Republic of Iraq

Ministry of Higher Education and

Scientific Research University of

Karbala College of Medicine

Department of Microbiology

The Role of Heparin Binding Protein and Virulence Genes of ESBL Uropathogenic Bacteria in Patients With Urinary Tract Infection

A Thesis

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

By

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1446 AH

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

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المجادلة: الآية 11

Committee certification

We, the examiners committee, certify that we have read the M.SC. thesis entitled: **(The Role of Heparin Binding Protein and Virulence Genes of ESBL Uropathogenic Bacteria in Patients With Urinary Tract Infection)**

We have examined the student (**Rusul Mohsen Hashem**) in it is contents. In our opinion itis meets the standards of thesis for the degree of Masters in Medical Microbiology and Immunology .

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/ /2024

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DEDICATION

To ….

God for completing the blessing that He bestowed upon me and for completing my message with pride and dignity.

To….

I influenced myself to be distinguished and to prevent myself from many of the obligations that I receive. This is because the ambition is great and the path is arduous and worthy of sacrifices, and because the goals are great, they are worth the effort.

To ….

my dear father, the icon of patience and tenderness, you have exhausted yourself so much so that you do not make me feel tired, so your patience has yielded profit for my giving. I congratulate you on mybehalf.

To….

The believing mother, through your prayers and supplications and yourlong wait, hope is fulfilled, O source of giving. I hope to bless you with something.

To….

Everyone who stood, supported, and the faces which the disease hide their smiles, endured, and asked every sincere and loyal person who helped me hard, I dedicate this success.

Rusul,2024

ACKNOWLEDGMENTS

First of all, I thank Allah for inspiring me with strength, patience, and willingness to accomplish this work.

I would like to express my deep and sincere gratitude to my supervisors **Assist. Prof**. **Dr. May Mohammed Ali** and **Dr. Abeer Thaher Naji Al-Hasnawi** for introducing me to the interesting field of science and for providing me with the opportunity to carry out this study. I also thank them for invaluable advice, patience and inspiring guidance throughout this work.

Thanks, and appreciations go to, clinician and the staff of hospital in Karbala province for their help in collection and diagnosis of patients.

All thanks and gratitude to participating patients and their relatives of their contribution to the study: I wish them fast recovery. It is a great pleasure to thank everyone who helped me to write my thesis successfully.

To all, please accept my truthful thanks

Rusul,2024

Summary :

Extended-Spectrum Beta-Lactamase (ESBL) producing bacteria are a group of bacteria that have developed resistance to a wide range of commonly used antibiotics, including many penicillins and cephalosporins. The presence of the *CNF, A/E, BLA OXA-48, BLA CTX-M,* and heparin binding protein in the urine of *Escherichia coli* -infected individuals suggests a multifaceted mechanism of virulence and antimicrobial resistance, where the cytotoxic, beta-lactamase, and adhesion-related factors may collectively contribute to the severity and persistence of urinary tract infections caused by this pathogen.

This case- control study was done at patients in hospital in Karbala province,. All patients collected were registered in UTI center in hospital from October (2023) to end of march (2024). Patients: 55 patients randomly recruited from the patients hospital in Karbala aged ranged between (20-63) years, who are diagnosed to have UTI based on clinical and laboratory findings)lymphocyte, neutrophils, eiosinophils) by the clinicians; the patients data collection were include residence area, Diabetes mellitus and gender.

One hundred fifty (150) participants were enrolled in this case control study. The first group includes 95 patients, females (50) and males (45),the second group includes 55 (29) males and (26) females as a healthy control group. All of the groups' ages range from 20 to 63 years.All specimens were analyzed by general urine examination (GUE) to identify the presence of bacterial cell, white blood cell, and other substances. For quantitative culture and presumptive identification from each urine specimen

Well-mixed urine specimens were cultured on MacConkey agar, and EMB and chromogenic agar orientation plates, separately. The DNA of *E.coli* isolates were extracted and PCR technique were used to detect some virulence

factor genes *CNF, A/E, BLA OXA-48, BLA CTX-M* , that responsible for ESBL-Producing *E.coli* pathogenicity.Heparin binding protein (HBP) was measurement by using Enzyme linked Immonosorbent assay for patients and control groups.The results showed the bacterial culture and the majority was 57.90% of samples culture having a bacterial growth, while 42.10% only presented with no growth. The highest percentage of bacteria isolated from patients was *Escherichia coli* at 70.90%, while the lowest percentage of bacteria isolated from patients was *Acinetobacter baumannii* and *Proteus* mirabilis at 3.60%. The results of the statistical analysis indicate a significant increase in HBP concentration in patients compared to the healthy control group. In the patient group, the average HBP concentration was 22.04 ± 0.96 ng/ml. In contrast, the healthy control group had an average HBP concentration of 7.78 ± 0.59 ng/ml. *Bla-CTM* gene was detected in 100% of UPEC isolates, conferring resistance to β-lactam antibiotics. *Bla OXA-48* gene and CNF gene were detected in 7.7% of isolates, associated with carbapenem resistance and virulence, respectively. EAE gene was detected in 20.5% of isolates, linked to the ability of E. coli to adhere to and invade host cells.

The Receiver Operative Characteristic Curve (ROC) analysis yielded a cut off value of HBP for prediction of disease activity. The overall AUC, sensitivity, and specificity for HBP were as follows: 0.936, 0.091, and 0.909, respectively.The results of current study revealed that Escherichia coli remains the protein (HBP) levels were significantly higher in patients as compared to healthy control suggesting that it may be a potential biomarker for UTI. In conclusion: the significantly higher levels of HBP observed in infected patients compared to healthy controls indicate that HBP may serve as a potential biomarker for the diagnosis and monitoring of UTIs.

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Code	Words
UTIs	Urinary tract infections
cUTIs	Complicated Urinary tract infections
CA-UTIs	community-acquired urinary tract infections
HA-UTIs	hospital-acquired urinary tract infections
EMB	Eosin Methylene Blue
HBP	Heparin-binding protein
EAE	Attaching and Effacing gene
ORENUC system	O-NO known factors; for R-Risk for Recurrent UTI; E-Extra urogenital risk factors; N-Nephropathy; U-Urological risk factors that can be resolved by therapy; C-catheter and the risk factors that cannot be resolved by therapy.
DEC	diarrheagenic *E.coli*
PAIs	pathogenicity islands
Omics	a biological analysis approach in which the data sets are multiple "omes", such as the genome, proteome, transcriptome, epigenome, metabolome, and microbiome
ELISA	ELISA Enzyme –Linked Immunosorbent Assay
S.G	Specific gravity
PH	potential of hydrogen

WBC	White blood cells
RBC	Red blood cells
RBC	Red blood cells
PCR	Polymerase chain reaction
MDR	Multi drug resistant
PDR	Pandemic drug resistant
DNA	Deoxy ribonucleic acid
ESBL	Extended spectrum beta-lactamase
UPEC	Uropathogenic *E.coli*

Chapter one

Introduction & literature Review

1.1Introduction:

Infections of the urinary system (UTIs) are a global problem that impair the standard of existence for both local residents and those in hospitals. Gram-negative bacteria belonging to the enterobacteriaceae family were the primary cause of urinary tract infections (UTIs). Of these, uropathogenic *Escherichia coli* (UPEC) contributes to eighty percent of cases of urinary tract infections globally. (Korbel *et al*., 2017).

Because of the various virulence characteristics it carries, the bacterium can proliferate and produce UTI. The worldwide distribution of UPEC strains' virulence factor-encoding proteins will render it possible for finding individuals depending upon the way these proteins influence the development about the infection (Desvaux & Bonnet, 2020). Pregnancy causes a number of physiological modifications that impact the female the organism, especially within the kidneys and bladder (Ahmed and Yosry ., 2021). These modifications increase the risk of UTIs.

Since a multidrug- resistant bacteria (MDR), *Escherichia coli* can withstand numerous different antimicrobial agents through a variety for mechanisms, among them the production of digestive enzymes like β-lactamase, leading to the effectiveness of β-lactam prescription antibiotics, as well as additional digestive enzymes which generate opposition to aminoglycosides, quinolones that and other medicines. (Kapoor *et al.*, 2017.)

Heparin-binding protein (HBP), also known as azurocidin or cationic antimicrobial protein of 37 KDa, is a promising biomarker to distinguish between patients with these conditions.it is biologically plausible that HBP is an early and predictive biomarker because it is prefabricated and rapidly mobilized from migrating neutrophils in response to bacterial

infections.HBP has a major role in the pathophysiology of severe bacterial infections and thus represents a potential diagnostic marker and a target for the treatment of sepsis (Fisher *et al*.,2017).

They are many studies deals with ESBL-producing bacteria in Iraqi patient which include (Raouf *et al*., (2022) who was found the emergence of *K. pneumoniae* strains harboring ESBL resistance genes necessitates the development of a regular surveillance program to prevent the spreading of these isolates more in Iraqi health care systems, and who was illustrated 39% of *E. coli* isolates from females with symptomatic genital tract infection, were surveyed phenotypically and genotypically for ESBL production in AlKut/Wassit Province/Iraq (Jabbar ,2013).

Another of the several major global health problems causing several thousand fatalities was microbial resistance to antibiotics, which is predicted to worsen gradually (Adzitey, 2020). Around the end of 2050, many predict that this phenomenon would result in 10 million fatalities yearly (Bengsston-Palme *et al*., 2018; Praveenkumarredy *et al*., 2020). The prevalence of ESBL-producing bacteria has been increasing globally, with significant variations across different regions and healthcare settings .In some parts of the world, ESBL-producing *E. coli* and *Klebsiella pneumoniae* have become endemic, posing a major public health threat. The spread of ESBL-producing bacteria is facilitated bythe widespread use of broad-spectrum antibiotics.(Mangin& Touati (2023).

Patients with UTIs caused by ESBL-producing bacteria have a higher rate of treatment failure, longer hospital stays, and worse clinical outcomes as well as ESBL-producing bacteria are resistant to many commonly used antibiotics, making UTIs caused by these organisms more difficult to treat effectively. So, the current study aims to remedy these

things by following the steps :

below:

1.Isolation and identification of ESBL bacteria from urine samples of adults patients bearing UTI symptoms by culture , bio chemical tests and vitek system.

2.Evaluate ESBL bacteria isolates' resistance towards antibiotic by vitek system.

3.Molecular detection of ESBL bacteria virulence genes by PCR techniques.

4.Detection of Heparin binding protein by Elisa test in patients and controlgroups.

1.2. Literature Review

1.2.1: Urinary tract infection:

Urinary tract infection (UTI) is one of the most common infections. (Husen *et al*.,2023). Urinary tract infection affecting the vast majority of people. UTI involves a simple infection caused by urinary tract inflammation as well as a complicated infection that may be caused by an inflammation of other urinary tract organs (Ilker *et al* ., 2018). A common health-care problem worldwide, urinary tract infection (UTI), represents a disease of significant impact on every country's economy, being the most common cause of hospitalization among elderly people and the most common cause of antibiotic prescription in primary care.(Heidar *et al*.,2019).

Uncomplicated infections most commonly occur in otherwise healthy women when uropathogenic bacteria, usually *Escherichia coli*, enter the bladder and overcome host innate immunity (Panel and Glover. 2019). Urinary tract infections (UTIs) are among the most common infective disease in the adult population. Enterobacteriaceae including a large, heterogenous group of Gram negative rods whose natural habitat is the intestinal tract of human and animals. Members of Enterobacteriaceae are aerobes or facultative anaerobes, ferment a wide range of carbohydrates, possess a complex antigenic structure, and produce a variety of toxins and other virulence factors (Brooks *et al*., 2007).

UTI diagnosis is based essentially on the presence of lower urinary tract symptoms (e.g., dysuria, urgency, and frequency) and the evidence of bacteriuria (by dipstick testing and/or urine culture). The diagnosis is not always easy because symptoms can be vague, or patient basal conditions can interfere negatively with the diagnostic process(Martino and Novara ,2022).Definitive diagnosis of UTI is performed through quantitative urine culture. Traditionally presence of 10^5 cfu/mL or more considered

diagnostic bacterial UTI. (Kumar *et al* ., 2015 : Davenport *et al* ., 2017)

1.2.2. Bacterial uropathogenic of Urinary Tract Infection :

Urinary Tract Infection (UTI) is one of the most common infections caused by bacteria (Ajmal *et al*., 2021). The vast majority of UTIs are caused by *Enterobacteriaceae* originating from the gut before entering the urethra (Dougnon *et al*., 2020). Gram-negative bacteria belonging to the genera *Escherichia* and Klebsiella are the most prevalent UTI-causing organisms; *Escherichia coli* causes about 70–90% of urinary tract infections (Behzadi *et al*., 2021).

Normally urine is sterile and free of bacteria, viruses and fungi but does contain fluids, salts and waste products (Brunze, 2021). An infection occurs when tiny organisms, usually bacteria from the digestive tract, cling to the opening of the urethra and begin to multiply (Komala and Kumar, 2013). The bacteria which are responsible for causing UTIs such as *E.coli*, *Citrobacter* spp., *Klebsiella* spp., *Proteus* spp., *Serratia* spp., *Providencia* spp., and *Morganella* spp, , possess more potent virulence characteristics that improve their capacity to adhere with, colonize, then invade the cells of their hosts.(Olier *et al*., 2017). With the aid of particular virulence- including might include different cellular elements including pili, capsules, lipopolysaccharides, and numerous additional cell surface constructions, these pathogens are able to evade the defenses of the host (Johnson and Johnson, 2018). Representatives from the family Enterobacteriaceae generate enzymes known as broad-spectrum beta-lac (ESBL), which have the ability to breakdown beta-lactam drugs such as these medications and penicillin's, giving organisms that generate these individuals an immunity to antibiotics. Globally, isolates of bacteria that produce ESBLs had proliferated. Numerous distinct genetic components

carried on chromosomes and plasmids generate various ESBLs. (*Dr. Uyanga et al ., 2020).*Recently, most of the Enterobacteriaceae and *Pseudomonas* sp. becoming a emerging health problem due to the ESBLs production including *Escherichia coli, Proteus mirabilis, Klebsiella pnemoniae, Enterobacter* sp., *Acinetobacter* (*N. Rajivgandhia Govindan et al .,2021)*

The organisms most commonly responsible for catheter-associated UTIs are *E.coli, Proteus mirabilis, P.aeruginosa* , and *Streptococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, Actinomycetes, Candida* etc can cause UTI. In addition *Mycoplasma* and *Chlamydia* may be associated with sexually transmitted UTI (Mane and Bhosale, 2023). Also, other risk factors such as recurrent infections, poor socio-economic status, ncreasing age, genetic defects, the number of intercourses per week, increasing births, diabetes, deficiency of the immune system, urinary tract abnormalities, and the use of contraceptives promote the colonization of coliform bacteria in the periurethral region, increasing the risk of UTI (Singh *et al*., 2014).

Certain human anatomical as well as physiological factors are also responsible for increasing the incidence of UTI, for example, length of the urethra is shorter in females as compared to males which leads to an increased chance of acquiring UTI (Vasudevan, 2014). Similarly, incomplete emptying of bladder particularly in old age results in accumulation of residual urine remaining in the bladder and vesicoureteral reflux which frequently occurs among pregnant women which is the vital factors that can predispose host to UTIs (Djahangirian and Wehbi, 2018).

Wide-ranging antibiotics are frequently utilized for treating UTIs, and therapy begins based only on feeling rather than doing an analysis or susceptibility test. Antimicrobial resistance among bacterium is growing

globally primarily a consequence from the improper and careless use of antimicrobial agents, giving the development of forms of bacterial infections (Abbo and Hooton, 2014). The widespread consumption that antibiotics has inevitably caused the emergence of resistant bacteria to antibiotics and the discovery of uropathogen isolates that generate ESBL (the extended Range Beta-Lactamase).(Perletti *et al*., 2018).

1.2.3 Epidemiology of Urinary Tract Infection:

UTIs are a common health problem in the Arab world, with prevalence rates ranging from 7% to 20% in the general population.(Alhamad *et al* ., 2022). In general english countries ,UTIs are one of the most common bacterial infections, affecting an estimated 150 million people worldwide each year. The prevalence of UTIs is higher in women, with an estimated 50-60% of women experiencing at least one UTI in their lifetime.(Foxman *et al* ., 2014).

Urinary tract infections (UTIs) are the second most common infectionpresenting in the community (Kovac.,2021).UTIs are amongst the most frequent bacterial infections. However, the clinical phenotypes of UTI are heterogeneous and range from rather benign, uncomplicated infections to complicated UTIs (cUTIs), pyelonephritis and severe urosepsis. (Wagenlehner *et al* .,2020).

Urinary tract infections are highly prevalent, lead to considerable patient morbidity, incur large financial costs to health-care systems and are one of the most common reasons for antibiotic use worldwide. (Sihra *et al*.,2018).

Urinary tract infections is one of the most common infections afflicting women.It is often accompanies vaginal infections and is frequently caused by pathogens originating in the digestive tract.(Czajkowski *et al* ., 2021).This infection affects all ages and both

sexes.Despite these, women are usually more susceptible to this infection and hasa higher prevalence compared to the men. Some of the risk factors responsible for this high prevalence is due to menopause, poor personal hygiene, pregnancy and the close anatomical relationship of the female urethra and the anus. Among the uropathogens involved in this infection, entrobacteriaceae especially the *E.coli* is usually the most prevalent and accounts for 80-85% of the total isolate. Most often this infection is usually neglected but it is capable of claiming life under severe circumstances.(John *et al* .,2016). Urinary tract infections (UTIs) commonly complicate pregnancy, present in approximately 10% of gestations. Asymptomatic bacteriuria (ASB) is encountered most frequently. Symptomatic infections, cystitis and pyelonephritis, complicate pregnancy (Allen ,2021).

1.2.4 Risk factors of urinary tract infections:

To develop urinary tract infection, there are many risk factors that contributed to the occurrence of infections.

1.2.4.1 Age:

UTIs are one of the most common infections in older adults, with an estimated 25-50% of women and 10-15% of men over the age of 65 developing a UTI each year, older adults, particularly those over the age of 65, have a higher risk of developing UTIs compared to younger adults. This is due to several factors, Weakened immune system: The immune system tends to decline with age, making older adults more susceptible to infections like UTIs. (Hanlon *et al* ., 2018).

1.2.4.2 Sex :

Having an average lifetime incidence of fifty to sixty percent in adult women, urinary tract infections (UTIs) are among the most prevalent

inpatient illnesses. The frequency of urinary tract infections rises with age, and among women who are over 65, it is around twice as high as in the general female population. The risk of infections and the majority of probable microorganisms are influenced with variables including catheterization, which is which changes depending upon the health of individuals in this age group. Increasing sexual behavior was an independent risk for developing urinary tract infections among younger women, and return before six months is typical(Medina and Castillo-pino.2019).The incidence of UTIs in adult males aged under 50 years is low, with adult women being 30 times more likely than men to develop a UTI. Appropriate classification of UTI into simple or complicated forms guides its management and the ORENUC classification can be used (Tan and Chlebicki ,2016)

1.2.4.3 Pregnancy:

Pregnant women have a higher susceptibility to developing UTIs compared to non-pregnant women. This increased risk is due to several physiological changes that occur during pregnancy, such as: Hormonal changes that can impair the immune system's ability to fight off infections, Mechanical changes, such as the growing uterus putting pressure on the bladder, leading to incomplete bladder emptying, Increased levels of progesterone, which can relax the ureters and allow reflux of urine(Schnarr*et al* ., 2008).

1.2.4.4 Contraceptives:

the use of certain types of contraceptives, spermicidal coated condoms significantly alter the normal flora, and cause the increase in colonization of the vaginal tract epithelium with UPEC, thus increase the risk of UTI (Braunwald *et al.*, 2001; Yadav *et al*., 2015).

1.2.5 Pathogenesis of urinary tract infection:

The bacteria can invade and spread within the urinary tract by two main routes: the ascending and hematogenous pathways. There is little evidence to support a lymphatic spread of infection to the urinary tract .

The first is hematogenous route; in which the renal parenchyma gets infection byblood-borne organisms occurs in humans, albeit less commonly than by the ascending route. The kidney is being frequently the site of abscesses in patient with bacteremia or endocarditis caused by a gram positive bacteria, like *Staphylococcus aureus*; infections of the kidney with Gram negative bacilli rarely occur by the hematogenous route .The second route is ascending Route ;urinary tract infections in women can be developed when uropathogens from the fecal flora colonize the vaginal introitus and displace the normal flora (*diphtheroids, lactobacilli*, coagulase-negative staphylococci, and *streptococcal* species) (Mussaed *et al*., 2018)

Colonization of the vaginal introitus with *E.coli* seems to be one of the critical initial steps in the pathogenesis of both acute and recurrent UTI (Dalawai *et al* .,2019) Most uropathogens originate in the rectal flora and enter the bladder via the urethra. The female urethras are short and proximal to the vulvar and perineal areas, making infection by contamination more likely (Rehman& Shrivastva, 2018). In women who have UTIs, the urethra has been colonized and the uropathogen gains entry to the bladder, presumably by means of the urethral massage that accompanies sexual intercourse. The evolution of infection depends upon the particularorganism, the size of the inoculum, and the adequacy of host defenses. Whenthe bacteria ascend into the bladder, they may multiply and then pass up to the ureters, particularly if vesicoureteral reflux is present, to the renal parenchyma (Cristina *et al*., 2017 : Mussaed *et al*.,2018)

1.2.6 Enterobacteriaceae:

Numerous Gram-negative microbes that constitute a natural part of the intestinal flora in human as well as animal intestine are members of the Enterobacteriaceae family. *Salmonella*, *Shigella*, *Escherichia*, the bacteria *Enterobacter Klebsiella*, *Proteous*, and other members of this heterologous family are included. This family of bacteria may cause a wide range of illnesses in both people and animals, such as infections of the vagina, respiratory tract infections, infections of wounds, urinary tract infections (UTIs), and nosocomial diseases. These are facultative or aerobic anaerobes, rod-like in shape and Gram-negative. The majority of the species are flagellated, non-spore-forming, lactose fermentation vessels, oxidation testing negative in nature, indole testing positive, and develop best at 37 °C. Bacteria possess a variety of pathogenicity factors, including digestive enzymes, poisons, capsule-like structures, and flagella. etc. (Oliveira *et al*., 2017 : Riedel *et al*., 2019;).

