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ISOLATION AND IDENTIFICATION OF BACTERIA CAUSING URINARY TRACT INFECTION AND INVESTIGATION OF INHIBITORY EFFECTS OF FENUGREEK SEEDS AND GINGER ROOTS AGAINST ISOLATED BACTERIA

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November 2023

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ABSTRACT

ISOLATION AND IDENTIFICATION OF BACTERIA CAUSING URINARY TRACT INFECTION AND INVESTIGATION OF INHIBITORY EFFECTS OF FENUGREEK SEEDS AND GINGER ROOTS AGAINST ISOLATED BACTERIA

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Master of Science in Biology

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Urinary tract infections (UTIs) pose a significant health concern globally, necessitating a thorough investigation into the causative bacterial species, their antibiotic resistance profiles, and potential alternative treatments. This study, conducted at Al-Alam General Hospital, aimed to provide a comprehensive understanding of UTIs by analyzing 200 urine samples collected from both male and female patients with acute and chronic infections spanning from December 2021 to July 2022. Bacterial isolates were cultured on MacConkey, blood, and mannitol agars, followed by gram staining and confirmation through the VITEK 2 system. Out of the total samples, positive growth was observed in 138 cases (69%), while 62 samples (31%) showed no bacterial growth on culture media. Further stratification revealed that among infected women, 95 (66.9%) exhibited positive bacterial growth, and 47 (33.1%) did not. In infected males, 43 samples (74.1%) showed positive cultures, while 15 samples (25.9%) demonstrated no bacterial growth. The study delved into the antibiotic sensitivity profiles of the bacterial isolates, uncovering significant variability in their response to the antibiotics under investigation. This variability emphasizes the critical need for understanding antibiotic resistance patterns, providing essential insights for the development of targeted treatment strategies tailored to the specific strains encountered. To explore alternative treatment modalities, alcoholic and aqueous extracts from ginger root and fenugreek bean were assessed for their inhibitory effects on bacterial growth at various concentrations. The results indicated that alcoholic extracts from both plants exhibited higher and more potent effectiveness against the isolated bacterial species compared to their aqueous counterparts. These findings suggest a potential role for alcoholic extracts from ginger root and fenugreek bean as promising candidates in the combat against UTIs, offering avenues for alternative or complementary therapeutic approaches. This study

contributes a nuanced understanding of the bacterial species responsible for UTIs, their antibiotic resistance profiles, and the potential antimicrobial efficacy of plant extracts. The findings not only shed light on the complexities of UTIs but also provide valuable insights for the development of more targeted and effective strategies for the management of urinary tract infections. Further research and clinical trials may build upon these findings, potentially paving the way for innovative and personalized treatment approaches.

2023, 82 pages

Keywords: Antibiotic resistance, Minimal inhibitory concentration, Plant extracts, Urinary tract infection

ÖZET

İDRAR YOLU ENFEKSİYONUNA NEDEN OLAN BAKTERİLERİN İZOLASYONU VE TANIMLANMASI İLE ÇEMEN OTU TOHUMLARI VE ZENCEFİL KÖKLERİNİN İZOLE BAKTERİLERE KARŞI İNHİBİTÖR ETKİLERİNİN ARAŞTIRILMASI

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İdrar yolu enfeksiyonları (İYE'ler), küresel olarak önemli bir sağlık sorunu teşkil etmekte olup, neden olan bakteri türlerinin, bunların antibiyotik direnç profillerinin ve potansiyel alternatif tedavilerin kapsamlı bir şekilde araştırılmasını gerektirmektedir. Al-Alam Genel Hastanesi'nde gerçekleştirilen bu çalışma, Aralık 2021'den Temmuz 2022'ye kadar akut ve kronik enfeksiyonu olan hem erkek hem de kadın hastalardan toplanan 200 idrar örneğini analiz ederek İYE'lerin kapsamlı bir şekilde anlaşılmasını sağlamayı amaçladı. Bakteri izolatları MacConkey'de kültürlendi. , kan ve mannitol agarlar, ardından gram boyama ve VITEK 2 sistemi aracılığıyla doğrulama yapılır. Toplam örneklerin 138'inde (%69) pozitif üreme gözlenirken, 62 örnekte (%31) kültür ortamında bakteri üremesi görülmedi. Daha fazla sınıflandırma, enfekte kadınlar arasında 95'inin (%66,9) pozitif bakteri üremesi sergilediğini, 47'sinin (%33,1) ise göstermediğini ortaya çıkardı. Enfekte erkeklerde 43 örnekte (%74,1) pozitif kültür görülürken, 15 örnekte (%25,9) bakteri üremesi görülmedi. Çalışma, bakteriyel izolatların antibiyotik duyarlılık profillerini araştırdı ve araştırılan antibiyotiklere verdikleri yanıtlarda önemli değişkenlikleri ortaya çıkardı. Bu değişkenlik, karşılaşılan spesifik suşlara göre uyarlanmış hedefe yönelik tedavi stratejilerinin geliştirilmesi için temel bilgiler sağlayarak, antibiyotik direnç modellerini anlama konusundaki kritik ihtiyacı vurgulamaktadır. Alternatif tedavi yöntemlerini araştırmak için zencefil kökü ve çemen otu fasulyesinden elde edilen alkollü ve sulu ekstraktlar, çeşitli konsantrasyonlarda bakteri büyümesi üzerindeki önleyici etkileri açısından değerlendirildi. Sonuçlar, her iki bitkiden elde edilen alkollü ekstraktların, sulu muadillerine kıyasla izole edilmiş bakteri türlerine karşı daha yüksek ve daha güçlü etkinlik sergilediğini göstermektedir. Bu bulgular, alternatif veya tamamlayıcı terapötik yaklaşımlar için yollar sunan, İYE'lere karşı mücadelede umut verici adaylar olarak zencefil kökü ve çemen otu

fasulyesinden elde edilen alkollü ekstraktların potansiyel bir rol oynadığını göstermektedir. Bu çalışma, İYE''lerden sorumlu bakteri türlerinin, bunların antibiyotik direnç profillerinin ve bitki ekstraktlarının potansiyel antimikrobiyal etkinliğinin ayrıntılı bir şekilde anlaşılmasına katkıda bulunmaktadır. Bulgular yalnızca İYE'lerin karmaşıklığına ışık tutmakla kalmıyor, aynı zamanda idrar yolu enfeksiyonlarının yönetimi için daha hedefe yönelik ve etkili stratejilerin geliştirilmesine yönelik değerli bilgiler de sağlamaktadır. Bu bulguların üzerine daha fazla araştırma ve klinik denemeler yapılması potansiyel olarak yenilikçi ve kişiselleştirilmiş tedavi yaklaşımlarının önünü açabilir.

2023, 82 sayfa

Anahtar Kelimeler: Antibiyotik direnci, Minimum inhibitör konsantrasyonu, Bitki özleri, İdrar yolu enfeksiyonu

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CONTENTS

LIST OF SYMBOLS

dL	Deciliter
°C	Degrees celsius
g	Gram
L	Liter
µL	Microliter
mg	Milligram
mL	Milliliters
-	Minus
%	Percent
+	Plus
m2	Square-Meters
U	Unit

LIST OF ABBREVIATIONS

CONs	Coagulase negative *staphylococci*
DNA	Deoxyribonucleic acid
MHA	Muller hinton agar
MIC	Minimum inhibitory concentration
MRSA	Methicillin resistant *S. aureu*s
WHO	World health organization

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1. INTRODUCTION

Medicinal plants are everything of plant origin, which is a plant that contains a substance or medicinal substances capable of treating a certain disease or reducing its incidence, or that contains raw materials used in the preparation of medicinal materials. Its importance lies in that it can be used in disease cases. They are used in pathological cases in which it is difficult to use chemical medicines for fear of deterioration of the patient's condition (Sumner 2000). They are considered safe for use, cheap and easy to apply without the need for special skills and experience in preparing and preparing them for use. They are available in most countries (Jamshidi-Kia *et al.* 2017).

It is also used easily in third world countries where there are few doctors, pharmacists and specialists. It treats more than one pathological condition and leads to psychological reassurance when used as a spice or drink and in the manufacture of insecticides and cosmetics (Koçyiğit and Sinanoğlu 2020) Useful in cases of anaemia and diabetes. Fenugreek seeds were chosen in this study because they are the medicinal part of this plant that is therapeutically important and widely used. The selection of fenugreek seeds in this study is due to the fact that it is the medicinal part of this plant, which is important for its treatment and widely used for symptomatic treatment (Muhammad 2006). As for the ginger plant, it is from the Zanzibar family, Zingiberaceae. Ginger thrives in moist, fruitful tropical soils. A thick, earthen stem adorned with a knot constitutes ginger. This subterranean stem is buried beneath the soil. This stem, which is approximately 30 centimeters in length, protrudes from the soil surface. A cluster of tall, green, ribbed leaves and white or yellowish-green flowers grow on this stalk (Smith-Hall *et al*. 2012). Both fully mature and partially mature types of ginger exist. The mature one requires the removal of a tough peel. Younger ginger can be purchased in grocery stores without being peeled (Ahmed, 2012). It is a plant species of the genus Ginger of the Zingiberaceae family, from plants in tropical regions. Its growing stems are used under the soil, which contain volatile oil, which have a pungent odor and pungent taste, and their color is either squirrel or yellowish-white (Ali *et al.* 2008). After drying, the rhizomes of ginger contain volatile oils and resins, the most important

of which are gingerols, starchy and gelatinous materials. Cases of urinary infections are among the common complications and diseases that affect people of both genderes, and their seriousness lies in their transformation from an emergency situation into a chronic condition, sometimes it causes impotence and kidney failure in the infected person. Urinary infections are both symptomatic and asymptomatic. It is a common and important disease, as infection occurs in both genderes and for all ages, and the incidence of urinary infections increases in women more than men due to physiological and hormonal factors (Kadnur and Goyal 2005). Urinary tract infections are treated using clinically valuable antibiotics, which damage microorganism cells without harming the host. However, widespread consumption of antibiotics without proper regimens remains a concern. Not only that, which led to the emergence of resistant strains of germs, but this led to the germs adapting to many antibiotics such as penicillin and other antibiotics (Afshar 2007).Therefore, the attention of many scientists and researchers turned to the use of plant extracts to find effective alternatives to the drugs that are used to treat infections in the urinary tract, as the plant extracts are characterized by their effect on pathogenic germs and have either limited or no collateral damage (Gupta *et al.* 2010).

Aims of study:

1. Isolation and diagnosis of bacteria that cause urinary infections for both genderes.
2. A procedure for detecting antibiotic sensitivity against bacterial isolates and determining their sensitivity to live antibiotics used as control samples.
3. Determination of the effect of plant extracts on pathogenic bacterial isolates isolated and diagnosed in the laboratory.
4. Comparison between the effect of antibiotics and plant extracts in terms of efficiency in inhibiting microorganisms.
5. Determination of the minimum inhibitory concentration (MIC) for the used plant extracts.

