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Isolation, Identification of *Escherichia coli* and Haematological Study for Iraqi Woman with Acute UTI

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Abstract: Urinary tract infection is one of the most common infections worldwide, especially in women, caused by different types of microbial agents. The purpose of this study was to focusing on infection of gram negative bacteria , haematological parameter and renal function in Iraqi women with acute UTI. Setting and design: 1-Sample collection from women with uti infection, 2- determine acute uti cases, 3- isoulation and identification of bacterial infection, 4- investigation for complete blood picture, 5- study renal function**.** A total of 220 samples (blood, serum and urine from the same case) were collected which included 120 samples that infected with UTI with mean age of (30-60) years old, in addition a 100 samples as controls were collected from healthy individuals. Morphological characteristics and biochemical tests were determined for acute women samples closely related to gram-negative bacteria that included microbiology that cultured on MaConky and EMB agar and examined by microscopic. The biochemical test included in this study were (Indole, Methyl-red, Voges-Proskauer, Citrate, Oxidase, Catalase), then used Api and Vitik for detected gram negative bacteria that presented. Finally used *16s rRNA* for detection of *E.coli* bacteria. Evaluation clinical biomarkers were determined for women samples includes WBC,TYM, platelets, MPV, CRP, Urea, creatinin and BUN. Statistical analysis used: The statistical package for social sciences (SPSS) application was used to observe the effects of various factors in research parameters. Least significant difference (LSD) test (ANOVA) or T-test was rummage-sale to significant compare among means. The results showed that 100 isolates gave typical phenotypic characteristics and biochemical tests as gram negative bacteria, that divided to the genus *Escherichia coli* was 96(80%), *Klepsiala* was 15(13%), *Enterobacterasea* was 15(13%) and *Protease* was 4(3%). Also confirmed the result for *E.coli* bacteria by using *16srRNA*. Clinical biomarkers were determined for all women samples that infected with UTI, the most common hematology and renal tests that studied in UTI infection women were WBC, MPV, LYM, PLT, CRP, Urea, BUN and Creatinine then compared with their levels in healthy control group. The results showed that there were significant increasing (p-value ≤ 0.05) in WBC, MPV and BUN, while there were non-significant increasing (p-value ≥ 0.05) in LYM, PLT, CRP, urea, Creatinine. It was concluded that many etiology that lead to UTI infection in women, it may be includes gram-negetive bacteria, gram-positive bacteria and fungi. The most causative agent of UTI infection was gram-negative bacteria, also haematological and renal function test may be good tool for diagnosis of acute UTI infection.

Key words: acute urinary tract infections, Gram-negative bacteria, Escherichia coli.

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Introduction

Gram-negative bacteria are among the world's most significant public health problems due to their high resistance to antibiotics. Two large groups, Enterobacteriaceae and the non-fermenters, are responsible for most clinical isolates; nevertheless, other

clinically concerning gram-negative organisms exist, including but not limited to *Neisseria, Haemophilus spp., Helicobacter pylori,* and *Chlamydia* trachomatis. *Escherichia coli* (UPEC) is the primary cause of urinary tract infections (UTIs), which are frequent infections. Age, sexual activity, family history, medical comorbidities, and a personal history of UTI are just a few of the many variables that affect an individual's risk for infection. Additionally, recurrent UTIs (RUTIs), which are defined as three or more UTIs in a 12-month period, significantly increase the incidence and mortality rates of UTI. There are several different pathogens that can cause urinary tract infections, including gram-positive, gram-negative, and fungal organisms. Patients who were if not healthy often suffer from uncomplicated urinary tract infections, as do the elderly and young children. Complicated urinary tract infections were frequently linked to indwelling catheters, immunosuppression, anomalies of the urinary system, or antibiotic exposure. *Uropathogenic Escherichia coli (UPEC)* was the most common causal agent for both simple and serious urinary tract infections. In order of occurrence, the additional causal agents for simple urinary tract infections were *Klebsiella pneumoniae, Staphylococcus saprophyticus, Enterococcus faecalis,* group *B Streptococcus (GBS), Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, and Candida spp. S. aureus, P. aeruginosa, P. mirabilis, Enterococcus spp., K. pneumoniae, Candida spp., S. aureus, P. aeruginosa,* and group *B Streptococcus* were the additional causal agents for severe urinary tract infections, in that order of prevalence (1). A urinary tract infection (UTI) is when uropathogenic bacteria colonize the urinary system and cause varying degrees of inflammatory reaction. The clinical spectrum was extremely variable and ranged from uncomplicated urinary tract infections, such as cystitis or pyelonephritis, in healthy, young women without urinary tract abnormalities to complicated urinary tract infections affecting weak people, such as urinary diversion, neurogenic bladder patients, elderly people, transplant recipients, and catheter-related urinary tract infections, which were a common cause of morbimortality in these populations with a high mortality rate (2). *Escherichia coli* is a gram-negative *bacillus* that is known to be a component of healthy intestinal flora but also has the potential to infect humans and cause extraintestinal and intestinal illnesses. Numerous *Escherichia coli* strains have been found, causing a range of illnesses from mild, self-limiting gastroenteritis to renal failure and septic shock. *Escherichia coli* can escape host defenses and acquire resistance to conventional antibiotics because of its virulence (3). According to several studies, the entry of pathogenic microorganisms like *Uropathogenic Escherichia coli (UPEC)* into the human urinary tract activated the immune system's innate and adaptive immune mechanisms. The urothelial cells serve as sensitive biosensors that trigger proinflammatory cytokines and other immune responses in the presence of pathogens. Additionally, the level of urinary tract infection severity, from benign to fatal and acute to chronic, is strongly correlated with the microbial virulence factors and the quality of the host's immune responses (4).

