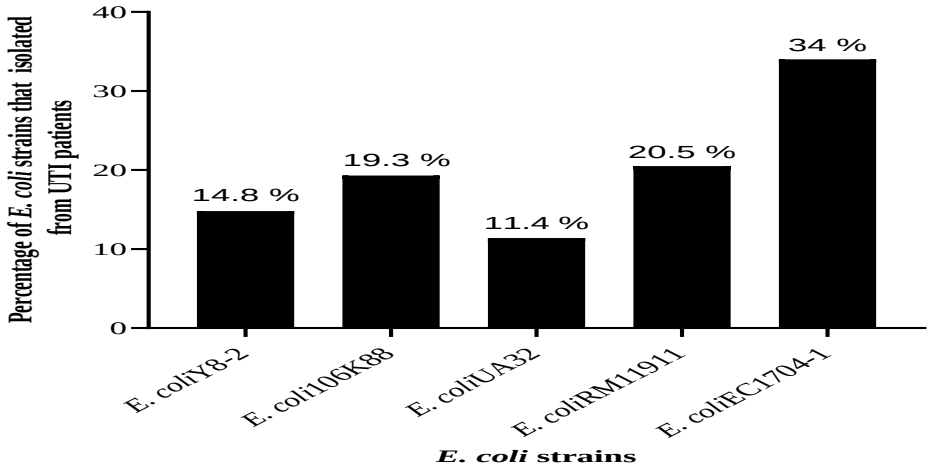


**Figure 5.** The percentages of *E. coli* strains, which they identified according to the DNA sequencing alignment results.

**Antibiotic susceptibility and biofilm formation of E. coli strains:** The results of this study showed that *E. coli* EC1704-1 and *E. coli* RM11911 were multi-drug resistant, which showed resistance against most of the tested antibiotics. However, *E. coli* Y8-2, *E. coli* 106K88, and *E. coli* UA32 appeared less resistant against the antibiotics that were used in this study compared with the first two strains, as shown in Table 1. The antibiotic sensitivity test was done for *E. coli* strains, and the results showed that *E. coli* EC1704-1 and *E. coli* RM11911 were multi drug resistant, which showed resistant against most of the tested antibiotics. However, *E. coli* Y8-2, *E. coli* 106K88, and *E. coli* UA32 appeared less resistant against the antibiotics that were used in this study.

**Table 1.** Percentages of antibiotic resistance (%) of *E. coli* strains that were isolated from UTI patients against the tested antibiotics.

| Antibiotic category | Antimicrobial agent | E.coli Y8-2 | E. coli 106K88 | E. coli UA32 | E. coli RM11911 | E. coli EC1704-1 |
|---|---|---|---|---|---|---|
| Non-Extended spectrum cephalosporin | Cefazolin<br>cefuroxime | 0.00<br>0.00 | 5.9<br>47 | 0.00<br>10 | 77.8<br>83.3 | 100<br>100 |
| Extended spectrum cephalosporin | Cefotaxime<br>ceftazidime | 7.7<br>0.00 | 11.8<br>11.8 | 10<br>10 | 83.3<br>72.2 | 100<br>93.3 |
| Fluoroquinolones | ciprofloxacin | 0.00 | 0.00 | 10 | 100 | 100 |
| Folate pathway inhibitors | Trimethoprim-sulfamethoxazole | 30.8 | 41.2 | 0.00 | 100 | 100 |
| Aminoglycosides | Gentamicin<br>Amikacin | 15.4<br>7.7 | 47.1<br>5.9 | 40<br>10 | 83.3<br>100 | 93.3<br>93.3 |
| Carbapenems | Imipenem<br>Meropenem | 0.00<br>0.00 | 5.9<br>0.00 | 20<br>0.00 | 83.3<br>44.4 | 83.3<br>50 |
| Monobactams | Aztreonam | 15.4 | 5.9 | 0.00 | 55.6 | 66.7 |
| Penicillins | Ampicillin | 23.1 | 41.2 | 70 | 100 | 100 |
| Penicillin-Betalactamase inhibitor | Amoxicillin-clavulanic acid | 23.1 | 5.9 | 0.00 | 100 | 100 |

