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Molecular Characterization of *algD* and *TspE4.C2* Genes in *Escherichia coli* Isolated from Urinary Tract Infection Patients

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Abstract

The current study collected 157 clinical samples from Iraqi patients suffering from urinary tract infections at various Baghdad hospitals, including Medical City/Educational Laboratories, Baghdad Teaching Hospital, Imamin Al-Kadhimiya Hospital, and the Central Children's Hospital in Baghdad, from 27 November 2022 to 11 January 2023. We identified and tested the formation of biofilms, which led to the detection of two genes, *algD* and *TspE4.C2*. Vitek 2 confirmed the final diagnosis after a biochemical and microscopic examination of 11 selected *E. coli* isolates. The Congo red method has detected the bacterial vulnerability to form biofilms. he findings of this study showed that all selected bacterial isolates formed the biofilm with a moderate degree (11 (100%)). The polymerase chain reaction (PCR) identified the *algD* and *TspE4.C2* genes, demonstrating their significant role in biofilm production. The polymerase chain reaction results revealed the presence of the *algD* gene in 9 (81.8%) out of 11 isolates, while the *TspE4.C2* gene was present in 10 (90.9%) isolates.

Keywords: *Escherichia coli*, *algD* gene, *TspE4.C2* gene, Virulence.

1. Introduction

The genus *Escherichia* takes its name from Theodor Escherich. *Escherichia coli* is extensively dispersed, and it is the predominant facultative anaerobic that lives in the human large intestine. Numerous pathogenic strains of *Escherichia coli* (*E. coli*) can cause intestinal and extraintestinal disorders, despite the fact that the majority of *E. coli* strains reside harmlessly in the colon (1, 2). The strains of extra-intestinal pathogenic *E. coli* (ExPEC), which infect humans, are fairly closely linked phylogenetically and share several virulence genes (3, 4, 5).Urinary tract infections UTIs are amongst the typical infections that affect people. Numerous microorganisms, including bacteria, are responsible for over 90-95% of UTI infections. Both negative and positive gram bacteria that cause UTIs are the foremost communal causative agents. *E. coli* accounts for 50–80% of the most prevalent gram-negative bacteria that cause these infections. (6, 7). The most frequent cause of urinary tract infections (UTIs) in both community-acquired and nosocomial settings, as well as associated diseases such as diarrhea and bacteremia, is *E. coli*. Due to its numerous virulence factors, including

adhesion, iron absorption, toxins, capsular polysaccharides, and proteins, *E. coli* can cause various infections. Other bacteria, including *Klebsiella*, *Proteus*, *Pseudomonas*, and *Enterobacter*, can cause UTIs (8). Finding the most sensitive drugs is essential for proper treatment since rising bacterial resistance to antibiotics has become the main worry as a result of antibiotic abuse. Sensitivity patterns should be developed in UTIs (9). One of the main issues with treating UTIs is the widespread occurrence of *E. coli* strains that are resistant to a number of antibiotics (coded by chromosomal or plasmid genes) (10).

Type fimbriae adhesions are important for uropathogenic *E. coli*'s (UPEC) pathogenicity because they allow the bacteria to stick to the uroepithelium. Researchers have tried to find the genes that make virulence factors like alginate (algD), which help bacteria attach to and colonize host cells (8, 9, 11). *Escherichia coli* inserts itself in a matrix of extracellular polymeric substances (EPS), which not only protects the bacterium from harmful ecological factors but also triggers infection. Moreover, it is the primary cause of recurrent urinary tract infections (12). Quorum sensing is a mechanism by which cells communicate with each other, leading to the accumulation of signaling molecules outside the cells and regulating the expression of specific genes. It is involved in the formation of biofilms (13).