Materials and methods

From October 2022 to January 2023, women with urinary tract

infections who visited AL-Yarmouk Hospital in Baghdad and healthy controls provided a total of 200 (blood and urine) samples. Urine samples were taken from females with a mean age of 30 to 60, and they were cultivated on (nutrient agar, macconkey agar, and EMP) before being examined under a microscope to find any microorganisms. Api and Vitik biochemical tests were then utilized to determine whether any gram-negative bacteria were present.

Media

The agar in the medium was chosen to support microbial growth, and it was prepared in accordance with the manufacturer's instructions before being autoclaved at 121°C and 15 Ib/In2 of pressure for 15 minutes to sterilize it.

Isolation of uropathogenic gram negative bacteria(5)

The colonies were then subjected to additional identification tests after being subcultured on EMB agar for further identification and incubated at 37°C for 24 hours. The urine sample was cultured on the media (blood agar and macconkey agar) by taking a loop under heat and cooling on blood agar by stricking from sample, and two possibilities on the macconkey agar (lactose fermented and non-lactose fermented colonies).

Identification of uropathogenic gram negative bacteria

Morphological characteristics (6)

After Gram staining the colonies and examining their morphological properties (size, color, shape, organization, and spore development) under a microscope, colonies that could grow on specific media were further identified.

Biochemical tests

The following biochemical assays were completed with the purpose of further identifying particular isolated colonies:

A. Indole production test (7)

Fresh cultures of all possible colonies were added to test tubes containing peptone water broth, and they were then incubated at 37°C for 24 hours. After adding 0.5 ml of Kovac's reagent, a positive outcome is indicated by the appearance of a red ring on the medium's surface.

B. Methyl red test (6)

Fresh cultures of each suspicious colony were added to test tubes containing MR-VP broth, and the test tubes were then incubated at 37°C for 24 hours. Five drops of the methylred reagent were then added, stirred, and readings started to appear right away. Bright red color is a sign of a favorable response.

C. Voges-Proskauer test (6)

Fresh cultures of each suspicious colony were used to inoculate test tubes containing MR-VP broth, which were then incubated at 37°C for 24 hours. After that, (5ml) of bacterial culture was mixed for 30 seconds with (1ml of VP1 and (3ml of VP2) before being added. A successful outcome is indicated when the color transitions from pink to red.

D. Citrate utilization test (6)

Fresh cultures of each putative isolate were stabbed into the bottom of Simmon's citrate slants to serve as an inoculum, which was then incubated at 37°C for 24 hours. If the medium changes from green to blue, it means the experiment was successful.

E. Triple sugar iron test (6)

Fresh cultures of each putative isolate were stabbed into the bottom of test tubes with triple sugar iron slants, and the test tubes were then incubated at 37°C for 24 hours. The results were then recorded as follows:

(At an Angle/Bottom Color)

Alkaline, acidic, and yellow.

Acid Yellow/Acid Red/Yellow

(Red / Alkaline / Alkaline)

When agar is forced to the top, black precipitation forms, which shows that CO_2 is being formed as well as H_2S being produced.

F. Oxidase test (8)

Selected colonies were transferred with a wooden stick to filter paper that had been combined with a few drops of an oxidase substance. Positive results are shown when a rich purple colour forms on filter paper within 30 seconds.

G. Catalase test (8)

Selected colonies were put onto a clean slide using a wooden stick. A few drops of catalase reagent (3%) H_2O_2 were then added, and everything was mixed. The emergence of gas bubbles denotes a successful outcome.

