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# INVESTIGATION OF OXA TYPE GENES IN MULTIDRUG RESISTANT *ESCHERICHIA COLI* ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTIONS IN BAGHDAD

**IN PARTIAL FULFILLMENT OF THE REQUIREMENTS**
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**BY**

**MUNA MAJEED ALI AL-MUSAWI**

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INVESTIGATION OF OXA-TYPE GENES IN MULTIDRUG-RESISTANT *ESCHERICHIA COLI* ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTIONS IN BAGHDAD

By Muna Majeed Ali AL-MUSAWI

October 2023

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**ABSTRACT**

# INVESTIGATION OF OXA TYPE GENES IN MULTIDRUG RESISTANT *ESCHERICHIA COLI* ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTIONS IN BAGHDAD

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October 2023

*Escherichia coli*, commonly known as *E. coli*, emerges as the predominant causative agent in urinary tract infections. The primary objective of this research is to meticulously isolate pathogenic strains of *E. coli* responsible for inducing inflammation in the urinary tract. The investigation aims to comprehensively evaluate the resistance of these strains to specific chemotherapeutic agents, particularly those linked to the oxa-type gene. In the examination of 150 urine samples, *E. coli* was discerned in 75 samples using the advanced Vitek 2 compact technology. The isolated strains of *E. coli* exhibited substantial resistance to a spectrum of antibiotics. The resistance percentages were notably high for various antibiotics, including Amoxicillin/Clavulanic Acid (90.7%), Piperacillin/tazobactam (86.7%), Imipenem (86.7%), Ticarcillin-clavulanic acid (74.7%), Meropenem (73.3%), Ampicillin-sulbactam (66.7%), Cefepime (66.7%), Ceftriaxone (66.7%), Cefixime (62.7%), Ceftazidime (62.7%), Cefoxitin (60%), Ciprofloxacin (60%), Doxycycline (56%), and Cefotaxime (54.6%). Conversely, resistance was comparatively lower against Imipenem+EDTA (45.3%), Cefalexin (40%), Tobramycin (33.3%), Levofloxacin (33.3%), Amikacin (22.6%), Colistin (16%), Tetracycline (9.3%), and Trimethoprim/sulfamethoxazole (9.3%). Moreover, the study uncovered a notable capacity of *E. coli* for biofilm production. Among the strains, 44.4% were classified as strong biofilm producers, 31.1% as moderate, and 24.4% as weak. The investigation also brought to light the presence of resistance genes, with 10% of the strains harboring the OXA-23 gene, 13.3% containing the OXA-24 gene, and

33.3% possessing the OXA-69 gene. This study underscores the prevalence of *E. coli* strains characterized by robust biofilm production and the existence of resistance genes, offering crucial insights into the formidable challenges posed by antibiotic resistance in the context of urinary tract infections.

**2023, 42 pages**

**Keywords:** *E. coli*, class D β-lactamases, carbapenem resistance, *OXA* type gene

# ÖZET

## BAĞDAT'TA İDRAR YOLU ENFEKSİYONLU HASTALARDAN İZOLE EDİLEN ÇOKLU İLAÇ DİRENÇLİ *ESCHERICHIA COLI*'DE OXA TÜRÜ GENLERİN İNCELENMESİ

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Ekim 2023

Yaygın olarak *E. coli* olarak bilinen *Escherichia coli*, idrar yolu enfeksiyonlarının önde gelen etkeni olarak karşımıza çıkmaktadır. Bu araştırmanın temel amacı, idrar yollarında iltihaplanmaya neden olan *E. coli*'nin patojenik suşlarını titizlikle izole etmektir. Araştırma, bu suşların spesifik kemoterapötik ajanlara, özellikle de oksa tipi gene bağlı olanlara karşı direncini kapsamlı bir şekilde değerlendirmeyi amaçlıyor. Gelişmiş Vitek 2 kompakt teknolojisi kullanılarak 150 idrar örneğinin incelenmesinde 75 örnekte *E. coli* tespit edildi. İzole edilen *E. coli* suşları, çeşitli antibiyotiklere karşı önemli direnç gösterdi. Amoksisilin/Klavulanik Asit (%90,7), Piperasilin/tazobaktam (%86,7), İmipenem (%86,7), Tikarsilin-klavulanik asit (%74,7), Meropenem (%73,3), Ampisilin dahil olmak üzere çeşitli antibiyotikler için direnç yüzdeleri oldukça yüksekti. -sulbaktam (%66,7), Sefepim (%66,7), Seftriakson (%66,7), Sefiksim (%62,7), Seftazidime (%62,7), Sefoksitin (%60), Siprofloksasin (%60), Doksisiklin (%56) ve Sefotaksim (%54,6). Bunun tersine, direnç, İmipenem+EDTA (%45,3), Sefaleksin (%40), Tobramisin (%33,3), Levofloksasin (%33,3), Amikasin (%22,6), Kolistin (%16), Tetrasikline (%9,3) karşı nispeten daha düşüktü. ve Trimetoprim/sülfametoksazol (%9,3). Ayrıca çalışma, *E. coli*'nin biyofilm üretimi açısından dikkate değer bir kapasitesini ortaya çıkardı. Suşların %44,4'ü güçlü biyofilm üreticisi, %31,1'i orta ve %24,4'ü zayıf biyofilm üreticisi olarak sınıflandırıldı. Araştırma aynı zamanda direnç genlerinin varlığını da gün ışığına çıkardı; suşların %10'u OXA-23 genini, %13,3'ü OXA-24 genini ve %33,3'ü OXA-69 genini barındırıyordu. Bu çalışma, güçlü biyofilm üretimi ve direnç genlerinin varlığı ile

karakterize edilen *E. coli* suşlarının yaygınlığının altını çizerek, idrar yolu enfeksiyonları bağlamında antibiyotik direncinin ortaya çıkardığı zorlu zorluklara dair önemli bilgiler sunmaktadır.

**2023, 42 sayfa**

**Anahtar Kelimeler:** *E. coli*, D sınıfı β-laktamazlar, karbapenem direnci, OXA tipi gen

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**CONTENTS**

## LIST OF SYMBOLS

| | |
|---|---|
| °C | Degrees Celsius |
| % | Percentage |
| ± | Plus minus |
| $m^2$ | Square meters |

## LIST OF ABBREVIATIONS

| | |
|---|---|
| MDR | Multidrug resistance |
| OXA | Oxacillinase |
| TBE | Tris/Borate/EDTA |

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## 1. INTRODUCTION

Clinical symptoms of urinary infections persist in exerting an important effect on millions of patients in the world, the most comprising women. Antibiotics treatment did not prevent of cystitis repetitions, which infect of one-fourth of females after initial urinary infections. The elevating antibiotics resistance in uropathogenic bacteria lead to complicate curative decisions, requiring new principles depended on the essential biological search (McLellan and Hunstad 2016).

Urinary infections represent by the most infections of bacteria, impacting 150 million of people in world every year (O'brien *et al.* 2017). Inspite of both males and females infect with urinary infections however it is traditionallly considered disease of women, where there are about 50% infected with it (Foxman 2014).There are approximate 25% of females having with a primary incidence of cystitis by bacteria, can suffer repetition urinary infection through 6 months, inaddition some women have six to more infections during year after the primary incidence (O'brien *et al.* 2015).Present treatments are less than optimal tratments, due to prevalence of resistant to multidrug uropathogenes increasing and antibiotic therabies for severe infections does not prohibit repeating infection.These rebellious infections become important health issue and decrease good-ness of life for infective male and females (Al-Badr and Al-Shaikh 2013). *Escherichia coli* consider a main world general health worry because it become resistance to antibiotics (Subashchandrabose *et al.* 2014).

*Escherichia coli* represents the most of reason for urinary infections acquired from hospitals in the community, where it causes more than 80% of the status urinary infections (Beatson *et al.* 2015). *E. coli* has virulence characteristics that are related to infection in urinary system involving the expression of specific adhesions, toxins (hemolysin), production of gelatinase, and fimbriae which represents a major virulent factor reason for pyelonephritis (Bahalo *et al.* 2013). However, new antibiotics are represent effective against enterococci antibiotic-resistance but are not periodic checkn although enterococci considers the causative factor pathogen in 10% of urinary

infections conditions and are establishing of nosocomial problems (Tandoğdu *et al.* 2016).

Aim of this study is isolation of pathogenic *E. coli* bacteria that cause inflammation of urinary tract and the determination of resistance to some chemotheraputic agents with related with present oxa type gene.

## 2. LITERATURE REVIEW

### 2.1 The Infection of Urinary System

The urinary system is the discharge system to eliminate extra water and wastes it involves the kidneys, ureters, the bladder, and the urethra. Kidneys are purified the blood, which have been reabsorbed the fluid filtered and secrete urine, that contains different chemical materials dissolved in the surplus water filtrated from blood via kidneys (Okonko *et al.* 2010).

