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Role of Chemokine CXCL10 and Virulence Factors of *E.coli* isolates From Patients with Urinary Tract Infection

A Thesis

Submitted to the Council of the College of Medicine/University of Karbala, in partial Fulfillment of the Requirements for the Master Degree of Science in Medical Microbiology.

By

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﴿يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ﴾

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Supervisors Certification

We certify that this M.Sc. thesis titled:

Role of Chemokine CXCL10 and virulence factors of *E.coli* isolates From Patients with Urinary Tract Infection

Was prepared under our supervision in the College of Medicine/ University of Kerbala, as a partial fulfillment of the requirements for the Degree of Master of Science in Medical Microbiology.

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Dedication

To My Master and Lord, Imam Al-Hujja Ibn Al- Hassan

To my mother, *who have overwhelmed me with her love and kindness, the main reason behind my success.*

To my father, *who was the father and the symbol, who taught me to be standing in the face of difficulties and paved the way for me and bear the pain. You will remain the sanctuary and the bond whom I be relied.*

To everyone helped and supported me.

To all my friends who support me.

*To my friend****(dr.naeema -aldulaimy)****who passed away early, I dedicate this work to here.*

Alaq-2024

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To all, please accept my truthful thanks.

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Summary:

Urinary tract infections (UTI) are one of the most prevalent bacterial infections affecting millions of individuals throughout the world each year.

The two types of urinary tract infections are categorised based on where they occur. The upper urinary tract is impacted by pyelonephritis, which affects the ureter and parenchymal kidney, and the lower urinary system is affected by cystitis and urethritis, which affects the bladder or urethra.

A case control study was conducted for period of 5 months, starting from September(2023) to January (2024) at Al-Hussein Teaching Hospital / Laboratory Microbiology in Karbala, Iraq; the total number of participants were 145 Subjects; they were divided into two groups: the first one includes 100 patients with UTIs, the second group includes (45) as healthy control.

Urine samples were taken from UTIs patients and performed for Aerobic bacteriological examination profile (microscopically ,culturing morphologically, and confirmative test by VITEK 2 compact system).C-X-C Chemokine ligand 10 levels detected in urine by serological techniques of sandwich ELISA.

Bacterial species isolated from UTIs group represented by the Gram positive bacteria were *Coagulase –v e staphylococcus* 9 (20%), the commonest isolated species followed by *Staphylococcus haemolyticus*4(8.9%), *Staphylococcus aureus* 4 (8.9%), *Staphylococcus epidermidis* 2(4.4), *Staphylococcus hominis* 1(2.2%), Enterococcus faecalis 1 (2.2%), and

Gram negative bacteria that represented by *Escherichia coli* (35.6%), were the most common followed by *Klebsiela pneumonia* 3(6.7%) and *Enterobacter aerogenes* 3(6.7%).

All study isolated of *E.coli* uropathogens were confirmed by molecular detection using specific *16srRNA.*

The percentage of virulence factors genes distribution in 16 isolates *E.coli* represented *FimH* gene 16 *(100%), OmpT* gene 8(50%), *Pap* gene 9*(*56.3%), *KpsmII* gene 13 (81.3%).

According to antibiotic sensivity which performed by vitek2 compact system some isolate *E.coli* showed a multi-drug resistance patterns (Multidrug-resistant, Extensively drug-resistant) level of resistance to the tested antibiotics, with a rate of 50%, where MDR(Multidrug- resistant) formed (25%),and XDR (Extensively drug-resistant) formed(25%).The common antibiotics sensitive for UTI treatment Meropenem, Imipenem, Gentamicin are used for treatment of UTI infected by *E.coli.*

Current study revealed that (C-X-C motif chemokine ligand 10)level was highly significant difference ($P < 0.001$) in all patients group incompared with healthy control.

This study showed that *E. coli* is the most predominant uropathogen of bacteria UTI(35.6%) followed by *Coagulase –ve staphylococcus*(20%). On the other hand, Urine chemokine 10 (CXCl10) have important roles in the diagnosis of UTI infection. *E.coli* uropathogen isolates showed many virulence factors and also showed Middle rates of resistance to different antibiotics (MDR,XDR).

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List of Abbreviations

Code	Words
AMR	antimicrobial resistance
AMP	antimicrobial peptide
BECs	Bladder epithelial cells
Bp	Base Pair
BPS	Bladder pain syndrome
CAUTIs	Catheter-associated urinary tract infection
CIAIs	Complicated intra-abdominal infections
CNS	*Coagulase-negative staphylococci*
Cuti	Complicated urinary tract infection
CXCl10	C-xc motif chemokine ligand 10
EMB	Eosin methylene blue
IBCs	Intracellular bacterial communities
IC	Interstitial cystitis
IM	Inner membrane
IFN-γ	Interferon- gamma
IP-10	Interferon-gamma inducible protein of 10 kDa
IPEC	Intestinal pathogenic E. coli
K	Capsular Antigens
LPS	Lipopolysaccharides
MDR	multi-drug resistant
µL	Microliter
Ml	Milliliter
NGAL	Neutrophil gelatinase-associated lipocalin

NK	natural killer
OD	Optical density
OM	Outer membrane
PAIs	pathogenicity islands
PCR	Polymerase Chain Reaction
PDR	Pan-drug resistant
PTX	Pentraxins
QIRs	quiescent intracellular reservoirs
RNA	Ribonucleic acid
SD	Standard deviation
Th1	Type-1 helper
TLRs	Toll-like receptors
TNF-α	Tumor necrosis factor alpha
uUTIs	Un Complicated urinary tract infection
UPEC	Uropathogenic Escherichia coli
US	United States
UTI	Urinary tract infection
VF	Virulence factor
WHO	World Health Organization
XDR	extensively-drug resistant

Chapter One

Introduction & literature Review

1.1 Introduction:

Urinary tract infections (UTIs) are common medical problems caused by bacteria invading and multiplying in the organs of the urinary tract system. These infections affect the kidneys, bladder, and urinary tract (Kahsay et al., 2024). The lower (which includes the bladder and urethra) and upper (which includes the kidneys and ureters) portions of the urinary system are further divisions. Because of its susceptibility to viral infections and inflammation, the lower urinary tract is significant. (Dickson, Zhou and Lehmann, 2024).

Many human diseases are caused by bacteria belonging to the family Enterobacteriaceae, which is a class of Gram-negative bacteria. *Salmonella*, *Klebsiella*, *Citrobacter*, *Enterobacter*, *Escherichia coli*, *Serratia*, and other species are included in it(Shetty *et al.*, 2023). The infection typically originates in the intestines and subsequently extends to the urethra as a result of the gradual proliferation of bacteria (Chahales and Thanassi, 2015).

Uropathogenic *Escherichia coli* (UPEC) is the name of the most prevalent bacterial strain responsible for urinary tract infections (UTIs)(Eulambius M. Mlugu et al., 2023). UPEC strains exhibit a variety of virulence factors that work together to create long-lasting infections, including toxins, fimbrial and , invasins, and iron-acquisition systems(Zagaglia et al., 2022).

The bacteria have the ability to establish and cause urinary tract infections (UTIs) due to the presence of virulence factors. These factors include adhesion (encoded by genes such as *fimH*, *sfa*, and *pap*), toxins (such as hemolysin, encoded by *hlyA*), Outer membrane protease(encoded by *ompT*), capsules (encoded by *kpsMII*), and iron-uptake systems that are encoded on plasmids or chromosomes' pathogenicity islands (PAIs)(Ahmed, 2021).

Urine culture is considered the most reliable method for diagnosing urinary tract infections (UTIs)(El-Refaey et al., 2020).Due to new advancements in 16S sequencing, which utilises the exact sequence of the highly conserved

16S ribosomal RNA (rRNA) gene to identify the bacteria in samples, we now have a sensitive and relatively objective reference standard for identifying organisms in urine(Shaikh et al., 2023).

Global public health authorities are encountering significant obstacles as a result of the widespread occurrence of antimicrobial resistance (AMR).

The presence of antimicrobial-resistant bacteria poses a significant risk to the effectiveness of commercially accessible antimicrobials due to their ability to acquire several pathways of resistance. There has been a global emergence and spread of bacteria that are resistant to multiple drugs (MDR). It is expected that these bacteria will become one of the main causes of death in the future(Sellera, Fuentes-Castillo and Furlan, 2023).

The cytokine response of cells, when triggered by a suitable stimulus, can function as a mechanism for both acquired and innate immunity, enabling the destruction or defense against infections(Pirdel and Pirdel, 2022).

One member of the CXC chemokine family, C-X-C motif chemokine 10 (CXCL10) is released in response to interferon gamma (IFN-γ) by activated B lymphocytes, endothelial cells, monocytes/macrophages, and fibroblasts(Oktay et al., 2022). Infections associated with CXCL10 include those caused by parasites, viruses,bacteria(Hussein, 2021).

Aims of study:

The aim of the study is:

1-To evaluate the level of CXCL10 in urine with UTI patients and healthy control .

2-Detect the common pathogenic bacteria that causes of UTI.

3-Study some virulence factor of *E.coli* like *fimH, pap, ompT* and *kpsMII* that are associated with infection .

4-Study the antibiotics resistance patterns of *E.coli.*

These aims achieved by the following objective:

1-Asses the level of CXCL10 by ELISA for patients and control.

2-Detect the pathogenic bacteria by culture

3-Profile the antibiotic sensitivity by VITEK-2 System

4-DNA extraction and molecular study the virulence factor *fimH, pap, ompT and kpsMII* by PCR

5-Confirmation of *E.coli* isolates *by 16srRNA.*

1.2. Literature Review

1.2.1.Definition of urinary tract infection (UTI):

Urinary tract infections (UTI) are one of the most prevalent bacterial infections affecting millions of individuals throughout the world each year(De Gaetano et al., 2023) Female suffer UTIs more frequently than male do for anatomical and physiological reasons. Because the female urethra is shorter than the male, bacteria entering it are more likely to ascend to the female bladder than the male bladder. Additionally, the vaginal cavity and rectal opening (where potential uropathogens reside) are closer to the female urethral opening (Silva et al., 2022). Also the male urethra's drier environment, pathogens may not be able to develop to their full potential compared to the urethra of female, Prostatic acid's antimicrobial action Men's secretions are another contributing cause that lowers male UTI risk(Khatri *et al.*, 2012).

Urinary tract infection can be classified according to the site of infection as(figure 1-1), pyelonephritis that affected the ureter and parenchymal kidney. when they affect the upper urinary tract , the second one cystitis and urethritis when they affect the bladder or urethra (lower infection tract) (de Llano, Moreno-Arribas and Bartolomé, 2020) .

The classification of UTIs according to acquisition settings and complication status is more commonly applied, despite the fact that there are other different classification schemes.

Include uncomplicated and complicated UTIs, as well as UTIs acquired in the community (CAUTIs) or UTIs linked with healthcare (HAUTIs)(Öztürk and Murt, 2020). Classical uncomplicated UTIs were considered infections in non-pregnant, healthy women often healed with antibiotic therapy .

while every other UTI, such as male cystitis, was categorized as complicated(Maisto et al., 2023).

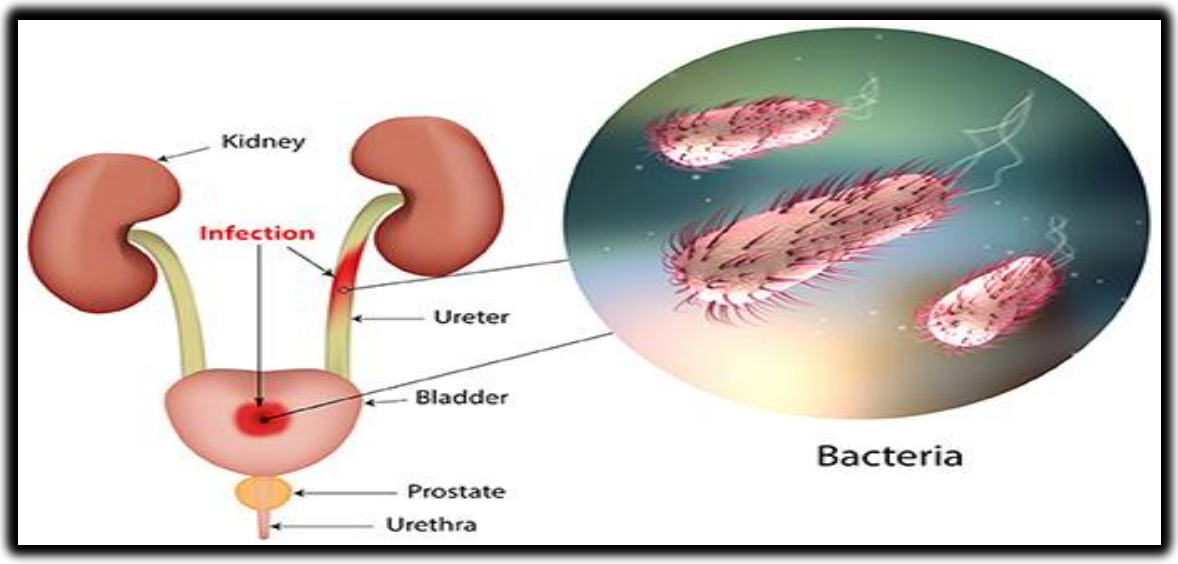


Figure (1 -1) The urinary tract and sites of infection.adapted from(Terlizzi, Gribaudo and Maffei, 2017).

1.2.2. Urinary tract infection epidemiology

In both community and hospital settings, urinary tract infections (UTIs) are among the most prevalent infectious clinical disorders. Despite their wide range of clinical severity, they have a significant epidemiological burden of morbidity and mortality, costing the healthcare system billions of dollars in treatment costs for patients and patients themselves(Harris, 2023). UTIs affect 50% of women at least once in their lifetime. UTIs are among the most prevalent infections during pregnancy7,8,9, with a reported frequency of 20% in expectant mothers.Compared to industrialised countries, developing countries have a higher prevalence of this infection(Salari *et al.*, 2023). In females of childbearing age, the percentage of asymptomatic bacteria in the urine ranges from 2–7%, but in older females, it can reach 50%(Dielubanza and Schaeffer, 2011). While men over 75 have between 7 and 10% of asymptomatic bacteria in their urine(Woodford and George, 2011). Urinary tract infections are most common in children under three months of age in uncircumcised boys, then in females under a year old(Bhat *et al.*, 2011).

UTIs pose a significant threat to the US healthcare system. Over 100,000

patients were admitted each year for this disease in the early 2000s, and over a million visits to the emergency department (ED) occurred due to UTIs. According to an analysis done in 2011, there has been a significant increase in the number of hospitalizations, with UTIs being the primary reason for admission in approximately 400 000 cases annually(Zilberberg et al., 2022).

1.2.3. Pathogenesis of urinary tract infection

A crucial factor that starts each stage of the progression of UTIs is adherence(Ana L. Flores-Mireles et al., 2015). Several adhesion proteins found on the cell surfaces of uropathogens that play a crucial role in the initial interactions between the host and pathogen(Govindarajan and Kandaswamy, 2022).A urinary tract infection (UTI) usually begins with periurethral contamination by a gut-dwelling uropathogen, which is then colonized by the urethra and migrates to the bladder, requiring appendages like flagella and pili to accomplish this Uropathogens' ability to colonize or be eradicated in the bladder is ultimately determined by the outcomes of complex host-pathogen interactions.(Ana L. Flores-Mireles et al., 2015).Figure (1-2) shown the Pathogenesis of Urinary tract infections(Mancuso et al., 2023).

When the inflammatory response of the host is unable to completely eradicate all of the bacteria, uropathogens begin to multiply and formation of biofilms, produce toxins and enzymes that help them to survive (Mancuso et al., 2023).

The following processes constitute the pathogenesis of UPEC during UTIs:

a -The urethra colonizes the periurethral and vaginal areas.

b- The cells ascend into the bladder lumen and proliferate as planktonic cells in the urine.

c- The cells adhere to the surface and interact with the defense mechanism of the bladder epithelium

d- The formation of biofilms .

e- Bladder invasion and replication Within intracellular bacterial communities (IBCs), quiescent intracellular reservoirs (QIRs) form and reminder the underlying urothelial tissue.

f -Kidney colonization and host tissue damage with an increased risk of bacteremia/septicemia(Kak Ahmed, 2023).

It doesn't take long for the number of bacteria growing within the IBC to reach 10^5 bacteria per cell. Furthermore, the bacteria in the IBC undergo morphological changes, exit the contaminated cell, and infect other cells(Spaulding and Hultgren, 2016).