2. LITERATURE REVIEW

2.1 Urinary Tract Infection (UTI)

Urinary Tract Infection (UTI) is an infection that affects any part of your urinary system the kidneys, ureters, bladder, and urethra and it is the most frequent medical infection, affecting all age groups (Addose 2000). UTIs, or infections of the urinary tract, are the most commonly seen medical condition in hospitals around the world. The global burden of UTIs is estimated to be over 150 million, resulting in significant morbidity and substantial healthcare expenditures. In the United States, urinary tract infections account for more than 10 million annual doctor visits, 2 million annual visits to the emergency room, and 100,000 annual hospital admissions (Flores-Meireles *et al.* 2015).

Most infections include the lower part of the urinary tract (bladder and urethra). An alternative definition of urinary tract infection is an inflammatory reaction of the urinary system triggered by the presence of pathogenic microorganisms in the urinary system's vessels, specifically in the fresh urine sample where the causative microorganisms are detected. The presence of bacteria in the urine, Bacteriuria, and it is difficult to distinguish and diagnose the bacteria if it is an external contamination or an internal microbial infection (Barini-García and Whitmore 2008), and the infection can be in any part of the urinary tract, and there may be symptoms or notany symptoms (Buys *et al.* 1994). Urinary tract infections occur when pathogenic microorganisms are present in the urine, urethra, bladder, or kidney (Stamm and Hooton 1993).

Under normal circumstances, urine is sterile and free of microorganisms, including bacteria, fungi, viruses, and others (Staiman and Lowe 2001), and the incidence of inflammation in females is higher than that of males for physiological and anatomical reasons (Forbes *et al.* 1998). The urinary tract is exposed to various common infections that amount to more than one million people annually. Approximately 30% of cases of urinary tract infections are cured, and most of them are acute infections that last for short periods and are accompanied by cases of renal failure (Avorn *et al.* 1994).

Urinary tract infections are a prevalent medical condition, affecting millions globally, varying in complexity depending on structural or functional abnormalities (Bacheller and Bernstein 1997). Most urinary tract infections begin with Urethritis and then the infection develops into cystitis and then Ureteritis. The infection is usually treated after taking a full course of treatment for ten days. As for the patient not recovering, the reason is either due to the resistance of the bacteria causing the sewage infection to the antibiotic used or due to the presence of congenital or structural abnormalities in the urinary system, which in turn increases the severity of the infection. Usually, most infections occur, especially after using contaminated devices in hospitals such as urinary catheters or Cystoscopies or after prostatectomy in men (Cruickshank *et al.* 1975), and that the presence of chronic diseases such as diabetes, chronic kidney disease, kidney transplantation, and the presence of stones enable microorganisms to exist in the urinary tract and thus enable them to cause inflammation. The incidence of kidney stones in men is 10%, and the ratio between men to women is 3:1. Stones occur more often at the age of 20-40, and if they occur outside this age group, it may be due to a disorder in the metabolism process, such as an increase in the ratio of Serum uric acid (Watterso *et al.* 2003, Warren *et al.* 1999).

2.2 Infection of UTI

Urinary tract infections usually occur when microorganisms move from the anal area and surrounding areas through the skin and stick to the opening of the urethra and then begin to multiply and grow inside the urethra, causing urethritis or as a result of the presence of bacteria in the genital tract, especially in the vagina, Or around the urethra. Bacterial species that cause infection often enter through the opening of the urethra and then leave towards the bladder to infect the mucous layer lining it and then cause cystitis (Kunin *et al.* 1993), or infect the upper parts of the urinary tract, including the kidney. And then it causes what is called glomerulonephritis (Schaeffer *et al.* 2007), and is helped by the wrong wiping method during urination and genderual intercourse, which in turn causes bacteria to enter the opening of the urethra and wait for a long time while keeping the urine inside the bladder as well as the use of effective contraceptives External use, and in most cases, the body resists the presence of this revival and does

not show any symptoms towards it (Barini-García and Whitmore 2008). In a study conducted by Todar (2002) in the United States, it was found that the causative agent of the disease prevailing in the urinary tract is due to the bacteria E.coli, and the infection rate ranged between 75-85%, and its virulence appears clear in all cases due to its clinical importance (Wawrysiuk *et al.* 2019), in the United States other study also showed that all types of the Enterobacteriaceae family cause urinary tract infections, especially urethritis and cystitis, and he also explained that other microorganisms such as fungi, chlamydia, mycoplasma, and others It has a role in the incidence of infection, but to a lesser extent (Tierney *et al.* 2003).

2.3 Epidemiology of UTIs

With the development of science in the last decades of the last century and the present century, especially in the field of radiography or using x-rays, and as a result of this development, doctors and researchers were able to diagnose cases of disorders that accompany the urinary tract, and it became easy to identify these disorders if they were epidemic or caused by inflammation Bacterial, parasitic, or dysfunction in the urinary system. The number of patients who visit outpatient clinics for urology in the United States of America alone is about 7 million infected, and the reviewers to government hospitals amount to about one million infected, which led to the admission of 100,000 injured to the hospital (Stamm and Hooton 1993).

The French epidemiological studies estimated in their annual bulletins that the number of cases diagnosed with infection reached 53,000 per million people during one year, which represents 1.05% to 2.10%. In the United States, the annual bulletins estimate that the number of women infected with glomerulonephritis reaches 250,000 (Pinson *et al.* 1994), and there are many ways of infection with urinary tract infections all over the world, and it must be noted that the difference in the process between people and between races is due to many factors that must be taken care of so that simple ones do not turn into an epidemic that poses a threat to societies, as studies show that The incidence of UTI increases in women and men after marriage. By the time they are 24 years old, between 40 and 50 percent of women will have experienced a UTI serious

enough to warrant antibiotic therapy from a doctor. More than half of married or pregnant women experience considerable bacteriuria within the first three months of marriage, and the gender factor plays a role in facilitating the transfer of germs from the vagina to the urethra, leading to urinary tract infections (Beckford-Ball 2006).

2.4 Classification of UTIs

Urinary tract infections can be classified according to their degree of severity into two types: complicated UTI and uncomplicated UTI, depending on the presence or absence of pathogens associated with UTI cases. Complex cases include urinary blockages arising from the presence of urinary stones or kidney function disorders. Either Uncomplicated UTI, including cystitis (Smelov *et al.* 2016).

2.4.1 Uncomplicated UTIs

Urinary tract infections comprise the most significant proportion of infections that manifest in the natural urinary tract, where no functional or anatomical abnormalities are present. This is due to the fact that the symptoms of urinary tract infections vary among individuals and age groups, making it difficult to distinguish between inflammation affecting the kidney or bladder (Kahlmeter 2003). Furthermore, these symptoms do not correlate with disorders that result in renal dysfunction (Emmons *et al.* 2004), illustrative instances of uncomplicated infections comprise urinary tract infections, bladder infections, and kidney and pelvic infections (Johansen et al. 2001). Among these, infections affecting the kidneys and pelvis, commonly referred to as Bright's disease, frequently manifest inflammation of the pelvis and cortex. This condition poses a significant risk to the patient's life and comprises the so-called inflammatory syndrome (Black 1999).

Bacterial infection is prevalent among women owing to the anatomical configuration of the female urinary system. It has the potential to affect certain women for the duration of their lives. Urethritis is an example of this infection, which is caused by bacterial

contamination along the urethra or a portion thereof. The infection is frequently transmitted through gender-specific contact or via urethral catheters (Cohn and Schaefrer 1998) which is a tube inserted into the bladder to drain pee in unusual circumstances such as urinary retention, infrequent urination, or prior to surgery (Ward and Jones 1996).

Every day, between 2% and 7% suffer from urinary infections as a result of their use (Pratt *et al.* 2001). The predominant category of infection is those who have been under the influence of catheter use for a long time, including the elderly or the infirm. Young people of either genderes or children are not excluded (Vickrey *et al.* 1999). The destructive effect of using catheters is attributed to the occurrence of erosion of the mucous layer of the urethra and bladder, which makes it a place for the colonization of bacteria (Krasinski 1998).

Bacterial inflammation that affects the kidneys and their presentation usually results from the rise of pathogens through the urethra, although it may be transmitted to it through the blood. 75% of cases result from *E.coli* and the rest from *P. mirabilis* 15%, as for streptococcal and staphylococcal bacteria, they constitute 5% of these cases, while Pseudomonas bacteria and Klebsiella app constitute 10% of all infections (Mevrier and Guibert 1992).

Infections of the kidneys and its acute pelvis include acute pyelonephritis, especially in women, as a result of their invasion by bacteria and parasites. This inflammation leads to enlargement of the kidney, and it may happen that some pus cells collect, forming an abscess, especially for those who have congenital defects or blockages, and chronic kidney and pelvic infections (Stapleton 2003). Pyelonephritis resulting from recurrent and persistent bacterial infections for long periods resulting from the irregular and incorrect treatment of the site of infection, which results when acute nephritis is not treated properly and its recurrence causes fibrosis and cysts of the tissues of the kidney, which makes its size smaller than normal and shrinks with it the functions that It may multiply until it reaches the stage of renal insufficiency or complete failure (Dennis *et al.* 2007).

2.5 Complicated UTIs

Urinary tract infection can lead to pathogenic complications, including new diseases, bladder stones, inflammatory shock, and kidney function issues, potentially causing renal failure (Black 1999). Inflammation in the urinary tract can develop in patients with functional and anatomical abnormalities, such as an enlarged prostate, abnormal structures, and kidney stones. These abnormalities can result from spinal cord injury, kidney transplants, or incomplete treatment, leading to strain resistance and worsening symptoms (Sim 2001).

Many people with urinary tract infections suffer from the uselessness of treatment after the infection has recurred twice or more and within a period of fewer than six months, as the treatment (antibiotics) does not work against the bacteria's resistance to it, or as a result of improper treatment of bacterial sites, and this enables the bacteria to enter Treatment resistance and infection of the kidney tissues by surrounding them with a layer of inflammatory organisms, especially gas-producing bacteria, usually E.coli that more than 50% of men who have a defect in the work of the prostate suffer from the return of urinary infections (Rozenberg *et al.* 2004).

Men are less likely to develop urinary tract infections than women, because the urinary tract of men is longer than the urinary tract of women, and men are affected at different ages. The incidence of urinary tract infection in adult males under the age of 50 is low, but the infection rate increases in elderly people who suffer from inflammation of the prostate gland, and genderual intercourse is one of the most important causes of infection resulting from the transmission of infection from the infected party to the other, so It is recommended to empty the bladder and fully form it after each intercourse to prevent ureteral syndrome or injury to the bladder (Nester *et al.* 2001).

