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College of Veterinary Medicine
Department of Microbiology**

Biochemical and Bacteriological Profile of Urinary Tract Infection in Patients with Infective Stones.

Thesis

**Submitted to the Council of Veterinary Medicine College/University
of Shatrah as Partial Fulfillment of the Standards for a Master's
Degree (M.Sc.) in Veterinary Sciences (Microbiology).**

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بسم الله الرحمن الرحيم

﴿قُلْ هَلْ يَسْتَوِي الَّذِينَ يَغْلُمُونَ وَالَّذِينَ لَا يَغْلُمُونَ

إِنَّمَا يَتَذَكَّرُ أُولُو الْأَلْبَابِ﴾

(الزمر: ٩)

Declaration

I hereby declare that the thesis entitled (**Biochemical and Bacteriological Profile of Urinary Tract Infection in Patients with Infective Stones**) is my original work except for quotations and citations, which have been fully acknowledged. Also, I declare it is not previously and currently submitted for other degrees at the University of Shatrah or other institutions

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Dedication

,To my beloved parents

Your unconditional love, sacrifices, and ****Father **** and **** Mother**** constant support have been the guiding light throughout my journey. You have shaped not only my education but also the person I have become. For that, I am forever gratefull To my dear Family and friends Thank you for your encouragement, patience, and belief in me—even when I doubted myself. Your presence made the hard days bearable and the good days even better

Ahmed

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Examining Committee Decision

We are members of the examining committee read this thesis (Molecular Study of Some Virulence Genes in Pathogenic Bacteria Isolated from Urinary Tract Infection Patients in The North of Thi-Qar Province) and after examining the student (Ahmed Rashed Sajit) in its content, we found it is adequate for award Master Degree in the Veterinary Medicine / Microbiology.

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Summary

Urinary tract infections (UTIs) are among the most common causes of urolithiasis. The frequency of infected stones has shifted dramatically during the previous 20 years. Several studies have revealed a link between urinary stones and urinary tract infections. This study compares the prevalence, antibiogram, and bacterial species diversity in the urine of patients with urinary tract infection and with those of renal stone formers. Accordingly, 150 urine samples were collected from two groups (75 UTI group and 75 UTI with stone group, attending Thi-Qar hospitals during the period extending from March 2025 to May 2025. Also, 10 renal stone samples were collected for biochemical analysis. Additionally, 80 blood samples were also obtained to investigate the Renal Function Tests(RFT) (blood urea, creatinine, and serum uric acid). Bacterial isolation and identification were conducted using traditional bacteriological and biochemical methods (IMViC, oxidase, catalase, and Gram staining), as well as molecular techniques (, 16S rRNA of *k pneumonia*, 16S rRNA of *E. Coli*, 16srDNA *S. aureus*, Urease proteus gene, OprL *Pseudomonas* gene). Antibiogram was assessed using the disc diffusion method.

The mean age $\pm$ SD of the entire study population was 38.8 $\pm$ 14.6, ranging from 13 to 68 years old. The mean age of the UTI group was 38.9 $\pm$ 14.1, while the average age of the renal stone former group was 39.2 $\pm$ 15.6.

Statistical analysis showed that there is no significant differences ($P\geq 0.05$) existed between the UTI group and renal stone former group for the three variables (age, sex, and residency). Bacteriological investigation revealed that only 53 (35.3%) showed positive bacterial growth on

culture media., Six bacterial species were successfully identified, with *E. coli* being the most prevalent, 30.19%, followed by *K pneumonia*, 22.6%, then *P. aeruginosa* (16.98%), and *S. aureus* 15.09% , *Protius . mirabilis* (9.4%), . The bacterial recovery rate in the UTI group was 38.7%, slightly higher than the renal stone group 32%. However, statistical analysis show no significant difference ($P>0.05$).

The antibiotic susceptibility assay showed among the 53 bacterial isolates, amoxicillin / clavulanic Acid and sulfa +trimethoprim were the lowest effect while amikacin, cefotaxime, ciprofloxacin, gentamycin, levofloxacin have moderate effect, however meropenem was the highest Susceptibility, moreover Out of 10 renal stones subjected to chemical analysis, three types (CaOX, Amorphous urate, and Amur+Uric acid stone) were identified, 6 (60%) were classified as infective stones. molecular techniques (, 16S rRNA of *k pneumonia*, 16S rRNA of *E. Coli*, 16srDNA *S. aureus*, Urease proteus gene, OprL *Pseudomonas* gene).were detected which was for 16S rRNA of *E. Coli*, (, 16S rRNA of *k pneumonia* (100%) for each studied samples , 16srDNA *S. aureus*, ureR gene, OprL *Pseudomonas* gene(20%,20%,10%) respectively

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List of Abbreviations

Abbreviations	Meanings Brain
µg	Micro Gram
BA	Blood agar
BHI	Brain Heart Infusion Percentage
BUN	Blood urine nitrogen
Ca_{ox}	Calcium oxalate
CLSI	Clinical & Laboratory Standards Institute
Co	Celsius Degree
CRP	C-reactive protein
DNA	Deoxyribonucleic Acid
ESBLs	Extended-Spectrum β-lactamaseLuria
ESR	Erythrocyte sedimentation rate
ESWL	Extracorporeal Shock Wave Lithotripsy
IL-6	Interleukin-6
MA	Mackonkey agar
MR	Mthyl red
PCR	Polymerase Chain Reaction Potential
PCT	Procalcitonin
UTIs	Urinary Tract Infections Ribosomal
V	Volt
VB	Voges-proskauer
XDR	Extensive Drug Resistance

Chapter One

Introduction

1.1 Introduction

The incidence of stone disorders, particularly kidney stones, has been reported to be growing globally, and these increases are evident across sex, age, and race. Urolithiasis is the third-most common urinary tract disorder. It has been a concern for humanity from the beginning of history(Ranjit and Singh , 2020). On the other hand, urinary tract infections (UTIs) are among the most prevalent bacterial infections in clinical practice, posing a significant health and economic burden on healthcare systems worldwide. A significant and clinically challenging subset of UTIs is associated with infective (or infection-induced) urinary stones (Alwan *et al.*, 2023). These stones form as a direct result of microbial activity, primarily by urease-producing organisms like *Proteus mirabilis*, *Klebsiella* spp., *Pseudomonas* spp. These pathogens hydrolyze urea into ammonia and CO₂, causing an increase in urine pH and the subsequent precipitation of magnesium ammonium phosphate (struvite) and calcium phosphate crystals, which aggregate to form calculi.(Flannigan *et al.*, 2014). Infective stones can be identified by their quick growth and proclivity to create enormous, branching structures known as staghorn calculi. These stones not only complicate surgical and medical therapy, but they also provide a haven for persistent bacterial colonization, which promotes recurring and chronic infections. This persistent microbial reservoir considerably raises the likelihood of serious consequences such as pyelonephritis, sepsis, and progressive renal parenchymal damage, which eventually leads to chronic kidney disease(Ali S. H. and Rifat, 2005). From a biochemical perspective, higher urease activity and increased ammonia concentration in urine are significant indicators associated with this form of UTI. Microbiological investigation often indicates positive urine cultures dominated by urease-positive

Chapter One introduction

bacteria with varying antimicrobial resistance patterns, especially in individuals with recurrent infections or previous drug exposure. Clinical problems include correct diagnosis, selecting suitable antibiotic therapy based on culture and sensitivity profiles, and preventing recurrence by metabolic and environmental control (Ranjit and Singh, 2020). Therefore, this cross sectional study investigates the biochemical and bacteriological profiles of urinary tract infections in patients with infective stones. By identifying the predominant microbial agents, their antimicrobial susceptibility patterns, and the underlying biochemical factors promoting stone formation, this research seeks to support evidence-based therapeutic strategies, improve clinical outcomes, and reduce the rates of recurrence and associated complications in this high-risk patient population, this aim was preformed by the flowing objectives which are ..

1.2 objectives

1. Finding the association between bacterial infections and types of stone
2. Prevalence of infectious stone
3. Bacterial Profiling the UTI with and without stone
4. Evaluate the effect of urinary tract infection caused by an infective stone on renal function by assessing key indicators such as serum creatinine, and blood urea nitrogen (BUN)leve

Chapter Two

Literature

Review

2. Literature review

2.1 Urinary tract infections (UTI)

Urinary tract infections (UTIs) are considered as the most serious and prevalent types of bacterial infections worldwide (Polse *et al.*, 2020), the infected urinary tract parts coming in different region and symptoms, Upper and lower urinary tracts. Cystitis (bladder infection) or pyelonephritis (kidney infection) are the names given to the infection based on the portion of the body affected. The symptoms associated with bladder and kidney infections are varied, including painful and frequent urination in the case of cystitis caused by a bladder infection (Al-joudi *et al.*, 2025). Bacteria are the principal organisms that cause UTI, and gram negatives cause 80-85% of infections, with *Escherichia coli* being the most frequent pathogen. But, in complicated UTIs the prevalence is for other antibiotic resistant bacteria such as *Klebsiella* and *Proteus* (Tawfeeq, *et al* 2014), Urinary tract infections induced by urease-producing bacteria can lead to kidney stones. Another factor to consider in urinary tract infections is the existence of a biofilm layer composed of microorganisms. These bacterial colonies adhere to inanimate surfaces, such as kidney stones,. Infections caused by bacteria that form a biofilm layer not only exhibit high resistance to treatment but also accelerate the formation of stones (M. Mohammed *et al.*, 2024). Urinary tract infections (UTIs) can impact individuals of any age, regardless of sex. A significant contributor to mortality is prostate infection, which predominantly affects older men, complicating bladder function and causing inflammation of the urinary system The primary causes of urinary tract infections in elderly women are hormonal changes brought on by ageing, immunosuppression pregnancy, and chronic conditions like diabetes or cancer, or HIV(human immunodeficiency virus) which cause

immunodeficiency and weakened immune systems. All these factors increase the risk of urinary tract infections (Alwan *et al.*, 2023)

2.1.1 Definition and classification

Urinary tract infection is one of the most common diseases worldwide (Saleh, 2024) Urinary tract infections can affect any part of the urinary system, including the kidneys, ureters, bladder, or urethra. Most infections affect the lower urinary tract, including the bladder and urethra. Women are more prone to urinary tract infections than men(. Hasan *et al.*, 2025). While a variety of variables contribute to UTIs, uropathogenic bacteria are the most commonly isolated species globally. Gram-negative bacteria, namely members of the Enterobacteriaceae family, are a major cause of UTIs. These infections are difficult to cure because of the high annual infection incidence and imprecise UTI diagnosis. UTIs are categorized into two categories: "**uncomplicated**" UTIs, Uncomplicated urinary tract infections (uUTIs) are one of the most frequent community-acquired illnesses, especially among women. A UTI is considered simple if there are no functional or anatomical abnormalities in the urinary tract and no renal functional impairment.t(Wagenlehner *et al.*, 2011), There is no concurrent condition that would lead to the UTI. The most common UTI symptoms are dysuria, increased urinary urgency and frequency, and lower abdomen pain. Symptoms may resolve within a few days with proper therapy. However, little research has been done on the emotional effects of this condition. The study conducted a qualitative interview-based study to better understand the emotional impact of uUTIs in women from China and Japan(Yun *et al.*, 2024). Which occur in healthy adults, non-pregnant women over the age of 14, with no abnormalities in the kidneys or urinary system, and "complicated" UTIs, which include males and all other women (Kamel and Ali, 2024) Antibiotic-resistant

bacteria such as *Klebsiella* and *Proteus* are frequently seen in complex urinary tract infections. However, *Escherichia coli* and other Enterobacteriaceae bacteria continue to be the most common causes of community-acquired illnesses. Their rising multidrug resistance in recent years has had a substantial impact on the prognosis and survival rates of many UTI patients (Tawfeeq, 2014). Gram-negative germs such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* are typically responsible for complex UTIs, which might include pyelonephritis, urine retention or obstruction in men, and urological abnormalities. These bacteria often exhibit multidrug resistance (MDR), especially extended-spectrum β -lactamases. Because of the failure of first-line treatments, the use of carbapenems has increased, leading to resistance (Sivanandy et al., 2024).

2.1.2 Epidemiology

Urinary tract infection (UTI) is one of the most common bacterial infections. UTIs account for 0.7% of physician office visits and 1.8% of emergency room visits in the United States, resulting in approximately 2.3 million visits per year. Because antibiotic therapy is effective, the fatality rate from complex UTI is minimal. In the United States, the age-standardized death rate is calculated at 3.44 per 100,000 people (Foxman et al., 2025). Urinary tract infections (UTIs) are common in both the community and health-care settings, with around 3 million people seeking medical attention for them each year. Severe UTIs can cause renal failure and sepsis. Although antibiotics remain the primary treatment for UTIs, they may not completely prevent recurrence and pose the risk of multidrug resistance (He et al., 2025). It is anticipated that bacterial antimicrobial resistance (AMR) in UTIs caused 0.26 million deaths, costing society an estimated \$3.5 billion annually. UTIs impose a huge

economic burden, disrupt daily life, and create psychological stress. More than half of people who get UTIs experience psychological problems. Anxiety and despair are two such examples. As a result, UTIs pose an increasing concern to public health(Kebede *et al.*, 2025)

2.1.3 Risk factor

Urinary tract infection is the most frequent bacterial illness in the community and in hospitals among people of all ages. Urinary tract infections (UTIs) are among the most common bacterial illnesses seen in clinical practice. They can be classed as upper UTIs (pyelonephritis) or lower UTIs (cystitis and urethritis). UTI development is regulated by a complex combination of anatomical, functional, physiological, behavioral, microbial, and immunological variables. Anatomical elements have a substantial impact, especially in women. The female urethra's small length makes it easier for germs from the perineal and stomach flora to enter the bladder(Kebede *et al.*, 2025), Hormonal variants, particularly those connected with the menstrual cycle, pregnancy, and menopause, have an important role in predisposing people particularly women to urinary tract infections. Estrogen, in particular, is an important regulator of the health of the urogenital tract, regulating mucosal integrity, local immunological function, and the composition of the vaginal microbiome.(Abdullah *et al.*, 2024). Women with diabetes get more frequent and severe urinary tract infections (UTIs). People over the age of 40 are more likely to have UTIs(Khalawe Tektook *et al.*, 2017). Personal and familial history of nephrolithiasis, poor 24-hour urine production, unusually low or high urine pH, repeated urinary tract infection episodes, deformity causing urinary stasis, and stone formation (Dobrek, 2020).

