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Prevalence of Drug Resistance and Some Pathogenic Factors in Uro Pathogenic Escherichia Coli (UPEC) Strains Isolated from Patients with Urinary Tract Infection

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Abstract:
Uropathogenic Escherichia coli is one of the most important causes of urinary tract infections. These strains possess various virulence factors, including adhesins, toxins, and iron acquisition systems. Virulence genes are situated on mobile genetic elements or in specific regions of the chromosome known as pathogenicity islands. In this study, 375 clinical samples from male and female patients suspected of having urinary tract infections were collected in the hospitals of Dhi Qar, Iraq, during the period from June 1, 2019, to November 1, 2019. Following the collection of 100 samples, bacterial isolation, DNA extraction, and antibiotic sensitivity tests were conducted using the disc diffusion method with the selected antibiotics. The presence of papC, aer, fimH, hly, cnf-1, and afa class genes was investigated using multiplex PCR. The results indicated that the highest frequency among the genes was associated with the fim gene (98%). The aer, papC, cnf-1, hly, and afa genes were also detected, with frequencies of 52%, 30%, 18%, 13%, and 11%, respectively. Additionally, the highest resistance and sensitivity among UPEC isolates were observed for amoxicillin (82.37%) and amikacin (92.35%) antibiotics, respectively.

INTRODUCTION

The emergence of resistance in pathogenic bacteria to antibiotics is one of the treatment problems all over the world. Currently, reports show that the level of resistance in UPEC bacteria is escalating. This issue is especially noticeable in countries where antibiotics are consumed inappropriately and indiscriminately. Determining the pattern of antibiotic resistance in common pathogenic bacteria is important to guide experimental and specific treatments against specific pathogens. Urinary tract infection is one of the most common infections among hospitalized patients and laboratory referees. Moreover, its frequency is classified after respiratory infection. The urinary system's susceptibility to infection, along with its potential for serious complications such as kidney failure, blood infection, and premature birth indicate the importance of diagnosing and treating this disease. Urinary tract infections (UTIs) are among the most common bacterial infections caused by the growth and colonization of pathogens in the urinary tract, which affect millions of people worldwide every year and cause a significant economic burden on the government. Due to the anatomical differences, the prevalence of this infection is much higher in women than in men, so more than half of the women suffer from UTI infections at least once (1). The epidemiology of UTI infection is not reported in many countries, which is why it is difficult to check its exact prevalence (2). However, due to the spread of resistance to antibiotics and the high level of virulence, there is evidence of scattered epidemics and the spread of the disease. According to the colonization range of UPEC (Uropathogenic *E. coli*) strains, UTI infections are known as cystitis (lower parts such as the bladder) and pyelonephritis (upper parts like the kidney), which are often acquired and related to the catheter. Symptoms and clinical complications of pyelonephritis can range from painful urination in the urinary tract or uncomplicated cystitis to severe systemic disease with abdominal or back pain, fever, sepsis, as well as bladder or kidney failure. Biological factors (such as age and gender) and behavioral factors of the host (such as sexual activities and the response of the host's immune system) as well as the type of infecting microorganism play an important role in the spread and prevalence of this infection (2, 3). Several reports show that bacteria play an important role in causing UTIs, among which E. coli is one of the most common pathogens associated with UTIs (1, 3). *E. coli* is a gram-negative, motile, rod-shaped bacterium that can grow at different temperatures and tolerate acidic conditions up to pH = 4.4. *E. coli* forms a part of the intestinal flora of humans and other mammals, which can turn into a pathogenic form under certain conditions. Pathogenic strains are also divided into two main categories: enteric *E. coli* (intestinal) and extraintestinal *E. coli* (ExPEC). Enteric pathogenic *E. coli* strains include EPEC, EIEC, ETEC, EHEC, EAEC and DAEC pathotypes, and the most important ExPEC strains are UPEC strains. Reports show that UPEC strains are responsible for about 90% of uncomplicated cystitis and pyelonephritis infections (4, 5). The purpose of this study is to investigate the prevalence of uropathogenic Escherichia coli and virulence factors among uropathogenic Escherichia coli strains and to investigate the antibiotic sensitivity of Escherichia coli isolates isolated from urine samples.

