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DIAGNOSIS OF *E. coli* WHICH IS ISOLATED FROM URINARY SYSTEM AND ANTIBIOTIC RESISTANCE OF BETA-LACTAM IN IRAQ

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April 2022

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

DIAGNOSIS OF *E. coli* WHICH IS ISOLATED FROM URINARY SYSTEM AND ANTIBIOTIC RESISTANCE OF BETA-LACTAM IN IRAQ

Nooruldeen Khalid Abed AL-ZOBAIE

Master of Science in Biology

Advisor: Asst. Prof. Dr. Emin BOZKURT

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This study aims to diagnose *Escherichia coli* bacteria isolated from urinary tract infections and their relationship to antibiotics. 50 clinical samples were collected from urine of patients with urinary tract infections and under the supervision of specialist doctors from several hospitals in Iraq, including Yarmouk Teaching Hospital, Al-Tifl Teaching Hospital and Abu Ghraib General Hospital in four months. Bacterial isolates were evaluated for sensitivity to 6 different antibiotics. As all bacterial isolates were multi-resistant to antibiotics, these tested antibiotics were ineffective.

Antibiotic resistance in *E. coli* isolated from urinary tract infections were found as 50 (100%) samples carbenicillin-resistant, 49 (98%) samples erythromycin-resistant, 26 (52%) samples penicillin G resistant, 43 (86%) samples ceftazidime resistant, 16 (32%) samples gentamicin resistant and 34 (68%) samples tetracycline resistant. The results showed that 45 (90%) bacterial isolates were biofilm-forming. Three (6%) of isolates were showed strong biofilm formation, when five (10%) of isolates showed medium degree biofilm formation. And 37 (74%) of isolates showed weak biofilm formation.

2022, 61 pages

Keywords: Urinary system, Antibiotic, *E. coli*, Resistance, Beta-lactam

ÖZET

IRAK'TA ÜRİNER SİSTEMDEN İZOLE EDİLEN *E. coli* TANISI VE BETA-LAKTAM ANTİBİYOTİK DİRENCİ

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Bu çalışma, idrar yolu enfeksiyonlarından izole edilen *Escherichia coli* bakterilerinin ve antibiyotiklerle ilişkisinin teşhisini amaçlamaktadır. Irak'ta Yarmouk Eğitim Hastanesi, Al-Tifl Eğitim Hastanesi ve Abu Ghraib General Hospital dahil olmak üzere çeşitli hastanelerden dört ay boyunca idrar yolu enfeksiyonu olan hastaların idrarından ve uzman doktorların gözetiminde 50 klinik örnek toplandı. Bakteriyel izolatlar 6 farklı antibiyotiğe duyarlılık açısından değerlendirildi. Tüm bakteri izolatları antibiyotiklere karşı çoklu dirençli olduğundan, test edilen bu antibiyotikler etkisiz olarak bulundu.

İdrar yolları enfeksiyonlarından izole edilen *E.* coli'lerde antibiyotik direnci 50 örnekte (%100) carbesilin direnci, 49 (%98) örnekte eritromisin direnci, 26 (%52) örnekte penisilin G direnci, 43 (%86) örnekte seftazidim direnci, 16 (%32) örnekte gentamisin direnci ve 34 (%68) örnekte tetrasiklin direnci olarak bulundu. Sonuçlar, 45 (%90) bakteri izolatının biyofilm oluşturduğunu gösterdi. 5 (%10) izolat orta derece biofilm oluştururken, üç (%6) izolatın güçlü bir biyofilm oluşturduğu gösterilmiştir. Ve 37 (%74) izolat zayıf biyofilm oluşumu göstermiştir.

2022, 61 sayfa

Anahtar Kelimeler: Üriner sistem, Antibiyotik, *E. coli*, Direnç, Beta-laktam

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Nooruldeen Khalid Abed AL-ZOBAIE

Çankırı-2022

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LIST OF SYMBOLS

α	Alfa
β	Beta
°	Degree
mL	mililiter
mm	milimeter
-	Negative
%	Percentage
+	Positive
pH	Power of Hydrogen
rpm	Round per Minute

LIST OF ABBREVIATIONS

ABC	ATP-Binding Cassete Family
BHI	Brain Heart Infusion
DAEC	Diffusely Adhering *E. coli*
EAEC	Enteroaggregative *E. coli*
EAST	Enteroaggregative Heat-stable Toxin
EHEC	Enterohemorhagic *E. coli*
EIEC	Enteroinvasive *E. coli*
EPEC	Enteropathogenic *E. coli*
ESBL	Extended Spectrum beta-lactamases
ETEC	Enterotoxigenic *E. coli*
LPS	Lipopolysaccharide
MATE	Multidrug and Toxic Efflux Family
MDR	Multi-drug Resistant
MFS	Major Facilitator Super Family
OMP	Outer membrane Protein
PBP	Penicillin Binding Protein
PDR	Pan-drug Resistant
QS	Sensing Quarum
RND	Resistance-Nodulation-Division Family
SMR	Small Multidrug Resistance Family
STEC	Shiga-Toxin Producing *E. coli*
TPN	Total Parenteral Nutrition
UPEC	Uro Pathogenic *E. coli*
WHO	World Health Organization
XDR	Extensively-drug Resistant

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1. INTRODUCTION

Escherichia coli is a member of the Gram-negative intestinal family of rod-shaped motile or non-motile, aerobic or non-aerobic, facultative. It ferments lactose, rhamnose and sorbitol and produces an enzyme. The optimum temperature for its growth is (36-37 °C). It is positive for catalase and negative for indole-producing oxidase, when it is non-citrate consuming test positive for methyl red and negative for fuchs procure (Hemraj *et al.* 2013).

It lives naturally in intestines of both humans and animals. They are opportunistic bacteria that cause more than one disease such as diarrhea for a relatively short period and also meningitis and septicemia. It is one of the most common variations of bacteria the cause of cystitis is in the urinary tract. It causes nearly 85% of urinary tract infections around the world including America and Germany. This bacteria's pathogenicity is attributed to its abundance of virulence factors, including iron chelates, and the presence of antigenic features like as flagella, capsular, and lipopolysaccharides on the bacteria's surface qualities due to its production of flagellated antigen, somatic antigen and capsular antigen. It also possesses cilia which helps them to stick to the tissues of the host, giving them the ability to form a biofilm (Hadi *et al*. 2014).

The urinary system is one of the important organs in the body, and any defect in the function of this system can be rest of the members cellular, and works to add toxic wastes from the blood in addition to its role in regulating the blood, and the concentration of the ions in it and its participation in the formation of red blood cells and the representation of vitamin D (Gordon *et al*. 2003).

A major cause of urinary tract infection is thought to be *E. coli* a member of the Enterobacteriaceae family of bacteria. Most people, regardless of their gender or age group, have urinary tract infections (UTIs), which are widespread in hospitals and the community (Alqasim *et al.* 2018). The uropathogenic *E. coli* (UPEC) group is capable of producing up to 90% of community-acquired UTIs, which is well-known as the primary causative agent of (UTIs) (Zhang and Foxman 2003, Kucheria *et al*. 2005).

Now, *E. coli* has developed and evolved resistance to a variety of drugs (Shrestha *et al*. 2019). As a consequence of its multi-resistant phenotype *E. coli* is unable to be effectively treated with antimicrobials, such as beta-lactams and fluoroquinolones.

The aim of this study is to diagnose *E. coli* which is isolated from urinary tract samples taken urinary tract infections (UTIs) patients from multiple Iraqi hospitals and also determine of its resistance to antibiotics especially the β-lactamase group of *E. coli*.

2. LITERATURE REVIEW

2.1 Enterobactriaceae

Intestinal bacteria are a big and diverse group of bacteria that naturally inhabit in the intestines of humans and animals. They might be rod-shaped, gram-negative bacteria that are aerobic or facultatively anaerobic. Its members can infect humans and animals with a variety of diseases, including wounds, hospital-acquired injuries, lung infections, urinary tract infections, and systemic genital injuries. It contains a variety of virulence factors, including poisons and enzymes. It also ferments a wide range of carbohydrates, the majority of which are converted into lactose; they are oxidase negative, catalase positive, and have the ability to reduce nitrate to nitrite for energy production; and the majority of them move by flagella rather than spores. 37 °C is the ideal temperature for its growth (Jawetz *et al.* 2016).

2.2 *Escherichia coli*

It is one of the most important members of the intestinal family, and it grows like a natural plant in the digestive system. It's also referred to as a pathogenic opportunistic bacteria, as it causes diarrhea as well as a slew of diseases from the outside its habitat the natural ones, about 90% of urinary tract infections are caused by bacterial infections, such as newborn meningitis, septicemia. Infections of the urinary tract are very common. It spreads readily from the anal area to the urinary system and bladder, and it is 40 times more common in females than males due to the shorter urethra in females (Levinson 2016).

E. coli was first diagnosed by the Escherich Theodore in 1885 during his research on commensal bacteria in newborns' faeces. Intestinal settling occurs soon after birth. Bray discovered that a strain of the bacteria *E. coli* was the most common cause of newborn diarrhea in England in 1945 (Bray 1945).

E. coli is related to other genera in the intestinal family, especially the genus *Shigella*. This genus includes five species that differ among themselves with some biochemical reactions. *E. coli* is the most important and common type of human disease (Olowe *et al.* 2017). Classification of *E. coli* was given in Table 2.1.

Table 2.1 *E. coli* bacteria was classified in the within the intestinal (Garrity *et al.* 2005)

Classification of *E. coli*	
Kingdom	Bacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	Enterobacteriales
Family	Enterobacteriaceae
Genus	*Escherichia*
Species	*E. coli*

2.3 Characterization of *E. coli*

They are gram-negative bacilli that are not spore-forming and are bundled by peripheral flagella that surround the entire organism. Its colonies are smooth and slightly convex, moist, not mucous or mucous when you have it to install the wallet full sharp edge, glossy pink on agar MacConkey, sheen metalic green on medium agar (EMB) eosin methylene blue. And it is also pink colonies on orientation cromagar agar, not fermented for cellulobiose sugar orientation cromagar agar, cellulobiose sugar is not fermented, but more than 89% of them are fermented to ramenose, and more than 90% are fermented to sorbetol. It's also gelatin that doesn't dissolve and doesn't release sulfide gas (H_2S). H_2S is mostly created in the TSI (agar iron sugar triple) media. The enzyme glucoronidase-β (GUD) does not develop in the presence of potassium cyanide (KCN), grows at a pH of 9 to 4.4 and grows best at 37 to 36 °C. It is catalase positive and catalase oxidase, urease, and indole cation negative, which is the best test for distinguishing it from other intestinal family members. It is not solely based on the consumption of knitwear. The red methyl test is also positive for carbon (Hemraj *et al*. 2013).

2.4 *E. coli* Types

E. coli bacteria that cause diarrhea are divided into six types based on their characteristics and specific virulence factors (Rivas *et al*. 2015, Malema *et al*. 2018);

1. Shiga-Toxin Producing *E. coli* (STEC) or Enterohemorhagic *E. coli* (EHEC)

2. Enteropathogenic *E. coli* (EPEC)

3. Enterotoxigenic *E. coli* (ETEC)

4. Enteroinvasive *E. coli* (EIEC)

5. Enteroaggregative *E. coli* (EAEC)

6. Diffusely Adhering *E. coli* (DAEC)

2.5 Epidemiology of *E. coli*

After birth, *E. coli* is the most common member of the intestinal family that invades and coexists in the intestine. However some of its strains have an opportunistic susceptibility to infecting the host with illnesses when ideal growth conditions are available (Gharajalar and Sofiani 2017).

2.6 Urinary Tract Infections

Urinary tract infections are one of the most diseases are caused by bacteria, and *E. coli* is the main culprit. About 90% of instances of urinary tract infections for age groups between 69-60 years and 39-30 years, as well as the fact that infection rates in females are higher than in men for age groups between 19-10 years, are considered one of the main difficulties in hospital injuries related to infection. Those who reside in nursing homes are referred to as convalescents (Foxman 2013, Forsyth *et al.* 2018).

The incidence of urinary tract bacteria (UPEC) varies from person to person, and the risk of infection rises when using urinary catheters allowing bacteria to enter the urethra. Infection rates vary by geographic location and health status, with infection rates rising in poor health and malnutrition. Injury infections of the urinary tract affect roughly half of the world's population, second only to urinary tract infections respiratory infections in poor areas, as about 150 million infections occur in the world each year. And there is no less than 10 bacterial cell per milliliter of urine, evidence of infection (Jaweetz *et al* 2016).

2.7 Virulence Factors *E. coli*

E. coli has several virulence factors that make it more vulnerable to disease (Figure 2.1). Urinary tract infections are the most common, and one of these factors is the ability to analyze red blood cells due to their presence of hemolysin, which comes in a variety of kinds depending on the type of lysis "alpha, beta and gamma", as the first type, hemolysin-α, gives bacteria the ability to degrade human red blood cells. Partially hydrolyzed, hemolysin-β gives the bacteria the ability to degrade blood cells in red, it is completely decomposed, The third type, hemolysin, offers bacteria the capacity to decompose other animals' red blood cells, not humans. Bacteria also possess cytotoxic necrosis factor necrotizing that breaks down red blood cells (Jaweetz *et al*. 2016).

