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Antibiotic Resistance, Biofilm Formation, and Identification of *FimH* and *FimA* Adhesion Genes in Uropathogenic *Escherichia Coli* (UPEC) Isolated from Patients in Baghdad Province

Safa A. Abdul Hamid *, Ramina M. Khoshaba
Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq

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Abstract

Urinary tract infections (UTIs) are one of the most common infectious disorders worldwide. The most frequent cause of UTIs is uropathogenic *Escherichia coli* (UPEC). The current study is designed to assess the resistance of antibiotics, the formation of biofilm, and the detection of adhesion genes in *E. coli* isolated from patients with UTIs. A hundred and fifty samples were collected from patients with confirmed UTIs in Baghdad province during the period of October 2022 to February 2023. Isolation and identification of *E. coli* were performed using cultural characteristics, gram staining, and biochemical examination, and the results indicated that 52 (35%) isolates were identified as *E. coli*. Eleven antibiotic discs were utilized to estimate the sensitivity of *E. coli* isolates. 98% of isolates were resistant to ampicillin, followed by 75.5% of isolates resistant to both trimethoprim/sulfamethoxazole and fosfomycin, followed by 73.5, 71.4, 67.3, 53.1, and 6.1% of isolates resistant to imipenem, ceftazidime, nitrofurantoin, cefepime, and amikacin. All isolates were sensitive to piperacillin-tazobactam, ciprofloxacin, and aztreonam at 100%, 100%, and 98%, respectively. The microtiter plate method was utilized to estimate the ability of *E. coli* to form biofilm. The majority of isolates (69.2%; n = 36) were moderate biofilm producers, while 19.2% (n = 10) and 11.5% (n = 6) of isolates were strong and weak biofilm producers, respectively. Polymerase Chain Reactions (PCR) were applied to determine the gene expression level of adhesion genes (*fimH* and *fimA* genes). Forty isolates harbored both *fimH* and *fimA* genes.

Keywords: UTI, Antibiotics, Biofilm, *fimH*, *fimA*.

مقاومة المضادات الحيوية وتكوين الأغشية الحيوية وتحديد جينات الالتصاق *fimH* و *fimA* في الإشريكية القولونية المسببة للأمراض البولية (UPEC) والمعزولة من مرضى في محافظة بغداد

صفا علاء الدين عبد الحميد*، رامينا ميخائيل خوشابه
قسم التقنيات الأحيائية، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة

تعد التهابات المسالك البولية (UTIs) واحدة من أكثر الاضطرابات المعدية شيوعا في جميع أنحاء العالم. السبب الأكثر شيوعا لعدوى المسالك البولية هو الإشريكية القولونية المسببة للأمراض البولية (UPEC). تهدف الدراسة الحالية إلى تقييم مقاومة المضادات الحيوية ، وتكوين الأغشية الحيوية ، والكشف عن جينات

