Archives of Razi Institute, Vol. 76, No. 4 (2021) 829-840

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DOI: 10.22092/ari.2021.355465.1689

Original Article

Molecular Analysis of *fimA* Operon Genes among UPEC Local Isolates in Baghdad City

Abdul Raheem Hasan, S[1 *], Sajid Al-Jubori, S[2], Abdul Sattar Salman, J[2]

1. Department of Biology, College of Education for Pure Science (Ibn al-Haitham), Baghdad University, Baghdad, Iraq
2. Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq

Received 6 July 2021; Accepted 8 August 2021
Corresponding Author: sazan.a.h@ihcoedu.uobaghdad.edu.iq

Abstract

Specialized *Escherichia coli (E. coli)* isolates, called uropathogenic *E. coli* (UPEC), cause most of urinary tract infections (UITs). Once bacteria reached the urinary tract of the host, they have to adhere to the host cell for the colonization. For this purpose, bacteria have different structures including fimbrial adhesins. Most of the UPECs contain type 1 fimbriae encoded by *fim* operon (*fimB, E, A, I, C, D, F, G, H*) which is responsible for the adhesive ability in these isolates. Ninety-four isolates of UPEC were obtained from UTI patients in Baghdad hospitals and their diagnosis were confirmed by the PCR method using *16srDNA* as a housekeeping gene. The UPEC isolates were tested for their ability of adherence to the urothelial cells obtained from the mid-stream urine from healthy women. Fifty isolates were subjected to detect type1 fimbriae genes (*fimA* operon) using specific primers followed by sequencing the amplified fragment which they were analyzed by Geneious software. The results confirm that all the isolates were *E. coli* according to the genetic analysis by the PCR test, and also, the ability of attachment for all isolates were approved (100%).For type 1 fimbriae, the findings figured out that 100% of the isolates harbored *fimA,fimI, fimC, fimD, fimG* and *fimH* genes; while 96% of them were positive for *fimB, fimF*,and 82% of the isolates were positive for *fimE*. This result exhibited a higher prevalence of *fim* genes, as the attachment ability was 100%. Approximately, all UPEC have type 1fimbrial genes, so it could be used as a genetic marker in the investigation of *E. coli* adhesion ability.

Keywords: *fimA* operon**,** Uropathogenic *Escherichia coli*, attachment ability

1. Introduction

Escherichia coli (*E. coli*) is a genus within the *Enterobacteriaceae* family that consist of a large number of species (1). It considered the most common causative agent for both the uncomplicated and complicated urinary tract infection (UTI) (2). Its rapid growing on chemically defined media and well-studied bacteria, make it an essential model organism, so it also called workhorse of molecular biology. *E. coli* is considered one of most essential pathogen within human body and about 70-95% of the community acquired UTI, beside 50% of nosocomial UTI were caused by the uropathogenic *Escherichia coli* (UPEC) infections (3). UPEC have many virulence factors (VFs), which prefer the adaptation in urinary tracts and allow them to disrupt the barriers of the immune system. Once bacteria reached surface of the host, for colonization, they have to adhere to host cell. The adherence ability of *E. coli* to host cells play an essential role in the process of colonization (4). In general, adhesins usually are exposed to the bacterial surface or they carried by filamentous structures fimbriae or pili and most of UPECs contain type 1 fimbriae encoded by *fim* operon (*fimB, E, A, I, C, D, F, G, H*) which are existed in the chromosome of most UPEC isolates (5). Therefore, current study was

designed to investigate the genetic marker and the potential of different isolates of *E. coli* adhesion ability.

2. Material and Methods

2.1. Collection and Diagnostic of UPEC Isolates

UPEC isolates were obtained from Hospital laboratories in Baghdad Iraq from UTI patients within the period from January 2019 to April 2019.

2.2. Attachment Ability to Pathogenic *E. coli*

Urine samples were taken from the healthy women (the midstream urine) as the main source for the uroepithelial cells. Due to the change in pH during the menstrual cycle, the sampling was done during the luteal phase of menstrual cycle. The samples were centrifuged for 5 min at 3000 rpm; the sediment that contained epithelial cells was washed with the phosphate buffer saline four times. The cells solution was considered a source for the uroepithelial cells for later use in adhesion assay (6). The concentration of bacterial cells was adjusted to 1.5×10^8 CFU/ml compared to McFarland (0.5), the epithelial cells were added. After the 60min of incubation time at 37°C, the disparate bacteria were eliminated by washing for several times with the phosphate buffer saline. The cells then were fixed and stained with the gram stain. Theadhered bacterial cells were counted directly under the light microscope (7).

2.3. Bacterial DNA Extraction

Broth of the bacterial isolates were cultured overnight in LB, then they were subjected for DNA extraction, by using ABIO pure TM kit. The concentration and purity of the extracted DNA were measured using Nanodrop.

2.4. Molecular Study

The housekeeping gene (HKG) 16S rDNA was used for the molecular diagnosis via PCR test(8, 9); *fim* operon specific primers were specially designed in current study, and they were used. Their sequence, and accession number beside the amplified molecular size, were itemized in table 1. The PCR mixture of the mentioned gene was composed of 12.5 µl of GoTaq®Green Master Mix (1x), 5µl DNA template; 1.5 µl for each of the forward and reverse primers (final concentration was 0.6 pmol/µl), and 4.5µl of deionized nuclease free water to reach the final volume of 25 µl. Then, the Eppendorf tubes were mixed shortly with vortex before being placed in PCR apparatus. For negative control, the mixture of the PCR without the template of DNA was used; the conditions whichwere used for the amplification process for all genes aredescribed in table 2. The final products subjected directly to the electrophoresis.

