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Detection of *yadN*, *ygiL*, and *draE* Genes in Ciprofloxacin-resistant Uropathogenic *Escherichia coli*

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Abstract

Background: In Uropathogenic *E. coli* (UPEC), Yad and Ygi fimbriae are associated with the colonization of the bladder.

Methods: A total of 200 urine samples were gathered from Urinary Tract Infection (UTI) patients to isolate *Escherichia coli* using Hichrome *E. coli* and Hichrome UTI agar media. Susceptibility of Uropathogenic *E. coli* isolates to 16 different antibiotics was determined by using the Disc Diffusion Approach and VITEK 2 compact system. Congo red agar and tissue culture plates were employed for the biofilm development test. By using Polymerase Chain Reaction (PCR) and gel electrophoresis, *yadN*, *ygiL*, and *draE* genes were detected.

Results: From 40 bacterial isolates, 21 (52.5%) were resistant to ciprofloxacin. The highest resistance of UPEC isolates was to Ampicillin (34/40; 85%) and Cefazolin (33/40; 82.5%), while the lowest resistance was to Amikacin and Tagicycline (0/40; 0%). However, none of the isolates were found to be extensively drug resistant (XDR) or pandrug-resistant (PDR) according to the VITEK 2 compact system, but 31 out of 40 bacterial isolates (77.5%) were multidrug resistant (MDR). The results of using microtiter plates revealed that (44%) of the isolates formed strong biofilms, while the remaining (14/25; 56%) were moderate biofilm-forming. When tested using the Congo red method, (9/25; 36%) gave black colonies (strong biofilm), (13/25; 52%) gave grey colonies (moderate biofilm), while only three isolates produced pink growth on medium (12%) (non-biofilm producing). The results of PCR showed that out of 22 UPEC isolates, (19/22 ; 86.36%) contained the *yadN* gene, (13/22; 59.09%) had the *ygiL* gene, and none had the *draE* gene.

Conclusion: The *yadN* gene was more frequent in UPEC Isolates, followed by the *ygiL* gene, which indicates that both have crucial roles in the virulence.

Keywords: *Yad* gene, *Ygi* gene, *draE* gene, Uropathogenic *E. coli* (UPEC).

التحري عن جينات *yadN, ygiL, draE* في الإشريكية القولونية الممرضة البولية المقاومة للسبروفلوكساسين

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الخلاصة

الخلفية: في الإشريكية القولونية المسببة للأمراض البولية، يشارك كل من خمل Yad وخمل Ygi في استعمار المثانة.

الطرق: تم جمع (200) عينة بول من مرضى التهاب المسالك البولية المحتملين للتعرف على عزلات الإشريكية القولونية باستخدام (Hichrome E. coli و Hichrome UTI.) تم تحديد حساسية عزلات

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UPECلـ 16 مضادًا حيويًا مختلفًا باستخدام طريقة Disc Diffusion وطريقة VITEK 2 Compact تم استخدام صفائح زراعة الأنسجة واكار احمر الكونغو لاختبار تطور الغشاء الحيوي،, وباستخدام PCR والرحلان الكهربائي للهلام، تم الكشف عن جيناتyadN ، ygiL , draE. باستخدام طريقة Kerby pour

النتائج: من بين 40 عزلة بكتيرية، كانت 21 عزلة (52.5%) مقاومة للسيبروفلوكساسين. ومع ذلك، لم يتم العثور على أي من العزلات XDR أو PDR حسب نظام VITEK 2 Compact.، ولكن 31 عزلة بكتيرية من 40 (77.5٪) كانت MDR .أظهرت نتائج استخدام اطباق المعاييرة الدقيقة أن (44%) من العزلات شكلت أغشية حيوية قوية، بينما في تقنية احمر الكونغو (25/9) (36%) أعطت مستعمرات سوداء (أغشية حيوية قوية). أظهرت نتائج تفاعل البلمرة المتسلسل أنه من بين (22) عزلةUPEC ، كانت 19 (86.36%) تحتوي على جينyadN ، و13 (59.09%) تحتوي على جينygiL ، ولم يكن أي منها يحتوي على جين draE.

الاستنتاج : كان جين yadN اكثر تواترا في عزلات الاشيريكية القولونية البولية الممرضة يليه جين ygiL مما يشير الى ان كلاهما لهما دور حاسم في الفوعة.