Urinary infection is the most infection after following the infections in upper respiratory system (Hryniewicz *et al.* 2001). Many various microorganisms cause urinary infections involving bacteria, fungi, and viruses. The bacteria represent the main organisms that cause infection and are causing than 95% of urinary infection conditions (Bonadio *et al.* 2001). Normally the bacteria are rapidly eliminating when which enter the urinary system by the body defenses.

Whereas at times bacteria conquer the body defenses and beginning infection. the urethra infection is known urethritis, whilst the infection in bladder is known cystitis. Also, bacteria can transmit up the ureters for proliferation and infect of kidneys, it is known pyelonephritis (Schappert and Rechtsteiner 2008).

Urinary infections become a pattern for pathophysiology studying of infections, like interactions between host and pathogen moreover the development infection mechanisms, and evolution new antibiotics are effective towards gram negative bacteria (Flores-Mireles *et al.* 2015). The utility experimental of urinary infcetion is essential where enormous quantities of pathogens have been isolated, the diagnosis of pathogen that related with the route of disease, also urine represents primary sample possible easily attainable. However, new antibiotics are represent effective against enterococci antibiotic-resistance but are not periodic check although enterococci considers the causative factor pathogen in 10% of urinary infections conditions and are establishing

of nosocomial problems (Tandoğdu *et al.* 2016).Recently, the virulent factors of uropath- ogenics bacteria and their effects in host also the invasion mechanisms of urothelial cells explored (Flores-Mireles *et al.* 2015).A complicated urinary infection is a high hazard than an un-complicated urinary infection as in gestation and the childhood. Hazard sometimes leads to an elevated chance of infection repeated. Also, the categorization takes the consideration various bacterial compositions in the infection. *E. coli* considers the main pathogen in uncomplicated urinary infection (Figure 2.1), while other pathogens are found in complicated urinary infections thus wide-spectrum antibiotics and therapy duration should be considered (Johansen *et al.*2011).

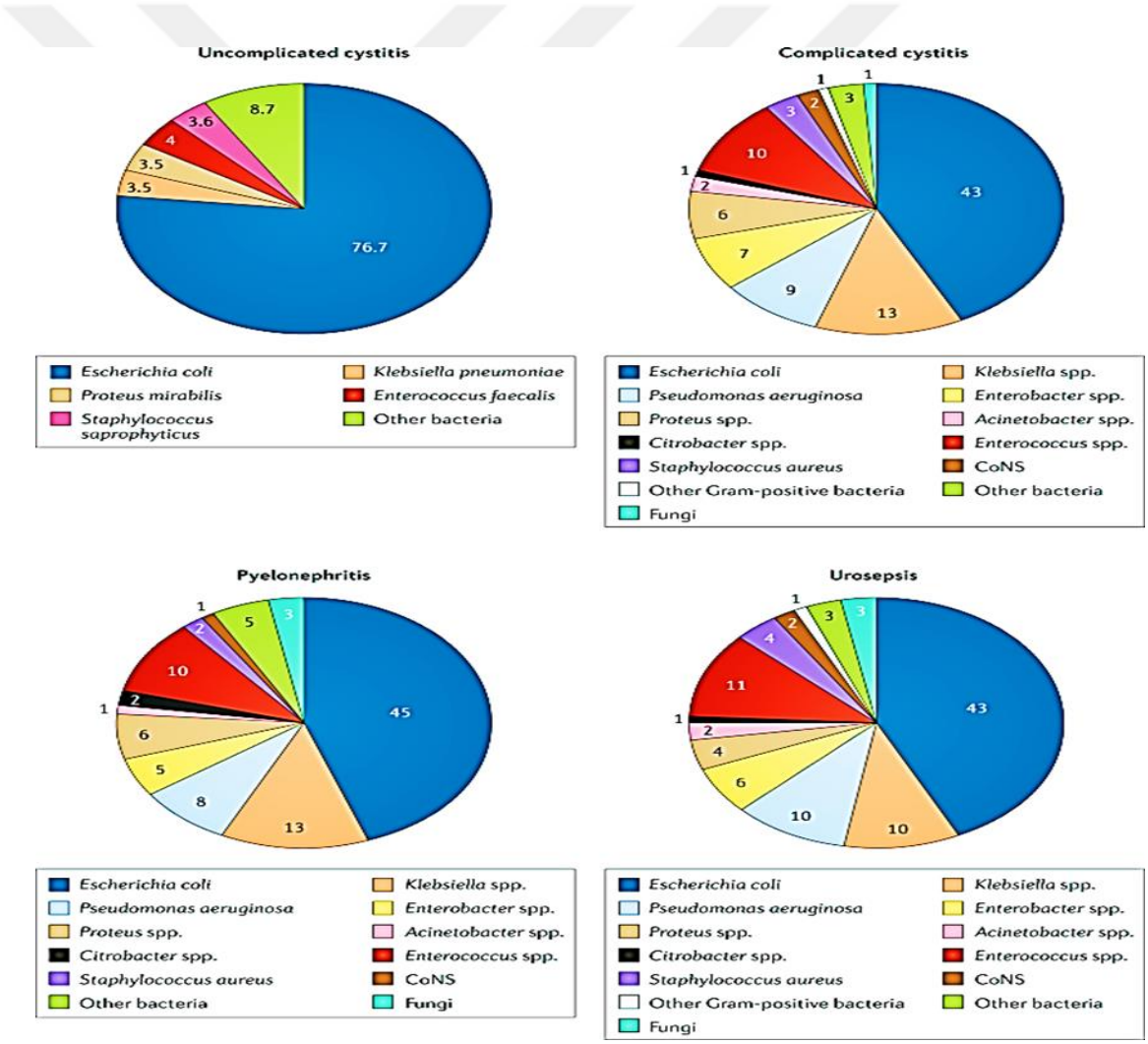


**Figure 2.1** Prevalence of pathogenic bacteria in urinary infection (Wagenlehner *et al.* 2020)

## 2.2 Pathophysiological of Urinary Infections

Urinary infection severity relied on an equlibrium between the mechanisms defense in host and the uropathogens virulent (Figure 2.2). Pathogenecity of bacteria is depended on the bacteria ability to conquer the mechanisms defence, biofilms formation and remain alive in various environment of the urinary system and bloodstream (Hibbing *et al.* 2016).

Urinary infections are one of the most infective diseases, with about 10% of people suffering from urinary infection throughout their life (Yang *et al.* 2022). It may appear symptoms or no symptoms, and where any type of infection led to a critical sequel if not treated (Flores-Mireles *et al.* 2015). Also, Urine contains pus (pyuria) as appears in individuals who have sepsis reason of urinary infections. Lower urinary system infections are indicated as bladder infections. Symptoms are urination burning and urinating frequently. People who suffer an upper urinary system infection, pyelonephritis may suffer waist pain, high temperature, nausea, and vomiting also the classical symptoms of lower urinary system infection (Arellano 2011).

Microorganisms come to the urinary system by the bloodstream or lymphatic system, there is clinical evidence of appearing that microorganisms' ascent from urethra represents a common path that causes urinary infection, specifically organisms that enteric origin such as *E. coli* and Enterobacteriaceae. It supplies a logical interpretation for the repeated infection in females than in males, also the increased hazard of infection after bladder catheterization. A catheter inserted into the bladder in patients led to urinary infections in (1-2%) of conditions (Salvatore *et al.* 2011).

