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Molecular Detection of Some Efflux Pumps Genes in *Escherichia Coli* Isolated from Patients with Urinary Tract Infections in Baqubah City, Iraq

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ABSTRACT

Background: Antibiotic resistance is among the major threats to health systems worldwide. Antibiotic resistance is often associated with efflux pumps, that distinguish many antibiotics are called Multidrug Resistance (MDR) efflux pumps.

Aim: investigate the prevalence of *Escherichia coli* bacteria that cause urinary tract infection by identifying the gene (*pap*E) and identifying the efflux pump genes (*acr*B, *Tol*C , *mdf*A) using the polymerase chain reaction.

Methods: 200 samples were obtained from the urine of patients with urinary tract infections from Baquba Teaching Hospital and from Al-Batoul Maternity Hospital in Diyala Governorate / Iraq during the period from January to April 2022. The nucleic acid DNA of the bacterial isolates under study was extracted and then the polymerase chain reaction (PCR) was performed through the use of specialized primers the gene (*acr*B, *mdf*A, *Tol*C) .

Results: The results of antibiotic sensitivity showed that all isolates were 100% resistant to each of Ampicillin and Augmentin, 98% Amikacin, 96% Tetracycline, 82% to Cefepime, and 50% to the results of the study showed that all bacterial isolates (20) isolates contain the gene (*mdf*A) 100%, and 19 isolates out of a total of 20 isolate targeting 95% contain both gene *Tol*C and *acr*B.

Conclusion: Most of the isolates of *Escherichia coli* bacteria under study, isolated from patients with urinary tract infections (UTIs) with multiple antibiotic resistance (MDR), and all bacterial isolates possessed the gene (*mdf*A) in a percentage of (100%), while the genes (*Tol*C, *acr*B) were found in a percentage(95%).

Keywords: *Escherichia coli*, Efflux pumps genes, MDR, antibiotic resistance.

INTRODUCTION

The intestinal family includes a large and heterogeneous group of Gram-negative bacteria that live in the intestinal tract of humans and animals naturally, with a sticky form and facultative anaerobic or aerobic [(1)] . This family includes several types, such as *Escherichia coli*, which live naturally in the intestines of humans and animals. Members of this family are considered the causative agent for several types of infections in humans, including urinary tract infections, respiratory infections, and bloodstream infections in hospitals or people who suffer from HIV [(2)].

Escherichia coli bacteria are Gram negative, facultative anaerobic or aerobic, rod shaped, non-spore-forming, producing pink colonies on the medium of MacConkey Agar As it produces circular colonies with distinct and convex edges and fermented for lactose, while it produces colonies with a distinctive green metallic color on Eosin Methylene Blue (EMB) medium, positive for the Iodole test, which is the preferred test that distinguishes it from other members of the intestinal family , and non-productive hydrogen sulfide gas H2S in the medium of triple iron agar (TSI), it grows rapidly as a result of its rapid cleavage and generation time of 20 minutes, and the optimum temperature for its growth is 37 °C [(3)] .

E. coli causes many common diseases, including meningitis, septicemia, and *E. coli*-associated diarrheal diseases. Urinary tract infections (UTIs) are one of the most common infectious diseases worldwide, with an estimated number of cases every year about 120-150 million cases. Depending on the anatomical location of the infection [(4)].

The excessive and improper use of antibiotics was one of the main reasons for the development of antibiotic resistance, as it is assumed that the rate of resistance spreads not only in hospitals but also in communities [(5)]. According to the type of effect of antibiotics, they are divided into two types: antibiotics with bactericidal effects, such as Penicillins, or antibiotics with bacteriostatic effects, such as Tetracycline antibiotics. Its effect is against a specific type of bacteria, while broad spectrum antibiotics have an effective effect against Gram-negative and Gram-positive bacteria [(6)].

Antibiotic resistance is often associated with a efflux pumps, as efflux pumps that characterize many antibiotics are called multidrug resistance (MDR) efflux pumps. There are genes that can encode the transfer of more than one substance of different chemical composition through a single efflux pump, as these are called multidrug resistance (MDR) efflux pumps, thus giving bacteria the characteristic of multiple resistance to antibiotics. Examples of MDR efflux pumps include AcrB in *Escherichia coli* and MexB in *Pseudomonas areoginosa*. Flow pumps can be classified into five families:, Small Multidrug Resistance Family (SMR) , ATP-Binding Cassete Family, (ABC) , Resistance-Nodulation-Division Family (RND) , Major Facilitator Super Family (MFS) , Multidrug and Toxic Efflux Family (MATE) . One of the most studied multi-antibiotic efflux systems is the AcrAB-TolC system in

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E. coli, this system is a tripartite complex consisting of AcrB, the cell's inner membrane protein that transports hydrophilic antigens, and AcrA, a protein found in the periplasmic space that covers the periphery of the bacterial cell and encoded by a gene called *acrA*, and an *acrB* protein that It is found in the inner membrane, while *TolC* is a funnel-like protein channel that is found in the outer membrane of the cell, as it works to transport most of the hydrophilic antigens [8] .

