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RESEARCH ARTICLE

Molecular Analysis of Type 1 Fimbriae Operon in *E. coli* Isolated from UTI

Duaa Al-Musawi[1*], Sawsan Sajid Al-Jubori[2]

Department of Biology, Biotechnology Branch College of Science, Mustansiriyah University.

***Corresponding Author: Duaa Al-Musawi**

Abstract

One hundred *E.coli* isolates wascollected from urinary tract infections. Patients aged between 2 and 73 years, 66 females, 34 males. E. coli's commitment to urinary epithelial cells appears to be 100% in local isolates and the ability of bacteria to form biofilm was detected by using conjured-plate method and result showed that 96% of these isolated capable to form biofilm and only 4 isolates non-producer. Types1 fimbrial adhesions are encode by a *fim*gene clusters, include nine gene essential for the biosynthesis. (*FimB, Fim E, fim A , FimI, Fim C, FimD, FimF, FimG, FimH*) according to this study all the above mentioned genes were detection using PCR technique but there percentage were differ with (93% , 85% , 98%, 98% , 100% , 100% , 100% ,100% ,98%) respectively . the results indicated that isolates were negative for *fim H* gene may be negative for biofim formation this indicated the importance of type 1 fimbriae in biofilm formation.

Keywords: *Uropathogenic Escherichiacoli (UPEC); Bioflim, (PCR); Type1fimbriae.*

Introduction

Fimbria (also called pili) is the hair-like protein structures protruding from the bacterial cell surface [1]. It is built on small and secondary subunits and has a fiber end with adhesion at the far end of the fibrillator which mediates the specific interaction with receptors [2]. Fimbria can be several microns and have a diameter of about 10 nm [3]. E. coli has the ability to express several different types of fimbria, with different adhesive properties. This ability enables them to adhere to different receptor structures, thereby increasing the possibility of incorporation in different environments [4].

The first type, pili, originally identified by their ability to mediate a sensitive manosecondesis, expressed by the majority of UPEC strain from a patient with cystitis, pyelonephritis and kidney disease, is still controversial. Most types of E. coli contain uropathogenic type pili encoded by the film opera [5]. It enables bacterial cells to stick to the epithelial cells of the host and play an important role in the creation of infections and colonization. The mannosylated receptors on the epithelial host cells are channeled through the FimH adhesive found in the tip of the bellus [6]. The type 1 pili are anchored inside the outer membrane of the bacteria, reaching extracellular space, and have a rod-like appearance with a length of up to 2 µm and a diameter of 7 nm. Type 1 pili of isolates (UPEC) are low-volume protein filaments, assembled by the Bečárová (CUP) pathway, and consist of 5 structural subunits: FimA, FimF, FimG, FimH, and FimI, which are together with the chaperone-usher pair encoded by FimC. The entry hole of the external membrane of FimD and fimB, fimE, located in the upstream direction of fimA, expresses the first type fimbriae by working on the phase key in opposite ways [7].

Material and Method

Collection and identification of E. coli

One hundred Gram negative bacterial isolated (primarily diagnosed like Escherichia coli) was obtained of patient suffering from urinary tract infection (UTI).Then diagnosed on genetic level use 16sRNA genes.

Adherence to Uroepithelial Cells

Samples from mid-stream urine was obtained from healthy women and considered as a source for epithelial cell .It was centrifuge at 3000 rpm for 5 minutes, the sediments containg the epithelial cells was washed 4 times with phosphate buffer saline .The sediment cells were suspended by the same buffer, and solutions were used as a source for human epithelial cells to be used in adhesion of bacteria to epithelial cell [8].

Bacteria (10^8 cells / ml) were added to 10^5 epithelial cells in phopshate buffer saline with dilute D-mannose final concentration of 0.5%.After incubation for 60 minutes at 37 ° C, unconnected bacteria were eliminated by repeated washing with phospate buffer saline. Finally, and the cells were fixed and stained by gram stain. Numbers of adhered bacteria were counted by the directed light microscope [9].

Kongo-red agar was prepared by dissolving 37 g from the heart of the brain infusion broth and 10 g agar agar and 50 g sucrose in 900 ml of distilled water then sterilized by sterilization and then cooled to 55 ° C. The dark red stain was prepared by dissolving 0.8 g in 100 ml of distilled water and sterilized by sterilization, then added to the previous media, and then poured into sterile Petri dishes. It was used to detect the production of biofilms [10]. The plates were immunized by a single pure isolated colony and incubated for 24-48 hours at 37 ° C. The positive results were black colonies with dry crystalline consistency. Typically, vulnerable producers remain pink.

Bacterial Genomic DNA Extract

Bacterial strains were cultured overnight in LB broth (Merck, Germany) and genomic DNA was extracted using the Promega-USA DNA collection according to the manufacturer's instructions.

Application of PCR

In order to confirm isolates such as E. coli, PCR scans it based on the sequence of 16sRNA genes with specific primers as described by Sal et al [11].Carried out in 25 reaction volumes consisting of 12.5 µl of Go Taq ® Green Master Mix, DNA 3µl template, front and back primers 1.5 µl each, and 6.5 µL of free deionized water were added to the PCR mix to obtain size The final of 25 µl. A PCR mixture was used without a DNA template as a negative control. PCR has been run according to the following conditions: Initial leveling step at 94 ° C for 5 minutes and starting 35 cycles by leveling step at 94 ° C for 30 seconds, steel at 58 ° C for 30 seconds, and 1 minute at 72 ° C as an extension step Followed by a final extension step at 72 ° C for 7 minutes.

Detection of Amplicon

After amplification, aliquots (10 liters) of each mixture were removed and the 100-bp phage ladder is examined by electrophoresis (70V, 60 min) in gels consisting of 1.5% agarose (w / v) (10 µl / 100 ml) or ethidium bromide (7 µl / 100 ml) was stained with green stain (10 µl / 100 ml), in 1X TBE buffer (40 ml, 20 ml boric acid, 1 mM EDTA, pH 8.3) Visualize the gel under UV illumination using a gel image analysis system.

Sequencing PCR Products

Uniplex PCR products were stored for all genes at -20 ° amplification, then the nucleotide sequence was performed by sending 25µl of primary product samples and PCR (forward or backward, separately) to NICEM Company, USA.

Data Analysis

The data were analyzed using the Genius Program (2019 prem) by comparing the result with NCBI control strains. The query was also queried and the identity alignment, with the same program.

