International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 15s,2025
https://theaspd.com/index.php

Fimbrial Detection In Escherichia Coli Isolated From Different Clinical Samples In Kirkuk City

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Abstract
Background: Escherichia coli has the ability to cause a range of infections, both in the intestines and in other parts of the body, which can vary in severity from moderate to life-threatening. The degree of infection severity is influenced by numerous virulence genes, including those that encode adhesion molecules such as fimbriae. These molecules aid in the invasion and colonization of epithelial cells.
Objective: To detect the presence of type 1, P, S, and F1C fimbriae in different clinical samples. **Method:** A total of 200 clinical samples (Urine, wound, sputum, and burn) were collected. The samples were diagnosed through culturing on differential and selective media, Biochemical tests and the Vitek 2 system. A hemagglutination test was performed. A polymerase chain reaction was performed for molecular detection of fimbrial genes.
Results: 40 E.coli isolates were obtained (28 from urine, 8 from wound, 2 from sputum and burn, respectively). The highest isolation rate was in females (75%) than in males (25%), with a higher percentage (42.5%) in the age group (17-36) years. For the hemagglutination test (52.5%) of the total isolates were positive, and (47.5%) were negative, (66.6%) were mannose resistant (MRHA), and (33.3%) were mannose sensitive (MSHA). Polymerase chain reaction revealed the percentage of detection was (90%) for fimH gene: (87.5%) from urine, (87.5%) from wound, (100%) from burns, and (100%) from sputum, (30%) for papC gene: (37.5%) from urine, (25%) from wound, (50%) from sputum, (10%) for sfaS gene: (12.5%) from urine, and (12.5%) from wound, (5%) for focG gene: (50%) of sputum sample.
Conclusion: The study concluded that Type 1, P, and S fimbriae were observed in urine and wound samples. In sputum samples, type 1, P, and F1C fimbriae were detected, while in burn samples, only type 1 fimbriae was detected.
Keywords: Fimbriae, Hemagglutination test, Clinical samples, E. coli

INTRODUCTION

Escherichia coli a collection of bacteria typically present in the microbial communities of the gastrointestinal tracts of humans and animals. They play a role in the process of digestion and the production of certain vitamins. But are implicated in the urinary tract infection (UTI), surgical site infection (SSI), hospital-acquired pneumonia (HAP), sepsis, hemolytic-uremic syndrome (HUS), meningitis and inflammation of the meninges (1). The infection induced by E. coli is initiated by the utilization of bacterial virulence factors, particularly the bacterial capsule, fimbriae, flagella, iron scavenger receptors, lipopolysaccharide (LPS), and toxins that alter the cellular processes of the host(2). Adherence is a crucial factor in the development of UPEC, as it promotes bacterial attachment, colonization, and allows interactions between the bacteria and the host cell matrix, thus leading to the production of biofilms. Type 1 fimbriae, P fimbriae, S fimbriae, F1C fimbriae, Dr. adhesins, and a fimbrial adhesions are the most common types of adhesions that are discovered in UPECs according to scientific research (3). Fimbrial adhesins are superficial peritrichous organized external proteinaceous appendages that have the ability to precisely bind to certain motifs on cell surface receptors, in a manner that is analogous to a key-and-lock function (4). The mannose-sensitive polymeric protein known as type 1 fimbriae, which measures 7 nanometers in width and 1–2 micrometers in length, has been reported to promote adhesion to other bacteria, host cells, and the surfaces of medical devices according to a study conducted by a group of researchers (5). P fimbriae are the most important form of adhesins, and they are also the second most common virulence factor of UPEC. They exhibit mannose-resistant haemagglutination, and they are highly connected with the ability of the bacteria to colonize the kidney and produce pyelonephritis (6). Mannose-resistant adhesins are encoded by the sfa operon of uropathogenic E. coli, which is responsible

for the formation of the S fimbriae. There is a correlation between the presence of S fimbriae and the pathogenicity of E. coli in cases of human meningitis and septicemia(7) . The F1C fimbriae nonhemagglutinating adherence factor is able to connect to their particular receptors throughout the entire zone of the urinary tract with the ability to do so. Both F1C and S fimbriae [4] have a number of characteristics that are very comparable. The presence of these chemicals makes it easier for bacteria to colonize tissues, which in turn causes harm to those tissues and weakens the host's defense mechanisms, which finally results in clinical manifestations(8).