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| | | | | | | |
|---|---|---|---|---|---|---|
| Phenicols | Chloramphenicol | 0.00 | 0.00 | 20 | 33.3 | 50 |
| Tetracycines | Tetracycine | 7.7 | 29.4 | 20 | 55.6 | 16.7 |
| Macrolide | Azithromycin | 7.7 | 11.8 | 0.00 | 11.1 | 33.3 |
| Nitrofurans | Nitrofurantoin | 0.00 | 5.9 | 10 | 22.2 | 16.7 |

Biofilm formation was done for *E. coli* strains identified in this study. The 96-well microtiter plate assay, the gold standard for estimating the biofilm formation capacity, was used to assess the biofilm formation capacity of *E. coli* strains in M63 broth. The strains were then separated into three groups including, weak, moderate, and strong. The results of this study showed that the strains, *E.coli* EC1704-1 and *E. coli* RM11911 created OD 0.5 and 0.7, respectively (weak biofilms), *E. coli* UA32 produced OD 1.1 (moderate biofilms), and *E. coli* 106K88 and *E. coli* Y8-2 produced OD 1.4 and 1.6, respectively (strong biofilms). This correlation was found to be statistically significant ($P < 0.0001$) (Figure 6).

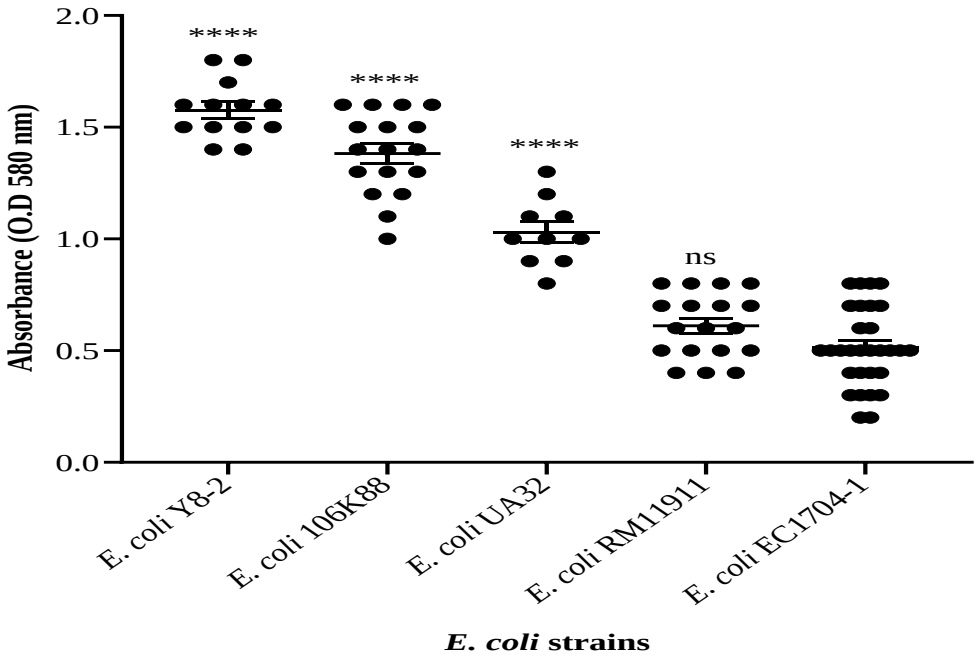


**Figure 6.** Biofilm formation capacity among *E. coli* strains. The experiment was repeated using three replicates of four independent biological samples. Significant differences were observed when comparing the biofilm formation of E. coli EC1704-1 with that of other strains using one-way ANOVA and Dunnett's multiple comparisons test. (**** $p<0.0001$, ns= non-significant, relative to the *E. coli* EC1704-1).

## Discussion

Several bacteria cause UTIs in children, and *E. coli* was chosen for antimicrobial susceptibility testing and molecular identification due to the high percentage of this bacterium that causes UTIs. Both the gender and age of the patients had a significant impact on the prevalence of the condition.