1.Introduction

Escherichia coli strains known as UPEC develop and remain in the urine tract, exhibit a great variety of virulence factors and mechanisms of action, and practice their role as commensal gut flora. They can hence infect the urinary tract and elicit diseases there [1, 2]. In the past ten years, managing Urinary Tract Infections (UTIs) brought on by *E. coli* has been a topic of widespread concern [3, 4, 5]. The severity of UTIs has increased due to the proliferation of virulent ESBL-producing MDR UPEC over the world [6,7]. There are important regional variations in the occurrence of resistance. The decision to use an antibiotic for the treatment of a UTI has been impacted by the magnitude of resistance [8, 9]. Numerous bacteria have pili and fimbriae, which are surface appendages of varying shapes and sizes. However, the term "pilus" should only be used to refer to appendages that participate in bacterial conjugation or the relocation of genetic material, whereas the term "fimbria" should only be used to refer to structures involved in bacterial adhesion to a variety of surfaces [10], chemotaxis, biofilm formation, and DNA transfer across cell membranes [11, 12]. Twelve putative fimbrial operons are found in the genome of *E. coli* CFT073. Patients with pyelonephritis have a prototype UPEC strain. Many of these strains have chaperone-usher fimbriae, whereas the other fimbriae are of the IV pili type, which is encoded by the yeh, yad, yfc, and ygi operons, as well as type 1, P, F1C, Auf, and F9 fimbriae [13,14]. In UPEC, Yad fimbriae are frequently observed. Yad fimbriae contribute to the development of biofilms, binding to bladder epithelial cells, and pathogenicity of the avian *E. coli* [15]. In comparison to 24% of fecal *E. coli* strains, 61% of UPEC isolates encode Ygi fimbriae, indicating that these fimbriae may represent urovirulence factors. *E. coli* CFT073's pyelonephritis strain was able to reduce the adhesion to the human kidney epithelial cell line HEK 293 and the development of biofilms on abiotic surfaces by deleting the ygi operon. Yad and Ygi fimbriae are involved in the colonization of the bladder [16]. Four genes (*draA, draC, draD,* and *draE)* from the Dr adhesin encoding operon are necessary for the complete expression of the mannose resistant haemagglutinin phenotype. The primary structural subunit that makes up each fimbrial appendage is encoded by *DraE* , which also functions as the sticky subunit for the DAF receptor [10,17] This study's objective was to examine the *yadN, ygiL* and *draE* genes in UPEC isolated from various UTI patients.

2.Materials and Methods

Isolation and Identification of UPEC

In order to isolate and biochemically identify *E. coli*, Hichrome E. coli and Hichrome UTI agar media (Himedia, India) were utilized[18]. A total of 200 urine samples from probable

UTI patients were collected. The VITEK 2 compact system was utilized to ensure obtaining accurate outcomes. The study was commenced after obtaining a clearance from the College of Science
Ethics Committee/ University of Baghdad (Ref.: CSEC/0922/0077).

Susceptibility test of UPEC

Kirby-Bauer disk diffusion susceptibility test was used to ascertain susceptibility to Ciprofloxacin antibiotic discs (Cipropharm, Pharma International). Susceptibility of UPEC isolates to 16 different antibiotics categorized into eight group (Pencillins, Cefalosporins, Carpenemes, Aminoglycosides, Quinolones, Tetracyclins, Nitrofurantion, and Trimethoprime/ Sulfamethaxazole) was tested by VITEK 2 compact system [19- 21].

Biofilm production

1. Congo red medium

For the biofilm development test, Congo red agar was employed. Culture medium constituents included 37g of brain heart infusion broth, 50g of sucrose, and 15g of agar-agar in 900 ml of D.W. The culture was sterilized, cooled at 55ºC, and 100ml of Congo red solution (0.8%) was added. After the streaking of bacterial isolates and incubation at 37 °C for 24 hr, pink colonies appeared to mark negative results (non-biofilm producers), while black colonies indicated strong biofilm producers and grey colonies indicated moderate biofilm producers [22, 23].

