Original Article

Molecular Detection of Genes Encoding for Adhesion Factors in Biofilm Formation among Uropathogenic *Escherichia coli* Isolates

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Abstract

Background: The ability of *Escherichia coli* to build biofilms leads to the development of numerous diseases and makes their removal challenging. In addition to being the most common cause of urinary tract infections (UTIs), *E. coli* has been linked to disease in almost every area of the human body. **Objective:** The objective of the study was to evaluate the uropathogenic *E. coli* biofilm development using molecular and biochemical methods. **Materials and Methods:** Out of the total 117 urine samples obtained from UTI patients and diagnosed by selective media EMB (eosin methylene blue agar) and Vitek2 system, antibiotic sensitivity test, biofilm formation assay, and molecular detection of genes encoding for adhesion factors using polymerase chain reaction (PCR) technique were done. **Results:** Fifty *E. coli* isolates from both sexes of different ages were isolated; the UTI rate in females was 82% and in males was 18%. The result of the antibiotic sensitivity test, in terms of the percentage of resistance, was as follows: ampicillin, 50%; amoxicillin-clavulanate, 98%; ceftazidime, 72%; cefotaxime, 68%; aztreonam, 26%; gentamicin, 48%; levofloxacin, 36%; and trimethoprim, 52%, so that a high percentage of multidurg resistance resulted in the current study was 88%. The results of the quantification of biofilm formation revealed that all isolates produced biofilm with the following percentages: five (10%) as strong adherents, 36 (72%) as moderate biofilm producers, and nine (18%) were weak producers. The prevalence of genes *fimH*, *csgA*, and *ag43* was 92%, 98%, and 92%, respectively, the result of detection of genes encoding for adhesion factors using PCR technique. **Conclusions:** The biofilm phenotype was indicated in all *E. coli* isolates and can confer virulence behavior and considered as a great challenging health problem and there is a significant association between adherent factor's genes (*fimH, csgA, ag43*) and the ability to produce biofilm within *E. coli* isolates.

Keywords: Antibiotic resistance, biofilm, *E. coli*, UPEC

Introduction

Urinary tract infections (UTIs) are one of the most common and pervasive bacterial diseases worldwide, affecting everyone from newborns to the elderly.[1] UTI refers to the presence of a certain number of bacteria in the urine (generally > 10^5/mL).[2] *Escherichia coli* has been linked to disease in almost every area of the human body, including the bloodstream, gastrointestinal tract, skin, intra-amniotic and puerperal infections in pregnant women, meningitis, and endocarditis. *E. coli* can also spread disease to people of all ages and result in both community-acquired diseases and infections associated to healthcare.[3]

A biofilm is a structured bacterial colony that is connected to either a live or inert surface and is shielded by a self-produced polymeric matrix.[4] *E. coli* biofilm formation frequently colonizes the gastrointestinal system of the human host within a few hours of birth and coexists with the host for decades, typically to the benefit of both parties.[5] The ability of *E. coli* to build biofilms leads to the development of numerous diseases and makes their

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removal challenging. The development of mature biofilms is influenced by elements such as various extracellular appendages that aid in *E. coli* surface colonization and their carefully regulated expression and activity. Initial adhesion or attachment (reversible), early growth of biofilm structure (irreversible), maturity of the established biofilm, and dispersion of cells from the biofilm to return to the planktonic state are the four main stages in the formation of a biofilm.[6] Several genes in *E. coli* have been associated with biofilm formation, including the genes for type 1 fimbriae (*fimH*), curli (*csgA*), and antigen 43 (*ag43*).[7]

The aim of the study was to evaluate the uropathogenic creation of *E. coli* biofilms using molecular and biochemical techniques.

Materials and Methods

Samples collection

A total of 117 urine samples were taken from both male and female patients with UTI using sterile containers from Al-Hilla Teaching Hospital and Al-Childhood and Gynecology Hospitals in Hilla city/Iraq, during the period from July to August 2022.

Identification of *E. coli*

After incubating a single colony of nutrient agar, MacConkey agar, and blood agar subcultured in eosin methylene blue (EMB) agar for additional identification and incubating at 37°C for 24 h, the pure culture was obtained with homogenous colonies (green metallic sheen). The identifications were confirmed by the Vitek2 system.