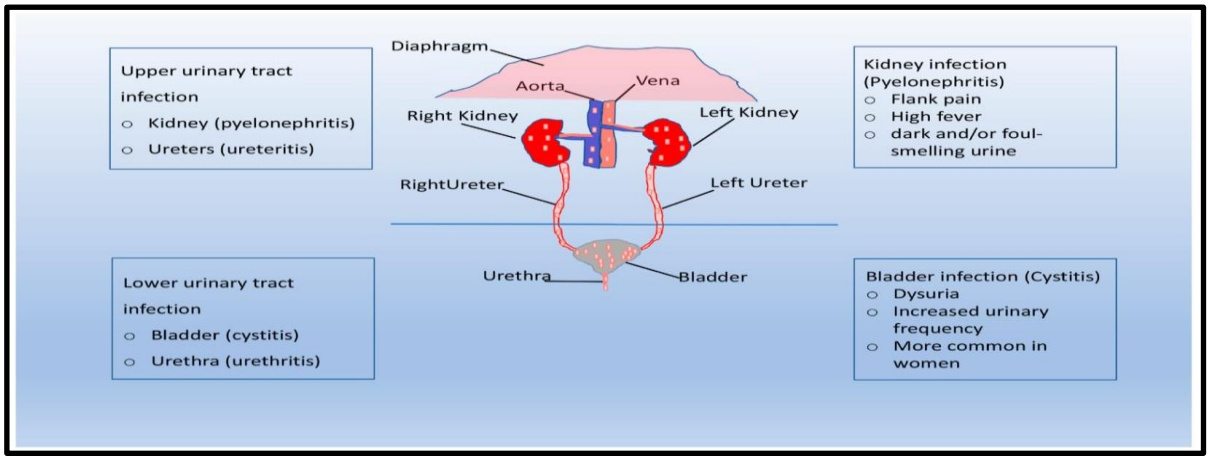


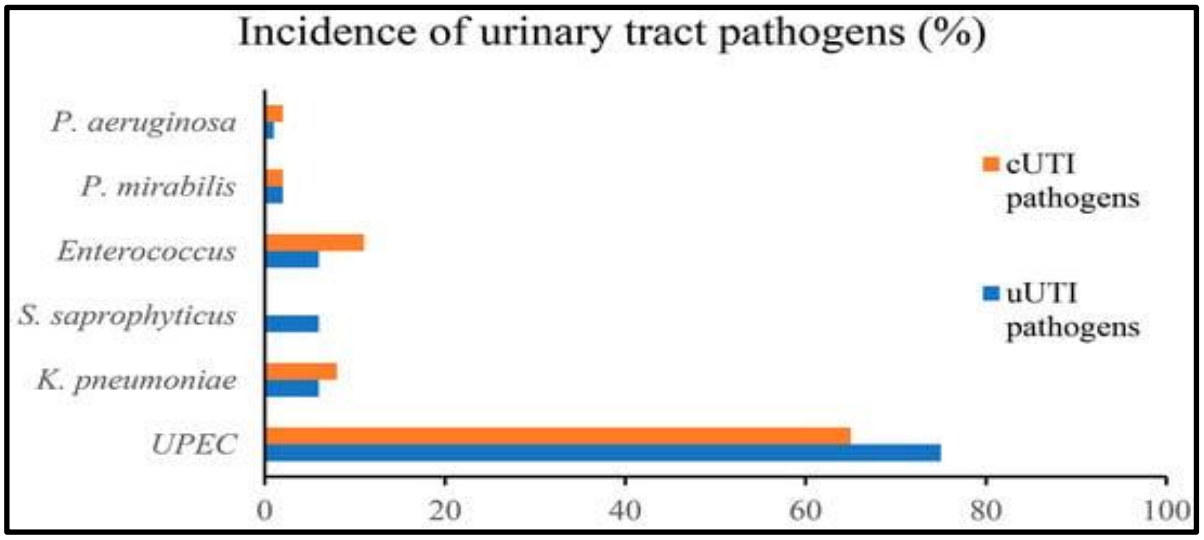
Figure (1-2) Pathogenesis of Urinary tract infections adapted from(Mancuso et al., 2023).

1.2.4. Causative agents of Urinary tract infection

Although fungi as well as viruses have been identified to occasionally cause UTIs, bacteria are the primary causal agents of these infections(Mancuso et al., 2023). Both Gram-positive and Gram-negative bacteria play a role in the occurrence of infections.Figure(1-3) shown the most frequent causative agents for both uncomplicated and complicated urinary infections include *Escherichia coli*, *Klebsiella pneumonia* , *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *and Staphylococcus aureus*(Ana L. Flores-Mireles et al., 2015).

Enterobacteriaceae represent one of the main families of Gram-negative bacilli responsible for serious urinary tract infections (Assouma et al., 2023)

The Enterobacteriaceae are an enormous family of Gram-negative bacteria that do not frame their spores. They are facultative anaerobic bacteria that can convert nitrate to nitrite at 37°C, and they have several virulence agents, including as toxins and enzymes . Certain species can be further identified through serological means using the flagella protein, lipopolysaccharide, and polysaccharide capsule. Some genera, including *Shigella* and *Klebsiella*, are non-motile, but the majority are motile due to peritrichous flagella. Other genera create capsules and develop quickly and readily on basic culture conditions(Alhadidi, 2021).



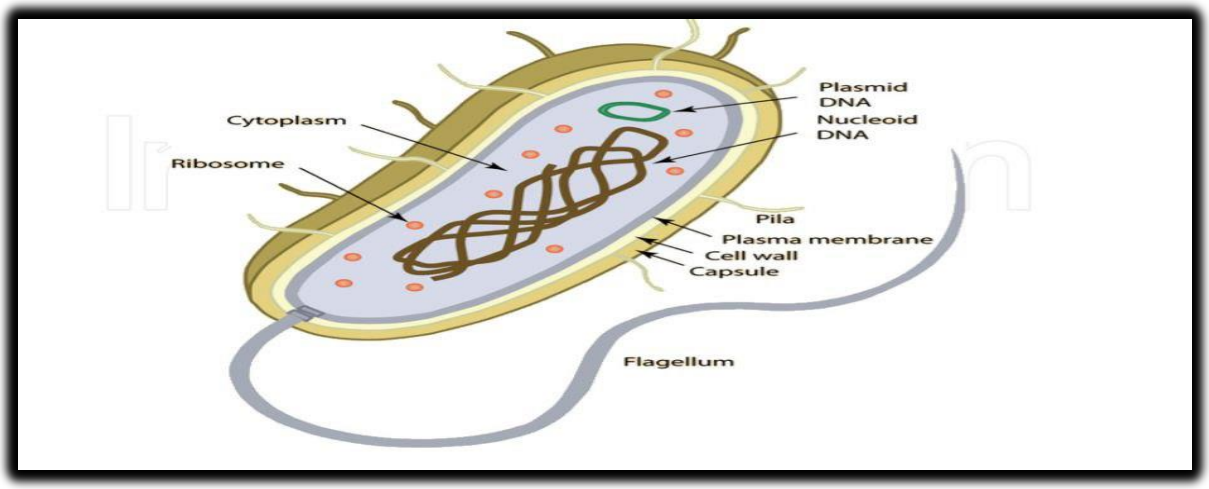
Figure(1-3): Epidemiology of uropathogens in uUTIs and cUTIs adapted from (Govindarajan and Kandaswamy, 2022)

1.2.5.*Escherichia coli*

For more than a century, *Escherichia coli* have been utilized as crucial model organisms to clarify fundamental concepts in molecular biology, disease, genetics, and evolution(Cobo-Simón, Hart and Ochman, 2023).

Figure(1-4) shown structure of *E.coli*(Basavaraju and Gunashree, 2022) .

Theodor Escherich, a German doctor (1857–1911), first discovered the bacteria *Escherichia coli* in 1885 after isolating it from the stool of. babies (Basavaraju and Gunashree, 2022).



Figure(1- 4) structure of *E.coli* adapted from (Basavaraju and Gunashree, 2022)

Under laboratory circumstances, *E. coli* cells can grow on a solid or in a liquid growth medium. It can be cultivated in the bare minimum of media, which contains other salts, trace elements, glucose as a source of carbon and energy, and ammonium salts as a source of nitrogen(Elbing and Brent, 2019). The nutrient agar, MacConkey agar, and EMB agar are typical media that can be used to cultivate *Escherichia coli* because of its low nutritional requirements(Bonnet et al., 2020).

Escherichia coli (*E. coli*) belongs to the family Enterobacteriaceae and is a Gram-negative , rod-shaped, motile, flagellated, facultative anaerobe, oxidase-negative bacteria (Kumar et al, 2022).Some *E. coli* strains can be given as probiotics and are recognized for improving host health. However, some strains are pathogenic and can infect humans and animals with extraintestinal and intestinal disease(Basavaraju and Gunashree, 2022) .

Intestinal pathogenic *E. coli* (IPEC) are the types of *E. coli* that cause diarrhea, while extraintestinal pathogenic E. coli (ExPEC) are the types that cause infections outside of the gut Such as urinary tract infections, sepsis, bloodstream infection and neonatal meningitis (Riley, 2020).

One of the most prevalent types of extra-intestinal pathogenic *E. coli* (ExPEC) is uropathogenic *E. coli* (UPEC). Uropathogenic *Escherichia coli* (UPEC) are among the most common causes of urinary tract infections (UTI)(Bunduki et al., 2021) .

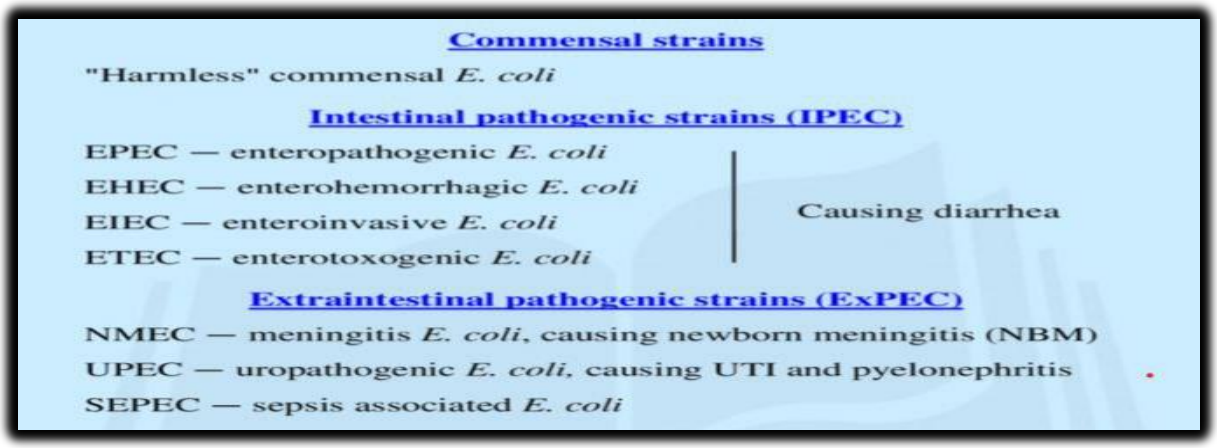


Figure (1-5) Different Escherichia coli strains(pathotypes) adapted from(Alhadidi, 2021).

1.2.6..Genetics and Factors of Virulence

The ability of a bacteria to cause illness is measured by its virulence, which is a measure of its pathogenicity. Numerous virulence factors present in *E. coli* species enable the bacterium to cause infection and diseases, urinary tract infections (UTIs) being the most significant of these infections (Parvez and Rahman, 2018).

A wide range of virulence factors (VF) are associated with the pathogenicity of Extraintestinal pathogenic *E. coli* (ExPEC). These include adhesins, toxins, iron acquisition factors, invasins, lipopolysaccharides, polysaccharide capsules.

The important *E .coli* virulence factors can be broadly classified into two groups: (i) bacterial cell surface and (ii) secreted virulence factor. Fimbriae particularly type 1 fimbriae and P fimbriae, are among the most frequent virulence factors found on bacterial cell surfaces. Additionally, flagellum, capsular lipopolysaccharide, and outer membrane proteins are bacterial cell surface virulence factors.Siderophores and haemolysin are secreted virulence factors (Huang et al., 2022).

1.2.6.1.Adhesion

Adhesion is the main factor that determines *E. coli's* virulence factor, and it is believed that the bacterial adherence to uroepithelial cells is the first step in the invasion process(Kallas et al., 2020) When UPEC pathotypes enter the human urinary system, they are able to attach to the urothelial cells' surface, initiating the colonization and biofilm-formation process. Fimbrial adhesins, which are part of superficial virulence factors, are therefore required to start this process. As an example, functional fimbrial adhesins such as P and type 1 fimbriae aid in bacterial adherence, colonization, invasion, and other processes (Khonsari, Behzadi and Foroohi, 2021).

More than 90% of UPEC isolates show the type I fimbriae adhesin factor FimH(Ribić *et al.*, 2018).

Uropathogenic *Escherichia coli* (UPEC) adhesin gene (*FimH*) is essential for mediating the initial interaction between UPEC bacterial strains and uroepithelial cells, which results in host cell colonization and invasion(Moubayed et al., 2023). P fimbriae have a significant role in determining the likelihood of bacterial colonization or invasion of the bladder in the absence of type 1 fimbriae and the upper urinary tract(Abdul-ghaffar, 2017).

1.2.6.2.Capsule

A capsule's primary function is to shield the bacterium from external factors and the host immune system, which is primarily composed of polysaccharides. The capsule offers antimicrobial resistance, antiserum activity, and protection against engulfment and complement-mediated bactericidal action in the host(Parvez and Rahman, 2018).

Based on the arrangement of their gene clusters and the processes involved in their biosynthesis and assembly, *E. coli* capsules are divided into four main types. Numerous K antigens (K1, K2, K5, etc.) are present in Group 2 capsules. K1 has a connection to meningitis. K2, a polysaccharide complex consisting of repeated oligosaccharide units, confers immune system protection due to its antiphagocytic function and confers serum resistance through the modification of the classical complement pathway by the bacterial outer membrane protein A (Omp A)(Ahmed, 2021).

1.2.6.3.Outer membrane protein(*OmpT*)

Two membranes surround Gram-negative bacteria: an outer membrane (OM) and an inner membrane (IM). Lipopolysaccharides (LPS), a highly negatively charged polymer, make up the outer leaflet of the outer membrane, which faces outward(Ahmed, 2021)

Molecule that is charged and extends into the surroundings of the bacteria. Because of its lipid composition, the outer membrane of bacteria protects them in places like the intestines by preventing hydrophobic molecules from entering the bacterium. However, it also keeps out hydrophilic compounds. Bacteria use unique protein channels known as pirons to get around this problem by letting low molecular weight hydrophilic molecules into the cell(Riedel et al., 2022) .

OMPs, or outer membrane proteins, are a crucial component of the outer membrane and play a variety of functions, including those of adhesion factors, nutrition absorption mediators, siderophore receptors, and enzymes like lipases

and proteases (Ahmed, 2021). Outer Membrane Protein T (OmpT) of *E.coli* belongs to family omptins, of outer membrane proteases(Chen et al., 2023). Proteases from bacteria are important components of virulence that are crucial at the host-pathogen interaction. By breaking down extracellular matrix proteins, host immune response proteins, and host hemostasis, they increase the pathogenicity of bacteria. These proteases are either embedded in the bacterial membrane, bound to the cell surface, or secreted (Brannon et al., 2015)

A variety of Gram-negative pathogens belonging to the Enterobacteriaceae family, such as *Escherichia coli* (*OmpT*), *Yersinia pestis* (*Pla*), *Salmonella enterica* (*PgtE*), *Shigella flexneri* (*IcsP*), and *Citrobacter rodentium* (*CroP*), contain omptins, a distinct group of integral outer membrane (OM) proteases implicated in pathogenicity (Brannon et al., 2015).

1.2.7.Resistance of antimicrobial

Antimicrobial resistance, as defined by the World Health Organization (WHO), is the inability of medications to treat diseases caused on by microorganisms. This has resulted in the spread of numerous infections, including urinary tract infections (UTIs), respiratory tract infections, and numerous other diseases that are challenging to treat because the causative microorganisms have changed their antimicrobial resistance capabilities(Adhikari, 2020).

1.2.7.1.The Evolution of Resistance in Bacteria

Mutations can confer resistance, as can acquisition of resistance from other organisms by conjugation, transduction (via plasmids or transposons), or transformation.Mutations typically result in a modification of the antimicrobial's target, which lowers its efficiency and activates efflux pumps, or they cause a shift in the metabolism through regulatory operons. There are various categories of resistance, such as extensively-drug resistant (XDR) (resistance to at least one

antibiotic in four or more groups), pan-drug resistant (PDR) (resistance to all antibiotics from all different groups), and multi-drug resistant (MDR) (resistance to at least one antibiotic from three or more groups of antibiotics. (Basak et al, 2016and Munita and Arias, 2016). Multidrug-resistant (MDR) bacteria have indeed surfaced and proliferated globally; it is anticipated that they will rank among the leading causes of death in the future (Sellera et al, 2023).

1.2.7.2 Antibiotic and antimicrobial agent resistance

Global public health authorities are facing enormous challenges due to the prevalence of antimicrobial resistance (AMR). Antimicrobial-resistant bacteria pose a threat to the efficacy of commercially available antimicrobials because of their capacity to develop numerous resistance pathways and mechanisms. Multidrug-resistant (MDR) bacteria have indeed surfaced and proliferated globally; it is anticipated that they will rank among the leading causes of death in the future (Sellera, et al, 2023).

1.2.7.3. Mechanisms of Resistance

Antibiotics are substances that specifically target certain parts of bacteria. In order for an antibiotic to be successful, it must target a specific site within the bacterium and disrupt a critical function that either stops the bacterium's growth (bacteriostatic) or kills it (bactericidal). There are numerous antibiotics on the market, each with a unique principle and mode of action.(Sebastian et al., 2021).

Mechanisms of antibiotic resistance in UPEC can include efflux pumps , which facilitate the increased evacuation of antibiotics from the inside of the cell, and chromosomal changes in target genes. Furthermore, it is possible to synthesize enzymes that either deactivate the antibacterial via breakdown or by a competitive exchange for binding sites. Figure(1-6) shows the cell targets of the antibiotics used to treat UTIs associated with UPEC(Whelan et al, 2023).