*Email: safa.aldeen87@gmail.com

الالتصاق في الإشريكية القولونية المعزولة من عينات عدوى المسالك البولية. تم جمع مائة وخمسون عينة من عينات الادرار من المرضى الذين تأكدت إصابتهم بعدوى المسالك البولية في محافظة بغداد ، خلال الفترة من تشرين الاول 2022 إلى شباط 2023. تم إجراء عزل وتحديد الإشريكية القولونية باستعمال الخصائص الزرعية والتصبيغ بصبغة كرام والفحص الكيميائي الحيوي وأشارت النتائج إلى أنه تم تحديد 52 (34%)عزلة فقط على أنها *E.coli*. تم استعمال أحد عشر قرصا من المضادات الحيوية لتقدير حساسية عزلات الإشريكية القولونية. 98٪ من العزلات كانت مقاومة للأمبيسيلين ، تليها 75.5٪ من العزلات المقاومة لكل من تريميثوبريم / سلفاميثوكسازول وفوسفوميسين ، تليها 73.5 و 71.4 و 67.3 و 53.1 و 6.1٪ من العزلات المقاومة للإيميبينيم والسيفتازيديم والنيتروفورانتوين والسيفيبيم والأميكاسين. كانت جميع العزلات حساسة لبيبيراسيلين–تازوباكتام وسيبروفلوكساسين وأزتريونام بنسبة 100 و 100 و 98٪ على التوالي. استعملت طريقة لوحة المعايرة الدقيقة لتقدير قدرة *الإشريكية القولونية* على تكوين الأغشية الحيوية. وكانت غالبية العزلات (69.2٪؛ عدد = 36) من منتجي الأغشية الحيوية المعتدلة، في حين أن 19.2٪ (عدد = 10) و11.5٪ (عدد = 6) من العزلات كانت منتجة للأغشية الحيوية القوية والضعيفة، على التوالي. تم تطبيق تفاعلات البلمرة المتسلسلة (PCR) لتحديد مستوى التعبير الجيني لجينات الالتصاق (جينات *fimH* و *fimA*) . اربعون عزلة احتوت على كل من جينات *fimH* و *fimA* .

1. Introduction

The most prevalent urological and renal condition is a urinary tract infection (UTI). Uropathogenic *Escherichia coli* (UPEC) is the most prevalent bacteria found in between 50 and 90 percent of all uncomplicated urinary tract infections [1]. *E. coli* is one of the most studied microorganisms due to its widespread use across medical, biological, and commercial fields [2, 3]. UPEC are *Escherichia coli* strains that have migrated from their commensal role as intestinal flora to cause urinary tract infections (UTIs) via several different virulence mechanisms. UPEC refers to *E. coli* strains that have been repeatedly linked to uropathogenicity [4, 5, 6, 7, 8].

Antibiotic resistance among gram-negative bacteria is a growing problem all over the globe [9]. Acquired resistance to three or more classes of antimicrobials, known as "multidrug resistance" (MDR), is now a significant barrier to effective treatment. With the advent of *E. coli* came a surge in resistance to many antibiotics, including fluoroquinolones, trimethoprim-sulfamethoxazole, and cephalosporins, which led to UPEC becoming an international public health problem. Uropathogens have recently become the most common antibiotic-resistant pathogens because of the widespread and careless use of antibiotics and the practice of prescribing antibiotics to treat UTIs without bacterial characterization, both of which have reduced the efficacy of oral therapies. Because of this, it is important to have access to reliable epidemiological data on the susceptibilities of uropathogens to antibacterial drugs if you want to start an appropriate empirical therapy quickly [10].

Indeed, biofilm-associated infections are linked to higher mortality and morbidity with infected indwelling medical devices in the hospital [11]. Biofilms are bacterial aggregations that are immersed in an extracellular matrix produced by the bacteria itself and consist of exopolysaccharides (EPSs), proteins, and micromolecules like DNA. They can grow on both biological and non-biological surfaces [12, 13].

Uropathogenic *Escherichia coli* strains have important virulence factors, such as type 1 fimbriae, which are responsible for bacterial pathogenicity and biofilm production. Type 1 fimbriae are expressed by more than 95% of all *E. coli* strains [14]. Noncovalent interactions between the four protein subunits that make up a type 1 pilus—FimH, FimF, FimG, and FimA—keep the pilus fiber together. The flexible, short fibrillar tip of the pilus is linked to

the rod, *FimA*, and contains the adhesin *FimH*, along with the two linker subunits FimF and FimG [15, 16, 17].

The objectives of this study were to evaluate the antibiotic resistance, formation of biofilm, and molecular detection of adhesion genes in *E. coli* isolated from patients confirmed with UTIs.