2. Tissue culture plates

In order to examine the adherence capability, UPEC isolates were grown in a nutritional broth containing 1% glucose in tissue culture plates [24- 26]. According to Atshan, *et.al*, the optical density cutoff (ODc) was calculated [27].

PCR protocol

1. DNA Extraction

Genomic DNA was extracted from bacterial growth according to the following protocol of ABIO pure Extraction:
- Overnight culture was re-suspended completely in Buffer BL to collect the cell pellet.
- For protein digestion and cell lysis, proteinase K solution was added.
- Absolute ethanol was added to the mixture, which was then transferred to the mini-column carefully and placed into a fresh 1.5 ml tube.
- Finally, Buffer AE was added and the mixture was incubated and then centrifuged (5,000 rpm for 5min).

2. **Quantitation of DNA**

The quantity of extracted DNA was measured using a Quantus Fluorometer. A diluted Quantifluor dye was combined with DNA. DNA concentration readings were found following 5 min of incubation period at room temperature.

3. Primers

Genes	{5' → 3'}	Annealing Tm	Product size (bp)	References
yadN	F : ATGCTGGCGTCTGAATGAC R : CATGTCGTTGTTCAAAGTCCC	55	185	28
ygiL	F : ACGCAAGTCCTGTTACGG R : GCCAGCAACAAGAAGTGAC	56	444	11
draE	F : TCATTTTGCCCAGTAACCCCC R :ATGAAAAAATTAGCGATCATGGCCG	60	463	29

4. PCR conditions

Steps	Temp ºC	Time	Cycle
Initial denaturation	95	5 m	1
Denaturation	95	30 s	
Annealing	55, 56, 60	30 s	30
Extension	72	30 s	
Final extension	72	7 m	1
Hold	10	10 m	

5. Agarose Gel Electrophoresis

Agarose gel electrophoresis was used to verify that amplification was present. Regarding the requirements for retrieved DNA, PCR is an entirely reliable methodology. A volume of 100 ml of 1X TAE buffer was mixed with 1.5 grams (1.5% agarose). After bringing the mixture to the boiling point, complete dissolve of all gel particles was achieved. A volume of 1µl of Ethidium Bromide (10 mg/ml) was added to the agarose gel. Samples of *E. coli* were separated and labeled with a 100 bp ladder marker [30].

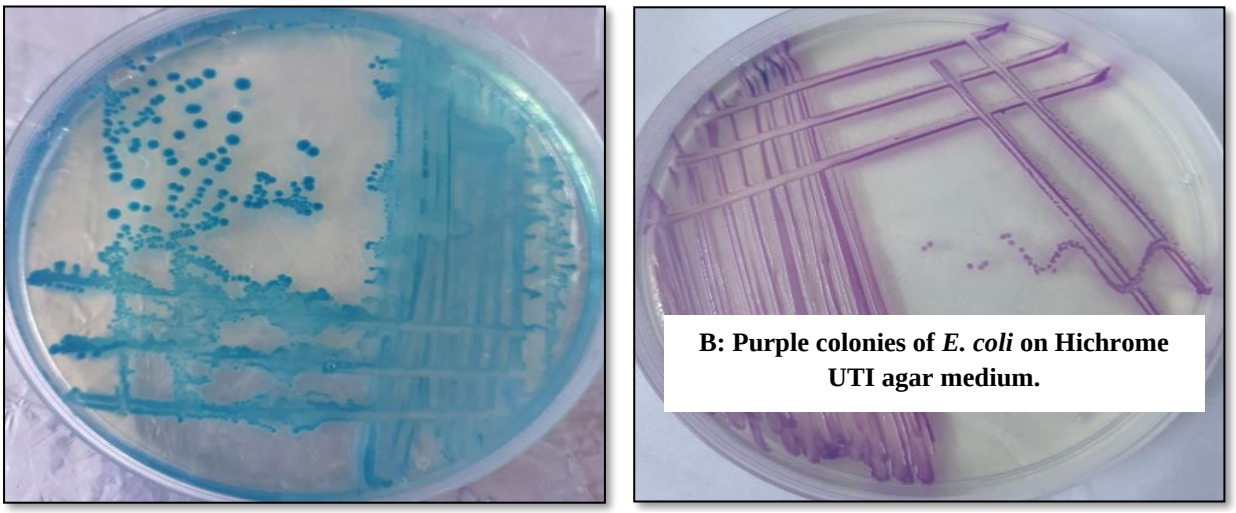
Statistical Analysis

Program: IBM SPSS version 27.0 was used to calculate the biofilm control mean and Standard deviation (SD) to determine the ability of adhesion for bacterial isolates [31].