Adults and those at a later age suffer from Diabetes, as they are more vulnerable to the risks and complications of urinary tract infection due to changes in immune activity or the immune system, as the incidence of asymptomatic bacterial environment increases with age. Risk factors for urinary tract infection in men include prostatic hyperplasia

and urological interventions, such as transrectal biopsy of the prostate (Karve *et al.* 2018), as it increases by approximately 40% in women and 30% in men in the absence of good care (Gomila *et al.* 2018). The majority of urinary tract infections in adults are nosocomial in nature, meaning they are acquired within healthcare settings. These infections pose a greater risk compared to those acquired from the community or the environment due to the high prevalence of multidrug-resistant bacterial species within hospital settings. Furthermore, the widespread availability and transmission of all strains of these bacteria within hospitals contribute to the increased difficulty in treating these infection (Mckane and Kandel 1985).

2.6 UTI in Women

Women with simple urinary tract infections are unlikely to recur, with one in five experiencing the infection annually, while men are infected once. Urinary tract infections provide a particular challenge for the individual in question, with around one-third of females experiencing such an illness at some juncture over their lifetimes. Appropriate treatment of UTIs requires careful classification including the site of infection, the complexity of the infection, and the possibility that the infection is due to an abnormality such as diabetes, stasis, or difficulty urinating (Schaeffer *et al.* 2007).

Weakness and aging also have a major impact on the infection. Postmenopausal women experience increased UTI incidence due to estrogen deficiency, promoting Lactobacillus milk ganglia proliferation, and reducing pH, preventing Enterobacteriaceae from invading the vagina (Raz 2001).

Bladder is susceptible to infections, causing dysuria, pain, frequent urination, and pus cells in 3/4 patients due to abnormal anatomical conditions and labia. The presence of a favorable and humid environment for the colonization of pathogenic bacteria responsible for these infections contributes to an 8-fold higher frequency of infection in women compared to men (Guay 2009). Acidic, with a pH ranging from 3-5, which is not suitable for colonization and growth of bacteria in this region (Prescott *et al.* 1999).

Pregnant women experience higher rates of urinary inflammation and bacterial infections due to urinary stagnation, uterine location, and increased amino acids. This growth increases pressure on the bladder, causing frequent urination and bacterial infections. Proper hygiene and care are crucial for maintaining a healthy urinary system during pregnancy (Gary 2006).

The hormonal and physiological changes that occur in the urinary tract during pregnancy are responsible for the increased infection of pregnant women with urinary tract infections. The uterus as a result of pregnancy (Anger *et al.* 2019) At this stage of pregnancy, the body of the urinary bladder expands and its ability to contract decreases, and the incidence of ureteral reflux increases (Partin 2021)

In addition to the fluctuation in the levels of estrogen and progesterone in the blood and their effect on the genital ducts and the resulting pressure on the bladder, all of which increase the possibility of urinary tract infections (Fowler 1998). Also, inflammation of the urinary tract can be transmitted through the lymph to the kidneys, while bacteria cannot Transmission through the lymphatic ducts of the colon and rectum and the occurrence of urinary tract infections (Tanagho and Jack 2000). The classification of the patient dictates whether hospitalization or urological imaging studies are required. Recurrent urinary tract infection in women warrants consideration of antimicrobial prophylaxis. Urinary tract infection prevention in postmenopausal women is significantly aided by self-administered vaginal estradiol cream applied topically. Asymptomatic bacteriuria merits antimicrobial therapy only in high-risk patients or those colonized with Proteus species (Lenger *et al.* 2020).

2.7 UTI in Children

Urine infections in children are very common, but they are neglected. Cases of urinary tract infection result from pathogenic bacteria that enter the urethra and up to the bladder and kidneys. In rare cases, bacteria enter the bloodstream, causing an infection, which may cause undesirable complications due to ignoring the infection or poor diagnosis. However, it is important to note that there may be instances of overdiagnosis

without the use of proper diagnostic methods. Additionally, the incidence of urinary tract infections (UTIs) in males with urinary tract inflammation is higher than that in females during lactation. However, during other stages of puberty, many children experience UTIs. Research suggests that approximately 3% of girls and 1% of boys under the age of 11 may be affected by urinary bladder injuries, urethritis, or asymptomatic pyuria. It is worth mentioning that these estimates may be unrealistic due to the challenges children face in expressing their pain, resulting in many cases of infection going unnoticed. Furthermore, uncircumcised males are at a higher risk of UTIs due to bacterial growth on the foreskin (Fahimzad *et al.* 2010).

In newborns, infections can lead to the migration of bacteria causing inflammation in the urinary tract. This differs from older children, where inflammation spreads vertically due to the accumulation of stagnant urine in the bladder and its subsequent ascent to the kidney. This process occurs as a result of abnormal urine reflux through the ureters, which can be attributed to congenital factors or weakened immune response in the child (Kennedy *et al.* 2010). This category refers to the occurrence of increased frequency of urination, accompanied by painful urination. It may be accompanied by urinary incontinence, wherein the child experiences a loss of control over urine. Additionally, abdominal pain and alterations in the odor of urine are commonly observed symptoms in such cases, particularly in the context of inflammatory bowel disease. The bladder, and the color of the urine in the child may change to red, especially when acute viral cystitis or with bacteria (Arandes 2010).

Statistics show that about 50% of infants and children under school age who have a urinary tract infection (especially those who also have a fever) have both a bladder infection and a kidney infection. If the kidneys are frequently infected and the reflux is severe, then about 5 -20% of children will develop scarring in the kidney. If the reflux is simple or non-existent, a small percentage of children will develop scarring of the kidneys. Scarring of the kidneys is a matter of concern because it can lead to high blood pressure and impaired kidney function in adulthood (Kennedy *et al.* 2010).

2.8 Microorganisms Causing Urinary Tract Infections

The infection of the urinary tract infections comes in the second degree after the infection of the respiratory tract infections, noting that it causes a high mortality rate, and the global spread of this infection is estimated at about 150 million people annually, as the organisms that cause these infections vary, as they are either bacteria or fungi, and sometimes parasites, and it may be more than two diseases. At the same time, the common ones are Gram-positive and Gram-negative bacteria that inhabit the intestinal tract as a natural environment for many of them (Sánchez-García *et al.* 2010).

In a study conducted in (1988) in Al-Rasheed Military Hospital, it was found that about (98.5%) of urinary tract infections are due to Gram-negative bacteria (Mohammed 1989). In Japan, during the period from 1989 to 1990, it was found that international sewage infections in hospitals are The percentage of Gram-negative bacteria was 69%, while the percentage of Gram-positive bacteria was 31% (Kumamoto *et al.* 1995). It must be noted that the most provoking infections are E. coli, which studies suggest is more than 80-95%. Of the infections are due to this bacteria, which is found naturally within the normal flora of the colon and vagina, which enables it to climb into the bladder, causing severe and recurrent infections (Stamm 1999a, Ronald 2002).

A study conducted by (Taneja *et al.* 2010) showed that in the Indian city of Amravati, for 174 urine samples, the infection rate with E. coli reached 59%, while Pseudomonas aeruginosa accounted for 15%, Klebsiella pneumonia, Proteus mirabilis 69, Staphylococcus aureus 6%, and Citrobacter frunedii 1%. He also indicated that the infection rate percentage of women is higher than that of men, at 63% for women and 37% for men.

And the bacteria are more resistant to antibiotics, which causes re-infection and difficulty in recovery (Braunwald *et al.* 2001). It has been noted that enterococcal bacteria come in the third degree of bacterial causes of hospital-acquired urinary tract infections after E. coli and Pseudomonas aeruginosa bacteria that do not cause (12%) of these cases (Tailor *et al.* 1993). A study was conducted on 363 patients with urinary

infections after a kidney transplant. Gram-negative bacteria accounted for 76%, one-third of the percentage was due to *E. coli*, and one-fifth was due to *Klebsiella*. spp and Enterobacter (Takai *et al.* 1998). It was also found out from the results of the research conducted on community-acquired urinary infections in a city located in southwestern Greece over the course of a year. 0.7% (Papapetropoulu *et al.* 1997). In statistics taken from the intensive care unit in a hospital in Riyadh, Saudi Arabia, the most common bacterial species causing urinary tract infections were Enterobacter, E. Coli, Klebsiella pneumonia, and Pseudomonas aeruginosa (Eltahawy 1997).

Wade (2000) indicated that the type E. faecalis is one of the most common types that cause urinary tract infections mainly, followed by E faecium and sometimes Favism E. casseliflavus, as it constitutes 15% of the bacterial groups that cause urinary tract infections in children's hospitals. These types of bacteria are known With its increasing resistance to antibiotics, the bacteria Proteus mirabilis causes inflammation of the bladder and kidneys because it possesses the urease enzyme, which has the ability to break down urea, causing high pH and thus the formation of stones in the kidneys and bladder (Wang *et al.* 2000, Coker *et al.* 2000), also works on the ion precipitation of calcium "Ca and magnesium" Mg, which soon ends with the formation of stones (Watterso *et al.* 2003), as well as bacteria (P. mirabilis P vulgaris, P. stuartii, *P. aeruginosa*. It constitutes a large percentage of infections in the urinary tract and plays an important role in the process of stone formation, as indicated by the study conducted by Abubakar (2009) in Nigeria. Gram-positive bacteria play a lesser role in causing urinary tract infections than Gram-negative bacteria, where the Staphylococcus group is one of the Bacteria that cause kidney failure, as it is possible for these bacteria to invade the skin, infect it, and these local infections may develop into systemic infections, especially when they reach the bloodstream, causing germs, and the transmission of these bacteria through the bloodstream to the urinary tract, leading to infections (Collee *at al.* 1996).

Staphylococcus saprophyticus-10 does not represent acute and symptomatic UTIs, especially in young women (Braunwald *et al.* 2001). Recent studies have shown that each of the bacterial genera belonging to the Staphylococcus group (S faecalis, S. souri,

S. aureus S, marcescenis, S. epidermicdis, S. hemolyticus, S. lentus) play an important role in the adhesion of bacteria to specialized tissues of the host and to the surfaces of medical prostheses such as intravenous catheters, in addition to possessing a capsule as an important virulence factor that helps them adhere to host cells (Yanagisawa *et al.* 2001, Abubakar 2009).

In rare cases, obligate anaerobic bacteria are known to cause UTIs such as Bacteriodes fragilis and Peptostreptococci spp. (Ronald *et al.* 2001) as well as aerobic and anaerobic hemolytic streptococcus type B such as Gardnerella vaginalis, Neisseria -gonorrhoea Salmonella spp and Shigella spp (Collee *et al.* 1996). The other 3 species are Mycobacterium tuberculosis, Chlamydia trachomatis Alcaligenes. spp. Nocardia, Trichomonas vaginalis 3 Mycoplasma spp. 3 Gardnerella vaginalis enterocolitica providencia spp arteriodes, this bacterium can cause UTI (urinary tract infection) (Salahaddin *et al.* 1996).