2.2 Infective stones

Urinary stones are one of the most prevalent illnesses of the urinary tract, with their composition and production influenced by metabolic, environmental, and microbiological variables. Infective stones, also known as struvite calculi, are distinguished from other forms by their high relationship with chronic or recurring urinary tract infections(Wu *et al.*, 2023). These stones account for approximately 10-15% of all urinary calculi and are typically formed in an alkaline urinary environment generated by the enzymatic activity of urease, which is secreted by specific microorganisms such as *Proteus mirabilis*, *Klebsiella* spp., *Pseudomonas* spp., and *Staphylococcus* bacteria(Al-Zaidi *et al.*, 2025)

Infection stones, which account for 10%-15% of all urolithiasis, are a kind of urolithiasis generated by organisms that produce urease. It can rapidly grow into massive staghorn stones within 4 to 6 weeks, and struvite is usually regarded as an independent risk predictor for infectious-related disorders, such as sepsis, in patients receiving percutaneous nephrolithotomy(Wu *et al.*, 2023)

2.3.1. Types of urinary stones

2.3.1.1.Calcium stones

Calcium oxalate stones (CaOx) can exist in either monohydrate or dihydrate form. This differential is significant because monohydrate stones are associated with underlying hyperoxaluric illnesses, whereas dihydrate stones are associated with hypercalcaemia. The vast majority of patients with CaOx stones have no systemic ailment, hence they are classified as idiopathic CaOx stone formers (Viljoen *et al.*, 2019). Calcium oxalate (CaOx) crystal accumulation within the tubules is a

typical finding in renal biopsies from both the original and transplanted kidney. Understanding the underlying causes may help with diagnosis and subsequent care. The most common cause of CaOx crystal deposition in the kidney is hyperoxaluria (Geraghty *et al.*, 2020)

Although calcium-containing crystal deposition has been occasionally seen in the renal pathology of individuals with acute kidney injury, it has not received enough attention. A common form of crystalline nephropathy, renal oxalosis, sometimes called oxalate nephropathy, is characterized by kidney damage brought on by an excess of calcium oxalate (CaOx). These crystals cause interstitial inflammation and fibrosis, harm kidney tubular epithelial cells, and ultimately lead to the progression of the illness with poor kidney outcomes (Yang *et al.*, 2024). Calcium oxalate crystals growing over Randall's plaque sites appear to be the predominant source of stones in people with hypercalciuria. Overgrowths of Bellini duct plug extremities have been reported in most stone phenotypes; do these overgrowths result in clinical stones? Microliths appear to form only in the dilated inner medullary collecting ducts of cystinuric stone formers. Finally, it is hypothesized that a number of tiny, yellow-looking, oval, smooth-looking calyceal stones are present when cystinuric stone formers produce their stones in free solution (Elgendy *et al.*, 2024)

2.3.1.2 Mixed or Struvite (Triple stones)

Infection stones, a type of urinary tract stone, are composed of minerals such struvite, carbonate apatite, and ammonium urate. Their etiology and formation methods are complex, with a strong association with urinary tract infections caused by urease-producing bacteria such as *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*,

Chapter Two Literature review

Staphylococcus, and Morganella. (Li et *al.*, 2024). It was discovered that *Proteus mirabilis* was a contributing factor in over half of all urease-producing infections. Urine pH rises as a result of bacterial urease breaking down urea into ammonia and carbon dioxide. Phosphates that precipitate to insoluble ammonium products—which typically manifest as big "staghorn" stones—are less soluble in alkaline urine (Dobrek, 2020). The infection causes the urothelium to secrete matrix material. This matrix offers the conditions and template necessary for the easy precipitation of carbonate apatite and magnesium ammonium phosphate in alkaline urine. When urea splits into ammonia and carbon dioxide, the resulting alkaline urine has sufficiently high supersaturation levels with the two salts from a physiochemical perspective. No precipitation of carbonate apatite or magnesium ammonium phosphate will occur in acid urine (Tiselius, 2011)

2.3.1.3.Uric acid stone

Uric acid is a consequence of purine metabolism; it is largely created by endogenous sources, with dietary purines contributing little to its production (Zheng et *al.*, 2024) Uric acid cannot be broken down in the human body and is removed mostly through the kidneys. For a variety of reasons, uric acid concentrations rise in the renal pelvis and crystallize, aggregating to form uric acid stones. High-throughput biotechnologies have eased the collection of omics data, shedding information on the pathophysiology, biomarkers, and potential therapeutic targets for many diseases (S. Xu et *al.*, 2024). It accounts for roughly 10% of kidney stones and has a high recurrence rate, since a person can get many stones in a single year. The recent increase in uric acid renal lithiasis could be due to the rising frequency of obesity and metabolic syndrome. As a result, uric acid renal lithiasis is a common, major public health issue.

Urinary alkalinizers, such citrate, and/or inhibitors of uric acid crystallization, like theobromine, can be administered orally to avoid uric acid renal lithiasis (Julià *et al.*, 2022)

2.3.1.4. Cystine stones

Cystinuria is a rare disease that results in the precipitation of cystine in the renal filtrate, which may cause acute kidney injury due to mechanical trauma (Noble and Kavanagh, 2024) Cystine stones are infrequent, accounting for 1% to 2% of adult urinary stones and up to 10% in pediatric instances. Cystine stones are more likely to require surgical intervention since they are normally hard and difficult to remove with shock wave lithotripsy. As a result, laser lithotripsy is the primary method for managing these stones. There have been few investigations on the dissolution of cystine stones using normal laser settings (Zeinelabden *et al.*, 2024) Other uncommon forms include those brought on by medications like guaifenesin, triamterene, atazanavir, and sulfa medicines, as well as hereditary abnormalities of enzyme metabolism. About 1% of all stone kinds are represented by this. Individuals(Mayank Bishnoi and Himadri Arya, 2024). Cystine stones are mainly caused by increased renal excretion of this extremely insoluble material. However, it is not commonly known that patients with cystinuria can produce stones made of a mixture of calcium oxalate and cystine, or even occasionally pure calcium oxalate (Martins *et al.*, 2002).

2.3.2. Pathophysiology of stone formation

Stone formation in the urinary system occurs due to a combination of biological and physicochemical mechanisms caused by oversaturated urine. This process is called "lithogenesis" and consists of multiple steps that occur sequentially or simultaneously (Kachkoul *et al.*, 2023). Renal stones (also known as nephrolithiasis or kidney stones) are caused by a complex interaction of metabolic, anatomical, and environmental variables, which result in the production of solid crystalline aggregates within the kidney. These stones arise when urinary solutes become supersaturated and precipitate, causing crystal formation that may expand and consolidate into bigger stones (Papatsoris *et al.*, 2024). Furthermore, dehydrated people are more likely to develop kidney stones, which can lead to a decrease in fluid intake and dehydration (Al-Tulaibawi *et al.*, 2024). Hypercalciuria can indicate a renal stone-forming diathesis. It has been over 30 years since the discovery of a link between painless, microscopic hematuria and increased calcium excretion in urine (McKay, 2010). However, Randall's plaque is a calcium phosphate deposit in the basement membrane of the narrow loop of Henle, which serves as a nidus for calcium oxalate stone development (Grases *et al.*, 2025).

Calcium stone production involves multiple phases of increasing accumulation of CaOx and calcium phosphate (CaP), namely nucleation, crystal growth, crystal aggregation, and crystal retention (Basavaraj *et al.*, 2007). Aberrant crystallization inside the human body can result in a variety of disease states or negative outcomes, but much remains unknown about the essential steps preceding these events, which can include crystal nucleation and growth, crystal aggregation, and crystal adherence to cells. Kidney stones, an aggregation of single crystals having physiological origins, are particularly demonstrative of

pathological crystallization (Shtukenberg *et al.*, 2022). Small crystals come together to produce larger particles. Encouraged by low doses of crystal growth inhibitors, Crystals must be kept in the kidney to form stones. Adhesion to renal epithelial cells, particularly in sites of damage or plaque. Inflammation and cell damage enhance crystal binding .Anatomical anomalies (such as blockage or stasis) Trap crystals(Baumann, 2014). Under each of these parameters, the nucleation and aggregation processes differ dramatically, allowing the stone to aggregate and develop under a variety of environments, resulting in physical and chemical variability. During the development of stone genesis, significant and repetitive dissolution can occur, which may modify the stone matrix at the ultrastructural level(Manzoor *et al.*, 2019)

2.3.3. Association between stone and UTI

Stones in the ureter, bladder, or renal pelvis can impede urine flow, causing urinary stasis. This stationary environment encourages bacterial colonization and growth by providing a nutrient-dense medium with low clearance. Urinary retention reduces the urinary tract's normal flushing function, making it substantially more susceptible to infection (Srirangam *et al.*, 2008). The surface of urinary stones provides an ideal substrate for bacterial adhesion and biofilm formation. Biofilms are organized microbial colonies wrapped in an extracellular polymeric matrix that protects microorganisms from human immune responses and drug penetration (Yaseen *et al.*, 2024). In investigations have showed that common uropathogens such as *Escherichia coli*, *Proteus mirabilis*, and *Klebsiella spp.* can cling to stone surfaces, generating tenacious biofilms that complicate therapy and contribute to recurring infections (Issler *et al.*, 2017). A specific type of stone, known as "infection stones" or

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struvite stones (magnesium ammonium phosphate), forms as a direct result of urease-producing bacterial infections (Grases *et al.*, 2025). Organisms such as *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* produce urease, an enzyme that converts urea to ammonia and CO₂. This mechanism alkalizes the urine (raises its pH), facilitating the precipitation of phosphate salts with magnesium and ammonium to produce struvite crystals (Flannigan *et al.*, 2014). These stones often grow quickly and may form a "staghorn" shape, filling the renal calyces. Their presence is a characteristic of chronic or recurring UTIs, and, if not treated, can lead to progressive kidney impairment and ultimately renal failure (Li *et al.*, 2024).

2.4 Biochemical profile

Infective stones, also known as struvite or infection-induced stones, are a type of urinary calculi formed in the presence of urease-producing bacteria. These stones are mostly composed of magnesium ammonium phosphate (struvite) and/or calcium carbonate apatite. Their production is directly related to urinary tract infections (UTIs), which cause major metabolic and biochemical changes in urine and serum. Understanding the biochemical profile of individuals with infective stones is critical for correct diagnosis, successful therapy, and prevention of recurrence (Shah *et al.*, 2020). Urinary tract infections caused by microorganisms that produce urease, such as: *Proteus mirabilis* (most frequent), *Pseudomonas aeruginosa*, *Staphylococcus* species. However, microbial ureases degrade urea to ammonia and CO₂. Infectious microorganisms with urease activity can cause infections such as stones, pyelonephritis, peptic ulcers, and hepatic encephalopathy (Sujoy and Aparna, 2013). The biochemical profile of infected stones shows both local urine alterations and systemic

responses to prolonged infection. Urinary Biochemistry Urine PH is markedly elevated (>7.2) (Albrich *et al.*, 2024). Serum creatinine and blood urea nitrogen (BUN) levels may be increased in obstructive uropathy or chronic kidney disease (Jan *et al.*, 2008). Compared to the regular diet, urine calcium and phosphorus levels increased significantly (Reddy *et al.*, 2002). In addition, systemic symptoms, inflammatory markers such as C-reactive protein, erythrocyte sedimentation rate (ESR) are often elevated during active infection.

2.4.1. urine analysis

Humans' early medical diagnoses were based on what ancient physicians could see and hear, which occasionally included examining human specimens. The ancient Greeks related all ailments to physiological fluid problems known as humors, and doctors used uroscopy on a regular basis in the late Middle Ages. Ancient physicians also began to examine patient specimens. The oldest known test for body fluids was performed on urine about 400 BC. Urine was thrown onto the ground and observed to determine if it attracted insects. If this happened, individuals were diagnosed with boils (Ahmad *et al.*, 2019). A complete urinalysis with urine test strips yields semi-quantitative biochemical data such as glucose, protein, albumin, pH, bilirubin, and ketones. Many factors affect urine test results, including age, sex, food, and environment. A thorough urinalysis can also aid in the diagnosis of non-urinary conditions such as bacterial cystitis, protein-losing nephropathy, and transitional cell carcinoma. Urinalysis can also reveal endocrine disorders like diabetes and systemic diseases such as intravascular hemolysis (Yousefi Ghadikolaei *et al.*, 2024). Urinalysis procedures included physical examinations of urine color, turbidity, and odor, as well as chemical

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properties such as pH, specific gravity, protein, blood, glucose, ketones, leukocytes, and nitrites, as well as microscopic examinations of collected samples for the presence of (cells, casts, bacteria, yeast, parasites and crystals) according to standard procedures(. *Mohammed et al.*, 2024).however Urinalysis involving a macroscopic evaluation of color and appearance and a microscopic inspection for the presence of urine WBCs, RBCs, and microorganisms(*Alshomrani et al.*, 2023).

2.4.2.Biochemical marker

One of the most common indicators of UTI is the presence of white blood cells in urine (pyuria). It is identified through urine and represents a local immunological response to infection. WBCs are counted using a dipstick test for "leukocyte esterase" or by microscopic examination(*Parikh et al.*, 2025).however CRP levels rise in the blood during inflammation, which might include systemic UTIs such as pyelonephritis. CRP is utilized as an indicator of infection severity and systemic involvement(*Shi et al.*, 2022). CRP levels rise in the blood during inflammation, which might include systemic UTIs such pyelonephritis. CRP is utilized as an indicator of infection severity and systemic involvement(*Xu et al.*, 2014). Calcium, Oxalate, and Phosphate in Urine These ions are important components in stone formation. Measuring their amounts in urine is an important diagnostic tool for determining the risk of stone development. Major risk factors include elevated oxalate or calcium levels, as well as insufficient citrate (*Evan et al.*, 2007). Citrate prevents stone formation by blocking calcium crystallization. Low urine citrate levels (hypocitraturia) are significantly linked to an increased risk of calcium oxalate stones(*Bialystok*, 2017). Oxidative stress promotes stone formation. Stone formers' urine contains more malondialdehyde (MDA), a lipid

peroxidation result from free radicals, and less glutathione (GSH), a critical antioxidant(Mo *et al.*, 2025)

2.4.3.Role of PH and crystallization

Urinary pH has a substantial influence on the formation of many forms of kidney stones. An alkaline pH encourages the crystallization of calcium and phosphate-containing stones, whereas an acidic pH stimulates the development of uric acid or cystine stones. various transport pathways involved in the control of calcium, citrate, and phosphate are sensitive to fluctuations in systemic or local pH, as demonstrated by various phosphate transporters.(Wagner and Mohebbi, 2010) Moreover Crystallization is the fundamental process in stone building, and it is heavily regulated by urine pH. Understanding the relationship between pH and crystallisation is critical for creating effective prevention and treatment measures(Kok and Khan, 1994). On other hand Urinary pH is a crucial factor in kidney stone development. When the pH of the urine exceeds 6, calcium phosphate supersaturation increases rapidly . The prevalence of calcium phosphate stones has lately increased, and urinary disorders that predispose to the production of these stones include hypercalciuria and hypocalciuria in alkaline urine(Carvalho, 2018). Nucleation is the first phase, in which dissolved molecules congregate to form stable nuclei that exceed a crucial size barrier. Nucleation can be either homogeneous or heterogeneous. Supersaturation, increased ion concentrations, and aberrant pH all contribute to this process(Qiao *et al.*, 2024). Following nucleation, ions or molecules adhere to the crystal surface, resulting in growth. Growth rate depends on urine supersaturation, which rises with increasing ion concentrations or pH alterations(Srirangam *et al.*, 2008). Small crystals do not necessarily form

stones until they bind to the renal tubular epithelium. Studies suggest that epithelial cell damage or altered expression of adhesion molecules enhances crystal binding, allowing accumulation and stone formation(Tsujihata, 2008)

2.5. Bacteriological Profile

Urinary tract infections (UTIs) are among the most frequent bacterial illnesses encountered in both community and hospital settings, impacting millions of people each year(Vazquez-Montes *et al.*, 2024), Bacteria are the primary cause of UTI, and they can infect people of all ages. UTI is the second most common bacterial illness affecting children. Every year, around 150 million people become infected with UTI, incurring medical costs of at least \$6 billion(Salman *et al.*, 2022). Gram-negative bacteria, particularly *Escherichia coli*, are a leading cause of urinary tract infections. *Escherichia coli* belongs to the Enterobacteriaceae family of bacteria. It is thought that within a short period of time after birth, it becomes the predominant component of the typical gut flora of both humans and animals, who benefit from one another. Aside from being an innocuous pathogen, *E. coli* can also be a well evolved pathogen that causes a variety of illnesses ranging from intestinal to extra-intestinal disorders, such as meningitis, septicemia, and urinary tract infection(Ibrahim *et al.*, 2021). *Klebsiella pneumoniae* is a prominent pathogen in nosocomial UTIs, particularly in catheterized patients and diabetics. It is increasingly linked to extended-spectrum β -lactamases production(Polse *et al.*, 2020).while *Proteus mirabilis* is known for its capacity to manufacture urease, which results in alkaline urine and struvite stone formation. It is often linked to complex UTIs and urinary tract instrumentation (Al-fahham & Kareem, 2022). Additionally