2. Materials and Methods

2.1 Collection, isolation and identification of clinical specimens

A total of one hundred fifty urine samples were obtained from patients with UTI during the period from October 2022 to February 2023, with the approval of the College of Science Research Ethics Committee based on CSEC/0123/0016. All these samples were supplied by Medical City Hospital in Baghdad. These samples were collected immediately from the patients, kept in sterile containers, and transferred into the laboratory for diagnosis. 50 µl of each sample were cultured on Eosin Methylene Blue (EMB) agar and MacConkey agar (Himedia/India) and placed in an incubator at 37 oC for 24 hours. In addition, morphological (gram staining) and biochemical examinations (chemical tests: Indole, catalase, oxidase, and citrate utilization tests) were performed in order to identify the bacterial isolates of E. coli [18, 19, 20].

2.2 Antibiotic susceptibility test

The disk diffusion method [21], as described in the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [22], was utilized in order to perform this test. The antimicrobial discs utilized were as Antibiotic Disc (Symbol; Disc content - µg/disc): Ampicillin (AMP; 10), Trimethoprim-Sulfamethoxazole (COT or SXT; 1.25/23.75), Ceftazidime (CAZ; 30), Imipenem (IPM; 10), Aztreonam (ATM; 30), Cefepime (FEP; 30), Fosfomycin (FOF; 200), Ciprofloxacin (CIP; 5), Nitrofurantoin (NIT; 300), Amikacin (AK or AMK; 30), Piperacillin-tazobactam (PIT; 100).

2.3 Estimation of biofilm formation

A colorimetric microtiter plate test was used to quantify biofilm development [23]. This method was carried out based on a previous report [24]. All bacterial strains were cultured for 24 hours at 37 degrees Celsius in brain-heart infusion broth. After adjusting the turbidity to McFarland standards (0.5), 100 µl of bacterial growth was transferred to a 2 ml tube of normal saline. A total of 180 µl of tryptic soy broth with 1% glucose was added to each well of sterile, flat-bottomed 96-well polystyrene microtiter plates. Twenty microliters of bacterial suspension (made in normal saline) were plated into three wells of a sterile 96-well polystyrene microtiter plate with a flat bottom. Six control wells received brain-heart infusion broth that had been treated to kill any microorganisms. Without disturbing the plates throughout their 24-hour aerobic incubation at 37 degrees Celsius, they were sealed with their lids. After incubation, all plates were washed with DW three times before being dried. After adding 200 µl of methanol to each well and letting them sit for 15 minutes at room temperature, the biofilms were fixed. The plates were stained for fifteen minutes at room temperature with 200 microliters of a crystal violet solution containing 0.1 percent concentration. In addition, the plate was let dry after washing the wells to get rid of any remaining color. For 10 minutes, 200 µl of 100% ethanol was used to dissolve the color. Using an ELISA reader, the optical density (OD) of each well was determined at 600 nm after three independent runs of each test. Table 1 displays the results of an ELISA reader set at 630 nm, which were interpreted as strong, moderate, or weak biofilm producers.

Table 1: Biofilm formation and adhesion: interpreting OD values

Biofilm Formation Adherence	Interpretation
4 * ODc < ODs	Strongly adherent
2 * ODc < ODs ≤ 4 * ODc	Moderately adherent
ODc < ODs ≤ 2 * ODc	Weakly adherent
ODs ≤ ODc	Non –adherent

* OD = optical density; ODc = optical density cut off.

2.4 Extraction of DNA

The EasyPure (R) Bacteria Genomic DNA Kit (TRANS/China) was utilized for the extraction of DNA from samples, and the procedure was performed according to the recommendations of the manufacturers.

2.5 PCR amplification for detection of adhesion genes

The PCR technique was utilized for the detection of adhesion genes (*fimH* and *fimA*) in isolates of *E. coli*. The total PCR reaction was 25 µl, which consisted of 12.5µl of 2×EasyTaq® PCR Super Mix, 1µl of forward primer, 1µl of reverse primer, 5µl of template DNA, and 5.5µl of nuclease-free water. A 2% agarose gel electrophoresis was performed to monitor the amplification of these genes at 7 v/cm for 90 min. The sequence of primers and their sizes of amplicon were included in Table 2.