Candida albicans is one of the common yeasts for diseases of the genitourinary tracts, as this budding yeast is part of the natural flora of the mucous membranes and is usually non-pathogenic. Infection with the disease when an imbalance in that balance (Muoz *et al.* 2000), in addition to Ureaplasma urealyticum and Herpes simplex virus, and in rare cases is one of the pathogens of the urinary tract (Braunwald *et al.* 2001).

2.9 Gram Negative Bacteria

2.9.1 Enterobacteriaceae

The different bacterial genera of this family are characterized as Gram-negative Bacillus bacteria that reside naturally in the intestinal tract of humans and animals, with dimensions ranging from 0.3-1.0 x 1.0-6.0 micrometres, moving with peripheral flagella or not, with or without a capsule. Aerobic or facultatively anaerobic, non-spore-forming, easily growing on meat broth medium, but some of them have growth requirements of their own, as they reduce nitrates to nitrites (except for some Erwinia

strains). The molar percentage of guanine and cytosine (GC%) in the DNA of most of its members ranges Between 38-60% (Holt 1979). positive for the catalase test and negative for the oxidase test, and not form spores and ferment for glucose and often produce CO2 gas (Carter and Wise 2004). This family includes approximately 31 genera and 139 species (Holt *et al.* 1994, Koneman *et al.* 1997).

Some of them are part of the natural flora, such as E. coli, and others are pathogenic to humans, such as Salmonella (Shigella) (Brooks *et al.* 2001). The intestinal family is found in several places on plants, soil, water, and in the intestines of humans and animals (Wound Infections) (Henry 2001, Lennette *et al.* 1985).

2.9.1.1 Escherichia coli

Bacteria are classified as follows (Garrity *et al.* 2005, Dworkin *et al.* 2006).

Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: *Escherichia*
Species: *E. coli*

It is a Gram-negative Bacilli, located at the head of the family Enterococcus, Escherich Theodor, non-spore-forming, facultatively anaerobic, and some of its strains have the ability to form covers or capsules (Al-Jubouri 1990). It is negative for the examination of urease and oxidase. It was named by the German bacteriologist Escherich, and is usually abbreviated as (Moffet 1980) E. coli and this type E coli comprises more than 99% of Escherichia isolates (Lennette *et al.* 1985). They are straight, measuring between 1.1 - 1.5 - 2.0 - 6.0 µm (Living) or 0.4 - 0.7 x 1.0 - 3.0 µm (Dried and Stained), single or double, without spores, moving by Peritrichous Flagella or not. Facultative anaerobes (Holt 1979, Holt *et al.* 1994).

It is characterized by its rapid fermentation of lactose with the production of acid and gas. It is fermented to a group of sugars (D-mannitol, D-mainose, L-arabinose, L-arabinose, L-maltose, L-maltose, Trehalose rhamnoge and D-xylose). Most of them produce gas from the fermentation of glucose (Finegold *et al.* 1978, Holt *et al.* 1994), and all strains are non-fermentative for Meso-inositol, negative for the gelatinolysis test, and do not produce HS gas in (Collee *et al.* 1996) Triple Sugar Iron Agar (TSI 1996), and the percentage to guanine and cytosine G + C in DNA 50 - 9651 (Holt 1979).

2.9.1.2 Klebsiella oxytoca

Bacteria are classified as follows (Garrity *et al.* 2005, Dworkin *et al.* 2006).

Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: *Klebsiella*
Species: *Klebsiella oxytoca*

General Characteristics and Cultural properties It was discovered by the German scientist Edwin Klebs in 1834 and named after him, but it was diagnosed for the first time by the scientist Von Frisch. It is a straight rod bacteria with a diameter of 0.3-1.0 micrometres and a length between 0.6-6 micrometers. Circular, appearing singly, in pairs, or in the form of short chains or diplococci Koneman *et al.* (1997), with large, sticky, macula-like mucous colonies fused together and surrounded by a capsule (Holt *et al.* 1994).

It is characterized by its inability to move and form spores, but at the same time it contains fimbriae, whose chances of growth under compulsory anaerobic conditions are very weak. The ideal temperature for its growth is 37 °C. It grows on the usual media. As for its growth on the medium of the Maconki agar, its appearance is bright pink because it ferments the sugar lactose, in addition to being fermented for a wide range of

sugars such as glucose, sucrose and ribose with its production of gas, and most of them reduce nitrates to nitrites (Edmondson *et al.* 1980).

They are negative for the Methyl Red test and positive for the Indole and Voges-Proskaur test and for the use of citrate utilization, and they are resistant to potassium cyanide (Cruickshank *et al.* 1975). It produces the enzyme catalase and does not produce the enzyme Cytochrome oxidase and has no ability to liquefy gelatin or produce H.S. It produces the enzyme urease (Finegold and Martin 1982).

This type of bacteria was isolated from the hospital environment in adults, from wastewater, from contaminated and uncontaminated surface water, and was also isolated from the soil, but in very low proportions (Bagley *et al.* 1981). This type causes bacteremia, soft tissue infections, and skin and meningitis (Tang and Chen 1995). It was also isolated from cases of urinary tract infection, which constitute 8% of hospital-acquired infections in adults (Podschun and Ullmann 1998). Bermudes *et al.* (1999) showed that 26% of Klebsiella isolates isolated from the intensive care unit belong to this type of bacteria, and in a study conducted by (Rasool *et al.* 2003) on 50 bacterial isolates belonging to this genus Klebsiella collected from different hospitals and laboratories in the city of Karachi They found that the highest infection rate was 52% of K. aryroca, 9642 of K. pneumoniae and 6% of K. azaemae.

2.9.1.3 Proteus vulgaris

Bacteria are classified as follows (Garrity *et al.* 2005, Dworkin *et al.* 2006).

Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: *Proteus*
Species: *Proteus vulgaris*

Hauser was the first to name this bacterium Proteus in 1885, and it is a Latin word of origin that means inversion in shape. This genus includes, in addition to the genus (Providencia and Morganella). These bacteria are widespread in nature. The members of this genus are distinguished from the rest of the species of this family of intestines in that they have the ability to remove the amino group from the amino acid phenylalanine, producing phenyl pyruvic acid. This test is one of the differential tests for this tribe (Brooks *et al.* 2007).

Bacterial species belonging to the genus Proteobacteria have distinctive cultural characteristics, as they are in the form of short, Gram-negative bacilli, ranging in diameter from 0.4-0.8 μm and a length between 1-3 μm, aerobic, mobile by peripheral flagella, non-spore-forming (Holt *et al.* 1994). In the field of biochemistry, the results indicate that the substance produces H2S gas, is negative for the oxidase test, catalase test, urea hydrolysis, and ammonia liberation, produces the urease enzyme, and is positive for phenylalanine deaminase. The sample passed the methyl red test with a positive result, the citrate utilization test and the Voges Proskauer test with heterogeneity, the indole test with a positive result, and the lactose and gelatin-forming tests without fermentation (Iwalokun and Akinwnmi 2002, Holt 1994). It does not ferment the lactose sugar, so it appears on the medium of Maconkey pale yellow, and its ideal growth is in temperatures ranging between 25-37 C, while its ideal pH is 7.4 (Mandell *et al.* 1995).

Members of this genus of bacteria cause many diseases in humans and animals, such as urinary tract infections, and are second only to E.coli in causing hospital-acquired UTIS (Garcia *et al.* 2007). This type is also one of the most important types of the genus Proteus and the most common in causing infections in male children and in adults of both genderes (Chaudhry 1982). Also, this type was isolated from people with diabetes or those suffering from structural abnormalities in the urinary tract and from hospital patients after using Contaminated devices such as long catheters used to withdraw urine from the bladder, and in general can isolate members of the genus Proteus from cases of wound infections, abscesses, otitis media, meningitis, septicemia, and osteomyelitis (Collee *et al.* 1996).

It also has the ability to cause various infections, especially in people who are immunosuppressed and users of antibiotics for a long time. These bacteria are considered opportunistic pathogens (Coker *et al.* 2000), as they cause chronic skin ulcers (Belas and Suvanusuthi 2005), as well as urinary tract infections. Chronic (Burall *et al.* 2004). Hospital-acquired infection is either alone or co-existing with Klebsiella spp. in the case of urinary tract infections, or with Staphylococcus spp. in the case of superficial surgical-site infections, with a rate of 33.34% (Filimon and Jacob 2007). Proteobacteria come in the third rank as a pathogen in cases of uncomplicated cystitis, pyelonephritis and prostatitis (Stamm 1999b).

P. vulgaris and P. perttari were easily isolated from hospitalized patients as the primary pathogen or immunosuppressed patients (Ohara *et al.* 2000). It also causes sepsis and bacteremia when it reaches the bloodstream, and is accompanied by a high fever, and a lack of white blood cells (neutrophilia) (Zhanel *et al.* 2000).

2.9.1.4 Serratia marcescens

Bacteria are classified as follows (Garrity *et al.* 2005, Dworkin *et al.* 2006).

Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: *Serratia*
Species: *Serratia marcescens*

Bacteria were named by this name in relation to the Italian scientist and physician Serrafino Serrati before 1787 (Buchanan *et al.* 1974), and they are short bacilli with a diameter ranging between 0.5 - 0.8 μm and a length between 2.0 - 0.9 μm. - Facultative anaerobes living, forming capsules in food media containing sugar, containing ciliates (Holt *et al.* 1994). It does not need special growth requirements, as it can grow on simple food media, and it can grow on synthetic food media using different compounds as a source of carbon. Its colonies growing after 24 hours of incubation have two diameters of 1.5-2 mm (Williams and Qadri 1980).

Likewise, its colonies growing on the medium of nutrient agar are often of a color that is either white, pink, or red, and the red pigment known as methyl-3-aml- (Prodigiosin-2 metho-oxy Prodigiosin-6) is one of the distinguishing characteristics of this genus. (Williams and Qadri 1980, Garrity *et al.* 1980). The ideal temperature for their growth is between 10°C-37°C, and a pH of 9-5=pH. A temperature higher than 45°C and a pH less than 4.5 is inhibitory for its growth (Geri *et al.* 2004).

Bacteria lack indole production, differentiate in methyl red and fux Proscore tests, except S. fornticola, which is citrate-consuming, catalase-positive, oxidase-negative, and produces urease, and do not produce hydrogen sulfide (H2S) (Holt *et al.* 1994). Nitrate is reduced to nitrite, thus decomposing corn oil. Most of its strains are fermented for many sugars, including maltose, trehalose, manose, manitol, sucrose, and salici. These bacteria are not fermentable for lactose (Dworkin *et al.* 2006, Koneman *et al.* 1997).