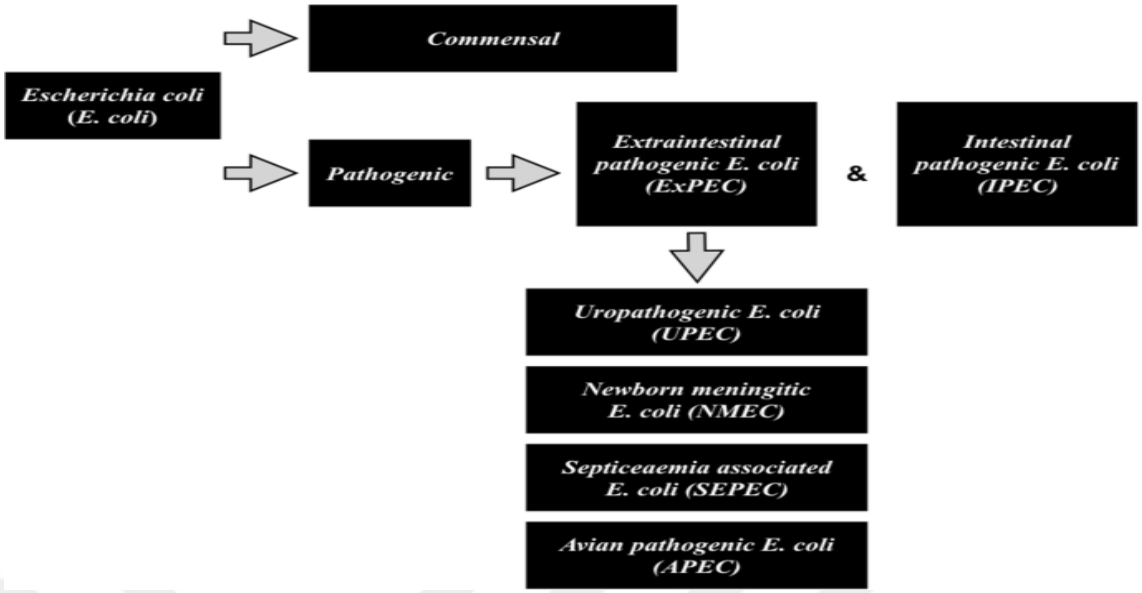

Figure 2.1 Virulence factors *E. coli* (Jaweetz *et al*. 2016)

The presence of surface structures such as endotoxins, which are made up of peptides, is another virulence factor of lipopolysaccharide (LPS) containing O (autosomal antigen O antigen somatic), allowing bacteria to colonize and lodge on host cells, particularly the bladder lining it also enables them to overcome the host's immunity, and for some of them the ability to produce multiple capsules polysaccharides that contain the capsular antigen (K antigen) as well as having surface structures. Flagella which are used as locomotion in motile organisms and contain similar structures on flagellar H antigen, are another example, as these antigens bacterium with the ability to overcoming the immunological system of the host. It also possesses corley fibers which possess qualities bacterial biofilm development is aided by physical and chemical factors. It also has bacteria vesicles located in the outer membrane that bind with toxins bacterial, enzymes and adhesion factors act as a facilitation system, allowing them to reach host cells more easily. Outer membrane proteins (OMPs) and released toxins are also present. Bacteria's ability to infect is increased by all secretions. Cilia are fimbriae or pili of the three types (F-fimbrial, S-fimbrial and P-fimbrial) and it has the ability to create a biofilm and thus boost its ability to acquire antibiotic resistance. Pilli type 1 is one of the most essential elements that help bacteria attach to the tissues of the host (Neamati *et al*. 2015, Spaulding *et al*. 2017).

This is in addition to colicin, a bacteriocin that kills both sexes. Other bacteria, and thus helps to protect microorganisms, as well as cyclomodulin toxins, which are inhibitory toxins. Other bacterial genera' genomic DNA also contains persistent toxins, according to EAEC. EAST1 (enteroaggregative heat-stable toxin1) is a heat-stable toxin that is released by the intestine. Stable-heat enterotoxin ETEC and heat-blocking enterotoxin ETEC enterotoxin labile-heat secreted by ETEC bacteria, encoded-plasmid toxin pet, which is toxic to red blood cells and intestinal cells, toxins similar to *Shigella* and vesicular toxins it also possesses the enzymes carbinimase that the bacteria confer resistance to carbenemes and beta-lactamases, which the cephalosporins and penicillins that give bacteria resistance to beta-lactams include and the enzymes acetyltransferase, adenyltransferase, phosphotransferase that confer bacteria resistant to anti-aminoglycosides (Zowawi *et al*. 2015) also chelate iron helps bacteria grow in a low-iron environment (Terlizzi *et al*. 2017).

2.8 The Ability of *E. coli* Create a Biofilm'

The biofilm is made up of bacterial cells that adhere to hard surfaces and are surrounded by an extracellular substance made up of polysaccharides and microorganism's DNA proteins. This extracellular substance help's to stabilize the biofilm, and this membrane plays a role in the infection of the host increases the bacterial population susceptibility to antibiotic resistance, and protects cells of the body's defense mechanisms, such as white blood cells (Soto 2013). The creation of a biofilm goes through numerous stages and as follows:

i Reverse adhesion this step is handled by flagella and chemical attractions for bacteria, and it involves cell attachment and adhesion to surfaces.

ii Non-reverse adhesion to surfaces: cell stability phase and low elongation, mediate this stage by the first type of cilia, the corley fibers a type of antigen is called 43 antigen.

iii The stage of extracellular substance formation, in which bacteria produce polysaccharides to aid in the collection and cellulose is the substance that allows

cells to adhere to surfaces, multiple amino-glucose, and cholic acid, as well as a variety of other substances such as nucleic acids, proteins, and so on.

iv Separation: the stage of biofilm maturation and separation from one surface to begin the process all over again on another (Soto 2014) (Figure 2.2).

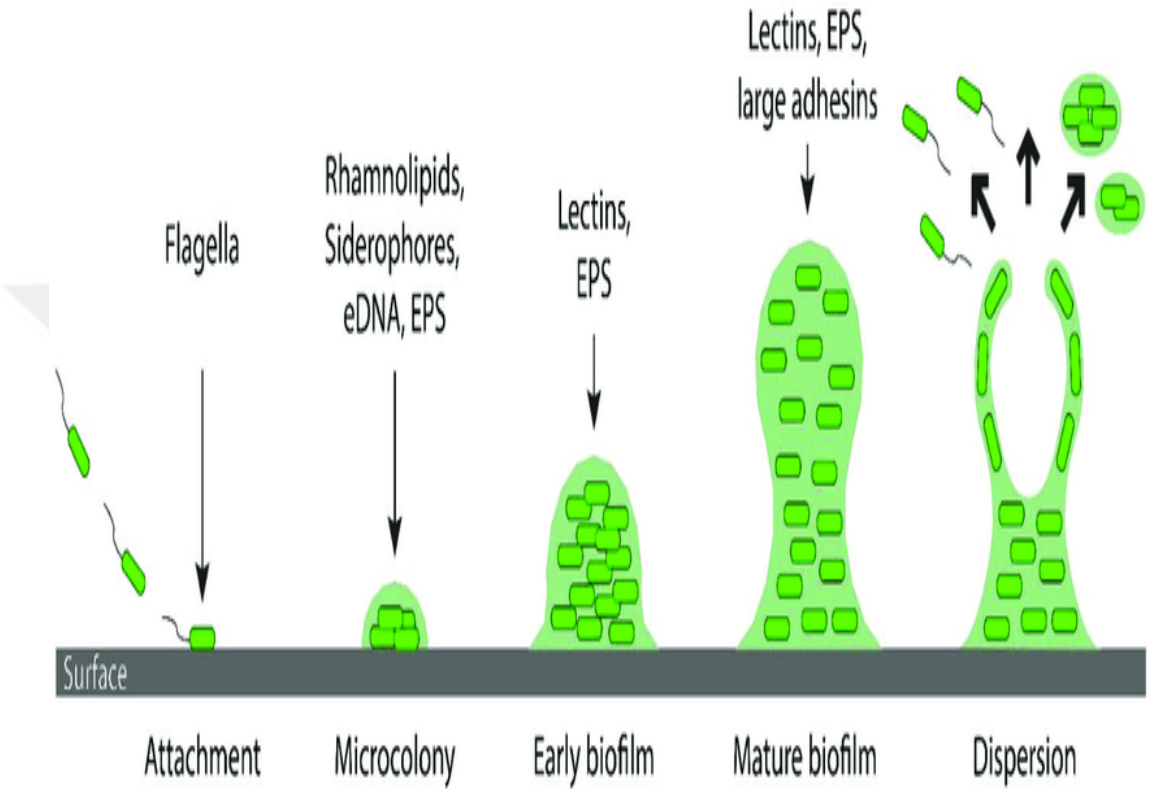

Figure 2.2 M carryover biofilm formation in *E. coli* (Silva *et al*. 2017**)**

According to studies, biofilm causes 89% of bacterial infections in the urinary system in humans, and it may be prevented by a variety of substances, including plant extracts and other chemicals, as its effect can be seen in the system for transmitting chemical signals between bacterial cells, known as the QS system (sensing quorum). Biofilm-producing bacteria are becoming more resistant to antibiotics. Antibiotics are unable to penetrate the biofilm, hence QS-encoded bacteria thrive genes are activated the effect of multiple efflux pumps that are resistant to antibiotic (Tajbakhsh *et al*. 2016, Poursina *et al*. 2018).

2.9 Pathogenicity of *E. coli*

These bacteria can cause a variety of disorders both inside and outside the intestine, including:

2.9.1 Enteric or diarrheal diseases

Bacteria manufacture enterotoxins during the time of attachment. There are three forms of enterotoxins, two of which induce watery diarrhea are heat agglomerated toxins cholera-like toxins and persistent toxins with heat. As for toxins similar to bacteria toxins called it causes bloody diarrhea and can be bloody diarrhea, caused by the bacteria themselves rather than its toxins, after it breached the gut lining, causing dysentery (Levinson 2016).

2.9.2 Septicemia and menengitis

E. coli causes meningitis in children. One of the captive antigens is also known as K1. It contains a toxin in terms of septicemia, caused by *E. coli*. Because newborns lack the M antibodies, the illness is more common in them, and it can emerge as a secondary infection after a urinary tract infections (Jaweetz *et al.* 2016, Soltani *et al.* 2018).

2.9.3 Urinary tract infection

It is one of the most frequent and significant diseases, since it is caused by bacterial infection and growth in the urinary system, which encompasses both the lower (urethra and bladder) and upper urinary tracts (ureters and kidneys). Inflammation of the lower urinary tract is known as cystitis urethritis, and typically develops after bacteremia and is asymptomatic. Catheter-associated urinary tract infection can be deadly. *E. coli* based urinary tract infections are the most prevalent cause of bacteremia (Foxman 2014, Forsyth *et al.* 2018).

E. coli caused urinary tract infections are one of the most common members of the intestinal family. 90% of urinary tract infections are caused by the following bacteria (Jawetz *et al.* 2016) as well as additional types of bacteria that cause urinary tract infections: *Enterobacter* spp., *Staphylococcus saprophyticus, Proteus mirabilis, Pseudomonas* spp., *Klebsiella pneumoniae, Serratia* spp. (Mirzarazi *et al.* 2013, Foxman 2014).

2.10 *E. coli* Antimicrobial Resistance

Antibiotics are secondary metabolites produced by microorganisms, which can be natural or synthetic. It's in the stationary phase and can stop other bacteria from growing without harming the host body's cells some has a narrow spectrum. They only act on a certain species or group of microbes, while others have a broad spectrum, indicating they work on a variety of microorganisms. Some of them are lethal, while others are not. It has an inhibiting action that was discovered by scientist Alexander Fleming in 1928 after he accidentally discovered the antibiotic penicillin, however this substance remained until the two scientists discovered it. The abstract was written by Flory Haward and Chain Ernst, and it was extensively circulated in 1941 (Ali *et al*., 2018).

Bacterial antibiotic resistance is one of the world's most important health and economic problems, prompting researchers to look for novel antibiotics to combat bacterial strain resistance. Illness with resistant bacteria results in a longer treatment time and a higher risk of infection. Antibiotic resistance can be classified as multi-drug resistant (MDR), extensively-drug resistant (XDR), or pan-drug resistant (PDR). At least one of the three medicines must be resistant to the MDR type. The type's resistance XDR shows that the bacterium is resistant to two or more antibiotics, whereas PDR resistance means that the pathogen is resistant to all antibiotics (Basak *et al*. 2016).

Characteristic of resistance can be inherited or acquired, and it can be acquired by chromosomal alterations or material transfer. The transfer of genes from one germ to another can happen in a variety of ways. Genetic material is directly transmitted between single cells in bacterial conjugation, bacterial genome is stripped of dead

bacteria, or through induction, genetic imputation mutations in plasmids, transposon genes, or transformation. Antibiotic resistance among bacteria is on the rise. It used to be resistant to around 39% of tetracyclines, streptomycin, and sulfisoxazole, but mutations and resistance plasmid transfer made it resistant to ampicillin, kanamycin, and ticarcillin as well. The bacteria have a variety of resistance strategies (Shariff *et al*. 2013, Afzal 2017). The mechanism of antimicrobial drugs was shown in Figure 2.3. The list of antibiotic resistance mechanisms are as follow:

i Target modification

This mechanism in nucleic acid building enhances bacterial resistance to anti-fluoroquinolones like ciprofloxacin and rifamycin, as well as its influence on ribosomes, which causes a deficiency in protein synthesis, increasing bacterial resistance to erythromycin and rifamycin (Kotsyuba *et al*. 2014).

ii Permeability of cell membranes changes

Modifying the type of the protein in the cell membrane changes the holes in the cell membrane or the transport processes, which alters the antibiotic's activity and renders it useless. This process imparts resistance to bacteria to tetracyclines and quinolines, such as ciprofloxacin, and some types of aminoglycosides, such as gentamicin, as well as antibiotics and sulfonamides antibiotics, as a result of these alterations (Kapoor *et al*. 2017).

iii Production of enzymes

This mechanism has an impact on cell wall formation. It is mediated by penicillins and cephalosporins β-lactamase enzymes have a role in the breakdown of aminoglycosides like chloramphenicol, as well as the acquisition of microorganisms resistant to these antibiotics, glycopeptides and quinolines which produces inhibitory enzymes for replication (Kotsyuba *et al*. 2014).

iv Alteration of metabolic pathways

Bacteria take folic acid from the environment to make ready-made modifications in their metabolic pathways, allowing them to develop resistance to antibiotics such as trimethoprim and sulfa, as well as possessing efflux pumps that pump toxic. Bacteria compounds allowing them to thrive in an unfavorable environment (Paltansing *et al.* 2015).