Table 2: Primer's sequences and amplicon sizes of adhesion genes (*fimH* and *fimA*)

Target gene	Primer's Sequence (5'-3')	Sizes of Amplicon (bp)	References
fimH	-GCTGTGATGTTTCTGCTCGT- -AAAACGAGGCGGTATTGGTG-	167	[25]
fimA	-TTGTTCTGTCGGCTCTGTCC- -AGGCAACAGCGGCTTTAGAT-	261	This study

The reaction mixture was amplified in a thermocycler (Eppendorf, Germany) with the following PCR conditions: initial denaturation at 94.0 ^{o}C for 5 minutes, denaturation at 94.0 ^{o}C for 30 seconds, annealing at 58.0 (*fimA*) and 56.0 (*fimH*) ^{o}C for 40 seconds, extensions at 72.0 ^{o}C for 45 seconds, and final extensions at 72.0 ^{o}C for 5 minutes.

3. Results and Discussion

3.1 Identification of E. coli.

A total of one hundred fifty urine samples were collected. Only 52 isolates were identified by culture on selective and differential MacConkey and EMB media. On MacConkey agar, the isolated *E. coli* showed up as pinkish colonies, while on Eosin Methylene Blue, they had a green metallic sheen. Biochemical tests indicated that isolates were indole-positive, catalase-positive, oxidase-negative, and citrate utilization-negative, whereas these isolates were identified as *E. coli*.

In a study conducted by Kulkarni *et al.*, only 395 isolates were obtained from 1000 samples [26]. Also, Driel *et al.* [27] collected 535 (78%) *E. coli* isolates from 696 urine samples. According to all these studies, urine is one of the best sources for the isolation of *E. coli.*

3.2 Antibiotic susceptibility test

Eleven antibiotic discs were utilized to estimate the ability of the *E. coli* isolate. As follows, *E. coli* isolates were found to have varying degrees of resistance to commonly tested antibiotics. Based on the results shown in Figure 1, 98% of isolates were resistant to AMP, followed by 75.5% of isolates that were resistant to both COT and FOF, followed by 73.5, 71.4, 67.3, 53.1, and 6.1% of isolates were resistant to IMP, CAZ, NIT, FEP, and AMK. All isolates were sensitive to PIT, CIP, and ATM at 100%, 100%, and 98%, respectively.