1.2.6.1 *Escherichia coli*:

The following are the genera of *Escherichia* that are members of the Enterobacteriaceae group of bacteria: *E. coli, E. fergusonii, E. vulneris, E. hermanii, E. blattae,* and *E. albertii*. The varieties listed are via biochemistry different from one another (Olowe *et al*., 2017). *E. coli* is a pathogenic opportunistic bacterium species of that thrives in the gastrointestinal tract as part of the normal microbe population. It is known for causing vomiting, where it is referred to as diarrheagenic bacterium or DEC, and infections of the urinary tract, where are brought through the form that is known as *E. coli* (UPEC). Roughly 90% of urinary tract infections in young women are caused by it (Levinson *et al* .,2019). Those belonging to the enter is a group of microbes that includes E. coli.(Castellani & Chalmers, (2022):

Domain: Bacteria

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gamma proteobacteria

Order: Enterobacterales

Family: Enterobacteriaceae

Genus: *Escherichia*

Species: *Escherichia coli* (*E. coli*)

1.2.6.2 Characterization of *Escherichia coli*:

a rod-like in shape Gram-negative in bacteria without spores that travels by using flagella. The bacterial populations often have smooth surfaces and usually conical in shape. The colonies it produces seem flat, dehydrated, as well as pinkish on MacConkey agar, but on Eosin Methylene Blue (EMB) agar, colonies appears to be covered in a vibrant, "metallic sheen." Sorbitol MacConkey agar is commonly used to distinguish the growth of ***E. Coli*** O157:H7 from other serotypes of the same bacterium since O157:H7 is unable to decompose sugar. Double Sugar Iron agar does not create H2S from ***E. Coli*** strains.

The majority strain have the capacity to produce the digestive enzyme β-glucuronidase. A pH value of 4.4 to 9.5 is ideal for growth, and the ideal temperatures to feed development is 36 to 37 °C**.** The bacteria gives a negative result to the oxidase, urease, nitrite tests while having a positive result in the catalase test, and as for the indole test, most strains give a positive result (Kodaka, *et al* ., 2004; Wanger *et al* .,2017; Riedel *et al* .,2019).

1.2.6.3 Extended spectrum beta-lactamases (ESBL) :

Representatives bacterium the Enter0bacteriaceae family generate proteins that have the ability to dissolve beta-lactam antibiotics such as these medications as well as penicillin, giving populations that produce them resistance to antibiotics. Globally, isolates of bacterial species that produce ESBLs had proliferated (Dr. Uyanga *et al* .,2020) recently, most of the Enterobacteriaceae and *Pseudomonas* sp. becoming a emerging health problem due to the ESBLs production including *Escherichia coli, Proteus mirabilis, Klebsiella pnemoniae, Enterobactersp., Acinetobacter* (Ramachandrana *et al* . ,2021).

1.2.6.4. Virulence Factors of *Escherichia coli* :

The bacterial infectiousness, or virulence, is an indicator of the bacteria's ability to bring about illness. A number of virulence factors present in *E. coli* types enable the microbes to cause illness as well as infection, with urinary tract infections (UTIs) constituting particularly significant among all these illnesses.

Some types of bacteria possess generating proteins that have mutations exclusive to certain sites called as island pathogenicity (PAIs), which give them the capacity to evade the immune system and cause illness. These islands range across sizes from 10 to 200 kb, and their G+C concentration sets them separate from other regions within the chromosome. Pathological bacteria have more PAIs that not pathogenic bacteria do, and these PAIs translate for pathogenicity features including adhesion, digestive enzymes, lipopolysaccharides, siderophores that toxins, and capsule-like structures, every single one which has its own unique coding gene (Parvez and Rahman, 2018).although the UPEC expresses several virulence factors that help it to invade, colonize the host issue and establish an infection. The common and major virulence factors are presented in Table (1).

***Table 1*: Virulence factors of the UPEC (Terlizzi *et al*, 2017)Major function**

Virulence factors	Major function
Flagella	Adhesion of *E. coli* in the urinary tract
Type 1 fimbriae	Adhesion to bladder epithelial cells
P fimbriae	Adhesion to kidney epithelial cells
Dr fimbriae Cell	Invasion
Haemolysin	Invasion, tissue damage
Secrete auto transporter toxins	Tissue damage
LPS	Immune response activator
Curli fimbriae	Adhesion, biofilm formation, invasion
Cellulose	Biofilm formation
Iron and zinc	acquisition Nutrition
Capsule	Resistance to phagocytosis
F1C fimbriae	Unknown

***1.2.6.4.1.cytotoxic necrotizing factor*:**

E. coli generate all types of extra-intestinal infections, such as neonatal meningitis, septicemia, and urinary tract infections (UTIs). *E.coli* strains involved in UTIs often produce exotoxins such as hemolysin, *cytotoxic necrotizing factor type 1* (*CNF1*), and colonization factors. The genes encoding exotoxins and colonization factors are located in the region of the chromosomal DNA called pathogenicity islands (Obaid *etal* 2014).Exotoxins, also including hemolysin is cytotoxic necrotizing protein variant 1 (*CNF1*), other colonisation proteins are frequently produced by *E.*

coli bacteria linked to UTIs. The section of the chromosomal genome known as the "their pathogenicity islands" contains gene sequences that encode exotoxins as well and colonization determinants (Hedayat *et al*.,2023).

The cytotoxic necrotizing factors (*CNFs*) are a family of Rho GTPase-activating single-chain exotoxins that are produced by several Gram-negative pathogenic bacteria. Due to the pleiotropic activities of the targeted Rho GTPases, the *CNFs* trigger multiple signaling pathways and host cell processes with diverse functional consequences. They influence cytokinesis, tissue integrity, cell barriers, and cell death, as well as the induction of inflammatory and immune cell responses (Chaoprasid *et al* ., 2021) . The *CNF1* has a cell-binding section located at its the N-terminal. Proteins 190 through 720 make up the primary region that makes up *CNF1* toxins, whose has a hydrophilic composition that may serve as a type of transmembrane transmitter (domain T).The 3042 bp genetic material, coding for around 1014 amino acid sequences, encodes the uropathogenic *E. Coli* (UPEC) the *CNF1* animal-derived ETEC isolates generate *CNF2*, and it shares 86% of the amino acid similarity with *CNF1*)(Gábor,2002). Uropathogenic *Escherichia coli* produce cytotoxic necrotizing factor-1 (*CNF-1*),which is mediates its effects on epithelial cells, and acts as one of uropathogenic *E. coli* virulence factors.(Almasaudi *et al* ., 2018).

1.2.6.4.2. A/E Lesions:

The *E. coli* Attaching and Effacing gene (*eae* gene) is located on the Locus of Enterocyte Effacement (LEE), which is a ~35-kb pathogenicity island where the main virulence genes of all strains of *E. coli* capable of inducing *A/E* lesions (Attaching/Effacing) are located. LEE is organized into 5 operons (LEE1 through LEE5). Located on LEE5, the *eae* gene is about 2800 nucleotides and encodes the adhesion protein intimin, a 94-kDa

protein required for the adherence of *E. coli* to host cells at the site of *A/E* lesion (Gomes *et al.*, 2016; Yang *et al*., 2020). A/E lesion are characterized by intimate adhesion of E. coli to the surfaces of enterocytes, on raised pedestals (pseudopodia), and destruction of nearby microvilli (Adhikari, ,2018).

The *A/E* gene is a crucial virulence factor found in certain strains of *E. coli*, including some uropathogenic strains. This gene encodes a type III secretion system, which allows the bacteria to attach to and efface (erase) the brush border of the host's epithelial cells. This attachment and effacement process facilitates the colonization of the urinary tract by the bacteria. In the context of UPEC, the *A/E* gene plays a significant role in the ability of these strains to cause UTIs. The attachment and effacement of the host's epithelial cells mediated by the *A/E* gene can lead to the disruption of the integrity of the bladder and kidney epithelium, allowing the bacteria to adhere, invade, and establish a foothold within the urinary tract. Furthermore, the *A/E* gene is often found in combination with other virulence factors, such as toxins and adhesins, which further enhance the pathogenic potential of UPEC strains. This synergistic effect of multiple virulence factors contributes to the increased ability of these strains to cause persistent and recurrent UTIs (Sokolova *et al* ., 2012).

Understanding the role of the *A/E* gene in the pathogenesis of UPEC-associated UTIs has important implications for the development of diagnostic tools and targeted therapies. Identifying the presence of the *A/E* gene in UPEC isolates can assist in the accurate diagnosis and targeted treatment of UTIs, potentially reducing the risk of complications and the development of antimicrobial resistance.(Manges & Johnson,2021)

1.2.6.4.3.BLA OXA-48:

Carbapenems have the broadest spectrum of all β-lactam antibiotics and are increasingly used to treat infections caused by otherwise multidrug-resistant Gram-negative bacteria. Consequently, emerging resistance to carbapenems is a major public health concern, especially when it involves acquired, horizontally transmissible carbapenemases. These enzymes are diverse, belonging to classes A, B or D of the Ambler β-lactamase classification. Acquired class D carbapenemases are encountered most often in isolates of *Acinetobacter* spp., where *OXA-23*, *-40* and *-58* variants are all important; however, the genes encoding the *OXA-48*-like subgroup, which originally escaped from environmental bacteria of the genus Shewanella (Talebi *et al.,* 2020: Jalal *et al*., 2023) are becoming more prevalent in Enterobacteriaceae.

Turkey is where *OXA-48* β-lactamase originated. *OXA-48*-producing Enterobacteriaceae expanded to a maximum of 118 individuals during a significant *OXA-48* epidemic in Belgium, indicating the horizontal transmission of the resistant gene via a number of plasmid. Underlying limited precision of traditional characterization techniques makes it difficult to understand the transmission patterns underlying resistant genes. Twenty-two non-outbreak-related *OXA-48*-producing The bacteria known as enter infections during the countries of the Netherlands, Libya, & Turkey have been selected in addition to the original *68 OXA-48*-producing samples gathered from the hospital outbreak

1.2.6.4.4.*BLA CTX-M* :

Extended Spectrum Beta Lactamases (ESBLs) in human medicine first came to prominence following the introduction of the extended-spectrum cephalosporins (cefotaxime, ceftazidime, ceftazidime,

ceftriaxone, etc.) in the 1980's. The term was applied to mutants of the already common plasmid mediated β-lactamases such as *SHV* and *TEM* . gene emerged in Enterobacteriaceae as a result of horizontal gene transfer from an environmental bacterium in the rhizosphere. The chromosomal homologs of the *CTX-M* genes in different species of *Kluyvera* have been mobilised into *Klebsiella spp* and *E.coli* on different occasions and global locations. Among some regions, *CTX-M* serves as the predominant βlactamase among gram-negative microbes also among the most prevalent ESBL specimens The pace at which ESBL expression among individual isolated will eventually stabilise is unknown. Given the widespread distribution about *CTX-M* gene variants between the gut microbes associated with food humans as well as animals, there are risks associated with human and livestock colonization as well as global foodborne transmission. The use of antibiotics that maintain their action towards *CTX-M* ESBL have become increasingly popular due to their growth; they are often carbapenems (e.g. meropenem, ertapenem etc.). A entire family of varied carbapenemases has emerged as a result of this selection pressure, and they pose a significant new danger to current medicine.

The increasing abundance of extended spectrum β-lactamase (ESBL) genes in E. coli, and other commensal and pathogenic bacteria, endangers the utility of third or more recent generation cephalosporins , which are major tools for fighting deadly infections. We found difference in bla CTX-M genes (and adjacent sequences) associated with different animal species; some *bla CTX-M* variants (*blaCTX-M-1*, *blaCTX-M-9*) were found only in human isolates and not in chickens in the community. Additionally, spacers flanking *blaCTX-M-15* in human and chicken isolates (in the same community) (Xavier *et al* .,2023).

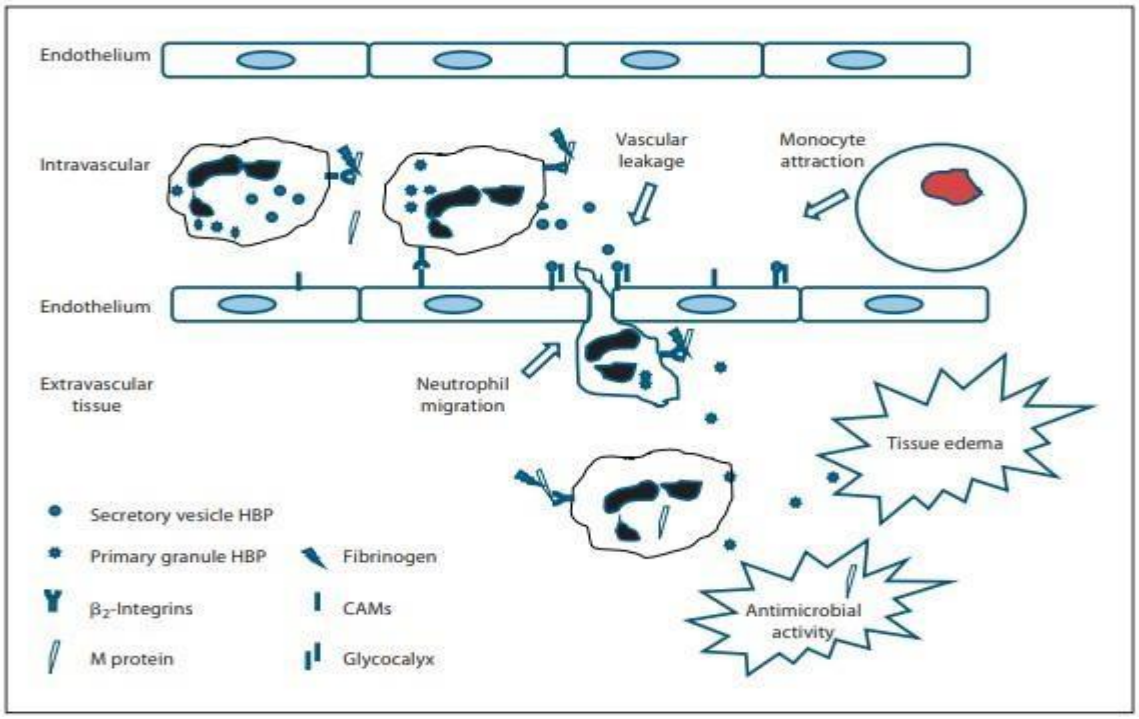
Escherichia coli is one of the major causes of urinary tract infections in primary healthcare, and treatment is more complicated due to the increase in antibiotic resistance. Extended-spectrum β-lactamases are the most common mechanism of resistance against third-generation cephalosporin, and *CTX-M*-like are among the most prevalent. These results show an increase in resistance to third-generation cephalosporin from 10.58% to23.96%.(Segarra *et al* .,2018).

1.2.7 Heparin Binding protein:

Also known as CAP37/azurocidin protein ,or cationic antimicrobial protein of 37 kDa (CAP37). A member of the serprocidin family of neutrophilcationic proteins. HBP induced Ca++-dependent cytoskeletal rearrangement and intercellular gap formation in endothelial-cell monolayers in vitro, and increased macromolecular efflux in microvessels in vivo. Polymorphonuclear leukocyte infiltration into tissues in host defense and inflammatory disease causes increased vascular permeability and edema formation through unknown mechanisms. Moreover, selective inactivation of HBP prevented the neutrophils from inducing endothelial hyper permeability .a paracrine mechanism in neutrophil-evoked alteration in endothelial barrier function.(Fisher & Linder, 2017).HBP is stored in two different granule subsets, azurophilic/primary granules and secretory vesicles. (Yang *et al* .,2019).

HBP causes vessel leakage along with the development of edema within along with acting as a chemoattractant & promoting the proliferation of monocytes and macrophage. The attachment of neutrophilic 2-the integrins, which can have been started by microbial constructions, causes the dissolution this particular enzyme. Strong cardiovascular leakage is the final result altogether. Research indicates significantly elevated HBP levels within the plasma are present in individuals with severe sepsis prior to the

first signs of hypertension. The cause of tissue inflammation disease involves HBP as well. With summary, this amino acid is an intriguing marker for diagnosis and a therapeutic target since it plays a significant role in the pathogenesis of serious bacteria-related infections. (Linder, Soehnlein and Åkesson,2010).During the establishment phase of inflammation, neutrophils are usually recruited before monocytes. This series of events is causally related and in fact HBP plays an important role in the transition from neutrophil to monocyte efflux (Soehnlein, *et al*.,2022) . Secreted from emigrating neutrophils, HBP binds to endothelial glycocalyx and is presented to leukocytes in the blood stream (fig. 1). In this location, HBP activates monocytes rolling along the endothelium and ultimately induces stable monocyte arrest. Adhesion of monocytes is followed by transendothelial extravasation and directed migration to the site of injury. Like other neutrophil-derived antimicrobial polypeptides, HBP chemoattracts monocytes (Fisher & Linder,2017) . The monocyte-attracting ability of HBP is about 80–100% of that of formyl-methionyl-leucyl-phenylalanine (fMLP), a strong enhancer of monocyte chemotactic migration. The monocyte population in humans and mice, however, is heterogeneous



Figure(1). Schematic overview of the effects of HBP in bacterial infections. *S. pyogenes*- derived M1 protein forms complexes with fibrinogen. These ligate neutrophilic 2 - integrin resulting in cellular activation and discharge of HBP from secretory vesicles. Due to charge interactions, HBP is deposited on endothelial cells where it induces increases in transendothelial permeability and edema formation. Furthermore, endothelial-bound HBP activates monocytes rolling along the endothelium promoting their firm arrest. Neutrophils migrated to the site of inflammation release HBP from primary granules which may primarily exert antimicrobial activities. In addition, HBP attracts inflammatory monocytes and activates macrophage antimicrobial activities. . (Kany & Relja ,2019)

HBP plays a crucial role in the immune response against bacterial infections, particularly in the urinary tract. In the context of UTIs, HBP has been studied extensively for its potential as a biomarker and diagnostic tool. Heparin Binding Protein has been observed to be elevated in the urine of individuals with UTIs caused by various bacterial pathogens, such as Escherichia coli, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.(Linder**&** Åkesson**,** 2009**).**

The mechanism by which HBP is involved in UTIs is as follows:

1. Bacterial invasion: When bacteria, such as *E. coli*, infect the urinary tract, they can adhere to the bladder or kidney epithelial cells, causing an inflammatory response.

2. Neutrophil recruitment: In response to the bacterial infection, the body recruits neutrophils to the site of infection. These neutrophils release HBP from their granules.

3. Antimicrobial effects: HBP has antimicrobial properties and can directly kill or inhibit the growth of the invading bacteria. It also helps recruit and activate other immune cells to combat the infection.

4. Diagnostic value: The presence and elevated levels of HBP in theurine of individuals with UTIs can be used as a biomarker to aid in thediagnosis and monitoring of the infection. Measuring urinary HBP levels can help clinicians distinguish between UTIs and other urinarytract conditions.

1.2.8. Diagnosis of Urinary Tract Infection:

Accurate diagnosis is the basis of all reactions to UTI, so it is essential that the diagnosis have to be correct and made using adequate methods. The diagnosis of UTI is based on clinical signs and/or symptoms, urinalysis (leucocytes, nitrite) and the presence of a significant growth of bacteria in the urine. The signs and symptoms depend on the level of infection and the age of the patient, but typical manifestations include fever, dysuria, frequency urgency, enuresis, abdominal pain, flank back pain and incontinence. Decision rules depend on symptoms and patient characteristicshave been developed and studied in order to select high-risk patients for urinalysis and treatment and low-risk patients for follow-up, For adult patients, a midstream clean catch urine sample is the recommended method for urine collection. Urine samples should be tested immediately after collection, but if urine cannot be examined and cultured

within 4 hours of collection, the sample should be refrigerated or preserved with boric acid (Martino ,2022).

Evaluation of UTI based on both lab investigations and clinical signs and symptoms. Lab investigations are including both urinalysis and urine culture. Diagnosis of UTI is not always straight forward because one cannot look at its appearance and conclude an infection(Henderson *et al*., 2019). Diagnosis follows the same algorithm used in younger patients, requiring the presence of genitourinary symptoms and a positive urine culture. Common urinary symptoms suggestive of cystitis include urgency, frequency, dysuria and supra-pubic tenderness. However, postmenopausal women may also present with nonspecific generalized symptoms, such as lower abdominal pain, back pain, chills and constipation (Chu & Lowder , 2018).

Asymptomatic bacteruria (ASB) in women is described as the presence of two consecutive urine specimens positive for the same bacterial strain in quantities $\geq 10^5$CFU/ml, in the absence of any signs or symptoms of a genitourinary tract infection. For men, ASB is defined as a single voided specimen with one bacterial isolate in quantities $\geq 10^5$ CFU/ml, in the absence of symptoms(Parnell, *et al*.,2023). For adults with an indwelling urethral, suprapubic or intermittent catheter, ASB is defined as a positive urinary culture for one bacterial isolate in quantities $\geq 10^2$ CFU/ml, in the absence of symptoms (Hooton et al, 2010). Also, there is application of multiple “omics” technologies aimed at investigating the UPEC genomic diversity, the global gene expression in different models of infection both in vitro and in vivo, and to define the occurrence of UPEC-specific proteins as new candidate therapeutic and vaccine targets (Lo *et al*.,2017).

1.2.9. Antimicrobial Treatment of urinary tract infections and resistant of uropathogens:

Antibiotics are considered the standard treatment for bacterial UTI. They aim to eliminate the causative organisms and provide symptom relief to patients (Hasan, 2021). One therapeutic issue among UPEC individuals who have urinary tract infections is resistance to antibiotics, particularly in female patients who have recurring urinary tract infections .

