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Research Article

*fim*H adhesin-related gene and biofilm formation in uropathogenic *Escherichia coli* isolates from AL-Hillah/ Iraq

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Abstract
Uropathogenic *Escherichia coli* (UPEC) is the main causative agent of urinary tract infection and their ability to build biofilms leads to increases its severity and makes its removal difficult: The objective was to validate the species- specific primers designed to amplify the target sequence in *E. coli* genes (*uid*A encoding β-D-glucuronidase, *yia*O encoding outer membrane protein and *16SrRNA*) in contrast to clinical isolates previously identified as *Shigella dysenteriae* and *Klebsiella pneumoniae* and to investigate the possible relationship between the prevalence and sequence variations of *fim*H gene and the intensity of biofilm formation. Primers were designed based on the database available for *E. coli* in the National Center for Biotechnology Information (NCBI) for *uid*A, *yai*O and 16SrRNA genes sequences. *fim*H virulence genes were investigated in fifty *E. coli* isolates of UTI patients by using PCR assay followed by sequence analysis of *fim*H among isolates with different degree of biofilm strength. Gel electrophoresis presented bands for *uid*A, *yai*O, and 16SrRNA genes in all *E. coli* isolates. However, no bands were detected among isolates of *Shigella dysenteriae* and *Klebsiella pneumoniae* species. DNA sequence analysis for *fim*H gene revealed three positions of variations were repeatedly detected among strong biofilm former isolates leading to changes in their amino acid types. The *uid*A, *yai*O, and 16SrRNA genes represented an ideal target for the identification of *E. coli* since all primers achieved exclusively related fragments. Variations of amino acid types among three biofilm phenotypic categories might be responsible for structural and functional heterogenicity of type 1 fimbria D-mannose specific adhesion protein.

Keywords: Uropathogenic *Escherichia coli, fimH,* uidA, yaiO

INTRODUCTION

Bacterial Urinary tract infections (UTIs) have become the most prevalent health issues and predominant bacterial nosocomial infections in developed countries. Every year, approximately 150 million people worldwide are diagnosed with UTI. Along with being a causal agent in different infectious diseases, *Escherichia coli* (*E. coli*) is the most prevalent etiological factor of UTI (Zhou *et al.*, 2023; Anton *et al.*, 2025). Both community-acquired infections and disease related to health care within individuals of different ages were associated with *E. coli* infections (Galindo-Méndez, 2020; Lilo *et al.*, 2020). Similar to other mucosal infections, uropathogenic *E. coli* (UPEC) strains have a multi-step pathogenesis process. These approaches enable bacterial cells to invade the mucosal surface and damage the tissue of the patients, wounds, and evasion defense mechanisms of the host as well as promote the inflammatory response (Kaper *et al.*, 2004). Bacterial persistence via the formation of a stable and complicated form known as biofilm seems to be the main pathogenesis factor and treatment failure (Alwan *et al.*, 2023).

Biofilm formation protects UPEC from antiviral drugs, host immune system, and environmental stress. Adhesion to the surface, cellular aggregation, the production of an extracellular matrix, and biofilm maturation are the four basic steps in forming a biofilm (Vetrivel *et al.*, 2021). Most coliform bacteria display type 1 and 3 (mannose-sensitive and mannose-resistant respectively) fimbrial adhesive molecules. Type 1 fimbria encoding by *fimH* gene has a high preference for the receptors of the urinary tract. As a result, *fimH* adhesion is essential for colonizing various niches of *E. coli* (Mahmood and Abdullah, 2015). Bacterial identification by molecular methods is another broad category of

diagnostic methods that faster their detection and classification and are frequently applied with high efficiency and sensitivity, as well as reducing the time spent in diagnosis. The sequencing of 16S rRNA gene for bacterial diagnosis is frequently depends on as one of the highly accurate methods used in laboratories. The 16S rRNA gene is unique for each bacterial species, thereby making it the typical objective in bacterial identification. Previous studies have employed the *uid*A gene, encoding of B-glucuronidase, to detect *E. coli* (Brons *et al.*, 2020; Alsanjary and Sheet, 2022; Osińska *et al.*, 2023) Despite the fact that both *uid*A and *uid*R genes are present in *E. coli* and *Shigella* spp., the enzyme's activity is only restricted to *E. coli* (Saleh *et al.*, 2022). The *yai*O gene [EcoGene:EG13297], which transcribed in both logarithmic and stationary phase of E. coli orphan ORFs (Alimi *et al.*, 2000; Alsanjary and Sheet, 2022), encoding a protein originally suggested by a bioinformatic prediction. It was subsequently discovered to be found and expressed in the outer membrane (Casadio *et al.*, 2003; AL-zuwainy and Abid, 2014). The present study aimed to evaluate molecular techniques to identify *E. coli* species based on species-specific genes (uidA encoding β-D-glucuronidase and yiaO encoding outer membrane protein, along with 16S rRNA) and to investigate the possible relationship between the prevalence and variations of the *fimH* gene sequence and the intensity of biofilm formation.