In the present study we aimed to investigate the prevalence of *Escherichia coli* bacteria that cause urinary tract infection by identifying the gene (*pap*E) and identifying the efflux pump genes (*acr*B , *Tol*C , *mdf*A).

MATERIAL AND METHODS

Specimens Collection

Two hundreds urine samples were collected from patients suffering from urinary tract infections (UTIs) of all ages and both sexes from Baquba General Hospital, Al-Batoul Teaching Hospital, and from educational laboratories after examining the specialist doctor during a period ranging from January to April 2022, which included the patients who were reviewed and hospitalized, then the samples were inoculated on different media and then incubated for 24 hours at a temperature of 37 °C for the purpose of isolating bacteria and their initial identification.

Bacterial isolation

The samples were cultured on MacConkey agar medium and methylene Eosin blue medium, then incubated for 24 hours at a temperature of 37 °C. Isolates were identified based on morphological trais such as shape, size, color, texture and edges of the bacterial colonies [9] . As well as the Biochemical diagnosis through several tests such as the catalase test,[10] the Urease enzyme production test [11] , Indole [12], the methyl red test[13] . and Voges – Proskaur Reagent [14] . In addition to the diagnosis using the Vitek 2 compact system.

Antibiotic susceptibility test

The susceptibility of isolates to antimicrobial agents was tested by using disks method of Mueller-Hinton agar based on CLSI (2021). The antibiotics in this study are Levofloxacin (5 µg), Ampicillin (10 µg) , Tetracyclin (30 µg) , Meropenem (10 µg) , Cefepime (30 µg) , Clavulanic acid/ Ampicillin (Augmentin) (30 µg) .

Molecular identification by *pap*E gene

Escherichia coli was diagnosed by using microscopic identification, cultural identification (**13**) as well as biochemical identification (**15**). To confirm the diagnosis at the species level of *Escherichia coli* at the molecular level by using a pair of DNA primers to amplify a specific *pap*E gene by polymerase chain reaction (PCR) , the nucleic acid (DNA) was extracted using the Nanodrop device, where the concentration of the nucleic acid ranged between (122-187) ng/µ1, while its purity was from (1,8-2,1) As the gene (*pap*E) was amplified to the size of (326) base pair.

Extraction of total DNA and PCR amplification

DNA was extracted from bacterial isolates of *Escherichia coli* ~~bacteria under study~~ , by using Geneaid Genomic DNA Purification , which were previously grown on nutrient broth medium, incubated for 24 hours at a temperature of 37 °C. the gene ***pap*E** (326bp) was amplified using the specific primer **F -** GCAACAGCAACGCTGGTTGCATCAT **R-** AGAGAGAGCCACTCTTATACGGAC The gene ***Tol*C** (1170 bp) was amplified using the specific primer **F-** (TGCTCCCCATTCTTCT TATCGGC) and **R-**(GCTCTTGCTTGGCGTTGTAC) and ***mdf*A** (523bp) gene using the specific primer **F-** (AAACCGGTCATTCATTAGGC) and **R –** (GTATTT GCGGCGGAACA) and ***acr*B** (761 bp) gene using the specific primer **F-** (GAA AGGCCAACAGC TTAAC) and **R –** (GAGCTGGAGTCAGGATCAAC) , the PCR Master Mix Components (Geneaid Accupower®Profi Taq PCR Premix) that Containing every other Component which necessary for Polymerase Chain Reaction (PCR) reaction, Taq DNA Polymerase , $MgCl_2$, Tris- HCl ,PH_9 , KCl as land sliding in Table (1) .

Table 1: Components for the polymerase chain reaction (PCR).

Components	Volume of reaction mixture for one tube (µL)
Master Mix PCR solution	5
Forward primer	1.5
Revers primer	1.5
Template DNA	3
Deionized Nuclease Free Water ~~14~~	14
Total volume	25

PCR tubes were prepared pursuant to the manufacturer's instructions. Then it was transferred to the centrifuge at a speed of 1300 revolutions per minute (rpm) for 3 minutes, after which it was placed in the PCR Thermocycler. PCR condition for *Tol*C gene included. The denaturation at 94°C for 5 min to *Tol*C followed by 30 cycles, Annealing at 65°C for 45 sec, Extension at 72°C for 2 min ,and final extension cycle for 7 min at 72°C . For the *mdf*A gene, the reaction conditions are included: denaturation at 95°C for 5min for *mdf*A followed by 30 cycles, Annealing at 50°C for 30 sec, Extension at 72°C for 2 min ,and Final Extension cycle for 7 min at 72°C . For the *acr*B gene, the reaction conditions are included : The denaturation at 94°C for 5min for *acr*B Followed by 30 cycles, Annealing at 65°C for 45 sec, Extension at 72°C for 2 min ,and Final Extension cycle for 7 min at 72°C . The PCR product

was transferred onto an agarose gel with a concentration 1% and then photographed by UV the image and documentation was captured by digital camera .