3. Results and Discussion

Isolation and Identification of UPEC

According to the results of cell growth on Hichrome media (Figure 1) and biochemical tests, 40 *E. coli* isolates from 200 urine samples of probable UTI patients were detected and the results were confirmed with VITEK compact 2 [32, 27].



B: Purple colonies of *E. coli* on Hichrome UTI agar medium.

A: Blue colonies of *E. coli* on Hichrome *E. coli* agar medium.

Figure 1: Growth of *E. coli* on Hichrome agar

Susceptibility test of UPEC

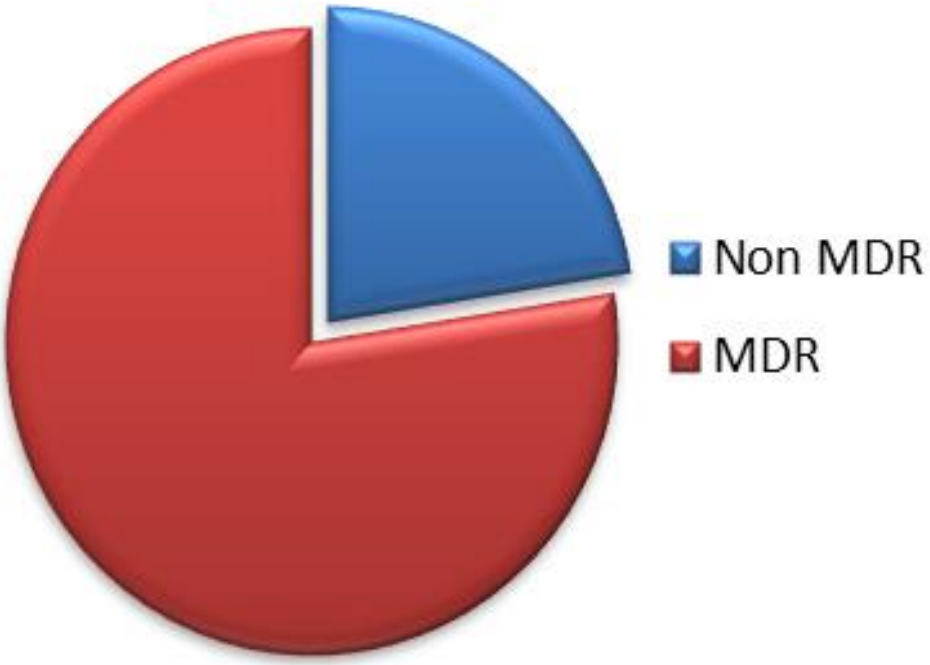


Figure 2: Susceptibility of UPEC to different groups of antibiotics

The Kirby Bauer technique produced identical results to those of the VITEK 2 compact system for examining the susceptibility of UPEC isolates to Ciprofloxacin discs. From 40 bacterial isolates, 21 (52.5%) were ciprofloxacin-resistant. The highest resistance of UPEC isolates was to Ampicillin (34/40) (85%) and Cefazolin (33/40) (82.5%), while the lowest resistance was to Amikacin and Tagicycline (0/40) (0%). Susceptibility of 40 bacterial isolates to 16 different antibiotics, which were grouped into eight groups by using VITEK 2 compact system, revealed that al isolates were neither XDR nor PDR, but 31 isolates (77.5%) were MDR (Figure 2, Table 1).

Table 1: Resistance of UPEC to antibiotic classes.