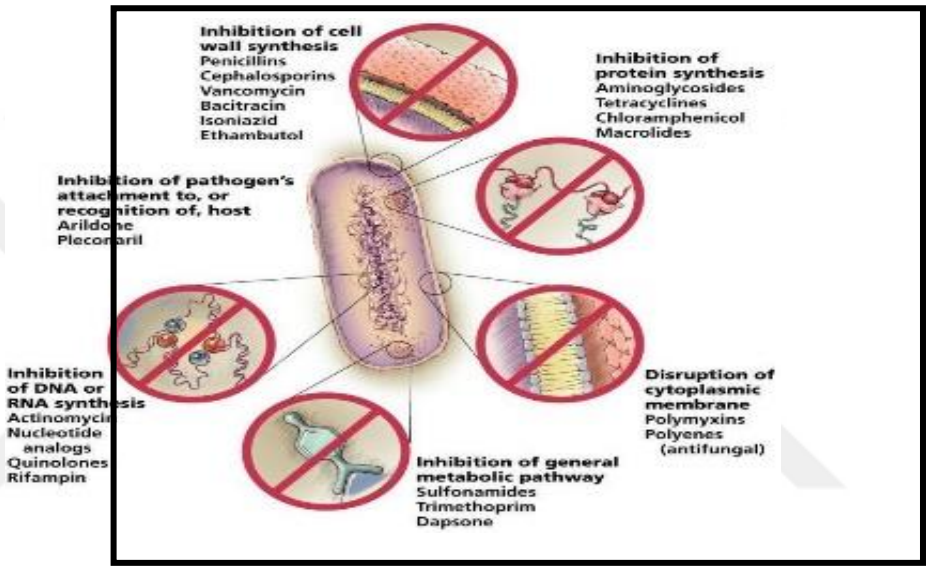

Figure 2.3 Mechanisms of antimicrobial drugs (Gillor *et al.* 2004)

2.11 β-Lactams and β-Lactamases

This class of antibiotics includes a wide range of antibiotics. The β-lactam ring in their molecular structure distinguishes them from other antibiotics. Penicillin is the most well-known β-lactam antibiotic, but in the previous 60 years, a wide range of new members of this class have been discovered and created. After ninety years after Fleming discovered penicillin, β-lactams still hold a significant place in our arsenal of antibiotics. Penicillins, cephalosporins, carbapenemas and monobactams are variations of the β-lactam ring. By attaching to penicillin binding proteins (PBPs) inside the bacterial cell wall, the drugs prevent the cell wall from being produced. Bactericidal antibacterial agents classified as β-lactams (Simonsen *et al.* 2017).

2.12 Resistance to β-Lactams

The β-lactams penicillin, carbapenems, and monobactams of cephalosporin are part of a category of antibiotics that act on the bacterial cell wall. These antibiotics target the enzymes carboxypeptidases and transpeptidases, which are responsible for the cross-linking of cell walls. In order to cross connect the peptidoglycan of the bacterial cell wall with the D-alal dipeptide, these enzymes may bind penicillin-binding proteins (Livermore and Paterson 2006).

By selectively eliminating vulnerable bacteria, the β-lactam medicines have allowed the antibiotic-resistant ones to thrive. As an example, in enterococci, resistance to β-lactams may be genetically predisposed in a species with a genetic predisposition (PBPs). Gene transfer or spontaneous mutation may also lead to the development of the condition. The generation of β-lactamases, impermeability, efflux, and target alteration are all possible causes of β-lactam resistance functionally. These modalities may be combined in many ways. Pneumococci and MRSA are two of gram-positive cocci resistance, which is caused by standard modifications PBPs and the acquisition of extra amounts of β-lactam-insensitive PBPs. Gram-negative bacteria often develop resistance owing to impermeability and efflux which were increased by a combination of endogenous acquired-lactamases and spontaneously elevated impermeability (Livermore and Paterson 2006).

2.13 Definition and Classification of β-Lactams

Antibiotics such as penicillin, cephalosporin, and azitreonam are hydrolyzed by the ESBLs, which may impart resistance to these antibiotics through hydrolysis. ESBL inhibitors such as clavulanic acid are able to prevent the hydrolysis of ESBL-producing bacteria, but not cephamycin or carbapenem (Paterson and Bonomo 2005).

Classes for β-lactamases are most typically based on ambler molecular coding and Bush-Jacoby-Medeiros functional coding (Ambler *et al*. 1991, Bush *et al.* 1995). In the

ambler technique, four primary categories of β-lactamases are identified (A to D). It is based on protein homology (amino acid similarity) rather than behavioral traits that this categorization system is built upon (Ambler *et al*. 1991).

Class A β-lactamases in the ambler categorization system include serine β-lactamases of the A, C, and D classes. Metalo β-lactamases belongs to class B. Aside from the class D OXA-type enzymes. All ESBLs are classified as molecular class A. β-lactamases are classified using the Bush-Jacoby-Medeiros categorization technique based on their functional similarities (profile of substrates and inhibitors). This categorization approach has four basic categories and numerous subcategories (Bush *et al.* 1995).

These therapeutically relevant β-lactamase and β-lactam substrates are of particular relevance to physician microbiologists working in diagnostic laboratories. *E. coli* ESBLs are classified into two groups: group 2be and group 2d (OXA-type). Group 2be enzymes are similar to group 2d enzymes, but they are more resistant to antibiotics (Bush *et al.* 1995). These enzymes (TEM-1, TEM-2, and SHV.1) are all descended from group 2b β-lactamases, and the 'e' in 2be indicates that they have a broader spectrum of activity. TEM1, TEM2 and SHV.1 related *E. coli* ESBLs may be separated from their forebears by a single amino acid change. It is now possible for the ESBLs to hydrolyze aztreonam or third-generation cephalosporins because of their changed enzyme activity. In contrast to AmpC-type β-lactamases group 1, group 2 ESBLs are inhibited cephamycins cannot be hydrolyzed because of clavulanic acid, which are their substrates but uninhibited. Treatment of infections with third-generation cephalosporins caused by AmpC-type β-lactamases that are hyper-produced mutants has resulted in clinical failure, according to studies. Cefepime, a fourth-generation cephalosporin, can treat AmpC-type β-lactamase-producing bacteria (Kaye *et al.* 2001, Cosgrove *et al.* 2002). EDTA, but not clavulanic acid, is a heavy metal chelator. Inhibits the hydrolytic enzymes group 3 produced by organisms such *Stenotrophomonas maltophilia*, which are capable of hydrolyzing third-generation cephalosporins (Yuan *et al.* 1998, Wachino *et al.* 2004). ESBL classification was shown in Figure 2.4.

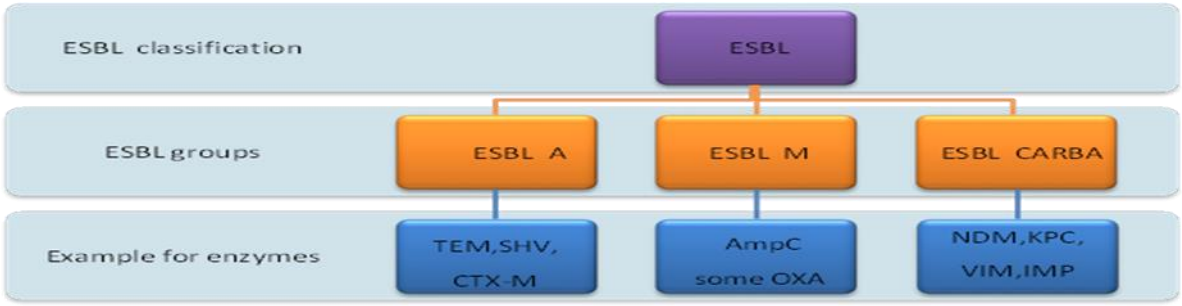

Figure 2.4 ESBL classification according to (Giske *et al*. 2009)

2.14 The Risk of ESBL Infection

Patients using medical equipment that are intrusive (urinary catheters, endotracheal tubes, central and venous lines) may get colonized or infected with ESBLs during long-term hospitalizations (Paterson and Bonomo 2005). Several studies have linked the use of nasogastric tubes as an extra risk factor (Asensio *et al.* 2000). Total parenteral nutrition (TPN) is necessary for patients who have gastrostomy or jejunostomy tubes, arterial lines, recently had surgery (de Champs *et al.* 1991), have hemodialysis (de Champs *et al.* 1991), or have decubitus ulcers (Mangeney *et al.* 2000, Weldhagen and Prinsloo 2004). Excessive use of antibiotics is another risk factor for ESBL acquisition (Ariffin *et al.* 2000). Several studies have associated the use of third-generation cephalosporins with the purchase of a strain that produces ESBLs (Ariffin *et al.* 2000, Pessoa-Silva et *al.* 2003). It's more likely that an ESBL-producing bacterium will spread between patients if they are kept in the same unit or room (Livermore and Paterson 2006).

A person's chance of getting an infection or colonization with an ESBL-producing bacterium increases if they have recently been hospitalized, have been treated with penicillins, cephalosporins, or quinolones, or are 65 or older, have dementia, or have diabetes (Livermore and Paterson, 2006).

2.15 Treating a β-lactamases Infection

When ESBL-producing organisms expose to oxyimino β-lactams, they may have developed resistance to some but remain susceptible to others. TEM and SHV-type ESBL-producing bacteria seem to be in vitro responsive to the antibiotics cefepime and piperacillin/tazobactam, however both medicines have an inoculum effect (Jacoby *et al.* 1997, Thomson and Moland 2001). Cefepime is more resistant to CTX-M and OXA ESBLs when tested with a conventional inoculum (Bonnet 2004). When tested in vitro, ESBL-producing bacteria are sensitive to cephamycins and carbapenems and show little or no inoculum effect (Jett *et al.* 1995). Carbapenem-resistant (AmpC) bacteria may also be resistant to oxyimino β-lactams, however this is not the case for the majority of (AmpC) strains. Cadpenemases of IMP, VIM, and OXA kinds are still vulnerable to aztreonamine's effects (Nordmann and Poirel 2002). All of these enzyme-producing strains are more likely to acquire resistance to non-lactam antibiotic treatments and thus need to be tested directly for their susceptibility. Antibiotics have a hard time killing fluoroquinolones and aminoglycosides (Jacoby and Carreras 1990). Patients with ESBL-forming enterobacteriaceae like *E. coli* are more likely to suffer from *E. coli* infections if antibiotics are not given quickly enough, resulting in higher hospital expenditures (Rodriguez-Bao *et al.* 2018).

2.16 Detection of β-lactamases Resistance

Researchers have explored the effect of several screening and confirmation methods on ESBL resistance in bacteria. Bacteria that have been isolated and put through a series of assays, including antibiotic susceptibility testing, are often used for this purpose (Ben-Ami *et al.* 2006). The detection of ESBL involves two phases. An indicative screening test for cephalosporin antibiotic resistance or sensitivity is required before additional testing can be carried out, and this must be done before any further testing can commence. ESBL isolates are differentiated from resistant isolates by testing for the synergy of oxyimino cephalosporin and clavulanate in the second stage (Ben-Ami *et al.* 2006).

2.17 VITEK β-lactamases Test

ESBL-producing organisms may be misidentified as cephalosporin-resistant if MICs are less than 8 g/mL when using standard VITEK cards. The FDA has recently approved a specific card for the detection of testing isolates' ESBL production. A combination of antibiotics, including cefotaxime and ceftazidime, as well as clavulanic acid (4 g/mL), is included on the VITEK ESBL card created by the biotechnology company (bioMerieux VITEK, Hazelton, Missouri). As soon as growth control is met, the well's data is examined automatically (4-15) hours of incubation (Steward *et al.* 2000).

2.18 Efflux Pumps

They are membrane-bound protein transporters that in cells. They play an important role in the transport and excretion of a variety of substances. Bacterial resistance to antibiotics is facilitated by this mechanism. Hydrophilic toxins, or hydrophobicity, as well as dyes such as red yellowish, crystal violet dye, and ethidium bromide dye fatty acids, disinfectants, and sterilizers may all be transported out of the cell via these transporters. Antibiotics, heavy metals, and organic solvents and number of examples of these classes of drugs: β-lactams, macrolides, and the antibacterial tetracyclines can also transport (Venter *et al.* 2015, Zhi Li *et al.* 2016).

2.18.1 Classification of efflux pumps

Flow pumps are classified according to three criteria:

- **Relying on the specificity of the transported materials**

General flow pumps, like the numerous efflux pumps in bacteria that may release antibodies of various chemical compositions, they are capable of pumping materials of various chemical structures outside the bacterial cell (Alav *et al.* 2018). So it contributes

to resistance multi-antagonists or specialized efflux pumps the cell, as in pumps specialized in stream antibiotics of one kind (Anes *et al.* 2015).

- **Source of energy**

Pumps that rely on a chemical source of energy to perform their functions, such as those that rely on efficient transport to dispose of materials, fall into one of two categories: 1. flow pumps that rely on primary energy sources, such as the energy produced by the hydrolysis of hydrocarbons a molecule is used and effluent pumps and 2. flow pumps that rely on secondary power sources which depends on the gradient of the hydrogen ion +H or on the gradient of the sodium ion +Na as a source energy in the process of excreting substances outside the cell (Delmar *et al.* 2014).

- **Relying on the genetics**

These pumps are classified into two main categories based on genetics, namely effluent pumps chromosome, which means that the genes encoded for them are carried on a chromosome in the cell. These are found in both eukaryotic and prokaryotic cells and confer natural or intrinsic resistance to germs, allowing them to persist in a certain environment, such as one with a high concentration of antibiotics. Plasmid efflux pumps and the genes that produce them are carried on genetic elements like plasmids or transposon genes, and they impart acquired resistance. It is found in prokaryotic cells, especially bacterial cells as in the MFS family of stream systems (Puzari and Chetia 2017).

2.18.2 Multi-flow pumps

There are genes that can code for the transfer of through a pump, more than one substance in the chemical composition. Multi-flow pumps are a type of single-flow pump this gives the bacteria multi-resistance to antibiotics. Five families of stream systems in the prokaryotic are following (Zhang *et al.* 2016):

A. Major Facilitator Super Family (MFS)

B. Small Multidrug Resistance Family (SMR)

C. Multidrug and Toxic Efflux Family (MATE)

D. ATP-Binding Cassete Family (ABC)

E. Resistance-Nodulation-Division Family (RND)

2.18.3 Flow pumps within the flow (ABC) family

When ABC type flow pump rely on the hydrolysis of the ATP molecule, other families depend on proton emitting energy (PMF) as an energy source. One of the most famous and most prevalent families is the RND family, which predominates in gram-negative bacteria such as *Pseudomonas* spp., *Proteus* spp., *Klebsiella pneumonia* and *Salmonella* spp. (Delmar *et al.* 2014).