Conventional feces biodiversity, whose acts as resistance reservoirs both prospective uropathogens, while uropathies are both significantly pressured to develop resistance by the empirical antibiotic therapy used in cases of recurrent urinary tract infections (Sihra *et al*., 2022). The global prevalence of antibiotic resistance in *Escherichia coli* strains that cause urinary tract infections has risen, with notable regional and temporal differences. (Sugianli *et al* .,2021).

Around eighty-five percent of the UPEC isolates collected individuals in india showed resistance to ampicillin, which is compared to 56% of UPEC isolates collected hospitalized individuals in Poland and Turkey (Kot *et al* .,2017), while above 85% of UPEC strains from patients in India were resistant to this antibiotic. High percentage (67.3%) of *E. coli* strains resistant to tetracycline was isolated from people with UTI from different parts of India (Mohapatra *et al*.,2022).suggested that the increase of resistance of UPEC to ciprofloxacin is a result of widespread use of this antibiotic in the treatment of uncomplicated UTIs in the early 2000s. The most published data mentioned that UPEC isolates are resistant to ampicillin, oral first-generation cephalosporins, TMP-sulfamethoxazole (Moya-Dionisio *et al*., 2016), cefuroxime (Chang *et al*., 2016), cotrimoxazole (Gharavi, M. J.,2021), amoxicillin-clavulanate, nalidixic acid, cefradine, and aminopenicillins (Terlizzi *et al* .,2017). In some cases,

the combined effect of various antibiotics prompted a significant increment in susceptibility, as found for triclosan with amoxicillin and gentamicin (Kampf *et al* .,2018).

The extended-spectrum beta lactamases (ESBLs) production by UPEC strains complicates treatment because these strains are resistant not only to β-lactam antibiotics but often area also-resistant to other classes of antibiotics-like aminoglycosides, quinolones, and cotrimoxazole, such as gentamicin, ciprofloxacin, and trimethoprim-sulfamethoxazole, respectively (Robles *et al*., 2020), so Carbapenems like imipenem which all UPEC strains (100%) are being sensitive to it,so it is representing the best option for the treatment of extended-spectrum beta lactamase (ESBL) strains (Idil*et al*., 2016). Antimicrobial resistance has been recognized as one of the world's most pressing public health problems. The intensive use and, especially, the misuse of antibiotics have been led to the development and selection of resistant bacteria in different settings. Beyond the use of drugs for therapeutic purposes in the human and animal settings, antibiotics are also used extensively as prophylactic agents and as animal growth promotersin agriculture. Therefore, resistant bacteria are not only confined to the human clinical setting, such as hospitals, where they were first recognized and studied. They have been also increased significantly in the community and in both farm and companion animals. Animals may act as reservoirs of resistant bacteria that can be transmitted to humans, or vice versa, by direct contact or indirectly, via the food chain (Pomba *et al*., 2017). Mammals can serve as repositories for resistance germs which individuals may acquire from them either personally or indirectly through their food chain. Furthermore, antibiotic-resistant microbes may propagate quickly the far-off territories and continents thanks to the expansion of trade and travel between countries (Van Boeckel *et al*., 2019).

AntimicrobialResistance to antibiotics in microorganisms might be acquired by horizontal gene transfer (HGT) or mutations in the DNA (Lerminiaux *et al.*,2019).

The prevalence of natural mutations varies based on the antibiotic and the bacteria. For example, in order to create fully effective therapeutic antibacterial fluoridation of bacteria might require a progressive accumulation of changes (Baquero *et al*.,2021).

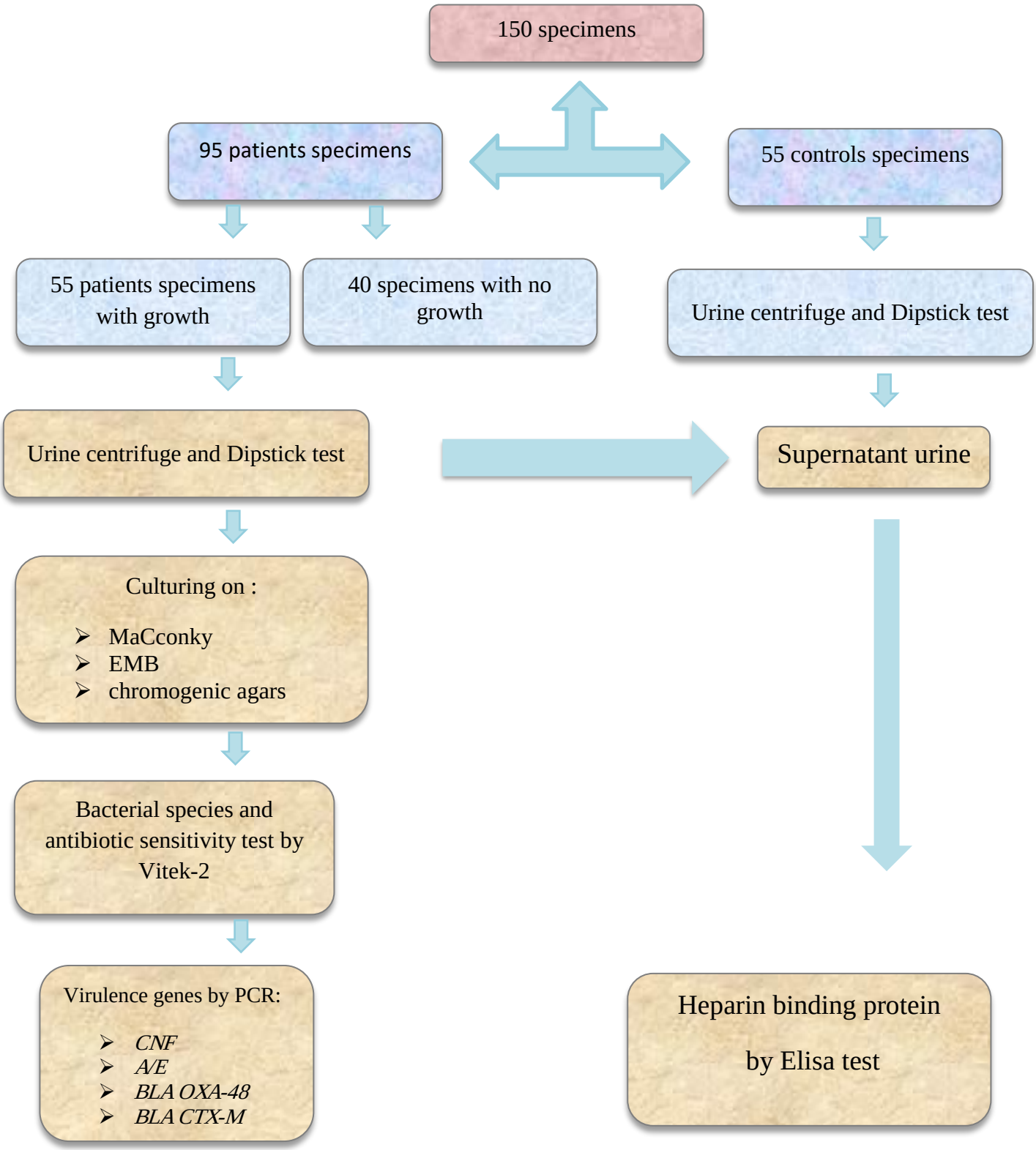
Horizontal gene transfer is play a key role in the evolution of bacteria and the spread of antimicrobial resistance genes (Virolle *et al*.,2020), It is involving the acquisition by the bacterial cell of foreign DNA, aphenomenon that may occur via three mechanisms: transformation (capture of free DNA), transduction (via bacteriophage DNA), or conjugation (Sharma *et al* .,2022).

Resistance traits located in genetic mobile elements like plasmids, transposons or integrons can be transferred to different strains or bacterial species (partridge *et al* ., 2018) The resistance acquisition or virulent traits may represent a survival advantage to the microorganism. It is conceivable that virulence genetic determinants, if located on the same genetic platform as antimicrobial resistance genes (plasmids, transposons, integrons) may be co-mobilized under antimicrobial selective pressure. *E. coli* has been one of the most versatile bacterial species and the diversity of its lifestyles is achieved through a high degree of genomic plasticity, via gene loss or gain, through lateral gene transfer so have seen increasing numbers of reports on the antimicrobial resistance acquisition by *E. coli* strains (Redondo-Salvo,2020).

Chapter Two

Materials and Method

2.1. Study design: Case control study:



Figure(2-1):Schematic diagram of current study

2.2. Study setting:

This case- control study was done at patients in hospital in Karbala province,. All patients collected were registered in UTI center in hospital from October (2023) to end of march (2024). Patients: 55 patients randomly recruited from the patients hospital in Karbala aged ranged between (20-63) years, who are diagnosed to have UTI based on clinical and laboratory findings(lymphocyte, neutrophils, eiosinophils) by the clinicians; the patients data collection were include residence area, Diabetes mellitus and gender.

2.3.Subjects Group:

One hundred fifty (150) participants were enrolled in this study including two groups involved in this case-control study according to clinical diagnosis by a clinician: the first one includes 95 patients [female(50) ,male(45)] and the second group includes [55 (29 male, 26 female)] healthy control group. All of the groups' ages range from 20 to 63 years. Detailed case information sheets involving age, gender, full history and other variables were carried out for each patient by a questionnaire as in (Appendix 1).

2.4. Ethical approval:

The study protocol will be sent to the relevant ethical committee in the health directorate. Also, verbal approval will be taken from each participants before taking the sample. During samples collection, health measures and safety will be taken.No.3284, date:19-11-2023.

2.5. Inclusion criteria:

All patients with urinary tract infection were diagnosed on the basis of clinical symptoms and other investigations. age from 20 to 63. Midstream urine and culturing on MacConky ,EMB and hicrom TM SEBL agars.

2.6.Exclusion criteria:

The patients who have autoimmune diseases except type2 diabetes, cardiovascular disease, sepsis cancer, congenital urinary tract anomalies, Acute Kidney Injury. UTI patients with Bacterial growth other than ESBL Enterobacteriaceae will be excluded. gram positive bacteria.

2.7. Materials:

2.7.1. Equipment and Instruments Utilized in the Study:

In the present study, the following equipment and instruments were used , as found in (table 2.1).

Table 2.1: **equipment and instruments of current study**

Equipment & Instruments	Manufacturing Company	Origin
Autoclave	Hirayama HVE-50	Japan
Biological safety cabinet	EuroClone Safemate	Italy
Burner	Amal	Turkey
Centrifuge	Kokusan	Korea
Cool box	VB	China
Deep freezer	Hettich	Korea
Electric oven	Olympus	Japan
ELISA Devices (washer & reader)	Human	Germany
ELISA printer	Epson	Japan
Eppendorf tube 0.5 ml	ALS	China
Flasks (different size)	Jlassco	India
Gel Tubes 6 ml	ALS	China
Graduated glass cylinder	Supc orior	Germany
Incubator	Bio base	China

Light microscope	Olympus	Japan
Loop	Himedia	India
Micropipette set	SLAMED	Germany
Multichannel micropipette set	SLAMED	Germany
Para-film	Bemis	USA
Pipette tip	ALS	China
Refrigerator	Panasonic	Korea
Sensitive balance	Sartorius	Germany
Slides	Himedia	India
Swab media	Himedia	India
VITECK® 2 compact system	Bio merieux	France
Vortex	Clay Adams	Germany
Water bath	Polyscience	USA
Water distillatory	GFL	Germany

2.7.2.Culture media:

The culture media used in the present study are in table (2-2).

***Table (2.2)*: Culture media used in the current study:**

Culture media	Company	Country of origin
Hicrom TM ESBL agar	Himedia	India
Eosine methylene blue	Himedia	India
Glycerol	Himedia	India
MacConky agar	Himedia	India
Nutrient agar	Himedia	India
Nutrient broth	Himedia	India

2.7.3. Laboratory Kits:

Laboratory kits are listed in table(2.3) :

Table2.3: **components of kit of current study**

Kit	Components	Manufacturer (Origin)
DNA Extraction kit	1. Nuclei lyses solution 2. RNase solution 3. Protein precipitation solution 4. GB Buffer 5.absolute Ethanol 6.GD Column 7.W1 buffer 8.Wash buffer 9. TBE buffer 10. CL buffer	Promega (USA)
Vitek 2 kit	1. Vitek strip 2. Normal saline 3. Vitek 2 GN card 4. Vortex 5. Vitek 2 Densichek	BioMerieux (France)

2.7.4. DNA marker:

DNA marker of current study , was demonstrated in tables(2.4):

Tables(2.4): **DNA marker of current study**

DNA Marker	Origin	Country
1 500bp DNA Ladder	Bioneer	Korea

The specific primers used in the present study were listed in table (2-5) and the condition in which PCR was performed for the four genes listed in table (2-5).

Table2.5: **primer sequence of current study**

Primer Name	Sequence 5-3	Annealing Temp. (°C)	Product size (bp)	References
EAE-F *EAE-R*	**CCCGAATTCGGCACAAGCATAAGC** **CCCGGATCCGTCTCGCCAGTATTCG**	**52 °C**	**877**	**Sousa, J. F. D. (2019**
Bla-CTM-F *Bla-CTM-R*	**CGCTTTGCGATGTGCAG** **ACCGCGATATCGTTGGT**	**65 °C**	**550**	**Poirel, L., *etal*.2001**
CNF-F *CNF-R*	**GCAGTCACCTGCCCTCCGGTA** **CATTCAGAGTCCTGCCCTCATTATT**	**66 °C**	**498**	**Nehmaa, S. A. (2023)**
Bla-OXA-48-F *Bla-OXA-R*	**CCAAGCATTTTTACC CGCATCKACC** **GYTTGACCATACGCT GRCTGCG**	**55 °C**	**438**	**Jalal Ahmed,*etal*.2023**

2.7.5. Commercial kits:

The commercial kits used in the present study are in table (2.6).

Table (2-6): **The Commercial kits which are used in the study.**

Kits	Company
Human Heparin Binding Protein ELISA Kit	BT LAB / China

2.8.method :

2.8.1. Sample Collection:

One hundred fifty of urine samples were collected from people with urinary tract infections (UTIs) from different places around kerbala Province. The people were chosen based on their general urine examination results. The samples were taken by standard mid-stream “clean catch” method from people with urinary tract infections and each urine sample was collected from patient into a sterile container (Karlowsky *et al*.,2006 ; Solberg *et al*.,2006) , all urine samples collection were classified in two to parts, first part cultured on MacConkey agar, EMB agar, chromogenic agar

using the streaking method, then they were incubated at 37 °C for 24 hours, and second part was used to determine Heparin binding protein by using Enzyme linked Immonosorbent assay.

2.8.2. Media Preparation :

a- Eosin Methylen Blue agar medium(Appendix 2).

b- MacConky agar medium: (Appendix 3).

c- Hicrom TM ESBL agar:(Appendix 4)

d- Nutrient Agar Medium :

Nutrient agar medium was prepared according to the method suggested by the manufacturing company. It was used for the cultivation of the bacterial isolates when necessary (Blane& Peacock (2016).

e- Nutrient Broth :

This medium was used to grow and preserve the bacterial isolates supplemented with 15% glycerol.(Blane& Peacock (2016).

2.8.3 Preservation and Maintenance of Bacterial Isolates:

The bacterial isolates were preserved on nutrient agar slant at 4˚C. The isolates were maintained monthly by reculturing on new medium. Nutrient broth supplemented with 15% glycerol was used for long preservation and the isolates were maintained frozen at -70˚C deep freeze for several months long term maintenance (Collee *et al*., 1996).

2.8.4.: Isolation and Identification of Microorganisms :

All specimens were examining by general urine examination (GUE) to identify the presence of bacterial cell, white blood cell, and other substances. For quantitative culture and presumptive identification from each urine specimen, Well-mixed urine specimens (50µl) were seeded on MacConkey agar, and EMB, chromogenic agar orientation plates, separately. They were incubated overnight at 37˚C in bacteriological incubators under aerobic conditions. The identification of *E. coli* was done depending on morphological features, and the pink color of the colonies on MacConkey agar plats, confirmed by metallic green sheen on EMB agar,will give Primary identification of *E. coli*. A pure culture on chromogenic agar plates were made from each single group of *E. coli* colonies. The pure cultures were prepared for biochemical tests IMVIC tests to confirm differentiation of *E. coli* from other lactose ferment Enterobacteriaceae. Bacterial colonies definitively identified depending on standard culture and biochemical characteristics of isolates by using Vitek-2 system (bioMerieux) according to the manufacturer's instructions.

2.9. Vitek 2 System :

vitek 2 System was used to confirm *E. coli* from a diagnostic group specific to the system, and this requires a diagnostic card specific to Gram-negative bacteria containing 64 slots and in each slot, a dried color-indicator. These indicators react to the sample given, and the System records these changes that were happening due to bacterial growth on the slots. According to the given changes in color, the System identifies the bacterial sample according to the guidance given by bioMerieux (Pincus, 2011).

2.10. Molecular Examination :

2.10.1.DNA isolation and Purification:

Bacterial isolates were used to extract chromosomal DNA. A single colony from each *E.coli* was cultured on solid medium and then inoculated into 5 ml of nutrient broth, the cultures were grown overnight at 37ºC. Genomic DNA was purified from the bacterial cells using a Genomic DNA Mini kit recommended by the manufacturer:

1- Cultured bacterial cells were transferred to 1.5 ml microcentrifuge tube, centrifuged for 1minute at 14-16,000 xg and the supernatant was discarded.

2- The lysis step was preceded for the cultured cell protocol.

3- GB Buffer was added (200 µl) to the sample and mix by shaking vigoroy for 5 minutes.

-The sample lysate was incubated at 37 ° C for at least 10 minutes. During incubation, the tube was inverted every 3 min. at this time, the required Elution buffer (200µl per sample) was pre heated to 70ºC (for step 5 DNA elution).

4- 5 µl of RNase a (10mg/ml) was added to the clear lysate and mixed by shaking vigorously, The lysate was incubated at room temperature for 5 minutes.

5- Absolute Ethanol (200µl) was added to the clear lysate and immediately mixed by shaking vigorously; the precipitate was broken up by pipetting.

6- GD Column was placed in a 2ml collection tube., all of the mixture was transferred (including any precipitate) to the GD column, centrifuged at 14-16,000 xg for 2 minutes,

7- The 2 ml collection tube was discarded containing the flow-through and the GD column was placed in a new 2 ml collection tube.

8- W1 buffer (400 µl) was added to the GD Column, Centrifuged at 14-16,000 xg for 30 seconds.

-The flow-through was discarded and placed the GD column back in the 2ml collection tube.

9- Wash buffer (600 µl, ethanol added) was added to the GD column, centrifuged at 14-16,000 xg for 30 seconds.

-The flow-through was discarded and placed the GD column back in the 2ml collection tube, Centrifuged again for 3 minutes at 14-16,000 Xg to dry the column matrix.

10-The dried GD column was transferred to a clean 1.5 ml centrifuge tube, - Preheated elution buffer or TE (100 µl) was added to the center of the matrix, centrifuged at 14-16,000 xg for 30 seconds to elute purified DNA.

11- reheated elution buffer or TE (100 µl) was added to the center of the matrix, centrifuged at 14-16,000 xg for 30 seconds to elute purified DNA.

2.10.2. Preparing the Primers :

The macrogen primers were prepared depending on manufacturer instruction for each one. The lyophilized primer dissolved with TE buffer to obtain 100 picomole per 1 µl as stock solution and the working primer tube was prepared by diluted with TE buffer to obtain final 10 picomoles per 1 µl depended on the procedure of each primer.

2.10.3.PCR Reaction Mixtures:

The DNA extract of E.coli isolates were subjected to different genes by PCR technique . The protocols used depending on manufacturer's instruction and final reaction volume (20 µl). All PCR components were

assembled in PCR tube and mixed on ice bag under sterile conditions as in table (2-7). Table (2-7): Contents of PCR reaction mixture.

***Table (2-7):* Contents of PCR reaction mixture**

PCR reaction mixture	Ammount/ µl
Forward Primer (10Pmole)	**1**
Reverse Primer (10Pmole)	**1**
Template DNA	**2**
Master Mix	**5**
Nuclase -Free Water	**11**
Final volume	**20µl**

Table (2-8):* PCR optimization of *E.coli

Gene Name	**Temperature (°C) / time Cycling condition**				
	Initial Denaturation/ time	Denaturation/time	Anneling/time	Extension	Final Extension
BLA-CTM	95 °C 5min	95 °C 30 Sec	52 °C / 45 Sec	72 °C / 1 min	72 °C / 7 min
BLA-OXA-48	95 °C 5min	95 °C 30 Sec	65 °C/ 45 Sec	72 °C / 1 min	72 °C / 7 min
CNF	95 °C 5min	95 °C 30 sec	66 °C/ 45 Sec	72 °C / 1 min	72 °C / 7 min
EAE	95 °C 5min	95 °C 30 Sec	55 °C/ 45 Sec	72 °C / 1 min	72 °C / 7 min
Cycle Number	1 x cycle	35 x cycles			1 x cycle

2.10.4.Preparation of Agarose Gel:

Agarose gel was prepared by adding agarose powder to 1X TBE buffer previously prepared in percent specific for each PCR products. The muddle was placed in boiling water bath until it become clear, allowed to cool to 50˚C, and 1.5 µl ethidium bromide at concentration of 0.5 mg/ml was added. The agarose poured kindly in equilibrated gel tray earlier set with its comb. The agarose allowed to solidify at room temperature for 30 minutes. The comb made wells used for loading DNA samples.

2.10.5.Agarose Gel Electrophoresis:

The amplified PCR products were detected by agarose gel electrophoresis and visualized by staining with ethidium bromide. PCR products were loaded to the agarose gel wells. 5µl from single product to single well in known sequence, followed by suitable ladder to one of the wells in the row. The gel tray was fixed in electrophoresis chamber. 1X TBE buffer was added to the chamber until covered the surface of the gel. The electric current was performed at 100 volt for 15 minute and then 70 volt for 30 minute.