MATERIAL AND METHODS

The objective of this research is to collect, isolate, and identify uropathogenic *E. coli* strains, as well as investigate the antibiotic resistance profile and prevalence of certain virulence genes (*hly, cnf-1, afa, fim, aer, and papC*) in samples obtained from patients suspected of urinary tract infections at hospitals in the city of Dhi Qar, Iraq.

Collection of samples

In this study, 375 clinical samples of male and female patients suspected of urinary tract infection were collected in hospitals in Dhi Qar city, Iraq from 01/06/2019 to 29/12/2019. After collection, the samples were transferred to the laboratory under sterile conditions and kept in the refrigerator.

Preparation of culture medium (NA)

To grow and multiply bacterial strains in this research, NA, NB and EMB media will be utilized. To prepare 100 ml of Nutrient Agar culture medium, 2.8 g of NA powder was added to an Erlenmeyer flask containing 100 ml of deionized water. After brief heating and dissolving the contents, the medium will be autoclaved at 121°C for 20 minutes for sterilization. After passing time, the culture medium was transferred into the plates and stored in the refrigerator.

Table 1. Antibiogram discs used in this research

Antibiotic	Antibiotic class
Amikacin	**AN**
Ciprofloxacin	**CIP**
Amoxicillin	**AMC**
Ceftazidime	**CAZ**
Trimethoprim	**TMP**
Ticarcillin	**TI**
Gentamicin	**GM**
Piperacillin	**PI**
Nitrofurantoin	**NIT**
Norfloxacin	**NOR**
Ceftriaxone	**CTR**
Cefixime	**CFM**
Nalidixic acid	**NA**

Culturing and recovery of clinical samples

In this research, approximately 100 clinical samples were collected from individuals suspected of urinary tract infection. To culture, revive, and purify the mentioned isolates, they were initially cultured on an NB culture medium. Subsequently, for purification and colony examination, they were cultured on an EMB medium. To achieve this, several colonies were aseptically removed from the solid culture using a sterile loop. These colonies were then placed individually in an Erlenmeyer flask containing 10 ml of NB liquid culture medium. The culture plates were incubated at 37ºC for 24 hours. Following the revival and refreshment of the samples, bacterial isolates that demonstrated growth were cultured on an EMB culture medium for purification and morphological examination.

Antibiotic resistance pattern analysis

In this study, the antibiotic discs listed in the table were utilized to evaluate the antibiotic resistance profile, following the protocol provided by the Clinical and Laboratory Standards Organization (CLSI). For this purpose, each of the isolates was cultured in the NB culture medium. Subsequently, from the logarithmic culture of each isolate, a 20 µl bacterial suspension with a concentration of 0.5 McFarland (equivalent to 1.5 x 10^8 CFU/ml) was added to the solid culture medium of Muller Hinton. Once inoculated, the bacterial solution was completely spread on the surface of the culture medium using a sterile swab. Finally, each of the antibiogram discs was removed using sterile tweezers and placed on the plate at specific intervals. After closing the lid of the plate, all the plates were incubated for 24 hours at 37°C. Finally, the diameter of the growth halo was measured, and the results were reported as resistant, semi-sensitive, or sensitive.

Genomic DNA extraction

To investigate the distribution of pathogenic genes among uropathogenic E. coli strains, the polymerase chain reaction (PCR) method was employed. Initially, the DNA genome of each isolate was extracted using the boiling method. To accomplish this, overnight cultures of each bacterial isolate were grown in a nutrient broth culture medium under optimal conditions. Subsequently, a specific volume of the bacterial suspension was inoculated into 1.5 ml vials. After centrifugation at 12,000 rpm for 5 minutes and washing the cell sediment with deionized water, 250 µl of distilled water was added to each vial. The samples were then subjected to vortexing, followed by placement in a bain-marie set at 100 ºC for 15 minutes. This step aimed to induce a temperature shock and disrupt the cell wall. Subsequently, the microtubes were transferred to a freezer set at -20 ºC for 1 hour to further enhance cell wall destruction. After the designated time had elapsed, the vials were removed from the freezer and subjected to centrifugation at 12,000 rpm for 10 minutes. This step facilitated the separation of genomic DNA from bacterial debris. Finally, the supernatant containing the extracted genomic DNA was carefully transferred to new sterile microtubes and utilized as a template for PCR analysis.