β-lactam antibiotics are among the most significant class of antibiotics in

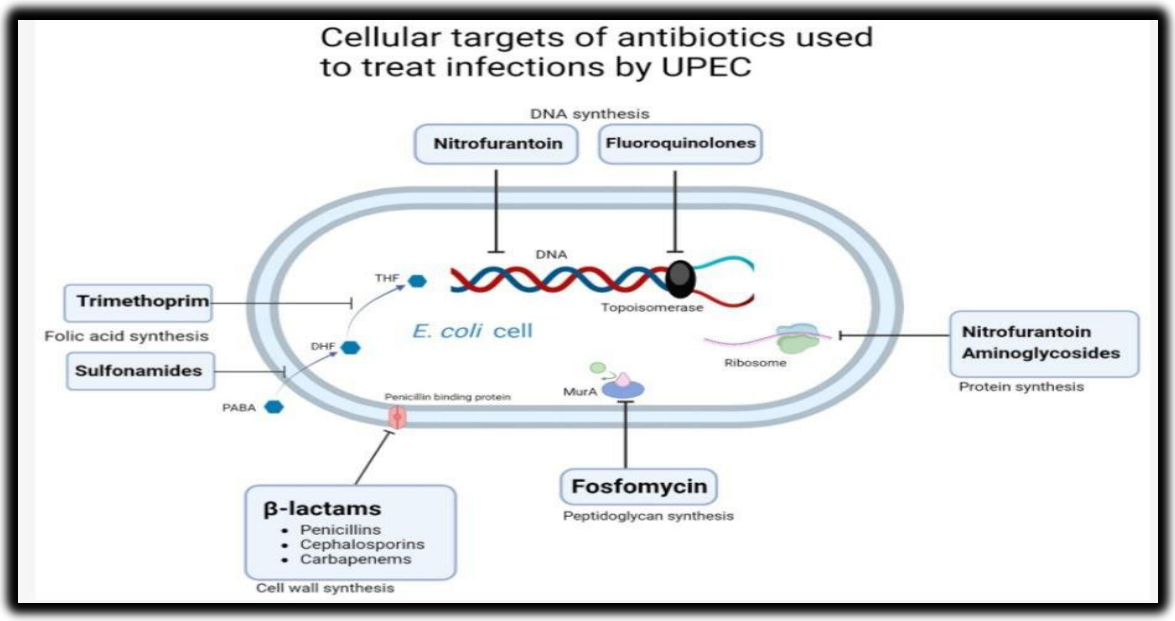
medicine. It contains cephalosporins and amoxicillin. used to treat urinary tract infections (UTIs) by binding to the penicillin-associated protein and preventing the production of the bacterial cell wall's peptidoglycan(Ny, 2019). *E. coli* produces β-lactamase, the most common and apparent resistance mechanism in *E. coli*, which contributes to the high rates of β-lactam antibiotic resistance that have been reported in numerous studies(Ahmed, 2021).

Other antibiotics target on the synthesis of proteins, RNA, and DNA. Examples of these include fluoroquinolone, which binds to DNA gyrase to prevent duplication, and topoisomerase IV, which helps uncoil DNA prior to duplication by causing double strand breaks that occur before DNA polymerase(Ny, 2019).

Because of their broad range of efficacy, fluoroquinolones have emerged as the preferred antibiotics for the treatment of complex and upper respiratory infections. It has recently been demonstrated that UPEC strains in the United States and Europe are becoming more resistant to the antibiotics fluoroquinolones and quinolones as a result of mutations or the acquisition of plasmids carrying the resistance determining genes(Alghoribi, 2015)

Targeting the 30S subunit, aminoglycosides cause misreading of the mRNA, which in turn causes an early termination of protein synthesis (Kapoor, Saigal and Elongavan, 2017)

Enzymatic drug modification, the employment of efflux pumps to lower the intracellular concentration of the antibiotic, and target modification (16S rRNA and ribosomal mutations) are the causes of *E. coli's* resistance to aminoglycosides(Ahmed, 2021).



Figure(1-6) Cell targets of antibiotics utilized to treat infections by Uropathogenic *E.coli.*

1.2.8.Response of the host immune system to uropathogenic *E. coli* infections

Most cases of UTIs occur in the bladder,strong barriers are present in the bladder epithelium, and bladder epithelial cells possess antibacterial qualities. BECs and the bladder epithelium are often disregarded by UPEC in spite of their advantages(Wu, Miao and Abraham, 2017).

The infection usually starts in the intestines and spreads to the urethra due to pathogens that grow slowly. Bacteria can develop rapidly in urine because it is a perfect growing substrate for them However, the majority of invaders are eliminated from the body by urination. In contrast, bacterial strains may adhere strongly to BECs lining the bladder by using the fimbriae organelles(Chahales and Thanassi, 2015)

One of the most important steps in the pathophysiology of UTI is the activation of the host immune response, which triggers the effector phase that is responsible for eliminating bacteria. The host's innate immune response is triggered by the introduction of UPEC into the normally sterile urine system. The process is rapid and effective, and it often involves Toll-like receptors

(TLRs), which recognise various microbial products and set off signalling pathways that ultimately result in the removal of the pathogen from the host and the creation of a memory response for potential attacks (Kak Ahmed, 2023).

1.2.8.1.Innate immune

The primary constitutive barrier in the urinary system against infection is the urothelium. It is composed of multiple layers of multinucleated umbrella cells that are resistant to infection, glycoprotein plates known as uroplakins, and mucus glycosaminoglycans, which inhibit pathogen adherence. By exfoliating the bladder lumen, apoptotic infected epithelial cells are discharged, which lowers the bacterial load. These apoptotic cells are replaced by fresh urothelium-producing inner basement stromal cells(Martell, 2020).

Epithelial cells express TLRs in addition to their barrier function to react to pathogens. When urothelium-expressed TLR4/5 is activated, pro-inflammatory cytokines, antimicrobial peptide AMPs, and numerous chemokines are released. These molecules attract bloodstream-derived neutrophils into the bladder lumen for phagocytosis. While mast cell-derived factors—such as histamine—cause vasodilation to facilitate cell migration, both macrophage and NK-cells release cytokines to support this process(Wu, Miao and Abraham, 2017)

The ability of UPEC to attach to epithelial cells can be inhibited by soluble substances like uromodulin. By targeting virulence factors, cathelicidin (LL-37), an antimicrobial peptide (AMP), is another soluble protein that plays a significant role in the immune system's response to infections, including UPEC, bacterial siderophores are bound by the neutrophil gelatinase-associated lipocalin (NGAL) protein, which inhibits bacterial growth, an evolutionary conserved protein family known as pentraxins (PTX) has the ability to act as soluble toll-like receptors (TLRs), it is believed that PTX3 binds to the surfaces of bacteria, causing complement-mediated death and enhanced phagocyte

intake. The interstitial compartments and the epithelium both contain immune cells. Dendritic cells, macrophages, neutrophils, and lymphocytes work together in the upper urinary system to fight off microbes. Natural killer (NK) cells, mast cells, macrophages, and neutrophils in the lower gastrointestinal tract (Martell, 2020).

1.2.8.2.Adaptive immune

The main component of adaptive immune responses is usually the lymphocyte, the bladder has seen an increase in several lymphocyte populations following infection, such as B cells and T cells, including CD4 and CD8 T cells(Sugiarto, 2016).The adaptive immune responses, especially in the bladder, are typically limited while the urinary tract's diverse innate immune responses are extremely susceptible to infections. Patients with bladder infections usually do not produce antibodies against the invading pathogen, Increased local IL-10 production has been identified as the fundamental cause of the bladder's incapacity to mount an adaptive response, a primary source of IL-10 in the bladder after a bacterial infection is mast cells, however UTIs that spread to the kidneys can cause the body to produce these antibodies. This amazing recurrence of UTIs, especially after bladder infections, may be largely attributed to this apparent deficiency in the bladder's antibody response(Martell, 2020).

1.2.8.3.Role chemokines inUrinary tract infections

Naturally, the cells' cytokine response in response to a suitable stimulus may represent both innate and acquired immunity to eradicate or defend against microorganisms(Pirdel and Pirdel, 2022). In the early 1970s and late 1980s, chemokines were discovered. These are a class of positively charged cytokines with molecular weights between 8 and 10 kDA. They are crucial to the immune system because they control the infiltration of immune cells and the release of inflammatory mediators (Li, Yu and Feng, 2023).

Chemokines and their receptors are essential for the activation, adherence, and recruitment of different kinds of leukocytes to inflammatory (Gao et al., 2020). The C-X-C motif chemokine ligand 10 (CXCL10) is another name for the inflammatory chemokine interferon-gamma inducible protein of 10 kDa (IP-10). The parasites, viruses, bacteria, and fungi that cause infections are linked to IP-10 (Hussein, 2021).

Type-1 helper (Th1) activation during inflammatory conditions triggers the production of IFN-γ and TNF-α, which in turn stimulates the release of CXCL10 by lymphocytes as well as other cells including neutrophils, monocytes, endothelial cells, fibroblasts, thyrocytes, and keratinocytes (Gao et al., 2020).

1.2.8.4. Treatment of UTI

1.2.8.4.1. Treatment with antibiotics

One of the most frequent reasons antibiotics are used in primary care is for UTIs and respiratory infections(Dolk et al., 2018). Nonetheless, it is well acknowledged that the unchecked, prolonged use of oral and systemic antibiotics has resulted in the rise of multidrug-resistant germs in recent years, which is concerning because there aren't many other options for treating urinary tract infections. The "golden era of antibiotics" is long over, and the world's antibiotic resistance continues to rise to alarmingly high levels (Ana L Flores-Mireles et al., 2015). Antimicrobial resistance has been identified by the World Health Organization as one of the top ten worldwide public health problems that humanity faces Thus, there is a critical need for alternate and logically designed UTI treatments(Vanderwall and Strine, 2022)

Although antibiotics can treat acute urinary tract infections in 75% of patients, their use negatively impacts the host microbiota's homeostasis when it is exposed to these medications. This, in turn, negatively impacts human health

by affecting metabolism, nutrients, pathogen resistance, and other processes(Morris et al., 2023).

1.2.8.4.2.Treatment without Antibiotics

Antibiotic treatment for UTIs is an efficient approach, although in the case of mild, uncomplicated infections, the body heals itself most of the time.When dealing with such mild cases, patients can utilize non-antibiotic approaches to speed up the healing process . UTIs can be prevented and treated by being hydrated, which means consuming lots of water and avoiding drinks that irritate the bladder (such alcohol and caffeinated drinks). Water aids in the elimination of waste from the body while maintaining the vital minerals and electrolytes that the body need. Getting enough water into the body causes the urine to become diluted and moves through the system more quickly, which hinders bacteria's ability to infect the urinary organs(Pulipati et al., 2017)

Similarly (Al-Badr and Al-Shaikh, 2013), consuming probiotics, or good bacteria, may contribute to the health and absence of harmful bacteria in the urinary system. A class of probiotics called lactobacilli aids in the treatment of urinary tract infections by preventing bacterial adherence to urinary tract cells in process called bacterial antagonism. The pH of urine is acidic because of lactobacilli, which makes it hard for bacteria to live. Additionally, they generate hydrogen peroxide in their urine. It has potent antimicrobial properties(Caretto et al., 2017).

Chapter Two
Materials and Methods

2.1.Materials

2.1.1.Equipments and instruments:

The following table (2-1) shows the instruments and equipment utilized in the study's methods as well as the references for each.

Table (2-1): The equipments and instruments used in the procedures of this study.

NO.	Name of equipment and instrument	Company	Origin country
1	Autoclave	Arnreold and Sories	U.K
2	Balance	Mettler instrument	Swiss
3	Centrifuge	Hettich	Germany
4	Cold centrifuge	Hettich	Germany
5	Deep freezer	GFL	Germany
6	Distillator (Water distiller)	GFL	Germany
7	Electrophoresis apparatus	Cleaver scientific	UK
8	ELISA instrument system	BioTeK	U.S.A
9	Incubator	Memmret	Germany
10	Micropipette tips	BIOBASIC	Canada
11	Micropipettes	BIOBASIC	Canada
12	Refrigerator	Memmret	Germany
13	PCR system	Bibase	China
14	Petri dish 9 cm	PLASTILAB	Lebanon
15	UV-transilluminator	Cleaver scientific	UK
16	Vitek 2 Compact	BioMerieux	France
17	Vortex mixer	Dargon	China
18	Water bath	Memmert	Germmany

2.1.2.Culture media

The table (2-2) below shows the culture media and their sources that were used in the study's procedures.

Table (2-2): The culture media used in the procedures of this study.

NO.	Name of culture media	Company	Origin country
1	Eosine methylene blue agar (EMB)	Himedia	India
2	MacConky agar	Himedia	India
3	Brain heart infusion broth	Himedia	India
4	Blood base agar	Himedia	India

2.1.3. Kits

The study's kits are shown in table (2-3) below.

Table (2-3): The kits used in this study.

NO.	Name of kit	Company	Origin
1	Human CXC-chemokine ligand10CXCL10 ELISA KIT	BT LAB	China
2	Presto™ Mini gDNA Bacteria Kit	Geneaid	Taiwan
3	Vitek 2 kit	Biomerieux	France

2.1.4. DNA Primers

The primers mentioned in (table 2.4) were used in the current study for detection of some virulence factors for *E.coli* study isolates.

Table (2.4): Primers used in the study.

N	Primer Name	Sequence	Product size (bp)	Reference
1	***kpsMII-F*** ***kpsMII-R***	5`-AAGTCAAAGCAGGGGTTGCCCG-3` 5`-GACGCCGACATTAAGACGCAG-3`	668	(Mostafavi et al., 2018)
2	***pap-F*** ***pap-R***	5`-GACGGTGTACTGCAGGGTGTC-3` 5`-ATATCCTTTCTGCAGGGATGCAA-3`	328	(Bahalo et al., 2013)
3	***ompT-F*** ***ompT-R***	5`-ATCTAGCCGAAGAAGGAGGC-3` 5`-CCCGGGTCATAGTGTTCATC-3	559	(Journal and Adwan, 2016)
4	***fimH-F*** ***fimH-R***	5`-AACAGCGATGATTTCCAGTTTGTGTG-3` 5`-TTGCGTACCAGCATTAGCAATGTCC-3`	465	(Bahalo et al., 2013)
5	***16SrRNA***	5`-AGAGTTTGATCCTGGCTCA-3` 5`-GGTTACCTTGTTACGACTT-3`	1400	(Mohammed, Sweedan and Flayyih, 2020)

2.1.5. Master Mix:

Table (2-5) The contents of master mix used in PCR

PCR Master Mix	COMPANY	ORIGIN
1-DNA polymerase 2-Reaction Buffer(PH 8.5) 3- dNTPs (dATP;dGTP,dCTP; dTTP). 4-MgCl2	Promega	U.S.A

2.2.Methods

2.2 .1.Subjects:

A one hunderat clinically diagnosed patients with urinary tract infection were included in the study. The patients were (22) males and (78) females with age ranged between (13-80).This is in addition to 45 healthy control participants with matched age and gender for patient group. Those patients were registered at Al-Hussein teaching hospital / Laboratory microbiology in Karbala city /Iraq were questioned with a specially designed questionnaire. This questionnaire listed in (appendix1).

2.2.2.Inclusion and Exclusion criteria

1. Inclusion criteria: All patients with urinary tract infection were diagnosed on the basis of clinical symptoms and other investigations.
2. Exclusion criteria: The patients who have autoimmune diseases, lung disease, pyelonephritis, asthma, pneumonia, cancer .

2.2.3. Ethical Approval:

All subjects involved in this work are informed and the aggremant will obtained verbally from each one before the sample were collected .This study was approved by the commite of puplication ethics at collage of medicine ,university of kerbala ,Iraq, as documented by reference number 31.

2.2.4. Study design:

This is a case - control study. The design of study was illustrated in figure (2-1).

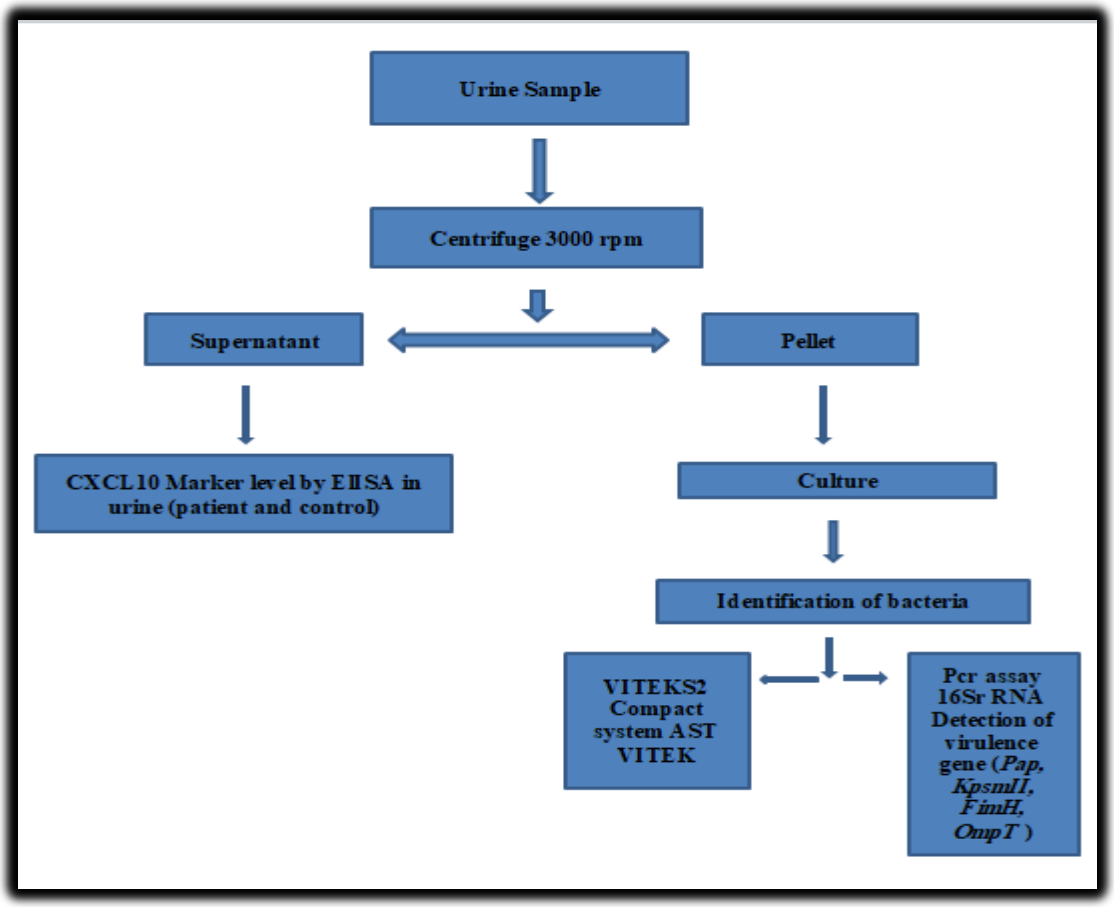


Figure (2-1): scheme design of study

2.2.5.Urine sample collection

All urine sample (100) were collected from patient with urinary tract infecction during the period September (2023) to January (2024) at Al-Hussein teaching hospital / Laboratory microbiology in Karbala city /Iraq. The urine samples with UTI were collected by sterile container directly, and then kept in a cool place by using a cool box because low-temperature act to inhibit bacterial replication until transported to laboratory then cultured on MacConkey Agar and incubated for 24 hours at 37°C.