Isolate NO.	Resistance to antibiotic classes	Isolate NO.	Resistance to antibiotic classes
1	Pencillines, Cefalosporins, Carpenems, Quinolones and Trimethoprime/Sulfam	26	Pencillines, Cefalosporins and Trimethoprime/Sulfam
2	Pencillines, Cefalosporins and Quinolones	27	Pencillines, Cefalosporins, Aminoglycosides, Quinolones and Trimethoprime/Sulfam
3	Pencillines, Cefalosporins and Trimethoprime/Sulfam	28	Pencillines, Cefalosporins, Aminoglycosides and Trimethoprime /Sulfam
4	Pencillines, Cefalosporins, Quinolones and Trimethoprime/Sulfam	29	Pencillines, Cefalosporins, Aminoglycosides, Quinolones and Trimethoprime/Sulfam
5	Pencillines, Cefalosporins and Trimethoprime/Sulfam	30	Pencillines, Cefalosporins, Quinolones and Trimethoprime/Sulfam
6	Non MDR	31	Non MDR
7	Pencillines, Cefalosporins, Quinolones and Trimethoprime/Sulfam	32	Non MDR
8	Pencillines, Cefalosporins, Aminoglycosides, Quinolones and Trimethoprime/Sulfam	33	Pencillines, Cefalosporins, Aminoglycosides, Quinolones and Nitrofurantion
10	Pencillines, Cefalosporins and Trimethoprime/Sulfam	34	Pencillines, Cefalosporins, Aminoglycosides, Quinolones and Trimethoprime/Sulfam
11	Pencillines, Cefalosporins, Aminoglycosides, Quinolones and Trimethoprime/Sulfam	35	Pencillines, Cefalosporins and Trimethoprime/Sulfam
14	Pencillines, Cefalosporins and Trimethoprime/Sulfam	36	Pencillines, Cefalosporins, Quinolones and Trimethoprime/Sulfam
16	Non MDR	37	Pencillines, Cefalosporins and Trimethoprime/Sulfam
17	Non MDR	38	Pencillines, Cefalosporins, Aminoglycosides, Quinolones and Trimethoprime/Sulfam
18	Pencillines, Cefalosporins, Aminoglycosides, Quinolones and Nitrofurantion	39	Pencillines, Cefalosporins, Aminoglycosides, Quinolones and Trimethoprime/Sulfam
19	Non MDR	40	Pencillines, Cefalosporins, Aminoglycosides, Quinolones and Trimethoprime/Sulfam
20	Non MDR	41	Pencillines, Cefalosporins, Aminoglycosides, Quinolones and Nitrofurantion
22	Pencillines, Cefalosporins and Trimethoprime/Sulfam	42	Non MDR
23	Pencillines, Cefalosporins and Trimethoprime/Sulfam	43	Pencillines, Cefalosporins, Carpenems, Quinolones and Trimethoprime/Sulfam
24	Pencillines, Cefalosporins and Trimethoprime/Sulfam	44	Pencillines, Cefalosporins, Aminoglycosides, Quinolones and Trimethoprime/Sulfam
25	Pencillines, Cefalosporins, Aminoglycosides and Quinolones	45	Non MDR

Ramírez-Castillo *et.al* stated that "multi-drug resistance (MDR) is spreading at an increasingly rapid rate, which results in complications, unsuccessful treatments, and higher rates of mortality and morbidity [33]. When studied against 10 classes of antibiotics, it was found that out of the 500 UPEC strains, 16.40% and 4.20%, respectively, were MDR and XDR strains [34]. While G. Awadallah *et.al* found that the prevalence of MDR in 50 UPEC isolates was 70% [35]. The results obtained by Al-Hasnawy *et.al* showed that 37 (88.09%) of the 42 UPEC isolates were determined to be MDR and 5 isolates (11.90%) were XDR [36]. When UPEC isolates were compared to test strains, it was discovered that the prevalence of MDR was significantly greater (51% vs. 9%) [1]. The findings shown by Ahmed and Ganjo