Antimicrobial susceptibility test

The sensitivity of the isolated organisms by EMB agar and Vitek2 system was tested against eight antibiotics. Tested antimicrobials belong to penicillin, β-lactams, aminoglycosides, quinolones and fluoroquinolones, folate pathway inhibitors, and cephems classes (ampicillin 10 mg, amoxicillin-clavulanate 20/10 mg, ceftazidime 30 mg, aztreonam 30 mg, gentamicin 10 mg, levofloxacin 5 mg, cefotaxime 30 mg, and trimethoprim 5 mg) as described by references [8,9] using the disk diffusion method.

Biofilm formation assay

The biofilm formation among *E. coli* isolates was investigated according to reference [10]. For growing bacteria for biofilm, bacterial cultures of the *E. coli* were cultivated in a rich medium overnight and then diluted into 1:100 by using fresh medium. Three milliliters of diluted culture were transferred into new fresh tube for biofilm assay (all cultures were fixed at optical density (OD) 0.06) and incubated the tubes for 24 h at 37°C. For staining the biofilm, after incubation, the cells were emptied out by gently shaking the liquid out of the tubes and flipping them over. The tubes were then placed in a small tank tub and shaken to remove any remaining liquid. The procedure was carried out once more, significantly less background staining results from the removal of unattached cells and medium components that could be stained in the following phase.

Each tube received 3 mL of a 0.1% solution of crystal violet (CV), which was then incubated for 10–15 min at a room temperature. The tubes were then flipped upside down and dried for a few hours or overnight after being cleaned 3–4 times with water submerged in a tank of water as described above. The tubes can be imaged when dry for qualitative testing. For each tube of the solubilized CV, 3 mL of 30% acetic acid was added to water to calculate the amount of biofilm, 15–20 min of incubation at a room temperature. After that, a fresh tube was filled with 1 mL of the solubilized CV, and the absorbance was measured at 550 nm with 30% acetic acid in water as the blank. The results were calculated according to reference[11] as follows: nonadherent if OD ≤ optical density control (ODC), weakly adherent if ODC < OD ≤ 2 × ODC, moderately adherent if 2 × ODC < OD ≤ 4 × ODC, and strongly adherent if OD > 4 × ODC.

Detection of adherent factors genes (*fimH, csgA*, and *ag43*)

Isolation of genomic DNA

DNA was extracted from the diagnosed isolates using the FavorPrep Genomic DNA Mini Kit for Gram-negative bacteria extraction.

Polymerase chain reaction procedure

The polymerase chain reaction (PCR) combination of 25 µL final volume was prepared from Go Taq Green Master Mix (2X) 12.5 µL, forward primer 1 µL, reverse primer 1 µL, DNA template 3 µL, and nuclease-free water 7.5 µL. The primers sequences for *fimH, csgA* and *ag43* genes were shown in [Table 1]. The PCR was performed with a PCR thermal cycler under the following conditions which are mentioned in Table 2.

Electrophoresis

One gram of agarose powder was dissolved in 100 mL of Tris Borate EDTA (TBE) buffer to create agarose gel (pH = 8). The solution was then heated in a microwave (Mbiano) for 30 s after being heated for 90 s at full power. Red safe dye was added after allowing the mixture to cool to 50°C. In order to create wells for loading DNA samples, a comb was connected to one end of the gel tray and a tape was placed across the end. It took 30 min for the agarose to completely solidify after being carefully put into the tray. The tap was then carefully removed from the tray's ends, followed by the comb. The latter was fixed in an electrophoresis chamber with a gel surface covered in

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

Primer name	Primer sequence 5′-3′	Product size (bp)	Reference
FimH	Forward: TGCAGAACGGATAAGCCGTGG Reverse: GCAGTCACCTGCCCTCCGGTA	508	[12]
Ag43	Forward: CGGCGGGCAATGGGTACA Reverse: CAGCTCTCACAATCTGGCGAC	384	[13]
CsgA	Forward: GATCTGACCCAACGTGGCTTCG Reverse: GATGAGCGGTCGCGTTGTTACC	178	[14]

Table 2: Program of polymerase chain reaction conditions

Step	Temperature (°C)	Time	No. of cycles
Initial denaturation	95	4 min.	1
Denaturation	95	30 s	30
Annealing	X*	30 s	
Extension	72	1 min	
Final extension	72	7 min	1
Cooling	4	4 min	

*X = annealing temperature for each primer of adherent factors genes *ag43*, *csgA*, *fimH* is 55°C, 60°C, 63°C, respectively

0.5× TBE running buffer. 5 µL of each DNA sample was transferred to Eppendorf tube. 5 µL of loading buffer was added to the tube, and the mixture was loaded into the wells in agarose gel. The electric current was allowed at 70 volt for 2 h. Ultraviolet transilluminator was used at 320–36 nm for the observation of DNA bands, and the gel was photographed using digital camera.