RESULTS

Isolation and Identification of *Escherichia coli*

Samples of urine were collected, as samples were collected from Baquba Teaching Hospital, Al-Batoul Maternity Hospital, and educational laboratories during the period from January to April 2022, from patients with urinary tract infections (UTI) of all ages.

90 samples, with a rate of (45%), showed a negative growth of the bacterial isolate, and 110 samples, with a rate of (55%), showed a positive growth of the bacterial isolate. Where 60 isolates were obtained, with a ratio of (%54,5) , belonging to *E. coli*, while 50 isolates showed (%45,4) belonging to species other than *E.coli* bacteria. The results of this study showed that *E.coli* bacteria are the most common Gram-negative bacteria that cause urinary tract infections.

Antibiotic susceptibility tests for bacterial isolates *E. coli*

All isolates of *Escherichia coli* under study were tested for antibiotic susceptibility pattern, was tested to 6 antibiotics are: Amikacin, Levofloxacin, Tetracycline, Ampicillin , Augmentin , Cefepime , Which are most commonly used to treat urinary tract infections (UTIs). The bacterial isolates were resistant to Ampicillin (100%), Tetracycline (96%), Cefepime (82%), Levofloxacin (54%), Augmentin (100%), Amikacin (98%).

Molecular diagnosis of *E. coli* using the *pap*E gene

The results of the current study showed that 26 (52%) bacterial isolates out of a total of 50 bacterial isolates carrying the (*pap*E) gene Which is considered the diagnostic gene for strain Uropathogenic *E. coli* (UPEC) depending on the primer used for the gene (*pap*E), as the gene has the size of (326) base pair compared with the DNA ladder that has bundles of known molecular size. As in Figure (1A,1B).