Staphylococcus saprophyticus is a prevalent cause of simple UTIs in young sexually active women, accounting for 5-15% of such cases (Hussein *et al.*, 2019). Diabetes mellitus, neurogenic bladder and urinary tract blockages, catheterization, and antibiotic exposure are all for atypical risk factors or resistant bacteria (Panaretto *et al.*, 1999)

2.5.1. Common pathogens in UTI

Urinary tract infections (UTIs) are among the most common community-acquired illnesses, affecting 50-60% of adult women throughout their lives and 150 million people annually. Dysuria, increased frequency and urgency of pee, and lower abdominal pain are all common symptoms of a kidney infection. With effective treatment, UTI symptoms may resolve during the course of treatment (Yun *et al.*, 2024) UTIs can manifest in a variety of ways, from benign with little symptoms to potentially lethal. UTIs are characterized as either uncomplicated or complex (Marantidis and Sussman, 2023) The most common organisms causing UTIs in the community are *Escherichia coli* from the gastrointestinal tract, *Proteus*, *Pseudomonas* species, *Streptococcus*, and *Staphylococcus*. Other pathogens could potentially cause UTIs. In fact, *E. coli* is responsible for more than 90% of acute infections in persons with normal urinary tracts. The factors responsible for this extraordinary monopoly are incompletely understood, but the relative resistance of *E. coli* to the inhibitory effects of vaginal fluids, its possession of pili that aids its attachment to the epithelial cells of the urinary tract, and its motility appear to contribute to its effectiveness as a uropathogen (Mattle and Hess, 2005) *Klebsiella pneumoniae*, on the other hand, is a high-priority pathogen that causes both hospital-associated illnesses such urinary tract infections and community-acquired infections. Clinical isolates from community-

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acquired infections commonly have a tacky, hypermucoid look, but urinary tract isolates are rarely mucoid, Mucoidy has been connected to capsule overproduction; however, recent investigations have demonstrated that *K. pneumoniae* capsule abundance and mucoidy are not necessarily associated. It has been discovered that human urine lowers *K. pneumonia* mucoidy and diversifies capsule polys(Khadka *et al.*, 2023) however. *P. mirabilis* causes 1% to 10% of all UTIs. Urease mediates virulence by producing urinary stones. A urease mutant is incapable of generating stones, which has a direct impact on *P. mirabilis'* ability to cause UTIs (Schaffer and Pearson, 2016). the most common causes of infection worldwide. While *E. coli* is primarily associated with the etiology of urinary tract infections, other organisms such as *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus*, Bacteria (gram negative and gram positive), and certain fungi have been found to contribute in UTI (Salman *et al.*, 2022)

2.5.2. Antibiotic Resistance

Antibiotic resistance is one of the most important worldwide health concerns of the twenty-first century. It refers to the ability of microorganisms, particularly bacteria, to adapt and resist the effects of antimicrobial medications intended to kill them, rendering normal treatments useless. Antibiotic abuse and misuse in human health, veterinary practice, and agriculture has hastened the development and spread of resistance strains(Read and Woods, 2014). Antibiotic resistance develops when bacteria gain the ability to survive exposure to antibiotics that were previously successful in killing or slowing their growth. As a result, traditional therapies become ineffective, infections continue, and the risk of serious disease and death rises. It is critical to distinguish

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between microbiological resistance and clinical therapy failure. Resistance is a biological phenomenon affecting microorganisms, whereas treatment failure refers to the inability to cure an infection caused by resistant bacteria (Munita and Arias, 2016). Bacteria don't just give up when faced with antibiotics they fight back using ingenious biochemical and genetic tricks. One common strategy is enzymatic degradation, where bacteria produce enzymes that literally break down the drug. For instance, many bacteria make an enzyme called beta-lactamase, which destroys antibiotics like penicillin and cephalosporins, rendering them useless. Another tactic is modifying the drug's target. Antibiotics usually work by latching onto specific parts of the bacterial cell, like ribosomes or cell wall components. But resistant bacteria can change the shape or structure of these targets, making it hard—or even impossible—for the antibiotic to bind. It's like changing the locks on a door so the key no longer works. These sophisticated changes enable bacteria to survive treatment and continue causing infections, making antibiotic resistance one of the most significant concerns in modern medicine (Chinemerem Nwobodo *et al.*, 2022)

2.5.3. Microbiological Techniques for Identification

Urinary tract infections (UTIs) are predominantly caused by bacteria, with *E. coli* being the most common uropathogen (accounting for 70–95% of community-acquired cases) (Arinzon *et al.*, 2012). Proper specimen collection is critical for accurate microbiological diagnosis. Midstream clean-catch urine, catheterized specimens, and suprapubic aspiration are all popular collection procedures. Contamination must be reduced, and prompt processing is crucial to prevent pollutants from overgrowing. (Yun *et al.*, 2024), However the gold standard for diagnosing UTIs is quantitative urine culture on solid medium (e.g., blood agar, MacConkey

agar) (Aljebory and Mohammad, 2019). Biochemical identification techniques have long been the foundation of microbiological diagnostics in clinical laboratories. These approaches use microorganisms' metabolic and enzymatic features to distinguish and identify bacterial species. Despite the advancement of molecular and proteomic technologies, biochemical tests continue to be an important part of normal microbiological workflows, especially in resource-limited settings (Systems, 2013) on other hand the VITEK 2 is an automated microbiology system that uses growth-based technology. The system is offered in three configurations (VITEK 2 small, VITEK 2, and VITEK 2 XL), with varying levels of capacity and automation. All three systems use the same colorimetric reagent cards, which are incubated and evaluated automatically (Pincus, 2010)

2.6. Diagnosis of UTI in Stone Patients

The diagnosis of UTI traditionally relies on a combination of clinical symptoms, urinalysis (pyuria, nitrite positivity), and urine culture, however Bacteria embedded in biofilms on stone surfaces are rarely discharged regularly into urine, resulting in sporadic or negative cultures despite active infection. Studies have shown that culture of crushed stone fragments gives higher bacterial detection rates than voided urine, underlining the limitations of standard urine cultures in this setting (Song *et al.*, 2024), on other hand imaging has a dual purpose in identifying UTI in stone patients. Non-contrast CT remains the gold standard for stone identification, although contrast-enhanced CT or ultrasonography can identify complications such as hydronephrosis, perinephric abscess, or pyonephrosis, (McKay, 2010) which are signs of obstructive pyelonephritis. MRI (magnetic resonance image) is increasingly used in

pregnant and pediatric patients. The appearance of gas in the urinary system (pneumaturia or emphysematous pyelonephritis) on imaging is a red flag for serious infection, particularly in diabetics.(Dobrek, 2020), Serum indicators such as C-reactive protein (CRP), procalcitonin (PCT), and interleukin-6 (IL-6) can help diagnose systemic infection, especially in cases of suspected sepsis. Elevated PCT levels correspond with bacterial burden and severity, which helps distinguish between sterile inflammation and actual infection(Xu *et al.*, 2014),moreover failure to diagnose UTI in stone patients might result in severe results such as septic shock and death. In contrast, overdiagnosis can lead to inappropriate antibiotic treatment, which contributes to antimicrobial resistance. A balanced, evidence-based approach that includes clinical judgment and modern diagnostics is required(Marantidis and Sussman, 2023)

2.7 Treatment Strategies

Treatment of renal stones involves a personalized approach based on stone characteristics (size, location, composition), patient considerations (renal function, comorbidities), and available technical resources. Medical expulsive therapy appears to be effective in treating patients with ureteral stones who are receptive to conservative management. Those with ureteral stones measuring more than 5 mm (distal) may benefit the most Insufficient(Türk *et al.*, 2023),on other hand fluid intake can be useful in preventing both primary and secondary nephrolithiasis if properly implemented. Maintaining enough hydration and urine output levels is critical for avoiding recurring stone development(Wang *et al.*, 2022) other line of treatment which increases spontaneous stone passage, especially for distal ureteral stones (5-10 mm). Alpha-blockers (e.g.,

tamsulosin 0.4 mg daily) are the most often utilized, with meta-analyses demonstrating increased stone expulsion rates (RR 1.67) and shorter transit times (Türk *et al.*, 2023). Extracorporeal shock wave lithotripsy (ESWL), a non-invasive therapeutic method that uses shock waves, is widely considered the standard treatment for upper urinary tract and kidney stones. Because of its non-invasive nature, ESWL has rapidly gained popularity as an alternative to invasive procedures since its first documented use in 1984. The ESWL is the first-line treatment for kidney stones under 2 cm (Safdari *et al.*, 2024)

2.8 Prevention Measures

The core of renal stone prevention is to alter urinary risk factors that induce crystallization. Stone forming salts are supersaturated and have low quantities of crystallization inhibitors. (Griffith, 1978). Prevention options for abnormal urine pH include water, diet, and medication (Menezes *et al.*, 2019). A high diet of animal protein increases urinary calcium, decreases citrate, and lowers urine pH, which promotes uric acid and calcium stone development. Many research discovered that people who ate the most animal protein had a 33% increased risk of acquiring urinary calculi (Muhbes, 2012). Citrate inhibits stone formation by binding calcium and preventing crystal aggregation (McKay, 2010). Obesity and metabolic syndrome increase the risk of getting stones. Weight loss through diet and exercise reduces risk (Issler *et al.*, 2017). Prevention of renal stones is an essential component of long-term therapy. The combination of appropriate hydration, dietary changes, and targeted pharmaceutical therapy based on stone composition and metabolic profile dramatically lowers recurrence. A tailored approach, supported by 24-hour urine testing and interdisciplinary care, is required.

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Patient education and adherence are crucial to success. Future research into gut microbiota, genetic risk assessment, and new medicines could improve preventive methods(Zhao *et al.*, 2024)

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Chapter

Three

Materials

and methods

3. Materials and Methods

3.1 Materials

3.1.1 Lab equipment Table (3-1): General laboratory equipments and instruments in the present study

NO	Instruments	Companies	Origins
1	Autoclave device	Human Lab	Korea
2	Burner	Amal	Turkey
3	Deep freeze	GFL	Germany
4	Disposable loops	Himedia	India
6	Disposable petri dishes	Himedia	India
7	DNA electrophoresis apparatus	Hettich	Germany
8	FTIR spectroscopy	Shimadzu	Japan
9	Eppendorf tube (2 ml)	Eppendorf	Germany
10	Hood-cabinet	BIO Hazard	Korea
12	Incubator	Memmert	Germany
13	Light microscope	Olympus	Japan
14	PCR thermal cycler	Agilent (8800)	USA
15	Micro pipette(different sizes)	Dragon_med	Spain
16	(UV) spectrophotometer	Shimadzu	Japan
17	Oven	Memmert	Germany
18	Plan Test tubes (10 ml)	AFMA	Jordan
19	Pipettes	DARWELL	China
20	Refrigerator	Hettich	Japan
21	Sensitivity balance	Sartorius	Germany

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Table (3.2) Dyes used in this study

Dye	Origin
Gram stain	Himedia (India)
Red Safe	Intron (Korea)

3.1.2 Chemicals and Kits

. .Table 3.3 shows the chemical components and kits utilized in this study

Chemicals and Kits	Company	Origin
DNA loading dye	Bioneer	Korea
G-spin Genomics DNA Extraction Kit for Bacteria	Intron	Korea
GoTaq® G2 Green Master Mix	Promega	USA
The Safe-Green 100bp Opti-DNA marker	Abm	Canada
10X TAE buffer (Tris Acetate EDTA)	Promega	USA
Agarose LE	Intron	Korea
Primers	Macrogen	Korea
Red-safe nucleic acid staining solution	Intron	Korea
Safe Green 100bp Opti-DNA Marker	Abm	Canada

3.1.3 Culture media

Table 3.4 shows the bacterial culture media used during this study.

no	Media	company	Origins
١	Aimes Transport medium	AFCO	Jardan

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٢	Blood Agar	Accumix	India
٣	Brain Heart Infusion Broth	Biolab	Hungary
٤	Buffered Glucose Broth	HIMEDIA	India
٥	MacConkey Agar	Accumix	India
٦	Mannitol Salt Agar	Accumix	India
٧	Mueller Hinton Agar	Accumix	India
٨	Peptone Water	Liofilchem	Italy
٩	Simmons Citrate Agar	Liofilchem	Italy

3.1.4. Antibiotics

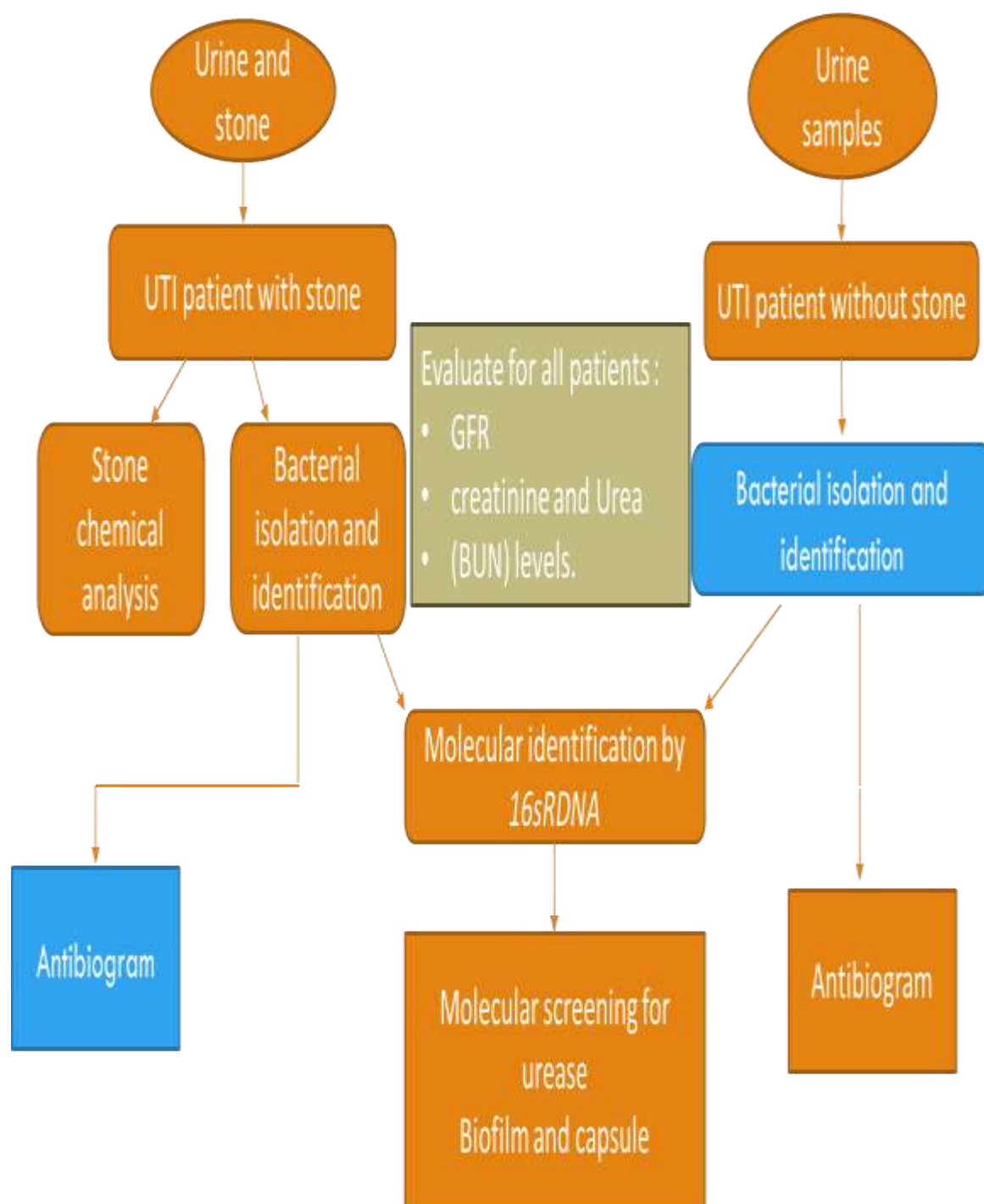
Table 3.5 presents the antibiotics used in this study to examine the antibiotic susceptibility test.