Another thing is that *E. coli* are a genetically diverse group of bacteria. Some subgroups of these bacterial species have acquired genes that allow them to cause disease outside of or inside the intestines, such as the DNA fragment *TspE4.C2* gene. However, we mainly divided *E. coli* strains into groups (A0, A1, B1, and B2) based on the presence of genetic markers like this gene and other genes, *chuA* and *yjaA* (14). The study's goal is to find two genes (*algD* and *TspE4.C2*) that control how dangerous *Escherichia coli* is when it is taken from people in Baghdad who have urinary tract infections.

2. Materials and Methods

2.1. Sample Collection

We collected urine samples from patients with UTIs midstream in the morning using a sterile container with a screw cap. From 27 November 2022 to 11 January 2023, we collected 157 samples from the Medical City/educational laboratories, Baghdad Teaching Hospital, Imamin Al-Kadhimiya Hospital, and the Central Children's Hospital in Baghdad. We then inoculated the culture medium (MacConkey, blood, and EMB agar) with a calibration loop of urine samples and cultured it aerobically at 37°C for 18 hours. We also used the Vitek II compact system to identify *Escherichia coli*. After clinically isolating the bacteria from patients in Iraq, we isolated 75 isolates of *Escherichia coli*, and selected 11 isolates for molecular tests to identify the virulence factor genes.

2.2. The Congo red agar (CRA) method to find the production of biofilm

This approach describes a simple way to identify biofilm formation using the Congo Red Agar (CRA) medium. Black colonies with a dry crystalline structure were suggestive of the formation of biofilms, while pink colonies show that no bacterial biofilms had formed (15).

2.3. VITEK 2 compact to test antibiotic susceptibility

A sufficient number of pure colonies suspended in 3 ml of physiological saline solution are placed in two transparent plastic test tubes, and the device's model number is input into the Vitek 2 compact system's database. The isolated bacteria suspension is measured using the Vetik2 (Densichek) turbidity device; the turbidity must equal (0.50-0.63), or around 1.5 X 10^8 CFU/m. Transfer 145 µL from the first tube to the second for the antibiotic susceptibility test. A two-test tube cassette containing a bacterial suspension was placed in it in accordance with company instructions (biomerieux). The transport tube was severed by the device, and it

was placed on the incubator card and incubated at 37°C. For each card that was in the reader, the outcome was read, and a diagnostic report with an antibiotic susceptibility test was printed (16).

2.4. Extraction of Genomic DNA

Using Fungal/Bacterial/Yeast DNA MiniPrep™, Catalog No. D6005, the DNA was isolated from the samples of UTI patients.

2.5. Agarose gel electrophoresis of DNA

In order to identify the size of bands on the consequence of the PCR interface on the Agarose gel (0.8%), electrophoresis was used to define DNA fragments later in the extraction procedure or to distinguish the consequence of the interaction of PCR when standard DNA is present.

2.6. Measurement of DNA purity and concentration

Detection of DNA concentration and purity Place 1-2 µL of mini-prepped DNA onto the pedestal and use a Nano-Drop to quantify your samples. The purity is measured (a good purity ranges from 1.80 to 2.00). Rehash each example.

2.7. Gene amplification

2.7.1. The specific primer *algD* of gene

The sequence 5′- ATGCGAATCAGCATCTTTGGT -3′ was used as a forward primer with the percentage of GC (66.93%) at 60°C, while the sequence 5′-CTACCAGCAGATGCCCTCGGC-3′ was used as **a** reverse primer with the percentage of GC (76.69 %) at 70°C, and both sizes of product are 1310 base pairs.

2.7.2. The specific primer *TspE4.C2.* of gene

The sequence 5′-GAGTAATGTCGGGGCATTCA-3′ was used as a forward primer with the percentage of GC (68.25%) at 60°C, while the sequence 5′-CGCGCCAACAAAGTATTACG -3′ was used as a reverse primer with the percentage of GC (68.25 %) at 60°C, and both sizes of product are 152 base pairs.

In both genes, the perfect state has been recognized for (initial denaturation and annealing); later, a work of a few examinations to acquire this state, the temperature has altered over the crafted by (gradient PCR) at entirely examples to choose the ideal state and furthermore altered the amount of DNA template amongst (1.5-2 µl), wherever it is viewed as these two elements from significant variables in primer annealing and complement.