Flow pumps were first discovered as a mechanism of resistance against tetracyclines in *E. coli*, where the RND family is divided into three classes based on their components. These include single-component efflux pumps represented by the inner membrane protein AcrB to the cell, which is encoded by a gene called AcrB, which transports hydrophilic antigens, and pumps it has two components represented by AcrB protein in the inner membrane and AcrA lipoproteins present in the vacuole plasma cells encoded by a gene called AcrA. Pumps with three components which are represented by AcrB protein in the inner membrane and AcrA proteins in the plasma vacuole and the channel a funnel-like protein called toic found in the outer membrane of the cell (Blanco *et al.* 2016) and that transports most of the hydrophobic the family in *E. coli* RND efflux pumps is also divided into two subfamilies depending on the material transmitted by:

Hydrophobic and amphiphilic efflux RND (HAE-RND) pumps fluids that transport toxic and harmful substances, neutral and hydrophobic, and include proteins (AcrAB. AcrEF. MdtAB. AcrAD. MdtC. MdtEF) (Figure 2.5) (Nies 2003).

Heavy metal efflux RND (HME-RND) pumps that transport heavy items and include one system that is (CusCFBA) copper- transporting efflux system (Delmar *et al*. 2014).

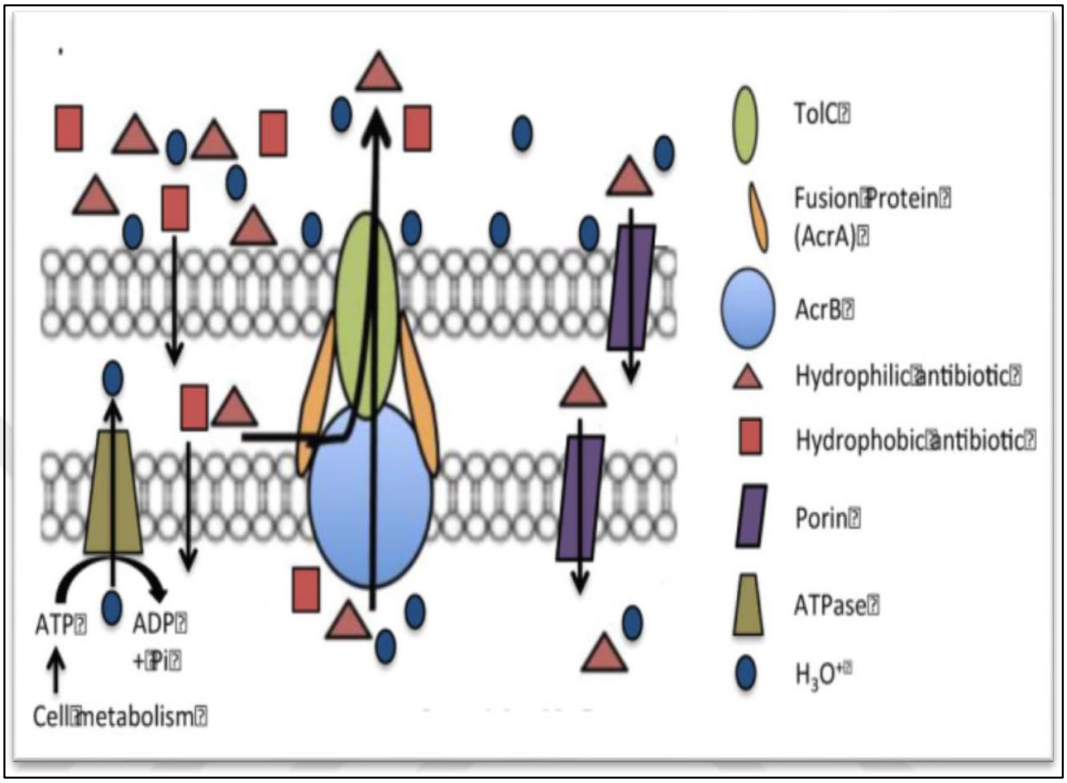

Figure 2.5 The efflux system (TolC-AcrAB) in *E. coli* that consists of a protein located in the inner membrane AcrB and proteins in the plasma vacuole AcrA and the Toic channel in the outer membrane (Amaral *et al*. 2014)

3. MATERIALS AND METHODS

3.1 Materials

The materials and laboratory equipment, culture media, the chemical and biological solutions, the reagents and stains listed in the Appendix 1.

3.1.1 Samples collection

50 urine samples collected from patients with UTI, samples were taken from the urine, taking into account the conditions of sterilization, and the patient's information was recorded, which is the name, age, sex, and medical history, and recorded it in a special paper found in the Appendix 2. The collection continued for a period of time from 10/3/2021 to 1/7/2021

3.1.2 Ethical considerations

For this study, let us obtain a permit from the hospitals Abi Ghraib General Hospital, Child Central Teaching Hospital, Yarmouk Teaching Hospital, and several accredited external laboratories in Baghdad. This permit was given in Appendix 3. Samples were collected with the consent of the patients and under the supervision of the experienced.

3.2 Principle of VITEK 2 System

The VITEK 2 is a microbiological system that uses growth-based technologies to automate the process. This style is mainly geared at clinical microbiology laboratories, and it offers better degrees of automation and capacity for higher volume laboratories. They also provide automated pipetting and dilution for antimicrobial susceptibility testing. Each of the reagent cards' (64 wells) can accommodate a different test substrate. System measure a variety of metabolic processes in the presence of inhibiting substances and substrates, including as acidification, alkalinization, enzyme hydrolysis,

and growth. An optically transparent covering on both sides of the card enables for the needed degree of oxygen transfer while maintaining a sealed vessel that keeps the organism-substrate admixtures out. Each card comes with an inoculation transfer tube already attached. The cards have barcodes on them.

3.3 Methods

3.3.1 Sterilization

The culture media and solutions were sterilized using two methods according to Brown and Smith (2017) and as follows

Heat sterilization. All ready-made, synthetic agricultural media and most of the solutions used in this study were sterilized, which did not influenced by heat, with an autoclave at a temperature of 121 °C and a pressure of 15 pounds/in for 15 minutes, and the glassware was sterilized in the oven at 180 °C for two hours.

Sterilization by filtration. Some of the solutions whose nature is affected by heat, such as urea, sugars and some dyes, have been sterilized using microfilters, the filtrate has a diameter of 0.22 μm.

3.3.2 Preparation of culture media

The ready-culture media was prepared, which is in the agar of leucine, the blue methylene, the MacConkey agar, the Muller-Hinton agar and blood agar and according to the company's instructions the manufacturer of it, which was installed on the package of each medium, sterilized the sterilizer at a temperature of 121 °C and pressure of 15 pounds/in for 15 minutes. The culture media was then poured into petri dishes and sterile tubes. It was incubated at 37 °C for 22 hours to ensure that it was not contaminated after which it was kept in the refrigerator at 2 °C until use.

Brain heart infusion (BHI) broth. In 1000 mL distilled water, dissolve 37.0 grams of broth. Dispense into bottles or tubes and autoclave for 15 minutes at 15 psi pressure in 121 °C (Sahm *et al.* 2002).

MacConkey agar. In 1000 mL purified/distilled water, suspend 51.53 grams of dehydrated medium. To completely dissolve the medium, it was heated to boiling. Sterilize via autoclaving for 15 minutes at 15 psi pressure in 121 °C, which is the verified cycle. Overheating should be avoided. Allow to cool to 45-50 °C. Before pouring into sterile petri dishes, make sure everything is well combined (Sahm *et al.* 2002).

Mueller Hinton agar. In 1000 mL purified/distilled water, dissolve 38.0 grams of agar. Bring to a boil to completely dissolve the medium. Autoclave at 15 psi pressure in 121 °C for 15 minutes to sterilize. Reduce the temperature to 45-50 °C. Fill sterile petri dishes halfway with the mixture (Sahm *et al.* 2002).

Nutrient Broth. In 1L distilled water, dissolve 13.0 grams. Dispense into tubes and autoclave at 15 psi pressure 121°C for 15 minutes to sanitize (Sahm *et al.* 2002).

Eosin methylene blue agar. Dissolve 40.0 grams of agar in 1000 mL purified/distilled water. To thoroughly dissolve the medium, bring to a boil. To sterilize, autoclave at 15 psi pressure for 15 minutes at 121 °C. Reduce the heat to 45-50 °C. Pour half of the mixture onto sterilized petri dishes (Sahm *et al.* 2002).

Urea agar. This medium was made following the manufacturer's directions by dissolving 24 grams of agar base medium urea in 950 mL of sterile distilled water, then sterilizing the medium using a sterilizer, and then left to cool to a temperature of 50 °C then 50 ml of 40% urea solution (made by dissolving 40 g of urea in 100 mL of water) was added milliliters of distilled water and then sterilized using filter units with a diameter of 0.22 microliters, then pour the medium into sterile tubes and store at 4 °C

until ready to use. Use this medium to detect the ability of bacteria to produce the enzyme urea (Tille 2017).

Blood Agar. Prepare the medium of the blood agar basis according to the manufacturer's instructions and sterilize with sterilization then cool it to 50 °C degrees, then add 5% blood to it, mix gently, and pour into dishes petri sterile and left to harden. This medium was used to investigate the production of hemolysin enzyme by the isolates under study (Forbes *et al.* 2007).

Hekton enteric agar. This medium was prepared after sterilizing the distilled water with an oxidizer and then cooled to a temperature of 51 °C, after which it was added mix it well, then pour it into sterilized petri dishes and leave to solidify. Use this is the medium in order to distinguish *E. coli* bacteria from the rest of the intestinal family (Brown and Smith 2017).

3.3.3 Reagents preparation

Oxidase reagent. Prepare this reagent by dissolving 1 gram of phenylenediamine-P-tetramethyl dihydrochloride in 100 mL of sterile distilled water in an opaque glass vial. The reagent used to test bacteria's capacity to produce the oxidase enzyme (Tadesse and Alem 2006).

Catalase reagent. This reagent was prepared from hydrogen peroxide at a concentration of 3% from the original solution at a concentration of 30% and kept in an opaque vial. This reagent was used to test bacteria's capacity to manufacture the catalase enzyme (Tadesse and Alem 2006).

3.3.4 Sample collection

Patients with symptoms of urinary tract infections had urine samples taken and under the supervision of the specialist doctor from different hospitals in the city of Baghdad. It

was used by tubes to collect the sample from the middle of the urine in the early morning for all patients, and then the samples were planted by taking 100 microliters of urine, cultured it on several differential media, and then incubated for 24 hours at 37 °C.

3.3.5 Microscopic examination

A total of 50 mid-stream urine samples were taken in early morning from uti patients (17 males and 33 females), and after conducting phenotypic, the samples were centrifuged with a centrifuge at 2000 rpm for 5 minutes. Then the precipitate was mixed with the homogenizer for half a minute, after which a drop of the precipitate was placed on the slide. It was examined under a light microscope using a magnification x 40, and it was considered the presence of 10 or more white blood cells in the field of the microscope, as well as the presence of 50-200 pure colonies growing in the dish, the result is positive. 50 isolations of *E. coli* bacteria have been detected Figure 3.1.

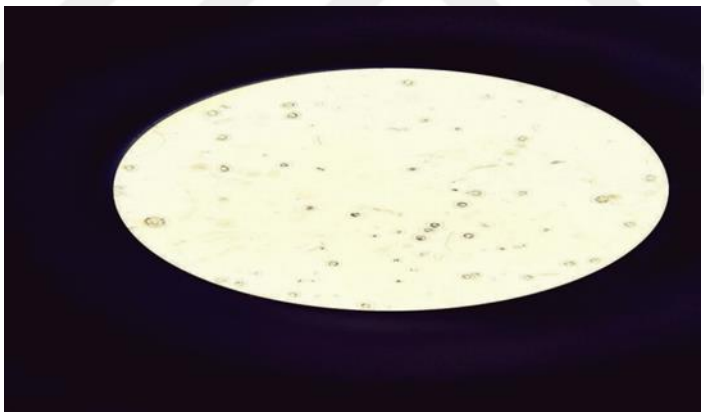

Figure 3.1 Microscopic examination

3.3.6 Culturing the samples

The phenotypic characteristics of isolated colonies were studied after culturing and purifying the bacterial isolates on culture media different of (eosin methylene blue, MacConkey agar, blood agar) the study included all of the shape, size, texture, color, edges and heights for isolated bacterial colonies (Wanger *et al.* 2017).

3.3.7 Diagnosis of bacteria by microscope

Swabs were prepared from bacterial isolates grown on MacConkey medium. It is 18-24 hours old, using CRAM technology, and then examined under a light microscope to see the shape, arrangement and colors of the cells depending on its interaction with the gram stain (Levinson 2016).

3.3.8 Biochemical identification

Oxidase test. Colony growed on MacConkey agar at 22 hour old was transferred onto filter paper with sterilized wooden sticks, then add a drop of oxidase reagent to it the change of color to purple in 30-60 seconds for evidence of the production of the oxidase enzyme (Figure 3.2) (Hemraj *et al.* 2013).

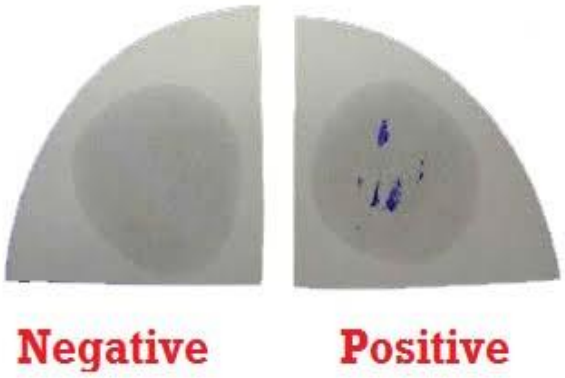

Figure 3.2 Oxidase test

Catalase test. Colony of MacConkey agar at the aged 22 hour old was transferred onto a dry, sterile glass slide with sterilized wooden sticks, then one drop of catalase reagent was added to it 3% the appearance the presence of bubbles on the glass slide's surface is proof of the production of the enzyme catalase (Figure 3.3) (Hemraj *et al.* 2013).

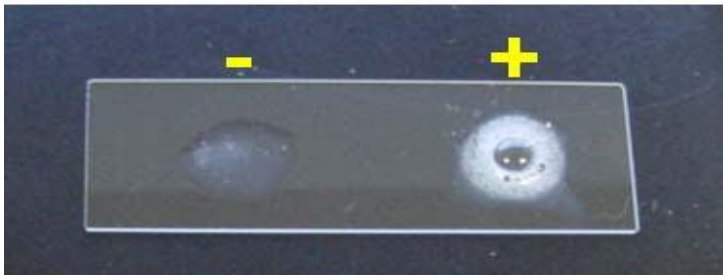

Figure 3.3 Catalase test

Indole test. This test was carried out by inoculating peptone water into test tubes. The bacteria colonies were then cultured at 37 °C for 24 hours. A few drops of Kovac's reagent should be added. The presence of a red ring on the medium's surface indicates that the test is positive (Figure 3.4) (Forbes *et al.* 2007).