Table-1: the following table illustrate primer used in the number current study with their sequence, molecular size and Accession number

Primer Name	Forward Primer	Reverse Primer	Predicted size (bp)	Reference Accesssion no
16sRNA	GGAAGAAGCTTGC TTCTTTGCTGAC	AGCCCGGGGATTTC ACATCTGACTTA	544	(Sabat *etal*.,2000)
fim H	GAGAAG AGG TTT GAT TTA ACT TAT TG	GCC GCT GTA GAA CTG AGG	559	(Abass*et al.,* 2015)
Fim D	TCATCTGCCGA ACTCTATTTT	TTTTGCTACCTGA TGATCTGT	595	Designed in this study
Fim E	AAACGTCGTTA TCTTACCGG	TTTCTTTCCCA TAATCCGGC	550	(Pusz *et al* .,2014)

recombinase				
fimC **chaperone**	GGGTAGAAAA TGCCGATGGTG	CGTCATTTTGGG GGTAAGTGC	477	Designed in this study
fimA	AGTTAGGACAGG TTCGTACCGCAT	AAATAACGCGCCT GGAACGGAATG	315	(Pusz *et al*)
fimB **recombinase**	CGAATCACTCC TTAAAGCA	GGCGTAACATGT GCGGA	379	(Pusz *et al*.)
fimG	GCGATCTTTATTC TTTCAGTCT	TGTAGGTATAGGTG ATGCTAAT	348	According to this study
fimI	GGTGCCTTTTGT TATTCATTTAC	GATATTTGGCGATG AAATGTAG	251	According to this study
fim F	CTGAATCAACCAA TTTTACTGTT	CATTAAGGGGTAT CTGATTTTG	261	According to this study

Table 2: PCR programs applied in the study

Amplified gene	Initial denaturation	No. of cycles	Denaturation	annealing	Elongating	extension
Fim E	94 ºC for 5min	35	94 ºC for 30 sec	55 ºC for 30 sec	72 ºC for 1 min	72ºC for 5 min
Fim B	94 ºC for 5min	35	94 ºC for 30 sec	60 ºC for 30 sec	72 ºC for 1 min	72ºC for 5 min
Fim D **usher**	94 ºC for 5min	40	94 ºC for 30 sec	55 ºC for 1min	72 ºC for 1 min	72ºC for 7 min
FimI	94 ºC for 5min	35	94 ºC for 30 sec	55 ºC for 1min	72 ºC for 1 min	72ºC for 5min
Fim G	95 ºC for 5min	40	94 ºC for 30 sec	54 ºC for 30 sec	72 ºC for 1 min	72ºC for 7 min
Fim C **chaperone**	95 ºC for 5min	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 5min	35	94 ºC for 30Sec.	60 ºC for 30 sec	72 ºC for 1min	72ºC for 7min

Results and Discussion

Identification of *E. coli*

Genotypic identification using 16s RNA revealed that all the isolated 100% were gave a positive results with amplified size of 544 as compared with DNA ladder. Figure (1-A) illustrated shine bands of positive isolates .It is reported that PCR-based molecular methods have been developed as an alternative method for accurate identification and classification of bacterial species.

Especially when bacteria are inflated 16s RNA genes [12].16s rRNA genes considered one of the basic criteria in the classification because of its regions were highly constant and unable to change over time, also contain areas of highly covariance among types of bacteria so that provides a specific sequence to each type, which means this gene might play important role in diagnosis, when the diagnostic methods is required [13].

The oversized DNA slice of the 16s RNA gene was analyzed by the Genius program and was 91% Pair wise identity which represents the percentage of residues that correspond in alignment including the gaps versus non-gap residues (Figure 1-B). Some differences were detected between local isolation and the recorded NCBI strain as is evident with a gap within the nucleotides.

Adherence to Uroepithelial Cells

The results showed the adhesion to epithelial cells occurred between all the isolated (100%).The epithelial cells have many nuts that help them catch bacteria and collect them an around the cell [14].Bacterial adhesion to host cells is an important virulence factor and represents the first step in colonization [15]. The adhesion of bacteriae to uroepithelial cells is used to differentiate between fecal strains and uropathogenic. recommended that uropathogenic *E. coli* might present a mean of 20 bacteria/ epithelial cell or more, while fecal strains may existing a mean of nearly 7 bacteria/ epithelial cell [16].

Biofilm Formation

A red plate used to detect the ability of E.coli bacteria to form a biosphere. The results showed that 96% of E. coli formed a strong layer of mud referred to by the formation of black colonies, while (4%) of E. coli did not produce a layer of mud referred to by the formation of pink colonies, the type fimbriae is generally necessary for adhesion.

The initial for E. coli on abiotic surfaces, where fimbrial mutations in E. coli were reported to be defective in the initial attachment of the abiotic surfaces [17].The biofilm formation has been linked to the survival of pathogenic bacteria in the hospital environment, leading to the colonization of sensitive bacteria, creating an important public health problem [18].

PCR Amplification Procedure for Film Genes Operon

Results showed a high frequency of fimbriae type 1 genes in local isolates. Site reconfiguration requiring phase variation requires two variable coefficients located at the top of it for film S of an inverse element, annotated with film B and film E [19]. A PCR study showed that 86% of E. coli had a firm introduction in a clear strip of 550 basis points on 1% agar gel (2-A). In addition, 93% of these isolates contain B film presented in a clear strip of 379 basis points on 1% Agar gel (3-A) film B and film E containing 48% of the amino acid similarity with each other Battaglioli et al [20]. It was originally suggested that film B and film E might act independently to switch a one-way fimS, either Phase-ON to Phase-Off, via inverse repeaters 9 bp, IRL and IRR.

For this reason, the percentage is relatively different. Movie B can connect the S movie element either by switching from Phase-ON to Phase-Off or vice versa. The sequences of the B film gene from E. coli were presented in Fig. 3-B. Pairwise was 98% with few differences between local isolation and the recorded NCBI E. coli strain (3 gaps only). The results showed that 98% of the isolated E. coli could contain A gene of magnitude 315bp (-4a). The promoter sequence of film A is subject to the site's reconfiguration, with the inverted element positioned in the direction of Phase-ON or Phase-Off. This switch phenomenon is known by phase [21].

Film A gene sequence (figure 4-B) was analyzed and compared with the standard strain CP025401. Pair wise identity was 96.7% which represent the percentage of residues that were identical in the alignment. Film I gene encodes a structural component (Schwan et al., 2011). For gene film I, the results showed that 98% of isolates contain this gene. The film I gene is a piles anchor termination subunit; Film I's function is not known [22]. Figure (5-A) showed the positive gene band for film I gene at amplified size 251 bp film I gene sequences of E. coli was presented in figure (5-B) the comparison was performed between this DNA segment and the standard strain CP030939: The Pair wise identity was (98%) with no clear differences between the local isolate and the recorded NCBI strain as few gaps appeared in the Query identity line.

There are two genes involved in the transport and assembly of type 1 fimbriae within the firm gene cluster: fimC and fimD. For fimC Results showed 100% of the isolates harbored Fim C gene, figure (6-A) showed PCR product of fimC gene with product size 477 bp. fimC is a member of the periplasmic chaperone family which functions in the chaperone-usher pathway and is indispensable in the biogenesis of the type 1 pilus fiber of Escherichia coli from C binds to and forms stable complexes with individual pilus subunits in a process called donor strand complementation (DSC) [23]. Fim C gene sequence of E. coli was analyzed as presented in Figure (6-B). The comparison was done between this DNA segment and the standard strain E. coli CP023386. The Pair wise identity was 99% which represent the percentage of residues that were identical in the alignment. No mutation was detected in the DNA sequence.