2.8. Maxime PCR PreMix Kit (i-Taq) 20µlrxn (Cat. No. 25025) and Diagnosis of Gene:

iNtRON's *Maxime*PCR PreMix Kit has not merely different types of PreMix Kit conferring to practice tenacity; nevertheless, it is a 2X Master combination solution. *Maxime* PCR Pre Mix Kit (*i*-Taq) is the product that is mixing all constituents: *i*-Taq DNA polymerase, dNTP mixture, reaction buffer, and so on—in one tube for 1 rxn PCR. This is a product that can give the finest finding through the maximum suitability system. The main purpose is that it has each product for PCR, so it can make PCR just supplement: a template DNA, primer set, and distilled water. Another purpose is to include a gel loading buffer for electrophoresis, which allows for gel loading without the need for any treatment. This method is suitable for processing numerous samples quickly and affordably.

In the Maxime PCR Pre Blend Unit (I-Taq), i-Taq DNA Polymerase, dNTP mixture, and reaction buffer are all mixed together in one tube for rxnPCR. You can combine this product with the finest and most accommodating system available. The subsequent explanation is that it contains a gel loading buffer for electrophoresis, which allows for gel loading with minimal treatment. The constituents of the Maxime PCR PreMix (i-Taq) kit are as follows: i-Taq DNA polymerase was in an amount of 5 U.µl-1, DNTPs were in an amount of 2.5 mM, the

reaction buffer was in an amount of 10X (1X), and the gel loading buffer was in an amount of 1X. While the combination of the specific contact for the identification gene was as follows: Taq PCR PreMix was an amount of 5 µl, the forward primer was an amount of 10 picomoles/µl (1 µl), the reverse primer was an amount of 10 picomoles/µl (1 µl), DNA was an amount of 1.5 µl, and distilled water was an amount of 16.5 µl; therefore, the final size equals 25 µl. The optimal state of detection was carried out as follows: The initial denaturation phase took place in 5 minutes at 94 degrees Celsius, involving one cycle. Denaturation (2), annealing, and extension (1) are each 45 seconds at 94°C, 55°C, and 72°C, respectively, with 35 cycles. While Extension: -2 phase in 7 min at 72°C with one cycle.

3. Results

3.1. Antibiotic resistance detection

An examination of the resistance of *Escherichia coli* bacteria to the following antibiotics was conducted: Ciprofloxacin, Amoxicillin, Nitrofurantoin, Cefotaxime, Gentamycin, Amikacin, Imipenem and meropenem Figure (1) showed that the above clinically isolated bacteria from Iraqi patients in Baghdad were resistant to multiple antibiotics as follows: Ciprofloxacin resistance 5(45.45%), Amoxicillin 1(9.09%), Nitrofurantoin 4 (36.36%), Cefotaxime 11(100%), Gentamycin 3(27.27%), Amikacin 9 (81.82%), Imipenem 1(9.09%) and Meropnem 2(18.185). In this study, results showed that all *E.coli* isolated were Cefotaxime resistance and many of the isolated bacteria were ciprofloxacin- and amikacin-resistant. The results of the *Escherichia coli* isolates were varied, as some of them were sensitive and others were resistant.