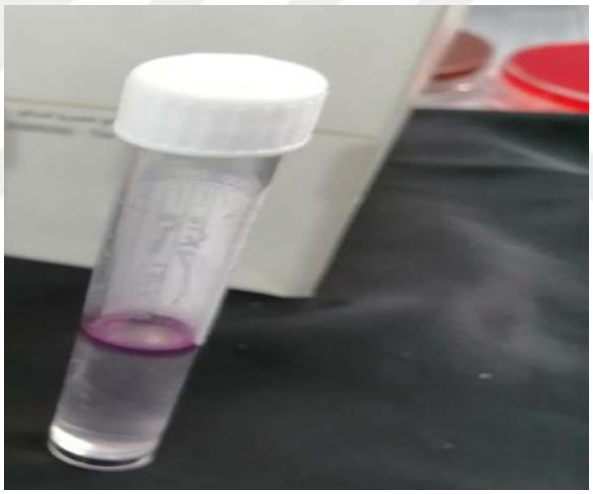

Figure 3.4 Indole test

Methyl red test. Inoculated tubes containing Methyl-Fox-Fuchs Proskawer medium (VP-MR) with colonies then add 2-5 drops of reagent to each tube bacteria, then incubated at 37 °C for 24 hours the red instance. The change in the color of the medium to red is an indication that the test is positive (Figure 3.5) (Hemraj *et al.* 2013).

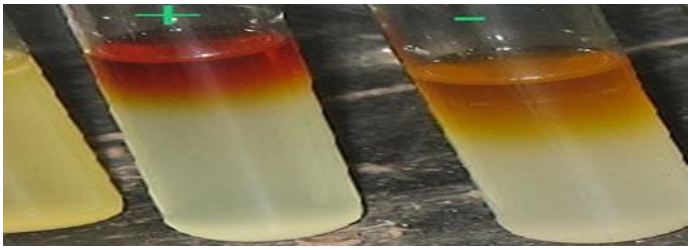

Figure 3.5 Methyl red test

Citrate utilization test. Inoculate simmons citrate agar with bacterial colonies that were cultured on MacConkey agar and incubated overnight at 37 °C. If the organism has the ability to use citrate, the medium change its color from green to blue (Figure 3.6) (Brown and Smith 2017).

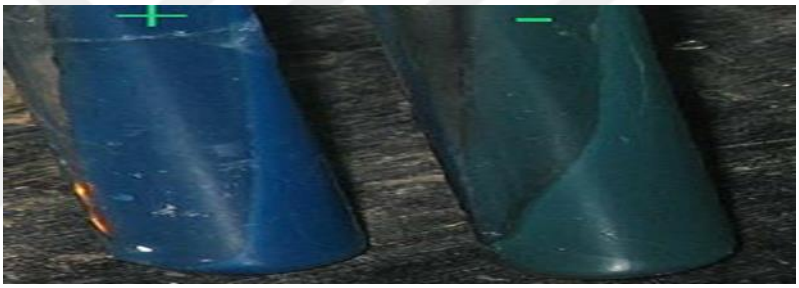

Figure 3.6 Citrate test

Urease test. The tubes holding the prepared urea agar medium were inoculated on the inclined media's surface, and then incubated for 22 hours at 37 °C. When the center color turns pink, the test is positive (Figure 3.7). The purpose of this test was to see if bacteria could create the enzyme urease (Tille 2017).

Figure 3.7 Urease test

Hemolysin production test. Bacterial isolates were cultivated on blood agar plates, and the plates were then incubated. The data were read by noting the type of breakdown after being kept at 37 °C for 24 hours (alpha, beta and gamma) (Figure 3.8) (Tille 2017).

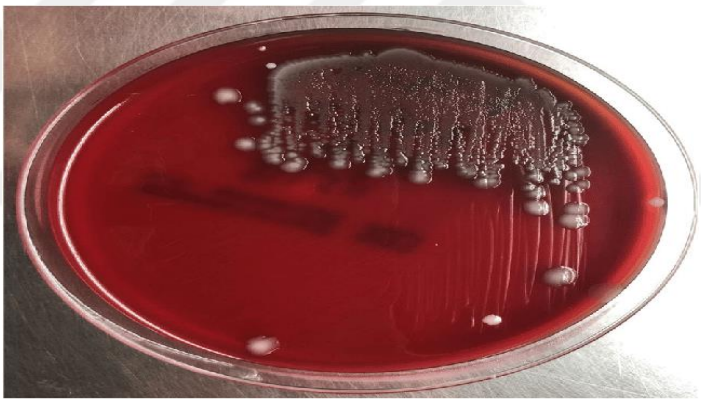

Figure 3.8 Hemolysin production test

3.3.9 Identification by VITEK 2

Suspension Preparation. Transfer a suitable number of colonies from a pure culture into a 12 x 75 mm transparent plastic (polystyrene) test tube containing 3.0 mL of sterile saline using a sterile brush (aqueous 0.45% to 0.50%NaCl, pH 4.5-7.0). A DensiChekTM turbidity meter is used to regulate the turbidity as needed. The suspension of turbidities was determined using the McFarland turbidity range (0.48-0.56).

Procedure. All steps were done according to the manufactures instructions (Biomerieux, France). As in following:

i Using a sterile swab or applicator stick, a sufficient number of colonies of a pure culture were transferred and suspended in 3.0 mL of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a 12 x 75 mm transparent plastic (polystyrene) test tube. The turbidity were adjusted accordingly by McFarland Turbidity Range (0.48-0.56) and measured using a turbidity meter called the DensiChekTM.

ii Identification cards were infected with microbe samples using an integrated vacuum system. A test tube containing the microorganism suspension was placed into a specific rack while the transfer tube was inserted into the matching suspension tube, and the identification card was placed in the next slot.

iii Once the vacuum was applied and air was re-introduced into the station, the organism suspension was pumped into micro-channels that filled all of the test wells.

iv The contaminated cards were passed via a machine that turned off the transfer tube and sealed the card before being deposited into the carousel incubator. The carousel incubator can hold up to 30 cards. All card kinds were incubated on-line at 35.5 + 1.0 °C. Each card was taken out of the carousel incubator every 15 minutes, delivered to the optical system, and then returned to the incubator until the next read time. Data was collected at 15 minute intervals during the incubation phase.

v The equipment took care of the rest; they controlled the incubation temperature, read the cards optically, and continuously monitored and transferred test results to the computer for analysis. The device automatically ejected the cards into a garbage receptacle after the test cycle was completed.

3.3.10 Antibiotic susceptibility test

Antibiotic discs used in this study were given in Table 3.1.

Table 3.1 The antibiotics used in this study and the manufacturer are listed

Antibiotics	Antibody concentration (µg)	Code	Company
Benicillin	25	PY	Bioanalyse (Turkey)
Penicillin G	30	P.G	
Ceftazidime	30	CAZ	
Erythromycin	10	E	
Gentamicin	10	CN	
Tetracycline	10	TE	

The sensitivity of *E. coli* to antibiotics was tested using the Baure-Kirby method as follows (Vandepitte *et al.* 2003) and my agencies:

A. 3-5 of the colonies that were grown on the medium of the MacConkey agar at 22 hours old were transferred to tube containing physiological saline 5 mL solution turbidity of the solution with the turbidity of McFarland's solution, which is equivalent to (10 x 1.5) cells/mL.

B. The sterile cotton swab was inserted into the tube containing the bacterial suspension and was rotated. Then pressed it against the inner wall of the tube to remove the excess inoculum, and then passed it on a plate contain Muller-Hinton medium several times and in different directions to obtain growth homogeneous.

C. Antibiotic tablets were placed on the culture medium's surface at equal dimensions the tablets were gently pressed using sterile forceps, and then the plates were incubated at 37 °C for 24 hours.

D. The results of the diameters of the damping areas around the discs were recorded in millimeters using a ruler, then the results were compared with standard tables (CLSI 2017).

3.3.11 Preservation of bacterial isolates as follows

Short-term preservation. The tubes containing the feeder agar were planted in a slanted manner bacterial isolates and were measured by schematic the isolates. Tubes were refreshed monthly using the procedure, and the tubes were incubated at 37 °C for 22 hours (Harley and Prescotte 2002).

Long-term preservation. Tubes containing 5 mL of heart and brain infusion broth medium supplemented with 15% glycerol were inoculated with isolates of bacteria *E. coli*. Then these tubes were incubated at 37 °C. The tubes were maintained at 21 °C for 22 hours before being used (WHO, 2003).

4. RESULTS AND DISCUSSION

4.1 Characteristic of Bacterial Culture

In our study, 50 samples of bacterial colonies were shown. Then they were grown on each of the MacConkey agar medium, the medium of eosin methyl blue agar, the medium of the blood agar, the medium of chromagen agar, and the medium of the hecton intestinal agar. The bacteria were found to be fermented lactose sugar, which produced pink, smooth, shiny, and sharp-edged colonies on the lacto-acid differential medium, which contains yellow salts and crystal violet dye. The isolates gave shiny green metallic colonies on the center of the eocene acre, in the blue methylene. Most of the bacterial isolates showed pink colonies on the chromagen agar media and small sharp-edged white colonies on the blood agar media bacteria produce glucuronidases-β enzymes that degrade conjugate chromatography when the carrier releases a β-glucuronid chromagenic substrate, it is called a chromagenic conjugate. The chromaphore gives a pink color to the bacterial colonies growing it on the middle of the blood agar was hemolysis-β, which indicates the production of the enzyme hemolysin. When bacterial colonies were grown on intestinal hecton agar medium, it was found that they showed yellow colonies. It turns orange in color as a result of bacteria fermenting the sugar lactose, as in the Figure 4.1

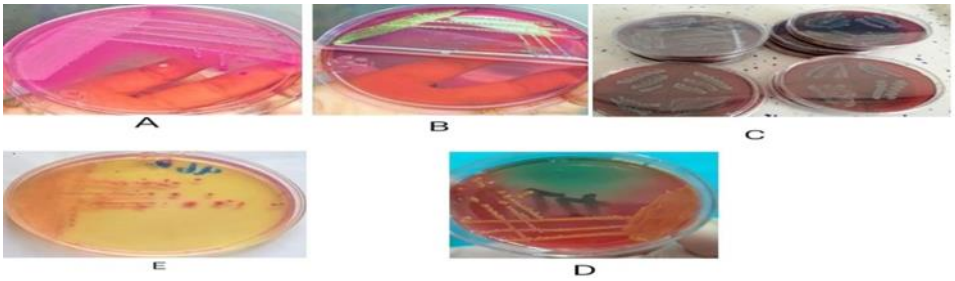

Figure 4.1 The phenotypic shape of colonies of *E. coli* on several diagnostic media, which are: (A: pink colonies medium MacConkey agar, B: glossy green metallic colonies on the methylene eosin agar medium, C: non-hemolytic milky white colonies on blood agar medium, D: pink colonies on chrome agar orientation medium and E: orange colonies on the medium of hecton intestinal agar).

4.2 Biochemical Identification

All isolates were tested for biochemical properties and all came back positive. *E. coli* used hydrogen peroxide reagent analysis to produce water and oxygen for catalase testing. The bacteria failed the oxidase test because they did not change color. Addition of the reagent turns the colonies purple because the bacteria lack the cytochrome enzyme. Oxidase bacteria lack the enzyme urease, and therefore cannot take up urea as a hydrogen acceptor, and the color of the medium did not change in the urease test, confirming that they did not consume urea. The results were given in Table 4.1.

Table 4.1 Diagnostic test results for *E. coli* (Negative; -, Poisitive; +)

Biochemical Test	Result
MacConkey agar	Lactose fermenting
EMB agar	green metalic sheen
Blood agar	variable
Chromagar orientation	small pink colonies
Hekton Enteric agar	Small orange colonies fermented for sugar Electro
Gram stain	-
Cell shape and grouping	rods bacilli
Urease	-
Catalase	+
Oxidase	-
methyl red	+
Indole	+
Citrate	-
Voges- Proskauer	-

4.3 VITEK 2 System Results

After the phenotypic results of the bacteria grown on the agar, the results of microscopic examinations and biochemical tests, 50 urine samples examined for men and women in VITEK 2 System. The results of phenotypic and biochemical test matched with VITEK 2-system results, and 50 isolates of *E. coli* were obtained.

4.4 Age Distribution of *E. coli*

In this study, *E. coli* infected UTI patients in females group were found to be 18 (54.5%) individuals in 10-29 age group, 10 (30.3%) individuals in 30-50 age group and

5 (15.2%) individuals in 51-70 age group, while it was found as 6 (35.3%) individuals in 10-29 age group, 8 (47.1%) individuals in 30-50 age group and 3 (17.6%) individuals in 51-70 age group in male patients (Figure 4.2). The total incidence of female cases is greater than males, so total number of infected in females was 33 (66%), and the total number of infected males was 17 (34%). Our results showed that *E. coli* isolates were lower in males under the 51-70 age group, which is in agreement with the articles documented by Gebremariam *et al.* (2019), which documented that *E. coli* isolation rates were lower in elderly 60 years males. It is preferable to conduct tests for females because they are more likely to be infected according to the results by taking samples of blood, sometimes urine, or other infected materials and sending them to the laboratory to be cultured. Finding bacteria in the sample confirms the diagnosis of the case. After determining the type of bacteria, another test may be done to see which antibiotics are effective for them.