MATERIALS AND METHODS

Isolation and identification of bacterial isolates

A cross-sectional study was conducted on patients. A total of 200 clinical samples were collected from various sources, including (urine, wound swab, sputum, and burn swab) in hospitals in the city of Kirkuk during the period from October 2023 to February 2024. After obtaining the colonies, they were inoculated onto blood agar, MacConkey agar, and EMB agar and incubated for 24 h at 37°C. The isolated bacteria were identified based on phenotypic colony characteristics, including size and shape. Subsequently, the bacterial colonies were subjected to Gram's stain and biochemical assays to confirm the presence of E. coli strains. These tests included catalase, oxidase, citrate utilization, indole synthesis, methyl red-Voges Proskauer, and triple iron sugar tests(9), The Vitek 2 Compact system identified all isolates

Haemagglutination Assay (HA)

The haemagglutination assay was conducted following the procedure described by(10), using the direct bacterial hemagglutination test-slide method to detect fimbriae in E.coli isolates phenotypically. The bacterial isolates were inoculated into Brain Heart Infusion broth and incubated at 37°C for 72 hours to achieve complete fimbriation. Afterwards, the mixture was subjected to centrifugation at a speed of 1500 revolutions per minute for 5 minutes. Triple sugar iron test, the mixture was subsequently mixed with phosphate buffer saline and then rinsed three times. Red blood cells, freshly obtained from human blood group "O", were washed three times in phosphate buffer saline and adjusted to a concentration of 3% vol/vol. These cells were then utilized for the haemagglutination test. The slide haemagglutination tests were conducted by combining 20 µL of RBC suspension with 20 µL of broth cultures on a cooled glass slide and then gently rocked for 5 minutes at room temperature. The test was repeated for every individual sample by introducing a small amount of 2% D-mannose to the mixture. The isolates were classified as positive if there was visible agglutination and classified as negative if no visible agglutination was seen.

Detection of fimbrial genes (fimH, papC, sfaS, focG)

DNA EXTRACTION

The DNA was isolated from the diagnosed isolates using the Add Prep Bacterial Genomic DNA Extraction Kit in accordance with the directions provided by the manufacturer.

Polymerase Chain Reaction (PCR)

A polymerase chain reaction was utilized to determine the fimH, papC, sfaS, and focG genes. Genes amplified using four primer pairs (Table 1).

Primers preparation

The primers utilized during this study were synthesized based on the instructions provided by the manufacturer, Macrogen Company. These primers were delivered in a lyophilized form. The lyophilized primers were reconstituted in nuclease-free water to provide a stock solution with a final concentration of 100pmol/µl . To create a functional solution of these primers, 10µl of primer stock solution (kept in a freezer at -20 C) was combined with 90µl of nuclease-free water. This resulted in a functioning primer solution with a concentration of 10pmol/µl. Table 2 presented the reaction conditions for molecular detection.

Table 1: primers for amplification of target genes in polymerase chain reaction

Primer name	**Sequence 5′- 3’**	**Product size (bp)**	**Ref.**

fimH	Forward: AACAGCGATGATTTCCAGTTTGTGTG Reverse: ATTGCGTACCAGCATTAGCAATGTCC	465	[11]
papC	Forward: GACGGCTGTACTGCAGGGTGTGGCG Reverse: ATATCCTTTCTGCAGGGATGCAATA	328	[11]
sfaS	Forward: GTGGATACGACGATTACTGTG Reverse: CCGCCAGCATTCCCTGTATTC	244	[12]
focG	Forward: CAGCACAGGCAGTGGATACGA Reverse: GAATGTCGCCTGCCCATTGCT	364	[12]

Table 2 Program of thermal cycler conditions

Gene	Initial denaturation (°C/min)	Denaturation (°C/s)	Annealing (°C/s)	Extension (°C/s)	Final extension (°C/min)	Cycles
fimH	94/10	94/2min	60/30	72/60	72/10	30
papC	94/10	94/2min	60/30	72/60	72/10	30
sfaS	95/4	94/30	60/45	72/60	72/8	33
focG	95/4	94/30	60/45	72/60	72/8	33

Electrophoresis

Following PCR amplification, agarose gel electrophoresis was utilized to confirm the presence of PCR amplification. PCR must completely rely on the extracted DNA. In a flask, combine 100 milliliters of 1X TAE and 2 milligrams (2%) of agarose buffer. After all of the gel particles had been dissolved, the solution was microwaved until it reached boiling point. Add 1 µl of safe dye (10 mg/ml) to the agarose was stirred to prevent bubbles and ensure proper mixing. The solution needed to cool at 50-60°C

Statistical Analysis

The SAS (2018) program was utilized to identify the impact of various factors on the research parameters through statistical analysis. The chi-square test was employed to examine the significance of the probabilities of 0.05 and 0.01 in this investigation. [Highly significant (P≤0.01), significant (P≤0.05), non-significant (NS).

