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Isolation and Diagnosis of Antibiotic-Resistant *Escherichia Coli* Bacteria from Urinary Tract Infection Patients in Mosul

Zainab Baqir Abbas*, Hasan Faisal Hussein

***Department of Biology, College of Education for Pure Sciences, University of Mosul, Mosul, Iraq**

*Corresponding Author: email: Zainab.22esp15@student.uomosul.edu.iq

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Keywords: Urinary Tract Infection, *Escherichia Coli*, Antibiotic Resistantce.

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Abstract:

In this study, 15 isolates of *Escherichia coli* were isolated from patients with urinary tract infections from Al-Khansa'a Teaching Hospital Nineveh/Iraq from May 2023 to July 2023. The isolates were studied and diagnosed biochemically. The result of microscopic tests showed isolates that are short Gram-negative bacilli, and the result of biochemical tests was that the isolates were positive for catalase, triple sugar iron, indole, methyl red, coagulation enzyme, fermentation of the following sugars maltose, sucrose, galactose and glucose, while *Escherichia coli* isolates showed negative results for urease, Voges-Proskauer, and citrate tests. The isolates also showed resistance to all antibiotics used, with high resistance to both ampicillin 100%, erythromycin 100% and cephalothin 100%. 60% for nalidixic, 53.33% for azithromycin and 53.33% levofloxacin and the lowest resistance rate shown by the isolates to gentamicin was 46.7%.

Keywords*:* Urinary Tract Infection, *Escherichia Coli*, Antibiotic Resistantce.

عزل وتشخيص بكتريا *Escherichia coli* المقاومة للمضادات الحيوية من مرضى التهابات المسالك البولية في مدينة الموصل

زينب باقر عباس*، حسن فيصل حسين

قسم علوم الحياة، كلية التربية للعلوم الصرفة، جامعة الموصل، الموصل، العراق

zainab.22esp15@student.uomosul.edu.iq, dr.hasankahya@uomosul.edu.iq

الخلاصة:

تم في هذه الدراسة عزل ١٥ عزلة من بكتريا الأشريكية القولونية *Escherichia coli* من مرضى التهابات المسالك البولية من مستشفى الخنساء التعليمي نينوى / العراق من أيار ٢٠٢٣ الى يوليو ٢٠٢٣. درست العزلات وشخصت كيموحيويا. أظهرت نتائج الاختبارات المجهرية أن العزلات عصيات قصيرة سلبية لصبغة كرام، كما أنها إيجابية لكل من اختبار الكتاليز Catalase، السكريات الثلاثية والحديد Triple Sugar Iron، الأندول Indol، المثيل الأحمر methyl Red، أنزيم تجلط الدم Coagulase وتخمر كل من السكريات الآتية مالتوز Maltose ، سكروز Sucrose ، كالاكتوز Galactose، كلوكوز Glucose، في حين أظهرت عزلات الأشريكية القولونية نتائج سلبية لاختبار اليوريز urease، فوكس-برسكاور Voges-Proskauer وسيتريت Citrate. وكذلك أظهرت العزلات مقاومة لجميع المضادات المستخدمة حيث وجد مقاومة عالية لكل من الأمبسلين ampicillin 100%,الأيرثرومايسين Erythromycin100% والسيفالوثين Cephalothin 100%. ٦٠٪ للنالدكسيك، 53.33% ازيثروميسين Azithromycin وبلغت مقاومة العزلات لليفلوفلوكساسين levofloxacin 53.33% واقل نسبة مقاومة أظهرتها العزلات للجنتامايسين Gentamycin بلغت 46.7%.

الكلمات المفتاحية: التهاب المسالك البولية، بكتريا الأشريكية القولونية، مقاومة المضادات.