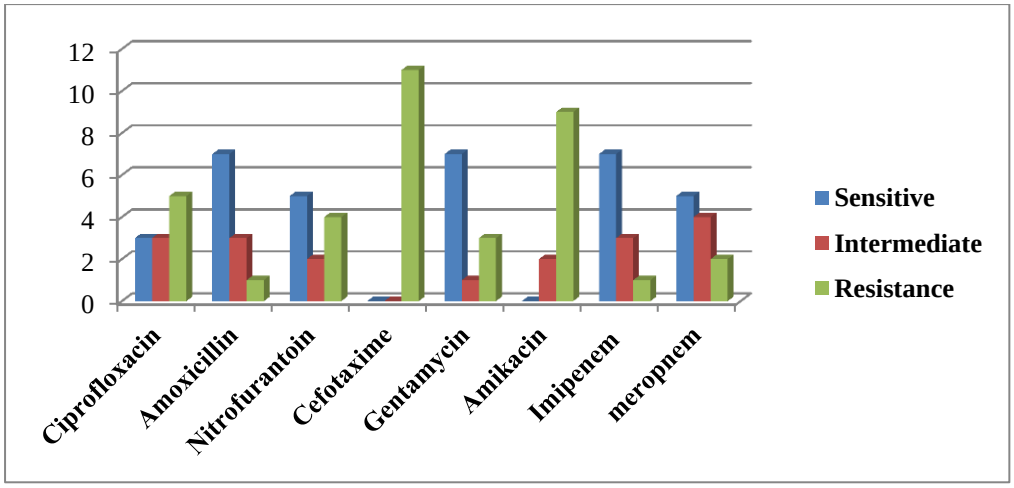

Figure 1. Antibiotics resistance of *Escherichia coli* in current study.

Bacterial resistance to antibiotics is the result of numerous factors working together. *Escherichia coli* acquires antibiotic resistance features through genetic processes such as horizontal gene transfer and clonal development of resistance isolates (17-20).

3.2. The capability to biofilm formation

The findings of the current study displayed moderate ability to form a biofilm. This result was detected by using the Congo red method to detect biofilm formation by *E. coli* bacteria clinically isolated, which showed moderate ability to form biofilm in all 11 (100%) selected isolates. This result is shown in **Table 1**.

Table 1. The ability of *E.coli* to biofilm formation.

NO.	Ability of Biofilm formation
7	Moderately
32	Moderately
67	Moderately
13	Moderately
80	Moderately
43	Moderately
14	Moderately
88	Moderately
65	Moderately
40	Moderately
51	Moderately

A variety of virulence factors present in *E. coli*, like adhesions, P-fimbriae, and additional mannose-resistant adhesions, offer an enhanced capacity for adaptation to novel environments and enable *E. coli* strains to cause a wide range of diseases (21, 22). These virulence traits are typically encoded on genetic components, and they can be deployed in various bacterial strains to produce original virulence factor permutations. Meanwhile, the bacterial extracellular matrix protects against antimicrobial drugs that might cause persistent infections and treatment issues, biofilm formation in *E. coli* is a significant factor in 60% of the severity of infection in people and antibiotic resistance (23, 24).

3.3. *algD* gene detection

The consequences of the molecular identification of the *algD* gene that add to the development of the alginate layer in *E.coli* were that nine isolates possessed *algD* gene and only two isolates did not possess the *algD* gene. These results are shown in **Table (2**) and **Figure (2**.)

3.4. Detection of *TspE4.C2* gene

Phylogenetic analysis was finished by PCR technique in light of the preserved gene *TspE4.C2* DNA fragment. The consequences of the recent study displayed the incidence of this gene in 10 of the 11 clinically isolated *Escherichia coli* isolates from Iraqi patients with urinary tract infection, as shown in **Table (2**) and **Figure (3)**.

Table 2. PCR product the size of band 1310 bp *algD* and band 152 bp *TspE4.C2* by electrophoresis on condition 2% agarose at 5 volt/cm^2.