For Fim, D results showed 100% of the isolates harbored this gene figure (7-A) illustrates PCR products for gene film D with amplified segment 595 bp as compared with 100 bp DNA ladder. Film D gene encodes an integral outer membrane protein that serves as an usher [24]. Film D gene sequence of E. coli was analyzed as illustrated in figure (7-B) the comparison between this DNA segment and the slandered strain CP006262. The Pairwise identity was 96 %, while 3 Gaps (3/544) were detected. Many differences were detected in the DNA sequence represented by gaps in the upper green identity line.

The two remaining genes in the film operon are fimF and fimG. FimF and FimG are associated with FimH adhesin, forming a fibrillum structure that anchors the adhesion to the pilus shaft and controls the length of the type 1 pilus [25]. The fimF gene was shown by expressed all the local isolates (100%) with the amplified size of approximately 261bp (figure 8-A). Sequencing of the fim F gene of E. coli was

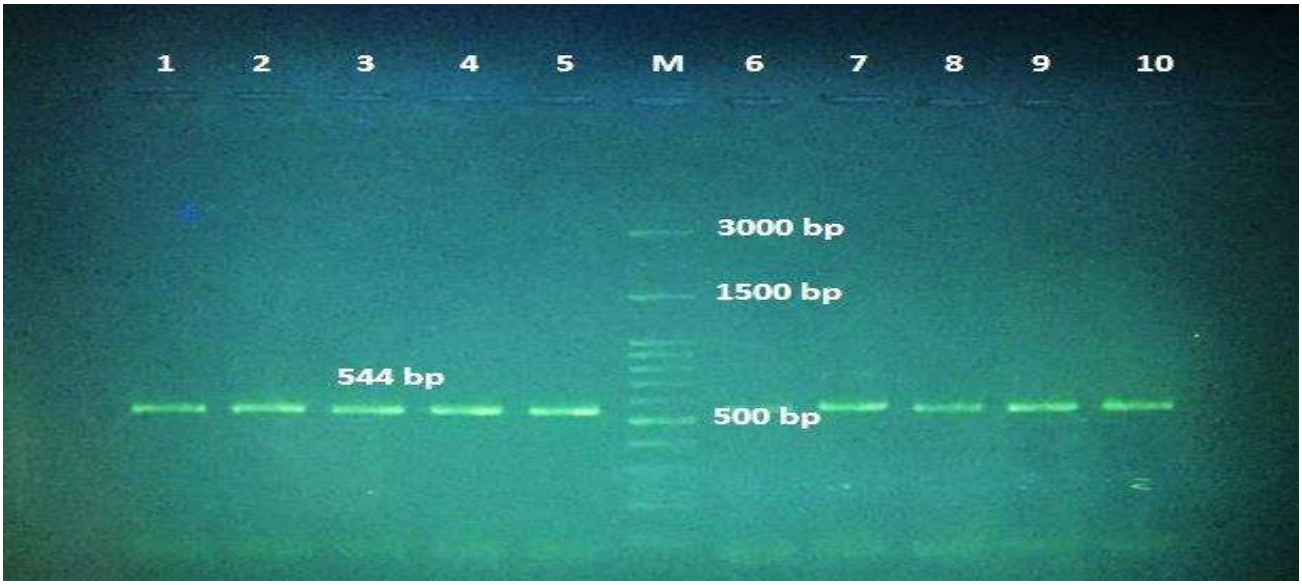
also analyzed as presented in figure (8-B) this time the comparison was checked between this amplified segment and the complete genome for standard strain CP024978. And the Pariwise identity was 98 %. And same prevalence rate was showed for fimG gene among all isolates.

Figure (9-A) illustrates PCR products for fimG gene with amplified segment equal to (348bp) as compared with 100 bp DNA ladder Sequence analyzes were illustrated in figure (9-B) the comparison between this DNA segment and the standard strain CP028110 No clear differences were noticed between the local and the NCBI strain as the Pariwise identity was 98% which represent the percentage of residues that were identical in the alignment. Finally with film H gene coding the mannose–the sensitive tip of fimbriae and coding for adhesion property, Figure (10-A) represent the positive results to the film H gene at the amplified size of 559 bp. The results showed 98% of the isolates of E. coli gave a positive result of Film H. Eto et al [26].

Showed that the addition of a Film H receptor analog to an inoculums of type 1-fimbriated E. coli significantly reduced bacteriuria in mice .Similarly, immune sera directed against type 1 fimbriae prevented colonization of the kidneys in a rat model of E. coli induced pyelonephritis, the film H gene once again was highly conserved in UTI isolates which confirms its crucial role during colonization of the urinary tract fimH gene sequence of E. coli was analyzed as presented in figure (10-B). And the Pariwise identity was 98% .few gaps were detected in DNA sequence in the upper green identity line as compared with the standard strain CP025401. One of interest found in this study the two isolates (E43 and E99) that showed a negative result for the fim H gene also showed a negative result for the formation of the biofilm. Such finding may indicate the importance of type 1 fimbriae specially fimH gene in the formation of biofilms. The current study showed that type 1 fimbriae was detected in 100 isolates but in a different manner, 79 % of E .coli contain all the genes of operon, 18% of isolates contain eight genes of type 1 fimbriae, 1% isolates contain 7 genes, 1% isolates contain 6 genes and one isolate contains 4 genes only. Several studies of experimental UTIs suggest that the first type of fimbria can help in the continuation of E. coli in the urinary system. Connell et al [5].

Showed that children with positive strains of type 1 showed a shorter period of symptoms prior to antibiotic treatment, higher fever, longer fever, and a higher blood cell count than children with negative isolates of type 1.The results showed that the expression of the first type by E. coli isolates led to increased severity of infection in children. The expression of the first type of fimbriae by UPEC enhances colonization and stimulates the host response in the UTI model and promotes the formation of the biomembrane and invasion of the host cell [4]. Also, a commitment to type 1mediation was suggested to play a role in inciting inflammation. Early laboratory studies have shown that bacteria of the first type are associated with phagocytic cells and urge a respiratory explosion [27].