Uropathogenic gram negative bacteria were also identified using the API 20 E system and Vitik

Molecular diagnosis

The *16S rRNA* gene was identified via PCR amplification. (Table 1) shows the bacterial isolates that were determined to be *E. coli* using certain phenotypic traits and tested by PCR using a specific primer for *16S rRNA*. The primer amplify area containing 228 bp of the *E. coli 16S rRNA* gene segment was highly conserved across species. (Table 2) shows an example of PCR programming.

Table (1): Primer used for detection of *E.coli* bacteria by 16s rRNA

Primer sequence (5´- 3´)				
16SrRNA* for *E.coli	**F**	GACCTCGGTTTAGTTCACAG	370 bp	(9)
	R	CACACGCTGACGCTGACCA		

Table (2): PCR program for amplification of 16S rRNA gene

Cycle No.	Stage	Temperature	Time
1	Initial Denaturation	94 C°	5 mins.
38x	Denaturation Annealing Extraction	94 C° 55 C° 72 C°	30 sec. 45 sec. 45 sec.
1	Final Extraction	72 C°	7 mins.

Maintenance of uropathogenic gram negative bacteria isolates

Maintenance of bacterial isolates was performed according to (10)

A. Short-term storage

Bacterial isolates were kept alive for a short period of time on MacConkey agar plates that were tightly coated in Para film and kept at 4C°.

B. Medium-term storage

By streaking on brain heart infusion agar slants and storing the container at 4°C with (5-8 ml) of the medium, bacterial isolates were kept alive for a few months.

C. Long-term storage

Brain-heart infusion broth was inoculated with a single colony and cultured at 37°C for 24 hours. Cell suspension (8.5 ml) and glycerol solution (1.5 ml) were also combined, and the mixture was then stored at -20°C.

Haematology and biochemical test for detection of recurrent urinary tract disease

For the complete blood count (CBC) test, 1.5 cc of blood samples were used. The following clinical parameters were assessed:

Complete blood count (CBC) test

The Complete Blood Count test was performed to determine the number of each cell type, platelets, and hemoglobin content in the blood. It provides the healthcare provider with details about the blood and general

health. CBCs aid medical professionals in the diagnosis, surveillance, and screening of a variety of illnesses, ailments, disorders, and infections.

According to age and gender as shown in (table 3) (which explains the test technique), results were (recorded) in that order.

Table (3): Protocol of Complete Blood Count test.

Sample	Cells	Normal Value	Assay
EDTA blood sample	White Blood Cell (Leukocyte)	4.0-10.0 billion cells/L	The test was performed automatically in accordance with the production protocol by entering the patient's data into the device and inserting the sample tube within the probe before pressing the start button. The findings were then displayed automatically.
	NEU	39.3 - 73.7	
	LYM	18 - 45.3	
	MON	2 – 8	
	EOS	7 – 6	
	BASO	0.0 – 1.70	
	Red Blood Cell (Erythrocytes)	3.80 - 5.80 trillion cells/L	
	Hemoglobin	11.00-16.50 grams/dL	
	Platelet Count	150- 450 billion/L	

Renal function test

For the chemical analyzer with photometric and electrolyte testing capabilities, the renal function (RFT) test was applied. a test where the levels of specific chemicals secreted by the kidneys are measured in blood samples. The results are shown in (Table 4) according to gender and age.

Table (4): Protocol of Renal function test

Sample	Normal Value	Westergren (mm/hour)
RFT sample	Urea	15-45 mg/dl
	Creatine	0.57-1.25 mg/dl
	BUN	8.8-22.8 mg/dl

Results and discussion

Subjects

In this study, 200 samples were taken from women at Al-Yarmouk Teaching Hospitals in Baghdad between October 2022 and March 2023 who were thought to have a urinary tract infection. The samples were taken from females with a mean age of 30 to 60, and the results were displayed in (Table 5), showing that 120 of the female subjects felt pain from a urinary tract infection and were diagnosed as having been infected with gram negative bacteria. Subsequent investigation and follow-up of the subjects for a minimum of three months revealed that approximately 50 of the subjects had a urinary tract infection. The study eliminated women with recurrent illnesses including diabetes and autoimmune disease from the control group, which was made up of healthy women with a mean age of 30 to 60 years old.