2.10.6.Electrophoresis Results:

The electrophoresis results were identified using gel documentation system. The base pairs of DNA bands were measured according to the ladder. The positive results were distinguished when there was DNA band equal to the target product size. Finally, the gel was photographed using gel documentation saving picture.

2.11.Immunological assay of HBP:

Enzyme linked immonosorbent assay for detection of HBP, The study was used ELISA techniques for determine the concentration of HBP from

patient and control with UTI according to manufacture company (BT LAB / China (Cat.No E4528Hu), appendix 5:

Assay Procedure:

1- All reagents, standard solutions, and samples should be prepared as instructed. The reagents should be brought to room temperature before use. The assay is performed at room temperature.

2- The number of strips required for the assay should be determined. The strips should be inserted into the frames for use. The unused strips should be stored at 2-8°C.

3- The standard should be added to the standard well (50µl). Note: Biotinylated antibody should not be added to the standard well because the standard solution already contains biotinylated antibody.

4- The sample should be added to the sample wells (40µl), followed by the addition of anti-AZU antibody to the sample wells (10µl). Then, streptavidin-HRP should be added to both the sample wells and standard wells (50µl each, excluding the blank control well). The mixture should be well-mixed. The plate should be covered with a sealer and incubated for 60 minutes at 37°C.

5- The sealer should be removed, and the plate should be washed 5 times with wash buffer. Each well should be soaked with 300µl wash buffer for 30 seconds to 1 minute for each wash. For automated washing, each well should be aspirated or decanted, and the plate should be washed 5 times with wash buffer. The plate should be blotted onto paper towels or other absorbent material.

6-Substrate solution A (50µl) should be added to each well, followed by the addition of substrate solution B (50µl) to each well. The plate should be incubated, covered with a new sealer, for 10 minutes at 37°C in the dark.

7- Stop Solution (50µl) should be added to each well, resulting in an immediate color change from blue to yellow.

8- The optical density (OD value) of each well should be determined immediately using a microplate reader set to 450 nm within 10 minutes after the stop solution is added.

Stastical analysis:

Statistical Package for the Social Sciences(SPSS) version 25.0 was used for analysis the data for current study.The findings were evaluated by using chi-square and LSD analysis. In addition, the probability P-value was estimated, in which values equal to or less than 0.05 were indicated as significant differences.

Chapter Three

Result

3. Results

3.1 Descriptive data for patients and control

Table (3-1) displays the descriptive data for study population (patients and control). Both patients and control were divided into four age groups including (20-30 y, 31-41 y, 42-52 y, 53-63 y). The results of statistical analysis revealed non-significant differences ($p>0.05$) among age groups in both study population; in the same context, age showed a non significant result ($p> 0.05$) in patients (36.11 ± 10.288) compared with control (37.16 ± 11.016). Patients and healthy control were also divided according to their residency into two groups (urban and rural), patients showed an increase percentage of the patients in urban areas was higher than in rural areas; while control percentage increase in rural areas. However, the results of the statistical analysis did not show any significant differences. Furthermore, non-significant ($P>0.05$) differences were found in study groups according to gender despite increased number of female patients (54.5%) compared with male (45.5%). Time collection for patients' samples also was recorded, so patients were dividing into three groups that including 1st group were collected from 8.30-10, 2nd group from 10.30- 11.30, and 3rd group was collected from 12.30, the highest percentage of patients (64.6%) was in the 1st group as displays in figure (3-1). Patients also were divided according to the hospitalization type, 64% of them were in community while 36% in hospital as shows in figure (3-2).

Table (3-1): **Descriptive data for patients and control**

Study population	**Age group (year)**					
	20-30 y	**31-41 y**	**42-52 y**	**53-63 y**	**Total**	***P value* (P ≤ 0.05)**
Patient	19(34.5%)	21 (38.2%)	8 (14.5%)	7 (12.7%)	55	0.882^{NS}
Control	17(30.9%)	21(38.2%)	11 (20.0%)	6 (10.9%)	55	0.882^{NS}
Total	36(32.7%)	42 (38.2%)	19 (17.3%)	13 (11.8%)	110 (100%)	0.772^{NS}
Study population	**Age (Year) Mean ± Std. Deviation**				**T- test**	***P value* (P ≤ 0.05)**
Patient	36.11 ± 10.288				0.388	0.146 ^{NS}
Control	37.16 ± 11.016					
Residency						
Study populatio N	**Urban**	**Rural**	**Total**		***P value* (P ≤ 0.05)**	**ODD (CI95%)**
Patient	35 (63.6%)	20 (36.4%)	55		0.056^{NS}	2.100 (0.978-4.508)
Control	25 (45.5%)	30 (54.5%)	55		0.085^{NS}	
Total	60 (54.5%)	50 (45.5%)	110 (100%)		0.055^{NS}	
Gender						
Study populatio N	**Male**	**Female**	**Total**		***P value* (P ≤ 0.05)**	**ODD (CI95%)**
Patient	25 (45.5%)	30 (54.5%)	55		.446^{NS}	0.747 (.353-1.581)
Control	29 (52.7%)	26 (47.3%)	55		.567^{NS}	
Total	54 (49.1%)	56 (50.9%)	110 (100%)		.445^{NS}	
*Significant difference at the 0.05 level by chi-square test and T-test. NS: Non-significant difference						

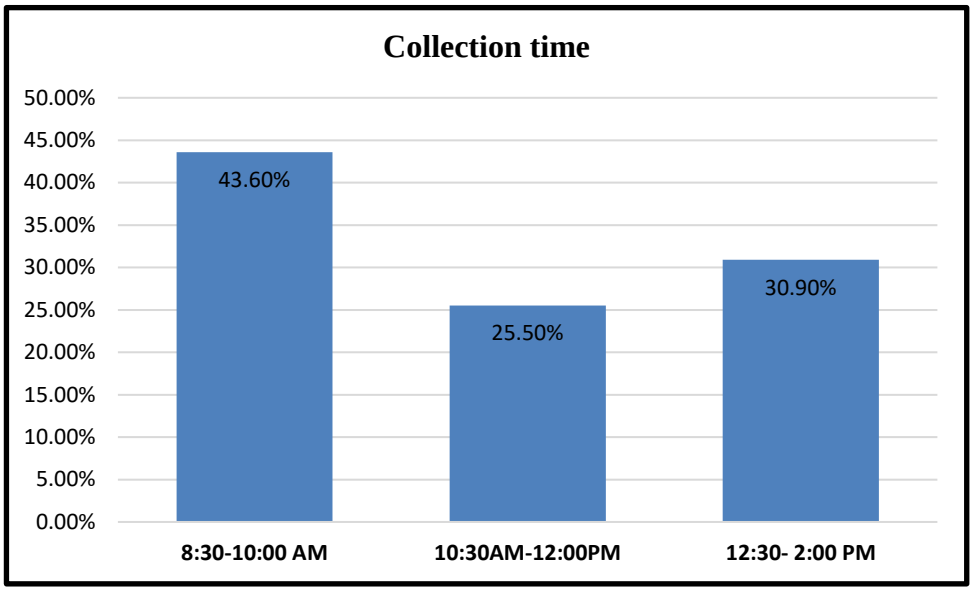


Figure (3-1): Time collection for patients' sample

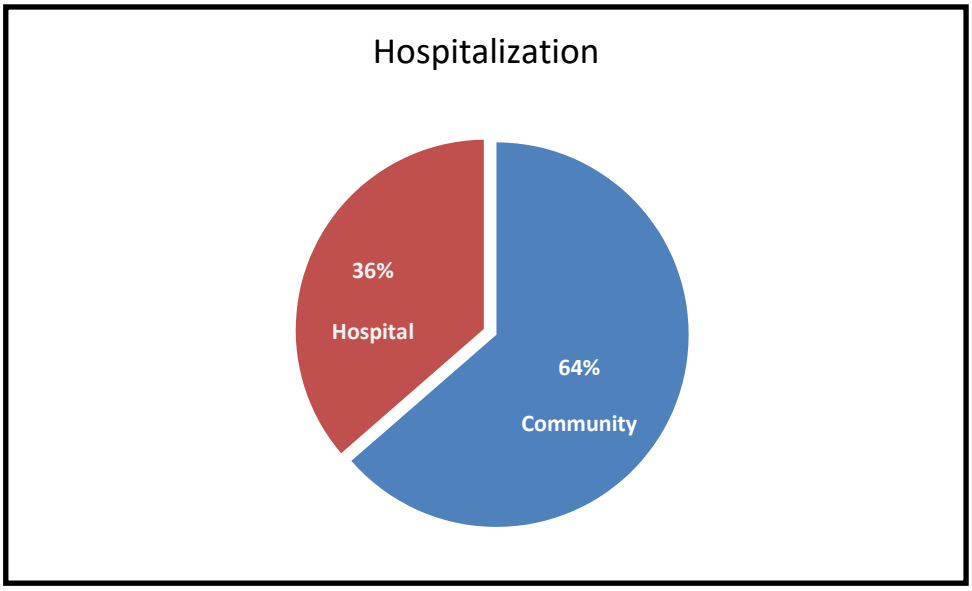


Figure (3-2): Distribution of patients according to the hospitalization type

3.2 Evaluation of laboratory parameters in patients according to diabetes disease

Table (3-2) shows the laboratory parameters in patients according to diabetic diseases, the results of statistical analysis showed that there were no significant differences ($p>0.05$) in the distribution of laboratory parametersin patients with and without diabetic disease.

Table (3-2): **Evaluation of laboratory parameters in patients according to diabetic disease**

Variables	Mean ± Std. Deviation			*P value*(P ≤ 0.05)
	With Diabetic	**Without Diabetic**	**Total**	
PH	6.38 ± .39	6.41 ±0.47	6.402 ± 0.44	0.779^{NS}
(S.G)	1.012 ± .005	1.01 ± 0.005	1.0124 ± 0.005	0.275^{NS}
Leucocyte	96.75 ± 16.33	91.26 ± 15.76	94.85 ± 16.22	0.236^{NS}
RBCS	50.61 ± 12.78	46.42 ± 12.05	49.16 ± 12.58	0.244^{NS}
NS: Non-significant difference under p ≤ 0.05 by T-test analysis.				

3.3 Bacterial culture from patients

Figure (3-3) shows the results of bacterial culture, the majority 57.90% of samples culture having a bacterial growth, while 42.10% only presented with no growth . The percentage of bacterial isolates from patients are displayed in Figure (3-4); the highest percentage of bacteria isolated from patients was *Escherichia coli*, at 70.90%, while the lowest percentage of bacteria isolated from patients was *Acinetobacter baumannii* and *Proteus mirabilis* at 3.60%, (appendix 6).

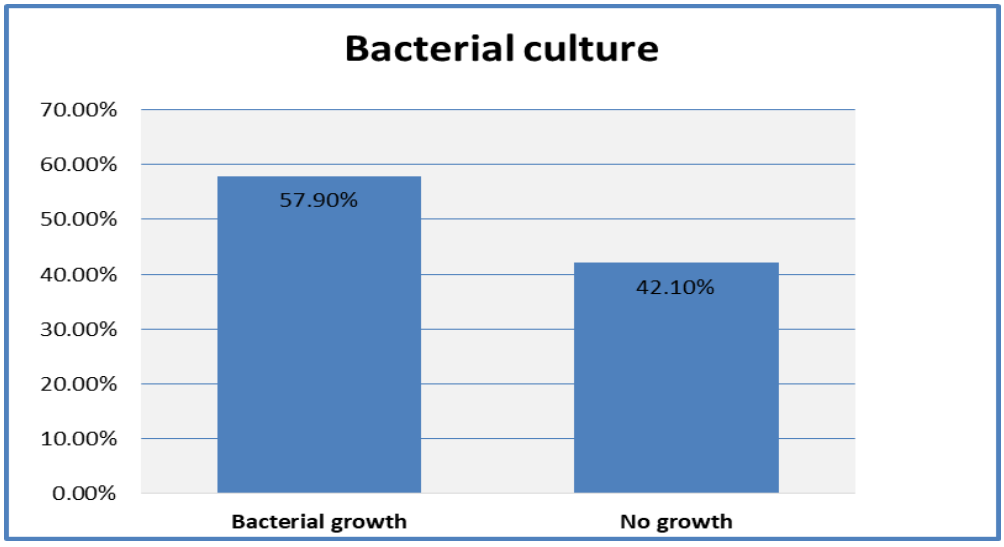


Figure (3-3): Type of bacterial isolate from patients

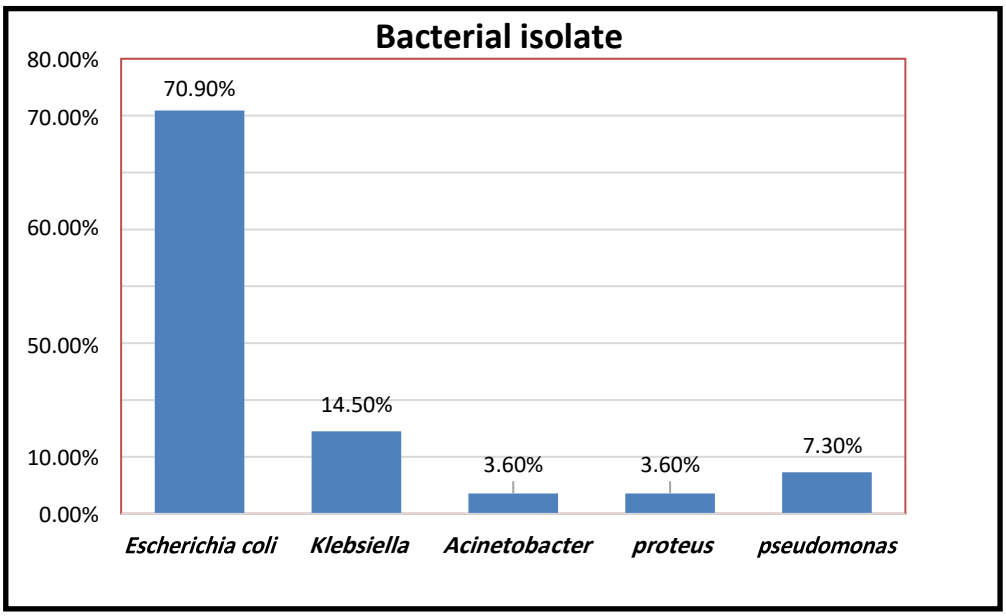


Figure (3-4): Percentage of bacterial culture from patients

Table (3-3): **Evaluation of Heparin binding protein in study population byELISA**

Study Population	HBP concentration (ng/ml) Mean ± Std. Deviation	P value ($p \leq 0.05$)
Patient	22.04 ± 0.96*	0.000*
Control	7.78 ± 0.59	
Total	14.91 ±0.89	
Highly Significant difference under $p \leq 0.05$ by T-test analysis.		

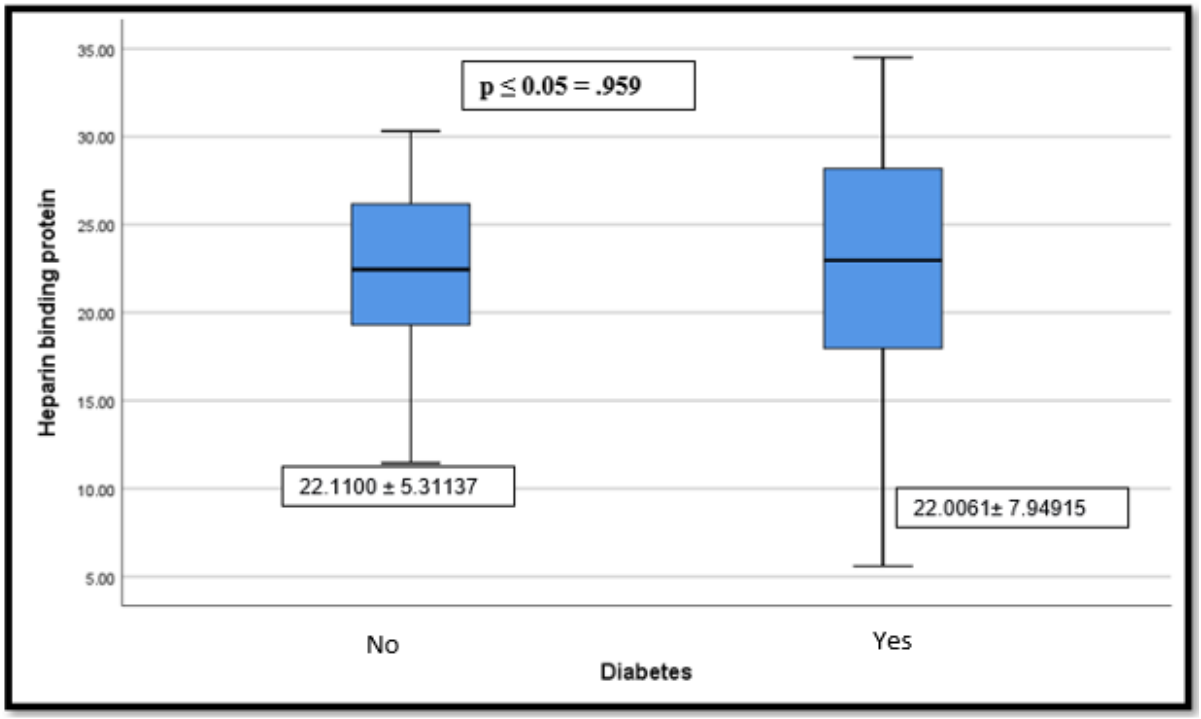


Figure (3-5): Boxplot for HBP concentration in patients with and without of Diabetes disease.

Table (3-4): **Evaluation of Heparin binding protein in patients according to bacterial isolate**

Bacterial isolate	NO.	HBP concentration (ng/ml) Mean ± Std. Deviation	*P value (p ≤ 0.05)*
Escherichia coli	39	[A] 38.88± 1.08*	0.000*
Klebsiella pneumonia	8	[B] 24.62 ± 6.48	
Acinetobacter baumannii	2	[C] 16.95± 3.99	
proteus mirabilis	2	[C]11.91 ± 1.46	
pseudomonas aeruginosa	4	[C] 17.84 ± 1.72	
Total	55	22.04 ± 7.1	
LSD	-	6.83	
Highly Significant difference under p ≤ 0.05 by One way – ANOVA			

Table (3-5): **Evaluation of Heparin binding protein in study populationaccording to gender**

Gender	HBP concentration (ng/ml) Mean ± Std. Deviation	
	Patient	**Control**
Male	19.95 ± 7.57	7.72 ± 0.92
Female	23.79 ± 6.29*	7.83 ± 0.70
Total	22.04 ± 7.1	7.77 ± 0.58
P value (p ≤ 0.05)	0.045 *	0.924 NS

***Significant difference under p ≤ 0.05 by T – test.**

NS: Non- significant difference.

3.4 Correlation analysis between Heparin binding protein and laboratory parameters

The current study included conducting a correlation between HBP andlaboratory parameters, which included: PH, S.G., leucocyte, and RBCs; in patients cases the results of the statistical analysis detected a highly significant (p≤0.01) positive correlation between heparin and all of S.G., leucocyte, and RBCs. On the other hand, the results of the statistical analysis did not show a significant correlation between heparin and any of laboratory parameters in healthy control, as shown in Table (3-6).

Table (3-6): **Correlation between Heparin binding protein and laboratory parameters**

Study population			PH	S.G	leucocyte	RBCs
Patients	arin bindingprotein	Pearson Correlation	-0.196-	0.767**	0.767**	0.774**
		Sig. (2-tailed)	0.151	0.000	0.000	0.000
		N	55	55	55	55
Control	arin bindingprotein	Pearson Correlation	0.008	-0.158-	.b	.b
		Sig. (2-tailed)	0.955	0.249	.	.
		N	55	55	0	0
**. Correlation is significant at the 0.01 level (2-tailed).						
b. Cannot be computed because at least one of the variables is constant.						

3.5Receiver Operative Characteristic Curve Analysis

The Receiver Operative Characteristic Curve (ROC) analysis yielded a cut off value of HBP for prediction of disease activity. The overall AUC, sensitivity, and specificity for HBP were as follows: 0.936, 0.091, and 0.909,respectively, as displayed in Table (3-7). Figure (3-7) illustrates the sensitivity and specificity values for HBP.

Table (3-7): **Receiver Operative Characteristic Curve-based analysis of HBP IN patients**

HBP	AUC	Sensitivity	Specificity	Cut-off	*P-value*
	0.936	0.91	0.909	10.8016	**<0.001**

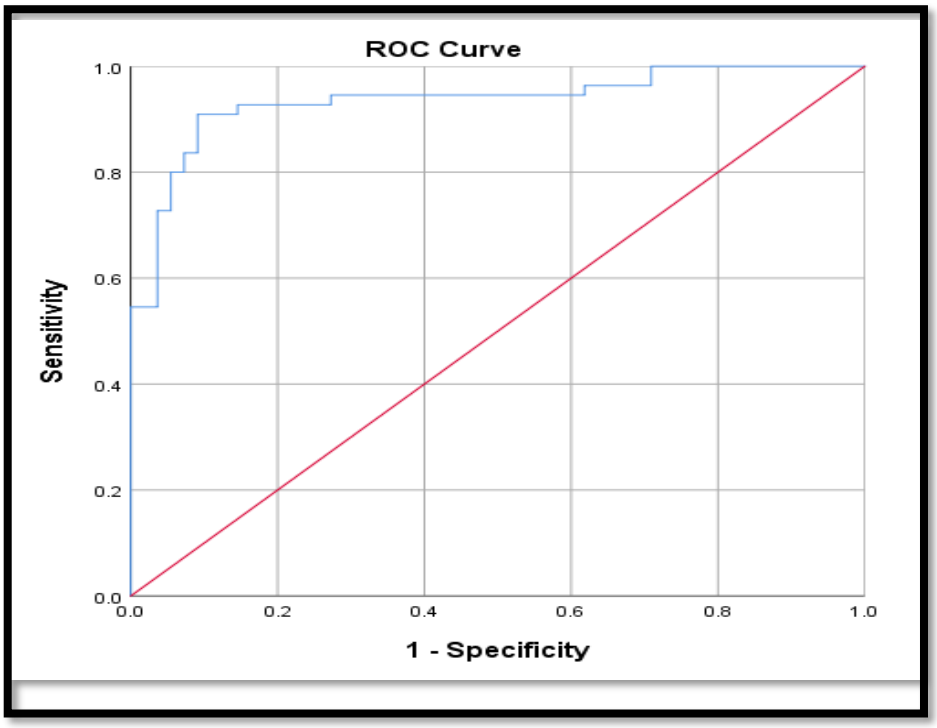


Figure (3-6): ROC curve for prediction of patients by HBP

3.6 Estimation the prevalence of ESBL bacterial isolate according to gender, residency and diabetes disease.

Figure (3-7) displays the distribution of ESBL *E. coli* according to gender, residency and diabetes. As the figure shows it was isolated at a higher percentage from females 53.8% than from males 46.2% and from urban areas 64.1% compared to rural areas 35.9%, and it was isolated at a higher percentage from patients with diabetes 69.2% compared without patients with diabetes.