A



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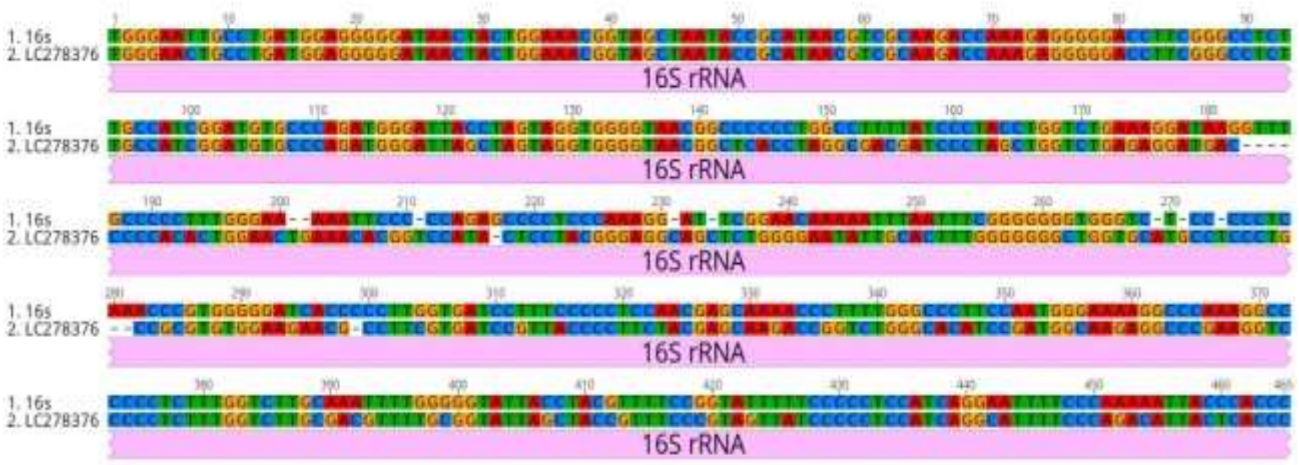


Figure 1-A :A:Agarose gel electrophoresis (1% agarose, 5 V/cm for 60 min) for 16S rRNA gene (amplified size 544bp) as compared with (100pb) DNA ladder lane (M). All lanes represent positive results. B: Pairwise identity and DNA sequencing for *16SrRNA*as compared with the standered strain *E. coli* taxon: 562; LC278376 (NCBI)

A

B

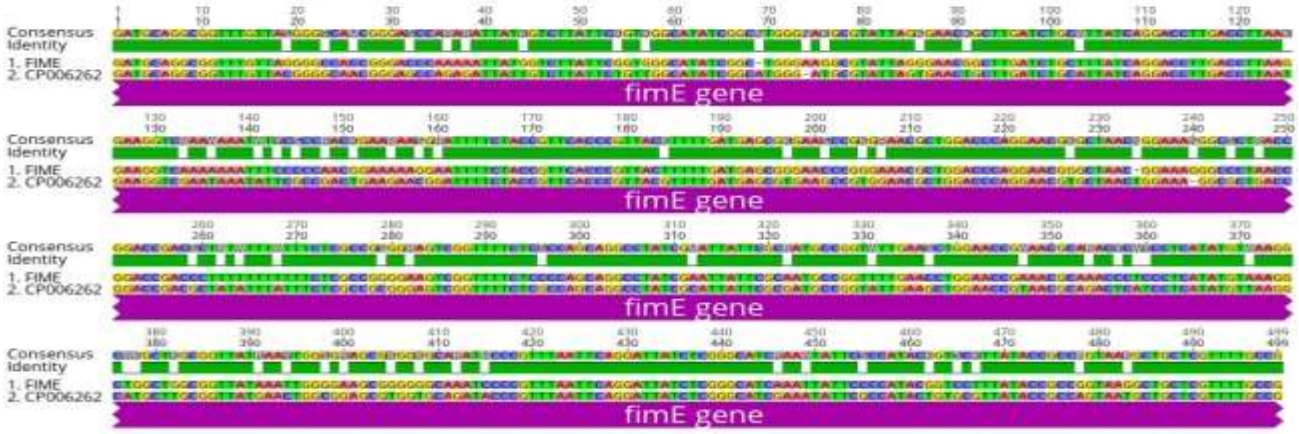
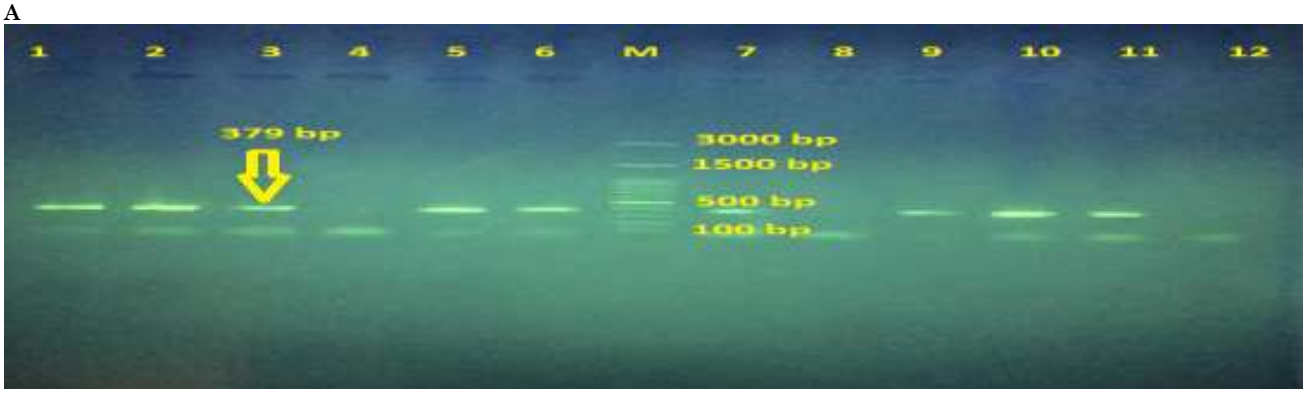
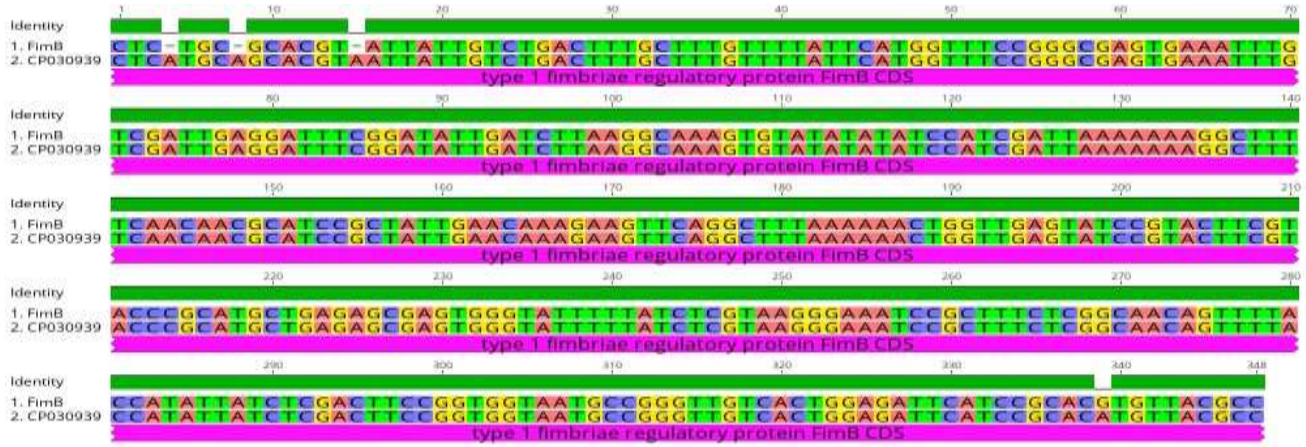