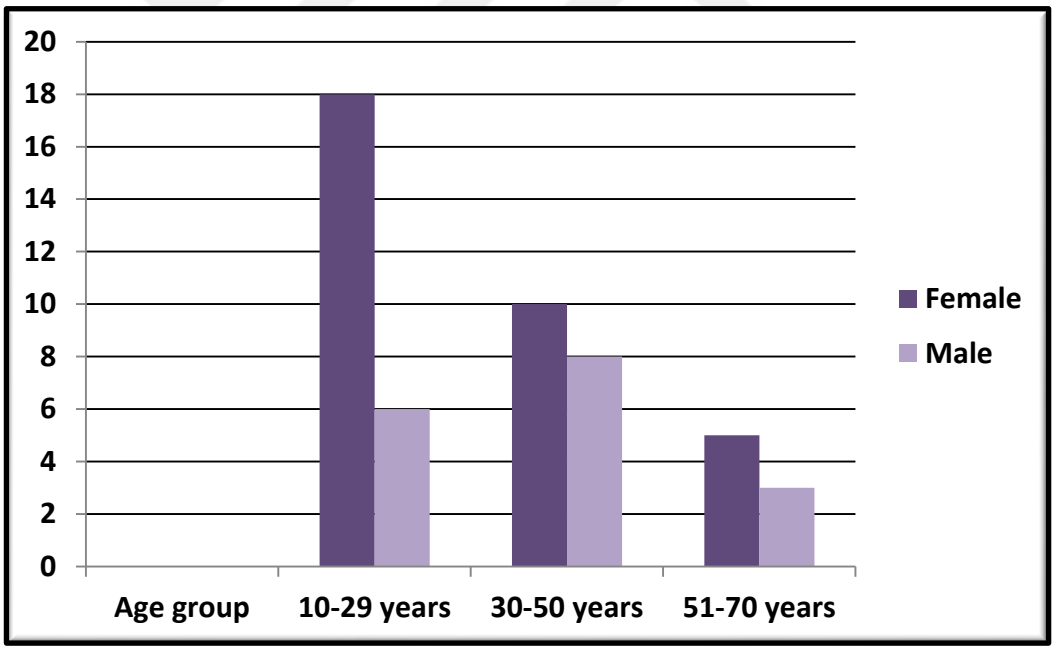

Figure 4.2 Descriptive statistics for the studied age group

4.5 Causes of Resistance to *E. coli* Bacteria

E. coli produces enzymes β-lactamases, including cephalosporinase and penicillinase, it is resistant to β-lactam antibiotics. Penicillins and cephalosporins, which work by breaking down the β-lactam ring, are inhibited by these enzymes. According to tetracycline resistance in *E. coli* may be attributed to a change in the outer membrane's (Kapoor, 2017).

4.6 *E. coli* Resistance to Antibiotics

Bacterial isolates were evaluated for sensitivity to 6 different antibiotics. All 50 isolates were found as carbenicillin-resistant bacteria (100%), while 49 (98%) samples were found to be an erythromycin resistant isolates. Antibiotic resistance were found as 26 (52%) of isolates penicillin G resistant, 43 (86%) of isolates ceftazidime resistant, 16 (32%) of isolates gentamicin resistant and 34 (68%) of isolates tetracycline resistant (Table 4.2 and Figure 4.3). As all bacterial isolates were multi-resistant to antibiotics, these tested antibiotics were ineffective.

Table 4.2 Antibiotic resistance patterns of *E. coli* isolates (E: Erythromycin, PY: Carbenicillin, CAZ: Ceftazidime, TE: Tetracycline, CN: Gentamicin, P.G: Penicillin G) (R: resistance, S: sensitive and I: intermedate)

No. of isolation	Source of infection	PY	E	TE	CN	CAZ	P.G
E -1	Urine	R	R	I	S	I	S
E -2	Urine	* R	R	R	R	R	R
E -3	Urine	R	* R	I	R	R	R
E -4	Urine	R	R	S	S	R	S
E -5	Urine	R	R	R	S	R	I
E -6	Urine	R	R	R	* S	R	R
E -7	Urine	R	R	R	S	R	R
E -8	Urine	R	* R	R	R	R	R
E -9	Urine	R	R	S	S	I	I
E -10	Urine	R	R	R	S	R	R
E -11	Urine	R	R	S	S	R	R
E -12	Urine	* R	R	R	R	I	S
E -13	Urine	R	R	R	* S	R	R
E -14	Urine	R	R	R	S	R	* S
E -15	Urine	R	R	I	S	R	R

E -16	Urine	R	R	R	S	R	R
E -17	Urine	R	* R	S	S	* R	R
E -18	Urine	R	R	R	R	R	S
E -19	Urine	* R	R	R	R	R	R
E -20	Urine	R	R	R	S	* R	S
E -21	Urine	R	R	S	S	I	S
E -22	Urine	R	R	S	S	R	S
E -23	Urine	R	R	R	S	R	S
E -24	Urine	R	R	R	R	R	R
E -25	Urine	R	R	S	R	S	S
E -26	Urine	* R	S	S	S	R	S
E -27	Urine	R	R	R	R	R	R
E -28	Urine	R	R	I	S	R	S
E -29	Urine	* R	R	R	S	R	R
E -30	Urine	R	R	R	S	R	S
E -31	Urine	R	R	* R	I	R	R
E -32	Urine	R	R	R	R	R	R
E -33	Urine	R	R	I	R	R	S
E -34	Urine	R	R	R	S	R	S
E -35	Urine	R	R	R	S	R	R
E -36	Urine	R	R	R	R	R	R
E -37	Urine	R	R	S	* S	R	R
E -38	Urine	R	* R	R	S	I	I
E -39	Urine	R	R	S	S	I	S
E -40	Urine	R	R	R	S	R	* R
E -41	Urine	R	R	R	R	R	S
E -42	Urine	R	R	R	S	R	* S
E -43	Urine	* R	R	R	S	R	S
E -44	Urine	R	R	R	R	R	R
E -45	Urine	R	R	* R	S	R	S
E -46	Urine	R	R	R	S	R	S
E -47	Urine	* R	R	R	R	R	R
E -48	Urine	R	* R	R	S	R	R
E -49	Urine	R	R	R	* S	R	R
E -50	Urine	R	R	S	R	* R	R

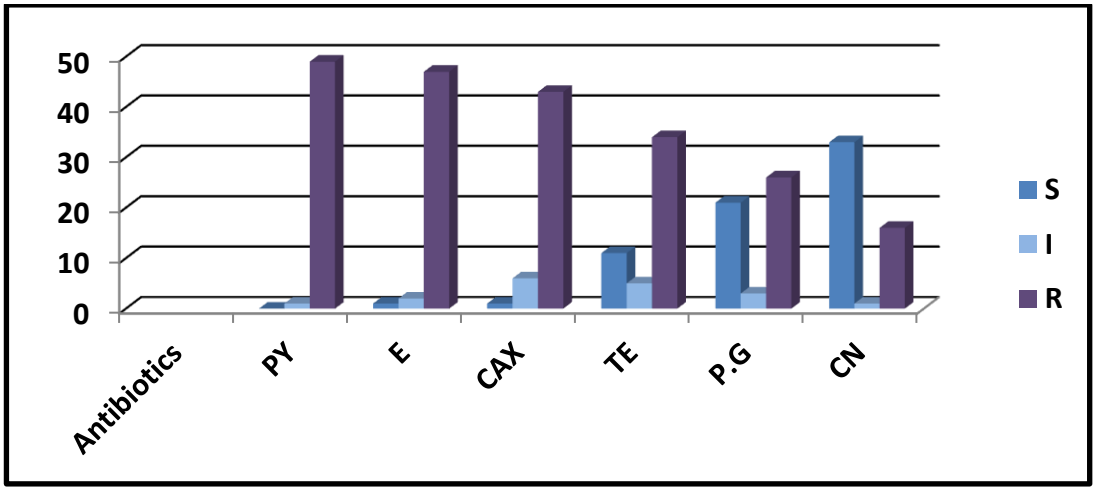

Figure 4.3 Distribution of antibiotic resistance (E: Erythromycin, PY: Carbenicillin, CAZ: Ceftazidime, TE: Tetracycline, CN: Gentamicin, P.G: Penicillin G) (R: resistance, S: sensitive and I: intermedate)

The results of the current study showed a very high resistance to the antibiotic erythromycin of the microlides, and these results were similar to the results reached by Kibret and Abera (2011) and Pricop (2015). They also found the antibiotic resistance as 89.4% and 81.8%, respectively. Kibret and Abera (2011) also reported that this results was agree with Onuoha and Fatokun (2014). Kafilzadeh and Farsimadan (2016) found bacterial resistance percentage as 52.8%, and this result is lower than our result. The results of the study for the antibiotic carbenicillin agree with previous studies, including the results of the researcher Ibrahim *et al.* (2014) in our study while 49 (98%) samples were found to be an erythromycin resistant isolates.

The results of the study for the antibiotic carbenicillin in which the proportion of bacterial isolates reached resistance to the antibiotic carbenicillin (100%) agree with previous studies, including the results of the researcher Ibrahim *et al.* (2014).

In our study 43 (86%) of 50 isolates were found as ceftazidime-resistant. Suresh *et al.* (2016) found the ceftazidime resistance in India as 98% and this result is agree with our result.

Also, this study agrees with the antibiotic tetracycline with the results reached by the researcher (Tajbakhsh *et al.* 2016) in Iran, where the rate of bacterial resistance to this antibiotic was (75%), as it was biofilm-producing as a result of having virulence factors.

In our study 16 (32%) of 50 isolates of were found as gentamicin resistant. And the results of the current study of the antibiotic gentamicin were consistent with the results reached by the researcher Suresh *et al.* (2016), which showed the rate of resistance to this antibiotic 37%.

In our study antibiotic resistance were found as 26 (52%) of isolates penicillin G resistant. Tajbakhsh *et al.* (2016) found penicillin G resistance as 25 (56%) and our finding is agree with Tajbakhsh *et al.* (2016).

4.7 Comparison Between Biofilm Production, Hemolysin and Urease in *E. coli*

The results of the biofilm were three (6%) of the isolates strong, five (10%) of isolates medium, and 37 (74%) of isolates weak, and five (10%) of isolates were negative. As for the results of hemolysin, it was three (6%) of the isolates were weak and 47 (94%) were negative. As for the urease results, they were completely negative. All these details are explained in the Table 4.3 and Figure 4.4.

Table 4.3 Factors of *E. coli* include the ability to form biofilm, hemolysin and urea enzyme production (Negative: –, Weak biofilm formation: +, Medium biofilm formation: ++, Strong biofilm formation: +++, Blood analyzer: β+)

Isolation symbol	Sex	Sources	Biofilm	Hemolysin	Urease
E1	Female	Abi Ghraib Hospital	+	-	-
E2	Female	Abi Ghraib Hospital	+++	-	-

E3	Female	Abi Ghraib Hospital	+	-	-
E4	Male	Abi Ghraib Hospital	+	-	-
E5	Female	Abi Ghraib Hospital	++	-	-
E6	Male	Abi Ghraib Hospital	+	-	-
E7	Female	Abi Ghraib Hospital	+	-	-
E8	Female	Abi Ghraib Hospital	+	-	-
E9	Male	Abi Ghraib Hospital	+	-	-
E10	Female	Abi Ghraib Hospital	+	-	-
E11	Male	Abi Ghraib Hospital	-	-	-
E12	Male	Abi Ghraib Hospital	+	-	-
E13	Female	Abi Ghraib Hospital	+	-	-
E14	Male	Abi Ghraib Hospital	+	-	-
E15	Female	Yarmouk Teaching Hospital	+	-	-
E16	Male	Yarmouk Teaching Hospital	+	-	-
E17	Male	Yarmouk Teaching Hospital	+	-	-
E18	Female	Yarmouk Teaching Hospital	+	-	-
E19	Male	Yarmouk Teaching	+	-	-

		Hospital			
E20	Male	Yarmouk Teaching Hospital	+	-	-
E21	Female	Yarmouk Teaching Hospital	-	-	-
E22	Female	Yarmouk Teaching Hospital	+++	-	-
E23	Female	Yarmouk Teaching Hospital	+	-	-
E24	Male	Yarmouk Teaching Hospital	+	-	-
E25	Female	Yarmouk Teaching Hospital	++	β+	-
E26	Female	Yarmouk Teaching Hospital	+	β+	-
E27	Male	Yarmouk Teaching Hospital	+++	-	-
E28	Female	Yarmouk Teaching Hospital	+	-	-
E29	Female	Yarmouk Teaching Hospital	+	-	-
E30	Male	Yarmouk Teaching Hospital	++	-	-
E31	Female	Yarmouk Teaching Hospital	-	-	-
E32	Female	Yarmouk Teaching	+	-	-

		Hospital			
E33	Female	Yarmouk Teaching Hospital	+	-	-
E34	Female	Al-Tifl Teaching Hospital	+	-	-
E35	Female	Al-Tifl Teaching Hospital	+	-	-
E36	Female	Al-Tifl Teaching Hospital	+	-	-
E37	Female	Al-Tifl Teaching Hospital	+	-	-
E38	Female	Al-Tifl Teaching Hospital	++	-	-
E39	Female	Al-Tifl Teaching Hospital	-	-	-
E40	Female	Al-Tifl Teaching Hospital	+	-	-
E41	Female	Al-Tifl Teaching Hospital	-	-	-
E42	Male	Al-Tifl Teaching Hospital	++	-	-
E43	Female	Al-Tifl Teaching Hospital	+	-	-
E44	Female	Al-Tifl Teaching Hospital	+	-	-
E45	Male	Al-Tifl Teaching	+	-	-

		Hospital			
E46	Female	Al-Tifl Teaching Hospital	+	β+	-
E47	Female	Al-Tifl Teaching Hospital	+	-	-
E48	Male	Al-Tifl Teaching Hospital	+	-	-
E49	Female	Al-Tifl Teaching Hospital	+	-	-
E50	Male	Al-Tifl Teaching Hospital	+	-	-

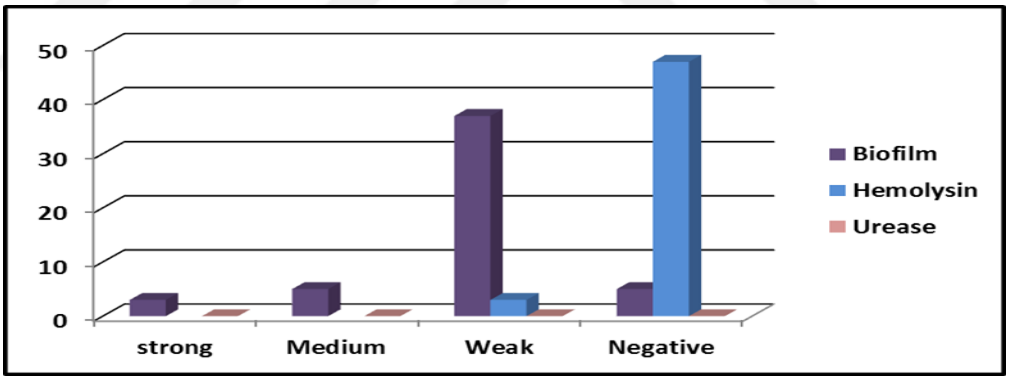

Figure 4.4 The virulence factors of *E. coli*

5. CONCLUSIONS AND RECOMMENDATION

•High incidence of urinary tract infection by the bacterium *E. coli* in hospitals Baghdad city.