MATERIALS AND METHODS

Sample collection and bacterial identification

One hundred and seventeen samples of urine were collected from patients infected with UTI from Al-Hilla Teaching Hospital and Al-Childhood and Gynecology Hospitals in Hilla city/Iraq. The samples were biochemically identified and the biofilm formation among *E. coli* isolates were investigated and analyzed in earlier published previous work (Abbood and Alwan, 2023).

Molecular identification

After the identification of *E. coli* bacterial species using biochemical methods, fifty isolates were reidentified through PCR detection for the *uid*A, *yia*O and 16SrRNA genes. DNA molecules isolation was achieved by using the FavorPrep Genomic DNA Mini extraction kit for Gram-negative bacteria. The mixture of PCR was prepared for up to 25 µl and included the following: Go Taq®Green Master Mix(2X) 12.5µl, each forward and reverse primer was 1µl, 3µl of the DNA template along with 7.5µl Nuclease free water. The PCR thermal cycler was set at the conditions: preheating at 95°C for 4 mins followed by 30 cycles of denaturation at 95°C for 30sec, extension at 72°C for 1 minute; and a final extension at 72°C for 7 min, primer sequence and annealing temperature for each target sequence of genes are shown in the (Table1). Nine clinical isolates of previously identified species for each *Shigella dysenteriae* and *Klebsiella pneumoniae* species (clinical isolates from stool and urine respectively, were supplied from Microbiology Lab. bank of identified bacterial species in Biology department/ College of Science/ University of Babylon) were also used to evaluate the specificity of the primer's amplification for the target sequence.

Sequencing of *fimH* gene

The relationship between the intensity of the virulence factor (adhesion factor encoded by *fim*H gene) and their sequence variation was investigated in multiple isolates of E. coli. The Polymerase chain reaction (PCR) products of *fimH* gene previously identified in all fifty isolates (Abbood and Alwan, 2023) were sequenced in different isolates representing three levels of biofilm formation degrees. The target sequence was amplified (Primers described in Table 1) and analyzed using Macrogene company in Korea. Homology search was conducted using the Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at (http:// www.ncbi.nlm.nih.gov) and BioEdit software for multiple alignments of *fimH* among different *E. coli* isolates.

Ethical approval.

The study followed ethical principles outlined in the Declaration of Helsinki. Before taking the sample, the patients provided verbal and analytical approval. A local ethics committee reviewed and approved the study protocol, subject information, and consent form under document ID M220603 in 22/06/2022 to obtain this approval.

RESULTS

Molecular identification of *Escherichia coli* species based on *uidA, yaiO, and 16SrRNA* genes

The presence of *uidA, yaiO,* and *16SrRNA* genes was identified among 50 *E. coli* isolates. Gel electrophoresis result of PCR products (Fig. 1 and 2) presented bands with sizes 242, 127, and 585 bp for *uidA*, *yaiO*, and 16S*rRNA* genes respectively. However, there was no PCR product for all these genes among isolates related to *Shigella* and *Klebsiella* species. The results showed that the frequency for all genes was 100% based on the presence of these genes in agarose gel. Depending on these findings, the *uidA, yaiO*, and 16*SrRNA* genes represented an ideal target for the identification of *E. coli* by PCR detection since all primers achieved specific fragment sizes exclusively related to the UTI isolates of *E. coli*.