2.2.6.Methods of sterilization

Using an autoclave at 15 bar/inch2 pressure and 121°C for 15 minutes, moist heat sterilization was utilized to sanitize media and some solutions that are not impacted by heat, while glassware was sterilized using dry sterilization for 1-2 hours at 160–180°C (By and Hato, 2019) .

2.2.7.Preparation of Culture Media

The manufacturer's instructions were followed in the preparation of all the culture medium utilized in this study by utilizing their defined procedures. as follows:

2.2.7.1.Blood Agar Medium

Blood agar base was made in accordance with the manufacturer's instructions, autoclaved for 15 minutes at 121 degrees Celsius and 15 bar/inch2, cold to 50 degrees Celsius, and aseptically added 5 milliliters of fresh, sterile human blood for each. After carefully mixing the material to achieve homogeneity,it was poured 95 ml into sterile Petri dishes(Monther, 2022).

2.2.7.2. MacConkey agar

The medium was made by dissolving 51.53 grams of powder in one liter of distilled water, and it was then autoclaved for 15 minutes at 121 degrees Celsius to sterilize it. Once the medium has cooled, it is transferred onto sterile Petri dishes and allowed to at room temperature to solidify after that medium refrigeted at 4c until used, this medium used to find the family the Enterobacteriaceae(Al-timimi, 2023).

2.2.7.3. Eosin methylene blue(EMB) agar

EMB was prepared by dissolving 36 g in 1000 ml of D.W, then autoclaved for 15 minutes at 121 degrees Celsius and distributed into plates after that medium refrigeted at 4c until used, EMB medium used to distinguish *E.coli* from other members of the enterobacteriaceae family. (Sabah and Aboshabaa, 2022).

2.2.8.Preservation of Bacterial Isolates

2.2.8.1 Short-term Preservation

After streaking the slant nutritional agar into the tubes, they were incubated for 24 hours at 37 °C and then stored at 4 °C till needed. (Ahmed, 2021).

2.2.8.2 Long-term Preservation

A long-term preservation media for isolates was created by mixing 15 mlof glycerol with 85 ml of brain-heart infusion broth. This mixture was then distributed among several sterile tubes that remained unaffected by heat before being sterilized by incubation. Following a period of cooling to room temperature, the tubes were nutrients agar-grown colonies that were injected and kept for 24 hours at 37 °C, after which the isolates were stored at -20 °C, with the knowledge that they might last for roughly 6–8 months. (Ahmed, 2021).

2.2.9. Laboratory Diagnosis

A number of morphological characteristics of bacterial growth on Eosin Methylene blue Agar, MacConkey Agar, and Blood agar were examined for primary identification tests. These characteristics included colony shape, color, and smell, edges, and texture(Alhadidi, 2021).

2.2.9.1. Microscopic Examination

Gram stain was applied to the bacterial isolates so that their shape could be seen under a microscope. On a sterile, microscopic glass slide, a tiny sample of the suspicious colony of the positive culture was put and fixed. Under oil immersion, every slide was examined (Hata and Thomson, 2017)and (Froböse et al., 2020).

2.2.9.2. Diagnosis Using VITEK- 2 Compact System

2.2.9.2.1.Identification of Bacteria with VITEK -2 System

Using the VITEK-2 system, a selected number of the bacterial isolates was chosen for identification and assessment of their susceptibility to antibiotics. The VITEK -2 system consists of the computer and instrument shown in (figure 2) as well as an instrument made up of the following five important.The results obtained from VITEK-2 system are shown in (appendix 2) and (appendix3).

Figure: (2-2) :VITEK 2 System (1) Fill Door , (2) User Access door,(3) waste door

A-Keypad.

B- Fill Door: The sample is transferred from tubes into the kit via a transmission pipeline within the kit; this procedure takes seventy-two seconds to complete.

C- Load Door: this procedure continues for for three to five minutes and involves cutting the transmission pipeline on the kit and transferring it into the incubator.

D- User Access door: This is where the kit's incubation and measurement changes as a result of bacterial growth in order to obtain a result.

E- Waste Door: utilized for kit collection following analysis finishing and result acquiring.

Using kits with specialized diagnostic capabilities, the VITEK 2 system is used to confirm isolate diagnoses and conduct sensitivity tests for antibiotics, which are utilized to diagnose the majority of types.

Every sample requires two kits, one for diagnosis and the other for a sensitivity test. The diagnosis kit has 64 wells with colored indicators and dried substances in each well. The VITEK 2 compact system records color changes caused by bacterial growth, and the sensitivity test diagnosis package includes 18–20 antibiotics spread throughout 64 wells. There are many concentrations of each antibiotic, and the VITEK 2 system records the turbidity changes during bacterial growth.

2.10.Molecular identification of *Escherichia coli*

To determine the additional identities of the isolates, molecular analysis was performed for all UPEC isolates. For this,uniplex conventional PCR experiment was employed to confirm bacterial identification for study isolates characteristic using the *16S rRNA* genes (Kak Ahmed, 2023) .

2.10.1 DNA extraction of *Escherichia coli*

The genomic DNA was extracted from the E. coli isolates using Presto™ Mini Gdna Bacteria Kit(Geneaid ,Taiwan) according to the manufacturer's instructions , as shown in the following protocol:

1-Sample Preparation:

A-Took small amount of bacterial cells to a 1.5 ml microcentrifuge tube contain 0.5 ml distilled water and was mixed together by vortex for 15 second.

B--The microcentrifuge tube was centrifuged at 14000 rpm for 2 minutes to pellet the cells and the supernatant was removed.

C- 180 µl of GT Buffer was added and gently re- suspended the cells pellet by vortex.

D-20 µl of Proteinase K was added and Incubated at 60ºC for at least 10 minutes. During incubation, was inverted the tube every 3 minutes.

2-Lysis:

A-200 µl of GB Buffer was added to the sample and mixed by vortex for 10 seconds.

B-The microcentrifuge tube was incubated at least 10 minutes to ensure the sample lysate was clear. During incubation, inverted the tube every 3 minutes.

C-Elution Buffer (200 µl per sample) was pre-heated at 70ºC (for step 5 DNA Elution).

3- DNA Binding:

A- 200 µl of absolute ethanol was added to the sample lysate and was mixed by shaking vigorously.

B-. Placed was GD Column in a 2 ml Collection Tube.

C- mixture was transfered (including any insoluble precipitate)

to the GD Column and was centrifuged at 14000 rpm for 2 minutes.

D-Discarded was the 2 ml Collection Tube containing the flow-through and was placed the GD Column in a new 2 ml Collection Tube.

4. Wash:

A-400 µl of W1 Buffer was added to the GD Column,and was centrifuged at 14000 rpm for 30 30 seconds then was discarded the flow-through.

B- Placed the GD Column back in the 2 ml Collection Tube.

C- 600 µl of Wash Buffer was Added to the GD Column, was centrifuged at 14000 rpm for 30 seconds then was discarded the flow-through

E-Placed the GD Column back in the 2 ml Collection Tube, then was centrifuged again for 3 minutes at 14000 rpm to dry the column matrix.

5- Elution:

A-the dried GD Column was Transferred to a clean 1.5 ml microcentrifuge tube

B-100 µl of pre-heated Elution Buffer was added into center of the column matrix, then was Centrifuged at 14000 rpm for 30 seconds to elute the purified DNA.

2.11. Primers Preparation

Macrogen provided the lyophilized primers utilized in this investigation. The lyophilized primers, as indicated in table 2.7, were mixed with 300µL of nuclease-free water to produce a stock solution with a final concentration of 100 pmol/µL. To make a working solution, 10µL of the primer stock solution was added to 90µL of nuclease-free water, which was stored in a freezer at -20°C.

2.12. PCR Mixture Preparation

The DNA taken from every isolate of *Escherichia coli* was subjected to a PCR procedure to identify various target genes for the investigation. Every PCR

reaction had a final volume of 25µL, with all the genes targeted and their appropriate size were shown in (table 2.6)

The GoTag Green Master Mix (2x) solution was melted at room temperature and mixed through a vortex to homogenize the mixture before use in the PCR. Prior to use, the primer's solutions were also mixed via a vortex

.

Table(2.6) : Primers and their proper volumes for PCR mixture

NO.	Gene	Vol. of master mix (2x)	Primer	N.F.W.*	Vol. of template DNA	Total Volume
1	***FimH***	12.5	4	3.5	5	25
2	***Pap***	12.5	4	3.5	5	25
3	***KpsMII***	12.5	4	3.5	5	25
4	***OmpT***	12.5	4	3.5	5	25
5	***16srRNA***	12.5	4	3.5	5	25

***N.F.W = nuclease free water**

2.13. Preparation of agarose gel and DNA loading

In order to prepare an agarose gel, 100 ml of TBE buffer (90 ml D.W. were added to 10 ml TBE buffer l0X, the final concentration was 1 X and pH 8) was first prepared.

Then, 1-1.5 g of agarose powder were added. After allowing the liquid to cool to 50°C and being placed in a boiling water bath until it formed clear, 0.5 mg/ml of ethidium bromide was added.. The agarose was generously poured into an equilibrated gel tray that had previously been set up with two combs linked in the middle and at the end.

The gel tray's two ends closed and the agarose was allowed to solidify for 30 minutes at room temperature.The seal and combs were carefully taken out of the tray Five microliters of the amplified PCR product were added to the agarose gel wells using the comb-made wells intended for loading DNA

samples, followed by DNA marker. (ladder) to one of the wells, the gel tray was put in the electrophoresis chamber, and IX TBE buffer was introduced to the chamber until it completely covered the gel's surface. 1.5 to 2 hours of electric current at 70 volts were used. (Sabah and Aboshabaa, 2022).

2.14. Thermal Cycling Condition

Prior to being placed inside the thermocycler, the components of each PCR mixture were vortexed together in the Eppendorf tube. The PCR thermal cycler apparatus was used for the reaction, and the manufacturer's instructions were followed. The procedure begins with the initial denaturation phase, which is carried out at 94 to 95 °C for varying lengths of time (minutes).

This is followed by repeated cycles that comprise the denaturation step (94 to 95 °C), the process of annealing at a temperature determined by the primer passing through the procedure, with the extension step carried out at 72 °C and the final extension step which was completed at 72 °C. as shown in (table 2.7).

Table(2-7): Genes and their optimum thermal cycling conditions

Gene	Initial denaturation (°C/min)	Denaturtion (°C/s)	Anneling (°C/s)	Extention (°C/s)	Final extention (°C/min)	Cycle
FimH	95/3 min	95/0.30 sec	63/30sec	72/1min	72/5min	32
KpsMII	95/3min	95/30sec	58/30sec	72/1min	72/5min	32
OmpT	95/3min	95/0.30sec	58/30sec	72/1min	72/5min	32
Pap	95/3 min	95/0.30 sec	59/30sec	72/1 min	72/5 min	32
16SrRNA	95/3 min	95/0.30	55/30sec	72/1 min	72/5 min	32

2.15. Biomarker Profile Assay by ELISA

urine level of CXCL 10 was determined by classic sandwich-ELISA using ELISA research kits.

2. 15. 1 .Principle of Sandwich ELISA technique

sandwiched with an antibody ELISA may represent the most effective immunosorbent technique for antigen detection. due to the fact that they are usually two to five times more sensitive than those in which the antigen is attached to the solid directly.

Micro titer plate wells are coated with a particular (capture) antibody and then incubated with test solutions containing antigen in order to detect antigen. After removing the unbound antigen, another incubation is carried out with an additional antigen-specific antibody conjugated to an enzyme (developing reagent). Substrate is introduced after the unbound conjugate has been removed Following. The level of substrate hydrolysis is assessed after a second incubation. The amount of antigen in the test solution is directly correlated with the amount of hydrolyzed substrate. (Hussein, 2021).

2.15.2. Test principle

Human Cxcl10 antibody has been pre-coated onto the plate.After being added to the sample, CXCL10 binds to the antibodies coated on the wells.and after that, CXCL10 in the sample was bound by the addition of biotinylated human CXCL10 antibody.The biotinylated CXCL10 antibody was subsequently bound by the addition of streptavidin-HRP.During a washing step following incubation, unbound streptavidin-HRP was removed.After adding the substrate solution, color developed in accordance to the concentration of human CXCL10. The addition of an acidic stop solution stopped the reaction, and the absorbance at 450 nm was measure.

2.15.3.Reagent preparation

1-Before use, all reagents should be brought to room temperature.

2-Standard reconstituted the 120µl of the standard (640ng/L) with 120µl of standard diluent to

generate a 320ng/L standard stock solution. Allowed the standard to site for 15 minutes with

gentle agitation prior to making dilutions. Prepared duplicated standard points by serially

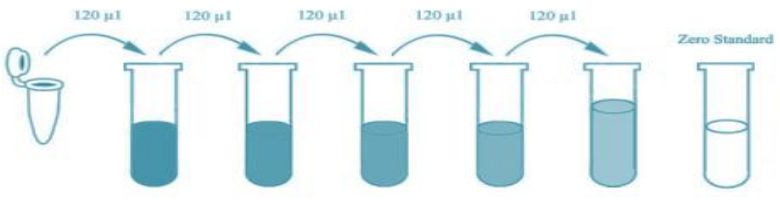
diluting the standard stock solution (320ng/L) 1:2 with standard diluent to produce 160ng/L,

80ng/L, 40ng/L and 20ng/L solutions. Standard diluent serves as the zero standard (0 ng/ml).

The remaining solution was used within a month after freezing at -20°C. The recommended

standard solution dilutions are as follows:

320ng/L	Standard No.5	120µl Original Standard + 120µl Standard Diluent
160ng/L	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
80ng/L	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
40ng/L	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
20ng/L	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
640ng/L	320ng/L	160ng/L	80ng/L	40ng/L	20ng/L

Figure (2.3): Concentration of standards ofCXCL10

Wash Buffer: 20 ml of diluted wash buffer 500 milliliters of 1x Wash Buffer can be obtained by concentrating 25 times in deionized or distilled water.

If crystals were observed to have developed in the concentrate, mixed gently until the crystals were fully dissolved.

2.15.4.assay procedure steps:

1- Before beginning the assay procedure, all of the reagents were prepared.

2- Each sample well received 50µl of standards, 40µl of sample, and 10µl of biotinylated antibody; the standard well did not get biotinylated antibody since it was present in the standard solution.

3- Each well (standard and sample wells) received 50µl of Streptavidin-HRP reagent; the blank well received no additions. The wells were then covered with a seal plate, gently shaken, and incubated for 60 minutes at 37C.

4-The color developed as follows: 50µl of Substrate Solution A was added to each well first, followed by the addition of 50µl of Substrate Solution B to each well. Shake well to combine. incubated in a dark, 37°C environment for ten minutes in order for color to develop.

5- 50µl Stop Solution to each well had been added to stop the reaction (The blue color immediately became yellow.

6-After applying the stop solution, the optical density (OD value) was measured at 450 nm in less than ten minutes.

2.15.5. Calculation of results

The results were calculated according to the standard curve showed in (figure 2-4).

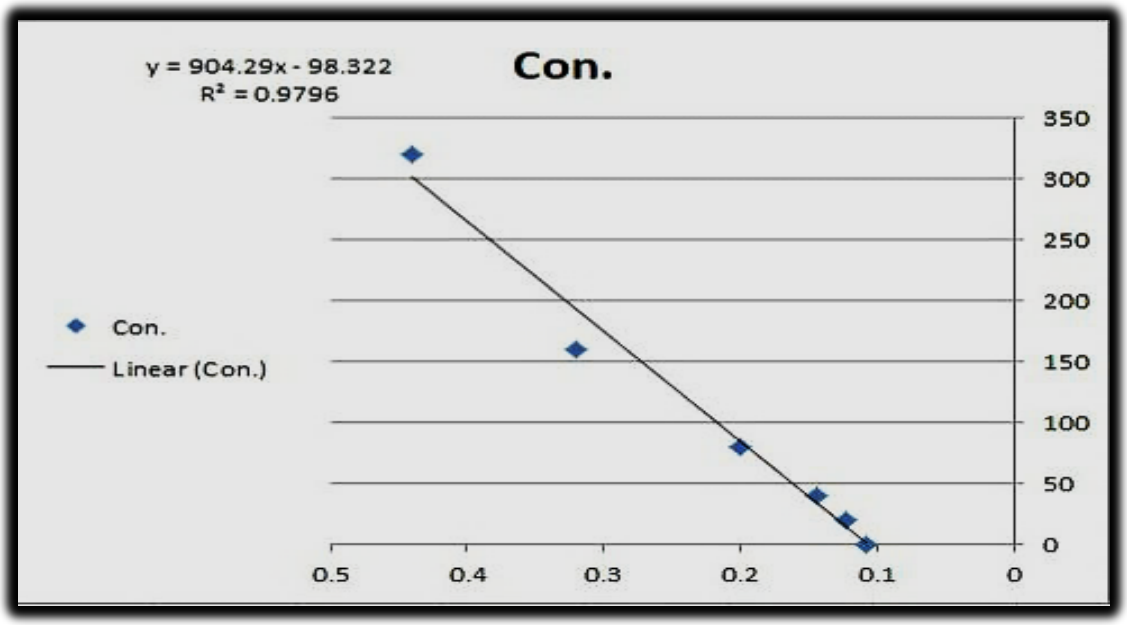


Figure (2-4): CXCL10 standard curve

2.16. Statistical analysis

IBM SPSS version 27.0 used to analyze the data statistically, the non-parametric data expressed as frequency and frequency percentages. While the parametric data calculated by Mean,SD, and Rock curve, the probability calculated through using chi-square. The probability is considered significant when it was less than 0.05.