These bacteria are found naturally in soil and water as well as in the human intestine (Acar 1986). It is an opportunistic nurse (Holt 1979). This type is the most common in clinical samples taken from humans, as recent years have witnessed increasing interest in its role in nosocomial infections in many countries of the world (VanOgotrop *et al.* 1997).

This bacterium is responsible for a large percentage of nosocomial infections represented in infections of the urinary tract and respiratory system, wound infections, burns, cystitis (Hartle 2005), septicemia, eye infections and meninges, osteomyelitis, as well as inflammation of the lining of the heart Endocarditic (Colles *et al.* 1996).

These Serratia bacteria are virulent when they enter the bloodstream as they produce an endotoxin that causes fever, platelet dissolution, and internal vascular thrombosis. The death rate due to bacteremia with these bacteria is high (Acar 1986). The non-chrome-producing strains cause pneumonia and endocarditis in hospitalized patients (Jawetz *et al.* 2006).

2.10 Gram-Positive Bacteria

2.10.1 *Staphylococcaceae*

Members of this family are characterized by the fact that their cells are spherical in regular shape, and their diameter ranges between 0.5-1.5 μm (Prescott *et al.* 1996). They are positive for Gram, aerobic or facultative anaerobes. Under the microscope, it requires growth factors, amino acids and vitamins in the environment. It is immobile. It produces water-insoluble dyes with colours yellow, red or orange. It grows in containers with a salt concentration of 5% NaCl. Little grows in the presence of 10-15%. It is active in its production of dyes. When grown in environments with a high concentration of NaCl, nitrates are reduced. Some species form groups of natural flora on the skin and mucous membranes of humans, so they are non-pathogenic. Other species cause pus, abscesses, and suppurations, and may lead to fatal poisoning (Delost 1997). The genus Staphylococcus is one of the most important genera in this family. Pathogenic species in the upper respiratory tract, skin, intestines, and vagina cause various diseases in humans. They analyze red blood cells, clot plasma, and secrete toxins, leading to food poisoning. Non-pathogenic species that do not cause blood clots are often found on the skin, skin glands, and in the mucous membranes of warm-blooded animals and on many foodstuffs. Some of its pathogenic strains are portfolio. The pathogenic types of staph are characterized by their emergence and resistance to many antibiotics, which causes a problem in the treatment (Foster 2000).

2.10.1.1 Staphylococcus saprophyticus

Bacteria are classified as follows (Garrity *et al.* 2005, Dworkin *et al.* 2006).

Order: Bacillales
Family: Staphylococcaceae
Genus: Staphylococcus
Species: *Staphylococcus saprophyticus*

It is a gram-positive bacteria that is negative for the coagulase test and is resistant to the antibiotic novobiocin, which is one of the diagnostic characteristics of this type of the genus Staphylococcus. It was discovered for the first time by Torres Pereira (1962) from 51 antigens of a woman with acute urinary tract infections (Torres Pereira 1962), and these antigens were later adopted as a diagnostic feature for this type (Raz *et al.* 2005). Individuals of this type appear under The microscope in the form of spherical clusters with a diameter of 5-8 micrometres, and their colonies are opaque, shiny and smooth, convex, grow anaerobically and selectively, positive for the catalase test, negative for the oxidase test and the DNase test, and not selective for salol (Kuroda *et al.* 2005). This type is not A phosphatase enzyme producer that does not contain pigments and does not dissolve blood in the medium of blood agars and is diluted for gelatin. One of the important diagnostic features of this type of bacteria is its production of the urease enzyme, which works to increase the pH in the alkaline direction, which in turn allows the formation of stones in the bladder (Kleine *et al.* 2010).

This type is one of the main causes of international sewage infections in humans after the E. coli bacteria, as it is the second most common pathogen in the urinary tract (Linda *et al.* 1985). The incidence of infection with this type of infection is large during genderual intercourse (Raz *et al.* 2005). The infection is transmitted from the rectum to the vagina in women, and infection abounds in the summer and spring seasons as a result of contact. It is frequently present in animals, especially sheep, cows and pigs. Most strains of this type cause direct contact with the red blood cells of sheep (Kuroda *et al.* 2005), and rarely infect patients. In hospitalized patients, Staphylococcus saprophyticus is noted for its ability to adhere to the epithelial cells of the urinary tract (Kuroda *et al.* 2005).

It is also found widely in patients who have undergone operations that require prosthetic devices for long periods of time, such as Vascular catheters and Continuous ambulatory peritoneal dialysis, which is the most common and constitutes 35% of hospital infections (Jarlov and Hoiby 1998). The bacteria Staphylococcus saprophyticus has a gelatinous layer, as it is considered an adhesion factor to the urinary tract, especially for women, to help it stick to the epithelial tissue lining the urinary tract. The adhesion

observed can be attributed to the existence of lithoichoic acid, a virulence factor that facilitates bacterial penetration of the urethral epithelial epithelium. Urinary excrement (Winberg 1993), inter-species contact, or human activity in rivers in close proximity to polluted areas (Marrie and Costerton 1983).

2.11 *Staphylococcus aureus*

Bacteria are classified as follows (Garrity *et al.* 2005, Dworkin *et al.* 2006).

Order: Bacillales
Family: Staphylococcaceae Genus: *Staphylococcus*
Species: *Staphylococcus aureus*

Gram-positive cocci, called Staphylococcus aureus, as their walls contain a layer of peptidoglycan and teichoic acid. It was discovered by Rosenbach (1884). These bacteria divide in more than one level and remain attached to each other in an irregular assembly, similar to the clusters of grapes (Elek 1959). They can be seen in the form of single cocci, pairs, or short chains, especially in liquid media (Cruickshank *et al.* 1975). They are non-motile, non-spore-forming, some of their strains contain a capsule, and are characterized by being aerobic and facultatively anaerobic. Growth-determining factors such as B vitamins and nicotinic acid (Cruickshank *et al.* 1975).

Staphylococcus aureus is the most perilous among the various and prevalent strains of staphylococcal bacteria. The emergence of penicillin resistance in 1950 gave rise to a coccal-shaped bacterial strain that presents a substantial risk to human life by inducing infections of the epidermis, lungs, heart valves, and bones. This is due to the ease with which these bacteria invade and spread in the tissues of the body, producing many toxins and enzymes that help them to cause these damages (Brook and Frazier 1998).

2.12 Medicinal plants

The terrible result was the increase in the world's population and the rise in medical and therapeutic awareness among the peoples of the world. The demand for drugs increased until it reached the point of a huge boom in science, especially in recent years (Jamshidi-Kia *et al.* 2017) The first: the materials extracted from medicinal plants, which are unfortunately few and do not meet human needs and requirements, due to the lack of interest in medicinal plants, their care and their multiplication (Atanasov *et al.* 2015). Second: Synthesized chemical compounds that have spread and diversified as a result of the great scientific development in all fields, including the different branches of chemistry, as well as in the field of extracting active substances from medicinal plants. The opposite happened, for man has known diseases that were not present in his human ancestors. Rather, we have entered the era of chronic diseases. The reason is due to several things, including that the treatments that people take in the jungle treat the symptoms that appear while the disease remains latent to turn into a chronic condition (Soejarto 2011).

After noticing the extent of the effect of manufactured chemicals on humans, man began to refer to the medicinal substances found in plants and worked to extract the effective part of them and identify the chemical composition of them. He built factories like their composition and gave the patient treatment in the form of tablets or syrup, and noticed the appearance of side effects on the patient. While God wanted to make the concentration of active substances in plants dilute and the body can interact gently in its natural form. In addition, each plant contains many active substances (Soejarto 2001) It is serious to mention that the world now tends more than before to treat with herbs and medicinal plants, after the results showed the effectiveness of treatment with them, and there is almost a global race to register as many patents as possible for the results of the research that is taking place, just as international drug production companies competed to produce medicines Herbal, in tablets or capsules, and put it into circulation after it was confirmed that many patients and doctors were keen to resort to treatment with natural extracts taken from medicinal herbs, and evidence of that (Jamshidi-Kia *at al.* 2017).

- Germany produced 300 kinds of herbal medicines
- The United States of America produced 1,800 types of herbal medicine
- England and Japan are striving to keep pace with the production of herbal medicines.

And according to a nationwide survey conducted in 1997, four out of every ten adults answered. that they prefer herbal medicine and alternative therapies. In statistics conducted by the American Medical Association, it showed that Americans spent $27 billion on herbal medicine and medicinal plants. Or with regard to our current study, we will present a medical study on two medicinal herbs widely known and used since ancient times, and these two herbs are: - Ginger and Fenugreek (Atanasov *at al.* 2015).

2.12.1 Ginger

Ginger is a plant of the Zingiberian family, Zingiberaceae, originating in India and tropical Asia. Ginger thrives in moist, tropical soils that are fruitful. A thick, earthen stem adorned with a knot constitutes ginger. This subterranean, earthy stem is comprised of an enormous, succulent root known as the hand. This stem, which is approximately 30 centimeters in length, protrudes from the soil surface. It is composed of a collection of branches that are annually renewed and bear elongated, ribbed green leaves along this stem. In general, ginger is offered in two varieties: mature and medium-ripe. The rind of the mature variety is tough and must be removed. The immature variety of ginger, which is readily available in markets, does not necessitate paring. The subsoil utilizes its growing stems, which contain volatile oils, resins (of which gingerol is the most significant), fibrous and gelatinous substances, and gingerol. In addition to possessing a pungent odor and flavor, they are either squirrel-shaped or yellowish white in color (Teleni *et al.* 2013), In addition, purple lips adorn the yellow blossoms of ginger (1-2). Without the decomposition of its uterine leaves is ginger extracted, and the root is not ground until it has desiccated. East India, the Philippines, China, Sri Lanka, Mexico, Pakistan, and Jamaica are among the countries where it is prevalent, with Jamaica producing the finest varieties of Jamaican ginger (Pattanittum et al. 2016).

The ginger plant is known to include volatile oils, which typically range from 2.5% to 3% in concentration. Additionally, it has a distinct group of oils referred to as Aryl alkanes. These oils are responsible for the pungent taste associated with ginger and may be categorized into two distinct groups: The first is (Gingerols), which contains (Gigenol), which gives it a spicy taste. It is an oily resin. It is anti-clotting and anti-inflammatory of all kinds (asthma, arthritis, migraine, colon and urinary system). The second is chocolate (Shogaols), of which one of the most important compounds is (shogol), which is a hot substance by nature, and contributes to the burning and digestion of fats, and paradols. It also contains starch by (50%), protein by (9%), fat by (7%), (Bellik 2014).