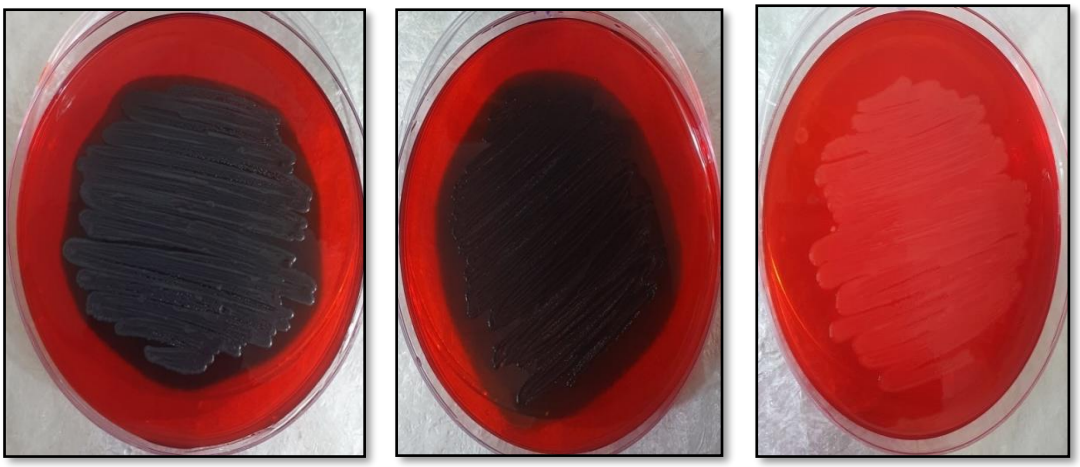
indicated that 42 of the 98 isolates of Enterobacteriaceae were MDR and 21 were non-MDR [37]. The majority of clinically effective antibiotics cause multifactorial resistance, which is primarily caused by drug efflux mechanisms, extrachromosomal plasmid-borne enzymes that are rendered inactive, and genomic changes. The pathogen's genomic plasticity which comprises a variety of mobile genetic components, like integrons, transposons, insertion sequences, and plasmids, also helps with this resistance. Continued clarification of the molecular processes behind resistance is required for clinical treatment to remain effective in the face of UPEC's ever-increasing multi-drug resistance [38].

Biofilm production

By using the Congo red and Microtiter plate methods, 25 UPEC isolates were investigated for their ability to produce biofilm; 21 isolates were Ciprofloxacin resistant, while 4 isolates were sensitive to all of the 16 antibiotics studied by the VITEK 2 compact system. According to Atshan, *et.al*., a cutoff value of 0.083 was computed [25]. According to the Congo red technique's results, only three isolates produced pink growth on the medium (12%) (non-biofilm producing), while (9/25) (36%) developed black colonies (strong biofilm) and (13/25) (52%) produced grey colonies (moderate biofilm) (Figure 3, Table 2). The results of using Microtiter plates exhibited that 44% of the isolates formed strong biofilms, whereas 56% formed intermediate biofilms.

Table 2: Biofilm production by the tested isolates as indicated by the Congo red technique.

Bacterial growth	Biofilm production	No. of isolates	Percentage
Black	Strong	(9/25)	36%
Grey	Moderate	(13/25)	52%
Pink	Non-producing	(3/25)	12%



A: Black colonies (Strong biofilm) **B: Grey colonies (Moderate biofilm)** **C: Pink colonies (Non biofilm producing)**

Figure 3: Growth of UPEC isolates on Congo red medium.

Recurrent, complex UTIs that are typically caused by MDR bacteria can also be brought on by biofilm-forming bacteria [39,40]. The color of colonies inoculated on CRA media changes, serving as a qualitative assay for the detection of microorganisms that generate biofilms [41]. A number of 200 *E. coli* isolates were tested for biofilm generation, and 125 (62.5%) of the isolates formed biofilms on Congo Red Agar [39]. Another work revealed that 69 (69%) of the 100 *E. coli* isolates tested for biofilm development were found to be positive by CRA [42,43]. These findings supported our investigation. Because biofilm-forming bacteria have a thick polymeric matrix that prevents antibiotic penetration, they often show better resistance

than planktonic cells. Biofilm-forming organisms show pronounced resistance to the majority of prescription antibiotics, including Gentamycin, Ceftriaxone, and Ciprofloxacin [44].

Detection of *yadN, ygiL,* and *draE* genes

In 22 UPEC isolates, including 21 isolates that were resistant to ciprofloxacin and one isolate that produced the strongest biofilm among the four isolates that were sensitive to ciprofloxacin and other antibiotics, the presence of the *yadN*, *ygiL*, and *draE* genes was detected after analysing PCR products on an agarose gel. The results showed that out of 22 PEC isolates, 19 (86.36%) contained the *yadN* gene (185bp) (Figure 4), 13 (59.09%) had the *ygiL* gene (444), and none had the *draE* gene (Figure 6).