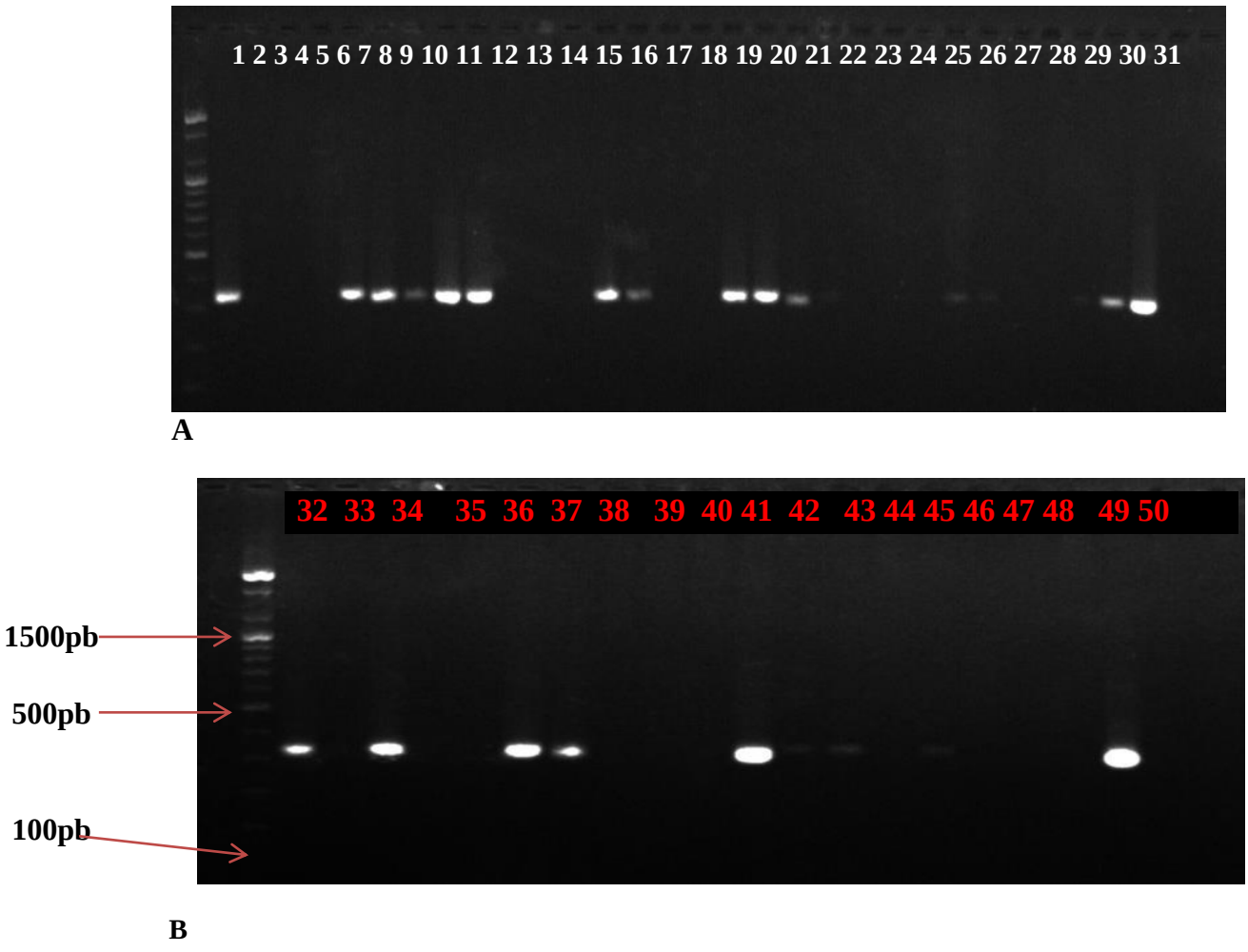

Fig 1 A, B : Electrophoresis of the *pap*E gene in *E.coli* bacteria by means of an agarose gel concentration of 1.5% containing 1µ of Ethidium bromide dye and by using a DNA ladder from (100-2000) base pairs, as the path is shown in the two figures The presence of the *pap*E gene (1,5,6,7,8,9,13,14,17,18,19,20,24,25,29, 30,31,32,34,37,38,42,43,44,46,50) .

Molecular detection of the DNA polymerase chain reaction product of the *Tol*C gene

The results of the molecular detection of the *Tol*C gene, which has a size of (1170) base pairs, using the PCR technique and using the Thremocycler, showed that 19 (95%) bacterial isolates possess the *Tol*C gene, as shown in the figure (2).

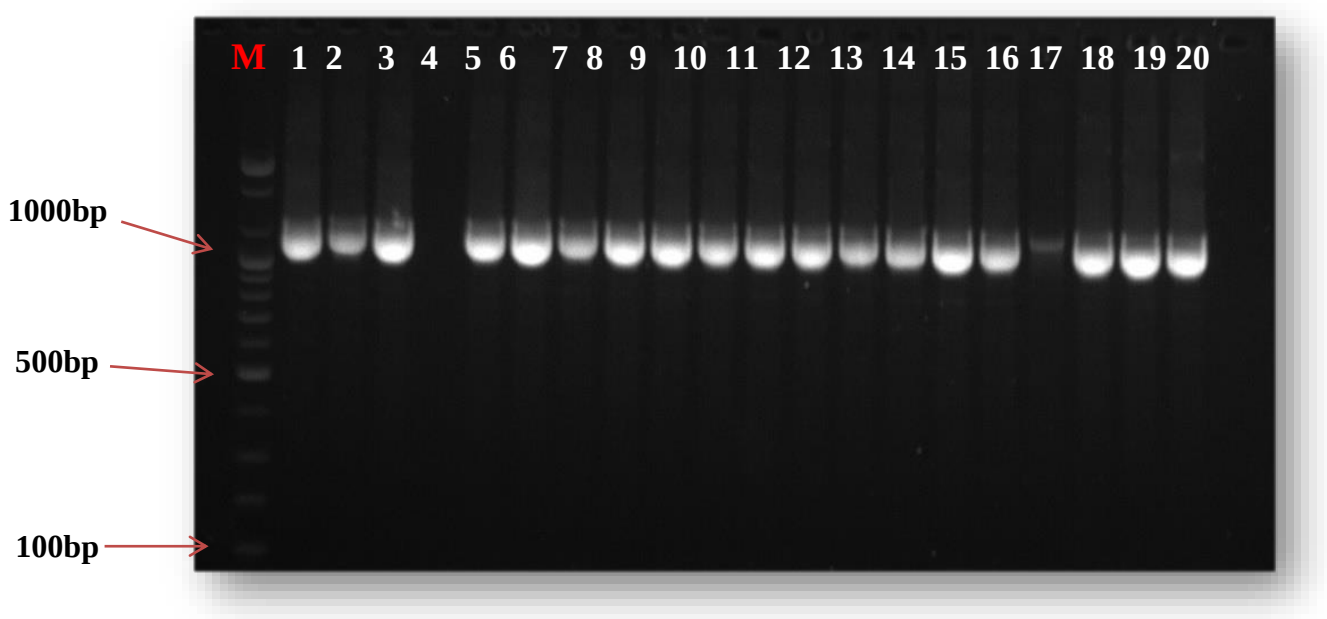

Fig 2: Electrophoresis of amplification products of the *Tol*C gene in 1% agarose gel and a voltage of 100 volts for an hour using a DNA Ladder (100bp-2000bp) as it appears in the M track and starts from 100 base pairs, the bands represent the gene packages of *E. coli* bacteria with a size of 1,170 bp.