Figure 2-A: Agarose gel electrophoresis of *fimE* gene (550bp amplicon). Electrophoresis was run at 1% agarose, 7v/cm² for90 min. lane M: DNA Ladder; lanes1-12 represent positive results. B: Pariwise identity and nucleotide sequence for *fimE*as compared with the standered strain *E.coli* CP006262 (NCBI)

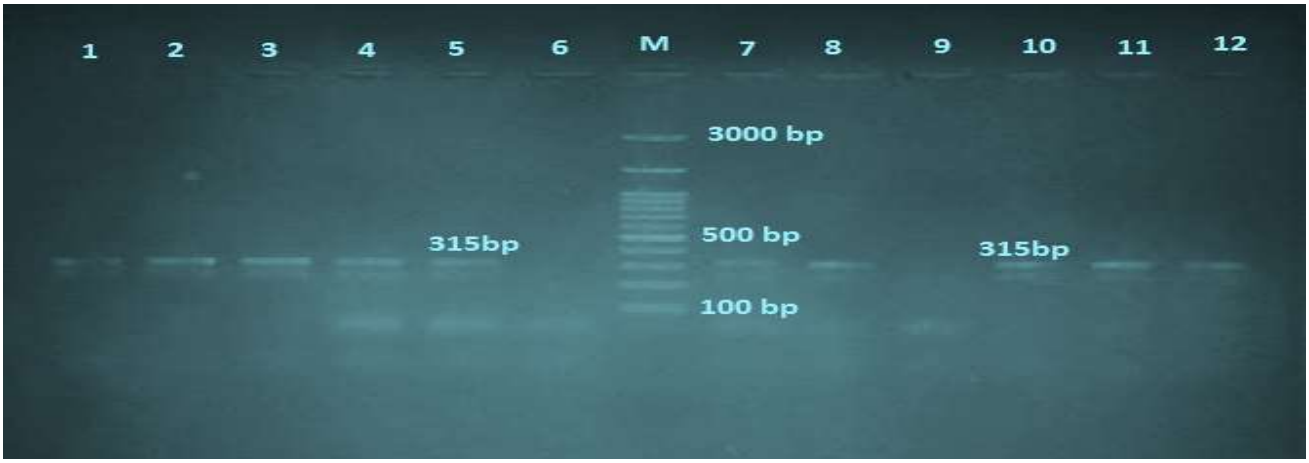
A





B

Figure 3-A: Agarose gel electrophoresis (1% agarose, 5 V/cm for 90 min) for *fimB* gene (amplified size 379 bp) as compared with DNA ladder lane (M). All lanes represent positive results for *FimB* gene: B: Pariwise identity and DNA sequencing for *fimB* gene illustrating few gaps in the local isolate



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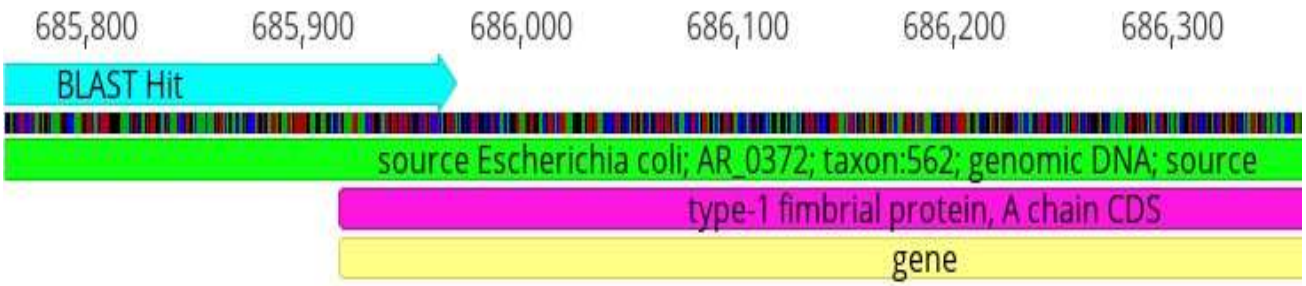
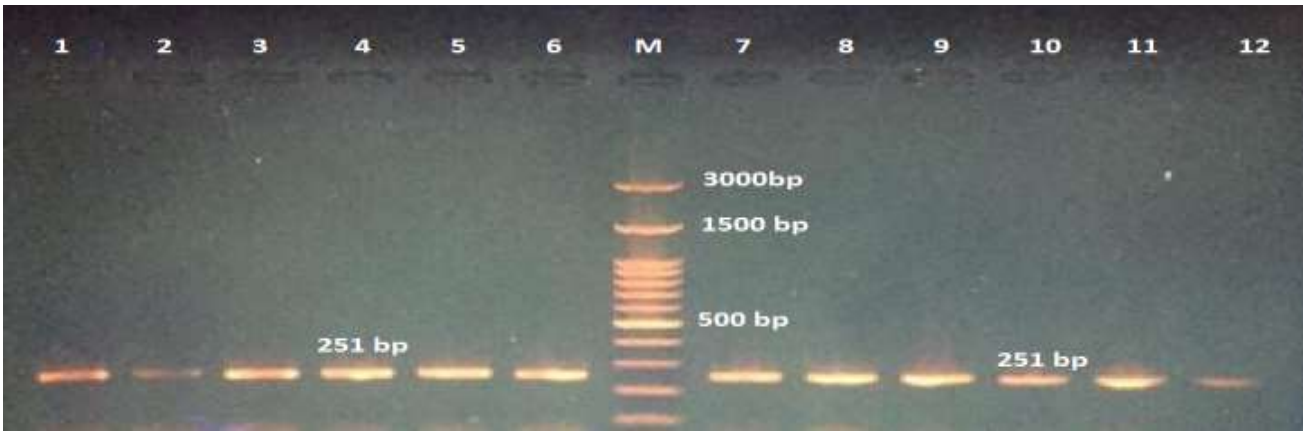


B

Figure 4-A: Agarose gel electrophoresis (1% agarose, 5 V/cm for 90 min) for *fim A* gene (amplified size 315 bp) as compared with DNA ladder lane (M). All lanes represent positive results to *Fim A* gene: B: Blast Hit of the amplified nucleotide sequence for the start open reading fram region related to *fim A as* compared with the standered strain *E. coli* (NCBI)