The PCR product for the amplified genes (stored at -20ºc) was sequenced by sending 25µl of the amplified product to Macrogen, Korea. Data were analyzed using Geneious software and the results were read by comparing them with the NCBI control standard strains. Query, pairwise alignment and identity, were anatomized with same software.

Table 1. Primers, sequence, accession number beside amplified molecular size; used in this study

Primer Name	Forward Primer (5'….. 3')	Reverse Primer (5'….. 3')	Siz (bp)	Accession number
***16Sr* DNA**	GGAAGAAGCTTGCTTCTTTGCTGAC	AGCCCGGGGATTTCACATCTGACTTA	544	LC278376
fimH	AATTGCCGTGCTTATTTTGCG	AGGTTTTGGCTTTTCGCACAA	204	LR134208
fimD	TCATCTGCCGAACTCTATTTT	TTTTGCTACCTGATGATCTGT	595	CP034595
fimE	AAACGTCGTTATCTTACCGG	TTTCTTTCCCATAATCCGGC	550	AP022362
fimC	GGGTAGAAAATGCCGATGGTG	CGTCATTTTGGGGGTAAGTGC	477	CP054371
fimA	AAATTAAAACTCTGGCAATCGT	TCCGTTATTCAGGGTTGTTT	446	LR883000
fimB	CGAATCACTCCTTAAAGCA	GGCGTAACATGT GCGGA	379	MK301554
fimG	GCGATCTTTATTCTTTCAGTCT	TGTAGGTATAGGTGATGCTAAT	348	CP061337
fimI	GGTGCCTTTTGTTATTCATTTAC	GATATTTGGCGATGAAATGTAG	251	CP059137
fimF	CTGAATCAACCAATTTTACTGTT	CATTAAGGGGTATCTGATTTTG	261	CP018252

Table 2. PCR programs used for amplification *E. coli* type 1 fimbrial genes

Amplified genes	Initial denaturation	Cycles No.	Denaturation	Annealing	Elongating	extension
***16Sr* DNA**	95 ºC for 5 min	30	94 ºC for 30 sec	55 ºC for 30 sec	72 ºC for 1 min	72ºC for 7 min
fimH	94 ºC for 5 min	40	94 ºC for 30 sec	58 ºC for 30 sec	72 ºC for 1 min	72ºC for 7 min
fimE	94 ºC for 5 min	35	94 ºC for 30 sec	55 ºC for 30 sec	72 ºC for 1 min	72ºC for 5 min
fimB	94 ºC for 5 min	35	94 ºC for 30 sec	60 ºC for 30 sec	72 ºC for 1 min	72ºC for 5 min
fimD	94 ºC for 5 min	40	94 ºC for 30 sec	55 ºC for 1min	72 ºC for 1 min	72ºC for 7 min
fimI	94 ºC for 5 min	35	94 ºC for 30 sec	55 ºC for 1min	72 ºC for 1 min	72ºC for 5 min
fimG	95 ºC for 5 min	40	94 ºC for 30 sec	54 ºC for 30 sec	72 ºC for 1 min	72ºC for 7 min
fimF	95 ºC for 5 min	40	94 ºC for 30 sec	54 ºC for 30 sec	72 ºC for 1 min	72ºC for 7 min
fimC	95 ºC for 5 min	35	94 ºC for 30 sec	60 ºC for 30 sec	72 ºC for 1 min	72ºC for 7 min
fim A	94 ºC for 5 min	35	94 ºC for 30 sec	60 ºC for 30 sec	72 ºC for 1 min	72ºC for 7 min

3. Results

3.1. *E. coli* Identification

16s rRNA was used as HKG for genotypic identification for UPEC isolates which were exposed, and all the isolates (100%) showed a positive result with expected amplicon size reached 544 bp as were matched with 100 bp DNA ladder; The pairwise identity was 96.6%, which represent exemplify residues percentage that were identical in alignment with gaps versus non-gap residue (Figure 1A and 1B). Some of the differences appeared between local isolate and recorded NCBI strain; as with a gap appeared within the nucleotides which it may be due to the sequencing process**.** The pairwise identity and the DNA sequencing for *16s rRNA* gene matched with the standard strain *E. coli* taxon: 562, LC278376 (NCBI) (Figure 1B).

3.2. Attachment Ability of UPEC

All the tested isolates were able to attach to the epithelial cells. The *fim* operon (Adhesive factor genes) can be used as a genetic marker in the investigations of UPEC that cause UTI.

3.3. *fimA* Operon Genes Result (Genotypic Detection and Data Analysis)

The results showed that all the isolates (100%) were positive for *fimA, fimI, fimC, fimD, fimG* and *fimH*; While 96% of them were positive for *fimB* and *fimF*, and 82% for *fimE*.