Table 3.5: Antibiotic discs utilized in this study

Antibiotic	Symbol	Potency (µg)	Company	Origin
Amoxicillin/ clavulanic acid	AUG	10/20	Liofilchem	Italy
Amikacin	AK	30	Himedia	India
Ciprofloxacin	CIP	5	Himedia	India
Gentamicin	GEN	10	Himedia	India
Levofloxacin	LE	5	Himedia	India
Imipenem	IPM	10	Himedia	India
Sulfamethoxazol-Trimethoprim	SUL-TR	35	Himedia	India
Cefotaxime	CFT	30	Himedia	India
Ceftriaxone	CFR	30		india

3.2 Methods

Study design



3.2.1 Sample Collection

Between September 2024 and February 2025, 150 midstream urine specimens were collected from patients in Thi-Qar Province attending the urology department of Al-Refaie General Hospital undergoing evaluation for urinary system disorders. Of these, 75 (50%) Urine samples were taken from patients who had been diagnosed with urinary stone disease, which was confirmed by a physician using ultrasound imaging. A total of 75 (50%) urine samples were obtained from individuals who were clinically diagnosed with UTI. Demographic information, including age, sex, and residency, was collected directly from patients by interview. Also, ten renal stones were taken surgically or naturally passed through urine in order to reveal their microbial and mineral components.

3.2.2 Preparation of Culture Media:

All media were prepared along with the manufacturer's directions

3.2.2.1 Blood Agar

To make the blood agar medium, follow the manufacturer's directions and dissolve 40 grams of blood agar base in 1000 milliliters of distilled water. The medium was autoclaved for 15 minutes at 121 °C, then cooled to 50 °C and 5% fresh human blood was added to the mixture. This medium serves two functions: first, as an enrichment medium for the cultivation of bacterial isolates, and second,

as a medium for assessing the bacteria's ability to hemolyze blood. **3.2.2.2**

MacConkey Agar

One liter of distilled water was used to dissolve 51 grams of MacConkey basic powder. For sterilization, the mixture was autoclaved at 121 °C for 15 minutes. After cooling, the mixture was poured over the plates.

3.2.2.3 Brain Heart Infusion Broth

Broth for infusion with glycerol medium as directed by the manufacturer, dissolve 31.45 grams of the ingredients in 850 milliliters of distilled water to make the brain heart infusion broth.. The medium was autoclaved for 15 minutes at 121 °C. After cooling to 55 °C, add 15% sterilized glycerol. This medium was used to enhance bacterial isolates that were held for an extended time

3.2.3 Isolation and identification of bacteria

One ml of mid-stream urine was centrifuged at 3000 rpm for 5 min. The sediments were inoculated onto selective and differential medium, such as blood agar (BA) and MacConkey agar (MAC), which had been prepared and sterilized beforehand. They were then incubated for 24 hours at 37°C. Colony morphology, pigmentation, Gram staining, and biochemical assays such oxidase, catalase, urease, indole, methyl red, Voges-Proskauer (VP), and citrate consumption were used to determine the phenotype.. All isolates were further confirmed by the automatic system VITEK II (bioMérieux, France).

3.2.4 Identification of bacterial isolates

3.2.4.1 Morphological Identification

Primary identification tests involved the morphological characteristics of bacterial growth on different culture media, including MacConkey agar and blood agar. Phenotypic identification included the colony shape, colony texture, color, and edges.

3.2.4.2 Microscopic examination

A bacterial smear was prepared from an overnight bacterial culture. After the smear's fixation step, Gram's staining technique was used to determine the shape, size, and arrangement of the bacterial cells.

3.2.4.3 Biochemical Tests

Traditional biochemical tests for bacterial identification were conducted using cardinal biochemical tests that specifically identify uropathogenic bacteria.

3.2.4.3.1 IMViC test

The IMViC test consists of four biochemical tests— Citrate, Voges-Proskauer, Indole, and Methyl Red are used to identify and separate Enterobacteriaceae species, particularly *Escherichia coli* and *Enterobacter aerogenes*. Each test evaluates a distinct metabolic capability: indole generation from tryptophan, acid production from glucose fermentation (Methyl Red), acetoin production (Voges-Proskauer), and the ability to use citrate as the sole carbon source. *E. coli* normally produces a ++-- pattern (positive for Indole and Methyl Red, negative for Voges-Proskauer and Citrate), whereas *Enterobacter* species usually produce a --++ pattern. The IMViC series is particularly effective in water microbiology for detecting fecal pollution..(Systems, 2013)

3.2.4.3.2 Oxidase Test

Bacteria that produce cytochrome c oxidase, an enzyme involved in the bacterial electron transport chain, can be identified using the Oxidase Test, a quick biochemical procedure. It aids in distinguishing oxidase-positive bacteria.

3.2.4.3.3 Catalase test,

The Catalase Test recognizes the existence of the enzyme catalase in bacteria, which breaks down hydrogen peroxide into water and oxygen. It helps differentiate catalase-positive bacteria.

3.2.5 Maintenance of the Bacterial Isolates

The bacterial isolates' maintenance was done as follows:

Short-term storage

The pure bacterial isolates were stored in screw-capped universal tubes containing brain heart infusion agar slant for a few months after being incubated at 37 °C for 24 hours. The slants were carefully wrapped with Parafilm and stored at 4°C for three months.

Long-term storage of culture

Pure colony picked by sterile loop to be inoculated on sterile brain heart broth with glycerol at concentration 20%, then incubated for 18hr at 37C , then the inoculum preserved at _20C for 2-8 months

3.2.6 Antibiotic sensitivity test

The standard disc diffusion method(Kirby-pour method) on Muller-Hinton plates was used to determine antibiotic sensitivity patterns for bacterial isolates in accordance with the Clinical Laboratory Standards Institute's guidelines (CLSI, 2021). Among the drugs studied were ceftriaxone (30 µg), amikacin (30 µg), and amoxicillin/clavulanic acid (10/20 µg). Levofloxacin 5 µg, ciprofloxacin 30 µg, sulfamethoxazole/trimethoprim 25 µg, gentamicin 10 µg, and imipenem 10 µg. The inhibitory zones were measured in millimeters using an

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ordinary ruler. CLSI (2021) states that the antibiotics disk utilized in this investigation had an inhibition zone diameter

Table (3.6) Antibiotics concentration used in this study

Antibiotics (Symbol)	Inhibition zone diameter (mm)		
	S	I	R
Amoxicillin-clavulanic acid (AUG)	$18 \leq$	$16-14$	$13 \geq$
Amikacin (AK)	$17 \leq$	$16-15$	$14 \geq$
Ciprofloxacin (CIP)	$26 \leq$	$25-22$	$21 \geq$
Levofloxacin (LE)	$21 \leq$	$20-17$	$16 \geq$
Gentamicin (GEN)	$15 \leq$	$14-13$	$12 \geq$
Imipenem (IMP)	$23 \leq$	$22-20$	$19 \geq$
Trimethoprim (TR)	$16 \leq$	$15-11$	$10 \geq$

3.2.7 Renal function assessment

3.2.7.1 Blood urea

To determine kidney function, the blood urea level is commonly measured using an enzymatic technique. A blood sample is drawn, and the serum was separated. In the laboratory, the enzyme urease degrades urea into ammonia and carbon dioxide; the resultant ammonia undergoes a detectable reaction, and the change in absorbance is measured spectrophotometrically to determine urea concentration. Results are expressed as Blood Urea Nitrogen (BUN) in mg/dL or urea in mmol/L, with normal BUN levels ranging from 7 to 20 mg/dL. (Baum *et al.*, 1975)

3.2.7.2 Blood Creatinine

Renal function is assessed by measuring the blood creatinine level. The Jaffe reaction, a colorimetric technique in which creatinine and picric acid in an alkaline solution react to form a red-orange complex, or more exact enzymatic procedures are frequently used to evaluate a serum or plasma sample. The creatinine content is proportional to the color intensity as measured by spectrophotometry. The usual adult level is between 0.6 and 1.2 mg/dL, and results are given in mg/dL or $\mu\text{mol/L}$. Increased levels may indicate impaired renal function.(He *et al.*, 2022)

3.2.7.3 Uric Acid

Blood uric acid levels are used to diagnose illnesses such as gout, kidney disease, and metabolic problems. An enzymatic approach is used to evaluate a serum sample. The enzyme uricase transforms uric acid into allantoin and hydrogen peroxide, which react to produce a colored product that is quantified spectrophotometrically. The results are expressed in mg/dL (normal range: 3.4-7.0 mg/dL for men and 2.4-6.0 mg/dL for women). Increased levels (hyperuricemia) may suggest overproduction or decreased excretion of uric acid.(Glantzounis *et al.*, 2005)

3.2.8 Bacteriological Isolates From Renal Stone

Renal stones were processed for bacteriological culture. The kidney stones were completely washed in sterile physiological saline and then smashed with a sterile hacksaw. The crushed stone core was cultured in 5mL of nutrient broth and incubated at 37°C for 18-24 hours before being sub cultured on blood agar and MacConkey's agar plates to isolate aetiological agents. The isolated organisms were identified using normal

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methods. Chemical investigation of renal stones for oxalate, calcium magnesium, ammonium, and phosphate was done.

3.2.9. Biochemical Analysis of Renal Stone

Kidney stone samples were taken from several places around Thi-Qar province. The stones were carefully cleansed with distilled deionized water, checked for physical features, and finally dried. The stones were ground into a fine, homogenous powder using a pestle and mortar. 100 mg of this powder was combined with potassium bromide, and the pellet was analyzed using Fourier transform infrared spectroscopy (FTIR). FTIR spectroscopy was performed using a Shimadzu 8700 FTIR Spectrophotometer in the frequency range..

3.2.10 Molecular Study

Preparation of buffers and working solutions used in PCR and agarose gel electrophoresis

3.2.10.1 Primers Preparation

The commercially obtained lyophilized primer (Macrogen, Korea) was dissolved in the required amount of sterile D.W. according to manufacturer instructions to achieve a final concentration of 100 pmol/ μ l (as a stock solution). After briefly spinning them down at 5000 rpm, they were placed in a freezer at -20 °C until they were used. To prepare the working primer solution, 10 μ l of primer stock solution was added to 90 μ l of D.W to achieve a final concentration of 10 pmol/ μ l. The primer working solution was then frozen before being used in the PCR reaction.

3.2.10.2 Bacterial DNA Extraction

The bacterial genomic DNA was extracted by the bacterial DNA extraction Kit **G-spin (Intron, Korea)** whose components are presented in Table 3.9

Table (3-7): The components of the Bacterial DNA Extraction Kit G-spin

Content	Amount
Binding Buffer	15ml
Elution Buffer	20ml
G-Buffer	20ml
Lysozyme, Lyophilized	20mg
Pre-Buffer	3ml
Proteinase K, Lyophilized	1.76mg
RNase A, lyophilized	3mg
Spin Column (Green color) & Collection tube	50 columns
Washing Buffer A	9ml
Washing Buffer B	10ml

Protocol of DNA extraction

1. 1-2 ml of cells (OD600: 2.0-1.0) was harvested by centrifuging at 1 min and remove supernatant.. 14,000 rpm for
2. 180 µl of G-Buffer solution with 20µl of proteinase K was added, and well by inversion. (For Gram-negative Bacteria) mixed
3. Incubated at 65°C for 15 min. Note: To help lyse cells, invert the tube every 5 min during the incubation.
4. Add 250 µl of Binding Buffer from Table (3-9) and mix completely by pipetting or gently vortexing
5. Cell lysates were placed on a column and spun at 13,000 rpm for 1 minute..

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6. Add 500 µl of washing Buffer A to the column and centrifuge for 1 minute at 13,000 rpm. Add 500 µl of washing Buffer B to the column and centrifuge for 1 minute at 13,000 rpm.
8. Removed solution and centrifuged for 1 minute at 13,000 rpm.
9. The G-spin™ column was inserted in a clean 1.5ml micro centrifuge tube, and 50-200 µl of elution buffer was directly applied to the membrane..
10. After 1 minute of incubation at room temperature, the sample was centrifuged at 13,000 rpm..

3.2.10.3 Preparation of the Agarose gel 1% for whole DNA Electrophoresis

The preparation of agarose gel was performed according to the protocol described by (Sambrook and Russell, 2006) Briefly, 1 g of agarose powder was dissolved in about 100 ml of a 1X TBE buffer and heated to boiling in a microwave oven. Following a cooling step at 45-50° °C, five µl of Red Safe nucleic acid staining solution was added to the gel and poured into the gel cast and left for about 30 minutes to be completely solidified. The gel plate was transferred into the gel tank and filled with 1X TBE buffer to the point of covering the gel surface.

3.2.10.4 Visualization of Extracted Bacterial DNA

Electrophoresis through agarose gel (1%) was performed to determine the success and quality of the extracted DNA (sharpness and thickness of the bands). Five microliters of extracted DNA were mixed with loading dye and loaded into each well of the gel. The electrophoresis apparatus was configured as follows: 70 volts,

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continuous current for 35 minutes. Finally, the gel was transferred to a UVP system to view the DNA bands under a 320nm light source.

3.2.10.5 Preparation of PCR reaction mixture

To prepare the PCR reaction mixture, all the components listed in Table 3.11 are mixed in a 0.2 ml heat-stable plastic tube.

Table 3.-8: The PCR reaction mixture components and amount

Components	Concentration	Volume (50 µl)
GoTaq® G2 Green Master Mix	1X	25 µl
Forward primer	10 µM/µl	4 µl
Reverse primer	10 µM/µl	4 µl
ddH ₂ O	\\	13 µl
DNA	40 ng	4 µl

Table (3-9): Primers' sequence of *16srDNA* E.coli. 16srDNA S. aureus. 16srDNA Klebsiella, Urease proteus, OprL Pseudomonas

Gene	Sequence (5'-3')	Annealing temperature	Product size	Reference
<i>16srDNA</i> E.coli	CCCCCTGGACGAAGACTGAC ACCGCTGGCAACAAAGGATA	58°C	401 bp	Abrar <i>et al.</i> , 2019
16srDNA S. aureus	AACCTACCTATAAGACTGGG CATTCACCGCTACACATGG	54°C	570 bp	Kiran) Kumar <i>et al.</i> , 2020
16srDNA Klebsiella	ATTTGAAGAGGTTGCAAACGAT TTCACCTCTGAAGTTTCTTGTGTT C	57°C	130bp	Abbas) and Ghaznavi-(rad, 2023
Urease proteus	GGTGAGATTTGTATTAATGG ATAATCTGGAAGATGACGAG	58°C	225bp	Kamil) abd Jarjes, (2021
OprL Pseudomonas	ATGGAAATGCTGAAATTCTCTGC T CTTCTTCAGCTCGACGCGACG	55°C	504bp	Chand <i>et al.</i> , 2021

3.2.10.6. PCR Reaction Conditions

Table 3-10 indicates the PCR conditions used for identification.