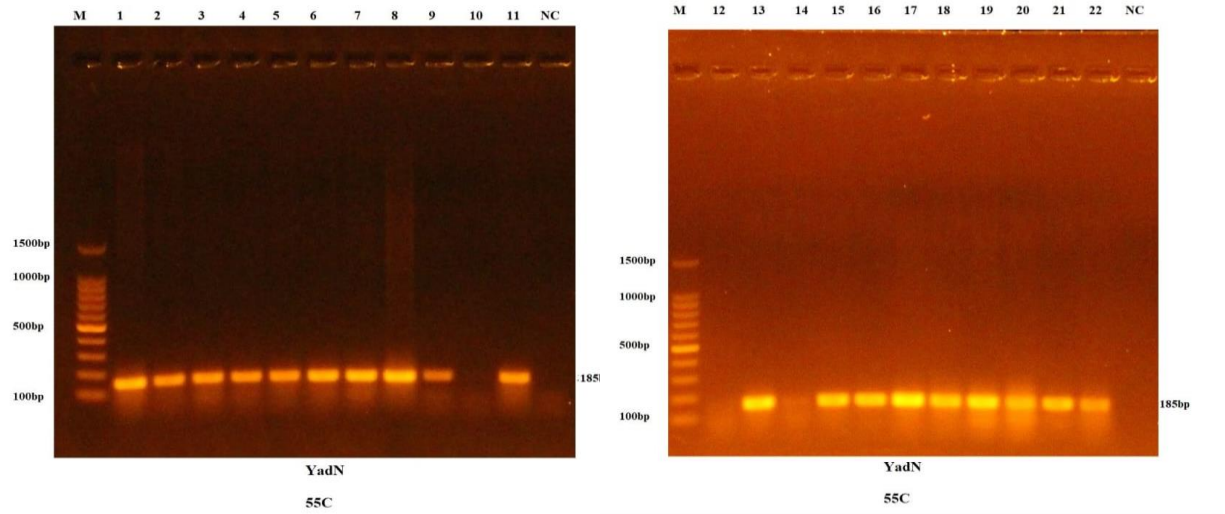


Figure 4: Results of *yadN* gene *(*185 bp) of *E. coli* samples on gel electrophoresis

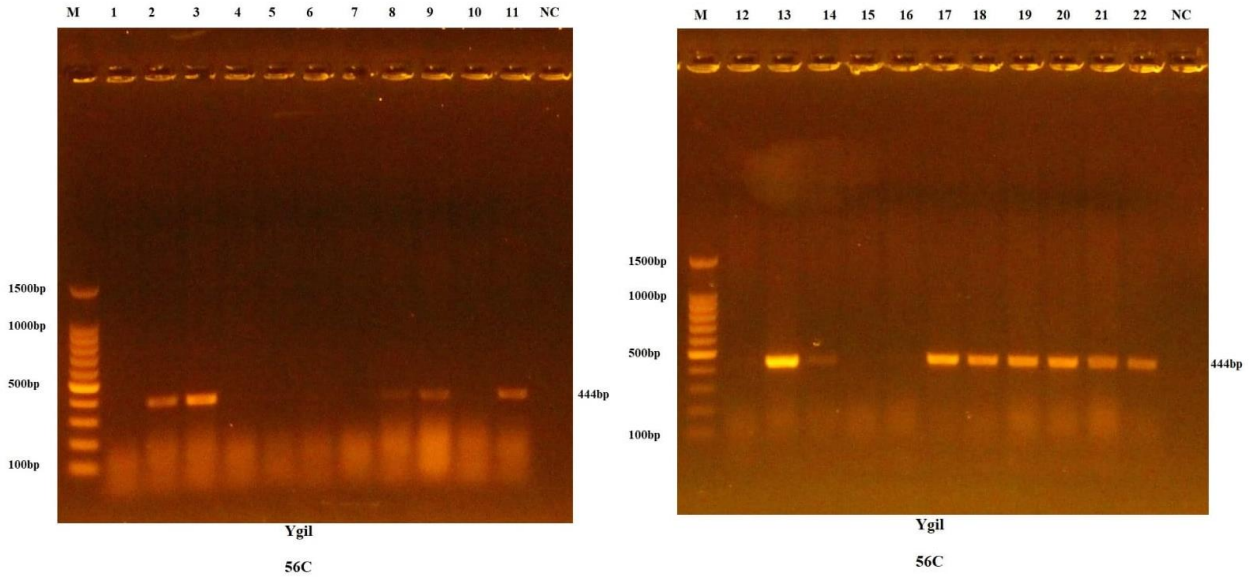


Figure 5: Results of *ygiL* gene *(*444 bp) of *E. coli* samples on gel electrophoresis**.**