Agarose gel electrophoresis

Horizontal agarose gel electrophoresis was employed to analyze the amplicons generated by the PCR reaction and assess the presence of bands corresponding to the targeted pathogenic genes.

RESULTS

Figure 1 shows the isolates grown on an EMB medium. In this environment, *E. coli* isolates by fermenting the lactose in the culture medium lead to the production of stable acetaldehyde and compounds of eosin Y and methylene blue, and the oxidation of

Fig1. Cultivation of E. coli isolates on EMB medium

these compounds in the vicinity of oxygen causes the appearance of dark-colored colonies with a shiny metallic lustre. Gram-positive bacteria were not able to grow and multiply on the culture medium due to the presence of eosin and methylene blue. The results showed that among the mentioned isolates, 100% of the samples can grow and survive on an EMB medium.

Figure 2 shows the distribution of uropathogens isolated by age group. Isolates were most frequent among patients aged 41-50 years. Over 58% of isolates came from patients between 30 and 60 years old.

In this research, the agar diffusion method (disk diffusion) was used to check the antibiotic sensitivity of UPEC isolates (Figure 3). The diameter of the non-growth halo around each antibiotic disk was measured and interpreted as resistant (R), intermediate (I), or susceptible (S) according to CLSI standards in Figure (5-4).

As can be seen in Figure 4, the highest resistance and sensitivity among UPEC isolates belong to amoxicillin (82.37%) and amikacin (92.35%) antibiotics, respectively. The resistance rates to ticarcillin, piperacillin, trimethoprim, nalidixic acid, cefixime, ceftriaxone, ceftazidime, norfloxacin, nitrofurantoin, gentamicin, ciprofloxacin, and amikacin were determined to be 81.37%, 80.39%, 78.43%, 74.0%, 55.92%, 62.74%, 55.88%, 48.03%, 40.19%, 27.45%, 28.41%, 36.27%, and 8.65%, respectively.

Research indicates that Enterobacteriaceae bacteria are usually resistant to antibiotics, and this resistance is caused by multiple inherited and acquired mechanisms. Beta-lactam antibiotics are often prescribed to treat Escherichia coli infections, but nowadays the emergence and spread of beta-lactamase-producing strains has also limited the effect of this antibiotic family. Figure 5 shows the frequency of each virulence gene based on gender. The prevalence of *fim, aer, papC, cnf-1, hly and afa* genes among the isolates isolated from male individuals was 38%, 19%, 11%, 6%, 6% and 3%, respectively. Among isolates isolated from female patients with UTI, the *fim* gene (60%) showed the highest prevalence. *aer, papC, cnf-1, hly* and *afa* genes were also in the next ranks with 33%, 19%, 12%, 8% and 7%, respectively. In general, the distribution of

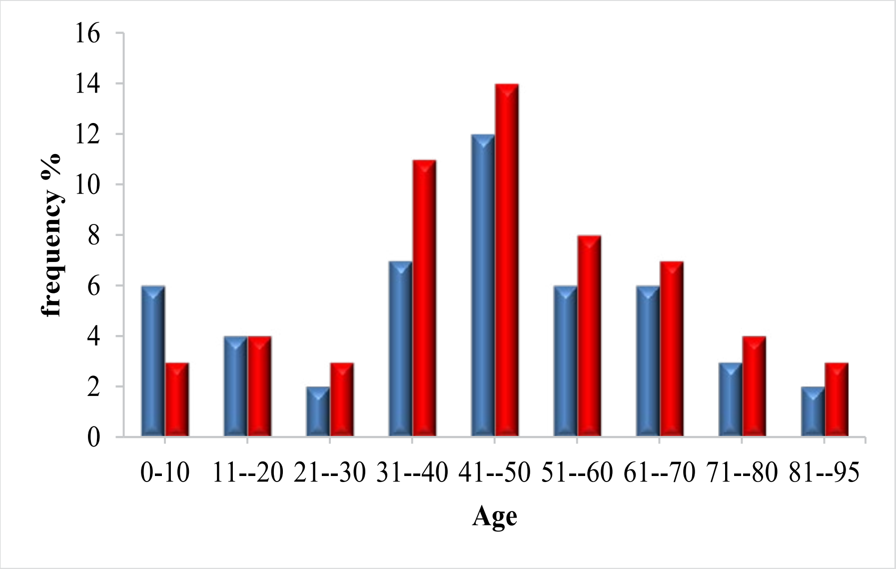

Fig 2. Frequency of isolates isolated from people with UTI according to age range

