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Antimicrobial Resistance

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Antibiotic Resistance and Virulence Gene Profiling of Uropathogenic *Escherichia coli* Isolated from Clinical Samples

Zahraa F. Al-Kabi, Hasan A. Aal Owaif*

Department of Plant Biotechnology, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq

Abstract

Background: Urinary tract infection (UTI) is the third most prevalent type of infection, following respiratory and gastrointestinal infections. Uropathogenic *E. coli* (UPEC) is a subset of extraintestinal pathogenic *E. coli* with a wide range of genotypes responsible for causing UTIs. This study aimed to detect the antibiotic resistance and virulence genes of local isolates of UPEC from UTIs and to determine biofilm formation by UPEC isolates.

***Methods and Results*:** One hundred urine samples were obtained from patients with UTIs from hospitals in Baghdad from September 2023 to December 2023 and then plated for 24 hours at 37°C on McConkey agar. Biochemical tests and the VITEK-2 system were used to identify UPEC isolates. The antibiotic susceptibility test was performed using the disc diffusion technique, and the results were identified according to the guidelines of CLSI 2023. The biofilm formation was determined by an ELISA reader at OD570 using the microtitre-plate technique. Detection of *csgA* and *fimH* genes was performed by PCR.

Fifty-two (52%) isolates of UPEC were identified from 100 urine samples. The results revealed that 100% of the isolates were resistant to ceftazidime, 92.3% to gentamicin, 90.4% to ciprofloxacin, 84.6% to azithromycin and aztreonam, and 46.1% to imipenem. Out of 52 isolates, 39(75%) have multiple resistance to more than three antibiotics. The results demonstrated that 12% of the isolates were strong biofilm producers, 40% - moderate biofilm producers, 36% produced weak biofilms, and 12% did not produce biofilms. The *csgA* and *fimH* genes were detected in 47(90%) and 49(94%) isolates, respectively.

Conclusion: This study demonstrated that elevated resistance rates were noted against administered antibiotics, signifying an increasing problem in effectively treating UTIs; 75% of the isolates showed an MDR phenotype. The study also verified the capacity of UPEC to produce biofilms (88% of isolates) and express virulence factors, enhancing their pathogenicity and treatment resistance.(**International Journal of Biomedicine. 2024;15(1):188-191.**)

Keywords: uropathogenic *E. coli* • antibiotic resistance • biofilm • virulence genes

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Introduction

Urinary tract infection (UTI) is the third most prevalent type of infection, following respiratory and gastrointestinal infections. UTI can occur in any component of the urinary system, such as the kidneys, ureters, bladder, or urethra. UTI is commonly caused by the proliferation of pathogenic microorganisms such as bacteria, fungi, and parasites[1] and can occur in individuals of any age or gender, whether they are in community or hospital environments.[2] UTIs are becoming increasingly challenging to manage because of the widespread antibiotic resistance observed in uropathogens.[3] Although UTIs can be treated, the rising resistance to antimicrobial drugs makes it harder to control these infections.[4] Uropathogenic *E. coli* (UPEC) is a subset of extraintestinal pathogenic *E. coli* with a wide range of genotypes responsible for causing UTIs. UPEC has developed from commensal *E. coli* by acquiring virulence factors via horizontal gene transfer. UPEC is widely recognized as the predominant bacteria responsible for complex (40%-50%) and simple (75%-95%) UTIs.[5] UPEC possesses numerous virulence-associated components, such as adhesions, toxins, iron acquisition factors, lipopolysaccharides, polysaccharide capsules, and invasions.[6,7] UPEC that exists within cells can mature into biofilm, a process linked to persistent and recurring urinary tract infections. Biofilm has a significantly higher resistance to antimicrobial drugs, including antibiotics, and to immunological responses from the

***Corresponding author**: Hasan A. Aal Owaif. Department of Plant Biotechnology, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq. E-mail: hasan.abdulhadi@nahrainuniv.edu.iq*

host, failing medical treatments.[8,9] This study aimed to detect the antibiotic resistance and virulence genes of local isolates of UPEC from UTIs and to determine biofilm formation by UPEC isolates.

Materials and Methods

One hundred urine samples were obtained from patients with UTIs from hospitals in Baghdad from September 2023 to December 2023 and then plated for 24 hours at 37°C on McConkey agar. Biochemical tests and the VITEK-2 system were used to identify UPEC isolates. The antibiotic susceptibility test was performed using the disc diffusion technique, and the results were identified according to the guidelines of CLSI 2023. The antimicrobial agents used in this study were: azithromycin (15 µg), aztreonam (30 µg), ceftazidime (30 µg), ciprofloxacin (10 µg), gentamicin (10 µg), and imipenem (10 µg). The biofilm formation was determined by an ELISA reader at OD570 using the microtitre-plate technique. Primers used in the detection of *csgA* and *fimH* genes by PCR were designed in this study (Table 1).