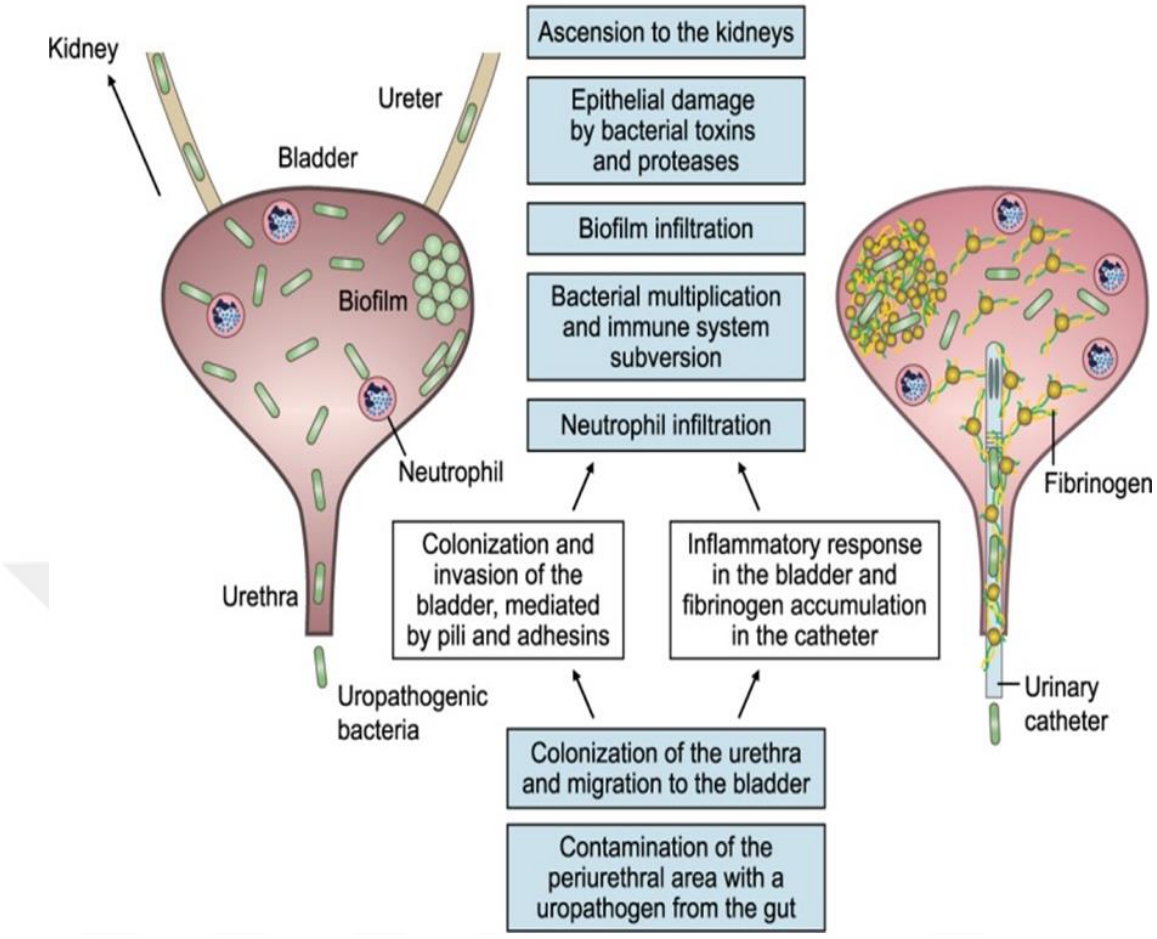


**Figure 2.2** Urinary system pathogenicity (Ortega Martell *et al.* 2019)

### *2.3 Escherichia Coli*

The genome of bacteria provide the information of diversity bacteria and their developing history (Cohan and Kopac 2011). *E. coli* genome sequence was completed and its publishing in 1997.Where It was DNA as a circular molecule contain 4.6 million bp in length, it has 4288 protein-encoding genes, also seven of rRNA operons, 86 tRNA genes. Although it has been the issue of intense genetical analysis about of 40 years, however an enormous number of those genes were already unknown. In addition, it has been observed the genome has several genetic transposable elements, repeating elements, bacteriophage remains, and cryptic prophages (Blattner *et al.* 1997). Many hundred genome sequences for *E. coli* and also Shigella spp are completed (Meier-Kolthoff *et al.* 2013). The sequences comparison appears a prominent number of

diversities where about of 20% of each genome appear sequences exist in each one of isolates, whereas about 80% of every genome differ among isolates (Lukjancenko *et al.* 2010). Each single genome has 4000- 5500 genes, while the overall number of various genes among all strains sequenced of *E. coli* surpass 16000 (Zhaxybayeva and Doolittle 2011).

## 2.4 Virulence of *Escherichia coli*

The strains of *Escherichia coli* encode several virulent factors, that permit colonizing of the bacteria in the urinary system and continue in the encounter of host defenses. *E. coli* shows high levels of genetic variation resulting from the posses of specific virulent genes found on genetic mobile elements known as islands pathogenic (Wiles *et al.* 2008). *E. coli* virulence factors can be categorized into two factors related to the surface of the bacterial cells and factors that related to excretion and load to the location of action (Oelschlaeger 2002).

### 2.4.1 Surface of virulence factors

It was included the various types of adhesion organelles represented fimbriae, that induce attachment of bacteria to tissues in the urinary system. The display of adhesins molecules by uropathogenic *E. coli* is a significant factor in pathogenicity. The adhesins participate to the virulent in various pathways such as directly inducing host and the bacterial cells signalings paths, aiding the transmission of other products of bacteria to tissue in the host , and inducing invasion of bacteria (Mulvey 2002). Fimbriae type 1 are consider as virulent factors in urinary infection (Bergsten *et al.* 2007). Role of fimbriae type 1 in human infections is difficult to determine due to expressed in pathogenic and nonpathogenic strains (Plos *et al.* 1991). Fimbriae P are the second virulent factor of plays a significant role in the pathogenicity of urinary system and pyelonephritis (Plos *et al.* 1995). Fimbriae P is responsible for attachting to mucosa and the tissue matrix also cytokines production by uropathogenic *E. coli* is a significant factor in pathogenicity (Hedlund *et al.* 1999). Moreover, the capsule is considered a virulence factor found on the bacteria's surface, it contains a structure from a

polysaccharide coat and protects the bacteria from host immunity. The capsule protects against phagocytosis and controls the bactericidal impacts. Also, the capsule appears as a molecular simulation of tissue composition (Sachdeva *et al.* 2017). Lipopolysaccharide is a major outer membrane composition for gram-negative bacterium which comprises about (75%) of the surface and (5-10%) of the dry weight for the gram-negative bacterium. Their principal structure includes of three portions: lipid A, repetitive polysaccharides, and core oligosaccharides classified as antigen O. Also, Lipid A is conserved highly and acts as an endotoxin, whereas the carbohydrate chains of antigen O are a polymer of repetitive oligosaccharides, that vary among species and are responsible for specificity the serological bacteria (Rezania *et al.* 2011). Besides, flagella represent an organelle that is responsible for the motility of bacteria and are participating in the interactivity of different strains of uropathogenic *E. coli* with epithelial cells. Flagellated uropathogenic *E. coli* give rise to (70-90%) of urinary system infections, and its pathogenicity includes attachment between bacteria and the epithelial cells surface of the urinary system. Many studies have indicated that the flagella of *E. coli* are important in permitting the bacteria to climb from bladder to begin renal infections in humans. Therefore, the antibody used against the flagellum prohibits the prevalence of uropathogenic *E. coli* in the kidney (Schwan 2008).

### 2.4.2 Secretion virulence factors

Toxins consider a significant virulence factor in different *E. coli*-diseases. The toxins produced by *E. coli* cause an inflammation response, which is a potential pathway for urinary infection symptoms. The excretion virulence factor of *E. coli* is the lipoprotein (α-hemolysin), that related to the upper urinary system like pyelonephritis (Bien *et al.* 2012).

The α-hemolysin toxins are found in gram-negative bacteria. It has appeared to act a double activity on epithelial cells arising from the proximal tubule in the kidney. α-hemolysin has a role in the lysis of red blood cells and nucleated cells, this process enables extra-intestinal pathogens such as uropathogenic *E. coli* to cross barriers such as

mucosa, injury immune cells, and stimulated oncoming to nutrients and iron repository (Ristow and Welch 2016).

The secretion of autotransporter toxin represents virulence factors in pyelonephritis of *E. coli*. Where, it has a toxic action against the cells originating from the bladder or kidneys, thus important for the pathogenicity of urinary infection. The cytotoxic necrotizing factor1 is a protein produced via *E. coli*, in vitro research has also appeared this factor interposes with phagocytosis and leads to apoptosis of epithelial cells in the bladder. In vivo, this factor causes the exfoliation of bladder cells and stimulated bacterial oncoming to the tissue (Bien *et al.* 2012).

## 2.5 Biofilm Formation

Biofilm forming consider a significant virulent factor in urinary infection and associated with specific hazard factors, like urinary catheter, stone, and uropathy. Biofilm is composed of communities of microorganisms covered within a self-polymeric matrix attach to the surface. The interaction between host and pathogen in complicated urinary infections varies from uncomplicated urinary infections. The response of the host can be abnormal in complicated urinary infections where the biofilm production in the pathogens with declined virulence may cause acute infections like *Pseudomonas aeruginosa* and *Enterococcus faecalis* (Flores-Mireles *et al.* 2015, Tandoğdu *et al.* 2016).

*E. coli* has abililty the formation of intracellular biofilm in bladder cells creating the hard reach to bacteria via antibiotics and mechanisms host defense (Anderson *et al.* 2003). Biofilm is formed by five steps involve: firstly, reversible linking of bacteria planktonic to surface, the bacteria is affected by appealing or expelling forces it differs based on levels of nutrient, temperature and pH (Donlan 2002). Also, flagella and chemotaxis have an important role in avoiding the hydrodynamic actions and expelling forces and surface selecting (Toutain *et al.* 2007, Schmidt and Kirschning 2012).