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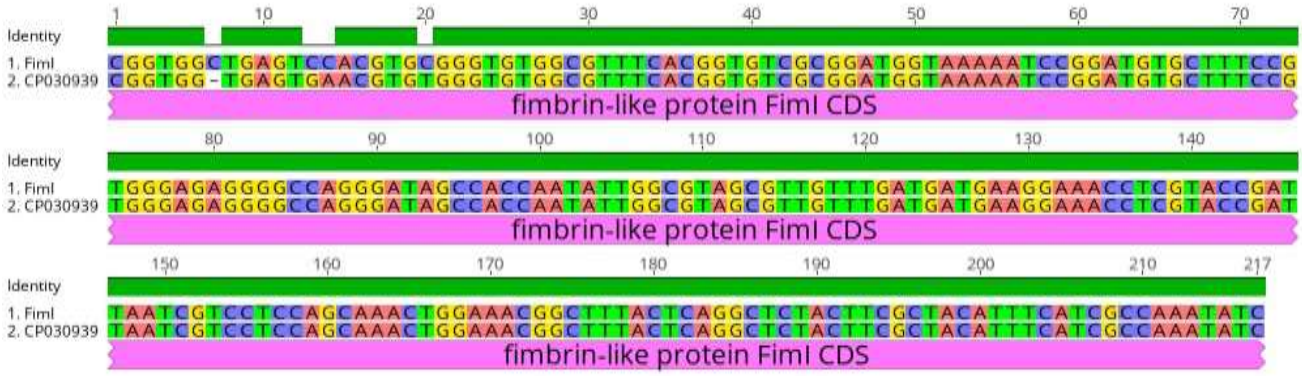
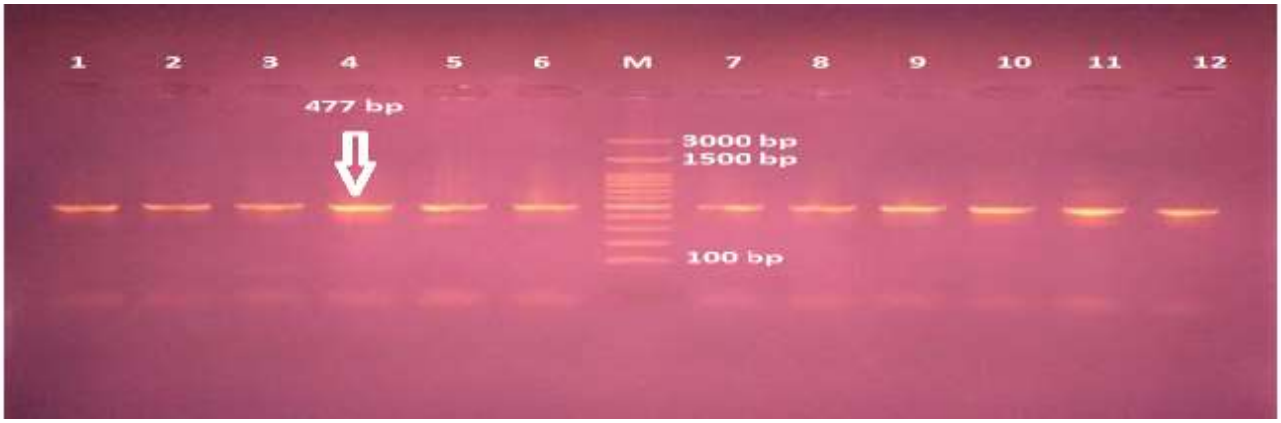


B

Figure (5-A):Agarose gel electrophoresis of *fimI* gene (251bp amplicon). Electrophoresis was run at (1% agarose, 7v/cm² for 90 min) lane M: DNA Ladder; lanes1-12 represent positive results. Figure (5-B): Pariwise identity and nucleotide sequence for *fimI*as compared with the standered strain *E.coli* NCBI strain



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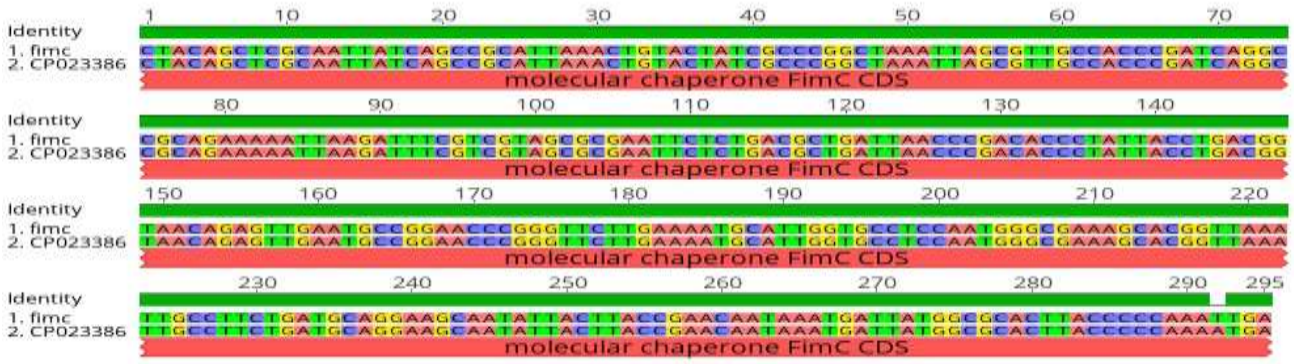
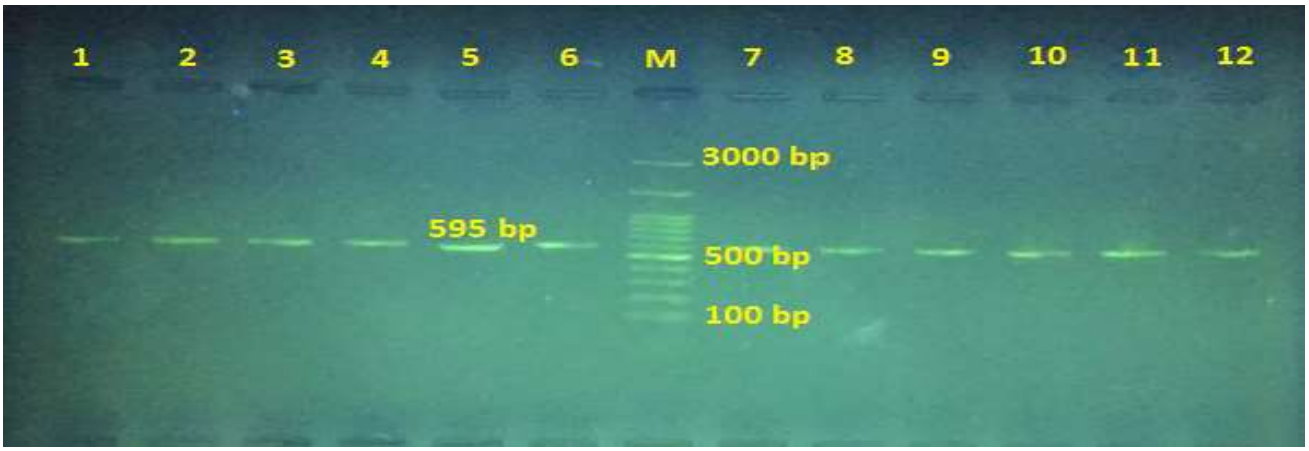


B

Figure 6-A: Agarose gel electrophoresis of *fimC* gene (477bp amplicon). Electrophoresis was run at (1% agarose, 7v/cm² for 90 min) lane M: DNA Ladder; lanes1-12 represent positive results. B: DNA sequencing for *fimC* gene illustrating high Pariwise identity between *E.coli* isolates and the standard strain