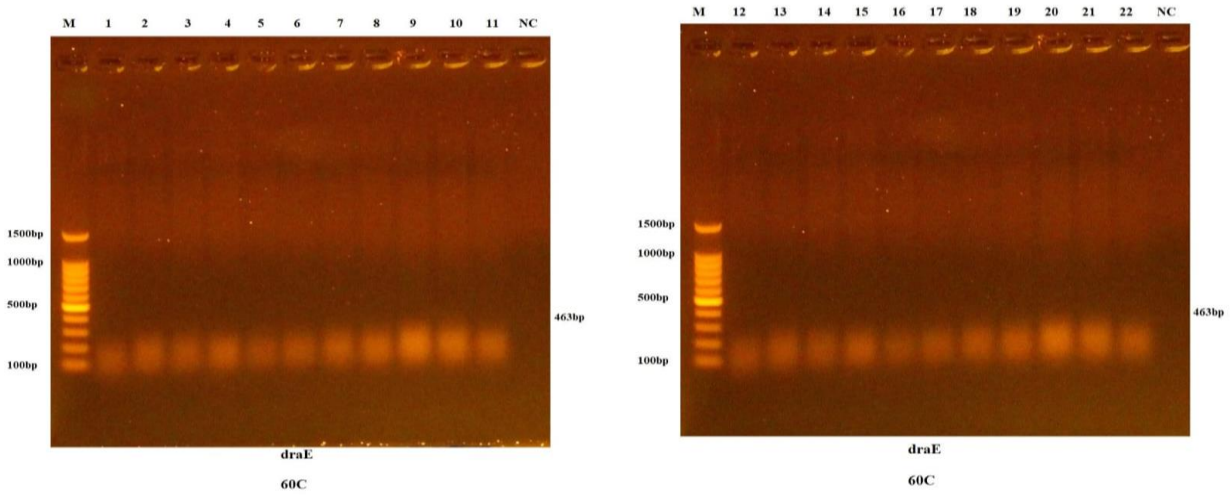


Figure 6: Results of *draE* gene *(*463 bp) of *E. coli* samples on gel electrophoresis.

A variety of virulence factors that are related to the bacteria's capacity to colonize the urinary system and cause disease are present in UPEC [45]. According to a previously published theory, ciprofloxacin-resistant bacteria may lose some virulence genes as a result of diminished gyrase and topoisomerase activity [46]. Dr fimbriae are made up of six subunits and controlled by many genes. The *draA* gene facilitates the regulation of transcription, *draB* and *draC* code for the chaperone, while ushe and *draD* code for the invasion of fimbriae . In addition, *draP* takes part in the mRNA cleavage mechanism and *draE* encodes the fimbriae tip subunit [47,48]. The association between fluoroquinolones and bacterial pathogenicity is particularly evident. In an earlier investigation, only the antibiotic ciprofloxacin caused the deletion of the urovirulence factor genes in all the six *E. coli* derivatives that have simultaneously lost *hlyA* and *cnf1,* which may be a sign that the PAI IIJ96 has been lost [49,50]. It is possible that Ygi fimbriae contribute to kidney colonization whereas Yad fimbriae adhere to bladder cells because several genes are related with isolates of pyelonephritis [51]. In contrast to commensal strains, UPEC isolates had a greater prevalence of *yqi*, *yadN*, and *ygiL*. Among the three UTI groups, there were no significant variations in the prevalence of the three genes [52]. It was revealed that the deletion of the yad operon from *E. coli* CFT073 reduced motility, biofilm development on abiotic surfaces, and adhesion to the tested human bladder cell line. This shows that Ygi and Yad fimbriae are both involved in bladder colonization [53].

Conclusions

In UPEC isolates, the yadN gene was more common than the ygiL gene, suggesting that both play important roles in pathogenicity.

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