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Detection of *afa* and *yqi* in Ciprofloxacin Resistant Uropathogenic *Echerichia coli*

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Abstract

Antibiotic resistance has dramatically increased among UTI patients with UPEC infections. They are crucial to the pathophysiology of UTIs because UPEC invades the bladder through a variety of virulence factors. Afa adhesins are produced by human-derived *Echerichia coli* and have been shown to bind to the (DAF, CD55) as a receptor, while Yqi adhesin recreates a denotative role in colonization, the initial stage of pathogenesis, during infection with *E.coli* . *E.coli* isolates from 200 urine samples of credible UTI patients were detected. By employing different media, VITEK, and the biochemical identification. Susceptibility to the Ciprofloxacin antibiotic was established by using the disc diffusion approach, while the susceptibility to Ciprofloxacin and other 15 antibiotics was achieved by the VITEK 2 compact system. A tissue culture plate was used in order to analyze the adherence ability of bacteria. Genomic DNA was pulled from cells according to the protocol of ABIO pure extraction. (Quantus Fluorometer) was used to get the concentration of DNA. *afa* and *yqi* genes were detected in 22 *E.coli* isolates amplified by PCR using certain primers. To ascertain whether the evaluated genes were present in the bacterial isolates, PCR products were checked on a gel. Twenty-one bacterial isolates from 40,52.5% 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%). The results appeared to indicate that (11/25,44%) of tested UPEC were strong biofilm-forming, while the rest of the isolates (14/25,56%) were moderate biofilm-forming. The analysis of PCR products on an agarose gel revealed that out of 22 UPEC isolates, 17/22,77.27%) had the *afa* gene and 7/22,31.81%) had the *yqi* gene. The aim of this research was screening of *afa* and *yqi* genes in UPEC isolated from different UTI patients.

Keywords: *Afa* gene, *yqi* gene, Urinary tract infection, Uropathogenic *E.coli*, Antibiotic resistance.

1. Introduction

Although antibiotics are currently the go-to therapy for bacterial illnesses, misuse of them may hasten the resistant strains from appearing and allow microorganisms to change their own

pathogenicity (1, 2). A common bacterial infection is urinary tract infection (UTI), and the organism that creates sharp infections most frequently is *E. coli* (3,4,5), especially among women (6). Wide-spectrum antibiotics like fluoroquinolones and quinolones are frequently used to treat UTIs caused by *E.coli* (7, 8). Ciprofloxacin is the most frequently given fluoroquinolone for UTIs due to its availability in both oral and intravenous formulations. Following oral administration, the digestive tract effectively absorbs it (9). Unfortunately, among UTI patients with UPEC infections, antibiotic resistance has significantly increased (10). Because UPEC applies a range of virulence managers to colonize the bladder, they are essential to the pathophysiology of UTIs (11, 12). Type 1 and 2 fimbriae, also known as P, Dr adhesin, S, and F1C fimbriae, are the two primary virulence agents that interact with the host cell adhesin (13, 14). *E. coli* that expresses Dr/Afa adhesins may predispose to the development of chronic or recurring infections; UPEC penetrated the epithelial cells through AfaD and AfaE, evading host immune surveillance and antibiotic therapy (3, 15). *Afa* determinants are ingredients of the (Afa/Dr family) of gene bunches, located on chromosomes that also encompass the (*dra* and *daa)* genes, which encode the (Dr and F1845) adhesins discretely (16, 17). An extremely conserved DNA section including the (*afaB, afaC,* and *afaD)* genes was found in *afa* gene clusters, whereas the *afaE* sequences showed variability, resulting in the development of adhesins that are antigenically unique (18, 19). The *Afa* operons reported in uropathogenic and diarrheal *E. coli* belong to *the Afa* family of gene clusters. The gene subtypes (afaE1, afaE2, afaE3, afaE5, dra, dra2, daa, and nfa) encode the Afa/Dr adhesins. Human-derived *E. coli* produces these adhesin, which bind to the DAF (CD55) as a receptor (20). a large number of new genes, including those that encode presumed adhesin like (*yad, yqi,* and *yeh*) (21). Now, it has been discovered that the adhesin, Yqi, recreates a denotative role in colonization, the initial stage of pathogenesis, during infection with *E.coli* (18). More than 50% of *E.coli*, including APEC and UPEC were discovered to carry the adhesin-encoding gene *yqi*, but none of the examined intestine pathogenic *E. coli* strains did (20). Multiple pilus systems are likely advantageous for niche adaptation, and the mixture of tissue-specific receptor synthesis and receptor specificity will eventually dictate the position of action for a particular pilus during attack (21).