Secondly irreversibl linking to surfaces via pili type 1 of *E. coli* (Cegelski *et al.* 2009). Thirdly, the complex layer from biomolecules is formed also polysaccharides production in biofilm to facilitate congregation, adhesion, and surface tension tolerance, permitting to colonize surface (Lappin-Scott and Bass 2001, Laue *et al.* 2006). Fourthly biofilms gain structure as a three-dimension when reaching mature. This structure with morphology of colony rely on product extracellular matrix compositions, adhesins,exopolysaccharides ,and forming amyloid proteins (Serra *et al.* 2013). Fifthly biofilms become mature, and separation occur. Separation permits cells to acquire a planktonic case and thus formation of biofilm in other location (Stowe *et al.* 2011).

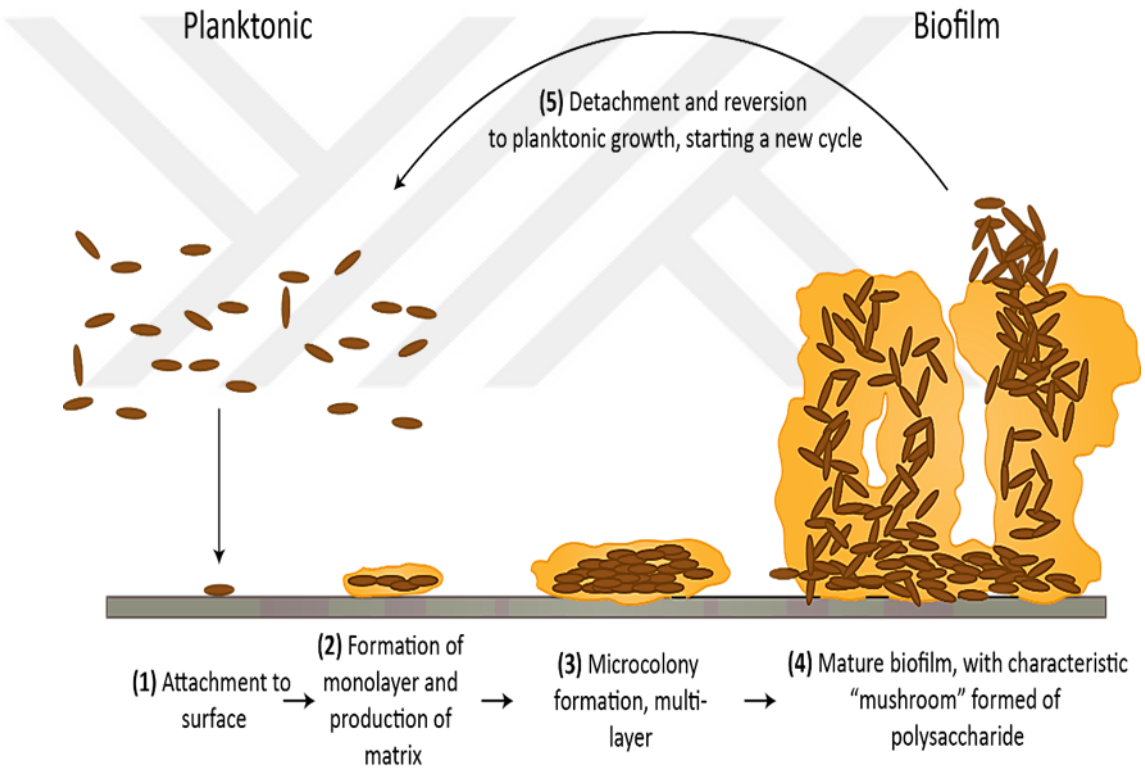


**Figure 2.3** Biofilm formation (Vasudevan 2014)

## 2.6 *E. coli* Resistance Genes

The production of β-Lactamase considers a significant mechanism because gram negative bacteria are resistant to the beta-lactams antibiotics group. Depending on the protein sequence similarity, antibiotics of beta-lactam are included in four main classes

(Class A–D) (Ambler 1980). Enzymes of A, C, and D classes include the serine active site to hydrolysis beta-lactams, while enzymes B class are hydrolase that depends on zinc. Different from most enzymes of class A, where beta-lactamases of class D accord a high resistance to a wide series of beta-lactams inhibitor (Poirel *et al.* 2010).

Beta-lactamases class D includes oxacillinases (OXA) which were qualified functionally as penicillinases able to hydrolysis for oxacillin and cloxacillin. However, the definition represents no longer exact because recently characterized enzymes are inadequate inactivating oxacillin and cloxacillin. Where all oxacillinases hydrolyze the carboxypenicillins and aminopenicillins (Mlynarcik *et al.* 2020).

OXA-24 represents an enzyme of class D that demonstrate an impedance to inhibitors of beta-lactamase such as sulbactam, tazobactam, and clavulanate (Bou *et al.* 2010). OXA-24 is an enzyme of class D that hydrolyzes carbapenem which owns an extended spectrum of cephalosporinase effectiveness. OXA-24 lead to critical issues in nosocomial contagions, like the infection of the bloodstream, wound, and pneumonia associated with a ventilator (Che *et al.* 2014).

OXA-23 belongs to the gene cluster of D class carbapenemase that represented the first group of OXA-type Beta-lactamases conferring carbapenem resistance, capable of hydrolysing broad-spectrum cephalosporins and carbapenems (Evans and Amyes.2014). The gene of OXA-23 is encoded on chromosomes, also on plasmids, and is related to the genetic components mobile (Mugnier *et al.* 2010). In other studies appeared Beta-lactamase OXA-69 found in Acinetobacter *baumannii* was similarly at 97% amino acid with OXA-51, also56 %and 62% of amino acid similar with OXA-23 and OXA-24 (carbapenem-hydrolyzing oxacillinases) respectively ( Héritier *et al.* 2005, Evans and Amyes 2014).

# 3. MATERIALS AND METHODS

## 3.1 Materials

### 3.1.1 Tools

Table 3.1 appeared the tools utilized in th present study.

**Table 3.1** Tools

| Tools | Company | Origin |
|---|---|---|
| PCR Cabinet | Bioair | Italy |
| Autoclave | Hirayama | Japan |
| Centrifuge | Memmert | Germany |
| Electrical balance | Kernpfb | Germany |
| Electrophoresis tool | ThermoScientific | USA |
| Incubator | Memmert | Germany |
| Light microscope | Olympus | Japan |
| Micropipette 0.5-10 ,2-20,100-1000 ul | Labnet | Germany |
| Microwave | Gosonic | China |
| Microcentrifuge | ThermoScientific | USA |
| Nano drop Spectrophotometer | ThermoScientific | USA |
| PCR Thermal Cycler | Analytik Jena | Germany |
| U.V. transilluminator | Scope 21 | Japan |
| Vitek 2 Compact | BioMérieux | France |
| Vortex | Labcoo | Germany |
| Water distiller | Tangshan Umg medical | China |

### 3.1.2 Chemicals

The chemicals used in the study are given in Table 3.2.

**Table 3.2** Chemicals

| Chemicals | Company /Origin | Catalog no |
|---|---|---|
| Agarose | Promega /USA | |
| DNA Extraction Kit | Promega /USA | |
| Gram stain | BD medical/ UK | |
| Loading dye | Promega /USA | |
| Master Mix PCR | Promega /USA | |
| Nucleases free water | Promega /USA | |
| Ethidium bromide | Promega/USA | |
| Primers | Alpha DNA/USA | |
| DNA Markers (1000bp) | Promega /USA | |
| Tris-Borate EDTA buffer(10x) | Promega/USA | |

### 3.1.3 Culture media

The culture media used in the study are given in Table 3.3.

**Table 3.3** Culture media

| Medium | Company /Origin | Catalog no |
|---|---|---|
| Blood agar | Himedia/India | |
| Nutrient Broth | Himedia/India | |
| MacConkey agar | Himedia/India | |
| Eosin Methylene Blue | Himedia/India | |

## 3.2 Sample Collection

It collected 150 urine specimens in sterile containers from patients with urinary infections from Baghdad hospital, and National Center for Educational Laboratories /Baghdad -Iraq in the period February 2022- April 2022. The samples were checked by microscope.

## 3.3 Methods

### 3.3.1 Prepartion media

Media used in this study prepared according to manufacturer companies.

### 3.3.2 Preparation solutions

Gram stain:

It was previously prepared according to the manufacturer company, it was used for staining bacteria isolates to examine by a microscope, it included crystal violet; iodine gram; ethanol 70 %; safranin stain.

Kovac's reagent:

It was consisted of 150ml of Isoamyl alcohol, ρ-Dimethyl aminobenzaldehyde 10 gram, HCl concentrated 50 ml. The aldehyde has been dissolved in acid(slowly added) and alcohol . This reagent has been prepared in little amount and stocked in refrigerator (Atlas *et al.* 1995).