Ethical Approval

The investigation was conducted in accordance with the ethical principles set forth in the Declaration of Helsinki. Consent was obtained from patients before sample collection. The study protocol was evaluated and approved by the local ethics committee under designated document 586 in 9/18/2023.

RESULTS

After collecting a total of 200 clinical samples distributed as (102 urine sample, 38 wound swab, 23 burn swab, 37 sputum sample), diagnosis was done by culturing on selective and differential media, and identification by vitek2 system[Figure 1]. 40 E. coli isolates were obtained (28) from urine samples, (8) from wound swabs, (2) from burn swabs and sputum samples, respectively. E. coli isolates were higher in women (75%) than in men (25%) among study isolates, with the highest percentage of E. coli isolates (42.5%) was in the age group (17-36) years.

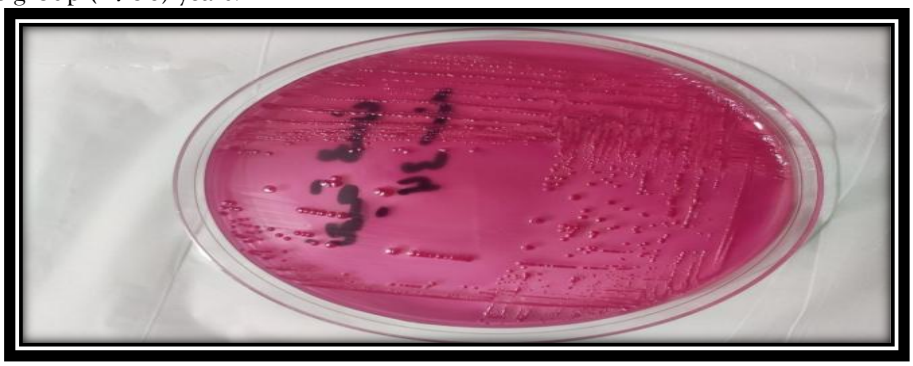

Figure 1 E. coli colonies on MacConkey agar

Haemagglutination

The phenotypic expression of fimbriae in E. coli isolates was monitored by testing the ability of the isolates to cause agglutination of erythrocytes [Figure 2] showed that 21(52.5%) of the total isolates were positive, and 19(47.5%) were negative, from the hemagglutination test positive isolates, 14(66.6%) were mannose resistant (MRHA), and 7(33.3%) were mannose sensitive (MSHA). [Table3].

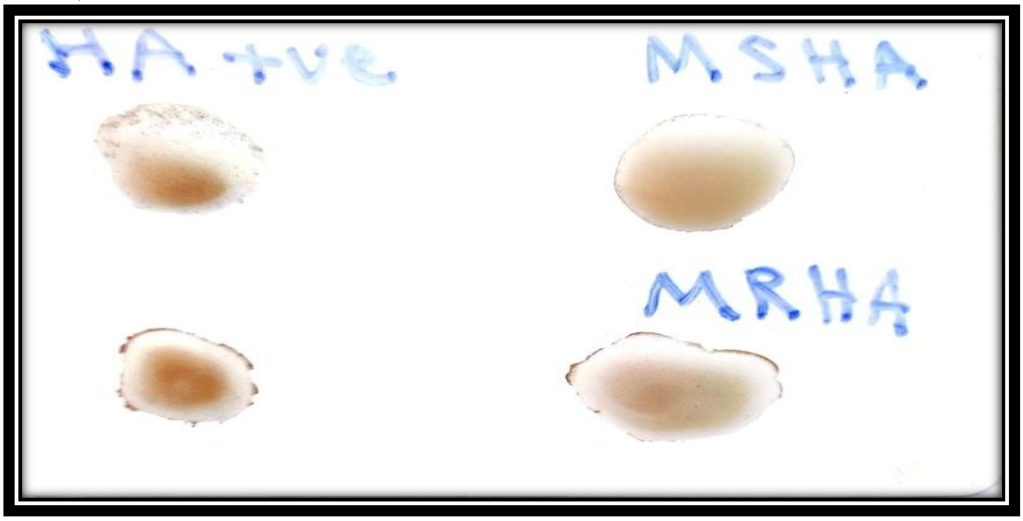

Figure 2 Hemagglutination test

Table 3 Hemagglutination test results among E.coli isolates

Source	Hemagglutination positive		Hemagglutination negative
	MSHA	**MRHA**	
Urine	7(100%)	9(64.2%)	12(63.1%)
Wound	0	4(28.5%)	4(21.1%)
Burn	0	0	2(10.5%)
Sputum	0	1(7.14%)	1(5.2%)
Total	7(33.3%)	14(66.6%)	19(100%)
P-value	0.0001 **		
** (P≤0.01).			