2. Materials and Methods

2.1. Isolation and identification of organism

By employing (MacConkey, EMB, Blood) agar and (Hichrome *E.coli*, Hichrome UTI) agar (Himedia, India), Gram stain, VITEK, and the biochemical identification, a total of *E.coli* isolates from 200 urine samples of probable UTI patients were detected (22, 23).

2.2. Susceptibility of bacteria to ciprofloxacin and other antibiotics

Susceptibility to Ciprofloxacin antibiotic discs (Cipropharm, Pharma International) was established by using the Disc Diffusion approach (24, 25). Overnight, the isolated colony was cultivated in nutritional broth. It was diluted to 1 x 10^8 (cell/ml) before being cultured on Muller-Hinton agar. After adhering the antibiotic discs, we stored the plates at 37°C overnight (26). The outcomes were then linked with CLSI data from 2020 (27). The VITEK 2 compact achieved susceptibility to Ciprofloxacin and other 15 antibiotics (Ampicillin, Piperacillin/Tazobactam, Cefazolin, Cefoxitin, Ceftazidime, Ceftriaxone, Cefepime, Ertapenem, Imipenem, Amikacin, Gentamicin, Levofloxacin, Tigecycline, Nitrofurantoin, and Trimethoprim/Sulfamethoxazole).

2.3. Biofilm formation

Bacterial isolates were cultured in nutrient broth containing 91% glucose on a tissue culture plate in order to analyze the adherence ability. After incubation, we used DDW to thoroughly clean the wells three times. then left overnight to dry. 200 µl of 0.1% crystal violet was used to color the affixed cells for 15 min; any extra stain was washed away with distilled water and then allowed to dry. 200 µl of 96% ethanol were used to dissolve the crystal violate, and a spectrophotometer was used to assess the absorbance at 490 nm. (28). Three triplicates of the experiment are run. As the negative control, the absorbance of wells bearing sterile N.B. was used. We used the optical density cutoff (ODc) to differentiate the isolates based on adhesion quantities (ODc = average OD of negative control + 3 standard deviation (SD) of negative control) (29). Optical density OD ≤ 2*ODc represents weak adherance, while 4*ODc ≤ OD refers to strong biofilm and moderate adherence between them (30).

2.4. DNA Extraction

Genomic DNA was extracted from cells according to the protocol of ABIO Pure Extraction (ABIO Pure, USA). (Quantus Fluorometer) (Promega, USA) was employed to gauge the quality of samples for use in subsequent applications by measuring the concentration of DNA that had been extracted. For 1 µl of DNA, add 200 µl of diluted Quantifluor dye. Incubated at room temperature (5 min); thereafter, DNA concentration values were found.

2.5. Amplification of *afa* and *yqi* genes of uropathogenic *E.coli*

The *afa* and *yqi* genes were detected in 22 *E.coli* isolates amplified by PCR using certain primers (Macrogen, Korea) (**Table 1**). The following were the PCR scenarios: early denaturing at 95 ºC (5 min), then 30 cycles, each for 30 sec; denaturation at 95ºC, annealing, and extension steps. Finally, one extension cycle at 72 ºC (7 min) and hold at 10 ºC (10 sec). After amplification, PCR yields were determined (31, 32).