The sequences of *fimB* gene for *E. coli* isolates were displayed in Figure 2A. Sequence comparison was done between DNA segment of the current study and the standard strain (MK301554) in which pairwise identity

reached 98.3% that represented the percentage of the residues identical in alignments including gap verses non-gap residues (Figure 2B). Few gaps were noticed between the local isolates and the standard strain.

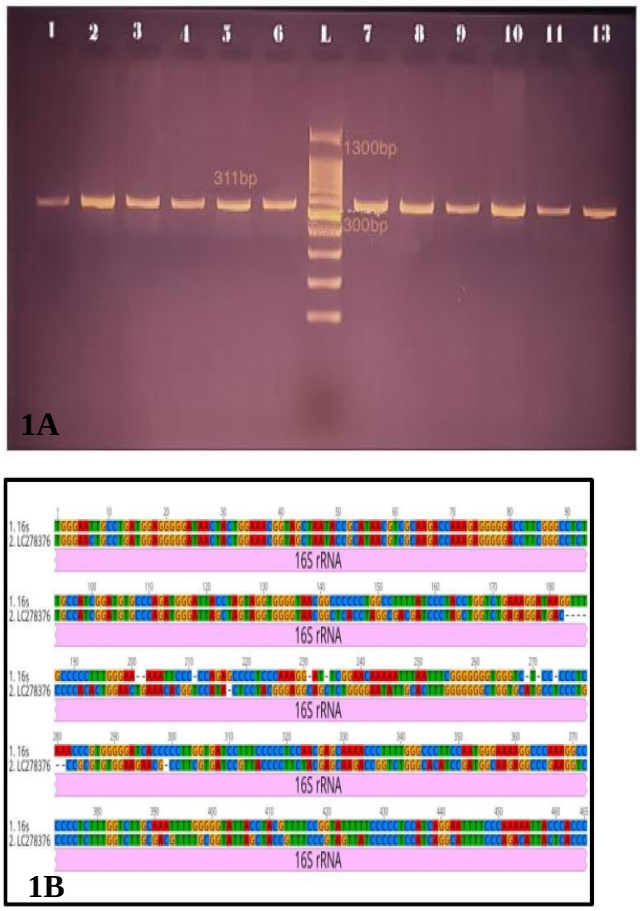

Figure 1. A: Genotypic identification for UPEC isolates, **B:** Pairwise identity and the DNA sequencing for 16s rRNA gene matched with the standard strain E.

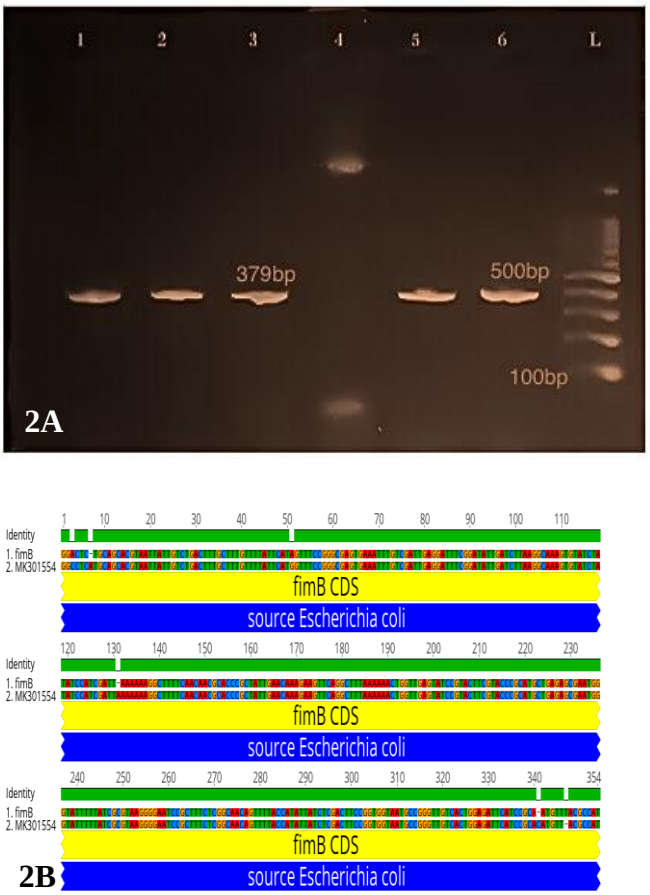

Figure 2. A: Gel electrophoresis of DNA segment of the *fimB* and the standard strain (MK301554) **B:** Pairwise identity and nucleotide sequence for *fimB* as compared with the standard NCBI *E. coli* strain MK301554

fimE gene percentage reached 82% in the current study, and some of the positive results are pointed in Figure 3A, the expected size in the agarose gel was 550 bp amplicon as compared with DNA ladder; Pairwise identity was 95% with few differences documented between local isolate and NCBI strain (AP022362) (Figure 3B).

fimA gene is another *fim* gene from this operon that was studied; It is encoding a major subunit. Results demonstrated that 100% of *E. coli* isolates harbored this gene with probable size of 446 bp amplicon (Figure 4A); Besides, the sequence of the amplified *fimA* gene was analyzed and compared with standard strain (LR883000) (Figure 4B). Pairwise identity was 100%, which represented the percentage of the residues identical in alignments.