Molecular detection

PCR technique was performed for 20 E. coli isolates in order to determine some of the genes encoding for type 1, P, S, and F1C fimbriae. The results of the molecular detection of the fimH gene showed that, 18 (90%) of the clinical isolates contain the fimH gene as shown in [Figure 3], 7 (87.5%) from urine, 7 (87.5%) from wound, 2 (100%) from burns, and 2 (100%) from sputum.The molecular detection of papC gene showed that 6 (30%) of the E. coli isolates contain the papC gene as shown in [Figure 4], 3 (37.5%) from urine, 2 (25%) from wound, 1 (50%) from sputum, and was not detected in burn samples. For the sfaS gene 2 (10%) of the clinical isolates gave positive results as shown in [Figure 5], 1 (12.5%) from urine, and 1 (12.5%) from wound. While focG gene was detected only in 1 (5%) of the clinical isolates as shown in [Figure 6], that was 1 (50%) of sputum sample. [Table 4], show the results of the molecular detection of the fimbrial genes.

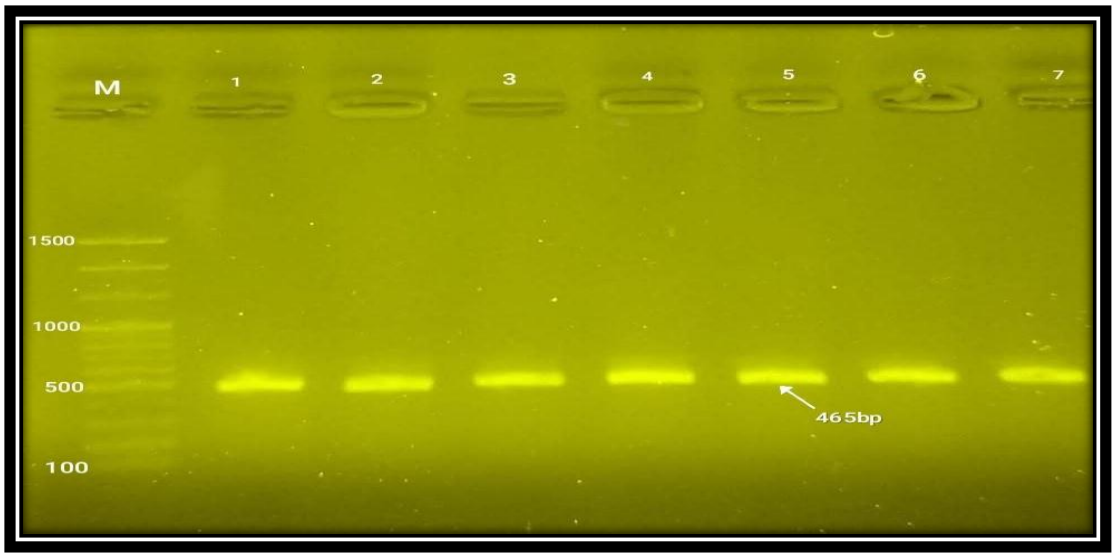

Figure 3 Agarose gel electrophoresis detection of fimH gene (465bp). Lane M: 100 bp DNA ladder, lane (1-7): Amplicons of the fimH gene.