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number M220603 (including the number and the date in 22/06/2022) to get this approval.

Results

After collecting a total of 117 urine samples obtained from UTI patients and diagnosed by EMB selective media and Vitek2 system [Figure 1], 50 *E. coli* isolates were obtained from both sexes of different ages and were isolated from different hospitals in Babylon province; the infection UTI rate in females (82%) was higher than in males (18%).

Antibiotics susceptibility testing

The antibiotic susceptibility testing was conducted by using the Clinical and Laboratory Standards Institute protocol (CLSI, 2021). Fifty Uropathogenic *E. coli* (UPEC) isolates were evaluated using the disc diffusion method for susceptibility to eight different antibiotic kinds as in Table 3. The pathogenic isolates tested showed varying degrees of resistance and patterns of susceptibility to these drugs.

Figure 1: *E. coli* colonies on EMB

The results of the antibiotic sensitivity test were as follows: the penicillins group, ampicillin, was 50% of the resistance, whereas 98% represented beta-Lactam (amoxicillin-clavulanate) as the highest resistance. The resistance percentage varied among the cephems group, where it was ceftazidime 72% and cefotaxime 68%, whereas the antibiotic aztreonam belonged to the monobactams group was 26%, and gentamicin 48% belonging to the group of aminoglycosides. In addition, 34% was the proportions of antibiotics levofloxacin, which belong to the group of quinolones and fluoroquinolones, whereas the proportion of the folate pathway antagonists trimethoprim group is 52%.

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Table 3: Antibiotic sensitivity profile for uropathogenic *E. coli* isolates

Group	Antimicrobial agent	Resistance	Intermediate	Sensitive
Penicillin	Ampicillin	50	2	48
Beta-Lactam	Amoxicillin-clavulanate	98	2	0
Cephems	Ceftazidime	72	14	14
	Cefotaxime	68	16	16
Monobactams	Aztreonam	26	6	68
Aminoglycosides	Gentamicin	48	20	32
Quinolones and fluoroquinolones	Levofloxacin	34	8	58
Folate pathway antagonists	Trimethoprim	52	2	46

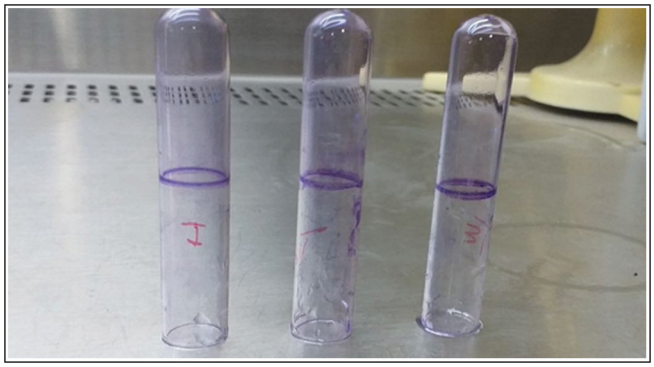

Figure 2: The production of the film by *E. coli*

Biofilm formation

The results of the quantification of biofilm formation revealed that all isolates produced biofilm with the following percentages: five (10%) as strong adherents, 36 (72%) as moderate biofilm producers, and nine (18%) were weak producers. The qualitative assay of biofilms producer was detected as a result of the occurrence of a visible film as in Figure 2.

Molecular study

PCR technique for the detection of adherent factors genes

FimH gene PCR was performed for 50 UPEC isolates in

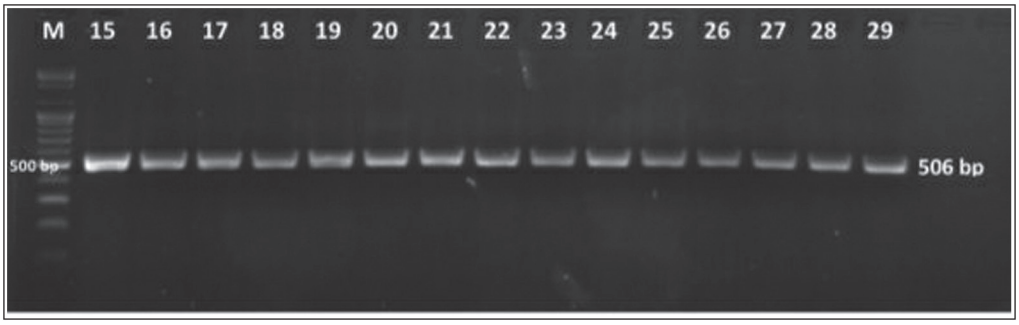

Figure 3: Agarose gel of *fimH* polymerase chain reaction product (506 bp) among *E. coli* isolates. M represents 100 bp DNA ladder; lanes 15–29 represent the isolates at 85 volt for 60 min