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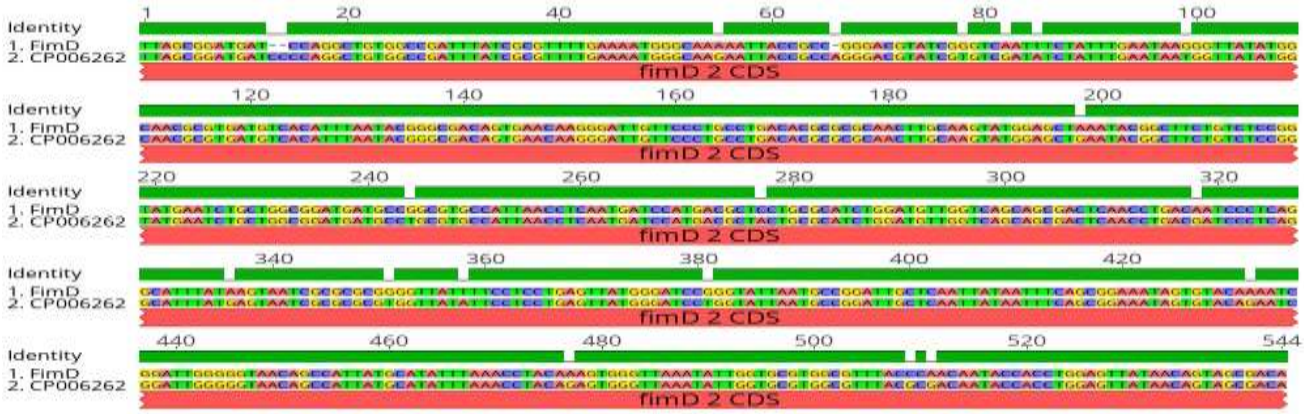
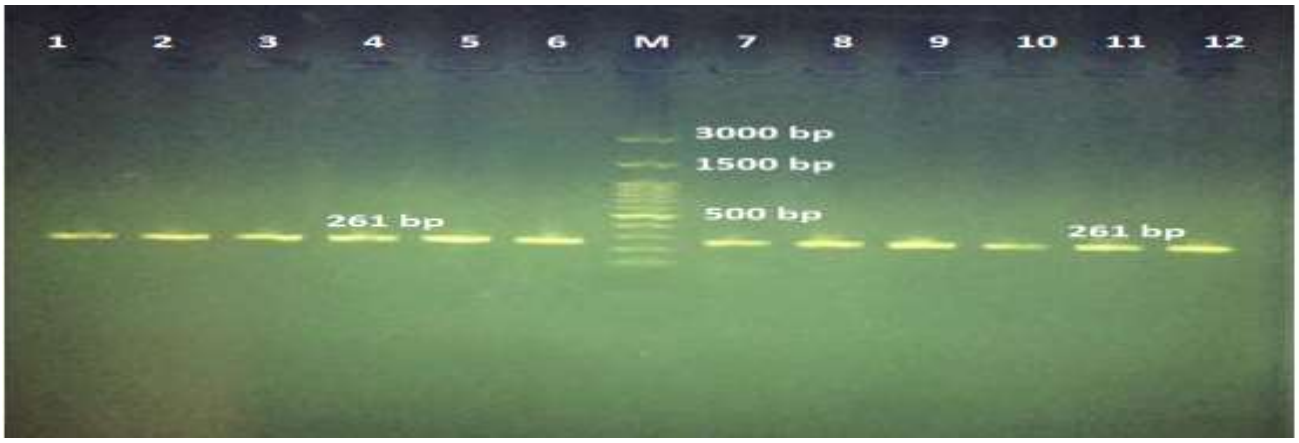


B

Figure 7-A: Agarose gel electrophoresis of*fimD* gene (595bp amplicon). Electrophoresis was run at 1% agarose, 7v/cm² for 90 min lane M: DNA Ladder; lanes1-12 represent positive results.B: DNA sequencing &Pariwise identity and nucleotide sequence for *fimD* as compared with the standered strain *E.coli* CP006262 (NCBI)



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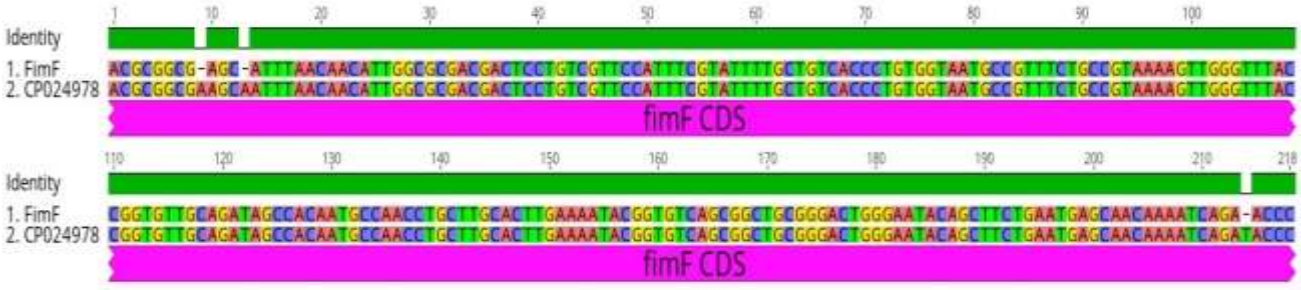
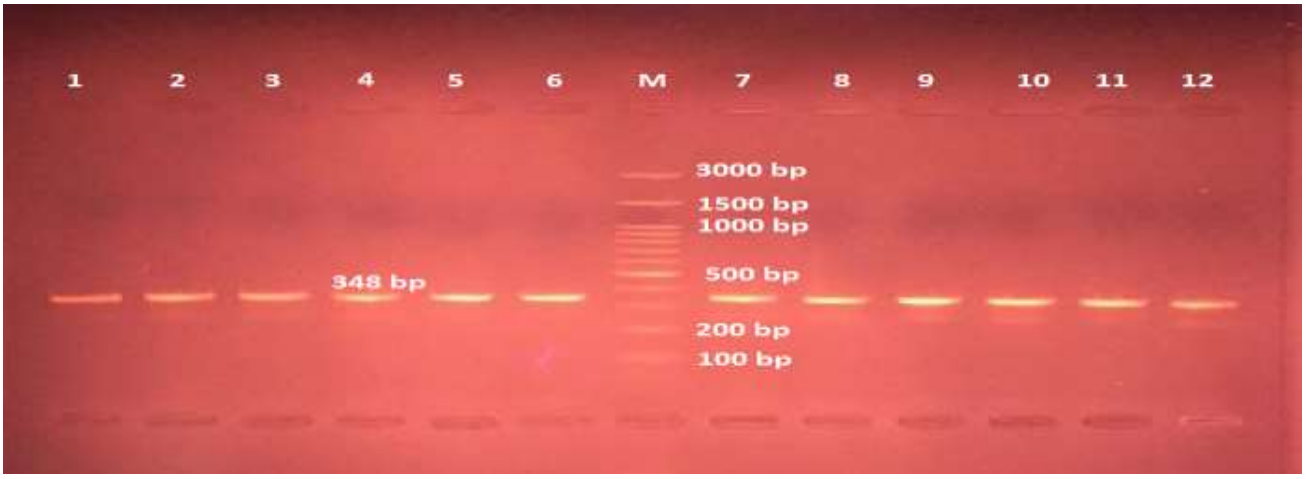