Chapter Three

Results

3.Results

3.1.Demographic Characteristics of study

Baseline characteristics of the studied groups showed in(table 1).The study was observed that females had a relatively higher prevalence rate than male,where the percentage of male (15.6%) and percentage of female was (84.4%). The study was conducted that People who live in the rural areas suffered from urinary tract infections more than those who live in urban areas, where the percentage in rural areas was (66.7%) and in urban areas it was (33.3%). According to the season, people suffered from urinary tract infections more in the summer season than in the winter season, where the percentage in summer (68.9%) and in winter was (31.1%). According to the age the distribution of UTI cases 42.2% belonged to (15-30) year age group followed by (26.7 %) who belonged to (46-60) year age group and 20% who belonged to (31-45) year age group,11.1% who belonged to >60 year age group.

Table (3-1): Demographic Characteristics of patients with UTI.

Variables		Patients	percentage
		NO.	%
Sex	Male	7	15.6
	Female	38	84.4
	Total	45	100
Residency	Urban	15	33.3
	Rural	30	66.7
	Total	45	100.0
	summer	31	68.9
Season	Winter	14	31.1
	Total	45	100.00
Age	15-30	19	42.2
	31-45	9	20
	46-60	12	26.7
	>60	5	11.1
	Total	45	100.00

3.2.Percentage of Bacterial isolates

Bacterial types isolated from UTIs group showed in (table 3-2) .

The Gram positive bacteria were 23 isolates (51.11%) represented by *Coagulase –ve staphylococcus* (20%) were the commonest isolated genera followed by *staphylococcus haemolyticus* (8.9%), *staphylococcus aureus* (8.9%), *Staphylococcus epidermidis*(4.4%),*Staphylococcus hominis* (2.2%), *Enterococcus faecalis*(2.2%),*streptococcus pneumonia* (4.4%) ,but 22 isolates (48.88%) were Gram negative bacteria that represented by *Escherichia coli* (35.6%) followed by *Klebsiela pneumonia* (6.7%) and *Enterobacter aerogenes* (6.7) .

Table (3-2): percentage of Bacterial study isolates that identification by vitek2

Bacterial species	No. of isolates	Percentage %
Escherichia coli	16	35.6
Klebsiela pneumonia	3	6.7
Enterobacter aerogenes	3	6.7
Streptococcus pneumonia	2	4.4
Coagulase –ve staphylococcus	9	20
Enterococcus faecalis	1	2.2
Staphylococcus epidermidis	2	4.4
Staphylococcus haemolyticus	4	8.9
Staphylococcus aureus	4	8.9
Staphylococcus hominis	1	2.2
Total	45	100.0

3.3. Uropathogenic *E.coli* isolates

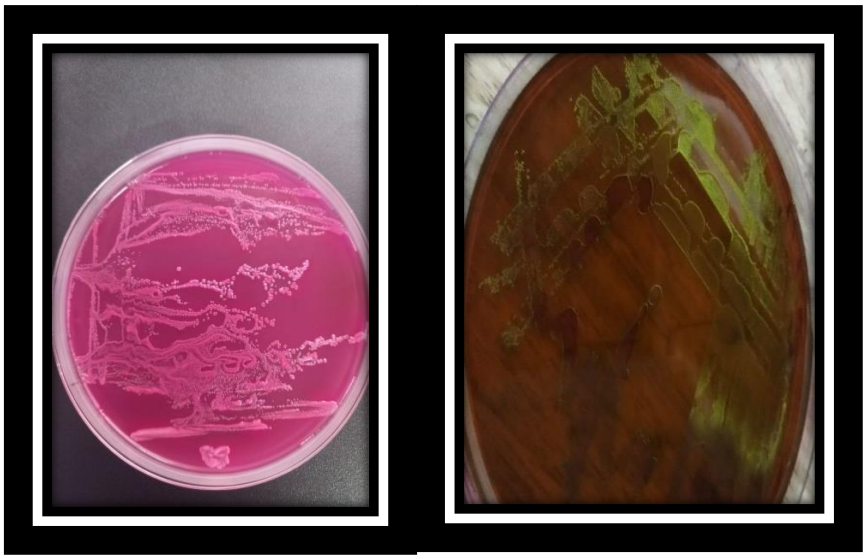
In all, sixteen *E. coli* isolates were chosen for the current study; these isolates came from individuals who presented to the laboratory with UTI symptoms. Six specimens (or 37.5% of the total) were obtained from male cases, whereas ten (or 62.5% of the total) were obtained from female cases. as shown in (table 3-3).

Table (3-3):The distribution of the 16 positive Uropathogenic *E. coli* isolates according to sex

Sex	Number(n)	Percentage%
Female	10	62.5
Male	6	37.5

3.4. Morphological identification of *E.coli*

E. coli isolates were identified depending on their morphological characteristics. The isolate appeared as green metallic sheen when cultured on EMB media and the colonies appeared as bright pink when cultured on Mac Conkey agar.as showed in (figure3- 1).



(A) **(B)**

Figure (3-1) : Bacterial growth on two different media. A: Bacterial colonies on MacConkey agar, bright pink colonies. B: metallic Sheen green colonies on EMB media

3.5. Molecular Detection of *E. coli*

3.5.1DNA Extraction

The total DNA of 16 *E.coli* isolates was successfully extracted by procedure mentioned in chapter 2. The results showed in (figure 3-2)

Figure (3-2):Electrophoresis of DNA *E.coli* isolates.Running conditions Gel agarose 1%, 75volt for 30 minute ,stained with ethidium bromide dye.

3.5.2.Identification of *E.coli* by *16srRNA* gene

The results of *16srRNA* Gene PCRclarified that all isolates of *E.coli.* Were successfully amplified and shown single band of *16srRNA* Gene of *E.coli* at (1400bp) as demonstrated in figure (3- 3) .

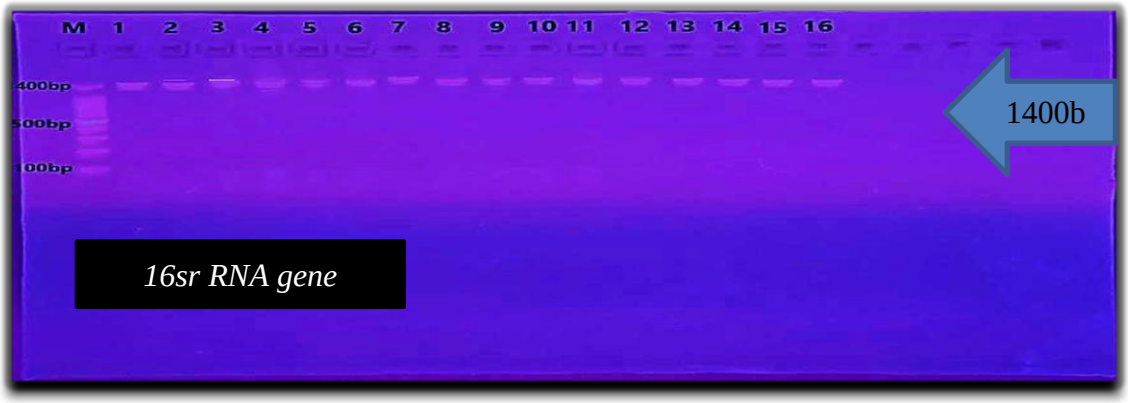


Figure (3-3): Identification speies specific of *16srRNA* gene of *E.Coli* PCR product band size (1400bp). Lane M = DNA ladder comb (100bp), lane (1-16) represent gene of *16 srRNA* of E.coli . The 16 isolates of *E.coli* is positive.

3.5.3.Detection of *E.coli* virulence genes

3.5.3.1.PCR amplification of *E. coli* virulence genes

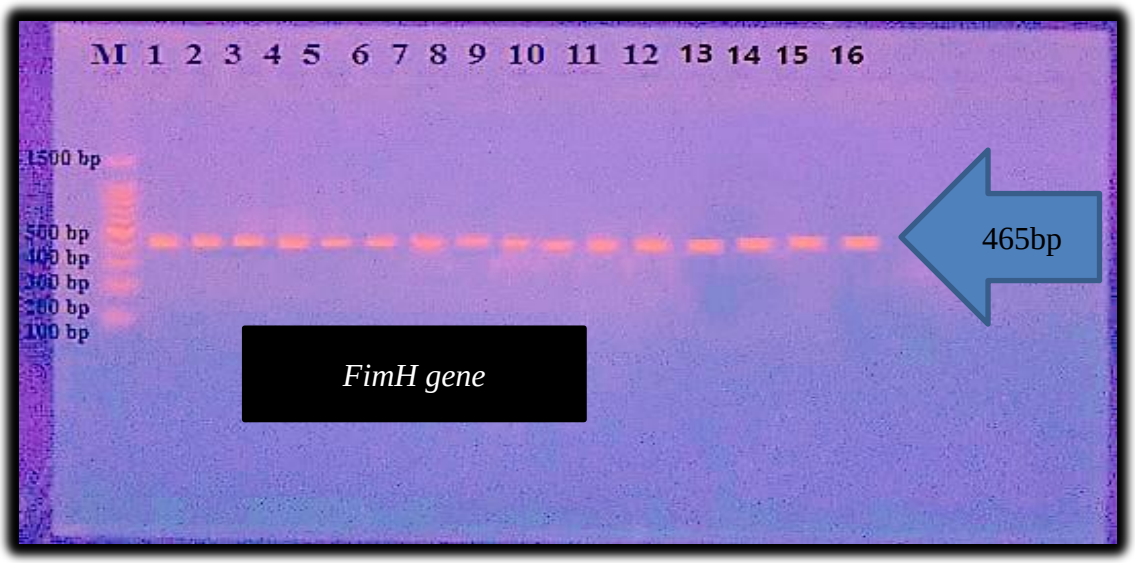
Conventional PCR was done in monoplex patterns.It were carried out to 16*E.coli* isolates, were amplify different fragments of *fimH,pap,ompT,KpsmII* genes encoding for Type 1 fimbria, P fimbria (pili), Outermembrane protease T, Capsule respectively in *E. coli* isolates with specific primers,all of the genes were detected in different percentages. Results are presented inand (table 3-4) .

Table (3-4) :Distribution of virulence genes in 16 isolates *E.coli*

Virulenc factor	Gene	Positivity rate (n/16) N= number of isolates	Positivity rate(%)
Type 1 fimbria	*fimH*	16/16	100%
P fimbria (pili)	*Pap*	9/16	56.3%
Outermembrane protease T	*ompT*	8/16	50%
Capsule	*KpsmII*	13/16	81.3%

3.5.3.1.1.Detection of *FimH* gene in *E.coli* isolates

PCR were carried out as previously described in chapter(2). the monoplex PCR assay for FimH gene revealed presence FimH gene in all 16 E. coli isolates. As shown in (figure 3-4).



Figure(3-4): Electrophoresis of amplified *fimH* (465bp).Agarose gel 1%,75volt for 1hrs,stained with ethidium bromide dye M: 100bp ladder marker. All isolates were Positive.

3.5.3.1.2 Detection of *Pap* gene in *E.coli* isolates

The adhesin *pap* fimbriae gene were showed a percentage 9 (56.3%)of 16 study isolates. As shown in (figure3- 5).

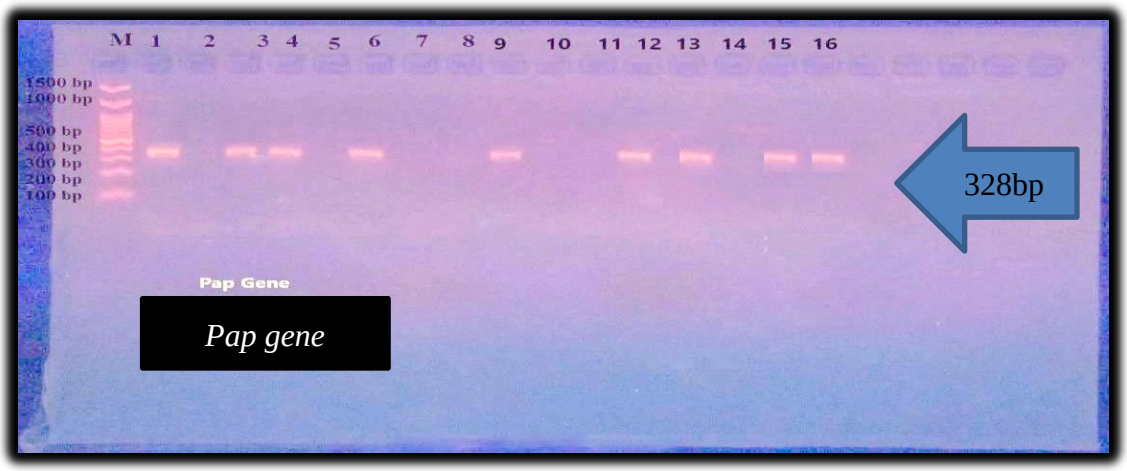
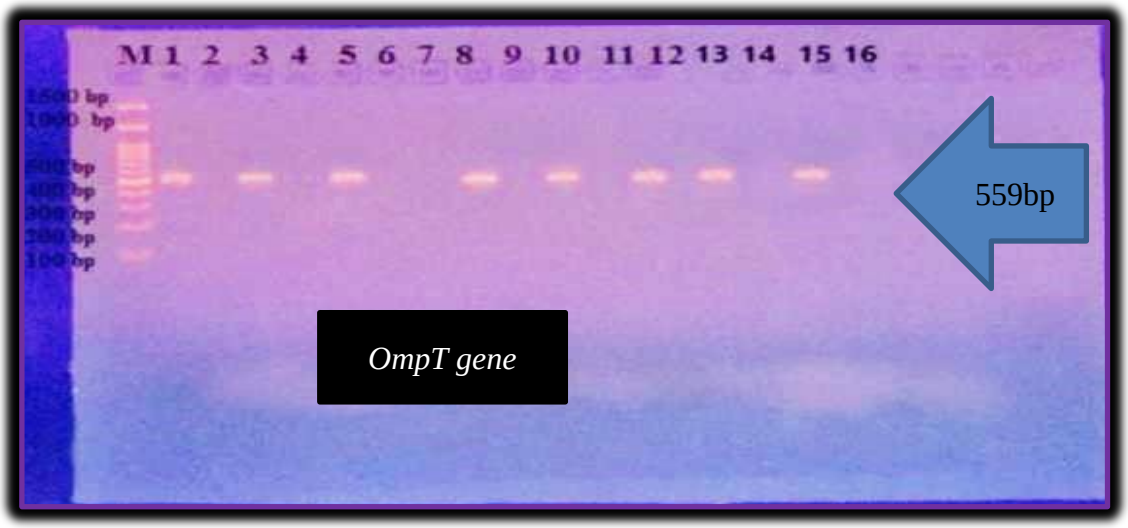


Figure (3-5): Electrophoresis of amplified *pap* (328bp).Agarose gel 1%,75volt for 1hrs, stained with ethidium bromide dye. Isolates 1,3,4,6,9,12,13,15,16 positive result, isolate 2,5,7,8,10,14,11 negative result

3.5.3.1.3. Detection of *OmpT* gene in *E.coli* isolates

In this study, detection of *OmpT* gene coding for outermembrane protease T revealed that 8 (50%) of 16 study isolates .Figure(3-6) showed result.



Figure(3-6):Electrophoresis of amplified *OmpT* (559bp).Agarose gel 1%,75volt for 1hrs,stained with ethidium bromide dye. Isolates 1,3, ,5,8,10,,12,13,15, positive result,isolate2,4,7, 11,6,9,14,16 negative result.

3.5.3.1.4 Detection of *KpsmII* gene in *E.coli* isolates

The PCR assay results identified *KpsmII* gene this study showed 13(81.3%of *E.coli* study isolates).The result showed in (figure 3-7) .