Table (5): The total number of clinical samples for UTIs

Suspected women with UTI	Women with UTI	Women with recurrent UTI	Health control women
200 with 50 years old	120	50	100

Identification of gram-negative bacteria

Morphological and microscopic characterization

About 200 urine samples were collected for this investigation, and each sample was subjected to morphological and microscopic analysis in order to determine whether samples were contaminated with gram-negative bacteria.For morphological examination depending on morphological characteristics, all samples were cultured. on nutrient and MaConky agar plat,)the results presented that just about 150 samples were positive culture on MaConky agar. Microscopically examination for these isolation showed the gram-negative bacteria appeared as oval shaped organisms, pink rods, short to medium length, straight or slightly curved, slender and non-sporulating occurring as pairs or) singled.

Biochemical test

About 120 isolates with growth on MaConky and EMB agar and closely related morphological characteristics to gram-negative bacteria were successfully subjected to biochemical tests. Methyl-red, Voges-Proskauer, citrate, oxidase, and catalase were among the biochemical tests used in this investigation. The outcomes of the biochemical tests are presented in (Table 6).

Table (6): Shows the outcomes of biochemical tests for gram-negative bacteria found in urinary tract infections

Biochemical Test	Indole	Methyl Red	Voges-Proskauer	Citrate	Oxidase	Catalase
Uropathogenic Escherichia coli	+	+	-	-	-	+
Klebsiella	-	-	+	+	-	+
Enterococci	-	-	+	-	-	-
Proteus	-	+	-	+	-	+

Therefore, *E. coli* demonstrated a positive reaction to indol, methyl-red, and catalase while a negative reaction to voges-proskauer, citrate, and oxidase was indicated. While *Klebsiella* had negative reactions to indole, methyl-red, and oxidase, positive reactions to voges-proskauer, citrate, and catalase were identified. Additionally, whereas voges-proskauer suggested a positive reaction, *Enterococci* displayed negative reactions to indole, methyl-red, citrate, oxidase, and catalase. Finally, *Proteus* displayed a negative reaction to indole, voges-proskauer, and oxidase while indicating a positive reaction to methyl-red, citrate, and catalase. One of the most prevalent illnesses in the world, particularly in women, is urinary tract infection. This infection is brought on by several microbial agents that enter the urethra via the skin or rectum and spread to the urinary tract. There are numerous factors, such as gram-negative bacteria, gram-positive bacteria, and fungus, that can cause UTI infections in women. The tests Vitiks and API 20 were then performed to ensure that the results were consistent.The findings revealed that various gram-negative bacteria were isolated and diagnosed as the cause of the UTI infection. According to (table 7), Escherichia coli accounted for 96 (80%) of these isolates, *Klepsiala* for 10(8%), *Enterobacterasea* for 10(8%), and *Protease* for 4 (3%).

Table (7): Types and frequency of microbes isolated from urinary cultures

Rows	Bacteria	Numbers
1	*Escherichia coli*	96 (80%)
2	*Klebsiella*	10 (8%)
3	*Enterococci*	10 (8%)
4	*Proteus*	4 (3%)

Molecular study

One selected UPEC isolate was used for total genomic DNA extraction in order to undertake a genetic detection of virulence and genes in UPEC isolates from UTIs patients. The collected DNA was then sent to Polymerase chain reaction for detection, and the diagnosis of this isolate was validated using *16srRNA* primers designed to detect *E.coli* bacteria. After electrophoresis of PCR product from UPEC isolate on agarose gel (0.1%), the result revealed DNA bands representing chromosomal DNA with size 370 bp, as illustrated in (Figure 1).

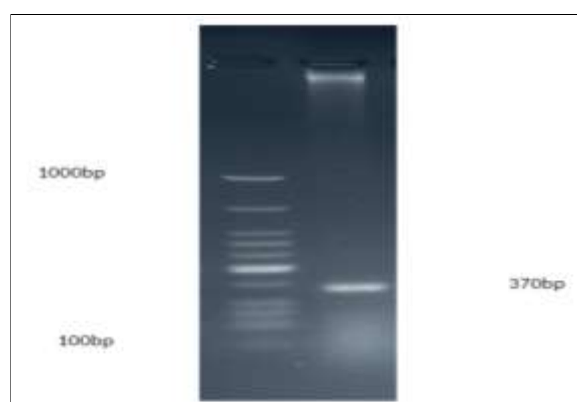

Figure (1): line 1 : DNA lader & line 2 : E.coli, Genomic DNA bands from UPEC isolates on agarose gel electrophoresis (0.1 % agarose gel, at 70 V, 1 h). Shown under UV light aRer staining with ethidium bromide

Laboratory test of the women study groups

(Table 4) displays the results of all women's laboratory tests for acute UTI. White blood cells, lymphocytes, platelets, mean platelet volume, C-reactive protein, urea, creatinine, and blood urea nitrogen are all included in the laboratory test.