Forty-six isolates (92%) among the total (50 identified *E. coli* isolates) biofilm forming categories were pre-

Table 1. Primers and annealing temperatures for the target sequence in 16SrRNA, uidA, *yai*O, and *fimH* genes of *Escherichia coli* species

Primer	Primer Sequence 5′-3′	Annealing temp.	Product Size/bp	Ref.
16S rRNA	F: CCTAACACATGCAAGTCGAA R: CATCTGACTTAACAAACCGC	60°C for 30 sec	558	Present study
uidA	F: CGAACTGAACTGGCAGACTA R: GCAGTTCAACACTGACATCAC	61°C for 30 sec	242	Present study
yaiO	F: GCGATGCAGGTGGTAGTTATA R: GTTTCTGGCGTCCAGTCATA	61°C for 30 sec	127	Present study
fimH	F: TGCAGAACGGATAAGCCGTGG R: GCAGTCACCTGCCCTCCGGTA	63°C for 30 sec	508	(Abdul-Ghaffar and Abu-Risha, 2017)

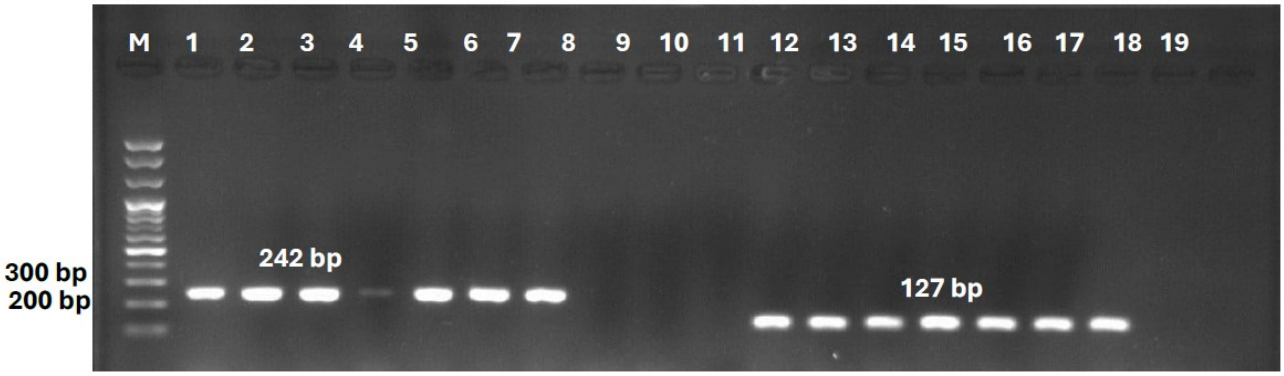

Fig. 1. *Agarose gel electrophoresis of uidA, yaiO PCR product (242 and 127 bp) among Escherichia coli isolates. M: represent 100bp DNA ladder, lane 1- 7 and 11- 17 represent uidA and yaiO genes respectively, lane 8-10 and 18, 19 represent negative control (no PCR product for Klebsiella sp. Samples run in 1% Agarose gel for 60 minutes and 85 V*

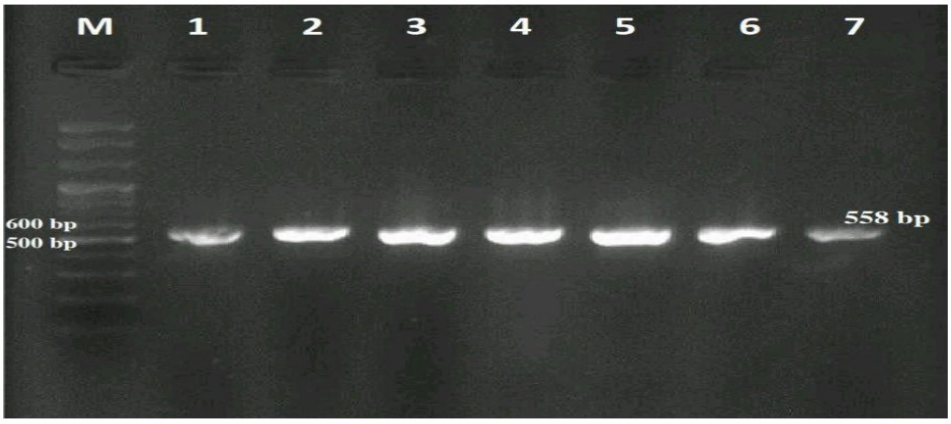

Fig. 2. *Agarose gel electrophoresis of 16S rRNA PCR product (558bp) among Escherichia coli isolates. M: represent 100bp DNA ladder, lane 1- 7 represent the isolates. Samples run in 1% Agarose gel for 60 minutes and 85 V*

sented with clear bands of size 508 bp for *fim*H gene. The positive relationship between the biofilm formation capacity and the presence adhesion factor genes is mentioned in Table 2.