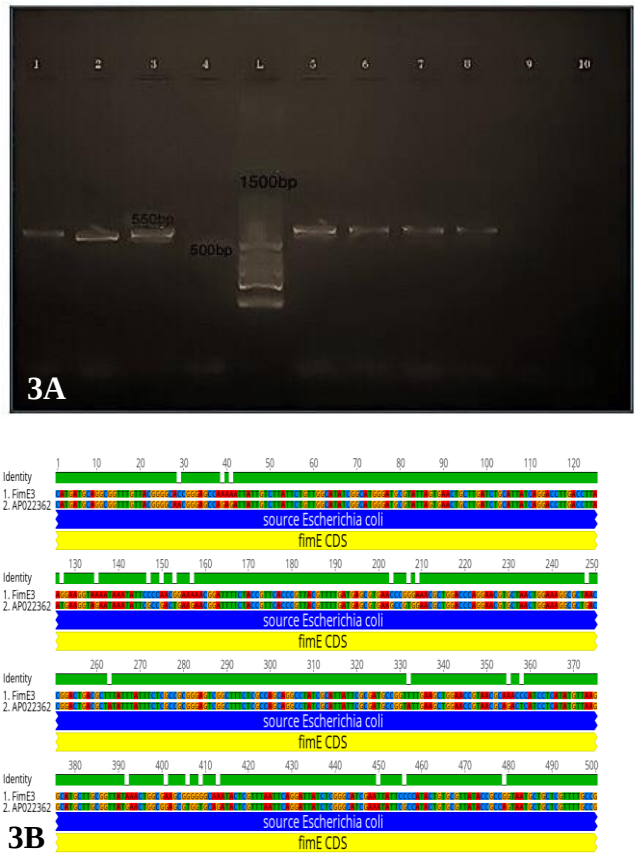

Figure 3. A: Amplified PCR amplicon of *fimE* gene with molecular size reached 550 bp, **B:** Pairwise identity and nucleotide sequence for *fimE* as compared with the standard NCBI *E. coli* strain AP022362.

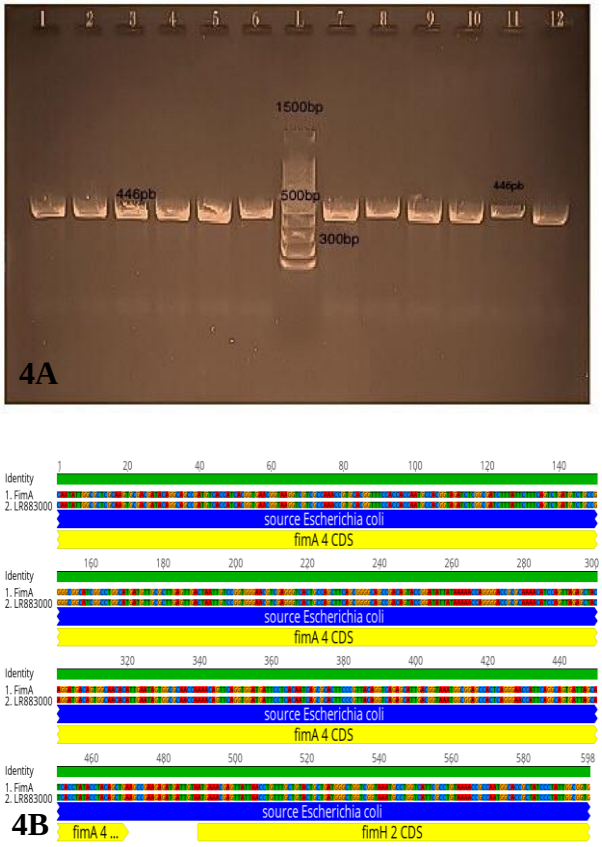

Figure 4. A: Amplified PCR amplicon of *fimA* gene with molecular size reached 446 bp**, B:** Pairwise identity and nucleotide sequence for *fimA* as compared with the standard NCBI *E. coli* strain LR883000.

fimI, which encodes the structural components, was the next amplified gene. Amplification of *fimI* gene showed that 100% of UPEC isolates comprised of this gene. Figure 5A displays positive shine band for *fimI* with probable amplified size of 251 bp; *fimI* sequence of UPEC was presented in Figure 5B. The comparison was performed between this DNA segment and standard strain CP059137. The Pairwise identity was 100% for precise size of 329 bp with indifferences between the local isolate and the recorded NCBI strain as no gaps appeared in the Query identity line.

Results of *fimC* exhibited that 100% of the isolates were harboring this gene. Figure 6A shows an amplified PCR amplicon of *fimC* gene with molecular size reached 477 bp. *fimC* gene sequence of *E. coli* was analyzed and is shown in Figure 6B. The comparison was done between DNA segment and standard strain (CP054371).