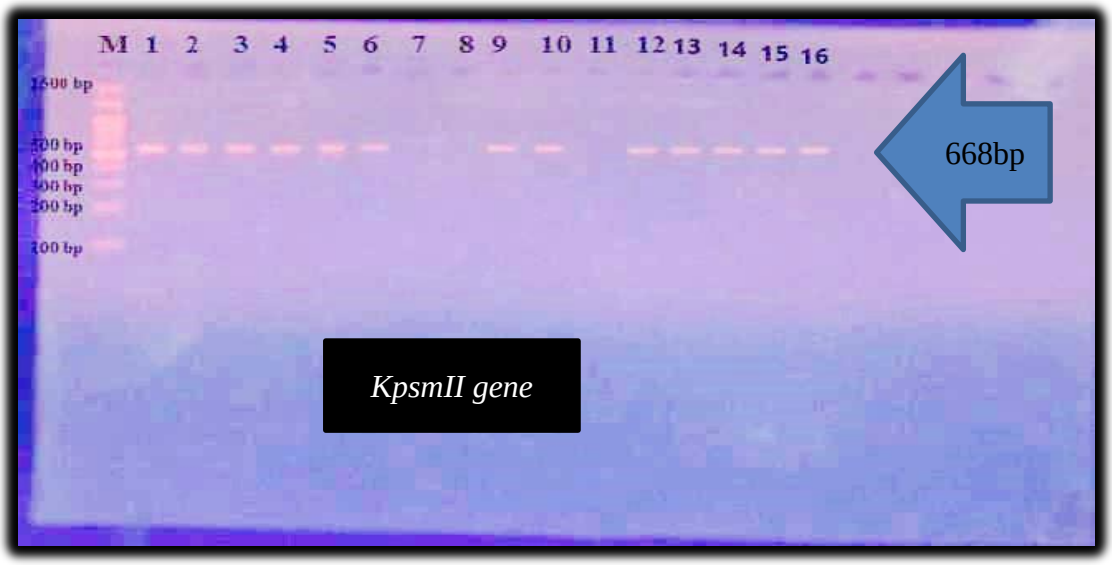


Figure (3-7): Electrophoresis of amplified *KpsmII* gene (668bp).Agarose gel 1%, 75volt for 1hrs,stained with ethidium bromide dye. Isolates 1,2,3,4,5,6 , 9,10, ,12,13,14,15,16 positive result,isolate,7,8, 11 negative result

3.6. Genetic Patterns

PCR results showed that 2 isolate (E11,E7) had 1 virulence genes ,3 isolate (E2,E8,E14) had 2 virulence genes,6 isolate (E4,E5.E6,E9,E10,E16) had 3 virulence genes, and 4 isolate (E1,E3,E12,E13,E15) had all of the virulence genes according to PCR results, as shown in (table 3-5).

Table(3- 5) :Genetic patterns of study isolates

Study Isolate code	No. of genes	Gene detected
E1	4	*FimH,KpsmII,OmpT,Pap*
E2	2	*FimH,KpsmII*
E3	4	*FimH,ompT,Pap,KpsmII*
E4	3	*FimH,Pap,kpsmII*
E5	3	*FimH,ompT,kpsmII*
E6	3	*FimH,pap,kpsmII*
E7	1	*FimH*
E8	2	*FimH,ompT*
E9	3	*FimH,pap,KpsmII*
E10	3	*FimH,ompT,KpsmII*
E11	1	*FimH*
E12	4	*FimH,ompT,pap,KpsmII*
E13	4	*FimH,KpsmII, OmpT, Pap*
E14	2	*FImH,KpsmII*
E15	4	*FimH,ompT,Pap,KpsmII*
E16	3	*FimH, Pap, kpsmII*

3.7. Antibiotic susceptibility of *Escherichia coli*

Isolates Antibiotics susceptibility test was conducted for 16 UPEC isolates against thirteen antibiotics by VITEK 2 system ; included:

1- Penicilline (Ticarcillin, pipracillin)

2- Cephalosporins(Ceftazidime, Cefepime)

3- Carbapenems(Meropenem,Imipenem)

4- Aminoglycoside(Tobramycin,Gentamicin,Amikacin)

5- Fluroquinolones(Ciprofloxacin)

6- Tetracycline(Minocycline)

7- Monobactams(Azteronam)

8- Sulfonamide(Trimethoprim/Sulfamethoxazole).

The results are shown in table(3- 6) as a clear variation in the extent among the isolates in the response to antibiotics used in this study.

The highest percentage of resistance among the *E. coli* isolates was recorded against Ticarcillin (87.5%), Pipracillin(81.3%).

Middle resistance appeared against some antibiotics Ciprofloxacin (56.3%), Aztreonam (50.0%), Trimethoprim\sulfamethoxazole(43.75%).

The lowest resistance was recorded against Ceftazidime (31.3%), Tobramycin (25.0%), Cefepime (18.75%), Meropenem (12.5%), Imipenem (6.3%), Gentamicin (12.5%), Amikacin (6.3%).

Table (3-6) :Antibiotic susceptibility results for 16 *E.coli* isolates

Antibiotic Group	Antibiotic	NO(%) Antibiotic Susceptible	NO.(%) Antbiotic Intermediate	NO.(%) (%)Antibiotic Resistance
Penicillin	Ticarcillin	2(12.5)	0(0.0)	14(87.5)
	Pipraciilin	2(12.5)	1(6.3)	13(81.3)
Cephalosporins	Ceftazidime	11(68.8)	0(0.0)	5(31.3)
	Cefepime	13(81.25)	0(0.0)	3(18.75)
Carbapenems	Meropenem	14(87.5)	0(0.0)	2(12.5)
	Imipenem	14(87.5)	1(6.30)	1(6.3)
Aminoglycosides	Tobramycin	12(75.0)	0(0.0)	4(25.0)
	Gentamicin	14(87.5)	0(0.0)	2(12.5)
	Amikacin	12(75.0)	3(18.8)	1(6.3)
Fluroquinolones	Ciprofloxacin	7(43.8)	0(0.0)	9(56.3)
Tetracycline	Minocycline	8(50.0)	7(43.75)	1(6.3)
Monobactams	Aztreonam	8(50.0)	0(0.0)	8(50.0)
Sulfonamide	Trimethoprim / Sulfamethoxazole	8(50.0)	1(6.3)	7(43.75)

3.8. Multi-drug Resistance pattern of *E.coli* isolates

Some isolate *E.coli* in the study showed a multi-drug resistance (MDR, XDR) level of resistance to the tested antibiotics, with a rate of 50% (25%XDR, 25%MDR). Results showed that isolates E1,E12were resistant to 7 antibiotic groups.Isolate E2 was resistant to 6 antibiotic groups.Isolate E6 was resistant to 5 antibiotic groups. Isolate E7,E10,E13 were resistance to 4 antibiotic groups.Isolate E4 was resistant to 3 antibiotic groups.Table (3-7) showed results.

Table (3-7):Resistance type for 16 isolates of *E.coli.*

Isolates	No.of antibiotic groups resisted	Resistance type
E1	7	XDR*
E12	7	XDR
E2	6	XDR
E6	5	XDR
E7	4	MDR*
E10	4	MDR*
E13	4	MDR*
E4	3	MDR
E5,E16,E8,E11	2	NON
E3,E9,E14	1	NON

*XDR=Extensively drug –resistant,*MDR =Multidrug –resistant.

3.9. Immunological Study

3.9.1. CXCL10 level in both the patient and control groups

The findings presented in (table 3-8)The analysis showed a high significant difference ($P < 0.001$*) in the total number of controls compared to the total number of patients.

Table(3-8): Measurement of CXCL10 levels in both the patients and the control group.

Parameter	CXCL-10 level mean ± SE (pg/ml)		Probability
	Patients	control	
CXCL10	137.02 ± 6.42	107.28 ± 4.99	P < 0.001*

3.9.2.ROC curve and AUC analysis for the CXCL10 in patients with Urinary tract infection compared to control groups

An ROC curve and AUC analysis were conducted to compare the CXCL10 levels in patients with those in the control group. The investigation of the receiver operating curve (ROC) curve and AUC for CXCL10 as a diagnostic parameter revealed that platelets exhibit a high level of accuracy in predicting such cases. The corresponding data can be found in (table 3-9).The sensitivity and specificity of CXL10 levels at a threshold of 131.795 are 55.6% and 82.2% respectively. The p-value for the Area Under the Curve (AUC) was less than 0.001, indicating a high level of statistical significance. The Sensitivity & Specificity results were validated by employing Youden's J statistics on the parameters.

Table(3-9): Receiver operating characteristic curve showing sensitivity and specificity of CXCL10 in patients with Urinary tract infection compared to control groups.

Test Result Variable(s)	**CXCl10**
AUC	71.4%
Sensitivity %	55.6%
Specificity %	82.2%
Youden index	0.378
Cut-off points	131.795
CI (95%)	0.607-0.822
PPV	76%
NPV	65%
Accuracy	70%
P value	<0.001[S]
S= Significant, PPV= Positive protective value, NPV= Negative predictive value, AUC= Area under curve, CI= confidence interval	

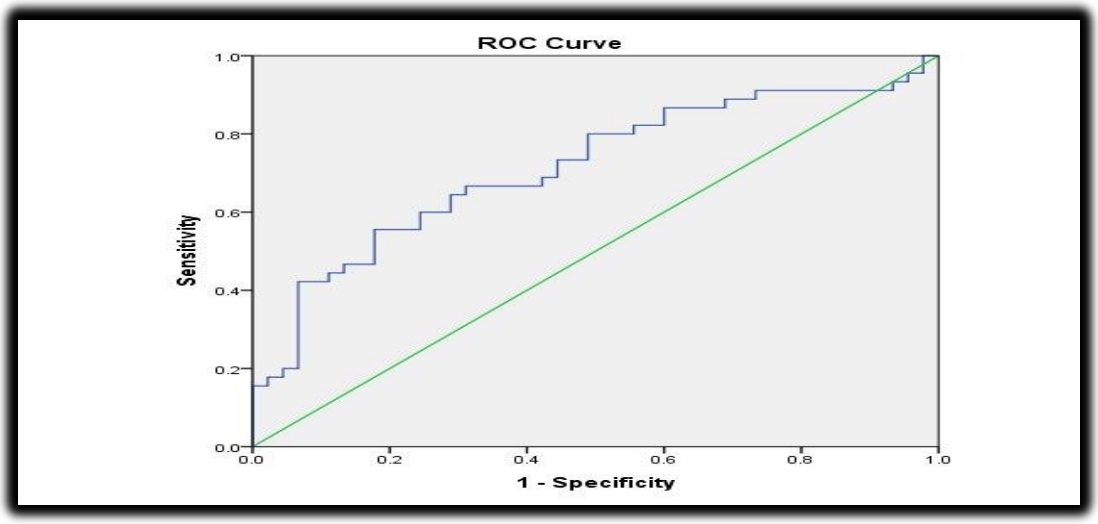


Figure (3-8) :Receiver operating characteristics (ROC) curve analysis of CXCL10 levels in patients with Urinary tract infection compared to control groups

Chapter Four

Discussion

4. Discussion

Urinary tract infections (UTIs) are a common type of diseases that can afflict individuals of any age or gender(Alhazmi et al., 2023). A urinary tract infection encompasses the presence of microorganisms in the urine as well as the invasion of any part of the urinary tract's tissues.Bacteria are the primary causative agents, although yeast and viruses can also play a role(Obeagu et al., 2023).

4.1.Demographic Characteristics of Studied Patients:

Based on the analysis of patient UTI distribution by sex, it was observed that females had a relatively higher prevalence rate than males. Specifically, the prevalence rate for UTIs was (84.4%) among females, whereas it was(15.6%) among males. Study by (Eulambius M Mlugu et al., 2023) reported that prevalence rate of UTI in male was (23.6 %) and in female was(76.4%), and these results agree with current study.

Also another results by (Zhan et al., 2024) , revealed prevelance of UTI in female more than men, where the percentage of prevelance UTI in men , (37.0%) and in female (63.0%).

While as study conducted by (Hanoon et al., 2023) found that The prevalence rate of UTIs in male had a higher prevalence rate of UTIs (54%) than female (46%) and this disagrees with current study.

The increased prevalence of urinary tract infections (UTIs) in females can be attributed to several factors, including the close proximity of the urethra to the anus, the wider and shorter length of the female urethra, sexual activity, incontinence, and vaginal factors. the surface's lower pH level and unhygienic living conditions (Alwan et al., 2023).

Due to the high prevalence of urine retention, urinary incontinence, long-term hospitalisations, the presence of comorbidities, concomitant urinary catheterizations, and waning immunological responses, older adults are more

susceptible to UTIs than younger people(Akhtar et al., 2021)

According to the present study's residents, a higher percentage of persons living in rural areas suffered from UTIs (66.6 vs. 33.3% in urban areas).

There is evidence from studies that showed that how a community's socioeconomic status and geographic location might affect the prevalence of urinary tract infections (UTIs) (Tadesse et al., 2014,Ayoyi et al., 2017). Current study agree with the study conducted by (Seifu and Gebissa, 2018) , in which the percentage of the prevalence of urinary tract infections in Urban area (37.1)% ,and in Rural (62.9)%.

While a study of prevalence rate of UTI by (Mwambete and Msigwa, 2017) who reported that UTI Percentage in (urban area) was very low (2 %), and in (rural area) was 12.5%.

Urinary tract infections (UTIs) can be affected by many individual-level variables. The variables contributing to urinary tract infections (UTIs) encompass participating in sexual activity, being of the female gender, having a prior history of UTIs, and maybe inadequate fluid intake or dehydration(Simmering et al., 2018).

According to the season,current study found that there was a higher prevalence of urinary tract infections (UTIs) during the the summer season, and this corresponds to the results reported by (Simmering et al., 2018),where urinary pathogens and hosts may be affected by temperature, or even both, from the perspective of the host, urinary tract infections (UTIs) could be more prevalent during the warm summer months if higher temperatures even subclinically diminish hydration levels, which would limit urine production and decrease the removal of possible urinary pathogens (Simmering et al., 2018).

Limited research has been conducted on the topic of infections across various age cohorts(Alwan et al., 2023).

Environmental risk factors for urinary tract infections (UTIs) may exist alongside human level risk factors. Undoubtedly, research conducted at a

particular location have demonstrated that urinary tract infections (UTIs) are more prevalent during the summer season, exhibiting a noticeable rise in occurrence ((Anderson, 1983),(Czaja et al., 2007)

According to age, Investigating the distribution of bacterial pathogens in urinary tract infections (UTIs) based on age is an important field of research(Zhan et al., 2024).

The current study revealed that the age range of 15-30 years had the highest prevalence of infection among both males and females, with a percentage of 42.2%. Based on the findings from study (Almukhtar, 2019), it was observed that 58.4% of patients within the age of (21-30) years expressed agreement with the current study.

The incidence of urinary tract infections (UTIs) in men rises as they get older, particularly after the age of 50. This susceptibility is attributed to the increased likelihood of developing prostate problems, which is caused by the reduction of prostatic fluid. An enlarged prostate gland can obstruct and reduce the urine flow, hence increasing the likelihood of infection(John, Mboto and Agbo, 2016)

(Nicolle, 2008) found that uncircumcised men had a higher susceptibility to urinary tract infections (UTIs) due to the increased accumulation of germs in the folds of the penile foreskin. This makes them more prone to getting UTIs.This finding consistent with that reported in provious studies ((Kasid, Alchalabi and Harith, 2023, and Alwan et al., 2023).

The high prevalence of urinary tract infections (UTIs) in the elderly population may be attributed to genito-urinary atrophy and vaginal prolapse following menopause in females.

These conditions increase the risk of bacteriuria by raising vaginal pH and reducing the presence of vaginal Lactobacillus, thus creating an environment favourable for the growth of gram-negative bacteria, which can act as uropathogens(Seifu and Gebissa, 2018).

A greater understanding of the variables contributing to the emergence and dissemination of urinary tract infections (UTIs) might facilitate the carry out of effective actions for public health, such as education programmes concerning preventive measures and hygiene practices(Eslami et al., 2023).

4.2. Bacteriology

4.2.1. Bacterial Isolates Percentage of the Study

Table(3-2) showed the results of the percentage of bacterial isolates.The Enterobacteriaceae family has been identified by numerous studies as the predominant etiological agent of urinary tract infections in human beings(Hussein, 2017 ,Odoki et al., 2019)

Escherichia coli had the highest occurrence among disease-causing microorganisms, with a total detection rate of (35.6%), as seen in table (2).The findings of the studies conducted (Alwan et al., 2023)and (Seifu and Gebissa, 2018)were similar with the results of the present investigation. Also results (Campos et al., 2022) agree with current study where the percentage was (34.75%).

While the findings of (Muharram et al., 2014) revealed contrast with current study, where identified *Klebsiella spp*. as the most common bacteria in patients with urinary tract infections (UTIs).In current study the percentage of *klebsiela pneumonia* (6.7%), Similar results have also been shown in Hargeisa, where the percentage *klebsiela pneumonia* (8.7%)(Ali, Reda and Ormago, 2022).also results of(Adlan et al., 2021) similar with current study , where the percentage of *Klebsiella pneumonia* was (8.08%).

In current study *Coagulase-negative staphylococci* (CNS) had an isolation rate of 20%, making them the second most prevalent pathogen, Similar results have also been shown in northern Ethiopia(Gebremariam et al., 2019), where the percentage of *Coagulase-negative staphylococci* (23%),also results of (Ali, Reda and Ormago, 2022) similar with present study , where the percentage of (CNS) was (16%).

In current study the percentage of *Staphylococcus haemolyticus* was (8.9%) agreement with study in Iraq , revealed the percentage of *S.haemolyticus*,was (8.9%)(Ahmed and Atiyea, 2022) This percentage was about the same as what (Luty et al., 2020)said about the infection rate, which was (11.5%).

In current study revealed the percentage of *Staphylococcus aureus* was (8.9%) agree with study in Najaf City, Iraq,where the percentage of this bacteria was (8.5%) (Mhana and Aljanaby, 2023).

In current study revealed the percentage of *Enterobacter aerogenes* was (6.7%), imilar with results(Alemu et al., 2012).where the percentage of *Enterobacter aerogenes* was (5%).

In current study,the percentage of *Staphylococcus epidermidis* was (4.4%),this study similar with results of (Shirvani, Keramati and Esmaeli, 2023),where the percentage of *Staphylococcus epidermidis* was(1.47%).In current study the percentage of *Staphylococcus hominis* was (2.2%),and this results similar with results (Ahmed and Atiyea, 2022)and (Luty et al., 2020) where the percentage was (0.89%) and (0.6%) respectively.