The current study also showed in Table (3-8) the distribution according to gender, residency and diabetes of all ESBL bacterial isolates, including *E. coli* for comparison. In regard to ***E. coli***, it was isolated at a higher percentage from females 53.8% than from males 46.2% and from urban areas 64.1% compared to rural areas 35.9%, and it was isolated at a higher percentage from patients with diabetes 69.2% compared with patients without diabetes. ***Klebsiella pneumonia*** was isolated from female

with percent 87% higher than in male 12.5%; it was isolated from patients resident in urban 75% higher than in rural 25%; it was also isolated from non-diabetic patients at a higher rate 62.5% than from those with diabetes 37.5%. Regarding to ***Acinetobacter baumannii,*** 100% of this bacteria isolated from male; while it was isolated in equal proportions from patients living in urban and rural areas and from patients with and without diabetes. ***Proteus mirabilis*** did not show any isolation bias according to gender, residence, or whether or not diabetes was present. Finally, ***Pseudomonas aeruginosa*** isolated with high percentage 75% from male compared with female 25%, while it was isolated in equal proportions from patients livingin urban and rural areas and from patients with and without diabetes. It is worth noting that although the distribution rates for bacterial isolates were differ according to gender, residence, and diabetes, but the results of the statistical analysis did not show any significant difference in these distribution.

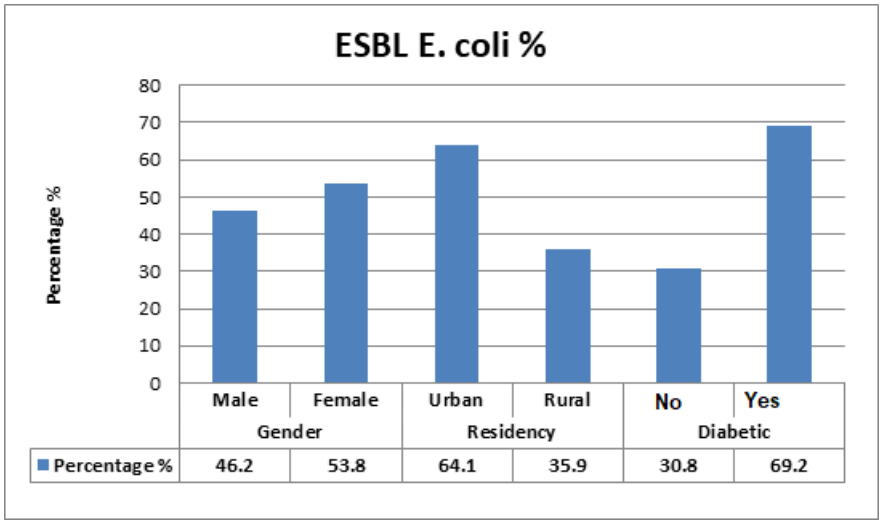


Figure (3-7): Estimation the frequencies of UPEC bacterial isolate according to gender, residency and diabetes disease

Table (3-8): **Distribution of ESBL bacterial isolate according to gender,residency and diabetes disease**

ESBL		Gender		Residency		Diabetic	
		Male	**Female**	**Urban**	**Rural**	**yes**	No
Escherichia coli	Count	18	21	25	14	27	**12**
	%	46.2	53.8	64.1	35.9	69.2	**30.8**
Klebsiella pneumonia	Count	1	7	6	2	3	**5**
	%	12.5%	87.5%	75.0%	25.0%	37.5 %	**62.5 %**
Acinetobacterbaumannii	Count	2	0	1	1	1	**1**
	%	100.%	0.0%	50.0%	50.0%	50%	**50%**
Proteus mirabilis	Count	1	1	1	1	1	**1**
	%	50.0%	50.0%	50.0%	50.0%	50%	**50%**
Pseudomonas Aeruginosa	Count	3	1	2	2	2	**2**
	%	75.0%	25.0%	50.0%	50.0%	50%	**50%**
Total	Count	25	30	35	20	4	**4**
	%	45.5%	54.5%	63.6%	36.4%	50%	**50%**
P value ($p \leq 0.05$)		0.303^{NS}		0.536^{NS}		**0.312^{NS}**	
NS: Non- Significant difference at the 0.05 level by chi-square test							

3.7 Distribution of uropathogens bacterial isolate according to age groups:

The current study also included the distribution of bacterial isolates according to the ages of the patients from whom these isolates were isolated. The patients were divided according to their ages into four age groups (20-30 y, 31-41 y, 42-52 y, 53-63 y) as illustrated in Table (3-9). In regard to ***E. coli***, the highest percentage 43.6% of them was isolated from

(31-41 y) age group compared to other groups. ***Klebsiella pneumonia*** the highest percentage 62.5% of them was isolated from (20-30 y) age group compared to other groups. Regarding to ***Acinetobacter baumannii,*** it was isolated in equal proportions 50% from age groups (20-30 y and 31-41 y), while it was not isolated from patients within the age groups (42-52 y, 53-63 y). ***Proteus mirabilis*** it was isolated in equal proportions 50% from age groups (31- 41 y, 42-52 y), while it was not isolated from patients within the age groups (20-30 y, 53-63 y). Finally, ***Pseudomonas aeruginosa*** it was isolated in equal proportions 50% from age groups from age groups (42-52 y, 53-63 y), while it was not isolated from patients within the age groups (20-30 y, 31-41 y) . The results of the statistical analysis showed significant differences in bacterial isolation according to age, as the 42-52 y age group showed a significant increase in the percentage of bacteria isolated from patients within this age group compared to isolates of other bacteria, as shown in Table (3-9).

Table (3.9): **Distribution of uropathogens bacterial isolate according to agegroups**

Types of isolates		Age group				Total
		20-30 y	31-41 Y	42-52 y	53-63 y	
Escherichia coli	Count	13	17	4	5	39
	%	33.3%	43.6%	10.3%	12.8%	100.0%
Klebsiella pneumonia	Count	5	2	1	0	8
	%	62.5%	25.0%	12.5%	0.0%	100.0%
cinetobacterbaumannii	Count	1	1	0	0	2
	%	50.0%	50.0%	0.0%	0.0%	100.0%
Proteus mirabilis	Count	0	1	1	0	2
	%	0.0%	50.0%	50.0%	0.0%	100.0%
Pseudomonas	Count	0	0	2	2	4

aeruginosa	%	0.0%	0.0%	50.0%	50.0%	100.0%
Total	Count	19	21	8	7	55
	%	34.5%	38.2%	14.5%	12.7%	100.0%
P value (p ≤ 0.05)		0.108^{NS}	0.077	0.030*	--	
NS:Non- Significant difference at the 0.05 level by chi-square test						

3.8 Phenotypic and genotypic characterization of ESBL UPEC isolates.

A various levels of susceptibility were detected in UPEC isolates towards (12) antibiotics belonged to different classes by using VITEK system. The results revealed that antibiotic susceptibility test were affected at High rates of sensitivity were seen with cefepime (78.8%), Gentamicin (78.6%), Amikacin (76.2%), pipera\Tazo and Nittrofurantoin (73.5%) and Amox/Clavu (72.2%); while High rates of resistance were seen with Ciprofloxacin (79.5%), Norfloxacin (76.9%), Trimeth\sulfamthoxazole (76.2%), Ceftazidime (73.8%), and Ampicillin (70.9%); in addition, 100% of E. coli isolates have intermediate sensitivity to Amox/Clavu, cefotaxime, Gentamicin Norfloxacin, and Trimeth\sulfamthoxazole as well as 70% intermediate sensitivity to Ceftazidime, as showed in Table (3-10) and Figure (3.8). appendix(7).

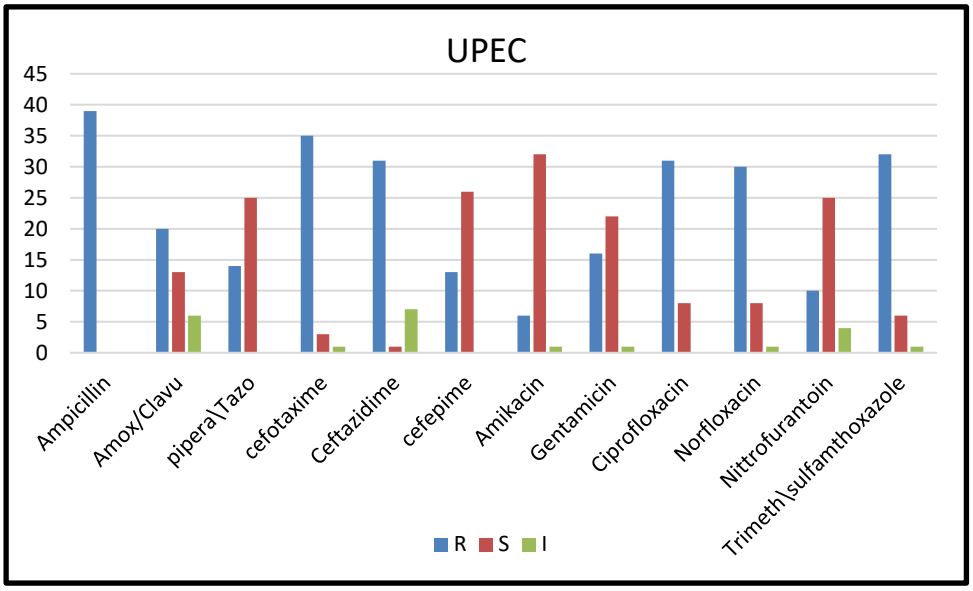


Figure (3.8): Type of antibiotic resistance for UPEC of study isolate

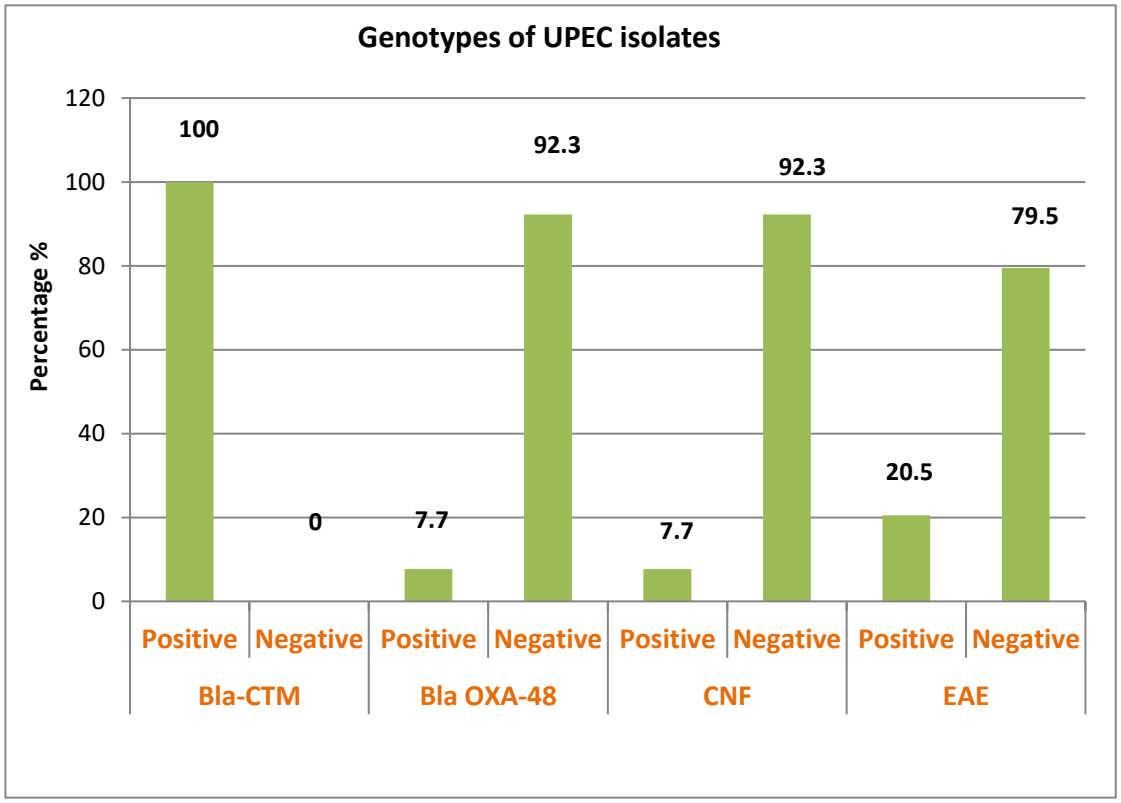


Figure (3-9): Virulence gene expression in *E. coli* isolate

Figure (3.10): Agarose gel electrophoresis of BLA- OXA 48 gene: the PCR product at band size438bp. (Lane M:100 bp DNA marker, Lane 1-3 represent BLA- OXA 48 gene of ESBLs isolates visualized under ultraviolet light after ethidium bromidestaining.

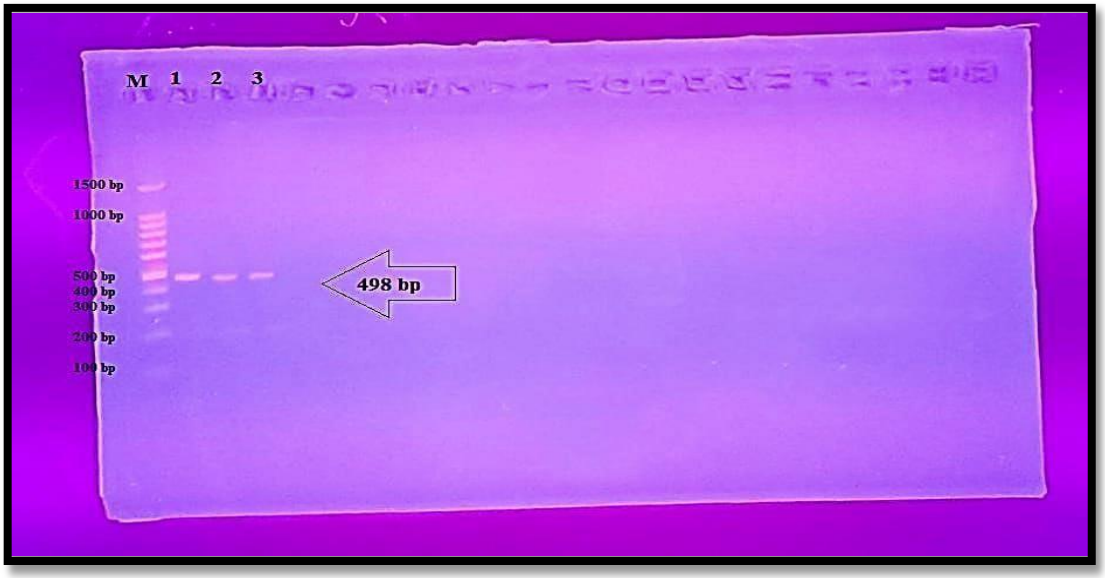


Figure (3.11): Agarose gel electrophoresis of *CNF* gene the PCR product at band size 498 bp. (LaneM:100 bp DNA marker, Lane 1-3 represent *CNF* gene of ESBLs isolates visualized under ultraviolet light after ethidium bromide staining.

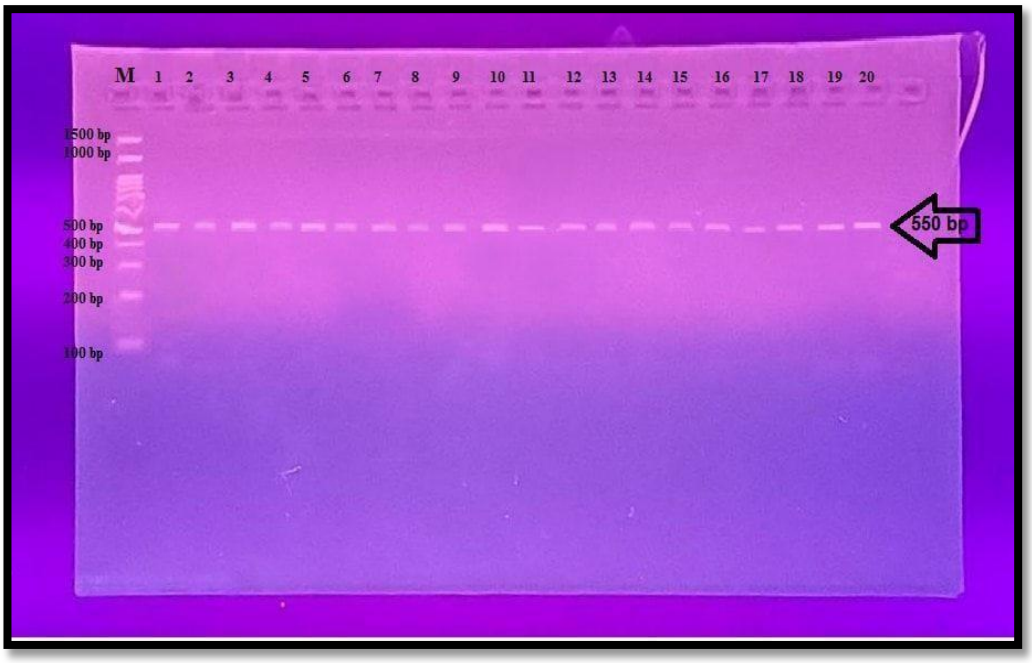


Figure (3.12): Agarose gel electrophoresis of *BLA-CTM* gene the PCR product at band size 550 bp.(Lane M:100 bp DNA marker, Lane 1-20 represent *BLA-CTM* gene of ESBLs isolatesvisualized under ultraviolet light after ethidium bromide staining.

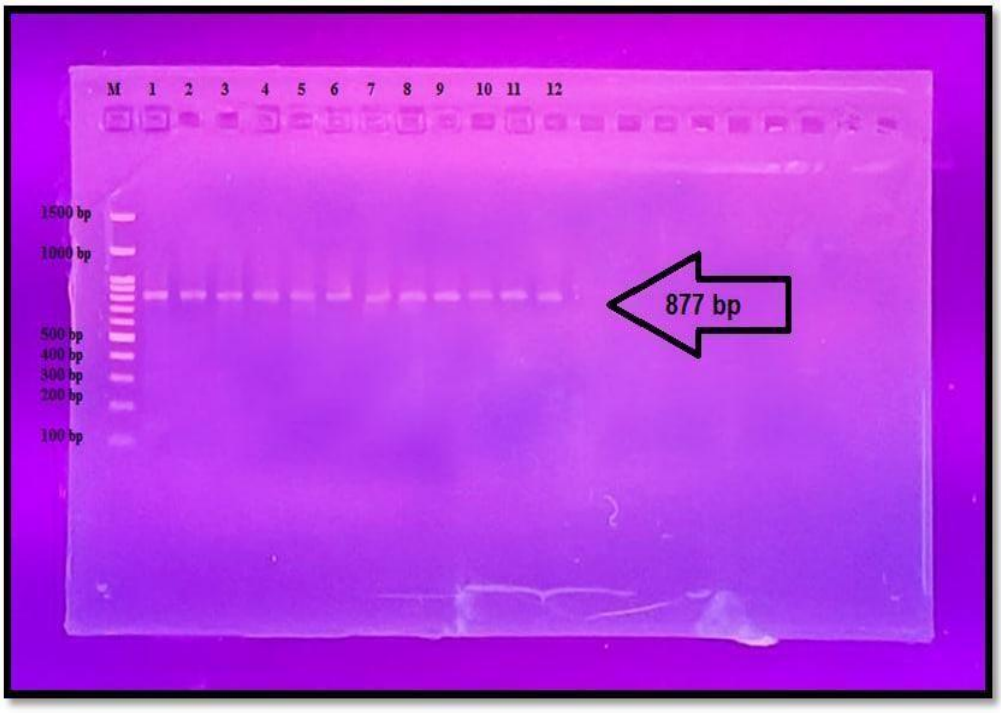


Figure (3.13): Agarose gel electrophoresis of *EAE* gene the PCR product at band size 877 bp. (Lane M:100 bp DNA marker, Lane 1-12 represent *EAE* gene of ESBLs isolates visualized underultraviolet light after ethidium bromide staining