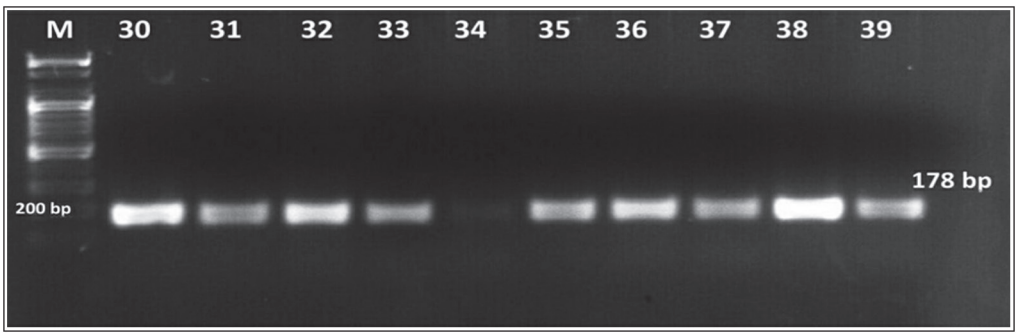

Figure 4: Agarose gel of *csgA* polymerase chain reaction product (178 bp) among *E. coli* isolates. M represents 100 bp DNA ladder; lanes 30–39 represent the isolates at 85 volt for 60 min

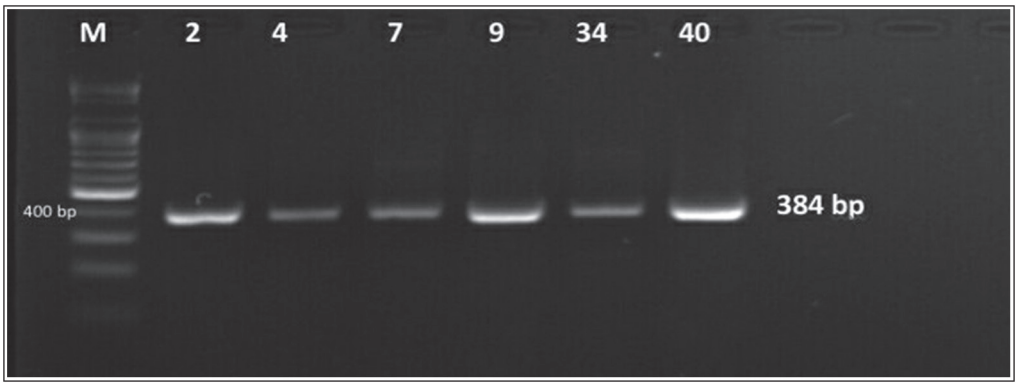

Figure 5: Agarose gel of *ag43* polymerase chain reaction product (384 bp) among *E. coli* isolates. M represents 100 bp DNA ladder; lanes 2, 4, 7, 9, 34, 40 represent the isolates at 85 volt for 60 min

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order to determine the presence of adherent factors genes encoded for type 1 fimbriae, or pili by the *fimH* gene are important for the initial attachment to abiotic surfaces. Forty-six isolates (92%) gave positive results as shown in Figure 3.

CsgA gene Curli fimbriae, encoded by the *csgA* gene, provide adhesion to abiotic surfaces by promoting cell–surface interaction. The result was that all isolates of PCR were subjected to the *csgA* gene with a molecular size of 178 base pairs, which gave 48 isolates with a percentage of 98% as shown in Figure 4.

Ag43 gene PCR detection for *ag43* was performed under optimal conditions by using a specific primer as shown in Figure 5. From a total of 50 extracted DNA samples, 46 isolates gave (92%) positive results.