According to the findings of the current study, the incidence of infections was significantly greater in female patients compared to male patients. Similarly, it was reported that the same prevalence in males and females within the first year of age increased the UTI infection in females compared with males after the first year of life (14). In addition, this study demonstrated that the risk of UTI increases after the age of 8. This data, similar to that of other studies,

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reports that UTIs are believed to be caused by a shorter distance between the anus (the typical source of uropathogens) and the urethral meatus, the longer length of the male urethra, and the antibacterial activity of prostatic fluid in males (15). This condition is more prevalent in the female population, particularly among young and middle-aged individuals. In accordance with the findings of earlier research, which revealed that *E. coli* was present in 75–90% of UTI isolates, it was demonstrated that *E. coli* was the bacterium that caused the majority of UTIs, and molecular identification for the *E. coli* isolates was done using *16S rRNA* that is the housekeeping gene of most types of bacteria (21- 23). Thus, these findings are crucial for identifying the strains of this bacterium, which in turn leads to the control and prevention of this infection in children. The antibiotic sensitivity test was done for *E. coli* strains, and the results showed that *E. coli* EC1704-1 and *E.coli* RM11911 were multidrug-resistant, which showed resistance against most of the tested antibiotics. However, *E. coli* Y8-2, *E. coli* 106K88, and *E. coli* UA32 appeared less resistant against the antibiotics that were used in this study. This may be due to the fact that the route of medication administration is simple, as well as the fact that bacteria in the juvenile population are sensitive to antibiotics. Because of the nature of antibiotics and treatment recommendations for the route of drug administration in children, almost all of the medications were given intravenously (16, 17). This may have been the basis for the medication administration. Before deciding on a treatment plan for urinary tract infections (UTIs), it is strongly recommended to do an antibiotic sensitivity test. It is the only method to ensure that the treatment plan remains on track, and it should be carried out on a regular basis in order to monitor the development of antibiotic resistance in the various clinical settings used.

Target gene mutations and the acquisition of resistance genes by mobile genetic elements such integrons and plasmids, which can confer co-resistance to many antimicrobial agents (32, 33), are the primary causes of antimicrobial resistance. Additionally, biofilm formation provides a further defense mechanism that enables the encased bacterial cells to evade harsh ambient conditions and the damaging effects of antimicrobial agents (34). A potential link between acquired antimicrobial resistance and virulence has been suggested by the fact that both virulence and antimicrobial resistance genes can be transferred together through plasmids or other transferable genetic elements, in addition to the ability of acquired resistance, such as fluoroquinolone (FQ) resistance, to influence gene expression among resistant isolates (16). This study demonstrated that biofilm formation is negatively correlated with antibiotic resistance. It has previously been documented that the biofilm-forming ability of uropathogenic *E. coli* is negatively impacted by acquired antibiotic resistance (35). Similarly, Poursina and colleagues (31) found that multidrug-resistant (MDR) isolates were present in negative and weak biofilm-producing UPEC isolates. In contrast, non-MDR isolates comprised 69.2% of the strong biofilm-producing isolates. The biofilm architecture uses a number of surface appendages, including fimbriae, as well as additional non-fimbrial proteins, as a supporting framework. The expression of these organelles may be impacted by the development of antibiotic resistance, which would be detrimental to the ability to create biofilms (31, 36). Similarly, it has been previously observed that the acquisition of genes producing ESBL enzymes negatively affects the ability of *E. coli* and *Pseudomonas aeruginosa* build biofilms (37). This implies that biofilm formation is a method that helps bacteria to get better survival, especially with bacteria that are less antibiotic-resistant, and this may be due to the reduced exposure to multiple

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antibiotics (31, 38). All of these findings support the theory that uropathogenic to *E. coli* isolates' ability to form biofilms, which is negatively impacted by the development of antibiotic resistance.