Molecular detection of the DNA polymerase chain reaction product of the *mdf*A gene

The results of the molecular detection of the *mdf*A gene with a size of (523) base pairs in all bacterial isolates amounting to (20) isolates using the polymerase chain reaction (PCR) technique and using a Thermocycler showed that all (20) isolates and (100%) possess this gene. As shown in Figure (3).

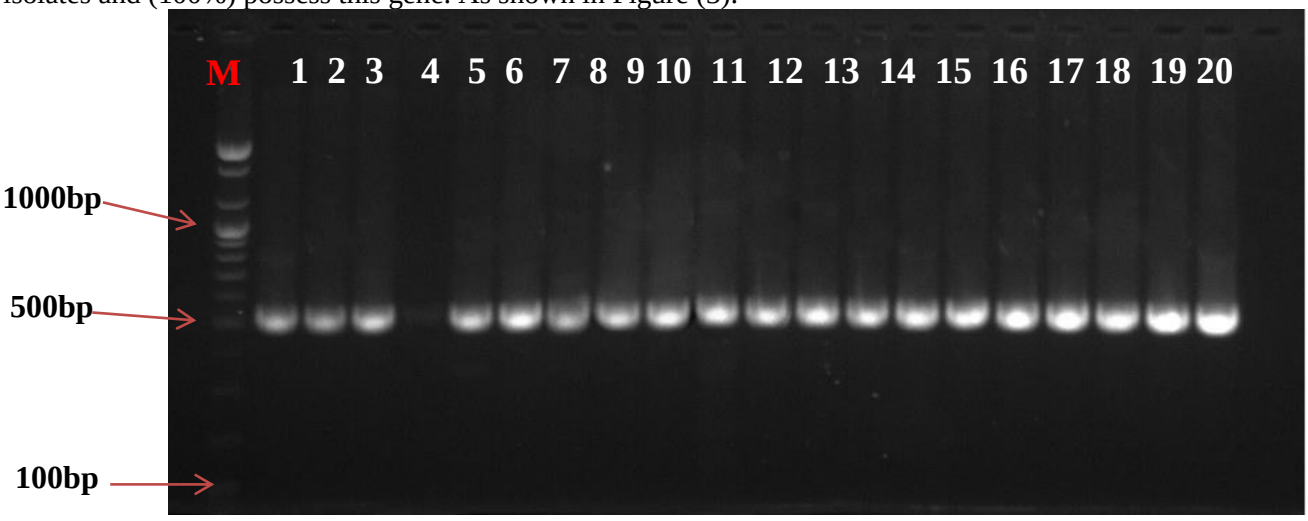

Fig 3: Electrophoresis of *mdf*A gene amplification products in 1% agarose gel and a voltage of 100 volts for an hour, using a DNA Ladder (100bp-2000bp), as it appears in the M pathway and starts from 100 base pairs, the bands represent the gene packages of *E. coli* bacteria with a size of 523 base pairs

Molecular detection of the DNA polymerase chain reaction product of the *acr*B gene

The results of the molecular detection of the *acr*B gene, which has a size of (761) base pairs, using the PCR technique and using a thermocycler, showed that the percentage of bacterial isolates that contain the *acr*B gene is (95%), i.e. (20/19) isolates, as shown in Figure (4**)** .

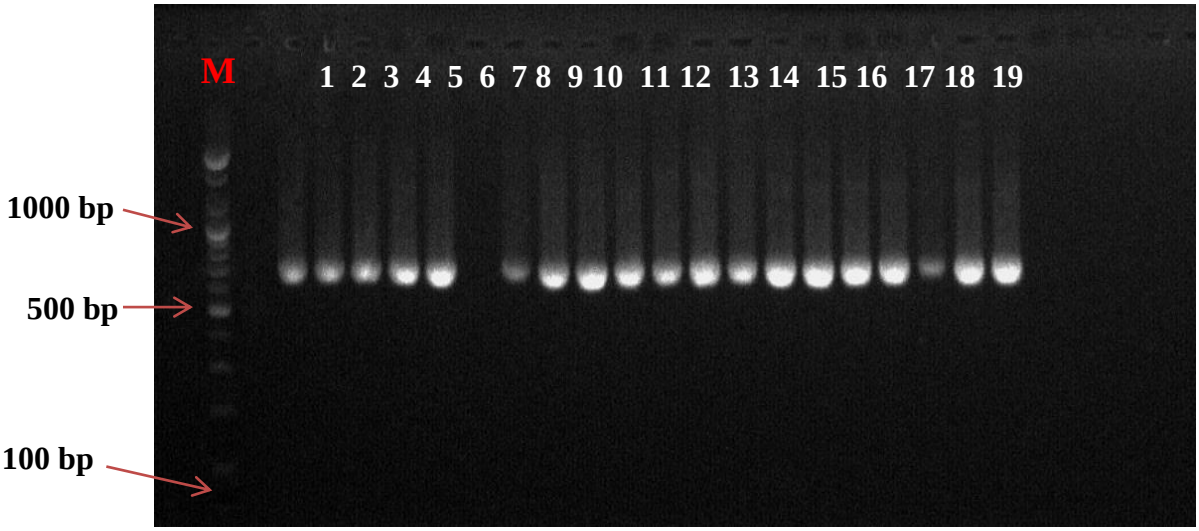

Fig 4: Electrophoresis of *acr*B gene amplification products in 1% agarose gel and a voltage of 100 volts for an hour, using a DNA Ladder (100bp-2000bp), as it appears in the M pathway and starts from 100 base pairs, the bands represent the gene packages of *E. coli* bacteria with a size of 761 base pairs.