Discussion

E. coli are the bacteria that are the first major cause of UTI, as the result of the current study, which agrees with many studies in which the incidence of *E. coli* was high.[15] And our results, which are in agreement with many previous studies, showed that the incidence of *E. coli* was higher in females than in males.[16,17] Despite the fact that both sexes are susceptible to infection, women are mostly susceptible because of their anatomy and reproductive physiology. The entry of infectious pathogens into the urinary tract causes infections that usually occur through the urethra.[18]

The results of the antibiotic sensitivity test were as follows: the penicillins group, ampicillin, was 50% of the resistance; the percentage of ampicillin is similar to a previous study,[19] whereas 98% represented beta-Lactam (amoxicillin-clavulanate) as the highest resistance reported in this study, which agreed with another study[20]; the reason for its increased resistance may be that most of the *E. coli* isolates contain beta-lactamase. The resistance percentage varied among the cephems group, where it was ceftazidime 72% and cefotaxime 68%,[21-23] which agreed with ceftazidime resulted in the current study, whereas the antibiotic aztreonam belonged to the monobactams group was 26%. Our study did not agree with the study reported.[22] The results also revealed that 48% of UPEC isolates were resistant to Gentamicin. This finding was compatible with Al-Taai.[24] In addition, 34% was the proportions of antibiotics levofloxacin, which belong to the group of quinolones and fluoroquinolones; the percentage resistance was agreed upon,[25] whereas the proportion of the folate pathway antagonists trimethoprim group is 52%.

The results revealed that quantification of biofilm formation by tube method and the productivity of the thin biofilm included all isolates with variation in the intensity of formation. This result was similar to that of previous studies.[19,24]

The genotypic detection of genes encoding adherent factors of our study was close to the results of previous studies regarding the adhesion factor gene *fimH*,[12,19,26,27] which confirms the widespread prevalence of virulence genes in patients with UTI. According to previous studies,[28,29] the results were similar to those of our study for the *csgA* gene. *Ag43* is the main autotransporter encoded by the flu gene.[13,30] These were studies close to the results of our study for the *ag43* gene, whereas the results of the study done by reference[12] did not agree with the results of our current study.

Conclusions

1 The biofilm phenotype was indicated in all *E. coli* isolates and can confer virulence behavior and considered as a great challenging health problem.
2 There is a significant association between adherent factor's genes (*fimH*, *csgA*, *ag43*) and the ability to produce biofilm within *E. coli* isolates.

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Conflicts of interest
There are no conflicts of interest.