**Conclusions**

*E. coli* strains that cause UTIs include *E. coli* Y8-2 (14.8%), *E. coli* 106K88 (19.3%), *E. coli* UA32 (11.4%), *E. coli* RM11911 (20.5%), and *E. coli* EC1704-1 (34%). This study demonstrated a negative correlation between antibiotic resistance and biofilm formation. The findings of this study contribute to a better understanding of the pathogenic potential of *E. coli* strains that can lead to severe cases of urinary tract infections. Additionally, it was recommended that identifying the expression of biofilm formation genes in this bacterium in the presence of antibiotics be crucial.

**Source of funding:** No source of funding.

**Ethical clearance:** The study protocol was approved by the Ethics Committee of Al-Batoul Teaching Hospital, a healthcare facility in Diyala, Iraq. The study was conducted, and samples were collected after receiving approval from the University of Diyala/College of Medicine's Research Ethics Committee (No.2024ASM878).

**Conflict of interest:** None.

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# تقييم تكوين الأغشية الحيوية في التعرف الجزيئي على سلالات الإشريكية القولونية المسببة لعدوى المسالك البولية عند الأطفال ومقاومة المضادات الحيوية

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## الملخص

**الخلفية:** تُعد الإشريكية القولونية (**E. coli**) العامل المسبب الرئيسي المسبب لألتهاب المسالك البولية، وهي من أكثر الأمراض شيوعًا، لا سيما بين الأطفال.

**الأهداف:** تهدف هذه الدراسة إلى تحديد السلالات من **E. coli** المسببة لألتهاب المسالك البولية لدى الأطفال، وتقييم العلاقة بين تكوين الأغشية الحيوية ومقاومة المضادات الحيوية.

**المرضى والطرق:** تم جمع ٢٩٠ حالة من مرضى التهاب المسالك البولية من مستشفى البتول التعليمي في محافظه ديالى ، العراق. تراوحت أعمار هؤلاء المرضى من يوم واحد إلى ١٢ عامًا ، من فبراير ٢٠٢٣ إلى يناير ٢٠٢٤. تم تحديد سلالات **E. coli** التي تسبب التهاب المسالك البولية باستخدام تفاعل سلسلة البلمرة (**PCR**) وطرق التسلسل. تم تقييم حساسية مضادات الميكروبات ، واستخدم اختبار لوحة **microtiter** لتقييم إنتاج الأغشية الحيوية.

**النتائج:** كانت البكتيريا السائدة المسؤولة عن التهاب المسالك البولية في الأطفال هي E. coli (٤٠ %) ، ولوحظ أن أدنى نسبة من البكتيريا التي تسبب التهاب المسالك البولية في هذه الدراسة كانت klebsiella oxytoca و pseudomonas aeruginosa ، كما ظهرت في ٥٪ من الحالات الاخرى. سلالات E. coli التي تسبب التهاب المسالك البولية في الدراسة الحالية هي E. coli Y8-2 (14.8%)، E. coli 106k88 (19.3%)، E.coli UA32 (11.4%)، E. coli RM11911 (٢٠,٣%)و E.coli EC1704-1 (٣٤%). اظهرت E. coli EC1704-1 مقاومة متعددة للادوية إلى سيبروفلوكساسين (١٠٠٪) ، سلفاميثوكسازول تريميثوبريم (١٠٠ %) ، السيفالوسبورين والبنسلين (١٠٠ %) ، والأمينوغليكوسيدات (٩٣,٣ %). ظهر E. Coli Y8-2 و E. Coli 106k88 و E. coli UA32 أقل مقاومة للمضادات الحيوية من E. coli EC1704-1 و E. Coli EC1704-1. بالإضافة إلى ذلك ، ثبت أن العلاقه بين تكوين الأغشية الحيوية ومقاومة مضادات الميكروبات كانت سلبيه بين العزلات.

**الاستنتاج:** أظهرت هذه الدراسة وجود صلة واضحة بين تكوين الأغشية الحيوية ومقاومة المضادات الحيوية ، مما يشير إلى أن هذه البكتيريا مع انخفاض المقاومة قد تعتمد على الأغشية الحيوية لتعزيز بقائها.

**الكلمات المفتاحية:** ***16SrRNA***، سلالات الإشريكية القولونية ، مقاومة المضادات الحيوية ، التهاب المسالك البولية ، تكوين الأغشية الحيوية.

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