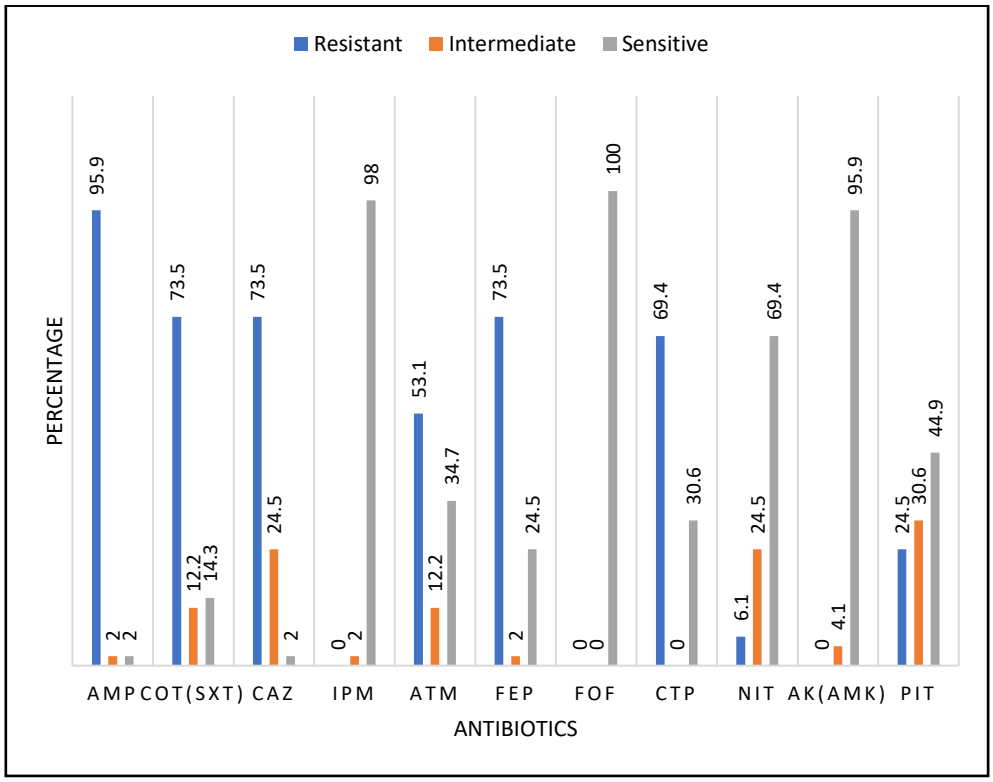

Figure 1: Antibiotic resistance frequency in E. coli isolates: ampicillin (AMP), trimethoprim/sulfamethoxazole (COT or SXT), ceftazidime (CAZ), imipenem (IMP), azatreonam (ATM), cefepime (FEP), fosfomycin (FOF), ciprofloxacin (CIP), nitrofurantoin (NIT), amikacin (AK or AMK), and piperacillin-tazobactam (PIT or TZP)

In China, the sensitivity of amikacin was 100%, and this result disagreed with the total sensitivity that was found in this study [28]. Also, it has been reported that the levels of resistance of *E. coli* against COT ranged between 40 and 80%, whereas the result of this study was included in this range [29]. In addition, it has been reported that 54.2%, 50.5%, and 49.5% of isolates were resistant to cefepime, trimethoprim/sulfamethoxazol, and ciprofloxacin [30]. Mechanisms such as upregulated efflux pumps, protection of the target structures, or reduced permeability of the outer membrane promote the spread of multi-resistant strains [31]. The prevalence of *E. coli* bacteria that are resistant to several antimicrobials in cases of urinary tract infection has been reported [32].

3.3 Biofilm formation

The microtiter plate method was utilized to estimate the capability of *E. coli* to produce biofilms. Based on the outcomes in Table 3, the majority of isolates (69.2%; n = 36) were moderate biofilm producers, while 19.2% (n = 10) of isolates were strong producers, and only 11.5% (n = 6) of isolates were weak producers, as shown in Table 3.

Table 3: The distribution of biofilm formation among isolates

Biofilm producer	N=52	Percentage (100%)
Non	0	0
Weak	6	11.5
Moderate	36	69.2
Strong	10	19.2

Some bacteria, including *E. coli*, are known to thrive in the unforgiving hospital environment because they produce extracellular polysaccharide (EPS) matrix [12]. In a previous study, 38% of *E. coli* were strong biofilm producers, while 22%, 32%, and 8% were moderate, weak, and non-biofilm producers, respectively [33]. In addition, it has been reported that 73 (45.9%), 35 (22%), 28 (17.6%), and 23 (14.5%) of the 159 (51.1%) UPEC strains were non-, weak, moderate, and strong biofilm-producing isolates, respectively [34].

Differential expression of antibiotic-resistance determinants, metabolic end-products, or virulence proteins may result from transcriptional alterations in these bacteria (often mediated by quorum-sensing [QS]-based processes) that occur during biofilm development. QS-mediated genes are involved in biofilm production, showing a close relationship between the two processes [35, 36, 37]. Because building a biofilm is an activity that benefits the whole bacterial population, it seems sensible that the genes involved in this process are QS-mediated. The inherent distinctions between the two modes of development, such as compositional variances in biomass, make it challenging to establish direct comparisons between planktonic cells and biofilm. During in vitro testing, bacterial biofilm producers and non-producers of the same species may show distinct resistance patterns due to these evolutionary trade-offs [35].