Conditions of the PCR program used to identify bacteria

Phase	T _m (°C)	Time	Cycles
Initial denaturation	94°C	5 min	1X
Denaturation	94°C	30 sec.	35X
Annealing	58 °C	30 sec.	35X
Extension	72°C	30 sec.	35X
Final extension	72°C	5 min	1X

3.2.9.5 Loading the PCR

Insert about 5 µl of each PCR product into the corresponding hole in the 1% gel. To determine the size of PCR products, add 5 µl of Safe-Green 100bp Opti-DNA Marker to the first hole in the gel lines. After loading the Safe-Green 100bp OptiDNA Marker and samples, the electrophoresis equipment was set up as follows: 70 volts of constant current for 35 minutes. Finally, the gel was transferred to a UVP system to observe the PCR products using a 320nm light source. Safe-Green 100bp Opti-DNA Marker

3.2.9.6. Statistical analysis

This study employed descriptive statistics. The categorical variables, including age categories, sex, residency, and bacterial isolation, were expressed as percentages (%). The numerical variables were expressed in terms of mean or median, depending on the normality test (Kolmogorov-Smirnov). Statistical comparison was conducted using the Chi-square test (χ^2) with two-sided for categorical variables and Student t-test or Mann-Whitney to compare the numerical variables. All statistical tests were conducted at the significance level of 0.05 using SPSS version 19. Graph-Pad Prism was used to generate statistical figures.

3.2.9.7. Limitations criteria

The main limitations of the study are a relatively small sample size , limitation of this study should be considered. Because all patients with mixed urine culture are excluded.

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Results

4.1 Demographic Characteristics of Study Populations

The demographic characteristics of the study groups are presented in Table 4-1. This study included a total of 150 patients divided into two groups: 75(50%) had urinary tract infections, designated as the UTI group, and 75 (50%) had renal stones or a history of recurrent renal stone, designated as the renal stone former group. The mean age $\pm$ SD of the entire study population (150 patients) was 38.8 ± 14.6 , ranging from 13 years old to 68 years old. The mean age of the UTI group was 38.9 ± 14.1 , while the mean age of the renal stone former group was 39.2 ± 15.6 . Regarding sex, 42 patients (65%) with UTI were male, and 33(44%) were female. On the other hand, among the renal stone former group, males constituted 47(62%), while females made up 28(37.3%). Concerning residency, the number of patients residing in urban areas was higher than that in rural areas for both groups (UTI and renal stones former). Statistical analysis show that no significant differences ($P \geq 0.05$) existed among the UTI group and renal stone former group for the three variables (age, gender, and residency).

Table (4-1). The demographic characteristics comparison of the two study groups, the UTI and renal stone former

CHARACTER	GROUPS	UTI (%)	STONE (%)	P-VALUE
AGE: MEAN $\pm$ SD		38.9 $\pm$ 14.1	39.2 $\pm$ 15.6	
SEX	Male	42 (56%)	47(62.7%)	0.5063
	Females	33(44%)	28(37.3%)	
TOTAL		75	75	
RESIDENCY	Rural	27(36%)	33(44%)	0.4048
	Urban	48(64%)	42(56%)	
TOTAL		75	75	

4.2 Prevalence of urinary tract infection versus Renal stone among different age groups

Table 4.2 presents a comparison of the prevalence of UTI and renal stone based on the age groups, both urinary tract infections and renal stones appear to share a similar pattern, as the prevalence among adolescents (11-20 years old) was lower (10.7% in the UTI group and 18.7% in the renal stone former group) than in the other age groups. However, the highest prevalence of UTI was found in the adult age group (30.7%), while the highest prevalence of renal stones was observed in the elderly age group (51-70 years old). Statistical analysis revealed that there was a significant difference ($P < 0.05$) in the distribution of the urinary tract infection among the UTI group. However, no such significant differences in renal stone prevalence were observed in the renal stone former group ($P > 0.05$).

Table (4-2). Prevalence of urinary tract infection versus Renal stone in different age groups of the two study groups

AGE GROUPS	UTI	RENAL STONE	TOTAL
11-20	8(10.7%)	14(18.7)	22(14.6)
21- 35	24(32%)	18(24)	42(28)
36-50	23(30.7%)	20(26.6)	43(28.7)
51-70	20(26.6)	23(30.7)	43(28.7)
TOTAL	75	75	150
P-VALUE	0.03	0.516	
X ²	8.680	2.280	
DF	3	3	

4.3 Diversity and Recovery Rate of Bacterial Species in the UTI and Renal Stone former groups

The bacterial isolation showed that out of 150 urine samples from the two groups, only 53 (35.3%) exhibited positive bacterial growth on culture media. The bacterial recovery rate in the UTI group was 38.7%, slightly higher than the 32% in the renal stone former group. However, statistical analysis exposed no significant difference ($P>0.05$) in the overall bacterial recovery rates. The recovery rate of *E. coli* and *K. pneumoniae* was advanced in the UTI group than in the renal stone former group; on the other hand, the isolation rate of *P. mirabilis*, *P. aeruginosa*, and *S. aureus* was higher in the renal stone former group than in the UTI group. Collectively, statistical analysis showed no significant differences in the distribution of the bacterial species in both groups. Table 4-3.

Regarding the bacterial species, this study's findings revealed that six bacterial species were successfully cultured and identified to species levels based on the morphological and biochemical tests. *E. coli* was the most prevalent bacterial species, 30.2%, followed by *K. pneumoniae*, 22.6%, and *P. aeruginosa*, 16.1%, while the lowest prevalent bacterial species was *E. faecalis*, 5.7%. Figure (4-1).

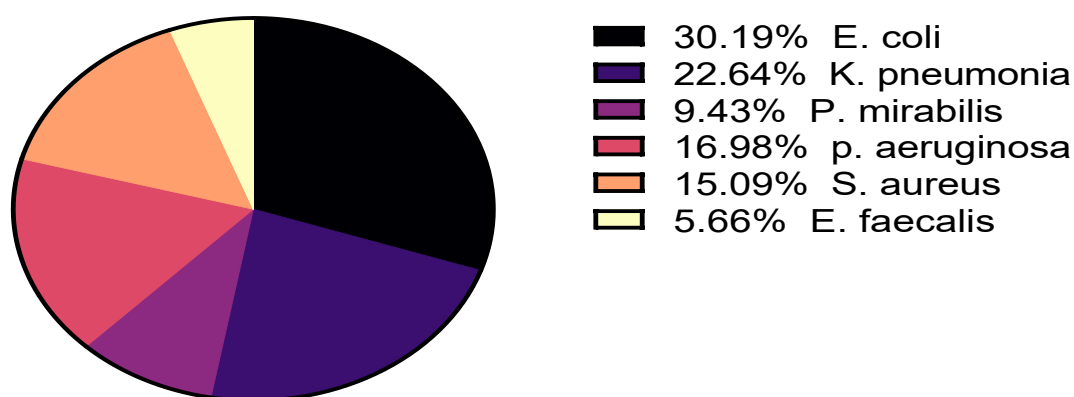


Figure 4-1: The prevalence of the six bacterial species isolated from urine samples of the two groups

Table(4-3). The isolation rate and distribution of various bacterial species isolated from UTI and renal stone populations.

Bacterial Species	No. isolates	UTI n(%)	Renal stone	P-value
<i>E. coli</i>	16(30.2)	10(34.5)	6(25)	0.332
<i>K. pneumonia</i>	12(22.6)	9(31)	3(12.5)	0.146
<i>P. mirabilis</i>	5(9.4)	0(0)	5(20.8)	----
<i>p. aeruginosa</i>	9(16.2)	4(13.8)	5(20.8)	0.999
<i>S. aureus</i>	8(15.1)	3(10.3)	5(20.8)	0.726
<i>E. faecalis</i>	3(5.7)	3(10.3)	0(0)	-----
Total	53(35.3)	29(38.7)	24(32)	0.4946

4.4moleculer study

4.4.1 Molecular detection of 16S rRNA of *E. Coli*

All isolates of *E. coli*(16) were detected by PCR amplification via 16S rRNA, and 16 isolates (100%) were positive for molecular detection of 16S rRNA gene at (410 bp) figure (4-2)

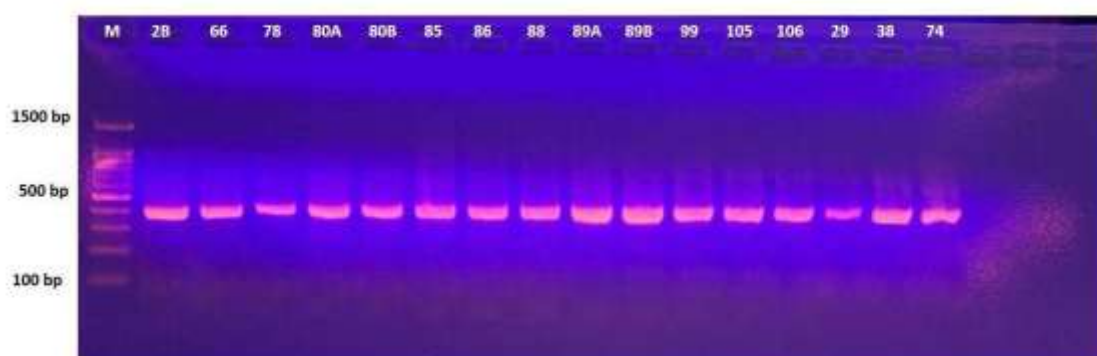


figure (4-2):PCR products of the amplification of partial region of gene 16S rRNA *E. coli*. The size of the PCR product is (410 bp). The gel was 1.5%. V: 80, Time: 70 minutes. M: DNA ladder (100 bp).

4.4.2 Molecular detection of 16S rRNA of *k pneumonia*

All isolates of 16S rRNA of *k pneumonia* were detected by PCR amplification via 16S rRNA of *k pneumonia*, and 12 isolates (100%) were positive for molecular detection of 16S rRNA gene at (130bp); figure (4.3)

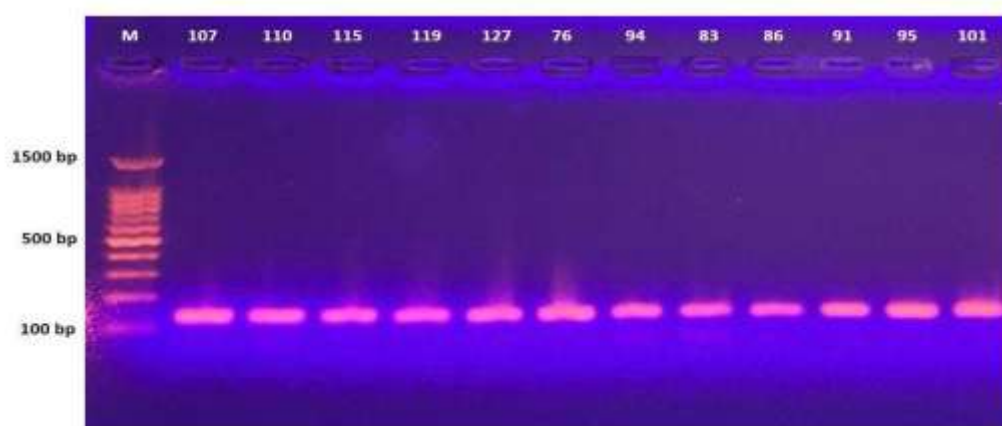


figure (4-3):PCR products of the amplification of partial region of gene 16S rRNA of *k pneumonia*. The size of the PCR product is (410 bp). The gel was 1.5%. V: 80, Time: 75 minutes. M: DNA ladder (130bp)

4.4.3 Molecular detection of *p. mirabilis* ,Urease proteus gene

Only 2 out of 5 isolates (40%) of *p. mirabilis* in the current investigation tested positive for Urease proteus gene, as shown in the figure below.

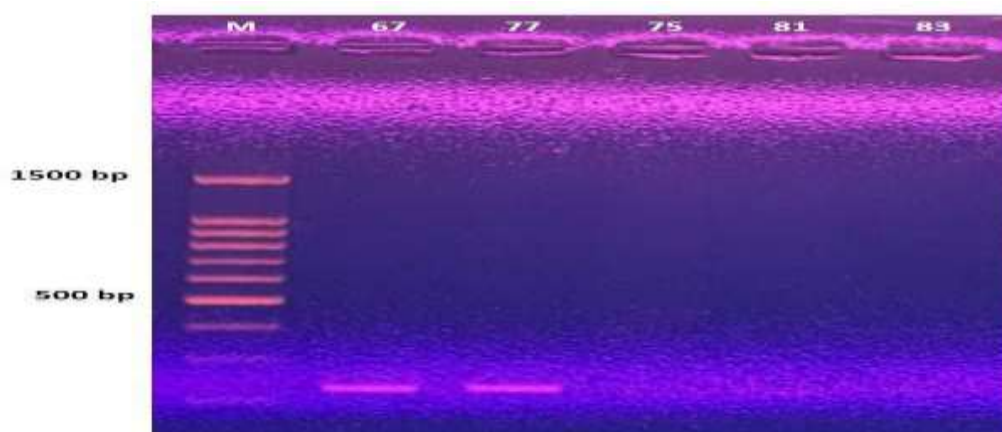


figure (4.4): PCR products of the amplification of partial region of gene Urease proteus in the isolates of *p. mirabilis*. The gel was 1.5%. V: 80, Time: 70 minutes. M: DNA ladder (225bp).

4.4.4 Detection of 16srDNA *S. aureus*

All five solates of *S. aureus* were examined for 16srDNA *S. aureus* by PCR (Figure 4.5, and only two isolates (50%) were positive. Data showed that the 16srDNA *S. aureus* was expressed and located at (570bp)in size

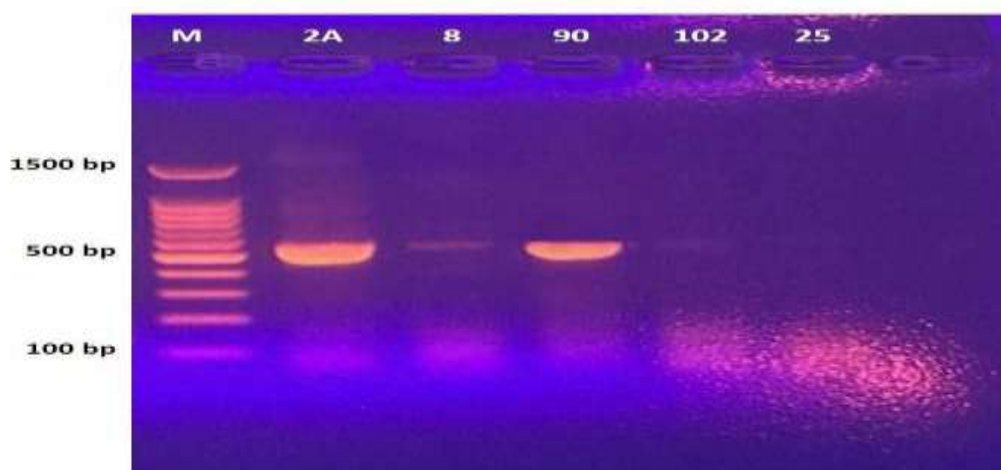


Figure (4.5): PCR products of the amplification of partial region of 16srDNA *S. aureus* in the isolates of *S. aureus*. The size of the PCR product is (570bp). The gel was 1.5% and. V: 80, Time: 70 minutes. M: DNA ladder (100bp).