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References

1. Tambekar D, Dhanorkar D, Gulhane S, Khandelwal V, Dudhane M. Antibacterial susceptibility of some urinary tract pathogens to commonly used antibiotics. Afr J Biotechnol 2006;5:1562-1565.
2. Sasirekha R. A study on comparison of different phenotypic methods for detection of extended spectrum beta lactamase production among Enterobacteriaceae in urinary tract infection in a tertiary care centre: Doctoral dissertation, Madurai Medical College, Madurai. 2017.
3. Galindo-Méndez M. Antimicrobial resistance in Escherichia coli. In: E Coli Infections—Importance of Early Diagnosis and Efficient Treatment. IntechOpen; 2020. Available from: http://dx.doi.org/10.5772/intechopen.93115.
4. Van Houdt R, Michiels CW. Role of bacterial cell surface structures in Escherichia coli biofilm formation. Res Microbiol 2005;156:626-33.
5. Kaper JB, Nataro JP, Mobley HL. Pathogenic Escherichia coli. Nat Rev Microbiol 2004;2:123-40.
6. Sharma G, Sharma S, Sharma P, Chandola D, Dang S, Gupta S, *et al*. Escherichia coli biofilm: Development and therapeutic strategies. J Appl Microbiol 2016;121:309-19.
7. Beloin C, Roux A, Ghigo JM. Escherichia coli biofilms. Curr Top Microbiol Immunol 2008;322:249-89.
8. Humphries RM, Ambler J, Mitchell SL, Castanheira M, Dingle T, Hindler JA, *et al*. CLSI Methods Development and Standardization Working Group best practices for evaluation of antimicrobial susceptibility tests. J Clin Microbiol 2018;56:e01934-17.
9. Borty SC, Rahman F, Reza AA, Khatun MS, Kabir ML, Rahman MH, *et al*. Isolation, molecular identification and antibiotic susceptibility profile of Aeromonas hydrophila from cultured indigenous Koi (Anabus testudineus) of Bangladesh. Asian J Med Biol Res 2016;2:332-40.
10. O'Toole GA. Microtiter dish biofilm formation assay. J Vis Exp 2011;47:e2437.
11. Stepanović S, Vuković D, Hola V, Di Bonaventura G, Djukić S, Cirković I, *et al*. Quantification of biofilm in microtiter plates: Overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS 2007;115:891-9.
12. Abdul-Ghaffar SN, Abu-Risha RA. Virulence genes profile of Escherichia coli isolated from urinary catheterized and non-catheterized patients. Iraqi J Sci 2017;58:820-35.
13. Restieri C, Garriss G, Locas MC, Dozois CM. Autotransporter-encoding sequences are phylogenetically distributed among Escherichia coli clinical isolates and reference strains. Appl Environ Microbiol 2007;73:1553-62.
14. Silva VO, Soares LO, Silva Júnior A, Mantovani HC, Chang YF, Moreira MA. Biofilm formation on biotic and abiotic surfaces in the presence of antimicrobials by Escherichia coli isolates from cases of bovine mastitis. Appl Environ Microbiol 2014;80:6136-45.
15. Ibrahim SA, Mohamed DA, Suleman SK. Microbial causes of urinary tract infection and its sensitivity to antibiotics at Heevi Pediatric Teaching Hospital/Duhok City. Med J Babylon 2020;17:109.
16. Shah T, Preethishree P, Ashwini PV. Bacterial profile of urinary tract infections: Evaluation of biofilm formation and antibiotic resistance pattern of uropathogenic Escherichia coli. J Pure Appl Microbiol 2020;14:2577-84.
17. Gunardi WD, Karuniawati A, Umbas R, Bardosono S, Lydia A, Soebandrio A, *et al*. Biofilm-producing bacteria and risk factors (gender and duration of catheterization) characterized as catheter-associated biofilm formation. Int J Microbiol 2021;2021:8869275.
18. Vasudevan R. Urinary tract infection: An overview of the infection and the associated risk factors. J Microbiol Exp 2014;1:00008.
19. Muhammad IA, Ghareb DJ. Biofilm forming capability, multidrug resistance and detection of associated genes in uropathogenic Escherichia coli isolated from catheterized patients. Zanco J Pure Appl Sci 2019;31:9-22.
20. Abass SK, Ali M, Authman SH. Isolation of multi antibiotics resistance Escherichia coli from urinary tract infection and the detection of PapC and fimH virulence genes by polymerase chain reaction technique. Diyala J Pure Sci 2014;10:112-27.
21. Shakhatreh MAK, Swedan SF, Ma'en A, Khabour OF. Uropathogenic Escherichia coli (UPEC) in Jordan: Prevalence of urovirulence genes and antibiotic resistance. J King Saud Univ Sci 2019;31:648-52.
22. Almutairy AAH, Alka'aby TA-A, Abdulla AA. Detection of metallo-β-Lactamases and their association with integrons among multidrug resistant clinical isolates of Escherichia coli. Int J PharmTech Res 2016;9:740-753.
23. Ghaffoori HA, Suleiman AA. Isolation, identification and antibiotic resistance profile distribution of clinical E. coli in Iraqi patients. Eurasian Med Res Periodical 2022;8:116-21.
24. Al-Taai HR. Antibiotic resistance patterns and adhesion ability of uropathogenic Escherichia coli in children. Iraqi J Biotechnol 2018;17:18-26.
25. Ebadi M, Askari N, Jajarmi M, Ghanbarpour R. Detection of fimbrial genes, antibiotic resistance profile and phylogenetic background of uropathogenic E. coli isolated from clinical samples in Karaj city, Iran. J Med Bacteriol 2017;6:15-20.
26. Merza NS. Prevalence and molecular characterization of fimH gene in Escherichia coli isolates recovered from patients with UTIs. Med J Babylon 2017;14:470-7.
27. Fadhil RA. Detection of fimH virulence gene in uropathogenic Escherichia coli using PCR. Journal of University of Anbar for Pure Science 2020;14:1-4.
28. Al-Yasi AAA, Al Saadi KA. Molecular detection of genes responsible for multidrug resistance in uropathogenic Escherichia coli. Iran J Ichthyol 2022;9:69-78.
29. Abd El-Baky RM, Ibrahim RA, Mohamed DS, Ahmed EF, Hashem ZS. Prevalence of virulence genes and their association with antimicrobial resistance among pathogenic E. coli isolated from Egyptian patients with different clinical infections. Infect Drug Resist 2020;13:1221.
30. Kadry AA, Al-Kashef NM, El-Ganiny AM. Distribution of genes encoding adhesins and biofilm formation capacity among uropathogenic Escherichia coli isolates in relation to the antimicrobial resistance. Afr Health Sci 2020;20:238-47.