Barritt's reagent:

It contains from solution A: Potassium hydroxide 40% (including 40 gram of potassium hydroxide and 100 ml distlled water) and solution B: 5 g of α-naphthol with dissolved with absoult ethanol 100 ml (Collee *et al.* 1996).

Methyl red:

It was contained from 0.05 g of methyl red, 150ml of ethanol (95%) and 100 ml of distlled water (Collee *et al.* 1996).

Oxidase reagent:

This reagent was prepared by tetramethyl-p-phenylenediamine dihydrochloride 1% was added to (10 ml) distilled water (Atlas *et al.* 1995).

Peptone water:

It was consisted of peptone 20 gram, sodium chloride 5 gram and one liter of distilled water adjusted pH to 7, and it was divided into tubes and sterilization by autoclaving (Atlas *et al.* 1995).

### 3.3.3 Diagnosis of *E. coli* and isolation

The samples of urine were cultivated on MacConky agar and incubation for 24 hr at 37 C. Bacteria colonies show in pinkish color due to lactose-fermentation and the colonies were re-ccultivated on MacConky agar to purification of colonies Inaddition, the bacteria were cultivated on Eosin Methylene Bluefor 24 hr at 37C to observe the metallic -green shiny of *E. coli*.

### 3.3.4 Biochemical assay of *E. coli*

Catalase assay

The colonies were put on glass slides. Drops of hydrogen peroxide (3%) had been used, the presence of bubbles indicated a positive reaction (Atlas *et al.* 1995).

Oxidase assay

Filter paper saturated by drops of tetramethyl-p-phenylene diamine dihydro chloride was applied in this test. Cells were selected by sterile stick from the slant growth and its

smearing on the wet paper. The changing color of a violet or purple during 10 seconds showed positive outcomes (Colle *et al.* 1996).

Indole assay

Kovac reagent (0.05 ml) was added to peptone water that has been inoculated with colonies and incubated at 37°C for 48 hours and mixed gently. The positive outcomes were by the observation of ring red on the surface (Collee *et al.* 1996).

Methyl Red Test

The broth tubes of MR-VP were implanted and incubation at 37°C for 48 hours. The reagent of methyl red (5 drops) was added and mixed. Positive outcomes have appeared shining red and negative results were yellow (Collee *et al.* 1996).

Voges-Proskauer assay

The broth tubes of MR-VP were implanted and incubated at 37°C for 24 hr. and added of Barritt's reagent A (1ml) and Barritt's reagent B(3ml) to 5 ml of culturing broth and shook for 30 seconds. The color of pink to red formation pointed to the positivite results (Collee *et al.* 1996).

Citrate utilization assay

The slant of Simmons citrate agar was implanted with bacteria and its incubation for 24 hr. The color royal blue pointed to a positive outcome whereas the color green pointed to a negative outcome (Atlas *et al.* 1995).

Urease assay

The activity of urease was detected by implanting the slant of surface of Christensen urea agar with bacteria growth and its incubation at 37°C for 24 hr. The color red-violet points to a positive outcome whereas the color yellow-orange points to a negative outcome (Atlas *et al.* 1995).

Fermentation of mannitol

Mannitol salt agar is implanted and its incubation at 37°C for 24 hr. The color change to yellow pointed to positive outcomes for fermentation mannitol (Collee *et al.*1996).

Test of production Hemolysin

It detected the production of hemolysin on blood agar cultivated with bacteria in this study that was incubated for 24h at 37ºC. A hemolysis that shows around the colonies indicating for positive results while no alteration around the colonies indicating for negative results (Collee *et al.* 1996).

### 3.3.5 API 20 E system to diagnosis *E. coli*

Prepare an inoculum by picking a single isolated colony of bacteria from a MacConkey plate. It is emulsified in saline to obtain a homogeneous bacteria suspension, using sterile pipettes, the VP, citrate, gelatin tubes, and cupules were filled with suspension, the remaining test tubes but no cupules are filled with the bacteria suspension. Tests of LDC, ADH, ODC, URE, and H2S were conducted by filling test tubes and adding of mineral oil to obtain anaerobic conditions then incubation for 24 h at 37 °C. Strip of API 20 E reads after 24 hr, according to read tables.

### 3.3.6 Detection of *E. coli* isolates and antibiotic-susceptible by Vitek 2

Vitek 2 compact system was utilized for the identification of the bacterial isolates and antibiotic-susceptible bacteria against antibiotics via using the diagnosis cards for gram-

negative (GN card 21341) and (AST-card GN76 413433). The isolates of bacteria were cultured on MacConky agar at 37°C and incubation overnight. Bacteria suspension was prepared in 1ml of normal saline by picking the colonies of bacteria from MacConkey agar and the turbidity was set at 0.5 for McFarland standard, then load the card through lower than 1 hr. The results were determined in 4-6 hrs.

### 3.3.7 Identification of biofilm

*E. coli* biofilm (45 isolates) was investegated depending on (Deka 2014) the method was explained through transplant *E. coli* from a nutrient agar to trypticase soy broth of (contain 1% of glucose) and incubation at 37°C for 24 hr. The suspension of bacteria was determined standard of turbidity to 0.5 of McFarland. Inoculum of *E. coli* (150µl) approximately $1.5\times10^8$ CFU/well, there after put in 96 wells (clean polystyrene plate), it was applied triplicate for each isolate. The plate was covered with a cover and incubation at 37ºC for 24 hr. Thereafter the culture liquid in wells decanted and nonadhesion of bacteria expel through washing for 2-3 times via normal saline. The adherence cells fixation was completed via the methanol absolute 200µl for 15 min. Wells was vacated to dry for overnight. The 0.5 % of crystal violet (200µl) was put to biofilms stain in wells for 15 min, after stain reaction completed the excess of stain is removed via rinsing (2-3) times via distilled water and drying in air to overnight. Lastely, 95% of ethanol absolute 200µl was put in wells to extract stain in 10 min. Crystal violet quantity in wells was estimated through a spectrophotometer in 630 nm using the microplate reader. The negative control was sterile tryptic soy broth. The results of biofilm interpretation were according to optical density (OD) when <0.12 mean No biofilm, OD = 0.12-0.24 mean moderate formation biofilm, OD >0.24 strong formation biofilm.

### 3.4 Detection of genes

#### 3.4.1 Extraction of DNA

The extraction of DNA bacteria was applied by DNA extraction kit (Promega/ USA) the protocol as follows:

1- *E. coli* were cultivated in the nutrient agar at 37ºC for 24hr. Thereafter, it was cultivated in nutrient broth. The bacteria culture (1 ml) was placed into the micro-centrifuge tube. The bacteria cells were sedimented through centrifugation (13000 rpm for 2 min), and the supernatant was removed.

2- The nuclei lysis solution (600µl) put to re-suspend cells and incubated in a microcentrifuge tube (80ºC / 5min) in the water bath to lysis of cells.

3- Three µl RNase solution put to the lysate cells and inverting 2-5 times to mix, incubated in (37ºC for 45 min).

4- Two hundred µl of protein precipitation solution was put into a lysate cell (which is treated with RNase) and vortexing strongly at 20sec, utilized ice to incubation (5 min) , after then centrifugated (13000 rpm / 3min).

5- DNA supernatant was transferred to the microcentrifuge tube which consists of six hundred µl isopropanol.

6-It was gently mixed microcentrifuge tube via inverting until visible DNA mass, thereafter centrifugated (2 min at 13000 rpm). The supernatant was decanted from the tube and placed on absorbent paper.

7- Six hundred µl of (70%) ethanol was utilized by putting into the tube and inverting it several times to wash DNA pellet, then aspiration of ethanol carefully. The tube was

placed on absorbance papers, thereafter pellet was dry on air in room temperature for 10-15min.

8- One hundred µl of the solution DNA Rehydration was placed in a tube consisting of a pellet and its rehydratation through the solution incubation at 4ºC for overnight. Finally, the DNA was saved at freezing - 20 ºC.

### 3.4.2 Estimation of purity and concentration of DNA

The purity and concentration of genomic DNA have been determined by Nanodrop device by taking two µl from DNA at (260 and 280 nm). 260nm measured the concentration of DNA while the ratio of 260/280nm represented the DNA purity. Also, the TE buffer is used as a blank.

### 3.4.3 Analysis PCR

The technique of PCR was applied to investigate the genes used in the current study involving oxacillinase gene OXA-23, OXA-24 and OXA-69. The sequence of primers show in Table 3.4. Primers were provided from Alpha DNA company/USA as lyophilized, then prepared depending on manufacturer company via the dissolving in free nuclease water to get 100 pmol/µL as stock solution. There after 10 pmol/µL prepared by putting 10 µl to 90 µl free nuclease water and storage at -20 C in freezer till to use later.