No. of sample	ID	DNA Result	*algD* / 1310bp	*TspE4.C2* /152bp
7	32 (1)	+	+	+
32	43	+	+	+
67	88	+	+	+
13	14	+	+	+
80	65	+	+	+
43	10	+	+	+
14	32 (2)	+	-	+
88	40	+	+	-
65	51	+	+	+
40	67	+	+	+
51	12	+	-	+

algD gene was found in 9 (81.8 %) isolates

TspE4.C2 gene was presence in 10 (90.9 %) isolates

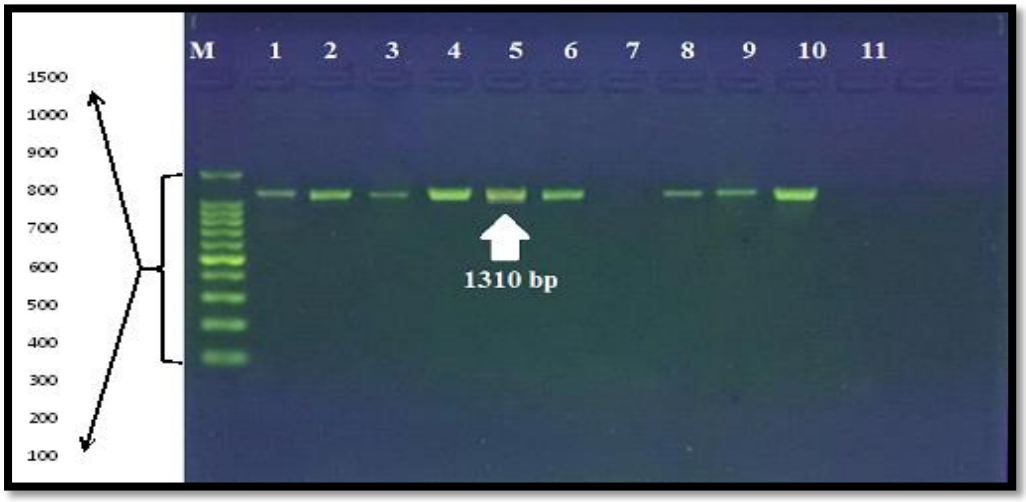

Figure 2. PCR product the size of band 1310 bp (*algD*). The product was electrophoresis on condition 2% agarose at 5 volt/cm^2.1x TBE buffer for 1hr. N: DNA ladder (100 base pair).

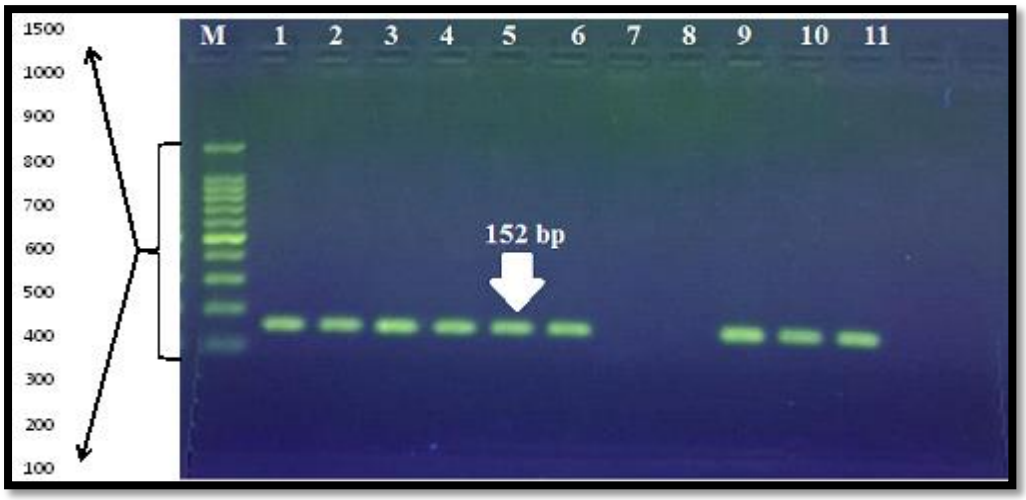

Figure 2. PCR product the size of band 152 bp (*TspE4.C2*). The product was electrophoresis on condition 2% agarose at 5 volt/cm^2. 1x TBE buffer for 1hr. was done for M: DNA ladder (100 base pair).