2.12.1.1 Therapeutic benefits and medicinal uses of ginger

There is a wide spectrum of diseases that ginger helps to cure or helps to strengthen the vital systems that the body contains and tonic for the nerves, and increases the ability of the immune system because it is a stimulant for the lymph nodes, and eliminates cancer cells, and prevents their spread in the body and their formation, anti-headache, and as a treatment For migraines or migraines (Crichton *et al.* 2022) it strengthens memory and does not forget, prevents Alzheimer's disease, strengthens the ability to see, treats night blindness, removes sore throat, clears and improves the voice, helps speech, also helps treat dizziness and maintain balance, treats cough, It is an expectorant, reduces stress and tension (Kiyama 2020).

2.12.2 Fenugreek

Fenugreek Botanical Description Fenugreek is a perennial herb, 20-60 cm high. It has a hollow stem and its branches branch out into beautiful plants, leaves that turn into fruits in the form of pods, about 10 cm long. Vegetable plants are somewhat similar in shape. Each pod is about 10 cm long, with a greenish-yellow color. The fenugreek plant consists of the following parts. The root is a strong stem that branches out to a large number of secondary branches, and there is a large amount of root nodes on all branches of the type meliloti (Barnes *et al.* 2005).

2.12.2.1 Therapeutic benefits and medicinal uses of the fenugreek plant

The global utilization of the fenugreek plant has expanded due to its inclusion in many therapies and its nutritional and medicinal advantages. Additionally, in certain locations, it is cultivated as a vegetable crop, further enhancing its benefits. The leaves, pods, or seeds of this plant are consumed either in their raw state or after being cooked, and are often used into various salad preparations. As a beverage that offers both refreshment and stimulation, or as a viable substitute for traditional options such as coffee and tea (Basu *et al.* 2004,Yoshikawa *et al.*1997), the use of fenugreek seeds as a spice is spread to food and drinks in most countries of the world (Snehlata and Payal 2012, Suresh *et al.* 2012).

3. MATERIALS AND METHODS

200 urine samples were collected from both genderes and of different ages ranging from (1 year-65 years old) in Al-Alam General Hospital for the period from December 15, 2021 to July 1, 2022. After transplantation and biochemical examinations, it was found that 138 samples gave positive growth, at a rate of 69%, while 62 samples showed a negative result for the transplantation test, at a rate of 32%. Patients who had taken antibiotics before the examination were excluded. The required laboratory analyzes were performed for both categories of patients to prove their infection with urinary tract infection, then 7-10 ml was taken from the patients' urine for both categories and then put it into the centrifuge.

3.1 Antibiotics

The antibiotic used in the study are shown in the Table 3.1.

Table 3.1 Antibiotics used in the study

Antibiogram Disck	Abbvration	Company and Origin
Ciprofloxacin	CIP	Bioanalyse (France)
Nitrofurantoin	F	Hi MEDIA (India)
Imepenem	IPM	Bioanalyse (France)
Oxacilin	OX	Bioanalyse (France)
Gentamicin	CN	Bioanalyse (Turkey)
Cefalexin	CTX	Bioanalyse (Turkey)
Vanvomycin	Van	Bioanalyse (Turkey)
Tobramicin	Tob	Bioanalyse (Turkey)
Sulphamethoxazole	SXT	Bioanalyse (Turkey)

3.2 Methods

3.2.1 Preparation of culture media

3.2.1.1 Blood agar medium

This Medium prepared, sterilized using Autoclave, cooled, and 5% human blood added, solidified in dishes, and cooled to 45°C. Then the dishes or tubes were placed (upside down) in the refrigerator at 4 °C (Cheesbrough 2006). This medium was used to grow bacteria and find out the type of hemolysis that causes them.

3.2.1.2 MacConkey agar

It was prepared according to the instructions of the company producing the medium (Himedia) and used to identify Gram-negative bacteria and lactose-fermenting bacteria in this medium (Lagier *et al.* 2015).

3.2.1.3 Nutrient agar

Prepared according to the manufacturer's instructions (Himedia) and used for bacterial growth and temporary storage (Eaton 2005).

3.2.1.4 Sensitivity test medium muller hinton agar

The experiment was conducted in accordance with the guidelines provided by the manufacturer (Himedia) to assess the susceptibility of bacteria to antibiotics (Eliopoulus 1989).

3.2.1.5 Indol test medium

This medium was prepared according to the manufacturer's instructions by dissolving 20 grams of peptone medium in 1 liter of distilled water and autoclaving it at a temperature of 121 ºC for 15 minutes. This medium was used to test the bacteria's ability to produce indole from tryptophan (Collee *et al.* 1996).

3.2.1.6 Methyl red test medium - fox proskauer mr - vp medium

The supplied company's guidelines were followed to prepare this medium. It was dissolved in 1 liter of distilled water and 5 milliliters of the solution were placed in each test tube. Subsequently, the tubes were autoclaved at 121 °C for 15 minutes, after which they were allowed to settle and monitored until ready for use (Madigan *et al.* 2008, Schumann *et al.* 2003).

3.2.1.7 Amid consumption of simmon citrate agar

The medium was sterilized in an autoclave set at 121 ºC for 15 minutes after preparation in accordance with the manufacturer's instructions (Alpha Biosciences). Subsequently, the sterile solution was transferred into sterile containers and allowed to solidify obliquely. It was utilized to assess the isolated bacteria's capacity to metabolize citrate as a carbon source (MacFaddin 2000).

3.2.1.8 Mannitol salt agar

Prepared according to the manufacturer's instructions (Himedia) and used to test the ability of some Staphylococcus species to ferment mannitol sugar present in this medium (Hitchens Tran and McCarron 1995).

3.2.1.9 Eosine methylene blue agar

The aforementioned medium was autoclaved at 121 ºC for a duration of 15 minutes subsequent to its preparation in accordance with the Oxoid manufacturer's guidelines. It was subsequently transferred into Petri dishes and allowed to solidify. By utilizing this medium, certain strains of intestinal bacteria were identified (Collee *et al.* 1996, Howard 1994).

3.2.2 Solutions and reagents used in biochemical tests

A- Kovac's Reagent
B- Methyl Red Reagent
C- Vogas - Proskauer Reagent

These reagents were commercially obtained from Hi Media (India) (Collee *et al.* 1996, Cruickshank 1975).

3.2.2.1 Oxidase reagent

It was obtained ready from the producing company and was used to detect the ability of bacteria to produce oxidase enzyme (Forbes *et al.* 2009).

3.2.2.2 Catalase reagent

It was prepared by dissolving 3 ml of hydrogen peroxide H_2O_2 at a concentration of 30% with 97 ml of distilled water for the purpose of obtaining a concentration of 0.3% of a H_2O_2 solution (Collee *et al.* 1996). This reagent was used for the purpose of detecting bacterial isolates that produce the enzyme catalase and to differentiate between positive staphylococci for this test from negative streptococci.

3.2.2.3 McFarland's solution

Obtained commercially prepared from Densi Chek Corporation (USA).

3.2.3 Microscopical exam of urine

The aforementioned method was used by Forbes *et al.* (2007) in examining urine output. Midstream urine samples were taken using ready-made sterile universal bottles, and the first part of the flow of urine was left to clean the lower urethra. Patients were instructed to wash the genital area in order to avoid Contamination with natural flora found on the skin, the urine sample was transferred for microbiological study and sterile preservation conditions were secured, and in the event of a delay in the treatment procedures for the sample, it should be cooled in the refrigerator at a temperature of 4 °C (Stapleton *et al.* 2008), and 5 ml was taken of urine, and placed in a centrifuge (M.P.3000 R) for 5 minutes. The filtrate was discarded and a drop of the sediment was taken. It was examined under a light microscope. Direct microscopy of the urination precipitate to see the inflammatory cells, red blood cells and white blood cells.

3.2.4 Culture of urine samples

Bacterial culture of urine samples was performed after collecting them on isolation media represented by blood agars and Maconkey agars using a loop, then the loop was sterilized by flame and then left to cool without touching anything. acre surface. The dishes were turned over and incubated at 37°C for 24 hours (Forbes *et al.* 2007), bacteria were identified by initial diagnosis based on their cultural and morphological characteristics and based on what was reported by (Goldman 2009).

3.2.5 Microscopical and cultural characteristics

Following the growth of colonies on the primary isolate's culture media, an initial diagnosis was made by assessing their morphological and cultural attributes,

encompassing factors such as colony size, color, edges, and height. Subsequently, the colonies were subjected to microscopic examination after being stained with Karam stain, as described by Forbes et al. (2007).

3.2.6 Methods of preserving farms

3.2.6.1 Medium-term preservation

A colony was taken and grown on slanted agar media by stabbing and planning method, then incubated at a temperature of 37° C for 24 hours, then kept at a temperature of 4°C. The process of perpetuating the isolates continued periodically every month by stimulating them in the brain - heart infusion medium - heart infusion broth, and it was re-grown on a new tilted culture medium to ensure its survival (Atlas 2010).

3.2.6.2 Long-term preservation

The preservation of bacterial isolates was achieved by transferring a portion of the colonies to brain-heart infusion broth supplemented with 15% glycerol in small glass tubes. These tubes were securely sealed and stored at a temperature of 4ºC for future utilization as required (Atlas 2010).

3.2.7 Biochemical tests

3.2.7.1 Test coagulase

The slide test method was used to test the enzyme activity, as a pure colony of Staphylococcus aureus was mixed with a drop of physiological solution on a clean glass slide by means of a carrier, and a drop of plasma was taken and mixed with the colony, and therefore coagulation occurred within 15 seconds, which is evidence of the positiveness of the test (Forbes *et al.* 2007).

3.2.7.2 Oxidase test

The process involves transferring a portion of a pure, 24-hour-old developing colony using a wooden stick onto a filter paper that has been soaked with a small amount of the reagent. The observation of a colony exhibiting a dark violet tint within a time frame of 10 seconds might be considered as indicative of a positive test, as stated by Forbes et al. (2007).

3.2.7.3 Catalase test

A portion of a 24-hour-old homogeneous and uncontaminated colony was carefully transferred using a wooden chopstick onto a sterile glass slide. Subsequently, a small volume of the catalase enzyme reagent, consisting of hydrogen peroxide (H2O2) at a concentration of 3%, was introduced to the colony. A positive outcome is indicated by the presence of air bubbles (Forbes *et al.* 2007).

3.2.7.4 Mannitol fermentation test

The experimental procedure involved the introduction of purified colonies of Staphylococcus aureus into a medium, followed by a 24-hour incubation at a temperature of 37 °C. The assay was employed to differentiate between various strains of Staphylococcus; therefore, a positive result is indicated by the medium's color transition from pink to yellow (Alfred 2005).

3.2.7.5 Indole test

To detect indole in this experiment, peptone water medium was utilized. A portion of the medium was inoculated with a purified colony that had been aged for 24 hours. After incubating the isolates for twenty-four hours at 37 °C, two droplets of Kovacs reagent were added to the tube. The presence of a crimson ring atop the medium signifies a positive outcome (Forbes *et al.* 2007).