**Table 3.4** Sequence primer of genes

| Primer | Sequence | Product size |
|---|---|---|
| *OXA-23* | F: GATGTGTCATAGTATTCGTCGT<br>R: TCACAACAACTAAAAGCACTGT | 1065bp ( Roberts *et al.*2001) |
| *OXA-24* | F:ATGAAAAAATTTATACTTCCTATATTCAGC<br>R: TTAAATGATTCCAAGATTTTCTAGC | 825 ( Roberts *et al.*2001) |
| *OXA-69* | F:CTTATAAGTCATATGAACATTAAAGC<br>R:CTCTATAAAAAGGGATCCGGGCTA | 922 bp ( Héritier *et al.* 2005) |

### 3.4.4 PCR amplification

PCR used to detect genes by amplify DNA and the mixture volume (25µl) involving 1.5 µl DNA, forward primer 1 µl and reverse primers 1 µl , master mix of PCR 12.5 µl (Cat NO: M7502, Promega ,USA) and 16.5 µl of free-nuclease water to reach final volume of 25µl. PCR tubes placed in a thermocycler of PCR device and the mixture reaction conditions (Table 3.5).

**Table 3.5** The condition reaction of PCR

| Genes | Initial Denaturation | Denaturation | Annealing | Extension | Final Extension |
|---|---|---|---|---|---|
| ***OXA-23*** | 95°C/5min 1cycle | 95°C/30sec. 35cycle | 55°C/1min 35cycle | 72°C/1min 35 cycle | 72°C/10min 1 cycle |
| ***OXA-24*** | 95°C/5 min 1 cycle | 95°C/30sec 35cycle | 55°C/1min 35cycle | 72°C/1min 35cycle | 72°C/10min 1cycle |
| ***OXA-69*** | 95°C/4 min 1 cycle | 95°C/30sec 35cycle | 55°C/45sec 35cycle | 72°C/1min 35cycle | 72°C/10min 1cycle |

### 3.4.5 Electrophoresis

Agarose gel was utilized ( 1.5 g agarose dissolved with 100 ml from TBE buffer 1X) Where TBE buffer prepared by diluting 10 ml from TBE 10X by 90 ml of distilled water. Thereafter boiling and cool at 45-50°C. Three µl of Ethidium bromide put to agarose solution, then poured the solution gradually in plate of electrophoresis to evade bubbles formation, and wells formed by comb fixing on the gel to put DNA. The comb eliminated carefully when solidification of gel. Agarose gel plate was placed horizontally in the tank, (1x) TBE buffer placed on the surface of gel till covered the surface of gel (Sambrook *et al.*1989). DNA or the PCR product (7µl) was put on 3 µl loading dye and put this mixture in wells of gel for electrophoresis. The power of electric is 5volt/cm2 (1 hours). The bands of DNA or amplicon showed on gel via UV transilluminator examination.

### 3.5 Statistic Analysis

SPSS Software (version 28.0) was utilized to estimate significant differences by Fisher test where probability value higher than 0.05 indicate to significant differences and less than 0.05 is non-significant.

# 4. RESULTS AND DISCUSSION

## 4.1 *E. coli* Diagnosis

*E. coli* was diagnosed in urine specimens when cultured on MacConkey agar, thereafter investigated bacteria by biochemicals tests, API 20 E system and Vitek 2 system. 75 isolates of *E. coli* were identified out of 150 specimens.

## 4.2 Antibiotics Susceptibility of *E. coli*

The antibiotics resistances for *E. coli* isolates were checked by Vitek 2 Compact. The outcomes are shown (Table 4.1). In this study, *E. coli* was resistant to most antibiotics applied in this study. *E. coli* isolates resistance was against Amoxicillin/Clavulanic Acid (90.7%), Piperacillin/tazobactam (86.7%), Imipenem (86.7%), Ticarcillin-clavulanic acid (74.7%), Meropenem (73.3%), Ampicillin-sulbactam,and Cefepime (66.7%), Ceftriaxone (66.7%), Cefixime (62.7%), Ceftazidime (62.7%), Cefoxitin (60%), Ciprofloxacin (60%), Doxycycline (56%), Cefotaxime (54.6%). While it was low resistance against Imipenem +EDTA (45.3%), Cefalexin (40%), Tobramycin (33.3%), Levofloxacin (33.3%), Amikacin (22.6%), Colistin (16%), Tetracycline (9.3%) , Trimethoprim/ sulfamethoxazole (9.3%).

**Table 4.1** Resistance of antibiotics for 75 isolates of *E. coli*

| N | Antibiotic* | Resistant | Intermediate | Sensitive | P value |
|---|---|---|---|---|---|
| 1 | Amoxicillin/Clavulanic Acid | 68(90.7%) | 7(9.3%) | 0(0%) | P < 0.01 |
| 2 | Piperacillin / tazobactam | 65(86.7%) | 10(13.3%) | 0(0%) | P < 0.01 |
| 3 | Ticarcillin-clavulanic acid | 56(74.7%) | 15(20%) | 4(5.3%) | P < 0.01 |
| 4 | Ampicillin-sulbactam | 50(66.7%) | 20 (26.6%) | 5(6.7%) | P < 0.01 |
| 5 | Cefalexin | 30(40%) | 21(28%) | 24(32%) | P > 0.05 |
| 6 | Tobramycin | 25(33.3%) | 15(20%) | 35(46.7%) | P >0.05 |
| 7 | Amikacin | 17(22.6%) | 35(46.6%) | 23(30.7%) | P > 0.05 |
| 9 | Colistin | 12(16%) | 40(53.3%) | 23(30.7%) | P < 0.01 |
| 10 | Imipenem | 65(86.7%) | 10(13.3%) | 0(0%) | P < 0.01 |
| 11 | Meropenem | 55(73.3%) | 10(13.3%) | 10(13.3%) | P <0.05 |
| 12 | Imipenem+EDTA | 34(45.3%) | 30(40%) | 11(14.7%) | P < 0.01 |
| 13 | Tetracycline | 7(9.3%) | 35(46.7%) | 33(44%) | P < 0.01 |
| 14 | Doxycycline | 42(56%) | 33(44%) | 0(0%) | P < 0. 01 |
| 15 | Trimethoprim/sulfamethoxazole | 7(9.3%) | 32(42.7%) | 36(48%) | P < 0.01 |
| 16 | Levofloxacin | 25(33.3%) | 10(13.3%) | 40(53.3%) | P < 0.01 |
| 17 | Ciprofloxacin | 45(60%) | 7(9.3%) | 23(30.7%) | P < 0.01 |
| 18 | Cefoxitin | 45(60%) | 0(0%) | 30(40%) | P < 0.01 |
| 19 | Cefotaxime | 41(54.6%) | 4(5.3%) | 30(40%) | P < 0.01 |
| 20 | Cefixime | 47(62.7%) | 28(37.3%) | 0(0%) | P < 0.01 |
| 21 | Ceftazidime | 47(62.7%) | 28(37.3%) | 0(0%) | P < 0.01 |
| 22 | Cefepime | 50(66.7%) | 25(33.3%) | 0(0%) | P < 0.01 |
| 23 | Ceftriaxone | 50(66.7%) | 25(33.3%) | 0(0%) | P < 0.01 |

## 4.3 Identification of Biofilm

The results revealed 20/45 isolates of *E. coli* had strong of the biofilm production (44.4 %), 14/45 isolates were moderate of the biofilm production (31.1%), and 11/45 isolates were weak of the biofilm production (24.4%) shown in Figure 4.1.

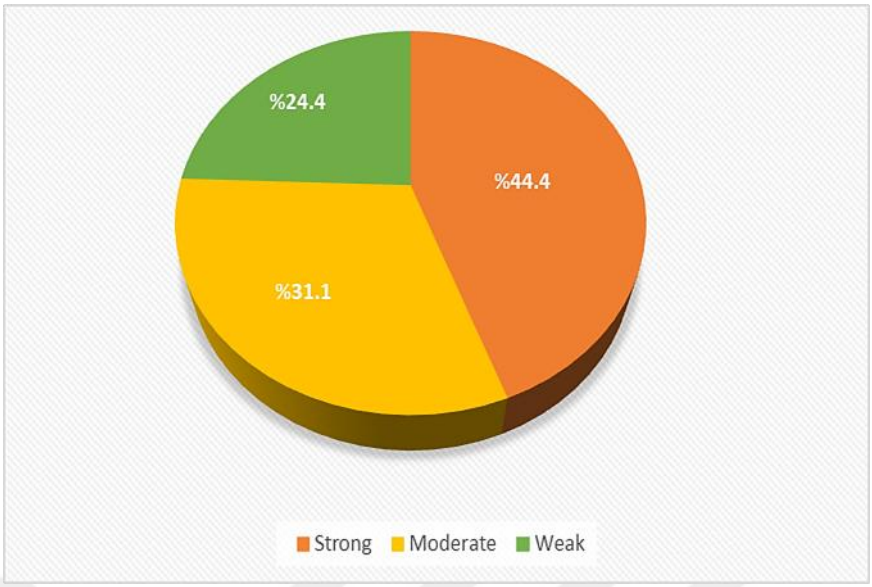


**Figure 4.1** The ratio of biofilm production in *E. coli* isolates

## 4.4 DNA Extraction Outcomes

The findings of DNA electrophoresis are shown for *E. coli* specimens in Figure 4.2. In addition, concentrations of DNA for specimens were 75-282 ng/ml, and also the ratio of purity ranged (from 1.7–2.0) within the accepted ratio (Figure 4.3 and 4.4).