4.Discussion

Alginates are polysaccharides that are viscous. two bacterial taxa, *Pseudomonas* and Azotobacter, can produce alginate as an exopolysaccharide during biofilm formation. It stops both opsonic and non-opsonic phagocytosis, defending the bacterial cell from the host's inflammatory reaction. A big group of genes at position 34 min on the *P. aeruginosa* chromosomal map make up most of the enzymes that are used to make alginate polymers and change them. Numerous genes, whose products are located in the 9 to 13 min region of the chromosome, transcriptionally control this process. *AlgR* and *AlgB*, two homologous genes, *algP*, a histone-like protein, *algQ*, and *algU* are all included. The crucial *algD* gene encodes GDP-mannose dehydrogenase, and these genes influence its expression. Alginate is also known as MEP (mucoid exopolysaccharide). When cultivated on solid media in the lab, *P. aeruginosa* that has this polysaccharide overexpressed has a mucoid look (25). Previous studies demonstrated that *Pseudomonas aeruginosa* produced the *algD* gene (26). In the current study, *E. coli* production of *algD* was determined in most isolates taken clinically from Iraqi patients, which reinforces the idea of virulence factors for this bacteria acquired from other bacterial strains and types.

These findings were consistent with a recent study that suggested that the ability of *E.coli* to form biofilm, which is the first step in the emergence of antibiotic resistance and is

accomplished through the mechanisms of transport of antibiotics resistance genes and communication between cells as well as the mechanisms of the sensor (QS), is a major factor in the development of antibiotic resistance (27).

The new work uses PCR in the same manner as earlier studies to identify phylogenetic groups of *Escherichia coli.* As a single gene responsible for the phylogenetic grouping of *E. coli* bacteria (A, B_1, B_2 and D), the genomic DNA of isolated strains was amplified through triplicate PCR utilizing targeted primers marker, *TspE4.C2* (21, 24, 28). It is well known that there are several different phylo-groups of *Escherichia coli*, and that the strains within each phylo-group differ in terms of their ecological niches, life-history traits, and propensity to cause disease. As a result, classifying an *E. coli* strain according to one of the known phylo-groups might reveal a lot about it. *E. coli* strains can be classified into phylo-groups using a PCR-based technique that is based on the presence or lack of two genes (*chuA* and *yjaA*) and an unidentified DNA fragment *TspE4.C2* (29). It is worth noting that the results of our current study agree to some extent with the results of a previous study that confirmed that the *TspE4.C2* gene is responsible for the classification of *Escherichia* isolated from urine and other samples. The previous study confirmed that the presence of *the TspE4.C2* gene is a major cause of the pathogenicity and virulence of *Escherichia coli* type A in the urinary system and the occurrence of urinary tract infection (30).

5. Conclusions

We discovered that all *E. coli* isolates produced biofilms, with the majority of these isolates using the microtiter plate method. Additionally, we employed PCR amplification to find two genes (*algD* and *TspE4.C2*) responsible for several virulence factors in *Escherichia coli* clinical isolates from Iraqi patients in Baghdad.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Funding

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Ethical approval

The study was conducted in accordance with the ethical principles. It was carried out with patients verbal and analytical approval before the sample was taken. The University of Baghdad, College of Education for Pure Science (Ibn Al-Haitham), a local ethics committee, reviewed and approved the study protocol, subject information, and consent form on 26 September 2022, using the document number CSEC/0922/0089.

References

1. Gomes TA, Elias WP, Scaletsky IC, Guth BE, Rodrigues JF, Piazza RM, Martinez MB. Diarrheagenic *Escherichia coli*. Braz J Microbiol. 2016;47:3–30. https://doi.org/10.1016/j.bjm.2016.10.003.
2. Abdulsalam AA, Saleh MK. Effect of colicin from *E. coli* product on some species of gram-negative bacteria isolated from urinary tract infections. J Educ Sci Stud. 2021;3:17.