DISCUSSION

The results of the current study were higher than the percentage obtained by the researcher [16] in Egypt as the percentage of Escherichia coli bacteria isolated from urine samples was (34%).

The results of the current study were less than the percentage obtained by the researcher [17]. The percentage of *E. coli* bacteria in urinary tract infections (UTIs) was (62%), which is close to the results of the current study. While the percentage of isolation of *Escherichia coli* in the current study was not consistent with the results obtained by the researcher [18] and it may be the cause of urinary tract infections in children under the age of two, as this indicates the presence of congenital defects in the urinary system or kidneys [19] . which reached the percentage of isolation from children and both sexes (64%), from females (80%) and from males [20] . The results of the current study were inconsistent with the results of the researcher [20] as the percentage of the presence of the *pap*E gene was (14%). The results reached by [18] the percentage of the presence of the *pap*E gene (37.5%), ie 15 isolates out of a total of 40 isolates. Also, the results of the study do not agree with the results reached by the researcher [21] as the percentage of bacterial isolates carrying the *pap*E gene was (42.6%) (64/26) (42.6%). There is diversity in the frequency of the *pap*E gene among UPEC breeds throughout the world and within the same geographic area.

The results of the current study regarding the antibiotic ampicillin were consistent with the findings of the researcher [22] in Diyala Governorate, where his study included 100 isolates of *E. coli* bacteria to verify their ability to resist antibiotics, and the results showed that the rate of resistance to the antibiotic is 100%. . The results of the current study also agreed with the results of the researcher [18] as the percentage of resistance to ampicillin was (97.5%). The antibiotic ampicillin targets the manufacture of the cell wall. While the results of the current study were inconsistent with the findings of the researcher [23] in Saudi Arabia where the rate of ampicillin resistance was (70%).

The results of the study regarding Amikacin were inconsistent with the findings of the researcher [24] as the rate of resistance to Amikacin was (61.7%). While it was in agreement with the results reached by the researcher [25] where the percentage of resistance to the antibiotic Amikacin was (93%).

The results of the current study for cefepime antagonist are consistent with the results reached by the researcher [18] where it was found that *Escherichia coli* was highly resistant to cefepime antagonist, with a resistance rate of (80%). While it did not agree with the results reached by the researcher [23] as the percentage of bacterial resistance to Cefepime was (20%).

The results of the current study with regard to the tetracycline antibiotic, the percentage of Escherichia coli resistance to this antibiotic was (96%), as the results of this study were not consistent with the results reached by the researcher [18] as the resistance rate to the tetracycline antibiotic in *Escherichia coli* was (57%). Also, the results of my current study were inconsistent with the results reached by the researcher [26] as the percentage of resistance to this antibiotic was (20.7%). coli resistance to tetracyclines is due to the presence of the efflux pump of the master facilitator superfamily (MFS) type efflux pumps drive tetracycline out of the cell.

The results of the current study were consistent with the findings of the researcher [27] regarding levofloxacin, as the resistance rate to this antibiotic was (50%) .

The results of the current study for the antibiotic Amoxicillin / Clavulanic acid (Augmentin) are (100)%. It was inconsistent with the findings of the researcher

[28] where the resistance rate to this antibiotic was (52%) , and the researcher indicated that the resistance to Augmentin was due to the beta-lactamase enzymes of the type TEM-1 and the enzymes SHV-5 and AMP that have vital role in Clavulanic acid resistance . The results of my current study were inconsistent with the findings of the researcher [25] as the percentage of resistance to this antibiotic was (83.3%).

The results of the current study were inconsistent with the results of the researcher [29] as the *Tol*C gene was present in *Escherichia coli* (70%). The results of the study were in agreement with many other researchers, such as the findings of the researcher [30] who discovered that the presence of the *Tol*C, *acr*A, and *acr*B genes in *Escherichia coli* is strongly associated with antibiotic resistance.