4.4.5 Detection of oprI Pseudomonas gene

All five isolates of Pseudomonas were examined for oprI Pseudomonas by PCR (Figure 4.6), and only one isolates (20%) were positive. Data showed that the oprI Pseudomonas gene was expressed and located at 504bp in size

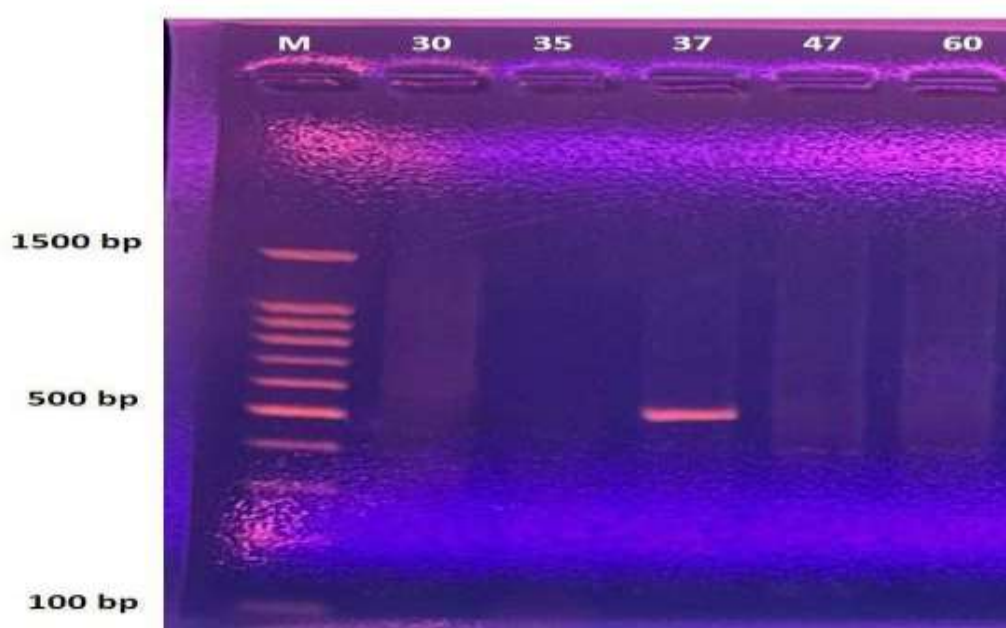


Figure (4.6): PCR products of the amplification of partial region of gene oprI Pseudomonas in the isolates of Pseudomonas. The size of the PCR product is 504bp. The gel was 1.5% and. V: 80, Time: 70 minutes. M: DNA ladder (100bp)

4.5 Antibiotic Resistance Profiling of Bacteria isolated from UTI and Renal Stone populations

4.5.1 *E. coli*

The antibiotic resistance profile of *E. coli* isolated from UTI patients and renal stone patients is shown in the figure (4-7). Generally, *E. coli* isolates showed higher resistance rates against amikacin 2(20%), cefotaxime, 5(50%), amoxicillin-clavulanic acid 8(80%), and sulfamethoxazole-trimethoprim, 7(70%), than those in renal stones which recorded the following resistance rate 1(16%), 3(50%), 2(30%), 3(50%), 0(0%), 1(16%), 5(83%), 4(66%), respectively. However, the isolates from renal stone patients showed a higher resistance rate than isolates of UTI patients, only for ciprofloxacin and gentamicin.

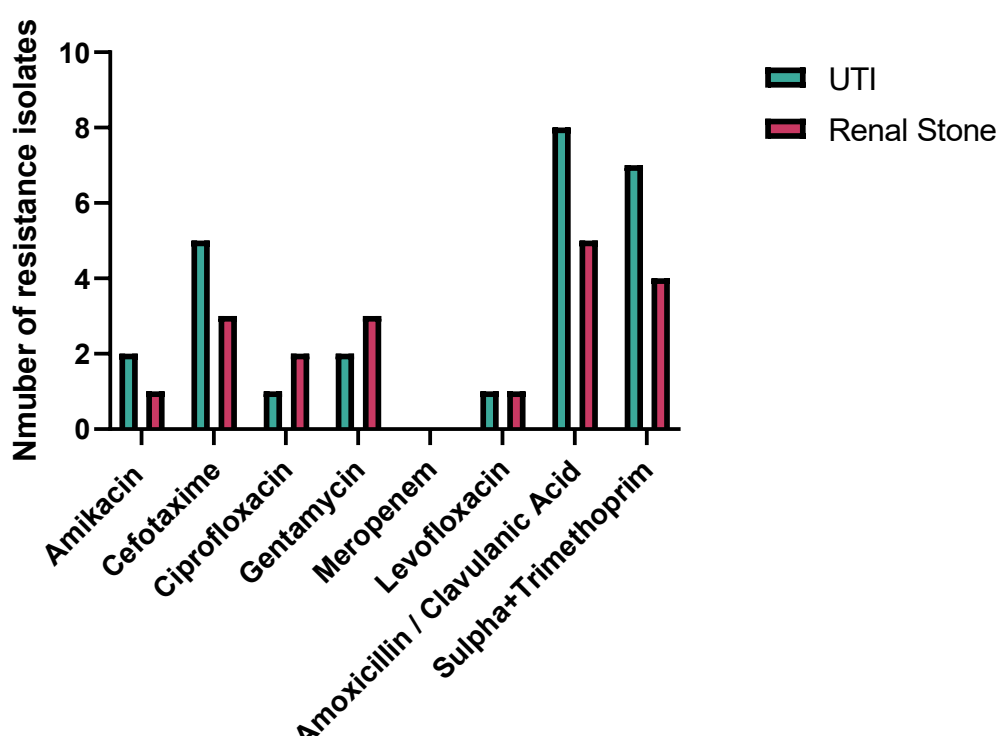


Figure (4-7) Antibiotic Resistance Profiling of Bacteria isolate of *E. coli* isolated from UTI and Renal stone former groups

4.5.2 *K. pneumoniae*

Figure (4-8) displays the antibiotics resistance profile of *K. pneumoniae* isolates from renal stone former group and UTI group ,which recorded the flowing resistance rates. amikacin2(22%), cefotaxime, 4(44%), ciprofloxacin 3 (33%), gentamicin 5 (55%), meropenem 1(11%), levofloxacin 3(33%), amoxicillin-clavulanic acid 7(77%), and sulfamethoxazole-trimethoprim, 7(77%). To be compared with bacterial isolates resistance from patient with renal stone as flowing, 1(33%), 2(66%), 1(33%),0(0%),0(0%),0(0%),3(100%),3(100%), respectively. Higher resistance rate in isolates from UTI agents sulfamethoxazole-trimethoprim and amoxicillin-clavulanic acid compered to isolates from renal stone patients .

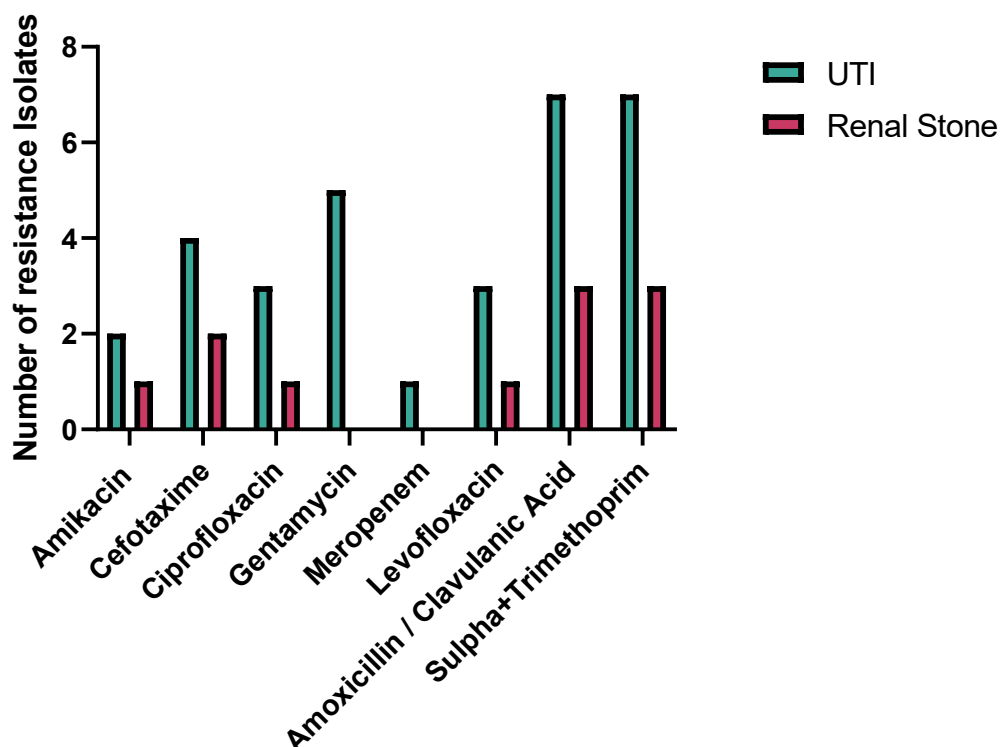


Figure (4-8): Antibiotic Resistance Profiling of *K. pneumoniae* isolated from urine samples of UTI and Renal stone former group.

4.5.3 *P. mirabilis*

Figure (4_9) displays the antibiotics resistance profile of *P. mirabilis* isolates from urine samples of patient suffering from renal stone which was as the flowing : amikacin3(60%), cefotaxime, 1(20%), ciprofloxacin2 (40%) ,gentamycin2(20%), meropenem0(0%),levofloxacin1(20%) amoxicillin-clavulanic acid 5(0%), and sulfamethoxazole-trimethoprim4(80%). However , there was no isolates of (*P. mirabilis*) from patient diagnosed with UTI.

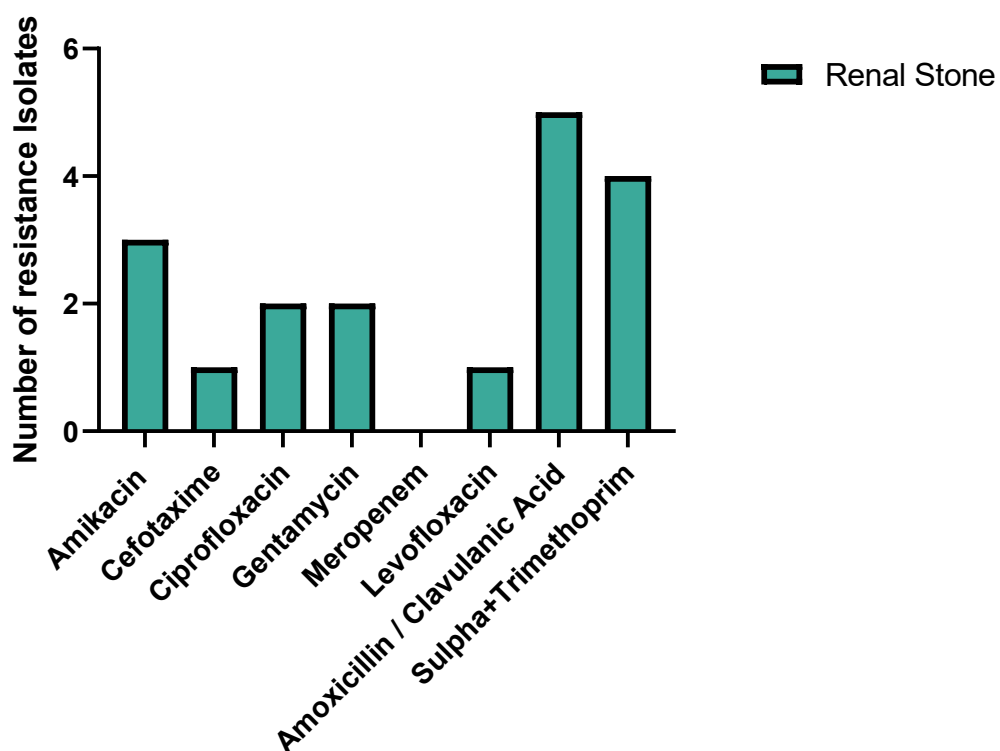


Figure (4_9) Antibiotic Resistance Profiling of Bacteria isolate for(*P. mirabilis*)

4.5.4 *p. aeruginosa*

Figure (4-10) displays the antibiotics resistance profile of *p. aeruginosa* isolates from patient suffering from renal stone and UTI ,with recorded the flowing resistance rates amikacin3(60%), cefotaxime, 3(60%), ciprofloxacin4(80%) ,gentamycin5(100%), meropenem1(20%),levofloxacin3(60%) amoxicillin-clavulanic acid 5(100%), and sulfamethoxazole-trimethoprim4(80%). While the isolates from patients with renal stone recorded the flowing resistance rate : 1(25%), 2(50%), 3(75%),2(50%),1(25%),3(75%),3(75%),2(50%), respectively. higher resistance rate may be observed in UTI samples agents gentamycin and amoxicillin-clavulanic acid compered with resistance rate of urine samples taken from patient with renal stones .

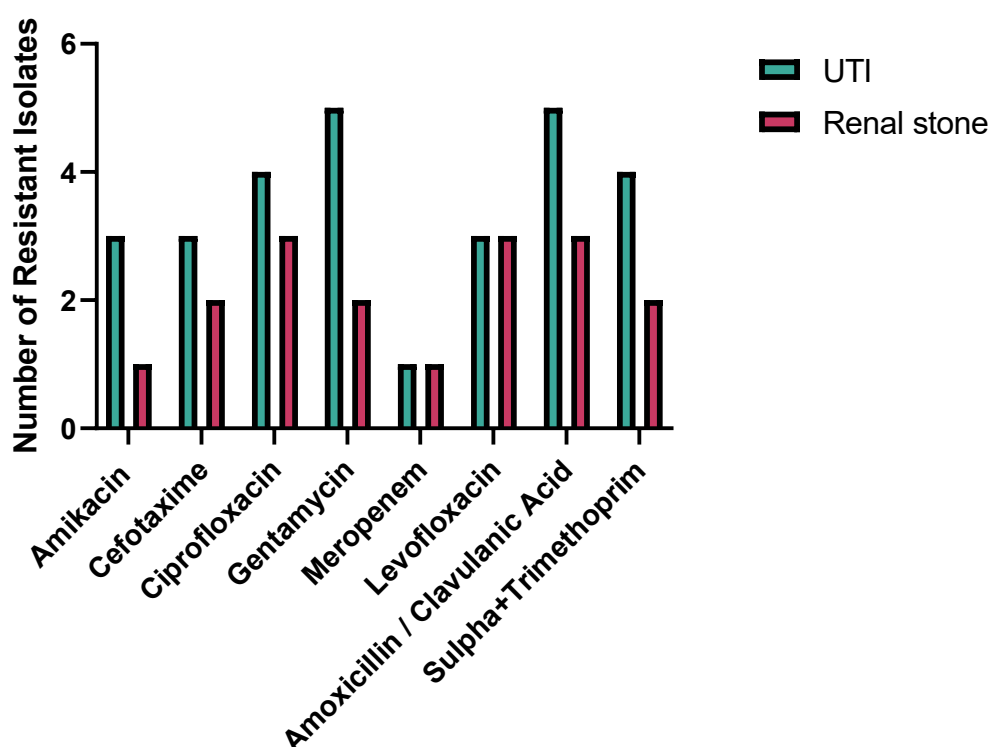


Figure (4_10)Antibiotic Resistance Profiling of Bacteria isolate for (*p. aeruginosa*)

4.4.5 *S. aureus*

Figure (4_11) displays the antibiotics resistance profile of *S. aureus* isolates from patient suffering from renal stone and UTI ,with recorded the flowing resistance rates amikacin2(66%), cefotaxime, 2(66%), ciprofloxacin1(33%) ,gentamycin1(33%), meropenem0(0%),levofloxacin2(66%) amoxicillin-clavulanic acid 3(100%), and sulfamethoxazole-trimethoprim3(100%). While the isolates from patients with renal stone recorded the flowing resistance rate : 3(60%), 2(40%), 2(40%),1(20%),2(40%),2(40%),4(80%),5(100%), respectively. resistance rate show elevation in samples from patient with renal stones agenst sulfamethoxazole-trimethoprim ,and amoxicillin-clavulanic acid compered with samples taken from patient with renal stone.