***Table (3-11):* Antibiotic susceptibility and MIC to bacterial isolate**

Types of isolates	Antibiotic	R/I/S	No.	%
Escherichia coli	Ampicillin	R	39	70.9%
	Amox/Clavu	R	20	64.5%
		S	13	72.2%
		I	6	100%
	pipera\Tazo	R	14	66.7%
		S	25	73.5%
	Cefotaxime	R	35	71.4%
		S	3	60.0%
		I	1	100%
	Ceftazidime	R	31	73.8%
		S	1	33.3%
		I	7	70.0%
	Cefepime	R	13	59.1%
		S	26	78.8%
	Amikacin	R	6	54.5%
		S	32	76.2%
		I	1	50.0%
	Gentamicin	R	16	61.5%
		S	22	78.6%
		I	1	100%
	Ciprofloxacin	R	31	79.5%
		S	8	50.0%
	Norfloxacin	R	30	76.9%
		S	8	53.3%
		I	1	%100
	Nittrofurantoin	R	10	71.4%
		S	25	73.5%
		I	4	57.1%
	eth\sulfamthoxazole	R	32	76.2%
		S	6	50.0%
		I	1	100%
Klebsiellapneumonia	Ampicillin	R	8	14.5%
	Amox/Clavu	R	7	22.6%
		S	1	5.6%

	pipera\Tazo	R	5	23.8%
		S	3	8.8%
	Cefotaxime	R	7	14.3%
		S	1	20.0%
	Ceftazidime	R	6	14.3%
		S	1	33.3%
		I	1	10.0%
	Cefepime	R	6	27.3%
		S	2	6.1%
	Amikacin	R	3	27.3%
		S	4	9.5%
		I	1	50.0%
	Gentamicin	R	5	19.2%
		S	3	10.7%
	Ciprofloxacin	R	5	12.8%
		S	3	18.8%
	Norfloxacin	R	5	12.8%
		S	3	20.0%
	Nittrofurantoin	R	3	21.4%
		S	3	8.8%
		I	2	28.6%
	neth\sulfamthoxazole	R	5	11.9%
		S	3	25.0%
cinetobacterbaumannii	Ampicillin	R	2	3.6%
	Amox/Clavu	R	1	3.2%
		S	1	5.6%
		S	2	5.9%
	Cefotaxime	R	1	2.0%
		S	1	2.0%
	Ceftazidime	R	1	2.4%
		S	1	33.3%
	Cefepime	S	2	6.1%
	Amikacin	S	2	4.8%
	Norfloxacin	R	2	5.1%
	Gentamicin	R	1	3.8%
		S	1	3.6%
	Ciprofloxacin	R	1	2.6%
		S	1	6.3%

	Nittrofurantoin	S	1	2.9%
		I	1	14.3%
	neth\sulfamthoxazole	R	2	4.8%
proteus mirabilis	Ampicillin	R	2	3.6%
	Amox/Clavu	R	1	3.2%
		S	1	5.6%
	pipera\Tazo	R	1	4.8%
		S	1	2.9%
	Cefotaxime	R	2	4.1%
	Ceftazidime	R	1	2.4%
		I	1	10.0%
	Cefepime	R	1	4.5%
		S	1	3.0%
	Amikacin	R	1	9.1%
		S	1	2.4%
	Gentamicin	R	1	3.8%
		S	1	3.6%
	Ciprofloxacin	R	1	2.6%
		S	1	6.3%
	Norfloxacin	R	1	2.6%
		S	1	6.7%
	Nittrofurantoin	S	2	5.9%
	neth\sulfamthoxazole	R	2	4.8%
pseudomonas aeruginosa	Ampicillin	R	4	7.3%
	Amox/Clavu	R	2	6.5%
		S	2	11.1%
	pipera\Tazo	R	1	4.8%
		S	3	8.8%
	Cefotaxime	R	4	8.2%
	Ceftazidime	R	3	7.1%
		I	1	10.0%
	Cefepime	R	2	9.1%
		S	2	6.1%
	Amikacin	R	1	9.1%
		S	3	7.1%
	Gentamicin	R	3	11.5%
		S	1	3.6%

	Ciprofloxacin	R	1	2.6%
		S	3	18.8%
	Norfloxacin	R	1	2.6%
		S	3	18.8%
	Nittrofurantoin	R	1	7.1%
		S	3	8.8%
	neth\sulfamthoxazole	R	1	2.4%
		S	3	25.0%

Table (3.12***):*** **Virulence gene expression with bacterial isolate**

Bacterial isolate		**Virulence gene**							
		Bla-CTM		**Bla OXA-48**		**CNF**		**EAE**	
		+	-	+	-	+	-	+	-
Escherichia coli	Count	39	0	3	36	3	36	8	31
	%	100.0 %	0.0 %	7.7 %	92.3%	7.7 %	92.3%	20.5%	79.5%
Klebsiella pneumonia	Count	8	0	0	8	0	8	1	7
	%	100.0 %	0.0 %	0.0 %	100.0 %	0.0 %	100.0 %	12.5%	87.5%
Acinetobacter baumannii	Count	2	0	0	2	0	2	0	2
	%	100.0%	0.0 %	0.0 %	100.0 %	0.0 %	100.0 %	0.0%	100.0%
Proteus mirabilis	Count	2	0	0	2	0	2	1	1
	%	100.0%	0.0%	0.0%	100.0%	0.0%	100.0%	50.0%	50.0%
Pseudomonas	Count	4	0	0	4	0	4	2	2
Aeruginosa	%	100.0%	0.0%	0.0%	100.0%	0.0%	100.0%	50.0%	50.0%
P value ($p \leq 0.05$)	***	0.356^{NS}	0.356 NS	0.210 NS					
*** No statistics are computed because Bla-CTM is a constant NS: Non- Significant difference at the 0.05 level by chi-square test									

3.9 Effect of antibiotic MIC on bacterial isolate

Effect of antibiotic MIC on bacterial isolate was displayed in Table (3.13). The results of statistical analysis revealed only in Trimeth\sulfamthoxazole (MIC) antibiotic, where a significant increase in *E. coli*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Proteus mirabilis* compared with *Pseudomonas aeruginosa.*

Table (3.13***):* Effect of antibiotic MIC on bacterial isolate**

Antibiotic	Bacterial isolate	N	MIC (µg/mL)		P value (p ≤ 0.05)
			Mean	Std. Deviation	
Ampicillin (MIC)	*Escherichia coli*	39	29.9231	6.29279	0.439^{NS}
	Klebsiella pneumonia	8	32.0000	.00000	
	Acinetobacter baumannii	2	24.0000	11.31371	
	proteus mirabilis	2	32.0000	.00000	

	Pseudomonas Aeruginosa	4	32.0000	.00000	
	Total	55	30.2364	5.70563	
Amox/Clavu (MIC)	*Escherichia coli*	39	20.2821	2.05293	0.571^{NS}
	Klebsiella pneumonia	8	28.5000	9.89949	
	Acinetobacter Baumannii	2	20.0000	2.00000	
	proteus mirabilis	2	20.0000	2.00000	
	Pseudomonas Aeruginosa	4	19.0000	7.54983	
	Total	55	21.3636	1.70903	
pipera\Tazo (MIC)	*Escherichia coli*	39	46.5897	9.05044	0.405^{NS}
	Klebsiella pneumonia	8	82.0000	22.44994	
	Acinetobacter Baumannii	2	4.0000	.00000	
	proteus mirabilis	2	66.0000	2.00000	
	Pseudomonas Aeruginosa	4	66.0000	5.79572	
	Total	55	52.3091	7.93067	

cefotaxime (MIC)	*Escherichia coli*	39	58.6154	14.70699	0.193^{NS}
	Klebsiella pneumonia	8	52.1250	8.30756	
	Acinetobacter Baumannii	2	32.5000	1.50000	
	proteus mirabilis	2	64.0000	.00000	
	Pseudomonas Aeruginosa	4	64.0000	.00000	
	Total	55	57.3091	17.13224	
Ceftazidime (MIC)	*Escherichia coli*	39	41.7949	20.95130	0.454^{NS}
	Klebsiella pneumonia	8	46.1250	9.20100	
	Acinetobacter Baumannii	2	16.5000	5.50000	
	proteus mirabilis	2	40.0000	24.00000	
	Pseudomonas Aeruginosa	4	52.0000	24.00000	
	Total	55	42.1818	2.20535	
cefepime (MIC)	*Escherichia coli*	39	21.1795	4.48643	0.215^{NS}
	Klebsiella pneumonia	8	44.2500	10.21335	

	Acinetobacter Baumannii	2	1.0000	.00000	
	proteus mirabilis	2	33.0000	3.84062	
	Pseudomonas Aeruginosa	4	26.5000	8.16026	
	Total	55	24.6182	3.88327	
Amikacin (MIC)	*Escherichia coli*	39	10.5641	3.13846	0.144^{NS}
	Klebsiella pneumonia	8	30.5000	10.35616	
	Acinetobacter Baumannii	2	1.5000	.70711	
	proteus mirabilis	2	32.5000	4.54773	
	Pseudomonas Aeruginosa	4	17.2500	1.17023	
	Total	55	14.4182	3.24243	
Gentamicin (MIC)	*Escherichia coli*	39	11.4615	3.64367	0.904^{NS}
	Klebsiella pneumonia	8	14.5000	5.35299	
	Acinetobacter Baumannii	2	16.5000	1.92031	
	proteus mirabilis	2	16.5000	1.92031	

	pseudomonas aeruginosa	4	17.0000	7.32051	
	Total	55	12.6727	4.14352	
Ciprofloxacin (MIC)	*Escherichia coli*	39	3.1731	1.50236	0.361^{NS}
	Klebsiella pneumonia	8	2.6563	.65625	
	Acinetobacter Baumannii	2	2.2500	1.75000	
	proteus mirabilis	2	2.2500	1.75000	
	pseudomonas aeruginosa	4	1.6250	.80039	
	Total	55	2.9182	.21818	
Norfloxacin (MIC)	*Escherichia coli*	39	14.6154	1.60133	0.662^{NS}
	Klebsiella pneumonia	8	13.7500	4.47912	
	Acinetobacter Baumannii	2	24.0000	11.31371	
	proteus mirabilis	2	17.0000	1.21320	
	pseudomonas aeruginosa	4	15.0000	7.50000	
	Total	55	14.5455	1.47649	

fosfomycin (MIC)	*Escherichia coli*	39	55.7179	13.69028	0.596^{NS}
	Klebsiella pneumonia	8	101.2500	38.36095	
	Acinetobacter Baumannii	2	16.0000	.00000	
	proteus mirabilis	2	16.0000	.00000	
	pseudomonas aeruginosa	4	72.5000	2.51122	
	Total	55	60.6727	12.00264	
Nittrofurantoin (MIC)	*Escherichia coli*	39	122.5897	30.42035	0.847^{NS}
	Klebsiella pneumonia	8	170.0000	75.58911	
	Acinetobacter Baumannii	2	40.0000	3.94113	
	proteus mirabilis	2	24.0000	11.31371	
	pseudomonas aeruginosa	4	137.0000	50.06399	
	Total	55	123.9455	25.50551	
Trimeth\sulfamthoxazole (MIC)	*Escherichia coli*	39	267.6154*	18.19417	0.05^{*}
	Klebsiella pneumonia	8	207.5000*	54.89438	

	Acinetobacter Baumannii	2	320.0000*	.00000	
	Proteus mirabilis	2	320.0000*	.00000	
	Pseudomonas Aeruginosa	4	95.0000	5.00000	
	Total	55	250.1273	17.10617	
*Significant difference at the 0.05 level by ANOVA test NS: Non-significant difference					

3.10. Evidence of MDR, XDR and PDR of ESBL of study isolate

As shown in Figure (3.10), among UPEC *E.coli* isolates there are 37 (94.9%), 2 (5.1%), and 5 (12.8%) were MDR, XDR, and PDR respectively.

On the other hand, Table (3.13) illustrates the presentation of other ESBL and type of resistance. The current investigation showed that eight of *Klebsiella pneumonia* (all cases of *Klebsiella pneumonia*) isolates were MDR. Two of *Acinetobacter baumannii* (all cases of *Acinetobacter baumannii*) isolates were MDR. All *Proteus mirabilis* isolates were MDR. All *Pseudomonas aeruginosa* isolates (100%), and 1 (25 %) were MDR, andPDR respectively.

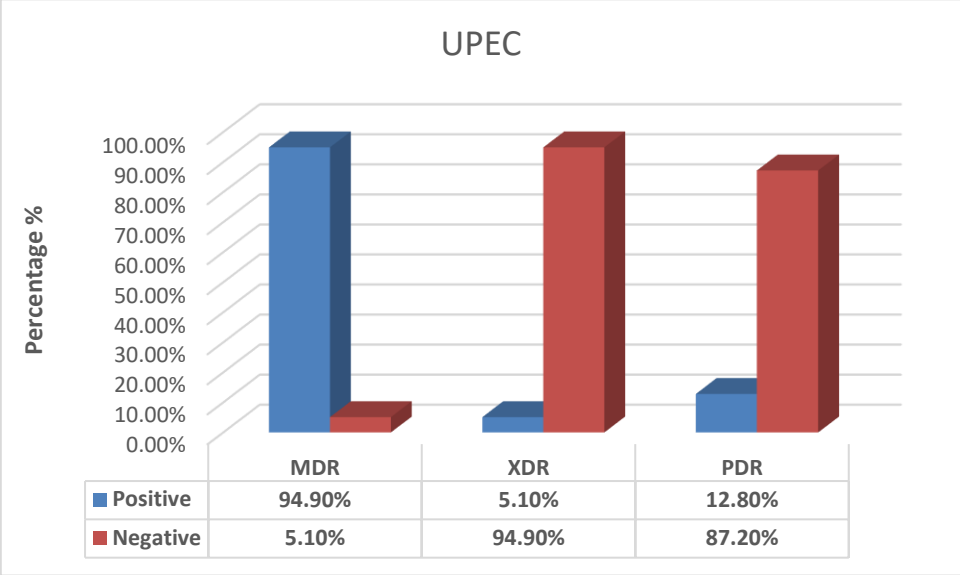


Figure (3.14): Evidence of MDR, XDR and PDR of UPEC of study isolate

Table (3.14***):*** **Evidence of MDR, XDR and PDR of ESBL of study isolate**

ESBL Bacterial isolate		ESBL					
		MRD		**XDR**		PDR	
		+	-	+	-	+	-
Klebsiella Pneumonia	Count	8	0	0	8	0	**8**
	%	100.0%	0.0%	0.0%	100.0%	0.0%	**100.0%**
Acinetobacter Baumannii	Count	2	0	0	2	0	**2**
	%	100.0%	0.0%	0.0%	100.0%	0.0%	**100.0%**
Proteus mirabilis	Count	2	0	0	2	0	**2**
	%	100.0%	0.0%	0.0%	100.0%	0.0%	**100.0%**
Pseudomonas Aeruginosa	Count	4	0	0	4	1	**3**
	%	100.0%	0.0%	0.0%	100.0%	25.0 %	**75.0%**
P value ($p \leq 0.05$)		.456^{NS}		.456^{NS}		**.916^{NS}**	
NS:Non- Significant difference at the 0.05 level by chi-square test							

Chapter Four

Discussion

4.1.Descriptive data for patients and control

The distribution of age groups among the patients in present studydoes not differ significantly from control group, as found in table (3-1). This results found that age may not be a significant factor in determining the risk or occurrence of urinary tract infection in the patient population, a *p-value* of 0.882 indicates that there is no statistically significant association between patient and control group.

Some studies have suggested that UTI incidence tends to be higher in certain age groups, such as infants, young children, and postmenopausal women. These age groups may have specific physiological or anatomical factors that increase their susceptibility to UTIs (Arinzon *et al.,* 2012; Chu & Lowder, 2018). On the other hand, other studies have not found a significant association between age and UTI rates. They may have observed that age alone is not a strong predictor of UTIs, and other factors, such as underlying health conditions, urinary tract abnormalities, or behavioral factors, may play a more significant role (Lo *et al.*, 2013).

In this table, the study found increased of UTI infection with non-significant in patient had 31-41 years old, this study was agreement with a study conducted by (Azab, 2021) who was found that age group of 31-41 years, the individuals may still be sexually active, which could potentially contribute to a higher incidence of UTIs compared to other age groups, sexual intercourse can introduce bacteria into the urinary tract, increasing thelikelihood of infection. Factors such as frequency of sexual activity, use of barrier contraceptives (e.g., condoms), and personal hygiene practices can also influence the risk of UTIs related to sexual activity (Mondal *et al.*,2022).

Among residence situation, it appears that there are no significant differences between patients and control. This suggests that the distribution

of residence group in patients with urinary tract infections (UTIs) is similar to the control group although the lack of significant differences suggests that residence may not be a strong factor in UTI development in this particular study , lifestyle factors may also be affected UTI also plays a role in its susceptibility (Hammod, 2023).

Some of these studies reported significant differences in UTI rates across residences, for example, if certain types of residence, such as a nursing home, long-term care facility, or hospital, were the highest risk there is a chance of getting a UTI in association It has an odds ratio of 2. This can be due to factors such as weak immune system, presence of catheters, high prevalence of UTI-causing bacteria in these cases (Demir *et al*., 2020). These studies have found mixed results, some showing higher UTI rates in rural areas due to lack of access to health care or sanitation facilities, while others have found no significant association (Lodhia and Foley,2020).

In the present study, there was no significant difference in the incidence of UTI between men and women. The odds ratio for gender was 0.77, indicating no difference in UTI risk between the two sexes. This finding suggests that sex may not be a significant predictor of UTI in the study population. It was different from other studies (Middelkoop *et al*., 2021; Gu *et al*., 2022), which found that gender is found to be an important factor in UTI risk. Generally, females tend to have a higher incidence of UTIs compared to males. This difference is primarily attributed to anatomical factors. Women have a shorter urethra, which allows bacteria to travel more easily to the bladder. Additionally, the proximity of theurethra to the anus in females increases the likelihood of bacterial colonization in the urinary tract and hormonal factors can also play a role in UTI risk. Estrogen, which is more prominent in premenopausal women, helps

maintain the health of the urinary tract lining, making it more resilient against infection, as women approach menopause and estrogen levels decrease, the risk of UTIs may increase (Deltourbe *et al.,* 2022).

4.2. Time collection for patients' samples

About figure (3-1),the study also interpreted the time collection for patients' samples; the best time to collect urine for detecting bacteria depends on the specific circumstances and the type of bacterial infection, a first-morning urine sample(also known as the first-voided urine) may be preferred for detecting bacteria.The first-morning urine is often more concentrated and may increase the chances of detecting low levels of bacteria. In certain clinical situations,such as suspected intermittent bacteriuria or infections with bacteria known to exhibit diurnal variation in urinary shedding, timed urine collections or specific timing instructions may be necessary(Collins *et al.,* 2020).

4.3. Distribution of patients according to the hospitalization type

Concerning figure (3-2), the higher percentage (64%) of community- acquired infections indicates that a significant portion of patients admitted to the hospital already had an infection prior to their hospitalization. This could imply that these infections were acquired in the community and were severe or progressed to a point where hospitalization was necessary (Ott *et al*., 2013). The presence of hospital-acquired infections in 36% of patients highlights the importance of infection control measures within healthcare settings. Hospital-acquired infections can have serious implications, including prolonged hospital stays, increased healthcare costs, and potential harm to patients already receiving medical care (Mun *et al*., 2022). The present study found that the rate of UTIs was lower in the hospital setting compared to the community setting, this is an

interesting finding that differs from some previous studies like a study published in the Journal of Clinical Microbiology in 2019 looked at UTI rates in a hospital versus the community, they reported that hospital-acquired UTIs were actually more common than community-acquired UTIs (Smith *et al.,* 2019).

Most of the UTIs were of community origin 611 (76.6%). Both community-acquired urinary tract infections (CA-UTIs) and hospital-acquired urinary tract infections (HA-UTIs) were more common in female patients (45.6% and 13.5%, respectively.(Lewis et al.,(2013),(Herbawi *et al*., 2024). said that exploring the factors contributing to hospital-acquired infections. These may include compromised immune systems of patients, invasive medical procedures, prolonged hospital stays, overcrowding, antibiotic resistance.

4.4.Evaluation of laboratory parameters in patients according to diabetes disease

Based on the statistical analysis, which indicates no significant differences ($p>0.05$) in the distribution of laboratory parameters between patients with and without diabetic disease as demonstrated in table (3-2), it suggests that the presence of diabetic disease may be does not have a significant impact on the values of these parameters. This results has important implications for the management and treatment of diabetic patients. It suggests that the laboratory parameters examined in this study, such as PH levels, S.G, Leukocytes and RBCs, may not differ significantly between patients with and without diabetic disease. This study was disagreement with (Burekovic *et al.,* 2021) who was found an elevated levels of leukocytes, or white blood cells, in the urine may indicate the presence of urinary tract infection (UTI) or inflammation in the urinary system. UTIs are common in individuals with diabetes due to

factors such as high blood sugar levels and immune system dysfunction in diabetic patients and have been illustrated as if blood cells are monitored the amount of red in the urine that can help diagnose and manage a UTI in patients with diabetes The presence of RBCs in the urine, known as hemorrhage, may indicate damage to the kidneys or other urinary tract. Diabetes, particularly when uncontrolled or associated with kidney complications (diabetic nephropathy), can increase the risk of kidney damage. RBCs in urine can aid in the early detection and management of kidney-related issues in diabetic individuals (Thipsawat, 2021).

The study specifically excluded individuals with diabetes mellitus, suggesting that diabetic patients without cognitive impairments were comparable to people without diabetes, in terms of non-significant differences between the two groups in blood of platelets, red blood cells (RBCs). The results , as well as the specific gravity of urine, may suggest that diabetes, in the absence of tumors, has no significant effect on these specific urine properties This suggests that the presence of diabetes alone may not be a factor leading to changes in these laboratory materials (Ejrnæs, 2011).

In the present study, the urinary specific gravity of diabetic (1.01 ± 0.005) and nondiabetic (1.01 ± 0.005) patients appeared to be similar, 1.01 specific gravity in the normal range found in urine reflects the amount of water vapor in urine and the physiological status of hydration, . Things like kidney function can be affected. end of the same specific gravity at the interface, diabetic and nondiabetic individuals show that, in this study, diabetes had no significant effect on water volume as measured by specific gravity (Kuiper *et al*., 2021).

4.5. Bacterial culture from patients

The study showed that 57.9% of the samples tested positive for urinary tract infections (UTIs), the remaining 42.1% of the samples as currently showed no increase in figure(3-3), a large proportion of bacterial infections (57.9%) indicates that the bacteria in the present study. is a major cause of the disease. However, a high percentage of cases (42.1%) indicate that there may be other or other causes of UTI in this population, such as bacterial, fungal, or non-infectious (Sabeer *et al* and colleagues,2020).