Table 1.

PCR primer sequence.

Gene	Sequence: 5' ⟶ 3'	Amplicon size (bp)
csgA	F:TGGTGCAGATGTTGGTCAGG R:CGGTCGCGTTGTTACCAAAG	212
fimH	F:GCCGTGCTTATTTTGCGACA R:GGTAGTCCGGCAGAGTAACG	148

Results

Fifty-two (52%) isolates of UPEC were identified from 100 urine samples. The distribution pattern of UPEC isolates according to age groups and gender is shown in Figure 1.

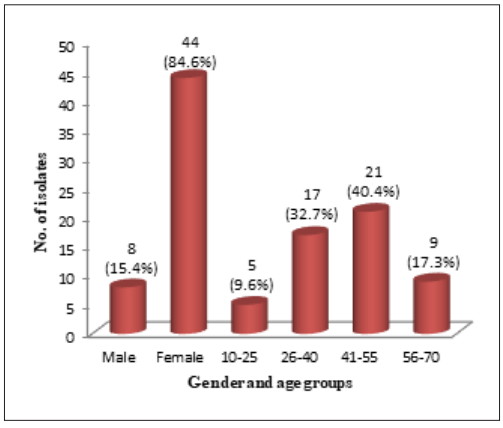

Fig. 1. *Distribution of UPEC isolates.*

The results revealed that 100% of the isolates were resistant to ceftazidime, 92.3% to gentamicin, 90.4% to ciprofloxacin, 84.6% to azithromycin and aztreonam, and 46.1% to imipenem (Table 2). Out of 52 isolates, 39 (75%) have multiple resistance to more than three antibiotics. The results demonstrated that 12% of the isolates were strong biofilm producers, 40% - moderate biofilm producers, 36% produced weak biofilms, and 12% did not produce biofilms. The *csgA* gene was detected in 47 (90%) isolates (Figure 2), and the *fimH* gene was detected in 49 (94%) isolates (Figure 3). The correlation between biofilm formation and antibiotic resistance is presented in Table 3.

Table 2.

Antibiotic resistance of UPEC isolates.

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistance n (%)
Imipenem	21(40.4)	7(13.5)	24 (46.1)
Azithromycin	5(9.6)	3 (5.8)	44 (84.6)
Ciprofloxacin	2(3.8)	3(5.8)	47(90.4)
Ceftazidime	0(0)	0(0)	52(100)
Aztreonam	4(7.7)	4 (7.7)	44(84.6)
Gentamicin	2(3.8)	2 (3.9)	48(92.3)

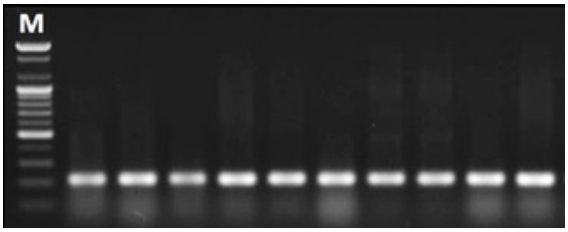

Fig. 2. *The csgA gene (212bp), M: 100bp DNA marker.*

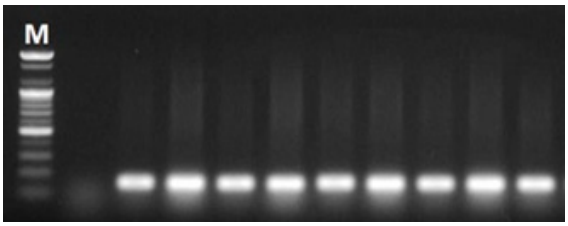

Fig. 3. *The fimH gene (148bp), M: 100bp DNA marker.*

Table 3.

The relationship between biofilm formation and antibiotic resistance in UPEC isolates.