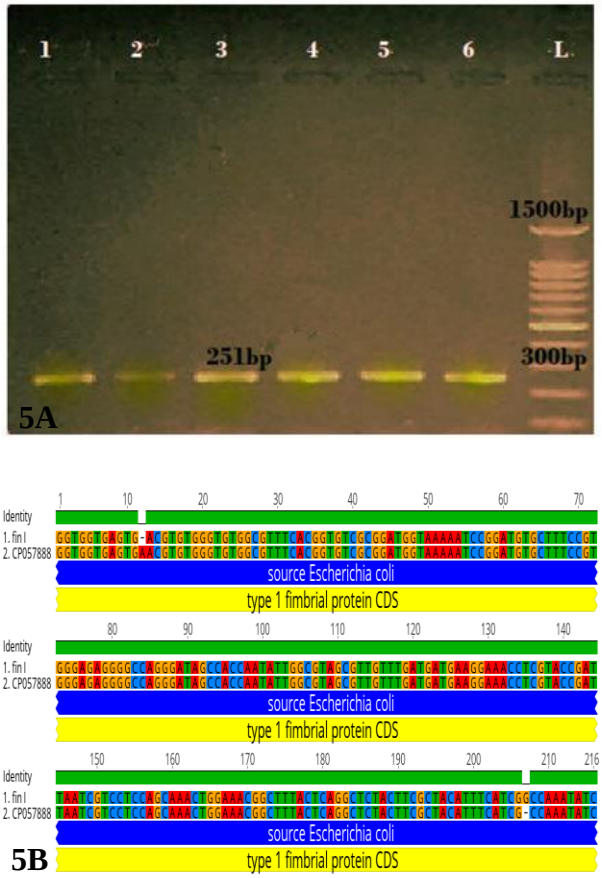

Figure 5. A: Amplified PCR amplicon of *fimI* gene with molecular size reached 251 bp, **B:** Pairwise identity and nucleotide sequence for *fimI* as compared with the standard NCBI *E. coli* strain CP057888.

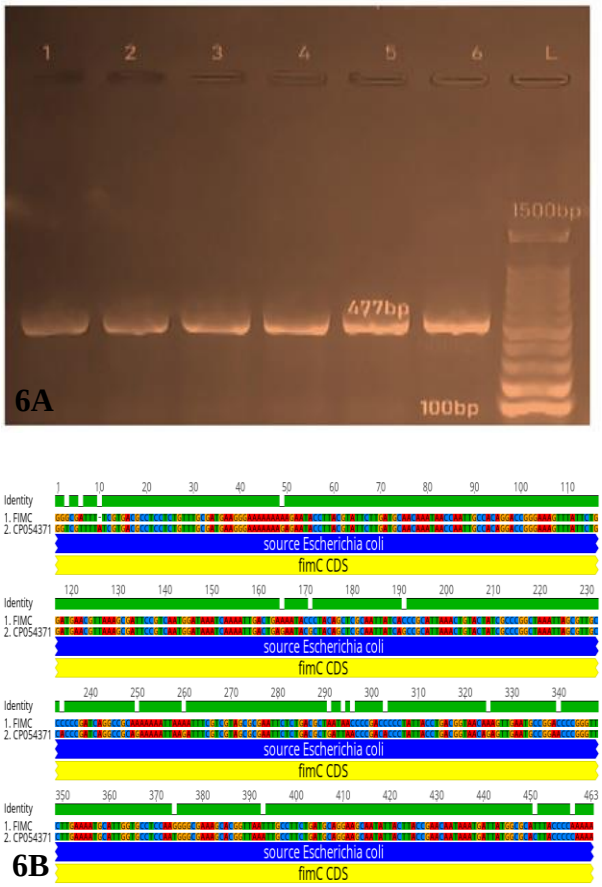

Figure 6. A: Amplified PCR amplicon of *fimC* gene with molecular size reached 477 bp, **B:** Pairwise identity and nucleotide sequence for *fimC* as compared with the standard NCBI *E. coli* strain CP054371.

fimD results revealed that it was appeared in 100% of the isolates as with *fimC*. Figure 7A demonstrates the PCR product for *fimD* gene with its amplified size 595 bp as compared with 100 bp DNA ladder. Pairwise identity and nucleotide sequence for *fimD* as compared with the standard NCBI *E. coli* strain CP034595 (Figure 7B).

fimF was expressed in most of the local isolates (96%,) and the product size was about 261 bp *fimF* sequence was analyzed and showed visually in figure 8A. The pairwise identity was 98% (Figure 8B). The occurrence rate for *fimG* gene was 100% in all UPEC isolates.