4.6. Percentage of bacterial culture from patients

Regarding figure (3-4), the results indicate that *Escherichia coli* remains the predominant causative organism, accounting for 70.9% of the UTI isolates. The other uropathogens identified and their respective frequencies include *Klebsiella pneumoniae* at 14.5%, *Acinetobacter* and *Proteus* both at 3.6%, and *Pseudomonas* at 7.3%. This distribution, with *E. coli* as the most common UTI-causing pathogen, is consistent with the trends reported in the existing medical literature on urinary tract infection epidemiology. *E. coli* is well-established as the leading uropathogen responsible for both community-acquired and hospital-acquired UTIs globally (Marrs *et al*., 2005).

Numerous studies have compatible with current study which reported that *E.coli* as the most common bacteria responsible for UTIs,(AL- Hasnawi and Al-Hasnawy, 2019),reported in a study of some Iraqi patients that *E. coli* was present in 44.64% of urinary tract infections,(Nji *et al.,* (2020) found on a study conducted in Cameroon that *E. coli* was the predominant pathogen isolated from community-acquired UTIs. It accounted for a significant proportion of the bacterial isolates in the study population and (Begum *et al.*,(2022) who was found in Bangladesh that *E.*

coli as the most common causative agent of UTIs. The study analyzed the microbial patterns and antibiotic susceptibility of urinary isolates and found *E. coli* to be most common and predominant pathogen., also, (Ronald, 2002) revealedthat *E.coli* remains the predominant uropathogenic at (80%) of isolates in acute community aquired uncomplicated infections. On the other hand, both *Acinetobacter baumannii* and *Proteus mirabilis* had the lowest percentage ofbacterial isolates at 3%. This suggests that these two bacteria were less commonly identified in the samples compared to the other types of bacteria.

This study was disagreement with (Uwingabiye *et al*., 2016) who was found the most common of bacterial isolates in urine sample of patient who admitted to intensive care unit is *Acinetobacter baumannii* as 52.08%.

On the other hand, the study was agreement with (Al-Bassam & Al-Kazaz, 2013) who was found in UTIs are the most common clinical manifestationof Proteus infections, Proteus infection accounts for 1-2% of UTIs in patient admitted to hospital.

4.7. Evaluation of Heparin binding protein in study population by ELISA.

The concentration of heparin-binding protein (HBP) was measured inboth patients and healthy control (table 3-3). The results of the statistical analysis indicate a significant increase in HBP concentration in patients compared to the healthy control group. In the patient group, the average HBP concentration was 22.04 ± 0.96 ng/ml. In contrast, the mean HBP level in the healthy control group was 7.78 ± 0.59 ng/mL. The significantly higher levels of HBP in the patient group compared with the healthy control group suggest that HBP may be associated with ESBL (Extended-Spectrum Beta-Lactamase) microorganisms.

Patients have developed elevated levels of heparin-binding protein (HBP) in their urine, suggesting that the kidneys account for much of HBP excretion. HBP is a protein that is primarily associated with neutrophils, a type of white blood cell involved in the immune system (Kjölvmark *et al.*, 2014). Elevated HBP levels can indicate a variety of conditions or diseases. For example, urinary tract infections (UTIs) or kidney disease can increase urinary HBP levels due to inflammation and immune reactions in the urine Other conditions such as kidney injury, glomerulonephritis, or interstitial nephritis may also be associated with increased urinary HBP (Kjölvmark *et al*., 2016).

4.8.Boxplot for HBP levels in diabetic and non diabetic patients:

The present study showed that there was no significant difference (P> 0.05) in heparin-binding protein levels in diabetic and nondiabetic patients admitted to Al-Hussain Teaching Hospital, which is unusual in a study a and effects obtained protein levels heparin affects the by diabetic plasma , but the exact direction of these effects and the regulatory proteins involved have not been fully characterized (Soerensen *et al*., 2021).

4.9.Evaluation of Heparin binding protein in patients according to bacterial isolate.

Analysis of heparin-binding protein (HBP) levels in patients with various bacterial isolates showed that levels were higher in patients infected with *E. coli* compared with those infected with *Klebsiella pneumoniae*, *Proteus mirabilis*, and that of *Acinetobacter baumannii* (Table 3-4). This observation suggests that there may be differences in the immune response and inflammation induced by different pathogens. HBP is a marker of neutrophil activation and is involved in the immune

response. Differences in HBP levels may be related to differences in infectious agents, host-pathogen interactions, or individual patient characteristics (Kjölvmark *et al*., 2012).

4.10.Evaluation of Heparin binding protein in study population according to gender.

Analysis of heparin binding protein (HBP) levels according to sex in the study population (Table 3-5), revealed that females have higher levels of HBP compared to males . The mean HBP level in females was 23.79 ± 6.29 ng/mL, while in males it was 19.95 ± 7.57 ng/mL. These findings suggest that there may be differences in HBP levels between men and women in the study population. A variety of factors, including metabolic and genetic differences, may contribute to the observed differences in HBP rates between men and women. It should also be considered that HBP levels may be influenced by other factors, such as inflammation or underlying health conditions, many previous studies have investigated possible differences in heparin binding protein (HBP) levels by gender between (Zhou *et al.*, 2019) This study aimed to To assess whether there are differences in HBP levels among males and females in patients with suspected sepsis and the results showed that female patients had Increased HBP levels compared to male patients.And (Sjöbeck *et al.* , 2021) described as a study Sex-related differences in HBP levels were investigated in patients with acute bowel disease The findings showed that female patients had significantly higher HBP levels compared to male patients and (Dankiewicz *et al*., (2013) who was HBP in patients with acute ischemic stroke. examined gender quantitative differences and association with clinical outcome The results showed that female patients had higher HBP levels compared to male patients.

4.11.Correlation analysis between Heparin binding protein and laboratory parameters

In the present study, correlation analysis was performed to evaluate the relationship between heparin-binding protein (HBP) levels and various laboratory parameters, including as defined pH, specific gravity (S.G.), leukocyte count, and blood cells red blood cell counts (RBCs) are listed in Table (3-6). Statistical analysis of the patients' data revealed a highly significant ($p \leq 0.01$) positive correlation between HBP and S.G., leukocyte count, total RBC, which were recorded as (0.76), (0.76) and (0.77).) respectively The positive correlation observed indicates that Hb There is a relationship between P concentration and measured laboratory parameters. Specifically, as HBP levels increase, SG, leukocyte count, and RBC increase correspondingly. This finding may have clinical implications and may provide insight into the potential role of HBP in the effects of these factors.This is consistent with the study (Kjölvmark *et al*., 2014) which found a correlation between some renal parameters S.G, leukocytes in urine patient and concluded that heparin-binding protein (HBP) is a product of neutrophils and consumed by the former is the topic of predictive identification and analysis of various pathogens as biomarkers.

4.12.Receiver Operative Characteristic Curve-based analysis of HBP in patients.

Based on the analysis of the ROC curve, it suggests that there is a significant difference in the HBP concentration in urine between the patient and control groups. The blue line, which likely represents the patient group, appears to be higher than the red line representing the control group. This indicates that the HBP concentration in urine is generally higher in patients compared to the control group. The ROC curve is commonly used to

evaluate the diagnostic accuracy of a biomarker. In this case, the curve helps assess the ability of HBP concentration in urine to distinguish between patients and controls. The higher positioning of the blue line suggests that HBP concentration may have good discriminatory power in identifying individuals belonging to the patient group. Additionally, the AUC value of 0.9 suggests that the biomarker has a strong ability to differentiate between patients and control, and the specificity of 90% suggests that the biomarker correctly identifies 90% of individuals who do not belong to the patient group. This indicates a low false-positive rate, meaning that the biomarker has a high ability to correctly classify controls. Furthermore, the sensitivity of 91% indicates that the biomarker correctly identifies 91% of individuals who belong to the patient group. This implies a low false-negative rate, indicating that the biomarker has a high ability to correctly classify patients.

4.13.Estimation the prevalence of ESBL bacterial isolate according to Sex, residency and diabetes disease.

Figure(3-7) and Table (3-8) showed that the isolation rate was reported to be higher in females (53.8%) as compared to males (46.2%). The higher incidence of bacterial UTIs in women than in men may be due to anatomic and physiologic differences between the sexes Women have shorter urinary tracts than men, which means a shorter distance for bacteria to reach the bladder and infection may increase the risk of bacterial infection from the gastrointestinal tract (Czajkowski *et al*., 2021) Furthermore, clearance was more frequent in urban areas (64.1%) than in rural areas (35.9). %), urban areas tend to have higher population densities, with many people living in close proximity to each other this increase in population may lead to the spread of infectious diseases including bacterial UTI. The high density of individuals in urban areas increases the chances

of infection and infection (Kubone *et al*., 2020).More recent studies still have emphasized the importance of density in urban areas and have accelerated the spread of infectious diseases. For example,(Rocklöv and Sjödin 2020) focus on the ways in which highly crowded urban areas can accelerate the spread of infectious diseases due to human contact and greater movement there. According to the study by (Zhang *et al*.,2021), since bacteria can spread rapidly in crowded healthcare facilities, high turnover of patients in urban hospitals raises the risk of the risk of developing a UTI in hospital is high Despite the fact that cities often have good infrastructure, sanitation and hygiene still have large disparities , especially in poorer cities (Ezeh *et al* .,2021) show that large populations and poor sanitation in informal settlements contributes to infectious diseases, such as UTIs.

Due to antibiotic overuse and misuse, antibiotic-resistant infections are more prevalent in urban areas, making UTI difficult to treat (Holmes et al. Na due to the high incidence of diseases, infectious diseases are easily spread by urban dwellers and migrants who frequently move in and out of cities.(Wu *et al*,2020) affect how the number of people moving to urban areas is important for the overall improvement in infectious diseases, the new study highlights that a number of urban characteristics, including population growth, health care utilization, hygiene differences, antibiotic resistance, and greater mobility, contribute significantly to the high incidence of UTIs in urban areas. For urban populations to experience successful disease prevention and control, several challenges must be addressed.

On the other hand, the UTI pathogens were isolated at a higher percentage (69.2%) from patients with diabetes compared to patients withoutdiabetes. This suggests that individuals with diabetes have a higher

likelihood of being affected by UTIs caused by this particular pathogen and this is attributed to Diabetes can weaken the immune system and impair the body's ability to fight off infections, making individuals with diabetes more susceptible to UTIs and other infectious diseases. Additionally, elevated blood sugar levels in diabetes can create an environment that is favorable forbacterial growth, further more increasing the risk of UTIs (Ramrakhia *et al*.,2020). In addition, diabetic cytopathy, a disorder that causes bladder dysfunction due to diabetic autonomic neuropathy, exists. These elements raise the risk of contracting infections. (Medicina, 2023). If the study results compared with other studies such as a comprehensive study and meta- analysis in Ethiopia revealed that the prevalence of UTIs in diabetes individuals was around 13.8%. This high incidence is caused by decreased immunological response, poor blood circulation, and bladder dysfunction (PLOS ONE, 2024).Similarly, a research in Saudi Arabia found a UTI incidence of 25.3% among diabetic patients, with significant risk variables including female sex, hypertension, and a higher BMI (Healthcare, 2024).

4.14.Distribution of uropathogens bacterial isolate according to age groups:

The present study observed a higher prevalence of urinary tract infections (UTIs) caused by uropathogenic bacteria in the age group of 42-52 years, compared to other age groups. This age group, which typically includes people in the menopausal and postmenopausal years, has unique physiological and behavioral characteristics that contribute to an increased susceptibility to UTIs These findings suggest that individuals in this age group have UTIs that such caused by this particular virus is more susceptible to, the hormonal changes during menopause and menopause can affect the urinary tract, making it more susceptible to infections These changes in hormone levels may affect the process of urinary safety,

increasing the risk of UTI (Aslam *et al*.,2020). (Raz and Stamm ,2018) found that UTIs are more common in postmenopausal women due to insufficient estrogen, which affects the urogenital flora and mucous defenses and also the ability of the immune system to weaken with age, increases middle-aged Adults susceptible to UTIs. (Hilt *et al*,2020) found that older individuals are more likely to develop UTIs due to immunosense and other age-related physiological changes. Also, there are age-related changes in the urethra, such as muscle tone reduction and thinning of the urethral lining , can affect the ability of the urethra to effectively expel bacteria. This may increase the risk of infection and subsequent UTI (McCloskey *et al*., 2024).Despite the decline in sexual behavior with age, sexual function remains a risk greater incidence of urinary tract infections (UTIs) in middle elderly individuals . This is supported by a recent study by (Ricoy-Cano *et al*., 2020) which found that people in their 42–52 years of age who are still able to engage in sexual activity continue to have this risk, as the association between these days understands activity and UTI in young people.

4.15. Type of antibiotic resistance for UPEC of study isolate

Uropathogens growing resistance is limiting the efficacy of prescription drugs in treating urinary tract infections. The treatment of UTIs is significantly hampered by antibiotic resistance. because it limits the possibilities for therapy, raises the chance of treatment failure, and has negative economic effects, such as higher healthcare costs, expenditures brought on by a rise in hospital admissions and drug use The results revealed that different antibiotics within the family of drugs showed varying rates of sensitivity and resistance in the tested *E. coli* isolates. Cefepime, Gentamicin, Amikacin , pipera/Tazo, and Nitrofurantoin exhibited high rates of sensitivity, while Ciprofloxacin, Norfloxacin,

Ceftazidime, and Ampicillin showed high rates of resistance, Cefepime belongs to the cephalosporin class of antibiotics, resistance to cephalosporins can occur through β-lactamase production, which enzymatically inactivates the drug.

Bacteria can produce different types of β-lactamases, such as extended-spectrum β-lactamases (ESBLs), which can confer resistance to cefepime (Mushtaq *et al*., 2021). Gentamycin and Amikacin antibiotics belong to the aminoglycoside class, the resistance to aminoglycosides can arise through enzymatic inactivation by aminoglycoside-modifying enzymes (AMEs) that chemically modify the drug, reducing its efficacy (Aishwarya *et al*., 2020).Several researchers reported that a high percentage of isolated uropathogens emphasized antibiotics resistance issue and this beside current study ,sending out a clear warning sign to optimize therapy in accordance with the resistance profile and carry out public interventions to contain the problem's spread. (Subramaniyan *et al*.,2021; Joya *et al*;2022; Herbawi, *et al*.,2024)

According to a research done in Sudan, 19.5% of patients had bacteriuria. The most common bacterial isolate, *Escherichia coli*, had a significant degree of resistance to several medicines, underscoring the difficulty of treating UTIs in areas where antibiotic resistance exists (Mohammedkheir *et al*. , 2024). The development of resistance emphasizes the need for the development of new antibiotics or alternative strategies to effectively combat these diseases, thus further complicating treatment options Com is . Ceftazidime resistance can be mediated by β- lactamases including ESBLs or AmpC β-lactamases. In some cases, the bacteria may even exhibit reduced external membrane permeability, limiting antibiotic penetration (Wang *et al*., 2020).

4.16.Virulence gene association with *E. coli* isolate

Table (12) shows that the *Bla-CTM* gene was detected in all UPEC isolates, indicating a 100% overlap. *Bla-CTM* is associated with the production of β-lactamases, which can lead to resistance to β-lactam antibiotics such as cephalosporins and penicillins, on the other hand, *Bla OXA-48* gene and *CNF* gene were detected in 3 isolates from humans, showing a multiple of 1000 with a mean of 7.7%. *Bla OXA-48* is associated with resistance to carbapenem antibiotics, while *CNF* (*cytotoxic necrotizing* factor) is a potential contributor to UPEC infection *EAE* gene was detected in 8 isolates in 1999, with a prevalence of 20.5%. *EAE* (intimin-encoding gene) *E. coli* is associated with the ability to adhere to and invade host cells, which contributes to its virulence (Obodoechi *et al*., 2020)..

The results show that the *Bla-CTM* gene is universally present in all bacterial isolates tested (100% across all species). This finding is consistent with several studies that reported a high prevalence of *Bla- CTM* genes in bacterial species, usually associated with extended-spectrum beta-lactamase (ESBL) production, which confers resistance against a wide range of ubiquitous beta-lactam antibiotics corresponds to *Bla-CTM* in our isolates The presence highlights the need for aggressive antibiotic management and alternative therapeutic strategies for resistance highlighting these antibodies. In contrast to *Bla-CTM*, the *Bla OXA-48* gene was detected in only a small proportion of Escherichia coli isolates (7.7%), and completely absent from the other bacteria tested This amount this low and other studies with *Bla OXA-48*, . a carbapenemage gene, is uncommon but plays a role in carbapenem resistance The limited presence in our sample, particularly in the Enterobacteriaceae, may be due to geographical diversity or the specific clinical setting from which the isolates were obtained.

The *CNF* (cytotoxic necrotizing factor) gene was present in 7.7% of *Escherichia coli* isolates and was universally present in the hosts of *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* . The distribution of observed variants suggests specific factors affecting the acquisition and retention of *CNF* genes, requiring further investigation into genetic mechanisms and environmental factors Finally, *EAE* (*E. coli attaching* and *effacing*) gene, which is associated with the ability to form adhesive and effacing lesions, was found in 20.5% *Escherichia coli* isolates This gene, which has been shown to have diversity in other species, is lacking *Acinetobacter baumannii* completely, and found in enterohemorrhagic *E. coli*. (EHEC) and *E. coli* pathogenic urinary tract infections (EPEC) is the major causative agent. The relative abundance of *Escherichia coli* is consistent with its known role in intestinal diseases, while its presence in non-Enterobacteriaceae species highlights the potential for upstream gene transfer and trait acquisition highlighting pathogenic species.

The current study found increase resisitance pattern of *E.coli* against Trimethoprim/sulfamethoxazole, this resistance may be return to Target Modification: Bacteria can develop resistance by altering the target sites of the antibiotics. Trimethoprim inhibits bacterial dihydrofolate reductase (DHFR), while sulfamethoxazole targets the enzyme dihydropteroate synthase (DHPS). Mutations in the genes encoding these enzymes can lead to structural changes, rendering them less susceptible to the inhibitory effects of the drugs (Eliopoulos& Huovinen, 2001).as figure(3-9) reveals that.

4.17.Antibiotic susceptibility and MIC to bacterial isolate.

The MIC values for in the table (3.13) showed all bacterial isolates have non-significant differences among antibiotics except trimethoprim/

sulphamethaxazol, the present study found increase in the mean ± SD of MIC in the *Acinetobacter baumannii* and *Proteus mirabilis* , it was recordedas 320.0 ± 0.024, followed by *E.coli* the study found mean ± SD of MIC was 267.6154 ± 18.194 against trimethoprim/ sulphamethaxazol, the ability of drugs to exert antimicrobial activity by blocking the reduction of dihydrofolate to tetrahydrofolate, the active form of folic acid, by susceptible organisms, it has inhibitory activity for most gram-negative aerobic bacilli (Gleckman *et al*., 2018).

4.18.Evidence of MDR, XDR and PDR of ESBL of study isolate.

The figure (3.14) reveals that MDR is the most prevalent resistance phenotype, with a positive rate of 94.90%, indicating that a significant proportion of the UPEC isolates are resistant to multiple types of antibiotics. This high level of MDR is concerning and suggests the need for comprehensive antibiotic stewardship programs and the development of alternative treatment strategies to manage infections caused by these resistant strains (Ibrahim *et al*., 2012).In contrast, the XDR phenotype, which represents an even higher level of resistance, has a relatively lower positive rate of 12.8%, although of the presence of XDR isolates is still a significant concern, as these strains are resistant to most, if not all, available antibiotics, leaving limited treatment options for clinicians, So the study disagreement with (Al-Hasani *et al*., 2023) who was found the percentage of XDR 1.17% of multiple drug resistance from *E.coli* isolated from Iraqi clinical isolates among patients in Baghdad city. The PDR phenotype, which denotes resistance to all tested antibiotics, has a positive rate of 5.81%. The emergence of PDR strains is particularly alarming, as they leave healthcare providers with no effective antibiotic options to treat the associated infections, potentially leading to increased morbidity and

mortality this study was agreement with (Datok *et al*., 2021) who was found PDR was 4.6% in Escherichia Coli isolated from barbecued beef (Suya) sold in a Nigerian City. .

The present study reveals the concerning prevalence of multidrug resistance (MDR) and pandrug resistance (PDR) among the bacterial isolates tested in Table(3.13).In particular *Klebsiella pneumoniae* (8/8). 8), all *Acinetobacter baumannii* (2/2). the divisions of the. , and *Proteus mirabilis* (2/2) were found to be MDR. Similarly, all *Pseudomonas aeruginosa* isolates were MDR, and one (25%) was identified as PDR.

These findings are consistent with worldwide reports of high rates of MDR in these bacterial species. For example, *Klebsiella pneumoniae* is well documented for its ability to acquire and harbor multiple resistance genes, contributing to the clinical superpathogen *Acinetobacter baumannii*, another notorious pathogen in healthcare settings in strains often exhibit MDR due to the remarkable ability to acquire resistance determinants through downstream genes transfer and clonal spread (Jain & Danziger, 2014). The universal MDR status observed in Proteus mirabilis and Pseudomonas aeruginosa aligns with their known resistance profiles. Pseudomonas aeruginosa, in particular, poses significant treatment challenges due to its intrinsic resistance mechanisms and ability to develop additional resistance through mutations and gene acquisition .The identification of a PDR isolate among Pseudomonas aeruginosa is particularly alarming, as PDR organisms are resistant to all available antimicrobial agents, leaving limited therapeutic options (Falagas *et al*., 2015). Also detected and explained in table (3-14)

Conclusions

&

Recommendations

Conclusions:

The current study concludes the following:

1. Most frequently isolated uropathogens included *E.coli* (70.9%) followed by *Klebsiella pneumoniae* (14.5%). The *E.coli* isolates showed high resistance rate especially to ciprofloxacin, norfloxacin, ceftazidime and ampicillin.
2. There was no significant difference in HBP levels between diabetes and non-diabetes
3. Patients with *E. coli* isolates had significantly higher HBP levels than those with other bacterial isolates
4. Female patients recorded significantly higher levels of HBP than male patients while there was no gender difference among controls.
5. Specific gravity, leukocyte count, and RBC count were all positively correlated to HBP in patient.
6. ROC analysis suggested HBP would be good marker for predicting disease status with high sensitivity and specificity.
7. Heparin binding protein (HBP) levels were significantly higher in patients as compared to healthy controls suggesting that it may be a potential biomarker for UTI.
8. The *Bla-CTM* gene in UPEC isolates were detected in all cases compared with other genes (*Bla OXA-48*, *CNF* and *EAE*)
9. About 94.9% of *E.coli* isolates were MDR while 5.1%were XDR and PDR is12.8%.