Table 1. Primers

Genes	{5' → 3'}	Annealing Tm	Product size	Reference
afa	F : CGGCTTTTCTGCTGAACTGGCAGGC R : CCGTCAGCCCCCACGGCAGACC	65	672	22
yqi	F : ATGCAATGGCAGTACCCTTC R : CTGGTGGCAACATCAAATTG	60	375	21

2.6. Statistical analysis

Program: IBM SPSS version 27.0 was used to calculate the biofilm control mean and Standard Deviation (SD) to determine the adhesion quantities for bacterial isolates.

3. Results and Discussion

3.1. Isolation and identification of organism

According to the results of cell growth on different media, Gram stain and biochemical tests (40 *E.coli* isolates) from 200 urine samples of probable UTI patients were detected, and the results were confirmed with the VITEK Compact 2 system (33, 34).

3.2. Susceptibility of bacteria to ciprofloxacin and other antibiotics

The results obtained by using the Kerby pour method for the susceptibility of UPEC isolates to Ciprofloxacin discs were identical with the results of the VITEK 2 Compact system: 21 bacterial

isolates from 40 (52.5%) were resistant to ciprofloxacin **(Figures 1 , 2)**. The results of the susceptibility of UPEC isolates to different antibiotics by using the VITEK 2 Compact system revealed that the most resistant of UPEC isolates was to ampicillin (34/40) (85%) and Cefazolin (33/40) (82.5%), while the least resistant were to Amikacin and Tagicycline (0/40) (0%). The results also showed that 31/40) (77.5%) of UPEC isolates were MDR, and 20/31) (64.5%) of these MDR isolates were resistant to Ciprofloxacin (**Table 2**).

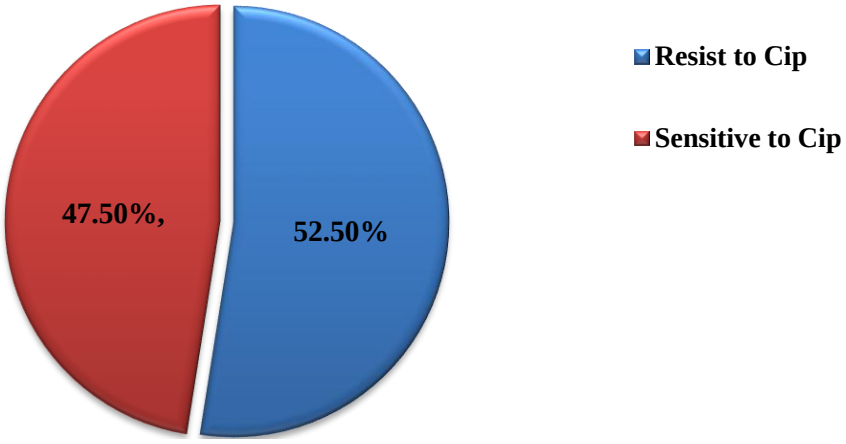

Figure 1. Percentage of Ciptofloxacin resistant UPEC.