PCR products of *fim*H gene analyzed by Macrogene company in Korea and the DNA sequencing results were first examined to confirm the nucleotide identity with others worldwide strains through the NCBI- Blast-query nucleotide–online application. The results showed that the identity percentages of 9 local *E. coli* isolates with other gene bank registered strains were ranged between 96- 98%. In the present study, genotypic variations were compared between 9 *E. coli* isolates representing three biofilm phenotypic categories (weak, medium, and strong) and three replicates were chosen for each type. Multiple alignment nucleotide sequences were performed to define the variations among these local isolates using BioEdit software (Fig. 3) and amino acids alignment (Fig. 4).

It is obvious that the OQ584342 local isolate, representing a strong biofilm former, was highly variable when compared with the rest of the isolates. Multiple alignment nucleotide sequences were performed to define the variations among these local isolates using BioEdit software (Fig. 3) and amino acids alignment (Fig. 4). Three positions (202, 214, and 230) of varia-

Table 2. Relationship between different categories of biofilm formation and prevalence of *fimH* gene in *Escherichia coli* isolates

Biofilm formation	Weak	Moderate	Strong	Total	P. value
	9 (18%)	36 (72%)	5 (10%)	50 (100%)	0.000
Fim H	8 (16%)	33 (66%)	5 (10%)	46 (92%)	

tions were repeatedly detected among strong biofilm former isolates (OQ584340, OQ584341, and OQ584342) which led to changes in the amino acid types of the sequence 68 into Tryptophan, 72 into Asparagine, and stop codon in 77 into Leucine respectively after changing their codons compared with other local isolates. However, the weak biofilm formers (OQ584335 and OQ584336, see Fig. 3) displayed unique nucleotide variation in sequence 185 along with one isolate of moderate biofilm former, which also resulted in a change of amino acid type at sequence 62 into Cysteine.

DISCUSSION

Previous medical records in Iraq showed that the most prevalent bacteria in urinary tract infections were *E. coli,* with a 51.70% percentage among different species of bacteria, leading to renal failure in long-term term untreated conditions. Accurate molecular detection of bacterial species is critical for further action to cure disease. PCR allows fast identification of genes of interest with great sensitivity and specificity (Nissen and Sloots, 2002; MA *et al.*, 2021; Alwan *et al.*, 2022; Alansary and Al-Saryi, 2024). Since significant fragments of important genes in the E. coli species have been preserved throughout the bacterial kingdom, determining primers for alternative E. coli identification is not straightforward. In the present study primers were designed for specific sequences in the target genes (*uidA, yaiO,* and 16*SrRNA*) which might be valuable for fast and accurate bacterial identification with less similarity with close bacterial species such as *Shigella* and *Klebsiella* species. Highly conserved parts within 16S rRNA gene are found throughout all prokaryotes, and variable regions are used to provide useful taxonomic information at different classification stages (Schmidt, 2002; Darwish *et al.*, 2004). The *E. coli uid*A gene encoding beta-D-glucuronidase enzyme is specific for *E. coli*, so it is frequently utilized in identifying kits and as a particular marker (Cleuziat and Robert-Baudouy, 1990). Amplification of *yai*O gene encodes a protein originally expressed and localized in the outer surface of membrane is recently used for rapid and specific detection of *E. coli* . In the present study, sequences of the designed primers taken from UTI *E. coli* gene bank exhibited the highest specificity and the frequency for all genes among all isolates was 100% based on their presence in agarose gel. However, there were no yielded amplicons of expected sizes for all these primer sets among isolates related to *S. dysenteriae* and *K. pneumoniae* species, which mean these probes represent a typical target for identification and are highly efficient for *E. coli*.

Results adapted by Bej et al., (1990) indicated that *uid*A primers obtained higher specificity, whereas (Fricker *et al.*, 1997) refer to misleading results for uidA amplification for *Hafnia alvei* as well as *Serratia odorifera* species. In agreement with other results, *E. coli* detection with *yai*O primers revealed more specificity (Molina *et al.*, 2015). In the biofilm mechanism, the growing range of *fim*H mutation could represent a natural strategy of variation in the ability of surface attachment of *E. coli*, protect against the undesirable impact of chemical and physical factors, and might offer a selective advantage for persistence during *E. coli* movement within the organism and the surrounding environment (Yoshida *et al.*, 2022).