Recommendations:

Here are the main recommendations in a more concise form:

1. Investigate if Heparin-Binding Protein (HBP) can be used as a urinary biomarker for UTIs.

2. Establish strict prescribing guidelines through antimicrobial stewardship and develop local antibiograms to control high rates of antibiotic resistance noticed mainly among *E. coli* isolates.

3. Improve infection prevention and control measures within healthcare facilities, with emphasis on the latter.

4. Advocate for development of other therapies against uropathogens that have become resistant to antibiotics.

5. Study how virulent factors such as *blaCTX-M*, *blaOXA-48*, *CNF* and *EAE* may contribute to UTI pathogenesis and antibiotic resistance, respectively.

6. Educate the public about finishing full antibiotic courses as well as the dangers of self-medication.

7. Carry out routine surveillance on uropathogenic strains for antibiotic resistance and virulence genes

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Appendix

Appendix1

Questionars:

1-gender : female ◯ , male ◯

2-residency: urban ◯ , rural ◯

3- diabetes: yes ◯ , No ◯

4- time of collect : ◯

5- PH : ◯ , S.G: ◯

6- WBCs: ◯ , RBCs: ◯

(Appendix 2)

A- Eosin Methylen Blue agar medium: This medium can be prepared by dissolving 40gm of agar in 1000 ml of D.W and then sterilization in autoclave at 121°Cfor 20 minute, this type media used selective and differential media when inoculated with UPEC give green metallic sheen phenomena (MacFadden, 2000).

(Appendix 3)

B- MacConky agar medium: This medium can be prepared by dissolving 40gm of agar in 1000 ml of D.W and then sterilization in autoclave at 121C°for 20 minutes, this type of media used selective gram-negative media (MacFadden, 2000).

(Appendix 4)

C- Chromogenic agar:

Composition:

Per vial sufficient for 500 ml medium :

Ingredient	concentration
Ceftazidime	1.50 mg
Cefotaxime	1.50 mg
Ceftriazone	1.00 mg
Aztreonam	1.00 mg
Fluconazole	5.00 mg

Directions:

Rehydrate the contents of 1 vial aseptically with 5 ml sterile distilled water. Mix well and aseptically add to 500ml of sterile, molten, cooled(45-50 **°C) hicrome ™ ESBL Agar M1829 , mix well and pour into sterile petri plates.(** Sambrook *et al* .,2001)

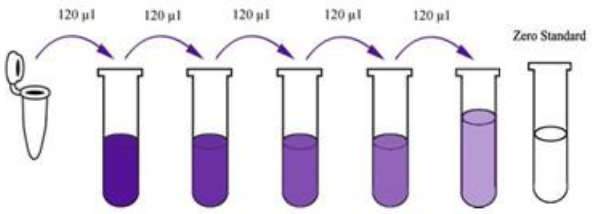
Appendix(5):

Reagent Preparation

1- All reagents should be brought to room temperature before use.

2-Standard Reconstitute the 120µl of the standard (48ng/ml) with 120µl of standard diluent to generate a 24ng/ml standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (24ng/ml) 1:2 with standard diluent to produce 12ng/ml, 6ng/ml, 3ng/ml and 1.5ng/ml solutions. Standard diluent serves as the zero standard(0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

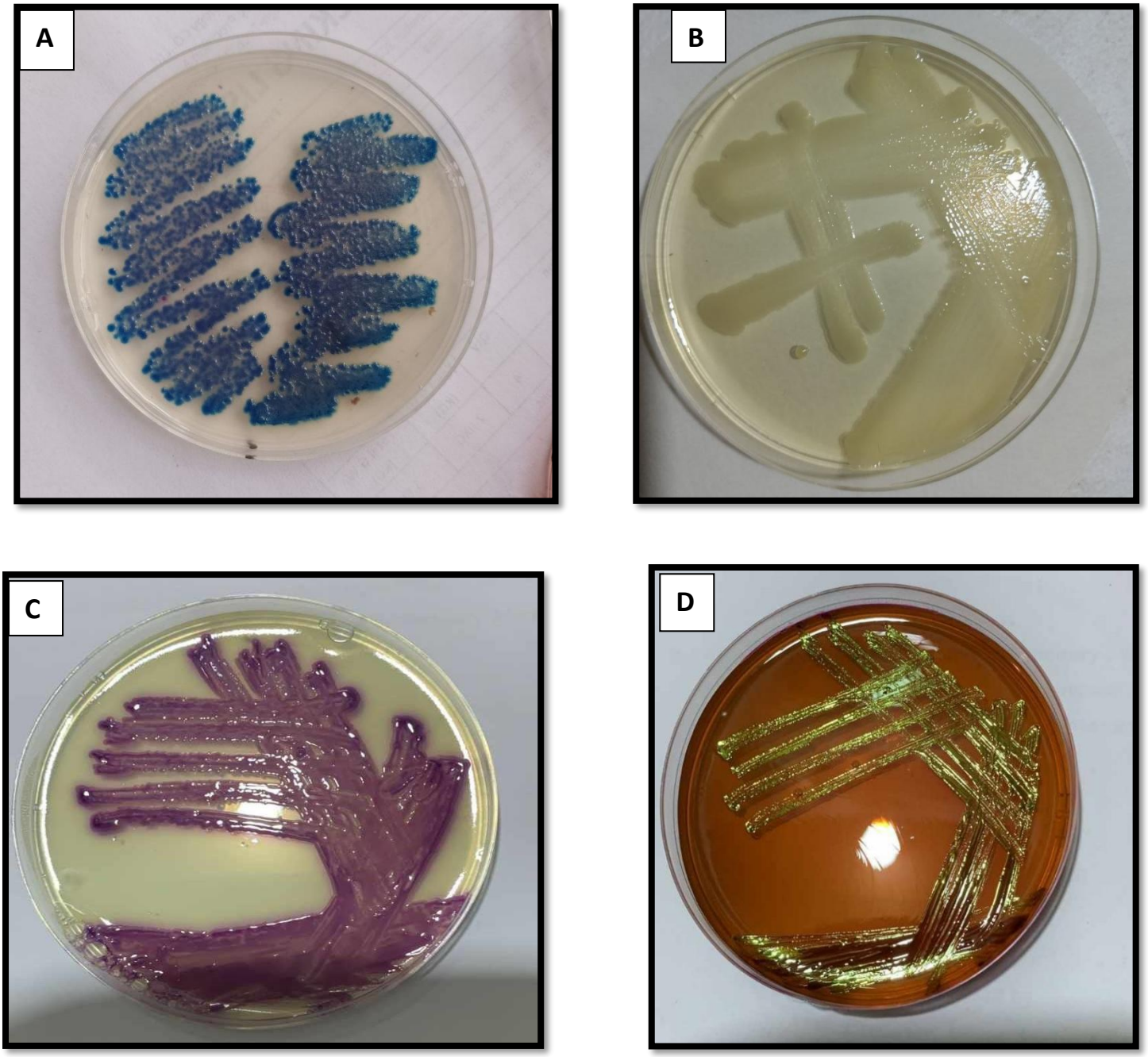
24ng/ ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
12ng/ ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
6ng/ ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
3ng/ ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
1.5ng /ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



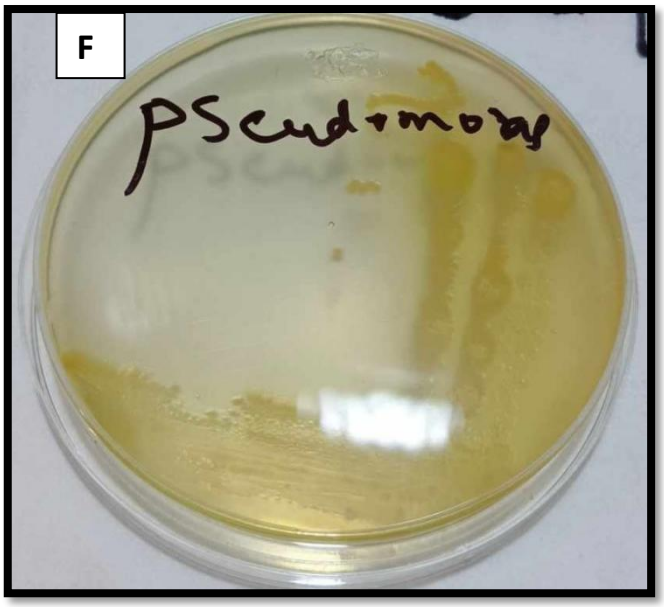
Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
48ng/ml	24ng/ml	12ng/ml	6ng/ml	3ng/ml	1.5ng/ml

Wash Buffer Dilute 20ml of Wash Buffer Concentrate 25x into deionized or distilled waterto yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

Appendix(6): ESBL producing bacteria on EMB agar and Chromogenic agar: A- *Klebsiella pneumonia* , B- *Acinetobacter baumannii* , C- *Escherichia coli* , D-*Escherichia coli on EMB agar,* E- *Proteus mirabilis* ,F- *Pseudomonas aeruginosa*




E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Appendix(7)

Table (10): Antibiotic susceptibility and MIC to bacterial isolate:

Types of isolates	Antibiotic	R/ I/S	No.	%	Bla-CTM		Bla OXA-48		CNF		EAE	
					+	-	+	-	+	-	+	-
Escherichia coli	Ampicillin	R	39	70.9 %	39 (100%)	0 (0%)	3 7.7 %	36 92.3 %	3 7.7 %	36 92.3 %	8 20.5 %	31 79.5 %
	Amox/Clavu	R	20	64.5 %								
		S	13	72.2 %								
		I	6	100 %								
	pipera\Tazo	R	14	66.7 %								
		S	25	73.5 %								
	cefotaxime	R	35	71.4 %								
		S	3	60.0 %								
		I	1	100 %								
	Ceftazidime	R	31	73.8 %								
		S	1	33.3 %								
		I	7	70.0 %								
	cefepime	R	13	59.1 %								

		S	26	78.8 %								
	Amikacin	R	6	54.5 %								
		S	32	76.2 %								
		I	1	50.0 %								
	Gentamicin	R	16	61.5 %								
		S	22	78.6 %								
		I	1	100 %								
	Ciprofloxacin	R	31	79.5 %								
		S	8	50.0 %								
	Norfloxacin	R	30	76.9 %								
		S	8	53.3 %								
		I	1	100 %								
	Nittrofurantoin	R	10	71.4 %								
		S	25	73.5 %								
		I	4	57.1 %								
	Trimeth\sulfamthoxazole	R	32	76.2 %								

		S	6	50.0 %								
		I	1	100 %								
Klebsiella pneumonia	Ampicillin	R	8	14.5 %								
	Amox/Clavu	R	7	22.6 %								
		S	1	5.6%								
	pipera\Tazo	R	5	23.8 %								
		S	3	8.8%								
	cefotaxime	R	7	14.3 %								
		S	1	20.0 %								
	Ceftazidime	R	6	14.3 %								
		S	1	33.3 %								
		I	1	10.0 %								
	cefepime	R	6	27.3 %								
		S	2	6.1%								
	Amikacin	R	3	27.3 %								
		S	4	9.5%								
		I	1	50.0 %								
	Gentamicin	R	5	19.2 %								

		S	3	10.7 %								
	Ciprofloxacin	R	5	12.8 %								
		S	3	18.8 %								
	Norfloxacin	R	5	12.8 %								
		S	3	20.0 %								
	Nittrofurantoin	R	3	21.4 %								
		S	3	8.8%								
		I	2	28.6 %								
Acinetobacter baumannii	Ampicillin	R	2	3.6%								
	Amox/Clavu	R	1	3.2%								
		S	1	5.6%								
		S	2	5.9%								
	cefotaxime	R	1	2.0%								
		S	1	2.0%								
	Ceftazidime	R	1	2.4%								
		S	1	33.3 %								
	cefepime	S	2	6.1%								
	Amikacin	S	2	4.8%								
	Norfloxacin	R	2	5.1%								
	Gentamicin	R	1	3.8%								
		S	1	3.6%								

	Ciprofloxacin	R	1	2.6%								
		S	1	6.3%								
	Nittrofurantoin	S	1	2.9%								
		I	1	14.3 %								
proteus mirabilis	Ampicillin	R	2	3.6%								
	Amox/Clavu	R	1	3.2%								
		S	1	5.6%								
	pipera\Tazo	R	1	4.8%								
		S	1	2.9%								
	cefotaxime	R	2	4.1%								
	Ceftazidime	R	1	2.4%								
		I	1	10.0 %								
	cefepime	R	1	4.5%								
		S	1	3.0%								
	Amikacin	R	1	9.1%								
		S	1	2.4%								
	Gentamicin	R	1	3.8%								
		S	1	3.6%								
	Ciprofloxacin	R	1	2.6%								
		S	1	6.3%								
	Norfloxacin	R	1	2.6%								
		S	1	6.7%								
	Nittrofurantoin	S	2	5.9%								
pseudomon	Ampicillin	R	4	7.3%								

as aeruginosa	Amox/Clavu	R	2	6.5%								
		S	2	11.1 %								
	pipera\Tazo	R	1	4.8%								
		S	3	8.8%								
	cefotaxime	R	4	8.2%								
	Ceftazidime	R	3	7.1%								
		I	1	10.0 %								
	cefepime	R	2	9.1%								
		S	2	6.1%								
	Amikacin	R	1	9.1%								
		S	3	7.1%								
	Gentamicin	R	3	11.5 %								
		S	1	3.6%								
	Ciprofloxacin	R	1	2.6%								
		S	3	18.8 %								
	Norfloxacin	R	1	2.6%								
		S	3	18.8 %								
	Nittrofurantoin	R	1	7.1%								
		S	3	8.8%								

Appendix(8)

Resistance of antibiotic											
pera\Tazo	cefotaxime	Ceftazidime	cefepime	Amikacin	Gentamicin	Ciprofloxacin	Norfloxacin	Nittrofurantoin	Trimeth \sulfamthoxazole	Bla-CTM	
	R	R	S	S	R	R	R	S	R	+	
	R	R	S	S	R	R	R	S	R	+	
	R	R	S	S	R	R	R	S	R	+	
	R	I	S	S	R	R	R	S	R	+	
	R	I	S	S	S	S	S	I	R	+	
	R	I	S	S	R	R	R	S	R	+	
	R	R	S	S	R	R	R	R	R	+	
	R	R	R	S	S	R	I	R	S	+	
	R	R	S	S	S	R	R	S	R	+	
	R	R	S	S	S	R	R	S	R	+	
	R	R	R	R	R	R	R	R	R	+	
	R	R	R	R	R	R	R	R	S	+	
	R	R	R	S	S	R	R	I	R	+	
	R	I	S	S	S	S	S	R	R	+	
	R	R	R	S	R	R	R	I	S	+	
	R	R	S	S	R	R	R	S	R	+	
	R	R	R	R	R	R	R	R	R	+	
	R	R	R	R	S	R	R	S	R	+	
	R	I	S	S	S	S	S	I	R	+	
	R	R	R	S	S	R	R	S	R	+	
	R	R	S	S	S	S	S	S	R	+	
	R	I	S	S	S	S	S	R	I	+	
	R	R	R	R	R	R	R	R	R	+	
	R	R	S	S	S	R	R	S	R	+	
	R	R	R	S	S	R	R	S	R	+	
	R	R	R	R	I	R	R	R	S	+	
	I	I	S	S	R	S	S	S	R	+	
	R	R	S	S	S	R	R	S	S	+	
	R	R	S	S	S	R	R	S	R	+	
	R	R	S	S	R	R	R	S	R	+	
	R	R	R	S	R	R	R	S	R	+	
	S	R	R	I	S	R	R	R	R	+	
	R	R	S	S	S	R	R	S	R	+	
	R	R	S	S	R	R	R	S	R	+	
	S	S	S	S	S	R	R	S	R	+	
	R	R	S	S	S	S	S	S	R	+	
	S	R	S	S	S	R	R	S	R	+	

	R	R	S	S	S	S	S	S	R	+	+
	R	R	S	S	S	R	R	S	S	+	+

الخلاصة

تعد البكتيريا المنتجة للبيتا لاكتاماز واسعة الطيف (ESBL) هي مجموعة من البكتيريا التي طورت مقاومة لمجموعة واسعة من المضادات الحيوية شائعة الاستخدام، بما في ذلك العديد من البنسلين والسيفالوسبورين. إن وجود جينات بروتين *CNF A/E* و*BLA OXA-48* و *BLA CTX-M* والهيبارين في بول الأفراد المصابين بعدوى الإشريكية القولونية يشير إلى وجود آلية متعددة الأوجه للفوعة ومقاومة مضادات الميكروبات، حيث ان تواجد بيتا لاكتاماز السام للخلايا ، والعوامل المرتبطة بالالتصاق قد تساهم بشكل جماعي في شدة واستمرار التهابات المسالك البولية التي يسببها هذا العامل الممرض.

عزلت مائة وخمسين (150) عينة من دراسة (Case-Control study). ضمت المجموعة الأولى 95 مريضا، تكونت من الإناث (30) والذكور (25) ، المجموعة الثانية ضمت 55 (29) ذكرا و (26) أنثى كمجموعة ضابطة اصحاء. تراوحت أعمار جميع الفئات من 20 إلى 63 سنة. اذ تم اختيار الأشخاص بناءً على نتائج فحص البول العامة. تمت زراعة جميع عينات البول التي تم جمعها على أجار ماكونكي وأجار EMB وأجار (Hichromogenic agar) باستخدام طريقة التخطيط. وتم قياس بروتين الرابط للهيبارين (HBP) باستخدام فحص مقايسة الامتصاص المناعي المرتبط بالإنزيم للمرضى ومجموعات المراقبة.

حللت جميع العينات عن طريق فحص البول العام (GUE) لتحديد وجود الخلايا البكتيرية وخلايا الدم البيضاء ومواد أخرى. من أجل التحديد الكمي والتعرف على الانواع كل عينة بول، تم زراعة عينات البول على أجار ماكونكي، و أجار EMB وأجار (Hichromogenic agar) ، بشكل منفصل. تم استخلاص الحمض النووي لعزلات الإشريكية القولونية وتم استخدام تقنية PCR للكشف عن بعض جينات عامل الضراوة المسؤولة عن إمراضية الإشريكية القولونية المنتجة للـ ESBL.

أظهرت النتائج وجود فروق غير معنوية ($P>0.05$) بين الفئات العمرية في مجتمعي الدراسة؛ وفي نفس السياق، أظهر العمر نتيجة غير معنوية ($P> 0.05$) في مجموعة المرضى.

كما وجدت فروق غير معنوية (P>0.05) في مجموعات الدراسة حسب الجنس. علاوة على ذلك، أظهرت نتائج التحليل الإحصائي عدم وجود فروق ذات دلالة إحصائية (P>0.05) في توزيع المعلمات المختبرية لدى المرضى المصابين وغير المصابين بمرض السكري.

من ناحية أخرى أظهرت النتائج أن الزراعة البكتيرية كانت في الغالبية العظمى من العينات المزروعة بنسبة 57.90% وجود نمو بكتيري، في حين أن 42.10% فقط ظهرت بدون نمو. وكانت أعلى نسبة للبكتيريا المعزولة من المرضى هي الإشريكية القولونية حيث سجلت نسبة 70.90%. في حين كانت أقل نسبة للبكتيريا المعزولة من المرضى هي Acinetobacter baumannii وProteus mirabilis بنسبة 3.60% على التوالي. أشارت نتائج التحليل الإحصائي إلى وجود زيادة معنوية في تركيز HBP لدى المرضى مقارنة بمجموعة الاصحاء. من جانب اخر في مجموعة المرضى، كان متوسط تركيز HBP 22.04 ± 0.96 نانوغرام / مل. في المقابل، كان لدى المجموعة الاصحاء متوسط تركيز HBP قدره 7.78 ± 0.59 نانوغرام / مل. تم اكتشاف جين Bla-CTM في 100% من عزلات UPEC، مما يمنح مقاومة للمضادات الحيوية بيتا لاكتام. وتم اكتشاف جين Bla OXA-48 وجين CNF في 7.7% من العزلات المرتبطة بمقاومة الكاربابينيم والفوعة على التوالي. تم اكتشاف جين EAE في 20.5% من العزلات، ويرتبط بقدرة بكتيريا E. coli على الالتصاق بالخلايا المضيفة وغزوها.

كشفت نتائج الدراسة الحالية أن الإشريكية القولونية بقيت الكائن المسبب السائد لعزلات التهاب المسالك البولية وأن مستويات بروتين ربط الهيبارين (HBP) كانت أعلى بكثير في المرضى مقارنة بالاصحاء مما يشير إلى أنه قد يكون علامة حيوية محتملة لالتهاب المسالك البولية. علاوة على ذلك، كان لدى المرضى الذين يعانون من عزلات الإشريكية القولونية مستويات HBP أعلى بكثير من أولئك الذين لديهم عزلات بكتيرية أخرى.

في الختام: لوحظت المستويات الأعلى بكثير من HBP في المرضى المصابين مقارنة بالأشخاص الأصحاء إلى أن HBP قد يكون بمثابة علامة حيوية محتملة لتشخيص ورصد عدوى المسالك البولية.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء / كلية الطب

قسم الاحياء المجهرية

دور البروتين المرتبط بالهيبارين وجينات الضراوة في البكتريا المنتجة لإنزيمات بيتا لاكتاماز واسعة الطيف بين مرضى عدوى المسالك البولية

رسالة مقدمة إلى

مجلس كلية الطب وهي جزء من متطلبات نيل شهادة الماجستير في الإحياء المجهرية الطبية

من قبل الطالبة

رسل محسن هاشم

بكالوريوس طب وجراحة بيطرية(2019)

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