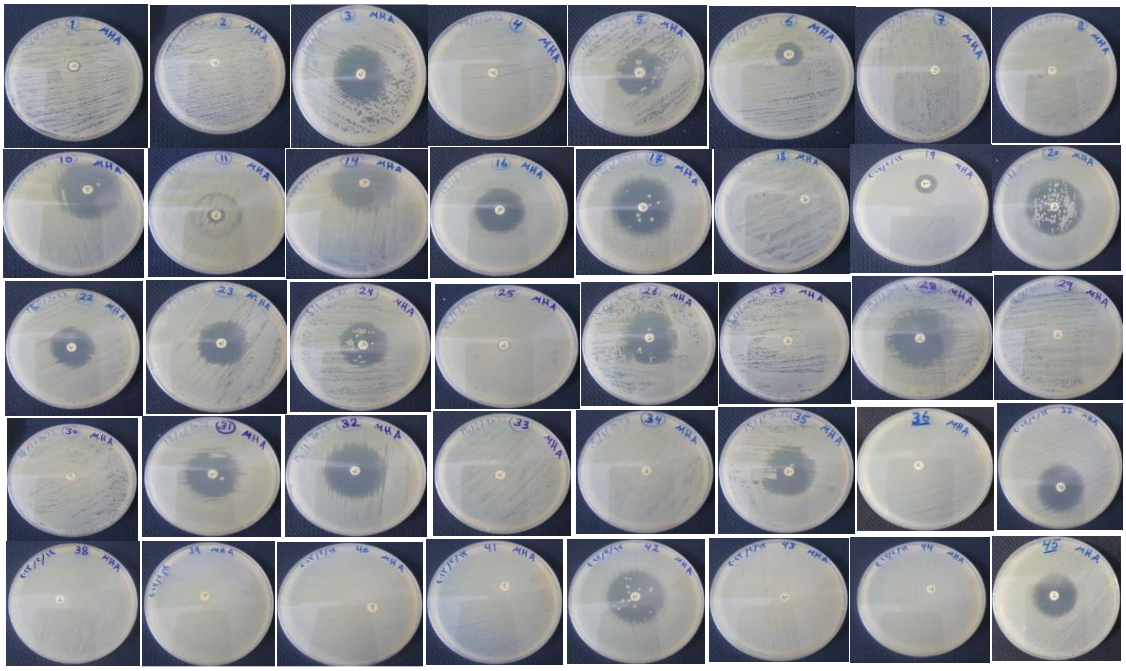

Figure 2. Susceptibility of UPEC to Ciprofloxacin discs on MHA by sing Kerby pour method**.**

Resistance transfer genes are simple for *E. coli* to obtain and can be carried on plasmids. (35,36). A wide-scale antibiotic named ciprofloxacin acts by averting DNA gyrase (topoisomerase II and IV) from performing on its target (37). Bacterial resistance to ciprofloxacin has been revealed to be on the rise (22.4%)) and male UTI patients were more likely to undergo this resistance (38). (39) reported that out of 324 UPECs analyzed, 61 (18.8%) were resistant to Ciprofloxacin (39). High resistance (76%) to Ciprofloxacin was obtained from testing 50 UPEC isolates (40). These findings showed that multidrug resistance was linked to ciprofloxacin resistance disseminated by UPEC that led to communicable UTIs. (38, 41).

Table 2. Percentage of resistant UPEC to different antibiotics.

Antibiotic	No. of resistant UPEC	%
Ampicillin	34	85
Piperacillin/Tazobactam	4	10
Cefazolin	33	82.5
Cefaxitin	7	17.5
Ceftazidime	20	50
Ceftriaxone	30	75
Cefepime	7	17.5
Ertapenem	1	2.5
Imipenem	2	5
Amikacin	0	0
Gentamicin	14	35
Ciprofloxacin	21	52.5
Levofloxacin	21	52.5
Tagecycline	0	0
Nitrofurantion	3	7.5
Trimethoprim/Sulfamethoxazole	28	70

(40) demonstrated that Ampicillin had the highest level of resistance (94%), whereas imipenem, Amikacin, and Nitrofurantoin had the lowest amount of resistance to UPEC (0%) (40, 42). (43) reported that the highest resistance rate (95.23%) among UPEC was to cefepime (43). Other findings revealed resistance to Ampicillin was the highest (85%), while Amikacin displayed a decreased frequency (38). Our study was able to identify all of these findings; thus, we can suggest Amikacin and Tagicycline as the best medicines to treat UTIs.

3.3. Biofilm producing UPEC

Twenty-five UPEC were explored for their talent to prompt biofilm by using Microtiter plates (21 isolates were resistant to Ciprofloxacin, and four isolates were sensitive to all 16 antibiotics tested by the VITEK 2 Compact). The cutoff value (0.083) was calculated according to (29), and the results appeared to indicate that (11/25) were strong biofilm-forming (44%), while the rest of the isolates (14/25) were moderate biofilm-forming (56%) **(Table 3**).