3.2.7.6 Methyl red test

Following incubation at 37°C for a duration of (24-48) hours with Peptone water containing a fraction of a pure colony that had been established for 24 hours, five droplets of methyl red reagent were introduced into the medium. The appearance of the medium turning pink signifies a favorable outcome (Alfred 2005).

3.2.7.7 Voges - proskauer test

The medium utilized in the methyl red test was supplemented with potassium hydroxide and alpha-naphthol reagents after being inoculated with a 24-hour-old pure colony and incubated at 37°C.The results were subsequently read 15 minutes later. A color transformation to red signifies a positive test result (Alfred 2005).

3.2.7.8 Citrate utilization test

The test was conducted by inoculating Simmon citrate agar slant with pure colonies aged for 24 hours by stabbing method and incubated at a temperature of 37 °C for a period ranging between (24-48) hours (MacFaddin 2000).

3.2.7.9 Growth of triple sugar iron (tsı) medium test

The test involved inoculating isolates on TSI agar medium and incubating at 37°C for 24 hours. The color change from red to yellow indicates glucose fermentation, while yellow indicates glucose and lactose fermentation. Gas bubbles indicate carbon dioxide production, while precipitated black indicates H2S production (Alfred 2005).

3.2.7.10 Novobiocin resistance test

Following the bacterial culture on solid Mullherton medium, sterile instruments were utilized to insert novobiocin antibiotic tablets, which were subsequently incubated at a

temperature of 37 °C for a duration of 24 hours. The diameter of the inhibition zone was quantified, and bacteria are deemed sensitive to the antibiotic if it exceeds 15-16 mm. Conversely, bacteria are resistant to the antibody if the diameter falls below that threshold (Collee *et al.* 1996, Vandepitte *et al.* 1991).

3.2.8 Microbial diagnosis using the Vitek 2 Compact device

The Vitec device is an automatic microbiology diagnostic system for identifying bacterial species, yeasts, and molds using special cassettes for Gram staining.. There are several systems of it, but all three systems accommodate the color reagent cards that It is incubated and interpreted automatically. The device is characterized by speed and accuracy in diagnosis, so it saves a lot of effort and time spent in the field of microbial diagnosis (Figure 3.1) (CLSI 2020).

Figure 3.1 Vitek 2 Compact Diagnostic Device (BioMérieux, France)

3.2.9 Steps of diagnosis in the device

Activation of the bacterial culture, and this was done by replanting the bacterial species to be diagnosed on the appropriate agar, where the positive species for Gram stain were activated on blood agar medium, while the Gram negative species were activated on Maconkey agar medium, then these media were incubated in the incubator at 37°C for a

period of 18-24 hours. Making a suspension by taking a single culture with distilled water in a tube and comparing its turbidity with DensiChek's solution, where the turbidity should be (0.5-0.6) and then the capillary tubes are installed in these tubes and fixed in their holder for the purpose of inserting them into the device from the upper door shown in the picture to start a series of operations inside the device that includes pulling and pushing air to generate the necessary pressure for the transfer of the bacterial suspension through the capillary tubes to the diagnostic cassette, so the holes in the cassette are filled with the suspension and then a series of reactions begins that ends with light signals for the purpose of moving the cassette to the loading door Where the device reads the results, and that takes 4-7 hours, and then shows them through a computer connected and programmed with the Vietek device (Branković *et al.* 2010).

3.2.10 Antibiotic sensitivity test

A test was conducted for the sensitivity of the isolates under study to antibiotics, where 9 antibiotics obtained in the form of open tablets from a company were used. Bioanalyase (Turkey) in accordance with World Health Organization recommendations. Muller Hinton Agar prepared from the company (oxoid) was used, and the modified Baure-Kirby method (1966) was followed and according to the recommendations of the World Health Organization (Finegold *et al.* 1978).

Bacterial suspension was prepared in physiological saline and compared to McFarland's standard 0.5 solution. Sterile cotton swabs were immersed in the suspension, spread onto Mueller Hinton Acar medium, and tablets distributed evenly. Dish plates were left at room temperature for 15-20 minutes, and incubated at 37°C for 18-24 hours. All results were recorded by measuring the diameters of the zones of inhibition using a millimeter ruler around each disc and compared with standard rates for diameters. Zones of inhibition for antigens, according to what was stated in CLSI (2020).

3.2.11 Preparation of the plant extract and the concentrations used in the study

A 200 mg/mml stock solution was created by dissolving dry plant in sterile distilled water, sterilized using filter papers, and used as a kit source at concentrations 25, 100, 75, and 50 mg/mml (Arjan 2017).

3.2.11.1 Preparation of the aqueous extract

A weight of forty grams of the desiccated plant sample was weighed, followed by the addition of 160 ml of sterile distilled water at a ratio of four times the weight by volume. The plant sample was positioned in a blender positioned in an ice bath, and the resulting mixture was agitated using a magnetic stirrer for a minimum of one hour in order to disrupt and rupture the plant cell wall. Following this, the mixture was placed in the refrigerator for twenty-four hours to facilitate soaking. Subsequently, the mélange underwent a filtration process involving multiple layers of gauze, followed by a second filtration using filter papers and a Buechner funnel. A vacuum apparatus was employed to extract any residual fibers and uncrushed plant parts. Following this, the extract was transferred into sterile glass containers and heated in an electric oven, maintained at a maximum temperature of 40 °C, in order to maintain the extract's active constituents until all of the water evaporated. Once the extract had dried, it was transferred to glass containers with tight-fitting caps and stored in the refrigerator until further use (Branković *et al.* 2010).

3.2.11.2 Preparation of the alcoholic extract

The alcoholic extract was prepared in the same way as the aqueous extract, except that the water was replaced by ethyl alcohol at a concentration of 95% (Devi *et al.* 2010). The modified method of Bauer (1966) was adopted, as 3-5 pure colonies growing on Maconki agar medium were transferred to the nutrient broth medium, incubated at 37 °C for 24 hours, and then diluted with normal saline and compared with the first tube (McFarland), which is equivalent to (1.5 x 810) cells / cm3, then 0.1 cm3 of the diluted

bacterial suspension was transferred to Mueller Hinton Agar and spread on the surface of the dish using a sterile cotton swab and incubated at a temperature of 37 °C for 30 minutes in order for the impregnation to take place and for the purpose of studying the inhibitory effectiveness For the extract of elderberry flowers, sage fruits and olive leaves on the growth of bacterial germs, holes were made with a cork borer and 1 ml each of the aqueous and alcoholic extracts were added for each of the concentrations used on Mueller Hinton Agar and incubated at a temperature of 37 °C for 24 hours and to demonstrate sensitivity The diameter of the resulting inhibition zone was measured.

3.2.11.3 Methods for detecting active compounds in plant extracts

The identification of glycosides was accomplished by combining 1 ml of the crude extract with 2 ml of Benedict's reagent in a water immersion for a number of minutes, or until a blue hue appeared, which signified the presence of glycosides. The presence of tannins was determined by observing the aqueous solution produced by adding 1% ferric chloride to an equivalent volume of plant extract; the formation of a bluish-green precipitate served as an indicator of the tannins' existence (Sasidharan *et al.* 2011).

The detection of flavonoids involved the addition of 4 ml of 95% ethyl alcohol to 1 ml of plant extract in a glass test tube. The test tube was then subjected to a boiling water bath for a duration of 25 minutes. Following removal from the water bath, 0.5 drops of potassium hydroxide solution were introduced to the mixture. According to Delazar et al. (2012), a positive test (indicating the presence of flavonoids) is indicated by the emergence of a dark color, which is considered the standard. The detection of phenols was carried out through the addition of 0.5 N plant extract to a small amount of 0.5 N ferric chloride solution. The observation of a dark green hue served as an indication for the existence of phenols (Patil *et al.* 2010).

The detection of terpenoids was conducted by combining 5 ml of the plant extract with a mixture consisting of 2 ml of chloroform and 3 ml of concentrated sulfuric acid. A positive test, indicating the presence of terpenes, was confirmed by the formation of a reddish-brown middle layer (Delazar et al., 2012). The alkaloid detection procedure

involved the addition of 5 ml of plant extract to 1 ml of Meyer's reagent. Meyer's reagent was made using two solutions, B and A, in the following manner: Solution A was produced by dissolving 1.58 grams of mercuric chloride (HgCl2) in 60 milliliters of distilled water. Solution B was made by dissolving a mass of 5 grams of potassium iodide (KI) in a volume of 10 milliliters of pure water. Prior to detection, the two solutions were combined without any intermediary steps and subsequently diluted with 100 cc of distilled water. The presence of a white precipitate serves as an indicator of a positive test result, indicating the presence of alkaloids (Ballard et al., 2010). The detection of saponins was conducted through the preparation of an aqueous solution using dry sample powder. Subsequently, the solution was transferred into a test tube and vigorously agitated.

3.3 Statistical Analysis

The results were analyzed statistically by applying the analysis of variance (ANOVA) test and the chi-square test, and the arithmetic means were compared with the Dunkin's multiple test with a probability level of $P \leq 0.05$, $P \leq 0.01$ (Quade 1979).

4. RESULTS AND DISCUSSION

Two hundred medial urine samples were collected for patients with acute and chronic urinary tract infection from Al-Alam General Hospital, for the period from the beginning of December 2021 to July 2022. After planting these samples on the culture media, 138 samples gave a growth rate of 69% when cultivated, while 62 samples did not show It grew on the culture media at a rate of 31%, as shown in Table 4.1. The positive growth divided into gram positive which constitutes 36 (26%) and gram negative which constitutes 102(74%) as shown in Table 4.2. Potential explanations for the absence of bacterial growth in the cultured samples include fungal, viral, or anaerobic bacteria pathogens, which are not amenable to isolation via conventional agricultural techniques. It was utilized in the research, which requires specialized growth conditions or culture media (Al-Douri 2011).

Table 4.1 The total number of isolates

The Growth	Number	Percentage
Positive Growth	138	%69
Growth Negative	62	%31
The Total	200	100

Table 4.2 The numbers and percentages of gram-positive and negative isolates

Growth	Number	Percentage
Gram-positive	36	%26
Gram- negative	102	%74
The Total	138	%100

4.1 Bacterial Isolation According to Age and Gender

A total of fifty years was the age range at which urine samples were collected from both sexes during the course of this investigation. Among the total of 200 samples, 142 were female, or 71% of the sample, whereas 58 were male, or 29% of the sample.

Table 4.3 The relationship between infection with bacteria and gender

Gender Bacterial infection	Females	Males	Percentage
infected	95	43	%66.9
Not infected	47	15	%33.1
Total	142	58	(% 100)

Previous studies have indicated that gender has an important role in increasing the incidence of urinary tract infection, as studies recorded a higher rate of infection in females than in males, because bacteria can reach the bladder more easily in females. This is partly due to the shortness of the urethra in the female and its proximity to the anus compared to the males (Farajnia *et al.* 2009). This contributes to its ongoing contamination with intestinal contents, and gender-based contact also plays a role in the transfer of pathogenic bacteria from the region encircling the urethra to the bladder. (Nicoll *et al.* 1998). Women during pregnancy are more likely to develop UTI (Al-Douri 2011).