Variations in amino acid types among strong biofilm former isolates resulted from nucleotide sequence alteration, insertion, and deletion (of the sequence 68 into Tryptophan, 72 into Asparagine, and stop codon in 77 into Leucine respectively) and that might have potential role in the functional heterogeneity of *fim*H protein. Similarly, weak biofilm formers isolates displayed unique nucleotide variation in sequence 185, along with one isolate of a moderate biofilm former, which also resulted in a change of amino acid type at sequence 62 into Cysteine. From the viewpoint of evolution, *Fim*H mutations influencing mannose-binding capability significantly rely on the environment being colonized by *E. coli*. *Fim*H may go through rapid microevolution, resulting in elevated nonspecific adhesion and independent development of biofilm from the mannose-binding ability . Study by (Sokurenko *et al.*, 1998) showed that natural variations of *Fim*H significantly alter the tropism of *E. coli* toward tissue and can play an essential role in the bacterial shift from the commensal to the adaptation of pathologic habitat. Therefore, the alteration to the virulent phenotype might be achieved not only through acquiring virulence genes, but also by the selection of the variations in genetic traits that are adaptive to a pathological condition.

Conclusion

The *uid*A, *yai*O, and 16SrRNA genes possibly represented an ideal target for the identification of *E. coli*

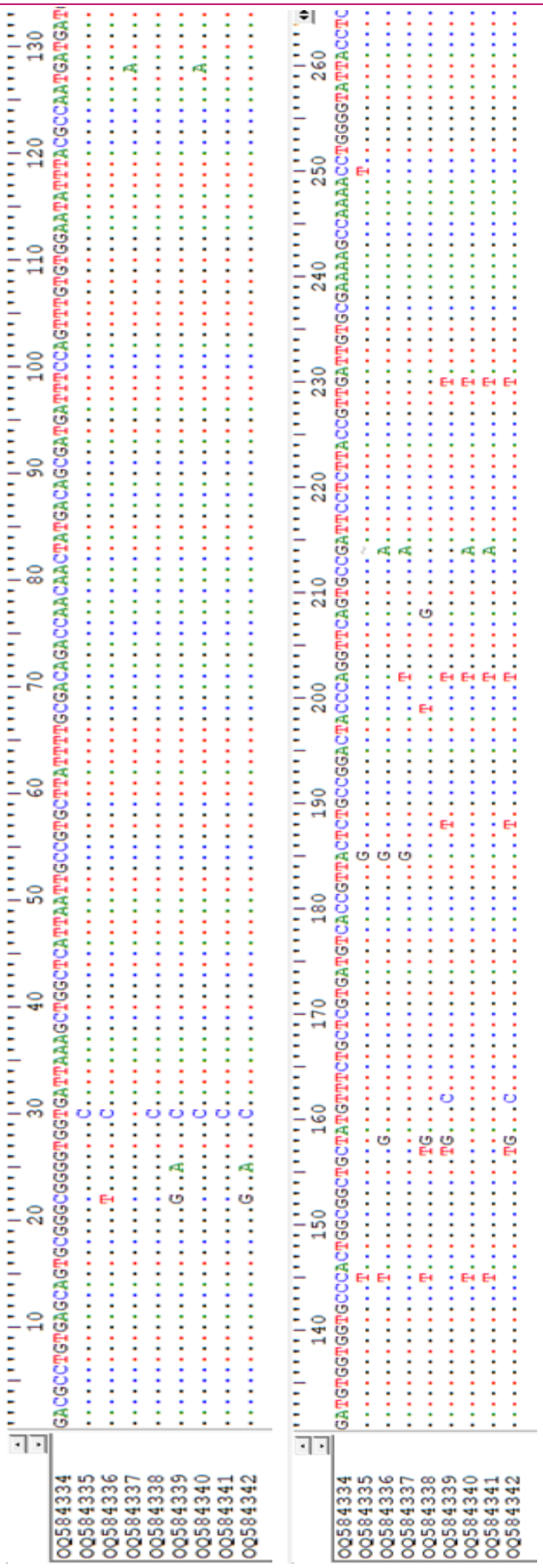

Fig. 3. *Nucleotides sequence alignment of the FimH1 gene among 9 local UTI isolates of Escherichia coli. Alignment carried out based on BioEdit sequence Alignment Editor software. The names of isolates are listed in the left side of the Figures which represent three replicates of weak biofilm formers (in the top), then the moderates and the strong biofilm formers are in the bottom*