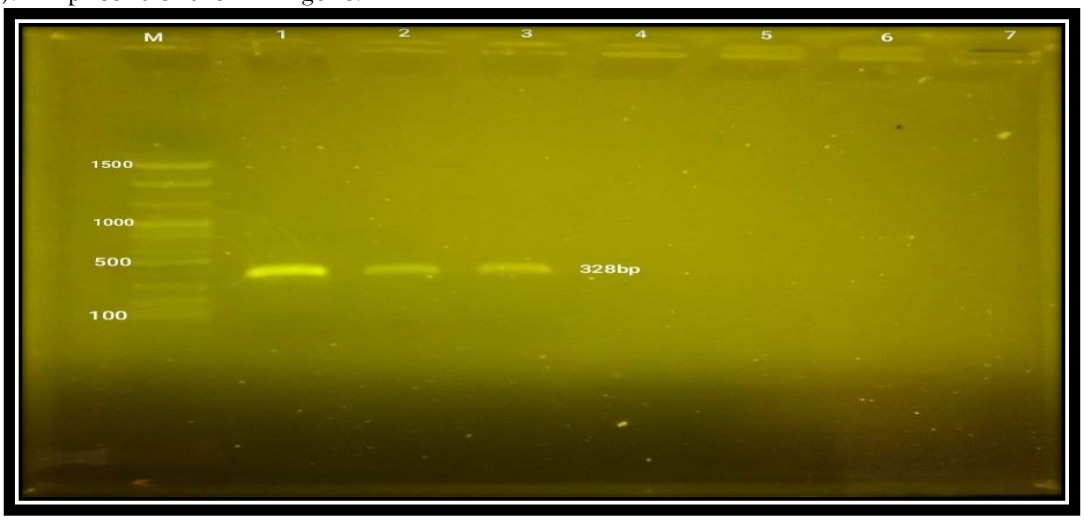

Figure 4 Agarose gel electrophoresis detection of papC gene (328). Lane M: 100 bp DNA ladder, lane (1-7): Amplicons of the papC gene.

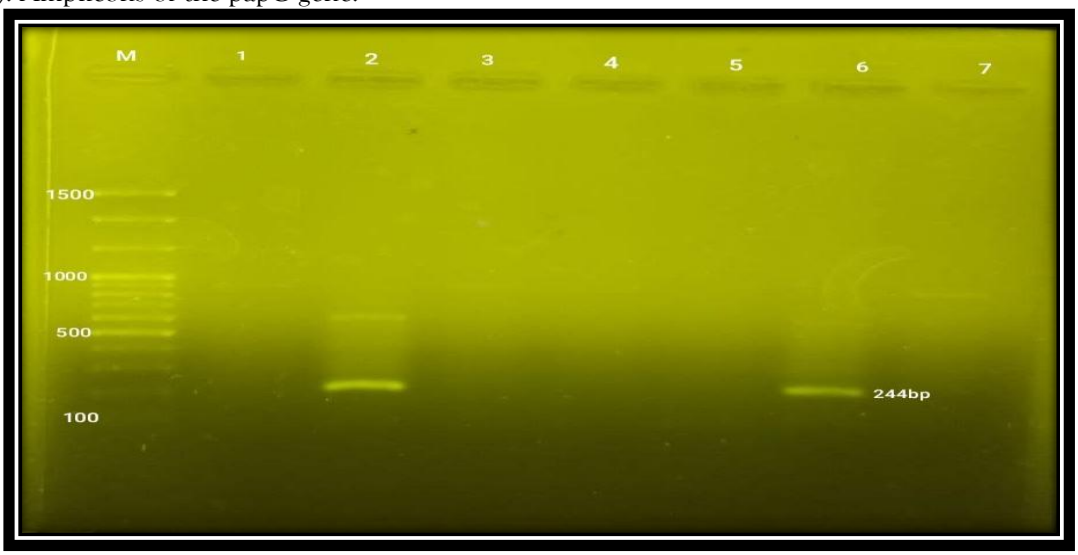

Figure 5 Agarose gel electrophoresis detection of sfaS gene (244bp). Lane M: 100bp DNA ladder, lanes (1-6): Amplicons of sfaS gene.