4.2.2. Isolating and Identifying 16 *E. coli* Isolates

In this study 16 isolates of *E. coli* were isolated . These isolates were obtained from patients who visited the laboratory with symptomatic urinary tract infections and were included in the study. Ten specimens (62.5%) obtained from female patients, whereas six specimens (37.5%) obtained from male cases.The female reported highest than male,as shown in table (3.3). AStudy counducted by (Kak Ahmed, 2023) recorded similar results with current study,where The majority of samples were obtained from females, with 33 (61.11%) specimens, while 21 (38.89%) specimens were gathered from male cases.

The gold standard for diagnosing urinary tract infections (UTIs) is urine culture,inaddition to the antibiotic susceptibility profile and pathogen

identification for better UTI mangment. However, bacterial growth can be adversely affected by prior antibiotic therapy or contamination during sample collection and this may causes false results(Ashkenazi-Hoffnung et al., 2021)

Results of culture showed colonies on MacCon key as bright pink colonies as shown in figure (3.1a). The obtained isolates exhibited the formation of rose-colored colonies when cultivated on MacConkey agar as a result of lactose fermentation(Kak Ahmed, 2023).

The Mac Conkey medium consists of lactose, bile salts, crystal violet dye, and neutral red (a pH indicator).One source of fermentation is the lactose present in the agar. The pH will drop when lactose-fermenting microbes create organic acids, especially lactic acid. When the pH falls below 6.8, neutral red, a pH indicator, changes from off-white to bright red or pink(Jung and Hoilat, 2022).

Eosin Methylene Blue is a differential medium used to distinguish *E. coli* from other members of the Enterobacteriaceae family. The colonies exhibited a sheen green metallic appearance.

Results of culture showed colonies on Eosin Methylene Blue as Sheen green metallic colonies , as shown in figure(3.1b).

The colonies were noted to exhibit organic acid production through fermentation of glucose and lactose, resulting in a distinctive sheen of green metallic color when eosin and methylene were present (Ahmed, 2021).

In order to further identify the *E. coli* isolates used in this investigation, the VITEK 2 system was employed. As a result, all 16 isolates were successfully confirmed and reidentified as E. coli. (Bitew, Molalign and Chanie, 2017) also ,employed VITEK 2 compact technology to identify E. coli uropathogns isolates.

Also, each strain of *E. coli* was genitically confirmed using polymerase chain reaction (PCR) assay , with the help of primers designed for that purpose. Regarding the results of the PCR assay utilizing *16S rRNA* 1400 to identify the

existence of the *E. coli* genome, all 16 samples successfully exhibited a 100% positive result as showed in (figure 3.3), results of (Sameer Jaaz, 2020) showed completely samples harbored the *16srRNA*, agreement with current study.also result (Himi et al., 2015), corresponded with current study.Actually *16srRNA* represents important and Specific tool for uropathogens identification.

The PCR approach is a precise method used to identify *E. coli* bacteria by detecting the *16srRNA* gene. This method is employed following traditional methods. Methods such as culture, morphological characterisation, staining procedures, and biochemical testing (Ramezanpoura, Bigdelia and Romingerb, 2020),Islam,(Islam, Kabir and Seel, 2016).

4.2.3. Identification of Virulence Genes by UniPlex PCR

The presence of certain virulence genes in *E. coli* isolates was determined through the use of uniplex PCR using a thermocycler. This method involved confirming the presence of the genes in concern by using gene-specific primers.

In current study, results of Genetic Patterns shown in table(3.4). The variation in the distribution of virulence genes may be attributed to disparities in geographical location, climate conditions, local behaviors, public health practices, food supply hygiene, and genetic variances across UPEC strains(Ahmed, 2021).

4.2.3.1. Identification of *fimH, pap, kpsmII, and ompT* Gene

Uniplex PCR was used to detect genes(*fimH, pap, kpsmII, and ompT*), The distribution of virulence gene associated with UTI was 16(100%) belong to *FimH* gene then *KpsmII ,pap* and *ompT* was 13 (81.3%),9(56.3%),8(50%) respectively.As shown in table(3.4) .

Related genes of *Escherichia coli* encode a wide variety of virulence factors, which provide the bacteria with the ability to evade human defences and cause UTIs. These elements are obtained by DNA transfer to bacteriophages, transposons, plasmids, and pathogenicity islands (PAI) (Ahmed, 2021).

The adhesive subunit of type 1 fimbriae, known as *FimH*, is a significant factor in the adherence of UPEC (Uropathogenic *Escherichia coli*). *FimH* has a strong affinity for receptors in the urinary tract, making it crucial for the colonization of various areas by *E. coli*(Hojati et al., 2015)

Furthermore, numerous studies have reported the significant involvement of *pap* adhesion genes in the pathogenesis of urinary tract infections (UTIs) caused by *Escherichia coli*(Rahdar et al., 2015).

Current study revealed that all of E. coli isolates had *FimH* gene at (100%),this results corresponded with study conducted in Iraq , Diyala, where the percentage of *FimH* gene (100%)(Ahmed, 2021). Study in Iran that also detected *FimH* gene was (100%) (Yazdi et al., 2020).

Results study in Ethiopia, explain that *FimH* was present in 82% of their isolates *E.coli,* and this disagree with current study(Dadi et al., 2020) .

In current study,revealed that isolates of *E.coli* had *Pap* gene was (56.3%), this study agreement with results(Rahdar *et al.*, 2015),where the percentage of *Pap* gene was (57%).

On the other hand, Researchers in Iraq (Salih, Nader and Rasheed, 2015) recorded that 51.7% of their isolates of *E. coli* had *pap.*

Results of (Yazdi et al., 2020) revealed the percentage *Pap* gene was (79%),this disagree with current study.

FimH encodes fimbriae H, which bind with α-D-mannosylated uroepithelial protein to stabilise adherence to the host's uroepithelial under high pressure, potentially resulting in urosepsis, on the other hand, *pap* encodes P fimbriae, which binds to α-D-galactopyronosol and is linked to pyelonephritis and cystitis (1-4),The upper urinary tract contains a receptor that contains β-D-galactopyronoside(Ahmed, 2021).

In current study the percentage of *OmpT*gene was 50%, and this results corresponds to the (54%) presence rate of *OmpT* gene that recorded by (Naziri et al., 2024).

On the other hand, results of(Baldiris-Avila, Montes-Robledo and Buelvas-Montes, 2020) that revealed the percentage of *OmpT* was (66.8%).

The results of the current investigation differ with those obtained in Iran by(Dehkordi et al., 2020).where they found *ompT* to be at (29.54%) . *OmpT*, which plays a role in invasion, adhesion, and inactivation, is frequently linked to cystitis and prostatitis.*Ompt* functions as a bacterial defense mechanism by enabling the bacterium to withstand the cationic peptides present in the urinary tract. This, in turn, enhances the bacteria's survival and increases its capacity to cause urinary tract infections(Sun et al., 2020).

Current study revealed the percentage of *kpsmII* gene at (81.3%),this study agreed with study in Iran counducted by(Haghighatpanah and Mojtahedi, 2019),where found the the gene *kpsmII* to be at 76%.

On the other hand, study conducted by (Baldiris-Avila, Montes-Robledo and Buelvas-Montes, 2020) ,found *kpsmII* to be at (66.8%). While results of(Farajzadah Sheikh et al., 2019), show disagreement with current study, where percentage of *kpsmII* gene was 23%. The *kpsMII* gene codes for the production of the capsule, a crucial component of *E. coli* virulence factors that protects the bacteria from phagocytosis and the immune system's complement system. The capsule also increases the likelihood of UTIs caused by *E. coli* because it provides additional protection(Ahmed, 2021).

Capsules provide protection for *E. coli* by preventing phagocytosis by the immune cells of the host. This is achieved by inhibiting the initiation of the phagocytic process by the reduction of opsonins binding and the concealment of ligands that facilitate attachment to phagocytic cells(Maruvada, Blom and Prasadarao, 2008) .Another role that the capsule can fulfill is in the process of adhesion and the creation of biofilms(Fleitas and Franco, 2016). Biofilms are aggregations of microorganisms that are surrounded by a complex structure called the extracellular polymeric matrix. This matrix is made up of nucleic

acids, proteins, and enzymes, and it adheres to both living and non-living surfaces. Biofilms enhance the viability of bacteria in the urinary system by shielding them from the purifying impact of hydrodynamic forces, host defence mechanisms, phagocytosis, and antibiotics. Hence, the development of biofilm plays a crucial role in the ability of UPEC strains to cause disease(Boroumand et al., 2021)

4.2.4. Antibiotic Susceptibility of *Escherichia coli* Isolates

Antimicrobial susceptibility was carried out for 13 antimicrobials by running the VITEK 2 system. The results of current study,showed in table (3.6)

The bacteria that cause UTIs are susceptible to drugs, but when they develop resistance over time, the disease becomes more severe and more difficult to treat(Maleki et al., 2016)

(The highest percentage of resistance among the *E. coli* isolates was recorded against Ticarcillin (87.5%), Pipracillin(81.3%).The result of (Alhadidi, 2021) in Iraq, who recorded that Ticarcillin resistance was (96.3%),and consistent consistent with the current investigation.

The middle percentage of resistance among the *E.coli* isolates was recorded against ciprofloxacin(56.3%).

The results of (Bahalo et al., 2013) in Iran, who reported that ciprofloxacin resistance was 56.25%, and this similler with the current investigation. Tajbakhsh and others were demonstrated that the bacterial biofilm development was the cause of the developed resistance to ciprofloxacin.

In current study the lowest percentage of resistance was recorded against Ceftazidime(31.3%), the results of (Altun, Ulu and Şengül, 2022), in Türkiye where they found the resistance to ceftazidime was recorded 32.6% are consistent with the current investigation.

The results of (Ahmed, 2021)in Iraq ,who discovered that resistance to

ceftazidime recorded (86.6%), which disagree with our study.The The lowest percentage of resistance was recorded against cefepime(18.75%),the result of(Tarverdi and Sadraddin, 2024),which agree with our study.

With a wide range of antibacterial action, carbapenems are the most effective beta-lactam antibiotics against both Gram-positive and Gram-negative bacteria. Their distinct molecular structure results from the beta-lactam ring and carbapenem present in combination. The combination of ampicillin and carbenicillin (AmpC) and the extended spectrum beta-lactamases (ESBLs) gives remarkable stability against the majority of beta-lactamases, which are enzymes that inactivate beta-lactams(Meletis, 2016) The highest of sensitive E. coli isolates was recorded to carbapenems group (meropenem 87.5% and imipenem 87.5%), a study conducted by (Kak Ahmed, 2023) they found that the sensitive to carbapenems group was recorded (meropenem 85.19%, imipenem 88. .89%) are consistent with the current investigation.

In current study ,the lowest percentage of sensitive was recorded against penicilline group(Piperacillin 12.5%) are disagrees with result of (Kak Ahmed, 2023),which percentage was(24.07%).

4.2.5 Patterns Multi –Drug Resistance of Isolates *E.coli*

Results Patterns of isolates *E.coli* Multi-drug Resistance in current study showed in table(3.7). The investigation found that (50%) of the isolates were multidrug-resistant (MDR, XDR),Where 4 (25%) were found to be MDR while 4 (25%) were found to be XDR , which might be attributed to either a mutation or the acquisition of a plasmid that confers a high level of resistance to the cell. The extensive and improper utilization of broad spectrum. The use of antibiotics has also resulted in the emergence of numerous multi-drug resistant strains of *E. coli* (Gawad et al., 2018).Current results were relatively comparable with the previous study conducted by (Et al., 2019), who reported that (88.09%) of *E.coli* were found to be MDR while (11.90%) were XDR, where the (Et al., 2019)

study's increase in MDR isolates and XDR appearance is a result of medicine's unchecked usage of antibiotics over the last many years.

The primary factor contributing to the formation of multidrug resistance (MDR) and the selection and spread of antibiotic-resistant bacteria in clinical practice is the negligent use of antibiotics without conducting antibiotic sensitivity tests (Et al., 2019).Only 7% of *E. coli* in Guinea were XDR strains, compared to 74.4% MDR strains(Shatalov, 2015).

The significant level of antibiotic resistance is seen as a crucial health issue, particularly in the case of *E. coli*, which is responsible for urinary tract infections (UTIs) - a major global health problem (Polse, Yousif and Assafi, 2016).

There are regional variations in the MDR rate between UTI isolates. For instance, it was reported that the prevalence of MDR UPEC was 92% in India ,compared to 7.1% and 42% in the USA and Slovenia, respectively

4.2.6. Immunological parameter Study

The CXCL-10 level mean ± SE (pg/ml) in patients and control group,was 137.02 ± 6.42, 107.28 ± 4.99 respectively,as showed in table (3.8).

One practical method for monitoring on bladder tissue inflammation is to analyse various urine proteins.Diverse pathophysiologies and intrinsic bladder states can be reflected in the protein profiles and biochemical contents of various lower urinary tract diseases(Jiang et al., 2020).

The inherent benefits of host protein biomarkers, such as the ability to reflect the body's reaction to an invasive pathogen, validate its application in helping physicians diagnose the cause of infection(Ashkenazi-Hoffnung et al., 2021)

When UPEC is adhered to mucosal surfaces, an inflammatory reaction occurs(Godaly et al., 2007)

During the inflammatory process in the bladder wall, cytokines and chemokines are released into the urine by the detrusor smooth muscle cells(Yu

et al., 2022).

Recent studies have shown that chemokines and their receptors are expressed by many nonhematopoietic cells, including urothelium and detrusor cells(Bouchelouche et al., 2006). In addition to inflammatory cell infiltration, chemokines have other uses.

The molecular weights of the secreted proteins belonging to the chemokine superfamily range from 8 to 10 kDa (Ragnarsdóttir and Svanborg, 2012).

They function by interacting with G protein-coupled receptors located on glycosaminoglycans within endothelial cell layers. Chemokines are characterized by their ability to interact with many receptors simultaneously, and receptors can bind to multiple chemokines(Ragnarsdóttir and Svanborg, 2012).

It is well known that CXCL10 is elevated and plays a role in chemokine communication during peripheral neuroinflammatory reactions. This cytokine was shown to be increased in urine specimens from patients with IC/BPS, indicating both neuropathic inflammation and afferent hypersensitivity in the bladder(Jiang et al., 2021).

The result of current study showed elevated CXCL10 level in UTI patient.

The result of (Tyagi et al., 2016) showed elevation level of CXCL10 corroborates with current study.

There were no statistically significant variations in the urine levels of CXCL10 between patients in the convalescent phase, patients with acute UTI, and healthy controls(Gorczyca et al., 2014).

According to(Niimi et al., 2018), there was no rise in urine CXCL10 in 25 patients with non-Hunner type IC/BPS when compared to 31 controls. Urine CXCL10 levels, however, were significantly higher in IC/BPS patients in our earlier investigation, which included 127 non-Hunner type interstitial cystits bladder pain syndrome IC/BPS patients and 28 controls. These levels were also

connected with the glomerulation grade and maximal bladder capacity in IC/BPS patients.(Jiang et al., 2020).

There are studies that used the same marker in viral infections,as it was used as diagnostic marker in viral disease ,such as study (Hussein, 2021),where Interferon Gamma Induced Protein 10kDa (IP-10) had important role in severity COVID-19 Infection.

Conclusions and Recommendations

Conclusions

The study concluded the following points:

1.The major pathogens among the gram-positive isolates were coagulase-negative staphylococcus, whereas among the gram-negative isolates, *E.coli* was the most prevalent pathogen based on the bacteriological profile of urinary tract infections (UTIs).

2.The common antibiotics sensitive for UTI treatment Meropenem, Imipenem, Gentamicin

3. In this study, *E. coli* isolates showed a middle rate of resistance to different antibiotics (MDR 25%, XDR 25%).

4. The study biomarker (CXCL10), had high level in patient group compared to control group which suggests an inflammatory state in these patients.

5. The (CXCL10) marker can be utilised to track the development, problems, and inflammation of UTI patients.

Recommendations

1.Raise public awareness of the potential consequences of overusing and improperly using antibiotics, which has been steadily worsening for all of humanity. Prescriptions should also take into consideration what Antibiotic-resistant microbes may indicate problems in the future.

2.Further studies with large sample size should be conducted to confirm the significant association of CXCL10 as indicator of severity UTIs patients.

3. Assessment the concentration of CXCl10 in the serum of individuals with urinary tract infections and compare concentration in urine.

4.Study other adaptive immune markers.