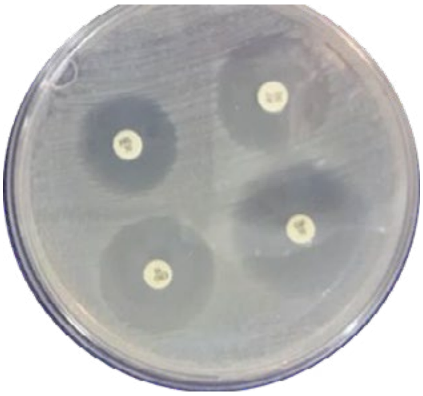

Fig3. Antibiotic sensitivity test results by disk diffusion method

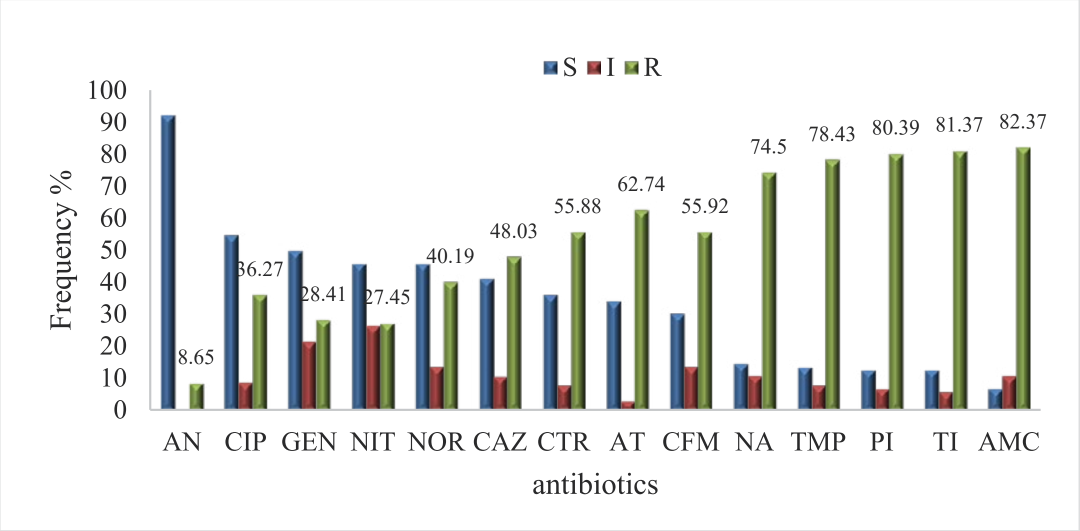

Fig4. Frequency of antibiotic sensitivity and resistance of UPEC isolates by disk diffusion method

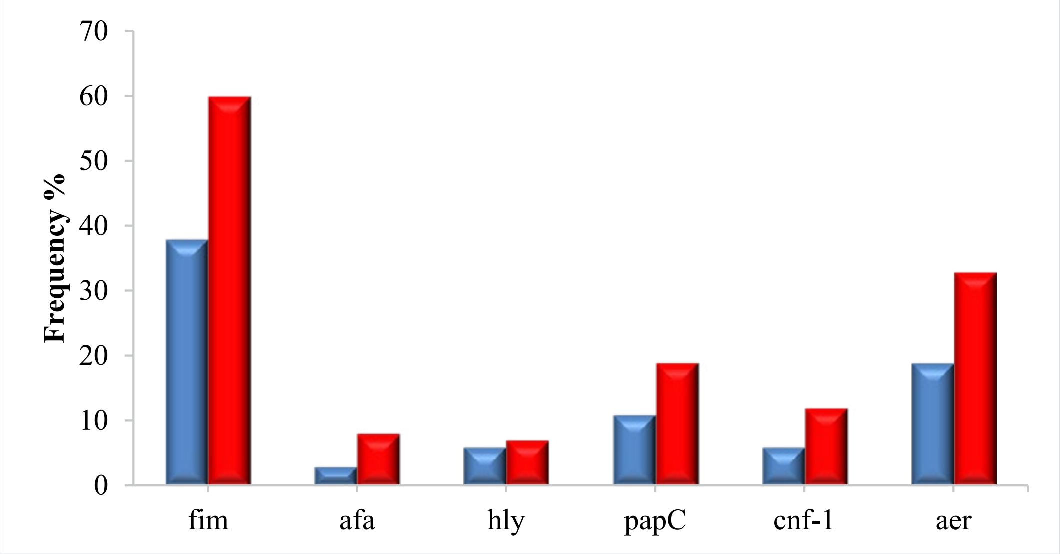

Fig 5. Prevalence of UPEC virulence genes isolated from male and female individuals

virulence genes was observed among female subjects more than male subjects.