Table (4): Different groups for UTI women (control and infected) with significant and p-value

Group / Parameter	Normal Range For Healthy Cases	Healthy Control group (n=100) mean±SE	Chronic UTI women group (general group) (n=120) mean±SE Patients	T-test	P-Value
WBC 10^3/ul	4-10	6.33±0.27	9.59±0.29	5.55	P≤0.05
LYM 10^3/ul	1.09-2.99	1.77±0.08	1.82±0.11	0.328	P>0.05
PLT 10^3/ul	155-450	303.5±15.9	284.1±15.9	-0.875	P>0.05
MPV (fl)	6.9-10.6	6.80±0.24	10.42±0.26	10.08	P≤0.05
CRP	0-5	4.0±0.23	15.72±0.26	-0.797	P>0.05
Urea	15-45 mg/dl	33.87±1.18	35.10±1.78	0.572	P>0.05
Creatinine	0.57-1.25mg/dl	0.86±0.04	1.09±0.07	1.726	P>0.05
BUN	8.8-22.8 mg/dl	16.45±0.62	21.56±1.73	2.769	P≤0.05
P>0.05= N.S., P≤0.05= Significant					

The results showed that WBC, MPV, and BUN exhibited significant increases (p-value ≤ 0.05), while LYM, PLT, CRP, urea, and creatinine showed non-significant increases (p-value ≥ 0.05). Another study found no statistically significant differences between individuals with Escherichia coli UTI and those without Escherichia coli UTI in the frequency of aberrant laboratory test results. The most frequent abnormal urine analysis findings were granular cylinders, proteinuria, leukocyturia, and cloudy urine. It is crucial to diagnose UTIs with and without Escherichia coli early in order to administer the proper empirical antibiotic treatment. This is crucial for preventing complications, extended therapy, recidivism, irreparable kidney damage, and chronic sickness (15). White blood cells observed (9.59±0.29) for chronic UTI women, while control group was (6.33±0.27). The results showed significant different between acute group with healthy control group (p-value ≤ 0.05)

On the other hand, the results showed that there was non-significant difference between chronic UTI women group and healthy control, which is for chronic group was (1.82±0.11) and healthy control group (1.77±0.08) p-value ≥ 0.05. Platelet count results showed also non-significant different between chronic UTI women group and healthy control (p-value ≥ 0.05). Mean platelet volume (MPV) results showed that there was non-significant difference between chronic group and healthy control (p-value ≥ 0.05) which is (10.42±0.26) respectively. The results of CRP in this study showed that significant difference between chronic UTI women and healthy control which is (15.72±0.26) and (4.0±0.23) respectively. In this study, it was found non-significant different in urea level in all patient group and healthy control group (p-value ≥ 0.05) (35.10±1.78) and (33.87±1.18) respectively. On other hand, there were non-significant different in the creatinine level between chronic group and healthy control was (p-value ≥ 0.05) which is (1.09±0.07);(0.86±0.04) respectively. Additionally, it was found that was significant different in BUN level between chronic UTI group and healthy control was (P≤0.05) which is (21.56±1.73);(16.45±0.62) respectively.

A few of them were lactose-positive pink colonies, which are what species that digest lactose will produce. Since lactose fermentation produces acidic byproducts that have a lower pH (9), the pH indicator turns pink as a result. (Escherichia coli, Enterobacteria, and Klebsiella) were lac positive species. Gram-negative bacteria will still form colonies even in the absence of lactose fermentation, but the colonies will appear white and there won't be any pH changes. Salmonella, Proteus, Yersinia, and Pseudomonas were lac negative species (11). On EMB agar, the majority of Escherichia coli strain colonies showed a distinctive green sheen. Rapid lactose fermentation and strong acid generation, along with a quick drop in the pH of the EMB agar, were major contributors to the development of the green metallic sheen that Escherichia coli was able to detect. Conversely, lactose non-fermenters may raise pH through protein deamination. This attests to the dye not being absorbed. The colonies will be light lavender or colorless, and other lactose non-fermenters are also colorless(12). The isolation and laboratory identification of the bacteria were necessary for the diagnosis of the gram-negative bacteria that cause UTIs. The samples were directly streaked on MaConky agar and Nutrient broth,