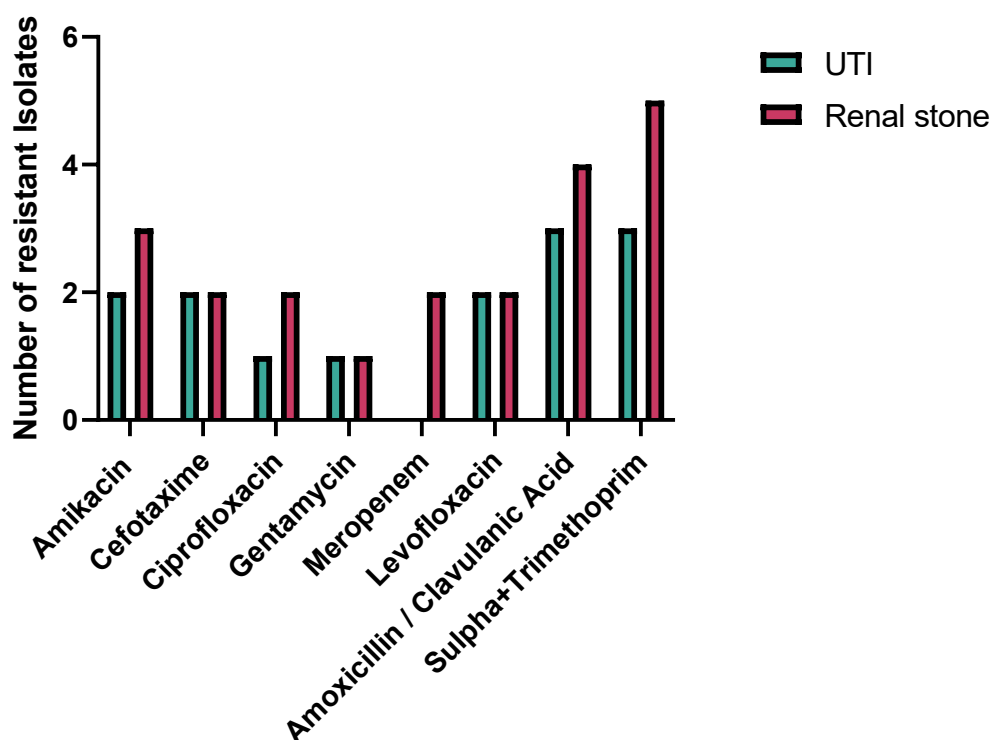


Figure (4_11) Antibiotic Resistance Profiling of Bacteria isolate for (*S. aureus*)

4.6. Profiling of renal stones

figure (4-12) presents the types of stones based on the chemical analysis of the stones. Out of 10 renal stones subjected to chemical analysis, three types (CaOX, Amorphous urate, and Amur+Uric acid stone) were identified. Regarding bacterial isolation, 6 (60%) were classified as infective stones, as they showed bacteria growth upon culturing, while the remaining four stones (2 Amorphous urate and 2 Amur+Uric acid stones) were sterile, with no bacterial growth. Calcium oxalate stones were highly associated with bacterial infections.

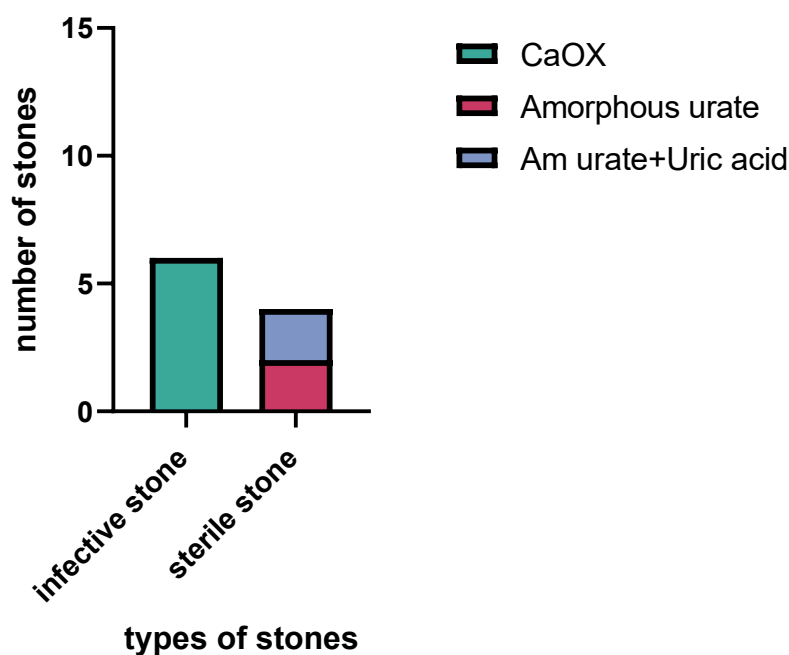


Figure (4-12) types of stone

4.7 Comparison of renal functions in Renal Stone and UTI populations

The comparison of renal functions of the UTI and renal stone populations illustrated in the Table (4.4). The median of the blood urea level in the renal stone population, 59, was higher than that recorded in the UTI

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population, 44.2. The difference was statistically insignificant ($P>0.05$). Nevertheless, the median of creatinine level in the renal stone population was 2, higher than in the UTI population, 1.4; the statistical analysis revealed that the difference was statistically significant ($P<0.05$). Similarly, uric acid and blood urea nitrogen (BUN) showed higher levels in the renal stone population than in the UTI population, as the mean of uric acid in the renal stone population was 5.985 compared to 4.823 in the UTI population. In the same context, the median of BUN in the renal stone population, 27.4, was higher than in the UTI population, 20.6. Statistical analysis displayed a significant difference ($P<0.05$) for both uric acid and BUN.

Table (4-4) comparison of renal functions of the UTI and renal stone former populations

Population	Urea ^A Mg/dl	Creatinine ^A Mg/dl	Uric Acid ^B Mg/dl	Blood-urine nitrogen ^A Mg/dl
UTI	44.2 ^A	1.4	4.823	20.6
Renal stone	59	2	5.985	27.4
P-value	0.0617	0.0218	0.0024	0.0342

A- Mann-Whitney U for Urea 573.5, Creatinine,532, BUN,549

B- T-value = 3.144, DF=76, F-value =1.745

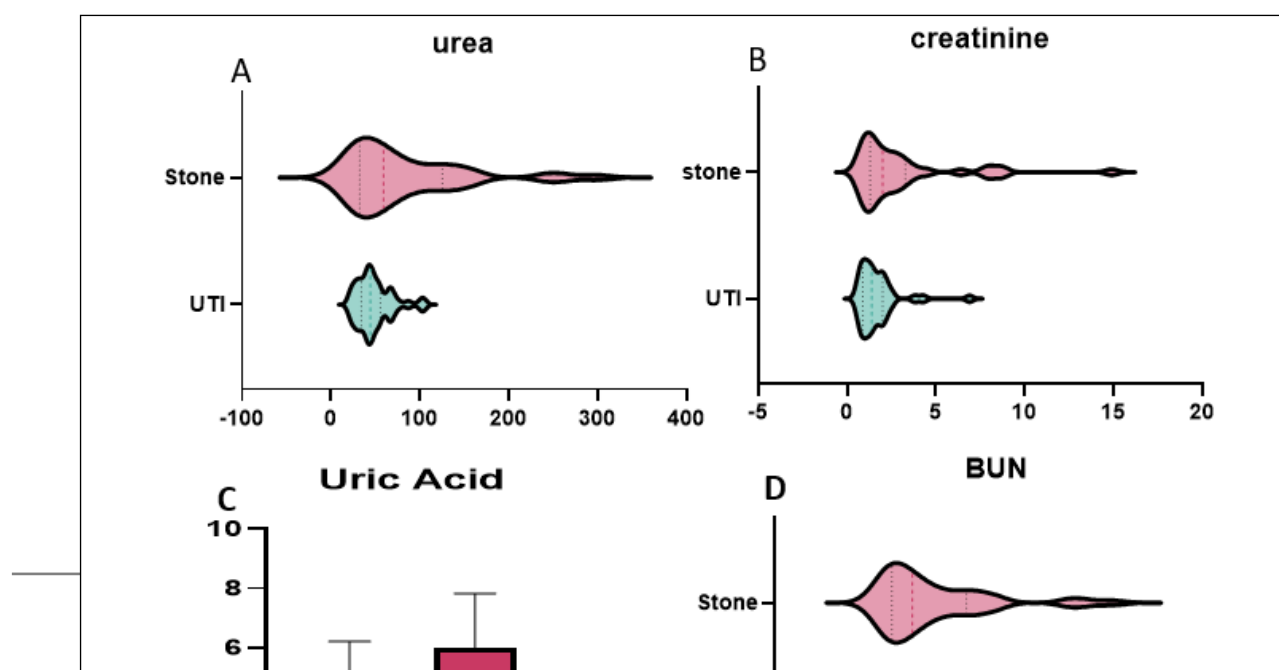


Figure (4-13): comparison of renal function between UTI group and renal stone former group

Chapter Five

Discussion

5.1.Demographic Characteristics of the study Population

In current study, the overall mean age of entire study population was (38.8) years, ranges from 13 to 68 years old. A similar population parameter to previous studies (Habeeb Al-Athari *et al.*, 2017)(Ranjit and Singh, 2020) ,the current study found that most of the patients were 13 to 68 years old, This is mostly connected to the high occurrence of renal stones in the elderly population. (Ismael, 2021) ,in other hand the main age of renal stone patient was at 39.2 These results contrast with(Sigurjonsdottir *et al.*, 2015) who found the median age of stone formers patient was 51 (range, 19–70) years , the average ages of the UTI patient at 38.9 this result is disagree with other studies (García-Agudo *et al.*, 2020) However, we did not found any statistical differences of main age of UTI population and renal stones patient population which is in the line with other study (Xie *et al.*, 2020,García-Agudo *et al.*, 2020) , The outcomes of the present investigation revealed that male patients were affected with renal stones. more than females, with male percentage (62.7%) and female (37.3%with a 1.6:1 ratio) . This result is in covenant with other studies, which showed that urinary tract stones affect men more than women(Kadir *et al.*, 2010) .Shah *et al.*, 2020, Shafi *et al.*, 2013; In this study, the percentage of males was also higher than that of females in the UTI group; this finding agreed with (Nicolle, 2014,(Alwan *et al.*, 2023)These differences could be attributed to the sample size examined, demographic characteristics, type of diets, and period of study. Findings of this study showed no significant differences between the two groups (UTI and Renal stone) for the thee

variables (age, sex, and residence), this finding was in agreement with (Brain *et al.*, 2021)

5.2. Prevalence of urinary tract infection versus Renal stone in different age groups

This study found that both UTI and renal stone occurrence was lower in adolescents age (11-20) (11-20 years), 10.7% and 18.7% respectively in both groups compared to other age groups, which was similar to finding of (Al-joudi *et al.*, 2024), also, in this study the high incidence of UTI in adults, 36-50 years, 30.7%, this finding was in agreement with (Ibrahim *et al.*, 2021), and in line with (Kamel and Ali, 2024, Alwan *et al.*, 2023) At the same time, it was disagreed with, may due to an increased sexual activity in this age or, it may related to other environmental factors, like region or Socio-economic factors This result is consistent with previous study (Al-Tulaibawi *et al.*, 2024), moreover Elderly individuals (51-70) years old are also prone to UTIs, possibly due to immunosenescence and comorbidities that impair bladder function. This result is in line with local study (A. Mohammed, 2025) on other hand the prevalence of kidney stone increased with age from 8.7% in adolescence to 24% in the age class 36-50 years this result is in line with study conducted in Tunisia (Alaya *et al.*, 2012). While other researcher performed a study in which is (Jan *et al.*, 2008) (Renal stones were most commonly encountered in the third and fourth decades of life. The findings contradict the present study, which found that the majority of renal stone patients were between the ages of 51 and 70. However, there are several causes for the differences in the reported prevalence rates in different countries, including the method of conducting epidemiological research, diagnostic criteria of the disease, definition of kidney stone disease, and the disproportionate age and gender groups under observation. (Moftakhar *et al.*, 2022).

5.3.Isolation rate and diversity of bacterial species of UTI and Renal stone

In this study, 53 (35.3%) of 150 urine samples showed microbial growth, similar to the result obtained in a previous local study (Mahmood et al., 2024), and in line with the isolation rate, 25.5% reported by (Ranjit and Singh, 2020), However, this study's overall isolation rate was much lower than the 78.5% obtained by (Alkadasi *et al.*, 2014) in Yamen and 79% in Pakistan (Jan *et al.*, 2008), this differences could be attributed to geographical variability (Habeeb Al-Athari *et al.*, 2017) The isolation rate in the UTI group was higher than in the renal stone group, 38. 7% vs 32%, although no significant differences were observed. This finding was in line with a local study (A. Mohammed, 2025), and with (Jan *et al.*, 2008) regarding the isolation rate in renal stone, 36.4%, while contradicting the isolation rate of UTI, 50%. This study's finding regarding the renal isolation rate of stone patients disagreed with a local study (Kadir *et al.*, 2010). This discrepancy could be attributed to the differences in the sample type, sample number, and diagnostic procedure. (Habeeb Al-Athari *et al.*, 2017)

Regarding the species diversity, this study indicated that only six species, namely, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *P. mirabilis*, and *E. faecalis*, were successfully identified, with *E. coli* being the most prevalent in both groups. This finding was agreed with many previous studies (M. A. Ali and Aljanaby, 2023, Al-Tulaibawi *et al.*, 2024). The high frequency of *E. coli* as the principal causative agent of UTIs in this study was consistent with previous studies, which support its role as the most frequent bacterium responsible for UTIs globally. In comparing the diversity of bacterial species in the urine of UTI and renal stone, this study revealed almost similar species in both groups (Mhana and

Aljanaby, 2023) except for *P. mirabilis*, which was found only in the renal stone group (Shah *et al.*, 2020). Regarding the diversity comparison, the findings of this study were similar to those obtained by (Jan *et al.*, 2008). Many previous studies also support this finding (Golechha and Solanki, 2001, Devraj *et al.*, 2016). The obvious association of *P. mirabilis* with the urine of stone formers in this study was consistent with many previous studies

5.4. Molecular study of bacterial isolates

In this investigation, the twelve isolates were first identified as *K. pneumoniae* using biochemical assays and traditional phenotypic criteria based on physical and biochemical traits. 16S rRNA gene sequencing by amplifying the V4 variable region of bacterial ribosomal RNA. Genomic DNA (gDNA) was extracted from stone homogenate and bladder urine samples using the Blood and Tissue kit according to standard techniques. 16S rRNA gene sequencing was positive for 12 (100%) of UTI patient and renal stone patient. This study is disagree with (Dornbier *et al.*, 2020), the result is in line with study conducted in china (He *et al.*, 2016). All 16 isolates of *E. coli* were recognized by biochemical tests, and *E. coli* bacteria were identified using molecular methods based on the 16S rRNA gene, with the appropriate PCR conditions. Polymerase chain reaction was performed on 16 bacterial isolates, with a gene appearance rate of 100%. Gene appearance rate was 100%. This result agree with local study which is conducted in Baghdad Iraq (Jasim *et al.*, 2019), Al-zubaidi, 2025). In this study, five *Pseudomonas* isolates were initially identified based on morphological and biochemical characteristics. However, further confirmation was required using molecular techniques. The presence of the *OprL Pseudomonas* gene was assessed via PCR, and the results showed that only 20% of the isolates tested positive for this

gene. This finding contrasts with a local study conducted at Al-Hakim General Hospital, which reported that 45 out of 57 isolates (78.9%) were positive for the *oprI* gene, (Alammar, 2025), however *P. mirabilis* isolates were initially identified through morphological and biochemical methods, but further confirmation was carried out using PCR. The presence of the urease proteus gene was investigated, and the results showed that 40% of the isolates tested positive for this gene. This finding contrasts with a local study that reported the presence of the ureR gene in 15 *P. mirabilis* isolates, with 14 of them (93.3%) expressing the ureR gene product. (Al-fahham and Kareem, 2022), In this study there is five *S. aureus* isolates that were initially identified based on morphological and biochemical characteristics, further confirmation was required using molecular techniques. This was achieved through the use of the 16S rDNA gene specific to *S. aureus*, however there is (40%) of 16S rDNA gene expression which is disagree with local study which have revealed that 7/40 (17.5%) isolates were *S. aureus* according to 16S rRNA (Farhood ad Hamim, 2024)

5.5.Profiling of renal stones

In this study, the most common type of stones was calcium oxalate 6/10 (60%) or a mixture of amorphous urate 2/10 (20%) and uric acid stone 2/10 (20%). Nearly similar results were found in other studies (Sabri Ammo, 2012, Afaj and Sultan, 2005). Renal stone is a frequent urological illness with many pathologies (Tsujihata, 2008). The mechanisms contributing to its increasing incidence remain unknown. It is unclear whether bacteria contribute to calcium-based stones, which account for the vast majority of kidney stones. Additionally, in this study, *E. coli* was the only bacterial species isolated from renal stone,

particularly from CaOx type, this finding is in agreement with (An *et al.*, 2021). One possibility is that bacteria adhere to crystals, which promotes their growth and aggregation. This is confirmed by results that bacteria such as Enterobacteriaceae selectively aggregated to oxalate calcium crystal and increased the number of aggregations..(Xie *et al.*, 2020)

5.6. Antibiotic Resistance Profiling of Bacteria isolated from UTI and Renal Stone populations

5.6.1 *E. coli*

The study found that *E. coli*, a member of the Enterobacteriaceae family, is the leading cause of UTIs, accounting for almost half of all cases.