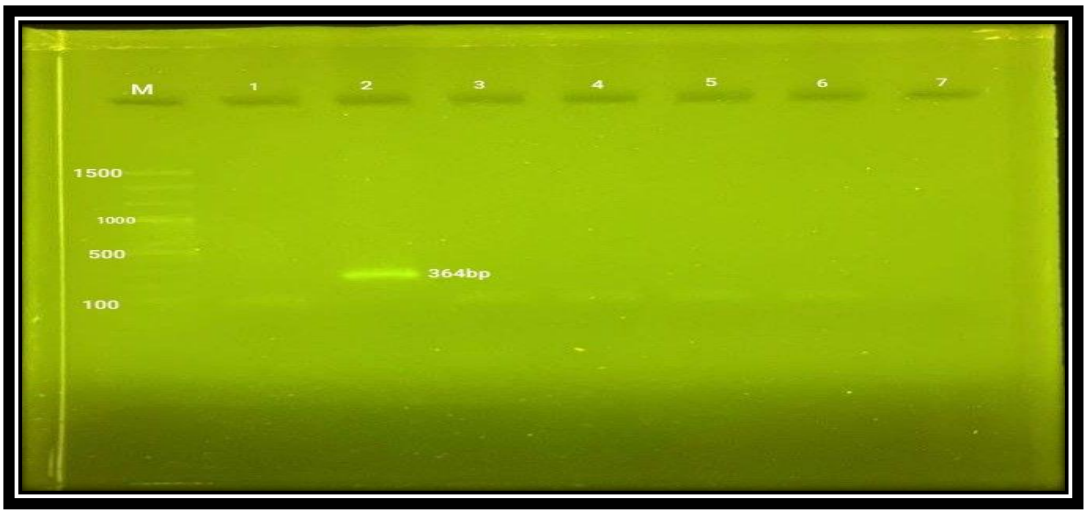

Figure 6 Agarose gel electrophoresis detection of focG gene (364bp). Lane M: 100bp DNA ladder, lanes (1-6): Amplicons of focG gene.

Table 4 Distribution of fimbrial genes in the clinical samples

Source &No. of isolates	**fimH**	**PapC**	**sfaS**	**FocG**
Urine (8)	7 (87.5%)	3 (37.5%)	1 (12.5%)	0
Wound (8)	7 (87.5%)	2 (25%)	1 (12.5%)	0
Burn (2)	2 (100%)	0	0	0
Sputum (2)	2 (100%)	1 (50%)	0	1 (50%)
Total	18 (90%)	6 (30%)	2 (10%)	1 (5%)
P- value	0.082 NS	0.226 NS	0.375 NS	0.389 NS
NS: Non-Significant.				

DISCUSSION

Escherichia coli possess the expression of many adhesion organelles, such as P, type 1, S, and F1C fimbriae, which is crucial for the colonization of the urinary system. This capacity makes it the primary pathogen responsible for urinary tract infections [13]. The results of the hemagglutination test obtained by the current study were (52.5%) of the total isolates were positive, and (47.5%) were negative this result was in agreement with [14] who reported (63.10%) of the isolates as hemagglutination positive and (36.8%) of the isolates were non-hemagglutinating, and MSHA were (28.1%), while disagreed the (34.9%) MRHA reported among the isolates of same study. But was in agreement with [15] in which MRHA in their study isolates were (63.6%).The molecular detection in our study for the fimbrial genes (fimH, papC, sfaS, focG) were presented as (90%, 30%, 10%, 5%), respectively, which was in line with the results obtained by [11,16].While was incompatible with what was mentioned by [12]. The study results were also similar to the results obtained by [17] in Isfahan city, Iran regarding papC, sfaS, and focG genes which reported papC as (30.3%), sfaS (17.1%), and focG (1.3%), while disagreed with the (68.4%) result of fimH presence reported by the same study. Regarding papC and sfaS genes the study results were compatible with [18] who reported papC as (26.1%) and sfa as (11.1%), but were incompatible with [19] in Diyala city, Iraq, in which papC was (94.44%), and sfaS was (72.2%), but was compatible with fimH gene result reported as (100%).Also the study results were close to the results of [7] which reported 95% positive for fim gene, 16% for foc gene, while disagreed with the results of pap and sfa genes which were 57%, and 81% respectively. Study results were also found to be similar to the results found in southern Iraq by [20] for fimH gene which was detected in (96%) of their isolates and papC genes which was detected in (36%) of their isolates. FimH, is a most important determinant of type 1 fimbriae, which has high tropism for urinary tract receptors; thus, fimH adhesion is vital in colonizing different niches of E. coli [21]. The expression of type 1 fimbriae can be regulated by various factors, such as environmental conditions and the genetic background of the isolates. Various environmental factors, as well as bacterial

phylogenetic group and host-associated factors, can influence bacterial gene expression, leading to variations in the genotypic and phenotypic traits of UPEC strains [22]. The results of the current study for the fimH gene was in agreement with many studies [23–27]**.** The variation in the prevalence of virulence genes between our study and various global studies may be attributed to differences in study population, sample size, and methods [11]. Some virulence genes can result from mutations, abnormalities in gene transcription and expression rates, or differences in gene copy numbers. Hence, a positive PCR result signifies the existence of genes. However, it is important to note that a negative result does not necessarily mean the lack of the corresponding genes, although this occurrence is uncommon [28].

CONCLUSION

1- The fimH gene which encodes for type 1fimbriae was observed in all clinical samples which indicate its role as an important virulence factor in extra-intestinal pathogenic E. coli.
2- Type 1 and P fimbrial genes have the highest percentage of detection in the clinical samples.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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