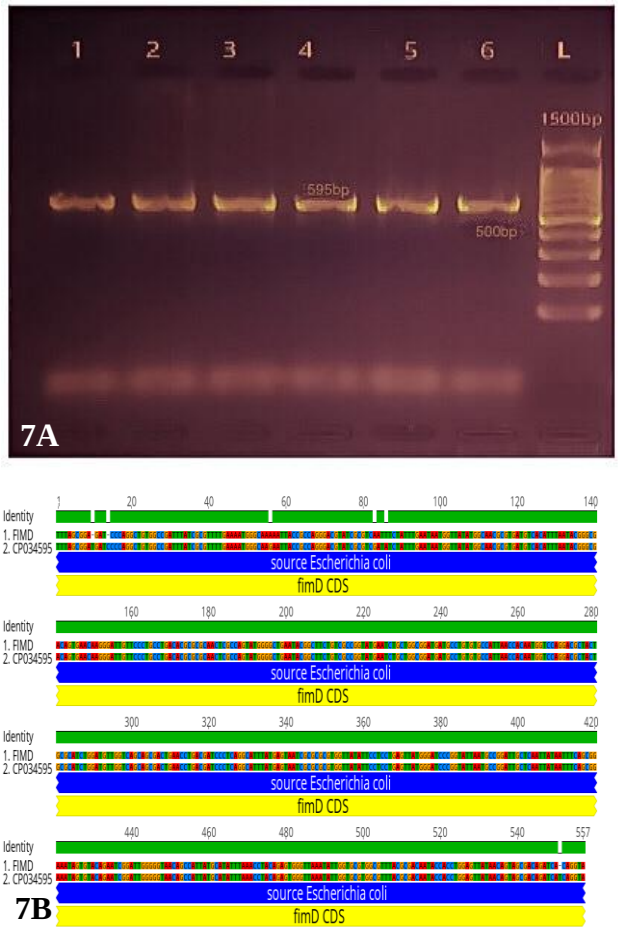

Figure 7. A: Amplified PCR amplicon of *fimD* gene with molecular size reached 595 bp, **B:** Pairwise identity and nucleotide sequence for *fimD* as compared with the standard NCBI *E. coli* strain CP034595.

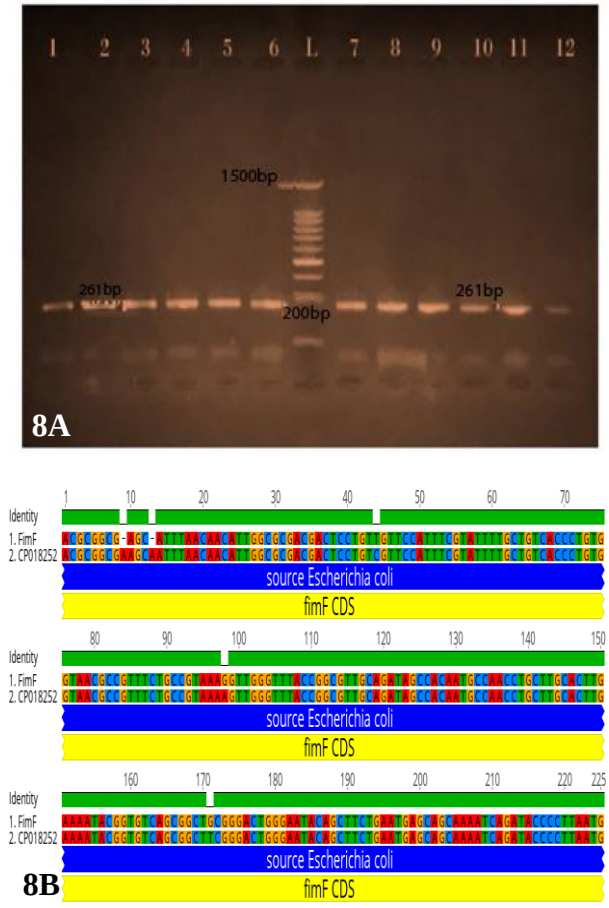

Figure 8. A: Amplified PCR amplicon of *fimF* gene with molecular size reached 261 bp, **B:** Pairwise identity and nucleotide sequence *fimF* as compared with the standard NCBI *E. coli* strain CP018252.

Figure 9A demonstrates PCR product for the amplified *fimG* fragment and it was approximately equivalent to 348 bp comparing to 100bp DNA ladder. For the alignment, the comparison was done between the amplified DNA segment in the current study and standard strain (CP061337) (Figure 9B). No much distinct changes were observed between local and NCBI strain, while pairwise identity reached 98.5% that represents the percentage of residues identical in their alignments.

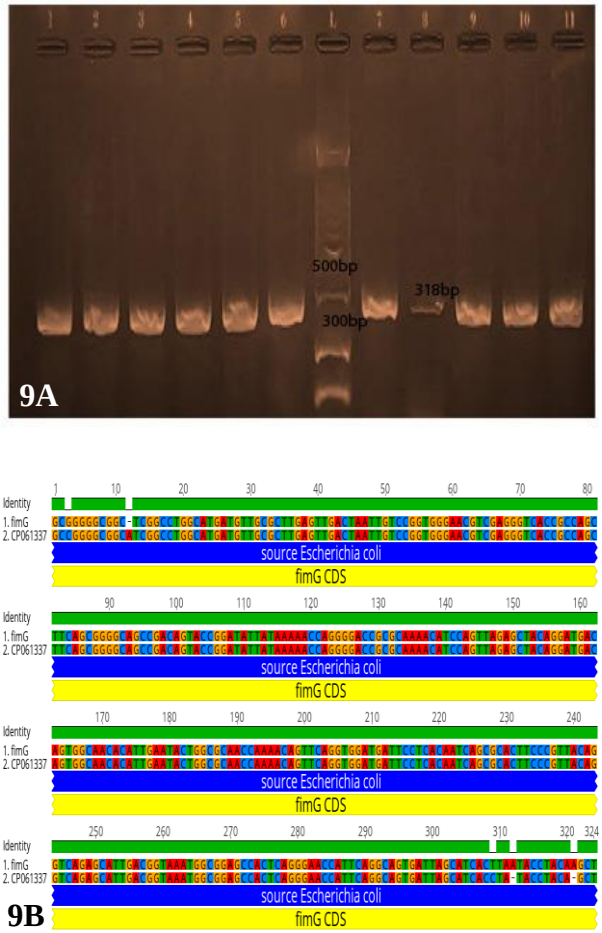

Figure 9. A: amplified PCR amplicon of *fimG* gene with molecular size reached 348 bp, **B:** Pairwise identity for *fimG* as compared with standard NCBI *E. coli* strain CP061337.

fimH was the last gene in type 1 operon genes coding fimbriae; Results indicated that all isolates harboring *fimH* gene (100%). Figure 10A epitomizes positive result for *fimH* gene with an amplified size of 204 bp, and its sequence was analyzed. Pairwise identity was 99.09%. Two gaps have been detected in DNA sequence in green lines as matched with the standard strain LR134208; First gap was at 73 bp and next one at 157bp (Figure 10B).