•The current study analyzed the phenotypic characteristics of *E. coli* with urinary tract infection (UTI).

•Women are more likely than males to get a UTI. Percentage of females was found as 66%. Women are more likely than men to get a urinary tract infection for a variety of reasons. Bacteria can enter the urethra during sexual activity. Certain forms of contraception can cause the UTI. Condom-wearing women are more likely to get a UTI. Pregnancy has a higher probability of contracting an infection. A urinary tract infection affects many hormone levels in postmenopausal women. Even girls and women who are not sexually active can get lower urinary tract infections because bacteria found in the female vaginal area can cause cystitis.

•It includes the most important methods of prevention. Water drinking helps dilute the urine and remove microorganisms. Until the infection clears up, avoid liquids that may irritate the bladder, as well as coffee and carbonated alcohol containing citrus juices or caffeine.

•The isolated *E. coli* bacteria were resistant to β-lactam antibiotics.

•The isolated *E. coli* bacteria had very dangerous virulence factors as biofilm and hemolysin.

REFERENCES

Abdelhamid, S. M., and Abozahra, R. R. 2017. Expression of the Fluoroquinolones Efflux Pump Genes Acra And Mdfa in Urinary Escherichia coli Isolates. PJM., 66: 25-30.

Afzal, A. M. S., 2017. Antibiotic Resistance Pattern of *Escherichia coli* and *Klebsiella* Species in Pakistan: A Brief Overview. J Microb. Biochem. Technol., 9: 277-279.

Ali, J., Rafq, Q. A., and Ratcliffe, E. 2018. Antimicrobial Resistance Mechanisms and Potential Synthetic Treatments. Future Sci. 4:1-6.

Alqasim, A., Abu Jaffal, A., and Alyousef, A. A. 2018. Prevalence of multidrug resistance and extended-spectrum β-lactamase carriage of clinical uropathogenic *Escherichia coli* isolates in Riyadh, Saudi Arabia. International Journal of Microbiology, 2018: 3026851.

Amaral, L. Martins, A. Spengler, G. and Molnar, J. 2014. Efflux Pumps of Gram-Negative Bacteria: What They Do, How They Do it, with What and How to Deal with Them. Front Pharmacol., 4: 168.

Alav, I., Sutton, J. M. and Rahman, K. M. 2018. Role of Bacterial Efflux Pumps in Biofilm Formation. J Antimicrob Chemother., 73: 2003-2020.

Ambler, R. P., Coulson, A. F., Frere, J. M., Ghuysen, J. M., Joris, B., Forsman. M., Levesque, R. C., Tiraby, G. and Waley, S. G. 1991. A standard numbering scheme for the class A beta-lactamases. Biochem J., 276: 269-270.

Anes, J., Mccusker, M. P., Fanning, S. and Martins, M. 2015. The Ins and Outs of RND Efflux Pumps in *Escherichia coli*. Front Microbiol., 6:1-14.

Asensio, A., Oliver, A., González-Diego, P., Baquero, F., Perez-Diaz, J. C., Ros, P., Cobo, J., Palacios, M., Lasheras, D. and Cantón, R. 2000. Outbreak of a multiresistant *Klebsiella pneumoniae* strain in an intensive care unit: antibiotic use as risk factor for colonization and infection. Clinical Infectious Diseases, 30: 55-60.

Basak, S. Singh, P. and Rajurkar, M. 2016. Multidrug Resistant and Extensively Drug Resistant Bacteria: A Study. Journal of Pathogens, 2016: 4065603.

Ben-Ami, R., Schwaber, M. J., Navon-Venezia, S., Schwartz, D., Giladi, M., Chmelnitsky, I., Leavitt, A. and Carmeli, Y. 2006. Influx of extended-spectrum β-lactamase-producing Enterobacteriaceae into the hospital. Clinical Infectious Diseases, 42: 925-934.

Bhattacharyya, S., Sarfraz, A., Ansari, M. A. A. and Jaiswal, N. 2015. Characterization and antibiogram of Uropathogenic *Escherichia coli* from a tertiary care hospital in Eastern India. Int J Curr Microbiol App Sci., 4: 701-705.

Blanco, P., Hernando-Amado, S., Reales-Calderon, J. A., Corona, F., Lira, F., Alcalde-Rico, M., Bernardini, M., Sanchez, M. B. and Martinez, J. L. 2016. Bacterial Multidrug Efflux Pumps: Much More than Antibiotic Resistance Determinants. Microorganisms., 4: 2-19.

Bonnet, R. 2004. Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes. Antimicrob. Agents Chemother., 48: 1-14.

Bray, J. 1945. Isolation of antigenically homogeneous strains of Bacteria *E. coli* neopolitanum from summer diarrhea of infants. J Pathol Bacteriol., 57: 239-247.

Brown, A. E. and Smith, H. R. 2017. Benson's Microbiological Applications Laboratory Manual in General Microbiology. 14th ed. McGraw-Hill Higher Education, 438 page, New York.

Bush, K., Jacoby, G. A. and Medeiros A.A. 1995. A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob. Agents Chemother., 39: 1211-1233.

CLSI. 2017. Performance Standard for Antimicrobial Susceptibility Testing. 27th ed CSLI Supplement M100.Wayne,PA:Clinical and Laboratory Standards Institute.32-41.

Cosgrove, S. E., Kaye, K. S., Eliopoulous, G. M. and Carmeli, Y. 2002. Health and economic outcomes of the emergence of third-generation cephalosporin resistance in Enterobacter species. Archives of Internal Medicine, 162: 185-190.

De Champs, C., Sirot, D., Chanal, C., Poupart, M.-C., Dumas, M.-P., and Sirot, J. 1991. Concomitant dissemination of three extended-spectrum β-lactamases

among different Enterobacteriaceae isolated in a French hospital. Journal of Antimicrobial Chemotherapy, 27:441–457.

Delmar, J. A., Su, C. C. and Yu, E. W. 2014. Bacterial Multidrug Efflux Transporters. Annu Rev Biophys., 43: 93-117.

Forbes, B. A. Saham, D. F. and Weissfeld, A. S. 2007. Baily and Scott's Diagnostic Microbiology. 12th ed. Mosby, Inc. an anffilliate of Elsevier, Inc.1031 page, China.

Foxman, B. 2013. Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. Infectious Disease Clinics of North America, 28: 1–13.

Forsyth, V. S., Armbruster, C. E., Smith, S. N., Pirani, A., Springman, A., Walters, M. S., Nielubowicz, G. R., Himpsl, S. D., Snitkin, E. S. and Mobley, H. L. T. 2018. Rapid Growth of Uropathogenic *Escherichia coli* During Human Urinary Tract Infection. mBio, 9: e00186-18.

Foxman, B. 2014. Urinary Tract Infection Syndromes Occurrence, Recurrence Bacteriology, Risk Factors, and Disease Burden. Infect Dis Clin N Am., 28: 1-13.

Garrity, G. M., Brenner, D. J., Krieg, N. R., Staley, J. T., Chairman, J., Boone, D. R., Chairman, V., Devos, P., Goodfellow, M., Rainey, F. A. and Schleifer, K-H. 2005. Bergey's Manual of Systematic Bacteriology. Vol Two. Part B. 2nd ed. Springer, 1106 page, USA.

Gebremariam, G., Legese, H., Woldu, Y., Araya, T., Hagos, K. and Gebreyesus Wasihun, A. 2019. Bacteriological profile, risk factors and antimicrobial susceptibility patterns of symptomatic urinary tract infection among students of Mekelle University, northern Ethiopia. BMC Infectious Diseases, 19: 1-11.

Gharajalar, S. N. and Sofiani, V. H. 2017. Patterns of Efflux Pump Genes Among Tetracycline Resistance Uropathogenic *Escherichia coli* Isolates Obtained from Human Urinary Infections. Jundishapur J Microbiol., 10: e40884.

Gillor, O., Kirkup, B. C. and Riley, M. A. 2004. Colicins and microcins: the next generation antimicrobials. Advances in Applied Microbiology, 54: 129-146.

Giske, C. G., Sundsfjord, A. S., Kahlmeter, G., Woodford, N., Nordmann, P., Paterson, D. L., Canton, R. and Walsh, T. R. 2009. Redefining extended-spectrum β-lactamases: balancing science and clinical need. Journal of Antimicrobial Chemotherapy, 63: 1-4.

Gordon, K. A. and Jones, R. N. 2003. Susceptibility patterns of orally administered antimicrobials among urinary tract infection pathogens from hospitalized patients in North America. Diagn. Microbiol. Dis., 45: 295-301.

Hadi, O. M., Al-Maliki, A. H., Al-Zubaidy, M. S. M. and Nihmah, Y. K. 2014. Prevalence of Uropathogenic *Escherichia coli* in Al-Hashymia District of Babylon Province. JUBPAS, 9: 2479-2488.

Harley, J. P. and Prescott, L. M. 2002. Laboratory Exercises in Microbiology. 5th ed. McGraw-Hill Education, 466 page, New York.

Hemraj, V., Diksha, S. and Avneet, G. 2013. A Review on Commonly Used Biochemical Test for Bacteria. IJLS, 1: 1-7.

Ibrahim, I. A., Al-Shwaikh, R. M. and Ismaeil, M. I. 2014. Virulence and Antimicrobial Resistance of *Escherichia coli* Isolated from Tigris River and Children Diarrhea. Infect Drug Resist., 7: 317-322.

Jacoby, G. A. and Carreras, I. 1990. Activities of beta-lactam antibiotics against *Escherichia coli* strains producing extended-spectrum beta-lactamases. Antimicrobial Agents and Chemotherapy, 34 5: 858-862.

Jacoby, R. F., Schlack, S., Sekhon, G. and Laxova, R. 1997. Del associated with juvenile polyposis. Am. J. Med. Genet., 70: 361-364.

Jawetz, E., Melnick, J. A. and Adelberg, E. A. 2016. Review of Medical Microbiology 27th ed. McGraw-Hill education, 851 page, New York.

Jett, B. D., Ritchie, D. J., Reichley, R., Bailey, T. C. and Sahm, D. F. 1995. In vitro activities of various beta-lactam antimicrobial agents against clinical isolates of *Escherichia coli* and *Klebsiella* spp. resistant to oxyimino cephalosporins. Antimicrobial Agents and Chemotherapy, 39: 1187-1190.

Kafilzadeh, F. and Farsimadan, F. 2016. Investigating Multidrug Efflux Pumps in Relation to the Antibiotic Resistance Pattern in *Escherichia coli* Strains from Patients in Iran. Biomed Res., 27: 1130-1135.

Kapoor, J., Saigal, S. and Elongavan, A. 2017 Action and Resistance Mechanisms of Antibiotics: A Guide for Clinicians. J Anaesthesiol Clin Pharmacol., 33: 300-305.

Kaye, S. B., Sims, G., Willoughby, C., Field, E. A., Longman, L. and Brown, M. C. 2001. Modification of the tear function index and its use in the diagnosis of Sjögren's syndrome. Br J Ophthalmol, 85: 193-199.

Kibret, M. and Abera, B. 2011. Antimicrobial Susceptibility Patterns of *E. coli* from Clinical Sources in Northeast Ethiopia. Afr Health Sci., 11: 40-45.

Kotsyuba, K. R., Voronkova, O. S., Vinnikov, A. I. and Shevchenko, T. M. 2014. Mechanisms of Antibiotic Resistance of Enterobacteriaceae Family Representatives. Vìsn Dnìpropetr Unìv Ser Bìol Med., 5: 33-38.

Kucheria, R., Dasguptaa, P., Sacks, S., Khan, M. and Sheerin, N. 2005. Urinary tract infections: new insights into a common problem. Postgraduate Medical Journal, 81(952): 83-86.

Levinson, W. 2016. Review of Medical Microbiology and Immunology. 14th ed. McGraw-Hill education, 821 page, New York.

Malema, M. S., Abia, A. L. K., Tandlich, R., Zuma, B., Kahinda, J. M. M. and Ubomba-Jaswa, E. 2018. Antibiotic-Resistant Pathogenic *Escherichia coli* Isolated from Rooftop Rainwater-Harvesting Tanks in the Eastern Cape, South Africa. Int J Environ Res. Public Health, 15: 1-14.

Mangeney, A., Heinrich, P. and Roche, R. 2000. Analytical Solution for Testing Debris Avalanche Numerical Models. Pure Appl. Geophys., 157: 1081-1096.

Mirzarazi, M., Rezatofghi, S. E., Pourmahdi, M. and Mohajeri, M. R. 2013. Antibiotic Resistance of Isolated Gram Negative Bacteria from Urinary Tract Infections (UTIs) in Isfahan. Jundishapur J Microbiol., 6: 1-5.

Neamati, F., Firoozeh, F., Saffari, M. and Zibaei, M. 2015. Virulence Genes and Antimicrobial Resistance Pattern in Uropathogenic *Escherichia coli* Isolated from Hospitalized Patients in Kashan, Iran. Jundishapur J Microbiol., 8: 1-6.

Nies, D. H. 2003. Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbiology Reviews, 27: 313-319.

Nordmann, P. and Poirel, L. 2002. Emerging carbapenemases in Gram-negative aerobes. Clinical Microbiology and Infection, 8: 321-331.

Olowe, B. M., Oluyege, J. O., Famurewa, O., Ogunniran, A. O. and Adelegan, O. 2017. Molecular Identification of *Escherichia coli* and New Emerging Enteropathogen, *Escherichia fergusonii*, from Drinking Water Sources in Ado- Ekiti, Ekiti State, Nigeria. J Microbiol Res., 7: 45-54.

Onuoha, S. C. and Fatokun, K. 2014. Prevalence and Antimicrobial Susceptibility Pattern of Urinary Tract Infection (UTI) Among Pregnant Women in Afikpo, Ebonyi State, Nigeria. AJLS, 2: 46-52.

Paltansing, S. 2015. Antimicrobial Resistance in Enterobacteriaceae: Characterization and Detection. Leiden University Press,155 page, Netherlands.

Poursina, F., Sepehrpour, S. and Mobasherizadeh, S. 2018. Bioflm Formation in Nonmultidrug Resistant *Escherichia coli* Isolated from Patients with Urinary Tract Infection in Isfahan, Iran. Adv Biomed Res., 7: 1-5.