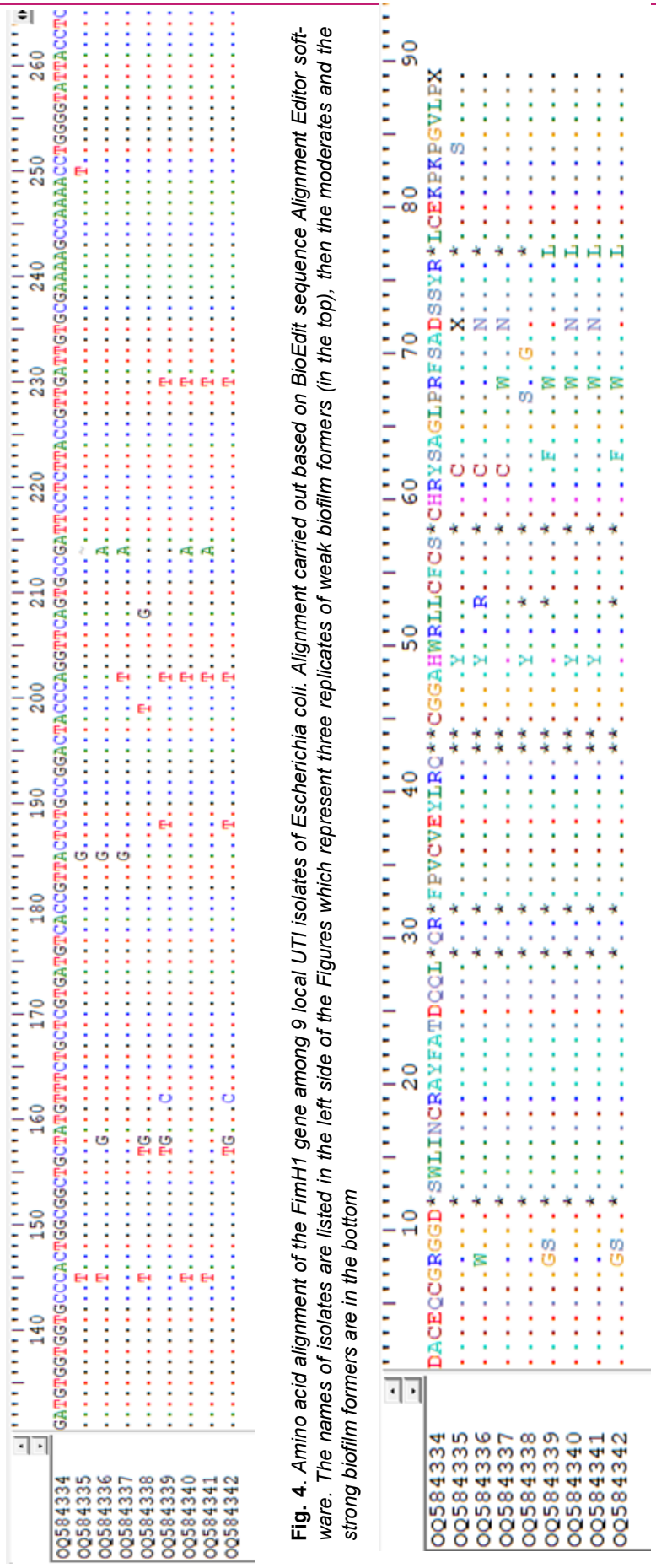

Fig. 4. *Amino acid alignment of the FimH1 gene among 9 local UTI isolates of Escherichia coli. Alignment carried out based on BioEdit sequence Alignment Editor software. The names of isolates are listed in the left side of the Figures which represent three replicates of weak biofilm formers (in the top), then the moderates and the strong biofilm formers are in the bottom*

since all primers achieved exclusively related fragments. The present study displayed the relationship between the variations of nucleotides sequences and amino acid types with different biofilm phenotypic categories (weak, moderate and strong). These variations may be responsible for the structural and functional heterogeneity of the type 1 fimbria D-mannose-specific adhesion protein, suggesting further experiments to elucidate the relationship between sequence variations and gene expression of the *fimH* gene among variable biofilm phenotypes in UTI isolates of *E. coli*.

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Conflict of interest

The authors declare that they have no conflict of interest.

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