3. AL-Nasrawi MM, AL-Hashimy AB. Molecular study of some virulence genes of *Escherichia coli* isolated from women with urinary tract infection in Al-Najaf City. Iraqi J Biotechnol. 2020;3:19.
4. Sarowska J, Futoma-Koloch B, Jama-Kmiecik A, Frej-Madrzak M, Ksiazczyk M, Bugla-Ploskonska G, Choroszy-Krol I. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: recent reports. Gut Pathog. 2019;11:1–16. https://doi.org/10.1186/s13099-019-0290-0.
5. Mohammed RK, Ibrahim AA. Distribution of dfrA1 and cat1 antibiotic resistance genes in uropathogenic *Escherichia coli* isolated from teen pregnant women in Iraq. Iraqi J Sci. 2022;63:3340–3353. https://doi.org/10.24996/ijs.2022.63.12.5.
6. Hammadi AH, Yaseen NN, Al-Mathkhury HJ. Molecular detection of some β-lactamases genes in uropathogenic *Escherichia coli*. Iraqi J Sci. 2015;56(3A):1925–1931.
7. Pirko EY, Ali MR, MR N. The relationship between phylogenic typing and antimicrobial susceptibility patterns for *Escherichia coli* isolated from UTIs at many hospitals in Baghdad City. Nurs Natl Iraqi Spec. 2017;30(2):1–2. https://injns.uobaghdad.edu.iq/index.php/INJNS/article/view/275.
8. Altaee AJ, Aldabbagh SY. Molecular identification of virulence genes of *Pseudomonas aeruginosa* isolated from fish (*Cyprinus carpio*) in Mosul City. Iraqi J Vet Sci. 2022;36(4):953–958. https://doi.org/10.33899/ijvs.2022.132967.2207.
9. Mohamad LS. The effect of alcoholic extracts of *Zingiber officinale* anti-*E. coli* isolates isolated from urinary tract infection. Iraqi J Sci. 2019;60(10):2136–2140. https://doi.org/10.24996/ijs.2019.60.10.18.
10. Faisal ZG, Al-Bakiri GH, Attawi FA. Role of plasmids in antibiotic resistance for *E. coli* strains isolated from patients with urinary tract infection. J Educ Sci Stud. 2013;1(1).
11. Al-Ganimi A, Al-Khafaji J. Some virulence factors genes and phylogenic groups of uropathogenic Escherichia coli (UPEC) isolated from Karbala patients. Kerbala J Med. 2016;9(1):2376–2385.
12. Sharma G, Sharma S, Sharma P, Chandola D, Dang S, Gupta S, Gabrani R. *Escherichia coli* biofilm: development and therapeutic strategies. J Appl Microbiol. 2016;121(2):309–319. https://doi.org/10.1111/jam.13174.
13. Hamady DR, Ibrahim SK. The study on ability of Escherichia coli isolated from different clinical cases to biofilm formation and detection of csgD gene responsible for produce curli (fimbriae). Biochem Cell Arch. 2020;20(2): 5553-5557.
14. Hussain A, Saleh M. Determination of phylogenetic groups and antimicrobial susceptibility patterns for *Escherichia coli* isolated from patients with urinary tract infection. J Coll Educ Pure Sci. 2019;9(1):71–81. http://doi.org/10.32792/utq.jceps.09.01.08.
15. Sultan AM, Nabiel Y. Tube method and Congo red agar versus tissue culture plate method for detection of biofilm production by uropathogens isolated from midstream urine: which one could be better?. Afr J Clin Exp Microbiol. 2019;20(1):60–66. https://doi.org/10.4314/ajcem.v20i1.10.
16. Karagoz A, Artun FT, Özcan G, Melikoğlu G, Anıl S, Kültür Ş, Sütlüpınar N. *In vitro* evaluation of antioxidant activity of some plant methanol extracts. Biotechnol Biotechnol Equip. 2015;29(6):1184–1189. https://doi.org/10.1080/13102818.2015.1053143.
17. Aljeboury GH. A comparative study of amoxicillin sensitivity against *Escherichia coli* isolates isolated from urinary tract infections. Iraqi J Sci. 2017;58(2B):417–426. https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/6115.
18. Olowe OA, Adefioye OJ, Ajayeoba TA, Schiebel J, Weinreich J, Ali A, Schierack P. Phylogenetic grouping and biofilm formation of multidrug resistant *Escherichia coli* isolates from humans, animals and food products in South-West Nigeria. Sci Afr. 2019;6:e00158. https://doi.org/10.1016/j.sciaf.2019.e00158.
19. Ali SA, Al-Dahmoshi HO. Detection of efflux pumps gene and relation with antibiotics resistance in uropathogenic *Escherichia coli* (UPEC) isolated from patients with cystitis. Iraqi J Sci. 2022;63(5):2388–2397. https://doi.org/10.24996/ijs.2022.63.5.26.