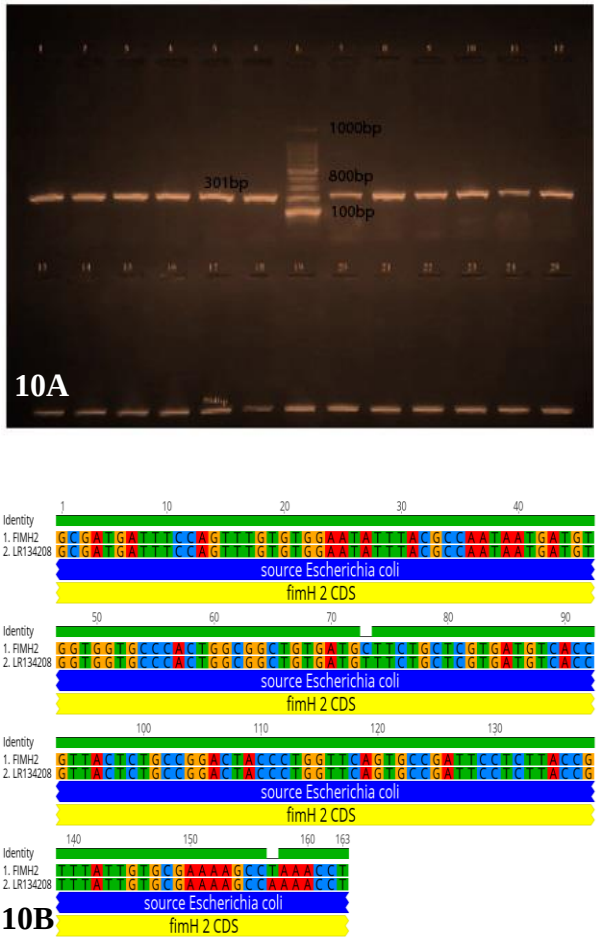

Figure 10. A: amplified PCR amplicon of *fimH* gene with molecular size reached 204 bp, **B:** Pairwise identity for *fimH* as compared with standard NCBI *E. coli* strain LR134208.

4. Discussion

Type 1 fimbria is a very essential in biofilm formation, principally *fimH* gene (10). As with current study, Al-Marjani (11) studied the adherence of *E. coli* that were isolated from the children suffered from diarrhea within hospitals in Iraq. The results of a study conducted by Al-Marjani (11) indicated that all tested isolates (100%) exposed an adhesion ability to epithelial cells. The results of Al-Musawi and Al-Jubori (12) presented the adhesion ability of *E. coli* isolates to the epithelial cells that have been occurred in all isolates and the rate was 100%. Bacterial adhesion to the cells of the host is the essential VFs and is considered the first step in colonization process (1).

The study of Al-Musawi and Al-Jubori (12) showed that 93% of the isolates contain *fimB;* Their PCR result explained that 85% of UPEC were harboring *fimE* gene. Their study revealed high frequency of type 1 fimbriae genes in isolates from Baghdad hospitals. These results are highly related to this study. Consequently, none of isolates in the current study was negative for both *fimB* and *fimE* genes, besides any UPEC isolate which did not harbored one of the two genes, should be absolutely contained the other one. In many cases, *fimE* exposed the switching of initiation phase from OFF to ON or might be when exact amino acid substitutions were make *fimS* orientation in phase OFF position which can lead to antisense transcripts production from *fimA* promoter (13). *fimE* and *fimB* might be act independently for switching *fimS* element from ON to OFF phase by9bp invertible repeated elements, thus, those genes percentage were relatively different in current study (14). Site-specific recombination allows the occurrence of phase variation which involves two transacting factors positioned nearly upstream *fimS* invertible element that are encoded by *fimB,* and *fimE* (15). Lower than the expression of *fimB* might mean the ratio of *fimB* to *fimE* will change to favor *fimE* and subsequent phase OFF orientation of *fimS* region., with *fimS* region being switched to phase OFF orientation combined with direct regulation of *fimA* transcription that losing type 1 fimbria over times in UPEC isolates from infected kidneys would be occur (16). Although some experiments showed that change in phase from OFF to ON switching increased at lower temperatures, some researchers revealed that *fimA* promoter element is biased in its switching from ON to OFF phase orientation in broth cultures that were grown at 20°C, however the switches favors *fimB* recombination at 37°C (17).

fimA is a pilin structural gene which encodes 158-159 amino acid polypeptides about 17 kD in molecular weight (14). Directly upstream of *fimA*, 314bp invertible elements (*fimS)*, which it contained promoter of *fimA* with 9bp inverted repeats (IRs), flanking this segment of DNA (18). The increasing *fimA* transcript levels have been attributed to the transcriptional attenuation between *fimA* and *fimI* (19). *fimA* promotor sequence undergo site specific recombination positioning invertible elements in piliated (Phase ON) or non-piliated (Phase OFF) orientations (20).