The results of the current study are not consistent with the findings of the researcher [31] in Egypt, where the percentage of the *mdf*A gene in *E. coli* isolates was (82.1%). Increased expression of efflux pump genes such as *mdf*A and *acr*A could lead to levofloxacin resistance in *Escherichia coli*, These findings contribute to the understanding of the molecular mechanisms of efflux pump systems and how they contribute to antibiotic resistance. Overexpression of the *mdf*A and *acr*AB-*Tol*C genes may contribute to antibiotic resistance to Flouroquinolones [32]

The results of the current study for the *acr*B gene were in agreement with the results reached by the researcher [33] as the percentage of the *acr*B gene in *E. coli* bacteria was (100%) . While the results of this study did not agree with the results reached by [34] in Hilla / Iraq if the proportion of this gene in *Escherichia coli* was (86%). This type of pump can pump large quantities of antibiotics as well as other substances such as metabolic wastes of bacteria, heavy elements, plant compound products, as well as organic matter. As a result, bacteria can confer acquired resistance to antibiotics in addition to the natural resistance to antibiotics [35].

On Conclusion, Most of the isolates of *Escherichia coli* bacteria under study, isolated from patients with urinary tract infections (UTIs) with multiple antibiotic resistance (MDR), and all bacterial isolates possessed the gene (*mdf*A) in a percentage of (100%), while the genes (*Tol*C,*acr*B) were found in a percentage(95%).

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REFERENCES

1- **Ramos-Vivas J, Chapartegui-González I , Fernández-Martínez M *et al.* (2019):** Biofilm formation by multidrug resistant Enterobacteriaceae strains isolated from solid organ transplant recipients. Scientific reports, 9(1): 1-10 .

2- **Hammoudi H, Ayoub M (2020):** The current burden of carbapenemases: Review of significant properties and dissemination among gram-negative bacteria. Antibiotics, 9(4): 186.

3- **Bourgeois A. Wierzba T, Walker R (2016):** Status of vaccine research and development for enterotoxigenic Escherichia coli. Vaccine, 34: 2880–2886.

4- **Gajdács M, Ábrók M , Lázár A *et al.* (2021):** Urinary Tract Infections in Elderly Patients: A 10-Year Study on Their Epidemiology and Antibiotic Resistance Based on the WHO Access, Watch, Reserve (AWaRe) Classification. Antibiotics, 10(9): 1098.

5 –**Esposito S , Gioia R , De Simone G *et al.* (2015): Bacterial** epidemiology and antimicrobial resistance in the surgery wards of a large teaching hospital in Southern Italy. Mediterranean Journal of Hematology and Infectious D4iseases, 7(1): e2015040

6- **Ali J, Rafiq Q , Ratcliffe E (2018):** Antimicrobial resistance mechanisms and potential synthetic treatments. Future science OA, 4(4): FSO290.

7- **Nishino K, Yamasaki S, Nakashima R *et al.*(2021):** Function and inhibitory mechanisms of multidrug efflux pumps . *https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8678522/*

8- **KornelsenV, Kumar A (2021):** Update on multidrug resistance efflux pumps in Acinetobacter spp. Antimicrob. https://journals.asm.org/doi/abs/10.1128/AAC.00514-21

9- **Wanger A. Chavez V. Huang R *et al.* (2017):** Microbiology and Molecular Diagnosis in Pathology. https://books.google.com/books?hl=ar&lr=&id=QpLUDQAAQBAJ&oi=fnd&pg=PP1&dq=Microbiology+and+Molecular+Diagnosis+in+Pathology&ots=QPA5yd2vnl&sig=LwFCcylzcgocWZBmbq0mY304pHs

10- **Cappuccino J, Welsh C (2018):** Microbiology: A Laboratory Manual https://lib.hpu.edu.vn/handle/123456789/29242

11- **Procop G. Church D. Hall G *et al.*(2017):** Koneman's Color Atlas and Textbook of Diagnostic Microbiology. http://dlib.sbmu.ac.ir/site/catalogue/166632

12-**Bott R (2014):** Brock Biology of Microorganisms. Igarss .https://doi.org/10.1007/s13398-014-0173-7.2

13- **Forbes B, Sah D, Weissfeld A(2007):** Bailey and Scott, S. Diagnostic Microbiology. https://books.google.com/books?hl=ar&lr=&id=D62ADQAAQBAJ&oi=fnd&pg=PP1&dq=Bailey+and+Scott,+S.+Diagnostic+Microbiology&ots=NBiG7OmZi7&sig=QllIDv88-h4fLmqZ3LSltwXJ5Yw

14- **Hemraj V,Diksh S, Avneet G (2013):** A review on commonly used biochemical test for bacteria. Innovare.J.Life.Scie.,1(1):1-7.

15- **Brown A, Smith H (2017):** Benson's Microbiological Applications, Laboratory Manual in General Microbiology. https://lib.hpu.edu.vn/handle/123456789/32674

16- **Abd El-Baky R, Ibrahim R , Mohamed D *et al.* (2020):** Prevalence of virulence genes and their association with antimicrobial resistance among pathogenic E. coli isolated from Egyptian patients with different clinical infections. *Infection and Drug Resistance*, *13*: 1221.