1. Introduction:

Urinary tract infections (UTIs) are one of the most common infections in humans across all age groups, from newborns to the elderly [1]. An estimated 150 million infections occur annually worldwide [2]. It is the second most common bacterial infection in humans after respiratory infections. The infection occurs in the urinary tract, whether in the urethra (urethritis), bladder (cystitis) or kidneys (pyelonephritis). Upper urinary tract infections can be fatal if bacteria from an infected kidney travels into the bloodstream, a condition known as sepsis [3]. *Escherichia coli* is one of the most important members of the intestinal family and grows as a normal house in the gastrointestinal tract, and it is also considered an opportunistic pathogenic bacterium as it causes watery diarrhea as well as three diseases outside its natural

habitat such as meningitis in newborns, septicemia and urinary tract infections, as it causes about 90% of urinary tract infections. It causes about 90% of urinary tract infections and can be easily transmitted from the anal area to the urinary tract and bladder and is about 14 times more common in females than males due to the shorter urethra in females **[4]**. Some studies have shown that *Escherichia coli* resistance is not limited to a specific group of antibiotics such as Beta-lactam but may extend to several types of antibiotics (quinolones, aminoglycosides, sulfa compounds, macrolides, tetracyclines, and vancomycin). The characteristic of multiple resistance to antibiotics is an indicator of the seriousness of infection with these bacteria, when the infection occurs, the pathogenic bacteria acquire high resistance and sometimes it is silent **[5]**. *Escherichia coli* has multidrug resistance due to its ability to accumulate resistance genes mostly through horizontal gene transfer, such as the acquisition of genes encoding Beta-lactam enzymes conferring resistance to cephalosporins, carbapenemases conferring resistance to carbapenems, 16SrRNA methylase conferring resistance to aminoglycosides, and quinolone resistance genes by(PMQR) plasmid conferring resistance to quinolones **[6]**. The main objective of this study was to isolate and diagnose *Escherichia coli* bacteria from UTI patients attending Al-Khansa'a Teaching Hospital and to determine the sensivity and resistanance isolates to a group of selected antibiotics and compare them with previous studies Furthermore, it encourages the use of antibiotics that combat microorganisms and implement preventive measures to minimize the emergence of resistance to these microorganisms

2. Material and methods:

2.1 Bacterial sample collection: *Escherichia coli* isolates were collected from the urine of patients with urinary tract infections at Al-Khansa'a Teaching Hospital in Nineveh/Iraq from 1/8/2023 to 1/10/2023. Sterile collection containers were used to collect urine samples early in the morning. Then 100 microliters of urine were taken and cultured on blood agar and MacConkey agar and incubated at 37°C for 18-24 hours.

2.2 Culture diagnostics: The phenotypic characteristics of isolated bacterial colonies were studied after cultivation and purification of bacterial isolates on different culture media, such as MacConkey agar, Eosin methylene blue, and blood agar (India, Himedia). The study included aspects such as shape, size, texture, color, edges, and heights of isolated bacterial colonies **[7]**.

2.3 Microscopic diagnosis: Swabs of bacterial isolates previously grown on MacConkey agar for 18-24 hours were prepared by Gram technique and then examined under a compound light microscope using a 100x oil immersion lens **[4]**.

2.4 Biochemical test of *Escherichia coli* isolates: Biochemical tests were performed on bacterial isolates isolated from patients with urinary tract infections and included the following tests: Catalase [8], methyl red [9], Voges-Proskaur, citrate utilization [10], urease [11], Coagulation enzyme, Indole [12], triple sugar iron [13] and fermentation of various sugars [14].

2.4 Antibiotic Disk Susceptibility Test: Antibiotic susceptibility test was performed for ampicillin 25µg, azithromycin 15µg, cephalothin 30µg, erythromycin 10µg, gentamicin 10µg, levofloxacin 5µg, and nalidixic acid 5µg (Turkey, Bioanalyse). Using the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards [15]. Pure single colony isolates were selected using a sterile inoculation loop and inoculated into a 5ml tube of sterile water, and then the turbidity of the bacterial suspension was matched with the turbidity standard (McFarland's 0.5 standard). A sterile cotton swab was dipped into the bacterial suspension to thoroughly culture the plates containing Muller-Hinton agar (India, Himedia). Using sterile forceps, place the tablets on the surface. The plates were then incubated for 24 hours at 35°C. The susceptibility pattern was then determined by measuring the zones of inhibition in millimeters [16].

3. Results:

3.1 Phenotype diagnostics: The bacterial isolates were diagnosed based on their morphological characteristics observed after cultivation in different media. The results revealed that Escherichia coli is capable of fermenting lactose, as it formed smooth, shiny, pink colonies on MacConkey agar, indicating its ability to ferment lactose and produce acids. The isolates also produced metallic green colonies on Eosin Methylene Blue (EMB) agar, which is a characteristic feature of this bacterium. On blood agar, the colonies appeared round, shiny, and transparent, reflecting the typical growth characteristics of E. coli on this medium. These morphological features serve as an important basis for the preliminary diagnosis of these bacterial isolates in the laboratory **Figure 1**.