Table 3. Biofilm producing UPEC**.**

Biofilm producer	Resistant UPEC	Sensitive UPEC	Total NO.	%
Strong	9	2	11	44
Moderate	12	2	14	56
Weak	0	0	0	0

More than 60% of human illnesses have documented biofilm formation in the environment (39). *E. coli* has the ability to aggregate and adhere to solid surfaces, creating intricate formations known as biofilms (44,45). Additionally, quorum sensing processes frequently regulate transcriptional alterations that correlate with these bacteria's creation of biofilms. This may result in the differential expression of distinct virulence factors and antibiotic resistance determinants (39). Biofilm-producing isolates displayed higher levels of antibiotic resistance than non-biofilm producers (46). Prostatitis, biliary tract infections, and urinary catheter cystitis are only a few of the major health issues that can result from biofilms comprised by clinical *E. coli* strains (47).

About 46% of UPEC isolates exhibited curli production. The strong collaboration between biofilm formation and the MDR phenotype leads to the recurrence of infections (48).

3.4.Detection of *afa* and *yqi* in uropathogenic *E.coli* isolates

After analyzing PCR products on an agarose gel, the appearance of the (*afa* and *yqi* 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, was discovered. Out of 22 UPEC isolates, the results showed that 17 (77.27%) had the *afa* gene **Figure 3** and 7(31.81%) had the *yqi* gene **Figure 4**.

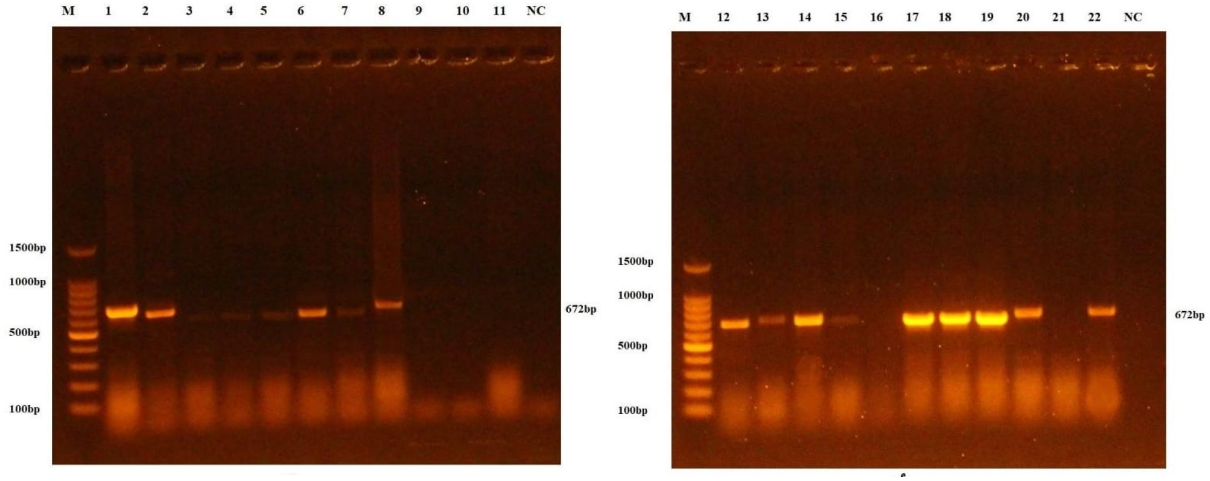

Figure 3. Results of *afa* gene (672 bp) of *E. coli* samples were fractionated on gel electrophoresis.