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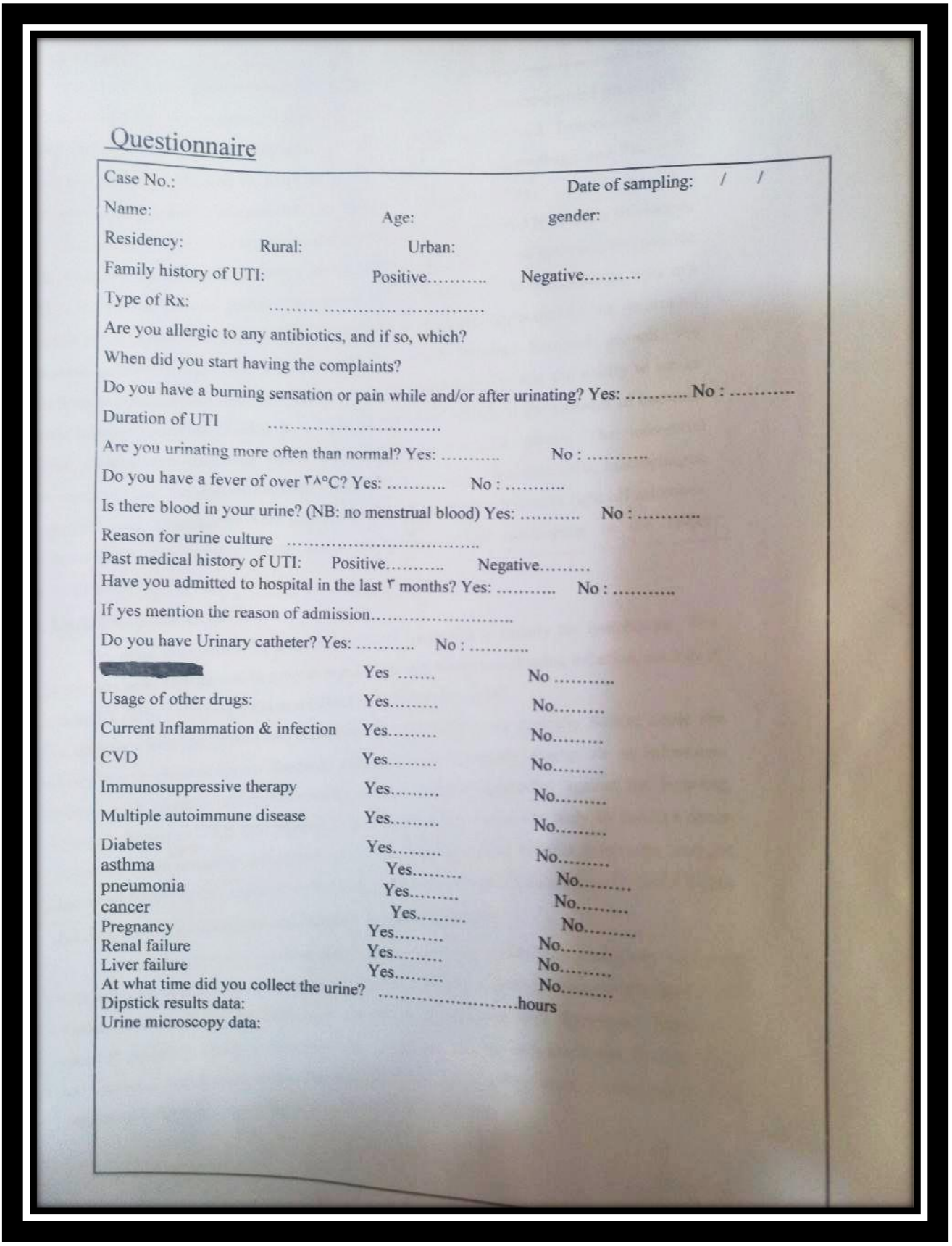
Apendices

Apendix 1: Questionnaire



Questionnaire

Case No.: Date of sampling: / /

Name: Age: gender:

Residency: Rural: Urban:

Family history of UTI: Positive……. Negative……

Type of Rx: …… …… ……

Are you allergic to any antibiotics, and if so, which?

When did you start having the complaints?

Do you have a burning sensation or pain while and/or after urinating? Yes: ……. No : …….

Duration of UTI ……

Are you urinating more often than normal? Yes: ……. No : …….

Do you have a fever of over ٣٨°C? Yes: ……. No : …….

Is there blood in your urine? (NB: no menstrual blood) Yes: ……. No : …….

Reason for urine culture ……

Past medical history of UTI: Positive……. Negative……

Have you admitted to hospital in the last ٣ months? Yes: ……. No : …….

If yes mention the reason of admission……

Do you have Urinary catheter? Yes: ……. No : …….

[redacted] Yes ……. No …….

Usage of other drugs: Yes…… No……

Current Inflammation & infection Yes…… No……

CVD Yes…… No……

Immunosuppressive therapy Yes…… No……

Multiple autoimmune disease Yes…… No……

Diabetes Yes…… No……

asthma Yes…… No……

pneumonia Yes…… No……

cancer Yes…… No……

Pregnancy Yes…… No……

Renal failure Yes…… No……

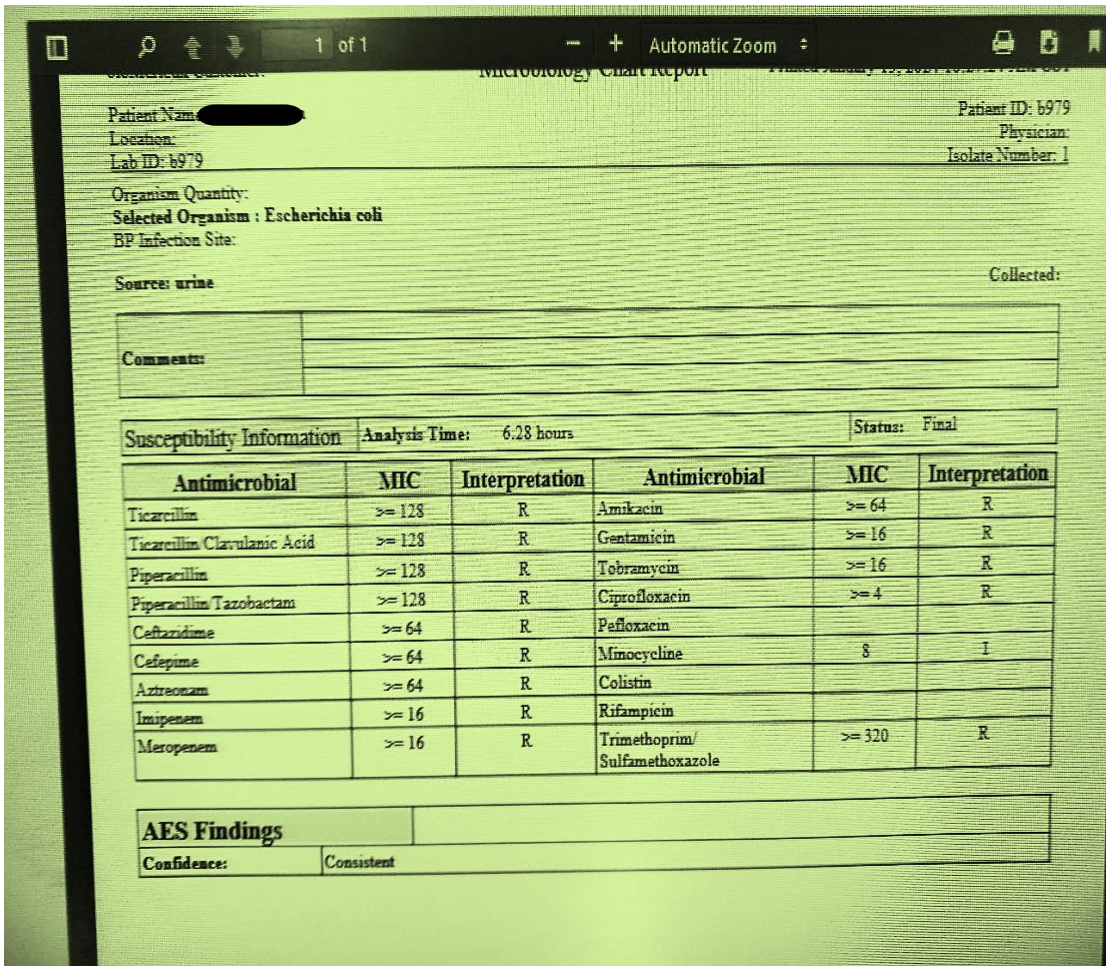
Liver failure Yes…… No……

At what time did you collect the urine? ……hours

Dipstick results data:

Urine microscopy data:

Appendix 2: vitek 2 system result showed samples of E.coli



1 of 1 — Automatic Zoom

Microbiology Chart Report

Patient Nam[redacted]
Location:
Lab ID: b979

Patient ID: b979
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli
BP Infection Site:

Source: urine

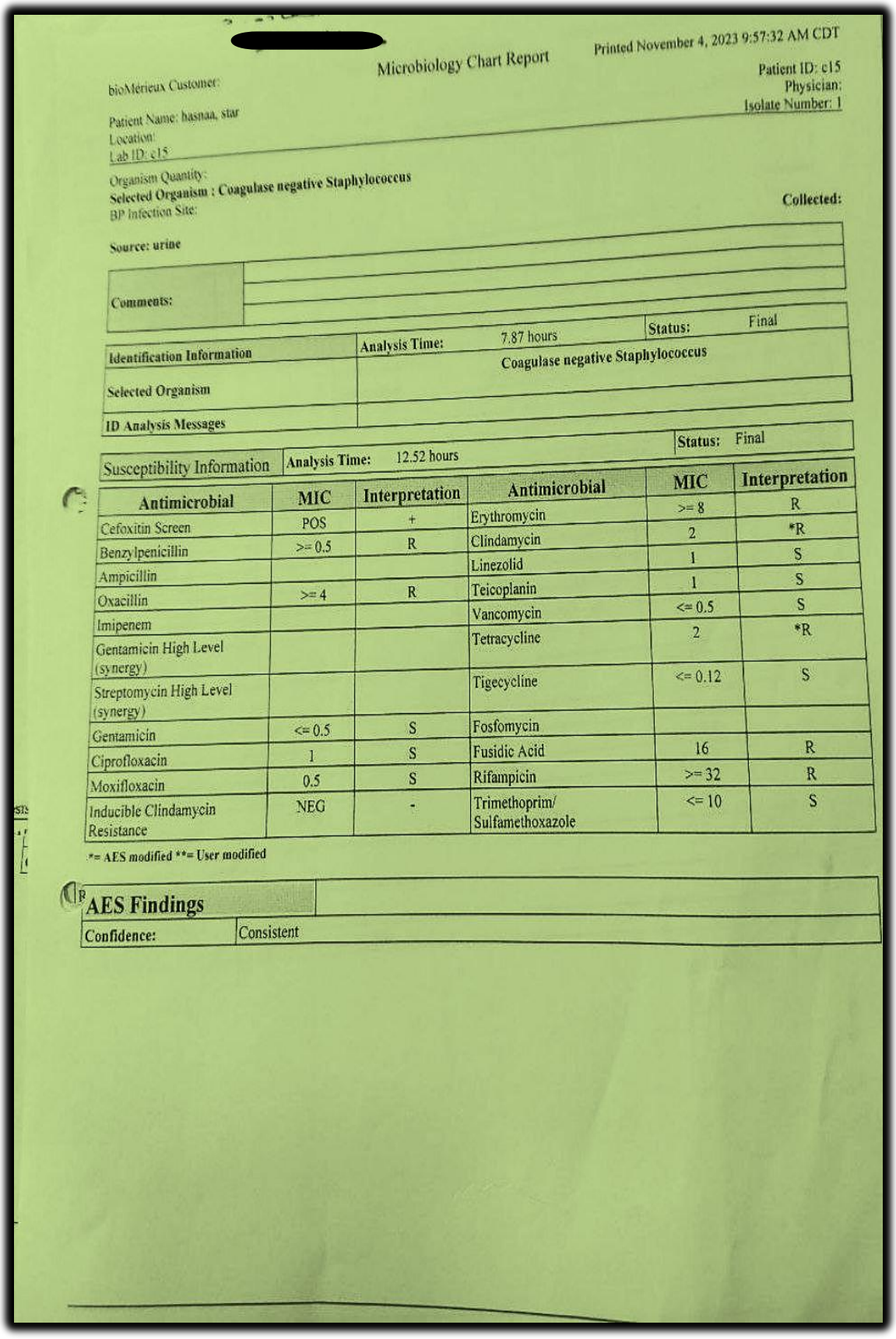
Collected:

Comments:	

Susceptibility Information	**Analysis Time:** 6.28 hours			**Status:** Final	
Antimicrobial	**MIC**	**Interpretation**	**Antimicrobial**	**MIC**	**Interpretation**
Ticarcillin	>= 128	R	Amikacin	>= 64	R
Ticarcillin/Clavulanic Acid	>= 128	R	Gentamicin	>= 16	R
Piperacillin	>= 128	R	Tobramycin	>= 16	R
Piperacillin/Tazobactam	>= 128	R	Ciprofloxacin	>= 4	R
Ceftazidime	>= 64	R	Pefloxacin		
Cefepime	>= 64	R	Minocycline	8	I
Aztreonam	>= 64	R	Colistin		
Imipenem	>= 16	R	Rifampicin		
Meropenem	>= 16	R	Trimethoprim/ Sulfamethoxazole	>= 320	R

AES Findings	
Confidence:	Consistent

Appendix (3) vitek 2 system result showed samples of Coagulase negative staphylococus



[redacted]

Microbiology Chart Report

Printed November 4, 2023 9:57:32 AM CDT

bioMérieux Customer:

Patient ID: c15
Physician:
Isolate Number: 1

Patient Name: hasnaa, star
Location:
Lab ID: c15

Organism Quantity:
Selected Organism : Coagulase negative Staphylococcus
BP Infection Site:

Collected:

Source: urine

Comments:	

Identification Information	**Analysis Time:** 7.87 hours	**Status:** Final
Selected Organism	**Coagulase negative Staphylococcus**	
ID Analysis Messages		

Susceptibility Information	**Analysis Time:** 12.52 hours	**Status:** Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Cefoxitin Screen	POS	+	Erythromycin	>= 8	R
Benzylpenicillin	>= 0.5	R	Clindamycin	2	*R
Ampicillin			Linezolid	1	S
Oxacillin	>= 4	R	Teicoplanin	1	S
Imipenem			Vancomycin	<= 0.5	S
Gentamicin High Level (synergy)			Tetracycline	2	*R
Streptomycin High Level (synergy)			Tigecycline	<= 0.12	S
Gentamicin	<= 0.5	S	Fosfomycin		
Ciprofloxacin	1	S	Fusidic Acid	16	R
Moxifloxacin	0.5	S	Rifampicin	>= 32	R
Inducible Clindamycin Resistance	NEG	-	Trimethoprim/ Sulfamethoxazole	<= 10	S

*= AES modified **= User modified

AES Findings	
Confidence:	Consistent

الخلاصة

التهابات المسالك البولية هي واحدة من الالتهابات البكتيرية الأكثر انتشارا التي تؤثر على ملايين الأفراد في جميع أنحاء العالم كل عام يمكن تصنيف التهابات المسالك البولية حسب موقع الإصابة بالتهاب الحويضة والكلية يؤثر على الحالب والكلى المتني عندما يؤثر على المسالك البولية العلوية الثانية التهاب المثانة والتهاب الإحليل عندما يؤثران على المثانة أو مجرى البول (عدوى المسالك البولية).

أجريت الدراسة لمدة 5 أشهر، ابتداء ايلول (2023) إلى كانون الثاني (2024) في مستشفى الحسين التعليمي / مختبر الأحياء الدقيقة في كربلاء، العراق؛وكان العدد الإجمالي للمشاركين 145 في الدراسة؛ تم تقسيمهم إلى مجموعتين: الأولى تضم 100 مريض مصاب بالتهابات المسالك البولية، والمجموعة الثانية تضم (45) شخصا من الأصحاء .

تم أخذ عينات البول من مرضى التهابات المسالك البولية وأجريت لهم الفحوصات البكتريولوجية (مجهريا، وزراعة شكليا، واختبار تأكيدي بواسطة نظام VITEK 2 المدمج).

تم الكشف عن مستويات CXCL10 في البول عن طريق التقنيات المصلية للساندويتش ELISA.

الأنواع البكتيرية المعزولة من مجموعة عدوى المسالك البولية ممثلة بالبكتيريا موجبة لصبغة جرام(20%) *Coagulase –ve staph* وهو أكثر الأنواع المعزولة شيوعاً يليه (8.9%)4 *Staphylococcus aureus 4(8.9), Staphylococcus haemolyticus* ثم *staphylococcus epidermidis 2(4.4 %) staphylococcus hominis1(2.2%))* و(2.2%) 1 *Enterococcus faccalis* . والبكتريا السالبة لصبغة جرام التي تمثلها(35.6%)*E.coli* هي الاكثر شيوعا وثم تاتي *Klebsiela pneumnia* و *Enterobacter aerogenes* حيث *كانت النسبة هي 3(6.7%)* .

تم تأكيد جميع الدراسات المعزولة لمسببات الأمراض البولية للإشريكية القولونية عن طريق الكشف الجزيئي باستخدام 16srRNA محددة.

النسبة المئوية لتوزيع جينات عوامل الضراوة في 16 عزلة ممثلة بالإشريكية القولونية جين 16 *FimH* (100%)، جين(50%) 8 *OmpT* ، جين(56.3%) 9 *Pap* ، جين.(81.3%) 13 *KpsmII*

أظهرت الدراسة الحالية أن مستوى كيموكاين(10) كان له فرق معنوي كبير في جميع مجموعات المرضى مقارنة مع الاصحاء في الختام، في دراستنا، تعتبر الإشريكية القولونية هي أكثر مسببات التهابات البولية يليها المكورات العنقودية سلبية التخثر، أظهرت عزلات الايكولاي المرضية العديد من عوامل الضراوة كما أظهرت معدلات مقاومة متوسطة للمضادات المختلفة، من ناحية أخرى، فإن الكيموكاين في البول له أدوار مهمة في تشخيص عدوى المسالك البولية.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي جامعة كربلاء
كلية الطب / فرع ألاحياء المجهرية الطبية

دور كيموكاين CXCL10 وعوامل ضراوة لبكتريا *E.coli* المعزولة من مرضى التهاب المجاري البولية

رسالة مقدمة الى
مجلس كلية الطب وهي جزء من متطلبات نيل شهادة الماجستير في ألاحياء المجهرية الطبية
من قبل الطالبة

ألق علي عبد الحسين
بكالوريوس كلية العلوم قسم علوم الحياة (2011)

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