Pricop, C., Suditu, N., Vrinceanu, R., Puia, D., Dimitriu, D. C., Ciuta, C., Todosi, L. and Checherita, I. A. 2015. Multidrug Resistant Urinary Tract Infections in Moldova, Romania: Focusing on Uropathogens and Their Antibiotic Susceptibility. Can We Do More?. Nobel Med., 11: 42-49.

Puzari, M. and Chetia, P. 2017. RND efflux pump mediated antibiotic resistance in Gram-Negative Bacteria *Escherichia coli* and *Pseudomonas aeruginosa*: A Major Issue Worldwide. World J Microbiol Biotechnol., 33: 1-8.

Rivas, L., Mellor, G. E., Gobius, K. and Fega, N. 2015. Detection and Typing Strategies for Pathogenic *Escherichia coli*. Springer, 110 page, New York.

Rodriguez-Baño, J., Gutiérrez-Gutiérrez, B., Machuca, I. and Pascual, A. 2018. Treatment of infections caused by extended-spectrum-beta-lactamase-, AmpC-, and carbapenemase-producing Enterobacteriaceae. Clinical Microbiology Reviews, 31: e00079-17.

Shariff, A., Shenoy, M. S., Yadav, T. and Radhakrishna, M. 2013. The Antibiotic Susceptibility Patterns of Uropathogenic *Escherichia coli*, with Special Reference to the Fluoroquinolones. J Of Clin And Dia Res., 7: 1027-1030.

Shrestha, R., Khanal, S., Poudel, P., Khadayat, K., Ghaju, S., Bhandari, A., Lekhak, S., Pant, N. D., Sharma, M. and Marasini, B. P. 2019. Extended spectrum β-

lactamase producing uropathogenic *Escherichia coli* and the correlation of biofilm with antibiotics resistance in Nepal. Annals of Clinical Microbiology and Antimicrobials, 18: 1–6.

Silva, D. P., Schofield, M. C., Parsek, M. R. and Tseng, B. S. 2017. An Update on the Sociomicrobiology of Quorum Sensing in Gram-Negative Biofilm Development. Pathogens, 6: 1-9.

Simonsen, G. S., Blix, H. S., Grave, K. and Urdahl, A. M. 2017. NORM/NORM-VET 2016-Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Norweigen Veterinary Enstitute, 151 page, Oslo.

Soltani, S., Emamie, A. D., Dastranj, M., Farahani, A., Davoodabadi, A. and Mohajeri, P. 2018. Role of Toxins of Uropathogenic *Escherichia coli* in Development of Urinary Tract Infection. JPRI, 21: 1-11.

Soto, S. M. 2013. Role of Efflux Pumps in the Antibiotic Resistance of Bacteria Embedded in a Biofilm.Virulence, 4: 223-229.

Soto, S. M. 2014. Importance of Biofilms in Urinary Tract Infections: New Therapeutic Approaches. Advances in Biology, 2014: 543974.

Spaulding, C. N., Klein, R. D., Ruer, S., Kau, A. L., Schreiber, H. L., Cusumano, Z. T., Dodson, K. W., Pinker, J. S., Fremont, D. H., Janetka, J. W., Remaut, H., Gordon, J. I. and Hultgren, S. J. 2017. Selective Depletion of Uropathogenic *E. coli* from the Gut by a FimH Antagonist. Nature, 546: 528-532.

Steward, C. D., Wallace, D., Hubert, S. K., Lawton, R., Fridkin, S. K., Gaynes, R. P., McGowan Jr, J. E. and Tenover, F. C. 2000. Ability of laboratories to detect emerging antimicrobial resistance in nosocomial pathogens: a survey of project ICARE laboratories. Diagnostic Microbiology and Infectious Disease, 38(1): 59-67.

Suresh, M., Nithya, N., Jayasree, P. and Kumar, M. P. 2016. Detection and Prevalence of Efflux Pump-Mediated Drug Resistance in Clinical Isolates of Multidrug-Resistant Gram-Negative Bacteria from North Kerala, India. Asian J Pharm Clin Res., 19: 324-327.

Tadesse, A. and Alem, M. 2006. Medical Bacteriology. EPHTI, 433 page, Ethiopia.

Tajbakhsh, E., Ahmadi, P., Abedpour-Dehkordi, E., Arbab-Soleimani, N. and Khamesipour, F. 2016. Biofilm Formation, Antimicrobial Susceptibility, Serogroups and Virulence Genes of Uropathogenic *E. coli* Isolated from Clinical Samples in Iran. Antimicrob Resist Infect Control., 5: 11.

Terlizzi, M. E., Gribaudo, G. and Maffei, M. E. 2017. Uropathogenic *Escherichia coli* UPEC Infections: Virulence Factors, Bladder Responses, Antibiotic, and Non-Antibiotic Antimicrobial Strategies. Front Microbiol., 8: 1-23.

Thomson, K. S. and Moland, E. S. 2001. Cefepime, piperacillin-tazobactam, and the inoculum effect in tests with extended-spectrum β-lactamase-producing Enterobacteriaceae. Antimicrobial Agents and Chemotherapy, 45: 3548-3554.

Tille, P.M. 2017. Baily And Scott's Diagnostic Microbiology. 41th ed. Elsevier, 1115 page, China.

Wachino, J., Doi, Y., Yamane, K., Shibata, N., Yagi, T., Kubota, T., Ito, H. and Arakawa, Y. 2004. Nosocomial spread of ceftazidime-resistant *Klebsiella pneumoniae* strains producing a novel class A β-lactamase, GES-3, in a neonatal intensive care unit in Japan. Antimicrobial Agents and Chemotherapy, 48: 1960-1967.

Wanger, A., Chavez, V., Huang, R. S. P., Wahed, A., Actor, J. K. and Dasgupta, A. 2017. Microbiology and Molecular Diagnosis in Pathology. Elsevier Inc., 304 page, USA.

WHO (World Health Organization). 2003. Basic Laboratory Procedures in Clinical Bacteriology. 2nded. Geneva. Switzerland.

Vandepitte, J., Verhaegen, J., Engbaek, K., Rohner, P., Piot, P. and Heuck, C. C. 2003. Bacteriological Investigations. In: World Health Organization.2nd ed. Basic Laboratory Procedures in Clinical Bacteriology. WHO, 167 page, Geneva.

Venter, H., Mowla, R., Ohene-Agyei, T. and Ma, S. 2015. RND-type drug efflux pumps from Gram-negative bacteria: molecular mechanism and inhibition. Front. Microbiol., 6: 377.

Yuan, M., Aucken, H., Hall, L. M., Pitt, T. L. and Livermore, D. M. 1998. Epidemiological typing of klebsiellae with extended-spectrum beta-

lactamases from European intensive care units. The Journal of Antimicrobial Chemotherapy, 41: 527-539.

Zhang, L. and Foxman, B. 2003. Molecular epidemiology of *Escherichia coli* mediated urinary tract infections. Front Biosci, 8: 235-244.

Zhang, C., Chen, X., Stephanopoulos, G. and Too, H-P. 2016. Efflux Transporter Engineering Markedly Improves Amorphadiene Production in *Escherichia coli*. Biotechnology and Bioengineering, 113: 1755-1763.

Zhi Li, X., Elkins, C. A. and Zgurskaya, H. I. 2016. Efflux-Mediated Antimicrobial Resistance in Bacteria Mechanisms, Regulation And Clinical Implications. International Publishing, 848 page, Switzerland.

Zowawi, H. M., Harris, P. N. A., Roberts, M. J., Tambyah, P. A., Schembri, M. A., Pezzani, M. D., Williamson, D. A. and Paterson, D. L. 2015. The Emerging Threat of Multidrug-Resistant Gram-Negative Bacteria in Urology. Nat Rev Urol., 12: 570-584.

APPENDICES

APPENDIX 1. Laboratory equipments, culture media, chemical and biological solutions, reagents and stains

APPENDIX 2. Patients information documents

APPENDIX 3. Permission of Ministry of Health for study

Appendix 1. Laboratory equipments, culture media, chemical and biological solutions, reagents and stains

Table of laboratory equipment used for work

Equipments and apparatus	Company	Origin
Autoclave	Hiramyama	Japan
Centrifuge	Memmert	Germany
Electric balance	Denver	Canada
ELISA reader	HS-Human Reader	Germany
Eppendorf tubes	Fischer	USA
Hot plate with magnetic stirrer	Labcoo	Germany
Incubator	Memmert	Germany
Micropipette	Brand	Germany
Oven	Beko	Turkey
Water bath	Memmert	Germany
Water distillatory	Buchi	Switzerland
Well flat bottom plate	Coastar	USA
Refrigerator	Concord	Lebanon
Flow air laminar (hood)	Labnet	USA
VITEK 2 System device	Bio mérieux	France

Table of chemical materials

The device	**Company**	**Origin**
Hydrogen peroxide	BDH	England
H_2SO_4	BDH	England
NaCl	BDH	England
Ethanol (70%)	BDH	England
Glycerol	Bioneer	Korea

Table of the agar culture media used in the study

Media	**Company**	**Origin**
Blood Agar Base	Himadia	India
Chromagar Orientation	CROMagar	
MacConkey Agar	Himedia	India
Urea Agar Base	Himedia	India
Blue Methylelen Eosin Agar	Oxoid	England
Trypton Soy Agar	Oxoid	England
Simmon Citrat Agar	Himadia	India
Muller-Hinton Agar	Oxoid	England

Table of other materials

Tools	Company	Origin
Petri dishes	AFCO	Jordan
Microtiter wells plates	AFMA	Jordan
Tubes eppendorf	AFCO	Jordan
Flasks, Beakers, Tubes	----	Jordan
Swabs cotton	AFCO	Jordan
Transporter swabs	Girenier	Jordan
Loop	Himedia	India
Millipore filter (0.22)	AFCO	Jordan

Table of pigments, reagents and solutions used in the study

Solutions, stains and reagents	Company	Origin
Normal saline solution	Himedia	India
Methyl red regent	Himedia	India
Kovac`s Regent	Himedia	India
Voges-Proskauer	Himedia	India
Gram stains Crystal violet Safranin stain Iodine Ethanol 69%	Buchi	Switzerland
EDTA	Biobasic INC	
Saline buffer phosphate	Chemical Point	

Appendix 2. patient information document

Number of Patients	Sample Type	Age	Sex	Date	Notes
1. Patient	**Urine**	**10**	**Female**	**10\03\2021** **01\07\2021**	**Isolates were collected from several hospitals in Iraq – Baghdad**
2. Patient	**Urine**	**13**	**Female**		
3. Patient	**Urine**	**16**	**Male**		
4. Patient	**Urine**	**15**	**Female**		
5. Patient	**Urine**	**19**	**Male**		
6. Patient	**Urine**	**17**	**Female**		
7. Patient	**Urine**	**20**	**Female**		
8. Patient	**Urine**	**23**	**Male**		
9. Patient	**Urine**	**22**	**Female**		
10. Patient	**Urine**	**26**	**Male**		
11. Patient	**Urine**	**29**	**Male**		
12. Patient	**Urine**	**24**	**Female**		
13. Patient	**Urine**	**30**	**Male**		
14. Patient	**Urine**	**28**	**Female**		
15. Patient	**Urine**	**33**	**Male**		
16. Patient	**Urine**	**36**	**Male**		
17. Patient	**Urine**	**27**	**Female**		
18. Patient	**Urine**	**37**	**Male**		
19. Patient	**Urine**	**18**	**Female**		
20. Patient	**Urine**	**40**	**Male**		
21. Patient	**Urine**	**29**	**Female**		
22. Patient	**Urine**	**25**	**Female**		
23. Patient	**Urine**	**24**	**Female**		
24. Patient	**Urine**	**43**	**Male**		
25. Patient	**Urine**	**19**	**Female**		
26. Patient	**Urine**	**16**	**Female**		
27. Patient	**Urine**	**49**	**Male**		
28. Patient	**Urine**	**17**	**Female**		
29. Patient	**Urine**	**22**	**Female**		
30. Patient	**Urine**	**50**	**Male**		
31. Patient	**Urine**	**23**	**Female**		
32. Patient	**Urine**	**30**	**Female**		

33. Patient	**Urine**	**33**	**Female**		
34. Patient	**Urine**	**35**	**Female**		
35. Patient	**Urine**	**37**	**Female**		
36. Patient	**Urine**	**39**	**Female**		
37. Patient	**Urine**	**70**	**Female**		
38. Patient	**Urine**	**41**	**Female**		
39. Patient	**Urine**	**44**	**Female**		
40. Patient	**Urine**	**46**	**Female**		
41. Patient	**Urine**	**67**	**Female**		
42. Patient	**Urine**	**70**	**Male**		**Isolates were collected from several hospitals in Iraq – Baghdad**
43. Patient	**Urine**	**53**	**Female**		
44. Patient	**Urine**	**48**	**Female**	**10\03\2021**	
45. Patient	**Urine**	**50**	**Female**		
46. Patient	**Urine**	**55**	**Female**	**01\07\2021**	
47. Patient	**Urine**	**59**	**Female**		
48. Patient	**Urine**	**51**	**Male**		
49. Patient	**Urine**	**66**	**Female**		
50. Patient	**Urine**	**62**	**Male**		

Appendix 3. Permission of Ministry of Health for study

Ministry of Health

Al-Karkh Health Directorate

Training and human development center

Research Committee

Resolution No. 15/6/2021

Research Committee Decision

The Research Committee of Al-Karkh Health Directorate studied the research project No. (6/2021) entitled:

Diagnosis of E.coli which is isolated from urinary system and antibiotic resistance of beta-lactam in Iraq

Submitted by the researcher (NOORULDEEN KHALID ABED AL-ZOBAIE) to the Research and Development Management Unit of the Training and Human Development Center in Al-Karkh Health Directorate

On June 14, 2021 and they decided:

"Approval of the research project as presented, and there is no objection to its implementation in the institutions of the Directorate"

CURRICULUM VITAE

Personal Information

Name and Surname **:** Nooruldeen Khalid Abed AL-ZOBAIE

Education

MSc Çankırı Karatekin University Graduate School of Science 2022 Present
Department of Biology

Undergraduate Al-Farabi University College, College of Science,
Department of Life Sciences 2015-2019

Work Experience

Year	**Institution**	**Position**
2017	Abu Ghraib Hospital	Analyzer