20. Raoof MAJ, Fayidh MA. Molecular study to detect *blaTEM* and *blaCTX*-M genes in ESβL *Escherichia coli* and their antimicrobial resistance profile. J Phys Conf Ser. 2021;1879(2):022051. https://doi.org/10.1088/1742-6596/1879/2/022051.
21. Barzan M, Rad M, Tabar GH, Azizzadeh M. Phylogenetic analysis of *Escherichia coli* isolates from healthy and diarrhoeic calves in Mashhad, Iran. Bulg J Vet Med. 2017;20(1):59–67. http://dx.doi.org/10.15547/bjvm.952.
22. Raoof MAJ, Fayidh MA. Investigation of biofilm formation efficiency in ESβLs of pathogenic *Escherichia coli* isolates. Int J Drug Deliv Technol. 2022;12(2):695–700. http://dx.doi.org/10.25258/ijddt.12.2.41.
23. Alrekaby SM, Alwendawi SA. *In vitro* evaluation of inhibitory activity of enteric Bifidobacterium isolates against Shiga toxin producing *E. coli* (STEC) O157:H7. Iraqi J Sci. 2014;55(3A):999–1005. https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/11370.
24. Javed S, Mirani ZA, Pirzada ZA. Phylogenetic group B2 expressed significant biofilm formation among drug-resistant uropathogenic *Escherichia coli*. Libyan J Med. 2021;16(1):1927483. https://doi.org/10.1080/19932820.2021.1927483.
25. Ra'oof WAM. Distribution of *algD, lasB, pilB* and *nan1* genes among MDR clinical isolates of *Pseudomonas aeruginosa* in respect to site of infection. Tikrit Med J. 2011;17(2):1–9.
26. Salah Al-Deen H, Ibrahim SK. Study the ability of *Pseudomonas aeruginosa* isolated from different clinical cases to biofilm formation and detection of *algD* gene. Indian J Forensic Med Toxicol. 2022;16(1):1368–1374. https://doi.org/10.37506/ijfmt.v16i1.17683.
27. Abdul Razzaq AH, Al-Kubaisi AWB, Aziz LM, Hussain AS. Bacteriological and molecular study of *Pseudomonas aeruginosa* strains isolated from different clinical cases in Erbil and Kurkuk. Iraqi J Vet Med. 2018;41(2):124–130. https://doi.org/10.30539/iraqijvm.v41i2.61.
28. Ebraheem AA, Alwendawi SA. Screening for in vitro biofilm formation ability of locally isolated uropathogenic *Escherichia coli* (UPEC). Iraqi J Sci. 2015;56(2B):1310–1314. https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/10081.
29. Gordon DM, Clermont O, Tolley H, Denamur E. Assigning *Escherichia coli* strains to phylogenetic groups: multi-locus sequence typing versus the PCR triplex method. Environ Microbiol. 2008;10(10):2484–2496. https://doi.org/10.1111/j.1462-2920.2008.01669.x.
30. Abdul-Razzaq MS, Abdul-Lateef LA. Molecular phylogeny of *Escherichia coli* isolated from clinical samples in Hilla, Iraq. Afr J Biotechnol. 2011;10(70):15783–15787. https://doi.org/10.5897/AJB11.1019/