17- **Masoud S, Abd El-Baky R , Aly S *et al.* (2021):** Co-existence of certain ESBLs, MBLs and plasmid mediated

quinolone resistance genes among MDR E. coli isolated from different clinical specimens in Egypt. *Antibiotics*, *10*(7): 835.

18- Al-Zuhairi A (2021): Molecular characterization of multi-resistant *Escherichia coli* isolates isolated from Iraqi children with urinary tract infections, Master Thesis, College of Education for Pure Sciences / Diyala of University.

19- Okarska-Napierała M, Wasilewska A, Kuchar E (2017): Urinary tract infection in children: Diagnosis, treatment, imaging - Comparison of current guidelines. J Ped Urol., 13(10):567-573

20- Badger-Emeka, L. Kausar N, Estrella E *et al.* (2022): A Three-Year Look at the Phylogenetic Profile, Antimicrobial Resistance, and Associated Virulence Genes of Uropathogenic Escherichia coli. *Pathogens*, *11*(6): 631

21- Dhahi M (2020): Sequencing Analysis of Pyelonephritis-Associated Pili Gene of Uro-Pathogenic E. Coli Isolated in From Patients-Baghdad, Iraq. *Biomedical and Pharmacology Journal*, *13*(2): 1011-1019.

22- Dawood W (2020): Molecular and susceptibility Study of Antibiotic Resistance Genes in *E. coli* Isolated from Selected Iraqi Patients. Sys Rev Pharm., 11(9):214-223.

23- Aabed K, Moubayed N, Alzahrani S (2021): Antimicrobial resistance patterns among different Escherichia coli isolates in the Kingdom of Saudi Arabia. *Saudi journal of biological sciences*, *28*(7): 3776-3782.

24- Al-Tamemi D (2021): Phylogenetic study of multidrug resistance uropathogenic *Escherichia coli* in children of AL-KUT city, Master thesis, College of Medicine / University of wasit.

25 – Eezzeldin H, Badi S, Yousef B (2022): The Antibiotic Resistance and Multidrug Resistance Pattern of Uropathogenic Escherichia coli at Soba University Hospital: A Descriptive Retrospective Survey. *Sudan Journal of Medical Sciences*, *17*(1): 56-69.

26- Shrestha A, Shrestha R, Koju P, Tamrakar S *et al.* (2022): The Resistance Patterns in E. coli Isolates among Apparently Healthy Adults and Local Drivers of Antimicrobial Resistance: A Mixed-Methods Study in a Suburban Area of Nepal. *Tropical Medicine and Infectious Disease*, *7*(7): 133.

27- Ahmed A (2021): Molecular detection of some virulence genes in E. coli isolated from women with urinary tract infections, Master Thesis, College of Science/ University of Diyala .

28- Al-No'aemy H (2018): Molecular diversity of Escherichia coli isolated from different local sources and its relationship to antibiotic resistance. Master thesis, Diyala University, Diyala, Iraq.

29- Chowdhury N, Suhani S, Purkaystha A *et al.* (2019): Identification of AcrAB-TolC efflux pump genes and detection of mutation in efflux repressor AcrR from omeprazole responsive multidrug-resistant Escherichia coli isolates causing urinary tract infections. *https://journals.sagepub.com/doi/abs/10.1177/1178636119889629*

30- Abdali N, Parks J , Haynes K *et al.* (2017): Reviving antibiotics: efflux pump inhibitors that interact with AcrA, a membrane fusion protein of the AcrAB-TolC multidrug efflux pump. ACS infectious diseases, 3(1): 89-98.

31- Abdelhamid S , Abozahra R (2017): Expression of the fluoroquinolones efflux pump genes acrA and mdfA in urinary Escherichia coli isolates. *Polish Journal of Microbiology*, *66*(1):25

32- Jalal S, Ciofu O, Høiby N *et al.* (2000): Molecular mechanisms of fluoroquinolone resistance in Pseudomonas aeruginosa isolates from cystic fibrosis patients. Antimicrobial Agents and Chemotherapy, 44(3): 710-712.

33- Al-Saadi Z (2019): Phenotypic and molecular detection of pumps efflux systems *Escherichia coli* isolated from urinary tract infections , Master thesis, College of Education for Pure Sciences/Ibn Al-Haytham , University of Baghdad.

34- Ali S ,Al-Dahmoshi H (2022): Detection of Efflux Pumps Gene and Relation with Antibiotics Resistance in Uropathogenic Escherichia Coli (UPEC) Isolated from Patients with Cystitis. https://www.iasj.net/iasj/download/a87cac42cbb32662

35- Blanco P, Hernando-Amado S, Reales-Calderon J *et al.* (2016): Bacterial Multidrug Efflux Pumps: Much More than Antibiotic Resistance Determinants . Microorganisms, 4(14):2-19.