3.4 Prevalence of adhesion genes (fimH and fimA)

The molecular detection of *fimH* and *fimA* genes was performed with specific primers using conventional PCR, as shown in Table 2. As shown in Figures 2a and 2b, only 40 isolates harbored both fimH and fimA genes in two band series with molecular sizes of 167 bp and 261 bp, respectively.

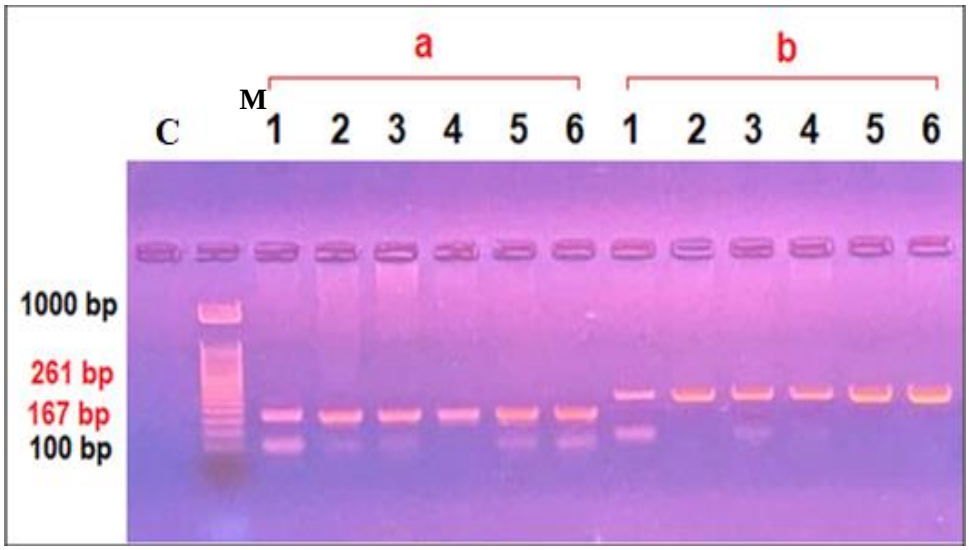

Figure 2: Gel electrophoresis in 2% agarose (90 min, 7 V/cm) for (a) 167 of the *fimH* gene and (b) 261 of the *fimA* gene amplification using PCR, C: negative control; M: DNA ladder

It has been reported that 100% of isolates harbored *fimH* and 31.6% harbored *fimA* [12]. Also, only 44% and 86.3% of isolates carried the *fimA* gene, as reported by [38] and [39], respectively. It is widely accepted that *E. coli*'s adhesions are the most important pathogenic factors. These molecules can activate pathways of communication between bacteria and host cells, thereby facilitating bacterial invasion and colonization of new tissues [40]. Given the importance of type I fimbria in the pathogenesis of extraintestinal illnesses caused by *E. coli*—through attaching to different kinds of epithelial cells, promoting biofilm formation, and avoiding extracellular antibiotics—the high frequency with which fimA is found is noteworthy [39]. Yazdi *et al.* (2018) reported that all isolates of *E. coli* from samples of urine from UTI patients carried the *fim* gene [25].

4. Conclusion

Our research showed a link between antibiotic resistance, the formation of biofilms, and the high number of *fimH* and *fimA* genes in UPEC. These genes play important roles in the disease process of UPEC. As a result, anti-virulence therapies may be the best alternative for UTI treatment.

5. Ethical Clearance

This study received approval from the Ethics Committee of Medical City Hospital and the University of Baghdad for Postgraduate Studies. Everyone present signed a form to show that they agreed in writing.

6. Conflict of Interest

There are no conflicts of interest between the authors.

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