3.2 Microscopic diagnostics: After a swab was taken from a single colony on an 18-24 hour old MacConkey agre and stained with gram dye, the bacteria appeared as short bacilli negative for Gram dye and non-spore-forming **Figure 2**, these results agree with the results of the researcher [4].

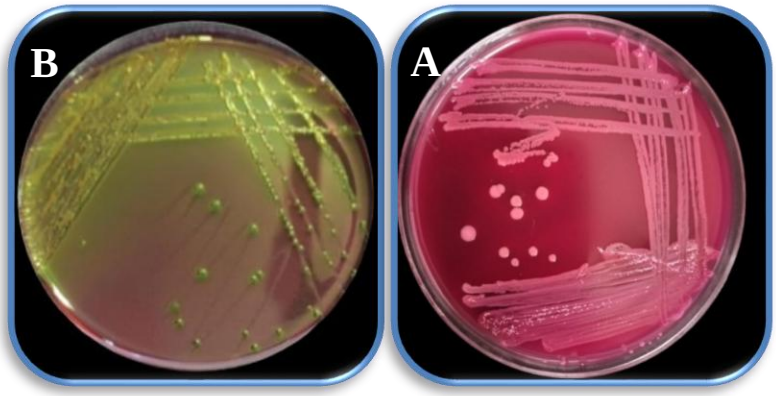

Figure 1: *Escherichia coli* colonies (Phenotypic diagnosis)
A: Colonies of pink *Escherichia coli* bacteria on MacConkey agar
B: Green *Escherichia coli* colonies with a metallic luster on Eosin methylene blue aga

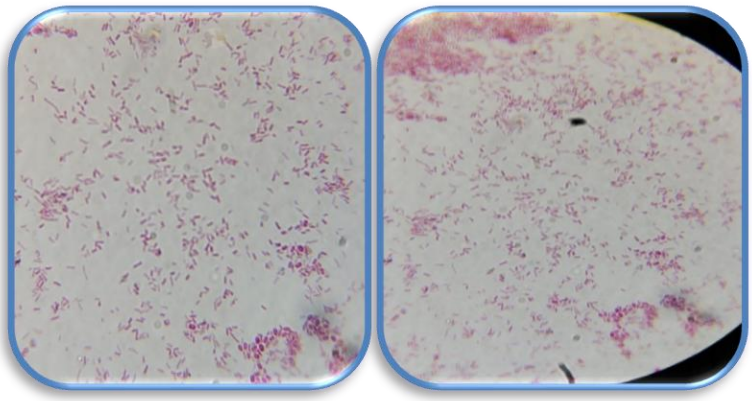

Figure 2: Microscope image for *Escherichia coli* bacteria stained with Gram stain

3.3 Biochemical diagnostics: Biochemical tests were performed on all bacterial isolates and the results are shown in Table 1 and Figure 3 It was found that all isolates were positive for the catalase test, it was negative for the urease test as there was no change in the color of the medium, isolates gave a positive result for the fermentation test of sugars such as sucrose, galactose, maltose and glucose and positive for the triple sugar iron test.

Table1: Biochemical Teasts Diagnosis of *Escherichia coli*

Results	Teasts
Catalase	+
Urease	-
Sucrose	+
Galactose	+
Maltose	+
Glucose	+
Triple sugar iron	+
Indole	+
Methyl red	+
Voges-Proskauer	-
Citrate	-
Coagulase enzyme	+

As for the results of the tests of the IMVIC group, the bacteria were positive for both the indole test. A positive result for the methyl red test, where it was observed that the color turned red,. As for the vogs- Perskauer test, the isolates gave a negative result where the medium appeared yellow and brown. For the citrate test, the medium did not turn blue-green, the isolates gave a negative. The isolates were positive for the coagulation enzyme test as a result of a clump appearing on the plasma droplet placed on a sterile glass slide within 10 seconds when a pure colony of bacteria was placed on It.