Figure 6 shows the products from the amplification of *afa* and *cnf-1* genes. As can be seen, the *afa* gene with a size of 750 bp appeared in more than 11% of the isolates. *cnf-1* gene with a size of 498 bp was expressed in more than 18% of UPEC isolates. Simultaneous expression of these two genes was observed in 3% of isolates (Ec-12, Ec-22, Ec-66).

The *cnf-1* gene is one of the virulence factors in *E. coli* strains, which is located on the bacterial chromosome and is often observed with *hly* genes and S and P fimbriae. This toxin is activated by Rho GTPases RhoA, Rac1 and Cdc42. CNF1 is a single-chain, 115 kDa AB toxin that deaminates the glutamine unit of the Rho family of GTPases. Reports indicate that CNF1 synthesis leads to long-term survival of UPEC in association with human neutrophils (Davis et al., 2005). Figure 7 shows the bands related to *hly* and *cnf-1* genes. The *hly* gene with a size of 1177 bp was present in 13% of UPEC isolates. The distribution of the mentioned gene among men and women was determined as 7% and 6%, respectively. Simultaneous expression of these two genes (*hly* and *cnf-1*) was observed in isolates Ec-2, Ec-11 and Ec-41.Figure 8 shows the bands related to *papC* and *fim* genes. The size of *papC* gene is about 200 bp. In 28% of the mentioned isolates, the above genes were present simultaneously.

Uropathogenic *E. coli* (UPEC) strains wield an arsenal of virulence factors, enabling them to establish a foothold within the urinary tract. These factors orchestrate a coordinated attack, facilitating accumulation, colonization, and immune system evasion through adherence to host epithelial cells.

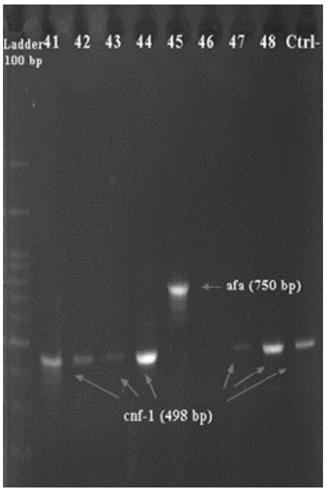

Fig 6. Electrophoresis pattern of PCR products for afa and cnf-1 genes

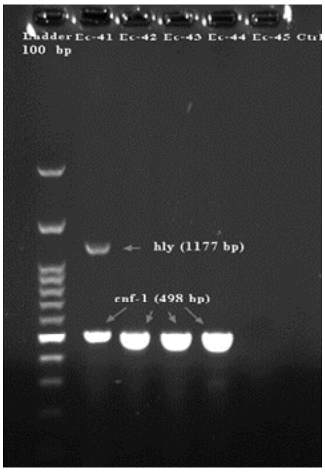

Fig 7. Electrophoresis pattern of PCR products for cnf-1 and hly genes

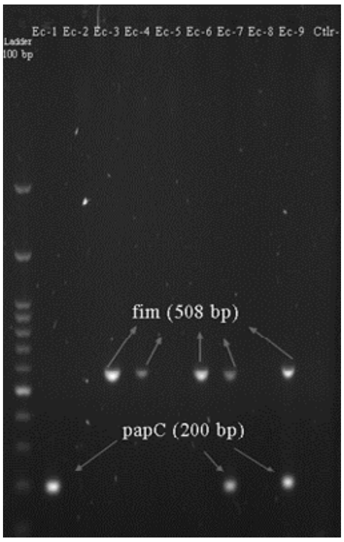

Fig8. Electrophoresis pattern of PCR products for fim and papC genes

Notably, fimbriae, key players in this pathogenic strategy, mediate adhesion to the urinary tract epithelium. Type 1 fimbriae (*fimH*) and P pili (*papC*) represent particularly potent adhesins within this class. This study identified a remarkably high prevalence (approximately 98%) of the *fimH* gene among the examined UPEC strains.