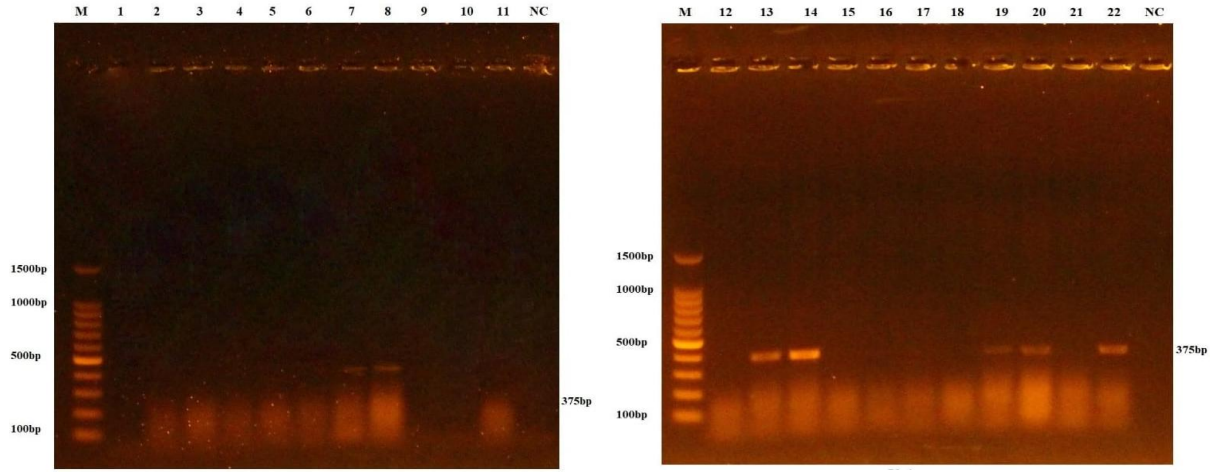

Figure 4. Results of *Yqi* gene *(*375 bp) of *E. coli* samples were fractionated on gel electrophoresis.

Dr. wFamily afimbrial adhesins have a particular renal tissue tropism associated with UTI. This characteristic may encourage the development of persistent and recurrent UTI (49). Many UPEC strains include the Dr Family afimbrial adhesins Afa-I and Afa-III, which attach to the receptor on the blood group Ags are formed on (DAF), preventing complement activity from lysing cells (22). afaA, afaB, afaC, afaD, and afaE represent transcriptional regulator, Periplasmic chaperone, Outer membrane usher protein, Afimbrial adhesion, and Adhesin protein, respectively (50). Out of the 212 UPEC isolates that were studied for the appearance of the (*afa* gene, 49 (23.1%) did (22). (51) reported that two of the 56 UPEC isolates had *the afa* gene (3.57%). Also, (52) reported that 12% of the *E. coli* isolates from 100 urine samples carried the

afa gene. Other findings indicated that none of the 10 UPEC isolates carrying the *afa* gene were from other UTIs or intestinal demeanors, and all were placed in the recurrent lower UTI group (3). The highly pathogenic Extraintestinal pathogenic *E. coli* (ExPEC) strains Avain pathogenic *E. coli* (APEC), Uropathogenic *E. coli* (UPEC), and Newborn meningitic *E. coli* (NMEC) are known to be associated with Yqi, also known as ExPEC adhesin I (21). *Yqi* may have a very certain role in the pathogenesis of ExPEC. Recently, it has been discovered that *Yqi* is crucial to colonization (18). The prevalence of the *yqi* gene was (65.9%), (54.4%), and (60.0%) in 138 UPEC, 406 APEC, and 25 NMEC, respectively, while none of the 153 intestine pathogenic *E. coli* isolates were discovered to have the *yqi* gene (21). In another study, it was found that the *yqi* gene was found in 7% of intestinal commensal isolates; however, the occurrence was much lower (27%) than in UPEC strains (3).

4. Conclusion

The highly pathogenic Extraintestinal pathogenic *E. coli* (ExPEC) strains Avain pathogenic *E. coli* (APEC), Uropathogenic *E. coli* (UPEC), and Newborn meningitis *E. coli* (NMEC) are known to be associated with Yqi, also known as ExPEC adhesin I. *Yqi* may have a very certain role in the pathogenesis of ExPEC. Recently, it has been discovered that *Yqi* is crucial to colonization. The prevalence of the *yqi* gene was detected in UPEC, APEC, and NMEC, while none of the intestine pathogenic *E. coli* isolates were discovered to have the *yqi* gene.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Ethical Clearance

The study advanced after receiving institutional ethical committee (IEC) approval. The College of Science Ethics committee approves the research proposal to be conducted in the presented form. None of the investigators and co-investigators participating in this study took part in the decision-making and voting procedure for this study.

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