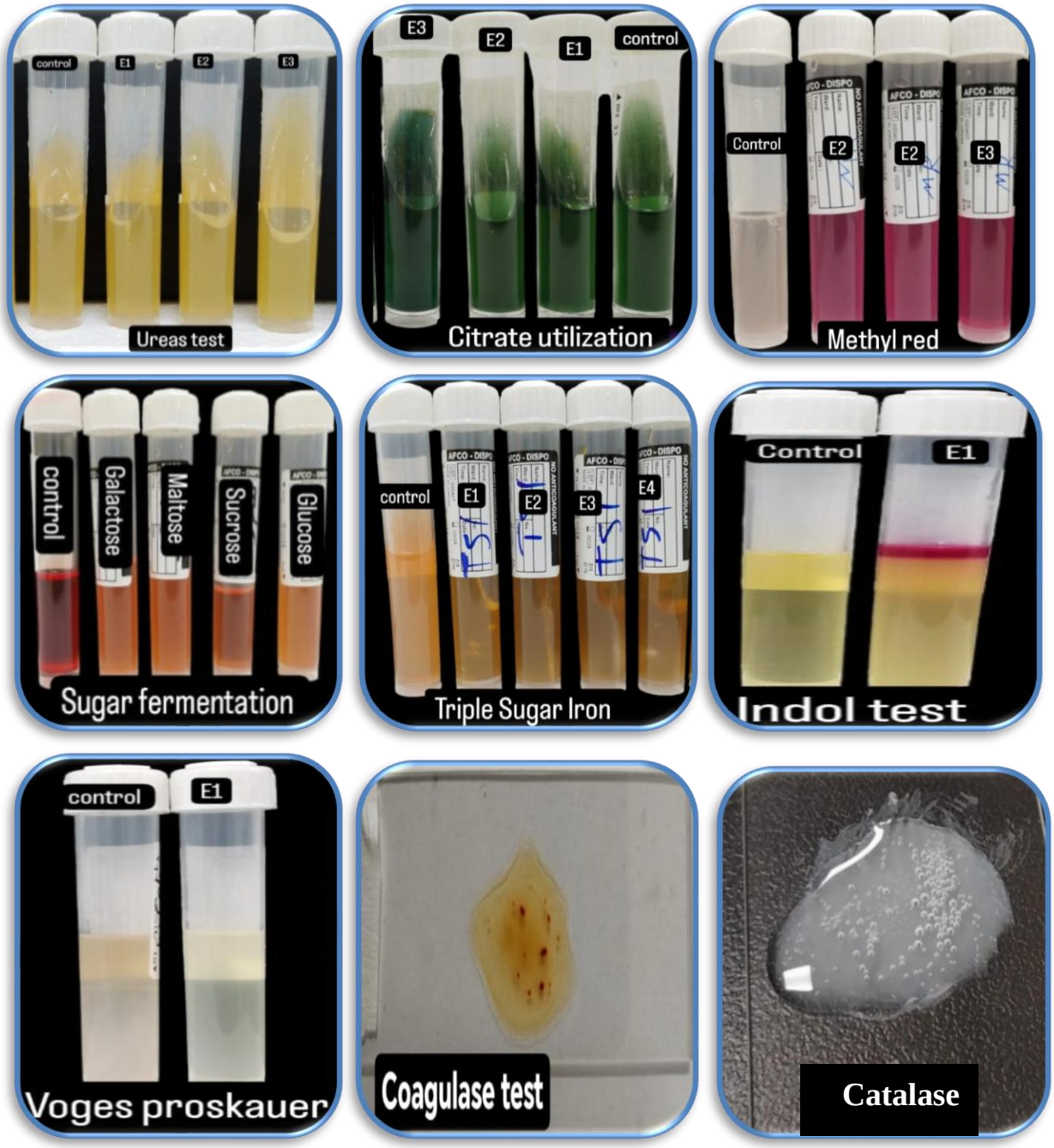

Figure 3: Biochemical tests for *E. coli* bacterial isolates

3.4 Antibiotic Disk Susceptibility Test: The results of the antibiotic susceptibility testing of bacterial isolates, interpreted according to the Clinical and Laboratory Standards Institute (CLSI) 2024 guidelines **[15]**, showed that all *Escherichia coli* isolates exhibited multi-drug resistance. The findings revealed that 15 isolates (100%) were resistant to the antibiotic Ampicillin, and 15 isolates (100%) were resistant to the antibiotic Cephalothin. Additionally, 15 isolates (100%) were resistant to the antibiotic Erythromycin, and 9 isolates (60%) were resistant to Nalidixic Acid. Twenty-one isolates were resistant to the antibiotic Azithromycin, while 8 isolates(53.33%) were resistant to Levofloxacin. The lowest resistance rate was observed for the antibiotic Gentamicin, with a resistance rate of 7(46.7%), **Table 2** and **Figure 4**.