Amplification of *fimI* gene showed that 100% of UPEC isolates comprised this gene, *fimI* products are necessary for *E. coli* during type 1 fimbriae biosynthesis. Graffeuil *et al*. (21) reported that the reduction of up to 43 fold in the expression of the *fim* machinery was related to the measurement of the expression of *fimI.* It is a pilus anchor termination subunit (22).

fimC and *fimD* genes are involved in transportation and the assembly of type 1 fimbriae within the cluster of *fim* gene, *fimC* gene was presented in all isolates. This result is very similar to Al-Musawi and Al-Jubori study (12) who reported that their results showed 100% of isolates were harboring *fimC*. While Li *et al.* (23) detected the *fim*C gene in 46.4% of *E. coli* tested isolates. This gene is considered as member of the periplasmic chaperone family. Besides, it works in chaperone usher pathway; it is indispensable in

biogenesis of type 1 pilus of *E. coli*. It binds to individual pilus subunits and then form stable complex in donor strand complementation process (24). Mutations in *fimC, apparently* affected the translocation of structural fimbrial components; *fimC* deficient in host cells periplasm contained the similar amount of *fimA* protein as in periplasm (25). Superficial cells had problem with coping with *fimA* presence, when *fimC*was unavailable, that it means the transcription of *fimB* in the infected bladders showed a trend similar to the *fimA* expression results. Unlike the *fimA* and *fimB* transcription results in UPEC infected bladders, *fimE* transcription was much lower than either *fimA* or *fimB* (16). *fimD* results revealed that it appeared in 100% of the isolates; Al-Musawi and Al-Jubori (12) subjected a percentage of 100% for the same gene, which agreed with this result. *fimD* gene encodes the integral outer membrane protein that serves as usher (26). The sequence of *fimD* demonstrated a high degree of homology to *papC* chaperone associated to the pilus system in UPEC (27).

fimF involved in regulation of length and mediation of adhesion of type 1 fimbriae (but not necessary for the production of fimbriae); It is involved in the integration of *fimH* (28). *E. coli* isolates lacking *fimF* and *fimG,* will produce an adhesive fimbria on their surface; although, *fimF* mutation in bacteria will give fewer fimbriae, as well as isolates missing *fimG* produce longer fimbriae. The fimbriae that were produced In both mutants, were functional and adhesive (29)**.** Jadhav *et al*. (30) reported that (45%) *E.coli* isolated from UTIs were expressed *fimH,*but their percentage was much lower than in current study, while the percentage of it inthis study is identical with Salih (9) who established that 100% of *E. coli* isolates were harbored *fimH* gene. *fimF, fimG* and *fimH* are notnecessary for fimbriae production, but they were involved in adhesive property and the longitudinalregulation of this structure (28). The existence of *fimG,* while either *fimF* or *fimH* were not existed, is sufficient for type 1 fimbriae assembly. However, the detection of surface-assembled fimbriae in *fimF, fimG* double mutant *E. coli* isolates indicated that these isolates need *fimA* and *fimH* only for the fimbriae assembly (31). One of the main and most important strategies for UPEC infection reduction was the targeting of bacterial adhesion via inhibition of *fimH* gene (32). Another researchers have been detected the changes in *fimH* gene expression and they have found that*fimH* suppression was leading to the loss of fimbriae (33).

This highest percentage in current study may prove the reason that it can contain the attachment of *E.coli* to mucosal surfaces and initiates the UTI infection. Bacterial ability of transition from reversible to irreversible adhesion is very critical for initiating microcolony formation. Irreversible attachment will lead to alteration in compositions of *E.coli* outer membrane proteins (34). Staerk *et al.*(5) found that the lack of type 1 fimbriae for the duration of planktonic growth in mouse and human urine specimens was a universal phenomenon for UPEC, which strongly reduced its adhesion to the bladder cell and the invasive potential. Many human commensals isolates that were positive for *fimH,* exposed lacking in type 1 fimbriae because of either deletion or lack expression of at least one of *fim* operon genes (4).

Approximately, all UPEC have type 1fimbrial genes thus it could be used as a genetic marker in the investigation of *E.coli* adhesion ability. The isolates harbored at least five to six of *fimA* operon genes that increase the efficiency for attachment ability to epithelial cells.

Authors' Contribution

Study concept and design: S. A. R. H.
Acquisition of data: S. A. R. H.
Analysis and interpretation of data: S. S. A.
Drafting of the manuscript: J. A. S. S.
Critical revision of the manuscript for important intellectual content: S. A. R. H.
Statistical analysis: S. S. A.

Administrative, technical, and material support: S. A. R. H.

Ethics

All procedures performed in this study involving human participants were in accordance with the ethical standards of the Baghdad University, Baghdad, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

Grant Support

The work was not funded.

Acknowledgment

The authors would like to thank the Department of Biology/College of Science, Mustansiriyah University/ Iraq (https://uomustansiriyah.edu.iq/) for their support to complete this work.

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