B

Figure (8-A) A: Agarose gel electrophoresis of *fimF* gene (595bp amplicon). Electrophoresis was run at (1% agarose, 7v/cm² for 90 min) lane M: DNA Ladder; lanes1-12 represent positive results. B: Pariwise identity and nucleotide sequence for *fimF*as compared with the standered strain *E.oli* CP024978 (NCBI)



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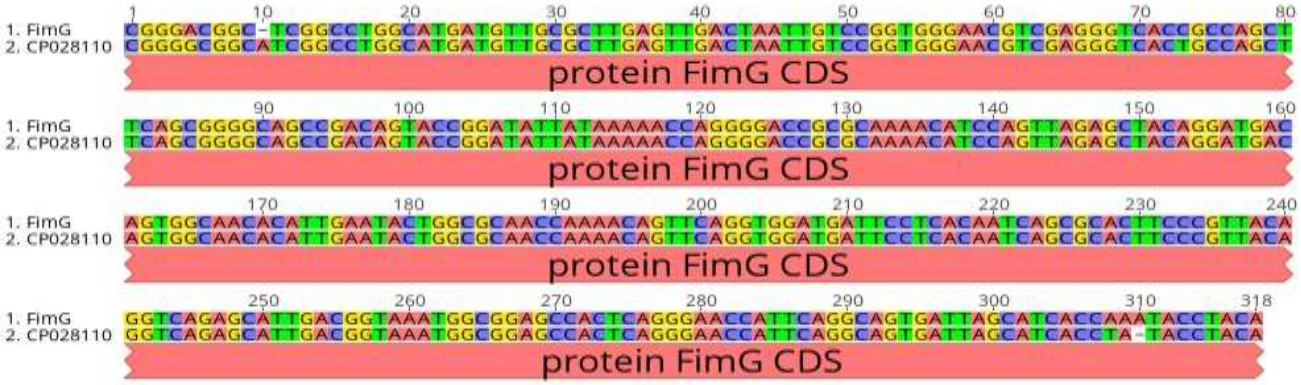
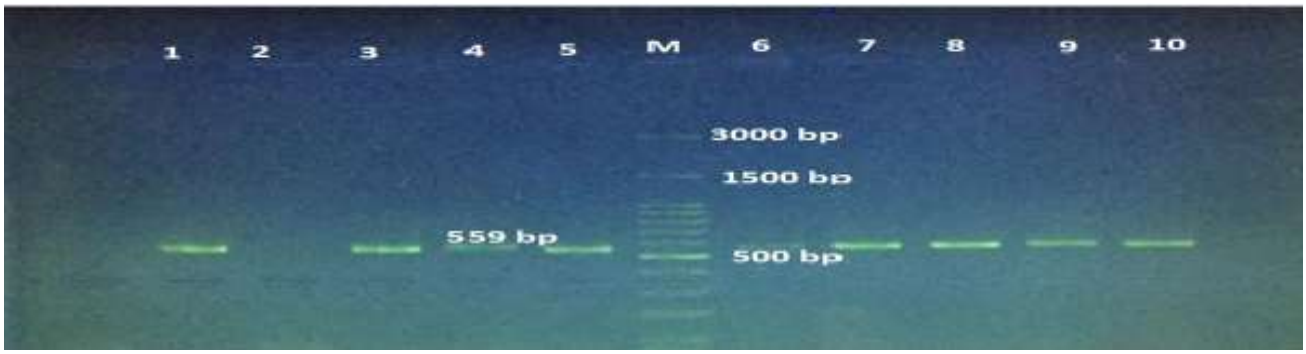


B

Figure 9-A: A: Agarose gel electrophoresis of *fimG* gene (384 bp amplicon). Electrophoresis was run at 1% agarose, 7v/cm² for 90 min, lane M: DNA Ladder; lanes1-12 represent positive results. B: Pariwise identity and nucleotide sequence for *fimG*as compared with the standered strain *E.oli* CP028110 (NCBI)



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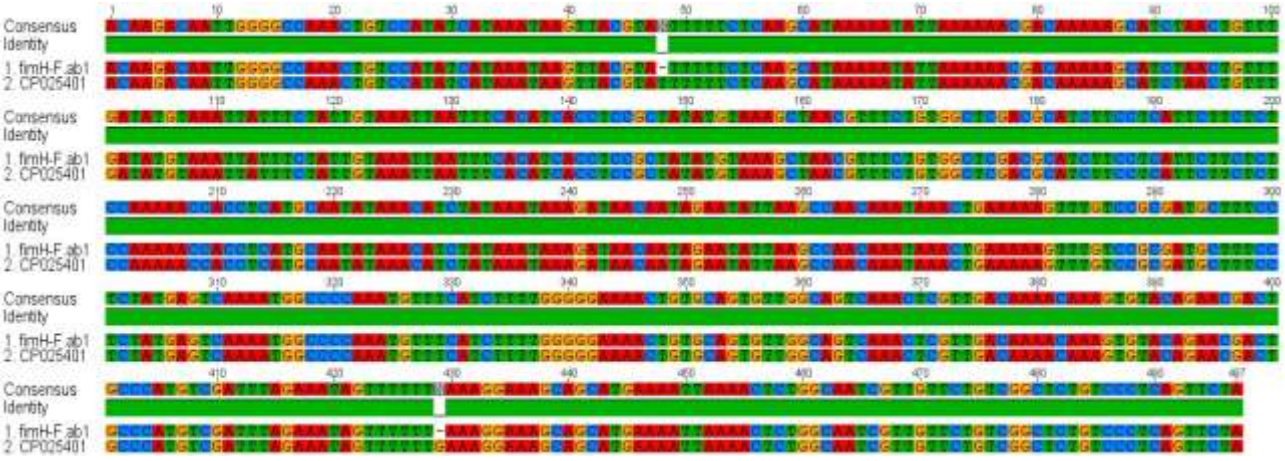

B

Figure 10-A: Agarose gel electrophoresis for *fimH* gene PCR product (559 bp amplicon). Electrophoresis was run at (1% agarose, 7V/cm² for 90min) Lane M, DNA ladder. Positive results in all the ten lanes except lane 2 (negative result). B: DNA sequencing for *fimH* gene illustrating some gapes in the green identity lane

Conclusion

Adhesive factor genes (fim operon) can be used as a genetic marker in the investigation of E.coli bacteria causing urinary tract infection and type 1 fimbria very important in the formation of the biofilm especially film H gene.

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