DISCUSSION

Since the discovery of bacteria, humans have continually sought effective drugs to combat the infections they cause, while bacteria have developed mechanisms to destroy antibiotics. The emergence of drug resistance among pathogenic bacteria has presented numerous challenges in treating infectious diseases. Reports of the widespread prevalence of antibiotic-resistant organisms in various hospital departments are now commonplace. Escherichia coli is a common bacterial agent found in human infections, particularly among hospitalized patients and is a major cause of urinary infections. Antimicrobial resistance in Escherichia coli has raised concerns globally due to the increasing rate of resistance. Studies have been conducted to differentiate strains by gender, with Amini and colleagues identifying UPEC strains from clinical samples, revealing that 69.98% of the total 122 samples collected from patients were gender-specific, with 30.33% being women and men (6). The average age of the patients was 47 ± 3.2, and E. coli bacteria were isolated from 36 infected individuals. The isolates were identified through various tests, yielding results consistent with previous findings. By isolating 450 urine samples from men and women suffering from UTI in Iraq (7), they determined that 392 isolates were female and 58 were male. The age range of affected people was also reported between 15 and 75 years. The majority of isolates were from married women (67 cases), unmarried girls (25 cases) and 15-year-old children (11 cases) for both sexes. In the report, out of a total of 364 strains isolated from different hospitals in Iraqi Kurdistan (8), about 81% were isolated from women and 19% from men. The results of the identification of the isolates also showed that more than 41.2% of the isolates (150 samples) belong to the uropathogenic Escherichia coli species (9). In Iraq, various studies have been conducted to evaluate the antibiotic resistance profile among Escherichia coli strains. In one of these researches, by examining the prevalence and pattern of antibiotic sensitivity among uropathogenic E. coli strains among people with urinary tract infection in Zakho, Iraq, they showed that out of 1120 urine samples, only 9.4% of the isolates belonged to UPEC species. The rate of resistance to ceftriaxone and ceftazidime antibiotics was determined to be 52%, which was within the range of our findings. However, the level of resistance to other antibiotics was inconsistent with the data of this research. In the study in the city of Najaf, Iraq, the results of antibiotic resistance profiles among UPEC strains were as follows: CAZ (9.76%), CIP (7.52%),

AN (5.50%), CTR (3.48%), GM (8.42%), NIT (5.17%), the prevalence of some of which (CIP, CTR, NIT) was almost similar to the results of the present study, and the percentage of isolates resistant to CAZ, AN And GM exceeded our findings (10). However, the level of resistance to other antibiotics was inconsistent with the data of this research. In the city of Zakho, Iraq, by examining 400 clinical samples, they showed that 25.35% of the isolates belong to UPEC species. The results of the antibiotic sensitivity pattern among the mentioned isolates showed that the percentage of resistance to amoxicillin, cefixime, ceftriaxone, amikacin, gentamicin, nalidixic acid, norfloxacin, ciprofloxacin and trimethoprim was 6.93%, 1.83%, 2.87%, 2.87%, 8.63%, 4.79%, 9.43%, 6.49% and 7.61%, which is the resistance of isolates to NOR, CIP, NA And TMP is almost consistent with the results of this research.

CONCLUSION

Considering the prevalence of antibiotic resistance of *E. coli* strains in different societies and the role of virulence genes in the occurrence of bacterial infections, choosing the appropriate treatment method and the type of antibiotic used can be particularly important in reducing the prevalence of antibiotic resistance. Afa-positive strains play an important role in the pathogenesis of urinary tract infection (UTI), especially in pregnant women, children and patients with frequent urinary tract infections. The present study showed that the prevalence of virulence genes, *fimH, aer* among uropathogenic Escherichia coli strains isolated from hospitalized patients in this region is high. Therefore, according to the gender, age and type of bacterial infection, determining the pattern of antibiotic resistance profile, screening of pathogenic bacteria, as well as the relationship of virulence genes with the rate of infection in people with UTI periodically can be of great help in improving the treatment conditions and epidemiology studies. Therefore, the above genes can be further studied as targets in therapeutic interventions.

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Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

Conflicts of Interest

The authors declare no conflict of interest.

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