Table 2: Antibiotic sensitivity pattern of *Escherichia coli* isolates.

Antibiotic	Code	concentration	Resistant n(%)	Intermediate n(%)	Sensitivity n(%)
Ampicillin	AM	25	15(100%)	0	0
Azithromycin	AZM	15	8(53.33%)	0	7(46.67%)
Cephalothin	KF	30	15(100%)	0	0
Erythromycine	E	10	15(100%)	0	0
Gentamycin	CN	10	7(46. 7%)	2(13.3%)	6(40%)
Levofloxacin	LEV	5	8(53.33%)	2(13.3%)	5(33.33%)
Nalidixic acide	NA	30	9(60%)	4(26.7%)	2(13.3%)

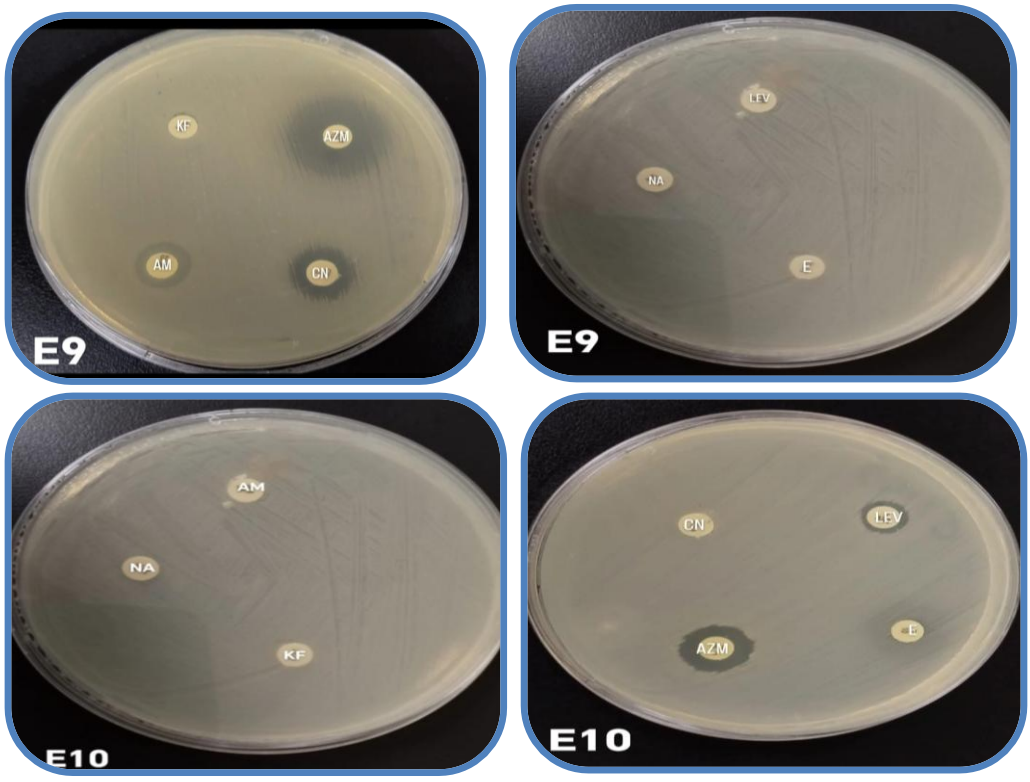

Figure 4: Testing the sensitivity of *E. coli* isolates to antibiotics.