which underwent a 24-hour incubation period at 370°C. On MaConky agar, colonies had a rich purple appearance as a result of lactose fermentation. On this medium, it is likewise depicted as moist, flat and circular with a partial edge. Associated with gram-negative rods, MaConky agar was a selective plating medium used for the isolation of Enterobacteriaceae.)Due to the conversion of neutral red indicator dye below (pH 6.8), lactose-fermenting bacteria produce colonies that are colored in various hues of red (9). A loopful of lactose-fermented colonies were streaked on EMB agar plates for confirmative identification, and they were then incubated at 370°C for 24 hours. For the isolation and identification of Enterobacteriaceae from a variety of specimens, EMB agar was a selective medium. An indication of acid generation from lactose is created when the aniline dyes eosin and methylene blue in this medium combine to form a precipitate with a green metallic shine at an acidic pH (13). Gram-negative bacteria were predominant in 43 out of 51 patients' urine cultures, accounting for 84.3% of all patient cases. The renal ultrasonography revealed that 13.5% of the 51 patients who were tested had abnormal) results. With a high rate of 80–90%, distant Escherichia coli is the most often isolated bacteria in infants and children with urinary tract infections. It is followed by Enterococcus species, Enterobacter, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus mirabilis, and Staphylococcus spp (14). The Escherichia coli isolates produced positive findings in the indole synthesis test because they can hydrolyze tryptophan into indole by producing the tryptophanase enzyme. They also produced favorable results in the methyl red test. Simmon citrate utilization testing and the Voges-Proskauer test both yielded negative results (15). Using VITEK2 dense automated system testing, which included a number of the biochemical assays, additional confirmatory identification)of the negative isolates offered information to the species level was carried out, Escherichia coli isolates underwent more identifications in the API 20 E system (16). The most regularly isolated bacteria were *Escherichia coli* species. The most frequent causative agent of UTIs is Escherichia coli, followed by Klebsiella, but other significant species include salmonella, Entrobacter, Proteus, and the line in this investigation. Since the clinical history and urinalysis with confirmation by a urine culture, the diagnosis of UTI was dependent (17). In various studies, Escherichia coli was the predominant bacterium that causes UTI, accounting for between 41% and 56% of cases.)Enterococcus faecalis (7.7%) and K. pneumoniae (18.5%) were two more bacteria that could be identified. For each sex, age, and location, current data on the prevalence of urine pathogens must be considered while choosing an observed antimicrobial therapy (18).

The results of isolation in this study agreed, UPEC was shown to be the most frequent cause of UTIs (67.21%), followed by Proteus species (9.83%) and Enterococcus faecalis (7.37%), in 122 urine samples from females. The Enterobacteriaceae family was regarded as the most significant human pathogens responsible for UTIs. They make for around 50% of all the isolates found in hospital laboratories in the US (19). According to a study conducted in the Iraqi city of Zakho, out of a total of 205 positive UPEC strains recovered from UTI cases, (57%) of the isolates came from females and (43%)

from males. In individuals older than 18 and younger than 60, UPEC isolate prevalence was high. Also a study in a Al-Karkh Surgery Hospital in Baghdad city found that of the 311 urine samples taken from patients with UTIs, 100 isolates from female patients and 25 isolates from male patients (68.75%) were returned to UPEC.A second study conducted in Iraq at the AL-Yarmouk Teaching Hospital in Baghdad found that 43 uropathogenic Escherichia coli isolates were found in 129 urine samples taken from UTI patients. One of the main agents responsible for UTIs in Iraq was thought to be UPEC strains(20). The increased prevalence of UTIs in women is associated with perineal contamination by pathogenic Escherichia coli, which is thought to be one of the major pathways for UTIs in women. Additionally, a smaller portion of the urethra that was close to the excreta route was thought to be a significant source of infections. Immune cells known as white blood cells (WBCs) perform crucial roles in serious illnesses such cancer, infections, and inflammatory disorders. White blood cells (WBCs) play a crucial function in the human immune system, and while the body typically maintains a certain level of each subtype of WBC, aberrant numbers are a key indicator of disease(21). Twenty to forty percent of leukocyte counts are made up of lymphocytes, which are agranulocytes and a component of the adaptive immune system. Lymphocytes are circulating immunological competent cells that have the capacity to detect, respond to, and move across different lymphatic tissues. In a number of illnesses, including infections, variations in neutrophil and lymphocyte cell numbers had been linked to the severity of the sickness. T-lymphocyte subsets represent the immunological health of the patient and have been linked to worse outcomes in a variety of disorders. However, there was no statistically significant difference between any of the groups since the relationship between T-lymphocyte subsets and the primary infection and renal prognosis in individuals with chronic kidney disease has not been adequately explored (22). The PLT numbers were considerably greater in the patient group compared to the control group. The smallest blood cells, platelets, played a role in hemostasis and coagulation and were produced by bone marrow megakaryocyte cells. It has been investigated how platelet factors affect many diseases and how these parameters affect uti infections, too. The MPV, or mean platelet volume, measures the platelet's stimulation and production rate. The utilization of platelet characteristics, particularly mean platelet volume (MPV), as an indicator of the immune system's inflammatory response during infections has been discovered.When there is a significant increase in MPV, platelet activity actually increases. The MPV was a valuable marker in the identification of viral and inflammatory disorders, according to earlier studies (23). Liver cells produce the pentameric acute-phase protein known as C-reactive protein. This protein's nineteen-hour half-life enables fast increases and decreases in its levels depending on the state of inflammation. CRP levels in the blood are normally less than 1 mg/L in healthy individuals, but they can rise to 100 times higher in acute inflammation(24).