E. coli is part of the natural microbial community in the human intestinal system, which may contribute to the increased risk of infection. As a result, it may quickly establish itself in the urinary system (Kamel and Ali, 2024). Females are more likely to develop a UTI than males because they have a shorter urethra, allowing bacteria to travel a shorter distance to the bladder. (Naqid, *et al.*, 2020), Antimicrobial resistance in uropathogenic *E. coli* is a big concern worldwide since it is increasingly resistant to various routinely administered medications., Beta lactamase enzymes are a global concern, contributing to antibiotic resistance in Enterobacteriaceae. however Antimicrobial susceptibility testing was done on Mueller-Hinton agar (using the disk diffusion (Kirby Bauer's) technique according to the CLSI standards... All isolates were highly resistant to augmantin and sulpha trimethoprim whereas, all isolates were highly susceptible to levofloxacin and meropenem. This results was comparable to different locally and worldwide studies (Polse *et al.*, 2016). Another study conducted in Iraq found that the majority of clinical *E. coli* isolates from urine samples were resistant to amoxicillin/clavulanic acid. The same investigation discovered that *E. coli* isolates were susceptible to

amikacin and imipenem.(Naqid, Balatay, *et al.*, 2020) which is totally in line with study .wile other study(. Hasan *et al.*, 2023) have found that Ciprofloxacin and Levofloxacin isolates have a moderate resistance rate to 37 (63.79%) isolates. which is agreed with study

In the present investigation, we discovered that the degrees of resistance to various antibiotics were higher in UTI samples than in renal stone samples, which could be related to inappropriate and excessive use of antibiotics over the previous years. This finding could be equivalent to local study. (Mohammed *et al.*, 2021)

5.6.2 *K.pneumonia*

The sensitivity of *K. pneumoniae* to antibiotics was determined based on the Kirby-Bauer method The test includes 8 types of common antibiotics

In the current study most of the 12 bacterial isolates showed strong antibiotic resistance, with varying resistance to beta-lactam & salphonamide antibiotics. For example, the resistance of *K. pneumoniae* to Augmentin & sulpha-trimetoprim reached 77% for both , lesser than the antibiotic resistance of 82.8% recorded by(. Mohammed *et al.*, 2023) showed the lowest effect towards *K. pneumoniae* isolates, As for meropnim and amikacin , both antibiotics recorded a resistance of up to 22%,11% respectively, which agrees with the findings of(Ahmed Hasan *et al.*, 2021). Our findings align with prior studies(Abd Fouad, 2025),.which reported high resistance to sulphanomide and amoxicillin-clavulanate, relative to carbapenems, In the same study, Klebsiella was observed to be somewhat sensitive to ceftriaxone, ciprofloxacin, and gentamicin, which is in line with local studies. (Naqid, Balatay, Hussein, Ahmed, *et al.*, 2020), The current study found that *K. pneumoniae* isolated from renal stone patients' urine was 33% and 100% sensitive to

ciprofloxacin and levofloxacin, respectively, but it was extremely resistant to augmantin. Furthermore, meropenem was found to be the most promising antibiotic against *K. pneumoniae* isolated from the urinary tract system, followed by cefotaxam and gentamicin, but it was highly resistant to amoxicillin/clavulanic acid.,

5.6.3 *P. mirabilis*

Proteus mirabilis is a Gram-negative rod-shaped bacteria that thrives in the natural world. It is well-known for its swarming motility and urease activity. *P. mirabilis* is the main bacterium causing complicated urinary tract infections (UTIs)..(Yuan *et al.*, 2021)

All *Proteus mirabilis* isolates were verified by using Cefoxitin, Amikacin, , Ciprofloxacin, , Gentamicin ,levofloxacin, Sulphamethazol-trimethoprim, Cefotaxim, Amoxicillin-clavulanic acid. Then Isolates were classified as resistant or sensitive according to previously published regulations.Local study (Salama *et al.*, 2025) have establish

The antimicrobial susceptibility patterns of *P. mirabilis* isolates were determined using the disc-diffusion method. Augmentin had the highest resistance level, followed by trimethoprim/sulfamethoxazole. There was moderate resistance to ciprofloxacin, cefotaxime, and ciprofloxacin. The study found limited resistance to amikacin and meropenem, which aligns with current findings. Another study (Abed Gumar *et al.*, 2022)have found *Proteus mirabilis* isolates include an aminoglycoside-modifying enzyme that functions as an inhibitor against antibiotics in this group, such as amikacin and gentamycin, therefore isolates of these pathogenic bacteria become resistant to antibiotics of the aminoglycoside group .this is un like what we have found in the current study which was moderate resistance to amikacin and gentamycin

5.6.4 *p. aeruginosa*

P. aeruginosa remains a critical health hazard, causing significant morbidity and mortality, particularly in ICUs and long-term care facilities.(Renolds and Kollef, 2021), This study sheds light on the microbicide resistance in major ways on *P. aeruginosa* as one of the primary causes contributing to UTI associated with antibiotic resistance. local study have found *P. aeruginosa* strains were identified. The results specified that the common of *P. aeruginosa* show high resistance to Amoxicillin + Clavulanic acid In divergence, there was significantly less resistance to Meropenem.:this study have concluded Meropenem was the encouraging antimicrobial agent used to treat the *P. aeruginosa* infections this result is in line with (Driscoll *et al.*, 2007) , (Al-Makhzoomy *et al.*, 2025) ,other local study have found that The growth in carbapenem resistance due to - β -lactamases, as well as resistance to aminoglycosides and quinolones, highlights the development of multidrug-resistant bacteria.(Hussein *et al.*, 2025) this is in contrast to what we have found in our study . The findings of our study indicate that the prevalence of ciprofloxacin resistance in *P. aeruginosa* isolates was 80 %, whereas for levofloxacin resistance this was 60 %; this was not in contrast with (Owaid and Al-Ouqaili, 2025). Finally our study have found high resistance rate to most antibiotics especially gentamicin this result is in line with study conducted in Libya (Abdelaziz *et al.*, 2025)

5.6.5 *S .aureus*

The spread of antibiotic-resistant forms, particularly MRSA, has made *Staphylococcus aureus* infections more difficult to treat. Infections caused by *Staphylococcus aureus*, which is resistant to several antibiotics, can be difficult to treat. In this study high resistance rate to most clinical samples especially the sample taken from renal stone compering to (Thari *et al.*, 2024) These findings highlight the necessity for the Health

Authority to develop adequate antibiotic treatment protocols in our institutions. The isolates in this investigation had a high resistance rate to cefoxitin and Amoxicillin-clavulanic, which was in line with the findings of a study by (Sami Awayid and Qassim Mohammad, 2022) This explains the widespread usage of these antibiotics in the treatment of various illnesses . This study used aminoglycosides gentamicin and amikacin, which resulted in varied resistance patterns for *S. aureus*, with both showing intermediate resistance. This result is in line with (Alwash and Aburesha, 2021). These variations in the resistant pattern could be caused by variances in resistant mechanisms depending on the plasmidial or chromosomal genetic elements (AL-Salihi *et al.*, 2023).

5.7. Renal Functions in Renal Stone Patients

This study found that levels of uric acid and blood urea nitrogen (BUN) were elevated in individuals with kidney stones. Specifically, the average uric acid level in this group was 5.985, and the median BUN level was 27.4. These findings contrast with those of another study, which reported that patients with uric acid and struvite stones had significantly lower valued glomerular filtration rates related to those with other types of stone conformations. (Chou *et al.*, 2011)

Conclusions and Recommendations

CONCLUSIONS

1. This study found that kidney stones are more common in men, and the majority of patients are in their fifth to seventh decade.
 2. Female patients were more prone to urinary tract infections.
 3. The most prevalent bacterium detected from the preoperative urine culture was *E. coli*, followed by *Klebsiella* and *Pseudomonas*..
 4. The most common kind of kidney stone is CaOx, followed by amorphous urate and mixed stones containing both uric acid and amorphous urate.
 5. Culture positive urine is associated with stone (CaOx), and the most .common organism identified with this type of stone was *E. coli*
- Thus, we would like to note that *E. coli*, albeit not generating urease, are important organisms recovered in the preoperative culture of urine.

Recommendations

- 1.Many aspects of renal stone development are uncertain. Understanding the processes of renal stone related with stone inhibitors or promoters is crucial for stone-removing medicines.
2. Furthermore, understanding the underlying genetic basis of kidney stone production should lead to the discovery of a method to control renal stones in the near future..
- 3.Use meropenem for treatment UTIs that caused by Enterobacteriaceae with prior antibiotic susceptibility testing, amikacin and levofloxacin for those caused by *E .coli* , however ,sulfatrimethprim and amoxicillin and claviolanic acid , have wide range of resistance

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وبلغت نسبة العزل البكتيري في مجموعة الالتهاب البولي ٣٨,٧٪، وهي أعلى قليلاً من النسبة في مجموعة الحصوات الكلوية (٣٢٪)، لكن التحليل الإحصائي بين عدم وجود فرق ذي دلالة إحصائية بين المجموعتين ($P > 0.05$).

أما اختبار الحساسية للمضادات الحيوية، فقد أظهر أن أموكسيسيلين/حمض الكلافولانيك وسلفاميثوكسازول/تريميثوبريم كانا الأقل فعالية، في حين أبدت المضادات الحيوية أميكاسين، وسيفوتاكسيم، وسيبروفلوكساسين، وجنتاميسين، وليفوفلوكساسين فعالية متوسطة، وكان ميروبينيم الأكثر فعالية من حيث الحساسية البكتيرية.

ومن بين ١٠ حصوات كلوية خضعت للتحليل الكيميائي، تم تحديد ثلاثة أنواع رئيسية وهي اوكسالات الكالسيوم وكانت بنسبة ٦٠٪ تليها حصوات حمض اليورك بنسبة ٢٠٪ وخليط من حمض اليورك وحصوات الامورفص يوريت وبنفس النسبة وكانت حصى الكالسيوم اوكسالات هي حصى مصابه وتم عزل الاشريشيا القولونية منها جميعا.

الخلاصة

تعد التهابات المسالك البولية من أكثر الأسباب شيوعاً لتكوّن حصى المسالك البولية قد شهدت نسبة الإصابة بالحصى تغيراً كبيراً خلال العقدين الماضيين. كما وكشفت دراسات عدة عن وجود علاقة بين الحصى والتهابات المجاري البولية. تهدف هذه الدراسة إلى مقارنة انتشار العدوى، وأنماط الحساسية للمضادات الحيوية، وتنوع البكتيريا في بول المرضى المصابين بالتهابات المجاري بولية مع أولئك المصابين بالتهابات بولية مصحوبة بحصى كلوية.

ولهذا، جُمعت ١٥٠ عينة بول من مجموعتين: الأولى مكونة من ٧٥ مريضاً مصاباً بالتهاب بولي فقط، والثانية من ٧٥ مريضاً مصاباً بالتهاب بولي مصحوب بحصوات كلوية، وذلك من مستشفيات محافظة ذي قار خلال الفترة الممتدة من آذار/مارس ٢٠٢٤ إلى أيار/مايو ٢٠٢٥. كما جُمعت ١٠ عينات من الحصوات الكلوية لتحليلها كيميائياً، إضافةً إلى أخذ ٨٠ عينة دم من المجموعتين اعلاه لفحص وظائف الكلى (اليوريا، والكرياتينين، وحمض اليوريك في مصل الدم).

أجري عزل البكتيريا وتحديدها باستخدام الطرق البكتريولوجية والكيميائية الحيوية التقليدية (مثل اختبارات، اوكسيديز، كتليز، وصبغة غرام اضافته الى اختبار IMVIC)، فضلاً عن التقنيات الجزيئية ضراوة معينة مثل 16S rRNA of k pneumonia, techniques (, 16S rRNA of E. Coli, 16srDNA S. aureus, Urease proteus gene, OprL Pseudomonas gene) اظهرت نتائج تفاعل البوليمريز المتسلسل ان بكتريا الاشيريشيا القولونية كانت موجبة لجين 16S rRNA of E. Coli بنسبة ١٠٠٪ وكذلك الامر بالنسبة لبكتريا الكلبسيلا الرئوية اما بالنسبة لبقية العزلات كانت

كما وتم فحص الحساسية الدوائية للعزلات البكتيرية باستخدام طريقة كيربي بور وتتم بطريقة الانتشار

بلغ متوسط أعمار المشاركين في الدراسة ($\pm$ الانحراف المعياري) $38,8 \pm 14,6$ سنة، وتراوح أعمارهم بين ١٣ و ٦٨ سنة. وكان متوسط عمر مجموعة الالتهاب البولي $38,9 \pm 14,1$ سنة، بينما بلغ متوسط عمر مجموعة مُشكّلي الحصوات الكلوية $39,2 \pm 15,6$ سنة. وأظهر التحليل الإحصائي عدم وجود فروق ذات دلالة إحصائية ($P \geq 0.05$) بين المجموعتين بالنسبة للمتغيرات الثلاثة (العمر، الجنس، والسكن وكشف الفحص البكتريولوجي أن ٥٣ عينة فقط (٣٥,٣٪) أظهرت نمواً بكتيرياً إيجابياً على وسائط الزرع. وتم تحديد ستة أنواع بكتيرية بنجاح، كان أكثرها انتشاراً

تم التعرف على ٦ أنواع من البكتيريا، وكان أكثرها انتشاراً بكتيريا الإشريشية القولونية، تلتها بكتيريا كليبسيلا، ثم الزوائف والعنقوديات. أما بكتيريا الإنتيروكوكس فقد كانت الأقل انتشاراً



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الخصائص الكيميائية الحيوية والبكتريولوجية للعدوى البولية المرتبطة بالحصوات المُعدية

رسالة ماجستير مقدمة الى مجلس كلية الطب البيطري- جامعة الشطرة وهي جزء من
متطلبات الحصول على شهادة الماجستير في اختصاص علوم الطب البيطري (الاحياء
المجهرية)

مقدمة من قبل
احمد رشيد ساجت

بكلوريوس طب وجراحة بيطريه (٢٠١٦)

تحت اشراف

ام د نبيل مهدي عبد

ام د مسلم ضاهر موسى

١٤٤٧ هجريه

٢٠٢٥ ميلاديه