4. Discussion

Escherichia coli bacteria are capable of fermenting lactose, leading to the formation of pink colonies on MacConkey agar, which contains bile salts and crystal violet dye. This composition allows the growth of Gram-negative bacteria, such as members of the Enterobacteriaceae family, while inhibiting the growth of Gram-positive bacteria **[17]**. On the other hand, the shiny green colonies on Eosin-Methylene Blue agar result from the presence of eosin and methylene blue dyes that precipitate in the acidic medium after binding with certain substances. This reaction gives the colonies a metallic green sheen, indicating that the bacteria have produced organic acids as a result of fermenting lactose and sucrose **[12]**. Garding the biochemical tests, all isolates were positive for the catalase test, indicating their ability to break down hydrogen peroxide into water. As for the urea test, the results were negative, as the isolates were unable to utilize urea due to the absence of the urease enzyme. The isolates were also positive for the test of the Triple sugar iron, glucose, maltose, galactose, and sucrose, as they demonstrated the ability to ferment these sugars. Regarding the tests of the enteric group, which distinguish *E. coli* from other genera of the Enterobacteriaceae family, the results were positive for the indole test, where a red ring appeared on the surface of the medium in the alcohol layer isoamyl, due to the breakdown of the amino acid tryptophan by the enzyme tryptophane. The results were also positive for the methyl red test, where the color changed to red due to the bacteria's fermentation of glucose. On the other hand, the result was negative for the Voges-Proskauer test, indicating that the isolates could not convert glucose into acetyl methyl carbonyl. Additionally, the result was negative for the citrate test, as the isolates did not use citrate as a sole carbon source, and therefore, the color of the medium did not turn bluish-green due to the absence of the citrate permease enzyme **[10,11,12]**.

As resistance to ampicillin Beta -lactam antibiotics reached 100%, consistent with the results of the researcher Al-Saadi which reported 100% resistance of isolates to ampicillin, Several studies have shown that 90% of *E. coli* are resistant to beta-lactam antibiotics due to the secretion of Beta-lactamase **[18]**. The bacterial isolates showed high resistance to the Cephalothin (100%) and were close to the results obtained by the researcher Ait-Mimoune *et. al,*. where the percentage of resistance of isolates to the antibiotic Cephalothin amounted to (85%) while there are high levels of resistance to ampicillin, tetracycline and cephalothin among *E. coli* and this may be related to the high rate of prescribing these antibiotics in the treatment of urinary tract infections and the excessive and inappropriate use of broad-spectrum antibiotics by patients is the main reason for the emergence of resistance caused by bacterial mutations **[19]**. While the results of the current study differed with the results of study

Hashemian *et.al,*. in Iran where the highest percentage of antibiotic resistance of isolates was for cephalothin (77.1%) and ampicillin (78.8%) **[20]**.

Gentamicin resistance in this study amounted to 46.7%. The result was close to the previous study Ismael *et. al,*. where the percentage of resistance of isolates in his study was 40% **[21]**. while it differed from the results obtained by searcher Ramirez-Castillo *et. al,*. who indicated that 28.2% of isolates are resistant to gentamicin and that the mechanisms of resistance to aminoglycosides are mutation Enzymatic site and ribosome site modification and the accumulation of decreasing intracellular antibiotic accumulation by altering the permeability of the outer membrane **[22]**. The isolates showed resistance to nalidixic and levofloxacin in our study 60%, 53.33% and was close to the study results Ghotaslou *et. al,*. (73%, 58%) **[23]**. Zaman *et. al,*. showed that *Escherichia coli* inhibits DNA synthesis through inhibition of the DNA gyrase enzyme or as a result of genetic mutations in it, which leads to resistance to antibiotics belonging to the quinolines group **[24]**. The isolates showed high resistance to erythromycin, reaching 100%, and agreed with the results of the Al-Saadi who found that 98% of the isolates were resistant to this antibiotic, and the resistance of the isolates to azithromycin amounted to 53.33, and differed from what Salhy found, as he indicated that 30.3% of the isolates were resistant to azithromycin **[17, 25]**. Zaman *et. al,*. pointed out that some of the reasons for the resistance of *Escherichia coli* isolates to macrolides are hydrolysis, which results in the degradation of the antibiotic and inhibition of its effectiveness, the production of glogsylation and phosphorylation enzymes that inhibit the action of the antibiotic and the acquisition of flow systems as well as a change in the targeting locations **[24]**.

5. Conclusions

In recent years, antibiotic resistance has become a global threat to health systems around the world, and E. coli poses the greatest threat to human health due to its increasing resistance to antibiotics, and all isolates in our study showed multiple resistance to the antibiotics used Ampicillin, Erythromycin, Cephalothin, Azithromycin. Gentamicin, levofloxacin and Nalidixic acid, as a result of the diverse resistance mechanisms exhibited by *E. coli* as a result of the overuse and misuse of antibiotics. and misuse, which may lead to serious health effects.

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