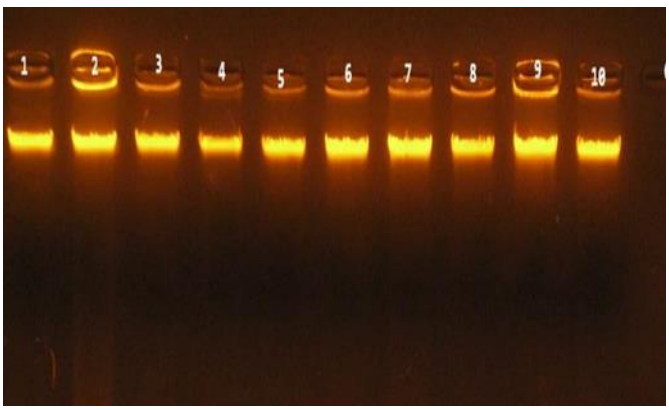


**Figure 4.2** DNA extraction for bacteria

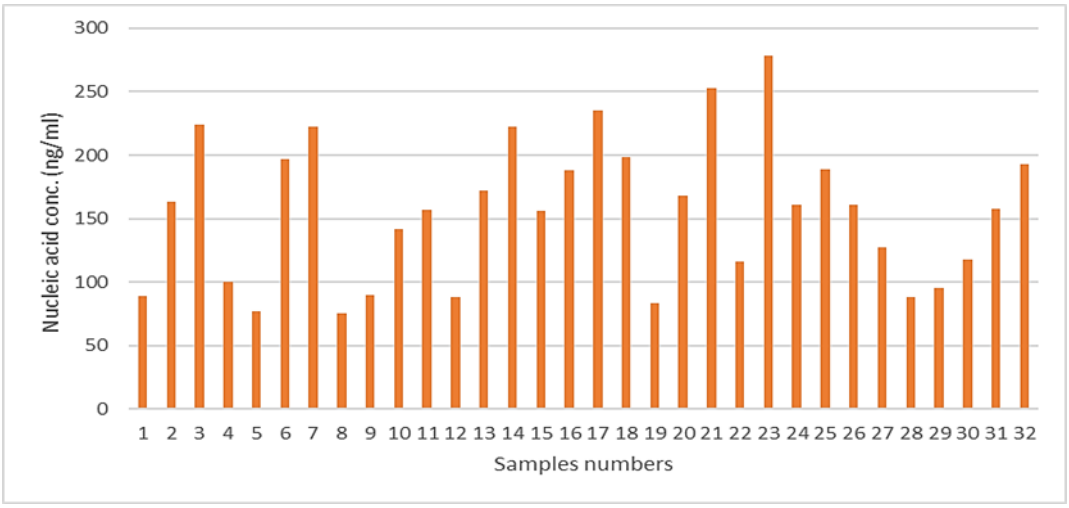


**Figure 4.3** Concentration of DNA samples

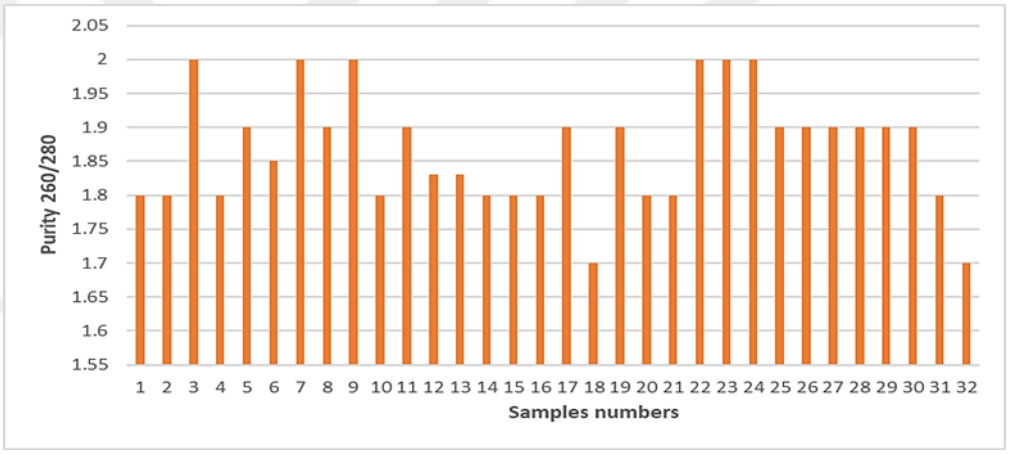


**Figure 4.4** DNA purity of samples

## 4.5 Genes Detection

It was confirmed the presence of *OXA-23* (10%) out of 30 isolates in 3 isolates of *E. coli* as shown in Figure 4.5. While *OXA-24* gene was present in 4 isolates (13.3%) of *E. coli* as shown in Figure 4.6. Also, it has appeared the presence of *OXA-69* gene in 10 isolates (33.3%) as shown in Figure 4.7.

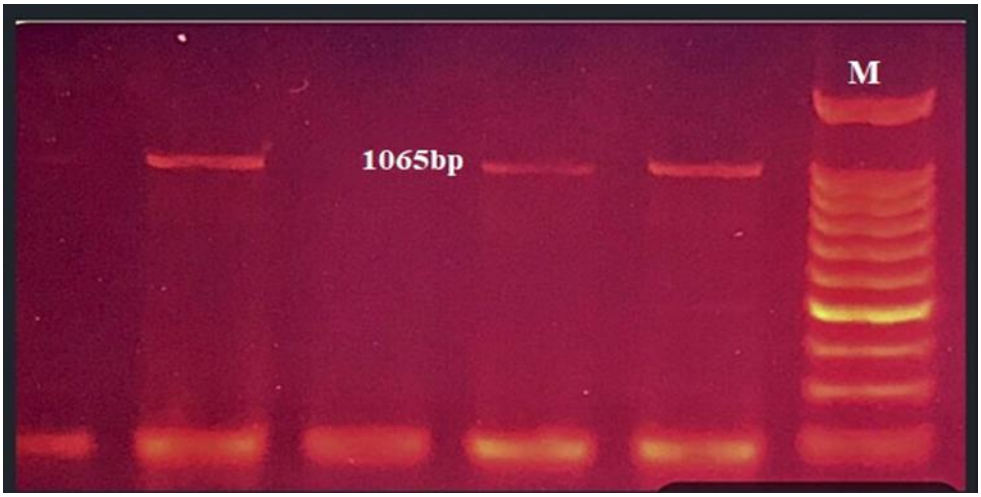


**Figure 4.5** Electrophoresis PCR product of *OXA-23* gene (1625bp) for isolates ( 2% agarose in TBE buffer) and 5 voltage/5cm$^2$ for (1:30) hour; M l =DNA ladder

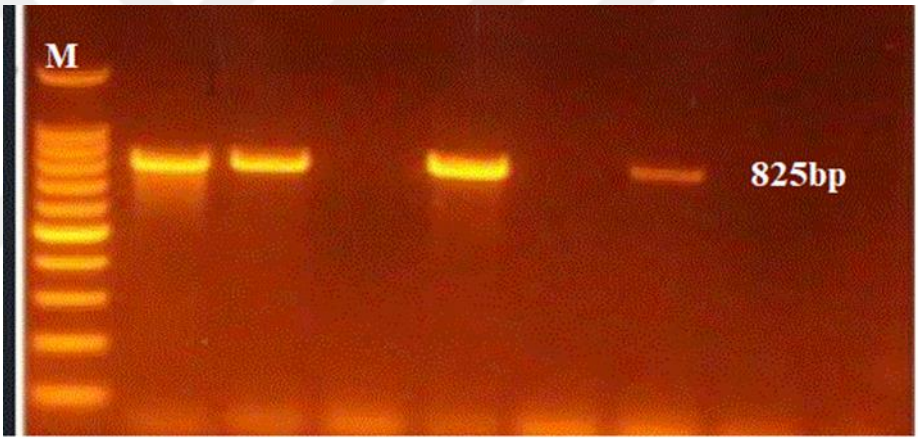


**Figure 4.6** Electrophoresis for PCR product amplification of *OXA-24* gene (825bp) for isolates ( 2% agarose in TBE buffer) and 5 voltage/5cm$^2$ for (1:30) hour;M lane =DNA ladder