Antibiotic	High (n=6)	Intermediate (n=21)	Low (n=19)	No biofilm (n=6)
	n (%)	n (%)	n (%)	n (%)
Imipenem	6(100)	13(61.9)	4(21)	1(16.6)
Azithromycin	6(100)	20(95.2)	16(84.2)	2(33.3)
Ciprofloxacin	5(83.3)	21(100)	18(94.7)	3(50)
Ceftazidime	6(100)	21(100)	19(100)	6(100)
Aztreonam	6(100)	18(85.7)	18(94.7)	2(33.3)
Gentamicin	6(100)	21(100)	17(89.4)	4(66.6)

Discussion

This study showed the prevalence of *E. coli* as a gram-negative bacteria that is considered a major cause of UTI, while *Staphylococcus* spp., as a gram-positive bacteria, was the most common; this result agreed with the study at the Banadir hospital in Somalia, which revealed the prevalence of *E. coli* and *Klebsiella* spp, contributing 56.4% and 30.6%, respectively, of total 171 urine samples collected from patients suffering from UTIs.[10] The higher presence of *E. coli* in women (84.6%) than in men (15.4%) could be caused by the close proximity of the anus to the urethral tube.

Also, the urethral tube of the female body is shorter than that of men, which shortens the distance microorganisms have to travel to get to the bladder.[11] The age group 41-55 years had the highest percentage, 40.4%, of the isolates. Older individuals are more prone to diseases such as urinary blockage, inadequate bladder emptying, diabetes mellitus, and prostatic hypertrophy. These variables promote the growth of bacteria and have a significant impact on UTIs.[12] The antibiotic susceptibility test revealed that all isolates were ceftazidime resistant, and 40.4% were imipenem sensitive. The resistance of UPEC to ciprofloxacin was 90.4%, which is a fluoroquinolone antibiotic that used to be one of the active agents as initial therapy for UTIs due to superior activity against uropathogens, specifically UPEC. Yet the immoderate medication for recurrent UTI, inadequate use of this antibiotic, and previous catheterization may lead to developing resistance.[13] In Pakistan, a study of UTIs indicated that *E. coli* represents 32% of the isolates, and their resistance against ciprofloxacin was 57.5%.[14] Resistance to imipenem was 46%, which is a beta-lactam antibiotic that belongs to a subgroup of carbapenems that inhibits the peptidoglycan synthesis of bacterial cell walls by inhibiting major enzymes (trans peptidases), contributed to the end stages of peptidoglycan biosynthesis.

β-Lactam resistance is associated with the presence of different types of β-lactamase enzymes. Beta-lactamases (*bla*) are the largest class of antibiotic-resistance genes.[15] The resistance to gentamicin was 92% of the isolates. In Nigeria, a study showed that resistance to Gentamicin by UPEC isolated from UTIs was 52.9%.[16] This antibiotic is related to the aminoglycoside group that acts as a protein synthesis inhibitor by cross-linking to LPS in gram-negative bacteria. Then is transported into the cytosol and disturbs the peptide elongation at the 30S ribosomal subunit that gives rise to inaccurate mRNA translations and misreading of the RNA message, leading to premature termination and to the inaccuracy of the translated protein product.[17] Our study revealed that out of 52 isolates, 39(75%) have multiple resistance to more than three antibiotics, and this is similar to a study in Iran, which found a high rate of multiple resistance among UPEC isolates to the commonly used antibiotics, which leads to considerable therapeutic problems.[18] Also, multiple antibiotic resistance of bacteria could be due to transferable plasmids carrying resistant genes that are transferred among pathogenic bacteria.[19] Antibiotic resistance may be associated with modifications in bacterial enzymes and the excessive use and misuse of antibiotics by patients.[20,21] The *csgA* gene was detected in 90% of the isolates, and this result agreed with a study in Iran that found that 82.1% of the UPEC carried the *csgA* gene.[22] This gene encodes the major subunit protein of the fimbriae, which is associated with biofilm formation.[23] The *fimH* gene was detected in 94% of the isolates, which agreed with a study in Iraq that highlighted the high prevalence of the *fimH* gene, 98.6% among UPEC isolates,[24] revealing its crucial role in enhancing the resistance of these bacteria to antimicrobial agents. Biofilm formation and antibiotic resistance were shown to be correlated in the current study. Planktonic bacteria are more susceptible to antibiotics than biofilm bacteria, according to these results, which are in line with previous studies.[25,26] On the other hand, horizontal gene transfer is much more common in biofilms because resistant bacteria can pass their resistance genes to other bacteria.[27,28]

Conclusion

This study demonstrated that elevated resistance rates were noted against administered antibiotics, signifying an increasing problem in effectively treating UTIs; 75% of the isolates showed an MDR phenotype. The study also verified the capacity of UPEC to produce biofilms (88% of isolates) and express virulence factors, enhancing their pathogenicity and treatment resistance. The formation of biofilms by UPEC isolates, along with the misuse and overuse of antibiotics, significantly contributes to the development and spread of antibiotic resistance.

Competing Interests

The authors declare that they have no competing interests.

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