In order to improve clinical outcomes, biochemical indicators are crucial for accurate diagnosis, risk assessment, and therapeutic implementation. The frequent use of serum analysis of renal function

markers such urea, creatinine, electrolytes, uric acid, and blood urea nitrogen has replaced urine testing, which was generally uncomfortable for the patient. The kidneys are responsible for excreting creatinine, which is a breakdown product of creatine phosphate in muscle, as well as a major nitrogenous end result of protein and amino acid catabolism. Urea and creatinine are effective markers of healthy kidney function, while increases in serum levels are signs of renal failure. The most common and commonly used markers to assess renal function are BUN and serum creatinine. The main indicators used to assess renal function are blood creatinine and urea nitrogen, and they are crucial in determining the severity. Males and females with lower creatinine readings in youngsters and those with decreased muscular bulk had different creatinine ranges. The glomerulus filters creatinine, and the serum creatinine level is used as an indirect indicator of glomerular filtration. Reduced glomerular filtration rate causes an increase in serum urea and creatinine. Since this increase denotes kidney disease development, serum creatinine has a better predictive capacity than urea for predicting unfavorable outcomes (24).

Conclusion

The various gram-negative bacteria were isolated and diagnosed as the cause of the UTI infection, that *Escherichia coli* accounted for 96 (80%) of these isolates, *Klepsiala* for 10(8%), *Enterobacterasea* for 10(8%), and *Protease* for 4 (3%). Also the results of all women's laboratory tests for acute UTI. included White blood cells, lymphocytes, platelets, mean platelet volume, C-reactive protein, urea, creatinine, and blood urea nitrogen are all included in the laboratory test. The *16SrRNA* for *Escherichia coli* that good method for diagnosis.

References

1. Mancuso, G.; Midiri, A.; Gerace, E.; Marra, M.; Zummo, S. and Biondo, C. (2023). Urinary Tract Infections: The Current Scenario and Future Prospects, Pathogens., 12(4): 623.
2. Yaaqoob, U. R. H. L. A. (2022). Antibacterial Activity of Cobalt and Titanium (Co: Tio2) Core-Shell Nanoparticles against *E. coli* Isolated From Urinary Tract Infections, Iraqi Journal of. Biotechnology., 21(2).
3. AL-Nasrawi, M. M. and AL-Hashimy, A. B. (2020). Molecular Study of Some Virulence Genes of *Escherichia coli* Isolated from Women with Urinary Tract Infection in AL-Najaf City, Iraqi Journal of Biotechnology., 3(19).
4. Shah, C.; Baral, R.; Bartaula, B. and Shrestha, L. B. (2019). Virulence factors of uropathogenic *Escherichia coli (UPEC)* and correlation with antimicrobial resistance, BioMed Central Microbiology., 19 :1–6.
5. Forbes, B. A., Sahm, D. F. and Weissfeld, A. S. (2007). Diagnostic microbiology, *Mosby St Louis.5(1) 842-855.*
6. Atlas, R. M.; Brown, A. E. and Parks, L. C. (1995). Laboratory manual of experimental microbiology. *Mosby St Louis*.
7. Ali, B. M. (2009). Identification and Sensitivity of Aerobic Bacteria Isolated from Urine of Chronic Renal Failure Patients. *Doctoral dissertation, Department of Microbiology, University of Khartoum.*6(5).
8. Garrity, G. M. and Holt, J. G. (2001). The road map to the manual, *in Bergey's manual® of systematic bacteriology, Springer,* 119–166.
9. Ema, F. A.; Shanta, R. N.; Rahman, M. Z.; Islam, M. A. and Khatun, M. M. (2022). Isolation, identification, and antibiogram studies of *Escherichia coli* from ready-to-eat foods in Mymensingh, Bangladesh, Veterinary World, 15(6) : 1497.
10. Maniatis, T.; Fritsch, E. F. and Sambrook, J. (1982). Molecular cloning: a Laboratory Manual Gold Spring Harber Laboratory, *New York, Biotechnology*, 5(6): 257–261.
11. Piljić, D.; Piljić, D.; Ahmetagić, S.; Ljuca, F. and Porobić-Jahić, H. (2010). Clinical and laboratory characteristics of acute communityacquired urinary tract infections

in adult hospitalised patients, Bosnian Journal of Basic Medical Sciences., 10(1) : 49.