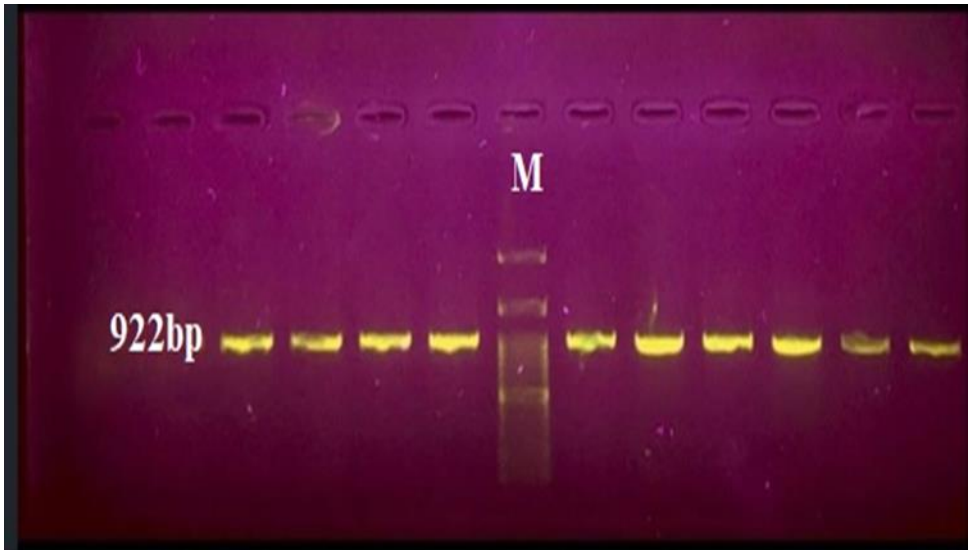


**Figure 4.7** Electrophoresis for PCR amplification of *OXA-69* gene (922bp) for isolates (2% agarose in TBE buffer) and 5 voltage/5cm$^2$ for (1:30) hour; M lane (DNA ladder)

## 4.6 Discussion

Urinary infection occurs due to different of microorganisms, involving gram-negative and gram-positive bacteria. The family of *Enterobacteriaceae* was common microorganisms that isolated from urinary infections in one of Iraqi studies was the most isolated bacteria in urinary infections, it was *E. coli* (Hussein *et al.* 2017).

Whilst it can the existence of *E. coli* in urinary infections was lower in previous studies such as in Al-Karkh Hospital in Baghdad /Iraq (Kareem and Rasheed 2011). The variation in findings with other studies is due to occur a variance in the sample size, area, community habits, imitations, personality clean level, and education levels (Al-Gasha a *et al.*2020).

In previous Iraqi study illustrated *E. coli* is dominant in urinary infections in the hospitals of Erbil/Iraq (Alsamarai*et al.*2016). Also, another global study in Saudi Arabia, Bang -ladesh, and Oman appeared similar outcomes in the prevalence of urinary infection by *E. coli* (Akbar 2001, Chowdhury and Parial 2015, Hassali *et al.* 2018).

*E. coli* represents the major bacteria that linked with urinary infections. *E. coli* has several of virulent elements and genes that cause increasing its pathogencity and antimicrobial-resistant .Nowdayes , there are recorded elevating rates of antibacterial-resistant and multi-drug phenotypes in *E. coli* which is a major issue in world ( Zagaglia *et al.*2022).

Concerning the production biofilm in urinary infection by *E. coli* the current study harmonized with several study. One of study indicated that 99% of uropathogenic of *E. coli* has ability to produce biofilm invitro and 27% from these bacteria was production of biofilm ranging from moderate to the strong (Naziri *et al.*2021). In Iraqi studies such as Kadhim Mohammed (2022) appeared in his study the ability of *E. coli* in urinary infection to form the biofilm with weak adherence (14%), moderate adherence (56%), and strong adherence (30%).

The virulent of uropathogenic *E. coli* mechanism demands the harmonius expression of virulence genes. Where the virulent- factors include adhesion structures (flagella, polysaccharide capsule, pili, outer membrane vesicle, and non pilli adhesins), outer membrane protein, lipopolysaccharide, and toxin that have a vital role in the *E. coli* ability to colonize in hosts (Karam *et al.*2019, Firoozeh *et al.*2022).

Biofilms confer the bacteria ability to resistance of antimicrobial drugs and immune response of human. Biofilm production is a significant virulent factors that has an essential role in urinary infections. uropathogenic *E. coli* produces biofilms on the bladder wall surface, and in the epithelial cells of bladder (Soto 2014, Eberly *et al.*2017).

In Iraqi study conducted by Kadhim Mohammed (2022) showed the resistance of uropathogenic *E. coli* against many antibiotics include Aztreonam (86%), Ceftriaxone (84%), Ciprofloxacin (80%), Ampicillin (72%), Chloramphenicol (50%).

Other Iraqi study appeared the high resistance of multi-drugs for *E. coli* (80.56 %) it was noticed resistance towards beta-lactamase and macrolides antibiotics (Assafi *et al.*2022).

While meta analysis in 13 research for 2504 infection found there were 1888 uropathogenic isolates of *E. coli*. Highest antibiotics-resistant avaerges were among the antibiotics class from tetracycline (69.1%), sulphonamides (59.3%), quinolones (49.4%) ,and beta-lactams (36.9%) ,in beta-lactams class was noticed high resistant in amino penicillins(74.3%),and cephalosporins (38.8%) (Bunduki *et al.*2021).

Regarding the outcomes of frequency *OXA-23, OXA-24,* and *OXA-69* genes in the present study, these outcomes approximated with other studies when revealing these gene in *Acinetobacter baumannii* while it was not found researches to detect of these genes in *E. coli*. Where in only one study conducted by Fang *et al.* (2008) appeared in their study the presence of the OXA gene encoded to OXA enzymes in 59% of *E. coli* isolated from Swedish hospitals.

Shoja *et al.* (2020) showed in their study 70% of *Acinetobacter baumannii* isolates contain *blaOXA-23*gene that was conferred *A. baumannii* the carbapenem-resistant.

Prevalence of resistant to carbapenem for *A. baumannii* bacteria has contain *blaOXA-23* discovered in many countries,38e41 and the rate of *blaOXA-23* prevelance was recorded from 31% -100% in the world (Irfan *et al.*2011, Ergin *et al.*2013).

Another study showed the prevalence of OXA-23 in A. baumannii isolates from hospitals and burns with a percentage of 94% and 100% respectively (Bakhshi *et al.*2022).

Beta-Lactam antibiotics involving penicillin, carbapenem, and cephalosporins are acting inhibition of bacterial cells via deactivating irreversible peptidoglycans transpeptidase and cause cell death (Walther-Rasmussen and Høiby 2006).

Beta-Lactamases enzymes originate from bacteria that degenerate Beta-lactams antibiotics into inactive antimicrobial compositions.These enzymes keep microorganisms from the destructive actions of beta-lactam antibiotics (Medeiros 1997),and the enzymes have a role in bacterial-resistant to drugs (Davies 1994 ).

Beta-lactamase is categorized into four groups (A,B,C, and D) depending on the sequence of amino acid identities (Walther-Rasmussen and Høiby 2006).

Carbapenems (imipenem, and meropenem) have a wide spectrum of action, and the antimicrobials resist hydrolysis via the majority of beta-lactamases, involving wide-spectrum beta-lactamases and activated chromosomal class A and C beta-lactamases (Bonfiglio *et al.* 2002). Metallo-beta-lactamases (class B) with some of class A and D beta-lactamases have ability to hydrolyze these compositions of antibiotics (Nordmann and Poirel 2002). Carbapenem-resistant became a problem for hospitals pathogen in the world (Irfan *et al.*2011).

There are various mechanisms for resistance carbapenems, involving changes of penicillins binding proteins, outer-membranes proteins loss, efflux pumps, and enzymes of carbapenems-hydrolyzing have participated in resistance of carbapenem (Huang *et al.* 2008).It has been observed that carbapenem-deactivate enzymes that belong to D class of carbapenemase including OXA enzymes (oxacillinase) considered major mechanism of impedance in *A. baumannii* (Poirel *et al.*2010).

## 5. CONCLUSIONS AND RECOMMENDATION

The present study isolated *E. coli* in urinary infection. *E. coli* isolated from urine showed resistance against penicillins, cephalosporins, and carbapenems. *E. coli* isolated from urine produced a strong biofilm (44.4%), moderate biofilm (31.1%), and weak biofilm (24.4%). Prevalence OXA-type gene noticed in *E. coli* for OXA-23 with (10%), OXA-24 with (13.3%), and OXA-69 with (33.3%).

Gene expression of OXA genes study in *E. coli* and compared with resistance to antimicrobial.OXA genes study in other species of bacteria in patients with urinary system infections.OXA genes study in species of bacteria isolated from different environments.

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