12. Vivijs, B.; Moons, P.; Aertsen, A. and Michiels, C.W. (2014). Acetoin synthesis acquisition favors *Escherichia coli* growth at low pH, Appled and Environmetal. Microbiology., 80 (19) :6054–6061.
13. Leininger, D. J.; Roberson, J. R. and Elvinger, F. (2001). Use of eosin methylene blue agar to differentiate *Escherichia coli* from other gram-negative mastitis pathogens, Journal Veterinary Diagnostic Investigation., 13 (3): 273–275.
14. Sokhn, E. S.; Salami, A.; El Roz, A.; Salloum, L.; Bahmad, H. F. and Ghssein, G. (2020). Antimicrobial susceptibilities and laboratory profiles of *Escherichia coli, Klebsiella pneumoniae,* and *Proteus mirabilis* isolates as agents of urinary tract infection in Lebanon: paving the way for better diagnostics, Medical Sciences., 8(3): 32.
15. Deshmukh, A.; Siddiqui, M.; Pathan, U. K. and Dhuldhaj, U. (2020). Microbial study of organisms isolated from nutritional fruit juices surrounded by local fruit market in Nanded, Maharashtra, India, Biodiversitas Journal of Biological., 21 (9).
16. Abo Kamer, A. M.; Amer, N. M.; Abdelmegeed, A. A.; El Maghraby, G. M. and Gamaleldin, N. M. (2023). Surfactant nanovesicles for augmented antibacterial activity against carbapenemase resistant enterobacteriaceae and extended spectrum beta-lactamases producing bacteria: in vitro and in vivo evaluation, BioMed Central Microbiology, 23 (1) : 1–14.
17. Lila, A. S. A.; Rajab, A. A. H.; Abdallah, M. H.; Rizvi, S. M. D.; Moin, A.; Khafagy, E. S., *et al*. (2023). Biofilm Lifestyle in Recurrent Urinary Tract Infections. Life (Basel, Switzerland), 13(1): 148.
18. Rostkowska, O. M.; Kuthan, R.; Burban, A.; Salińska, J.; Ciebiera, M.; Młynarczyk, G., *et al*. (2020). Analysis of susceptibility to selected antibiotics in *Klebsiella pneumoniae, Escherichia coli, Enterococcus faecalis* and *Enterococcus faecium* causing urinary tract infections in kidney transplant recipients over 8 years: single-center study, Antibiotics, 9 (6): 284.
19. AL-Jubouri, S. S., & Shami, A. M. (2022). Molecular Detection of Cephalosporin Resistance Genes in *Escherichia coli* Isolated from Urinary Tract Infections in Baghdad Hospitals. Iraqi Journal of Biotechnology, *21*(2), 145-152.
20. Wang, D.; Wang, S.; Zhou, Z.; Bai, D.; Zhang, Q.; Ai, X., *et al*. (2022). White Blood Cell Membrane-Coated Nanoparticles: Recent Development and Medical Applications. Advanced Healthcare Materials, 11(7): e2101349.
21. Xiong, J.; Qiao, Y.; Yu, Z.; Huang, Y.; Yang, K.; He, T., *et al*. (2021). T-Lymphocyte Subsets Alteration, Infection and Renal Outcome in Advanced Chronic Kidney Disease. Frontiers in Medicine, 8, 742419.
22. Budak, Y. U.; Polat, M. and Huysal, K. (2016). The use of platelet indices, plateletcrit, mean platelet volume and platelet distribution width in emergency non-traumatic abdominal surgery: a systematic review., Biochemia Medica, 26 (2): 178–193.
23. Sproston, N. R. and Ashworth, J. J. (2018). Role of C-reactive protein at sites of inflammation and infection, Frontier in Immunology, 9: 754.
24. Luo, H.; Yang, X.; Chen, K.; Lan, S.; Liao, G. and Xu, J. (2022). Blood creatinine and urea nitrogen at ICU admission and the risk of in-hospital death and 1-year mortality in patients with intracranial hemorrhage, Frontiers in Cardiovascular Medicine., 9: 967614.