Table 4.4, shows the number of bacterial infections according to age groups. In the age group 1 day - 10 years, the infection rate among females was higher than it was among males, and this is consistent with what the researcher Jamil *et al.* (2017) stated, where the infection rate of females was higher than that of males, males in the age group less than 5 years. In the age group 11-20 years, the results obtained in this study showed that the incidence of urinary tract infection in females is much higher compared to males, This is due to the duration of menstruation at this age, as females are more susceptible to infection due to the presence of menstrual blood and the accompanying hormonal changes during this period (Melekos and Kurt 2000), and in the age group 21-30 years, the incidence of UTI disease among females recorded a clear increase, and this is consistent with the results of the study carried out by some researchers, as it recorded an increase in the percentage of infection among women compared to male, and this is due to the fact that these ages represent the peak of genderual activity for females and represent the appropriate age for marriage Pregnancy and the associated interactions (Maheshwari *et al.* 201), and in the age group (31-40) years, the incidence of UTI disease showed an increase in females compared to males, and this is due to hormonal

changes that occur in women after menopause, as there is a decrease in the level of estrogen, which makes females more susceptible to UTI disease (Beuth *et al.* 1988).

Table 4.4 Number of bacterial infections by gender and age group

Gender Age	The number of infected females		The number of infected males	
	number of isolates	**percentage (%)**	**number of isolates**	**percentage (%)**
1 day - 10 years	12	12.6	4	9.3
11-20 years	28	29.4	3	9.6
21-30 years old	37	38.9	6	14.0
31-40 years old	12	12.6	9	20.9
41-50 years	6	6.5	21	48.9
the total	95	100	43	100

Likewise, in the age group 41-50 years and over, UTI infections in males were higher compared to females, and the reason may be due to an enlarged prostate (prostatic hypertrophy) in males at this age and a narrowing of the urinary tract, which causes urine to remain confined to the bladder, thus increasing the chance of Bacterial proliferation and infection (Kasper *et al.* 2015).

4.2 Relationship of Urinary Tract Infection and Pregnancy

Pregnant women are more susceptible to urinary tract infection, with 54.5% of infected pregnant women as shown in Figure 4.1. This results consistent with previous studies. There are numerous causes for the enlargement of the urinary tract during pregnancy, but the most significant are the physiological and hormonal changes that take place in the pregnant woman. Specifically, during the final months of pregnancy, fluctuations in hormone levels cause the ureters to dilate due to the expansion of the uterus at the pelvic boundary (Al-Douri 2011). This impedes the flow of urine through the ureters, leading to pooling of urine in the bladder, which leads to providing a suitable environment for bacteria to grow, causing urinary tract infection (Sheikh *et al.* 2000).

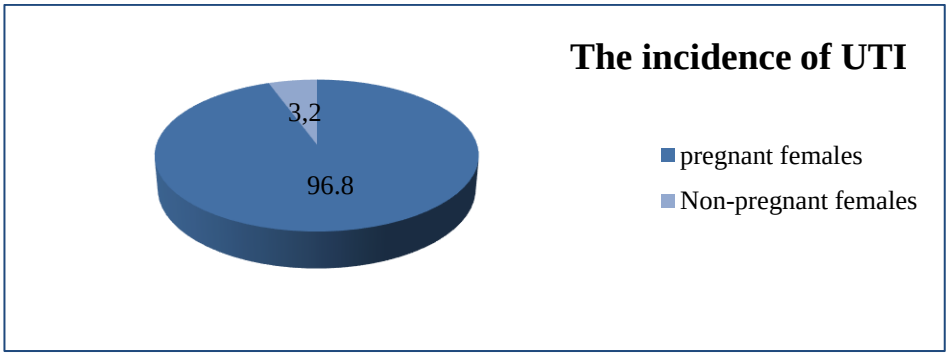

Figure 4.1 The incidence rate of UTI during pregnancy

4.3 Relationship of Urinary Tract Infection and Diabetes Mellitus

The findings of the study revealed a significant association between urinary tract infection and diabetes, with a majority of individuals diagnosed with urinary tract infection also having diabetes. Specifically, the data indicated that 33.3% of males with urinary tract infection were found to have diabetes, while 36.5% of females with urinary tract infection were also diagnosed with diabetes (Figure 4.2). These results are consistent with what was reported by Al-Hamdani (2019) in Tikrit, where the percentage of women infected with urinary tract infection and diabetes was 28.5%, but it differed with what was stated by Abdul-sahib (2008) in Baghdad, where the infection rate was 74% in women. It agrees with what was stated by Al-Bayaty *et al.* (2020), where the infection rate in males was 33.3%. Diabetes mellitus increases urinary tract infection risk, with over 20% suffering due to pathological bacteria exploiting glucose excreted in urine for reproduction (Ibrahim 2010).

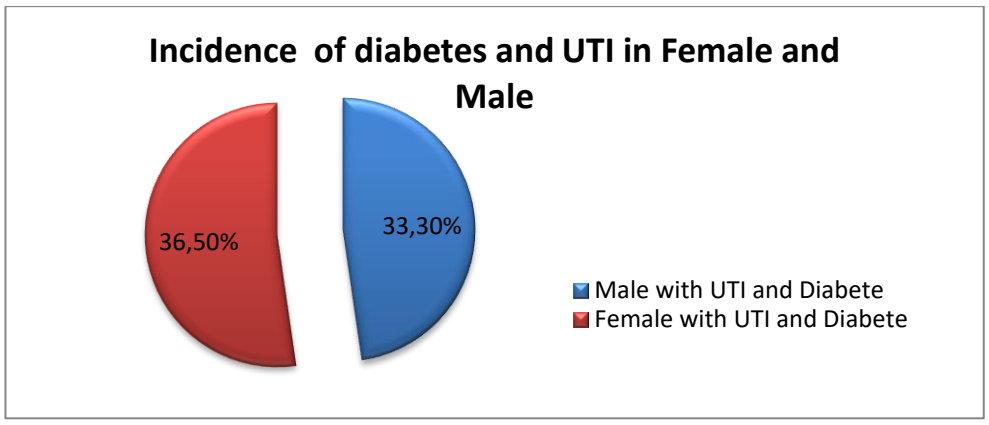

Figure 4.2 The incidence of UTI and diabetes

4.4 Isolation and Identification of Bacteria

It is clear from Table 4.5, that the most isolated and dominant types of bacteria are Escherichia coli with an isolation rate of 43.5%, followed by Klebsiella pneumonia with a percentage of 21.8%, Proteus mirabilis with a percentage of 11.6%, Staph. aureus with a percentage of 5.8%, Enterobacter cloaca with a percentage of 5.8%, and Serratia bacteria. marcescens by 2.9%, Pseudomonas aeruginosa by 2.9%, Saprophyticus Staph by 2.9%, Micrococcus luteus by 1.4%, and Citrobacter freundii by 1.4%. The primary characteristics of the cultured bacterial isolates depended on MacConkey agars, blood agars, and mannitol agars, then staining with Gram stain, studying the microscopic and morphological characteristics, and biochemical tests according to what was stated (Mahon *et al.* 2007). The bacteria species that were identified and isolated are detailed in Table 4.5. Due to the fermentation of lactose, E.coli colonies on macConkey medium were pink and desiccated (Figure 4.3) (Alfred, 2005). In contrast, E.coli colonies on EMB medium were brilliant metallic green (Figure 4.4). This finding aligns with the results reported by Soomro et al. (2002). The diagnosis of colonies that exhibited a pale pink hue was established using biochemical assays and culture characteristics. Klebsiella, on the other hand, exhibited mucous pink colonies due to the presence of a capsule (Sun et al. 2000). In contrast, S. aureus was isolated from mannitol-agar, which resulted in yellow colonies and a yellowing of the medium (Figure 4.5) caused by mannitol fermentation. S. saprophyticus generates diminutive pink colonies in which the color of the medium remains unchanged (Atlas 2006).

Table 4.5 Types and number of bacterial isolates

Isolated bacteria	Number of isolates	Percentage
Escherichia coli	60	43.5
Klebsiella pneumonia	30	21.8
Proteus mirabilis	16	11.6
Enterobacter cloaca	8	5.8
Pseudomonas aeruginosa	4	2.9
Serratia marcescens	4	2.9
S. aureus	8	5.8
S. Saprophyticus	4	2.9
Citrobacter freundii	2	1.4
Micrococcus luteus	2	1.4
Total	**138**	**100**

Figure 4.3 *E.coli bacteria on macConkey medium*

Figure 4.4 *E.coli* bacteria on EMB medium

Figure 4.5 *S.aureus* bacteria on mannitol medium

4.5 Biochemical Tests

In order to allocate the isolates to species, an extensive battery of biochemical assays were performed. The results presented in Table 4.6 and Table 4.7 were further validated by employing the VITEK 2system technique. Every biochemical assay was evaluated in relation to the methods suggested by Holt et al. (1994). The results of isolating 70 samples revealed two forms of growth: one that was positive for gram stain and the other that was negative for gram stain. In varying proportions, ten different bacterial species were isolated. The results of the current study differ from the results of the researcher Nasciment (2000), where the percentage of isolation of Escherichia coli was 22.8% and Saprophyticus bacteria. Staph was 23.8%, but it was relatively consistent with what was stated by Egbuna and Ifemeje (2016), where the percentage of Escherichia coli was isolated at 38%, Serratia marcescens at 4%, and Proteus mirabilis at 8%. and Citrobacter freundii by 2% and Klebsiella pneumonia by 12%. It also differs with the results of Al-Obaidi (2017), where the percentage of Staph bacteria was isolated. Aureus 3.2% and the percentage of Saprophyticus bacteria 8% (Figure 4.6).

Table 4.6 Biochemical tests for gram-positive isolates

Tests / Isolates	Novobiocin resistance	Mannitol	Coagulase	Catalase
Staph. Aureus	-	+	+	+
Saprophyticus . Staph	+	+	-	+

Table 4.7 Biochemical tests for Gram-negative isolates

Tests	Triple-sugar Iron				Catalase	Methyl red	Citrate	Indole
bacterial isolates	Gas	H_2S	Butt	Slop				
E.coli	+	-	A	A	+	+	-	+
K.pneumoniae	+	-	A	R/K	+	-	+	-
Serriatia marcessens	-	-	A	A	+	-	+	-
Citrobactera freundii	+	+	A	A	+	+	+	+
P. aeruginosa	-	-	R	K	+	-	+	-

A :Acid , K :Alkaline , R :No change

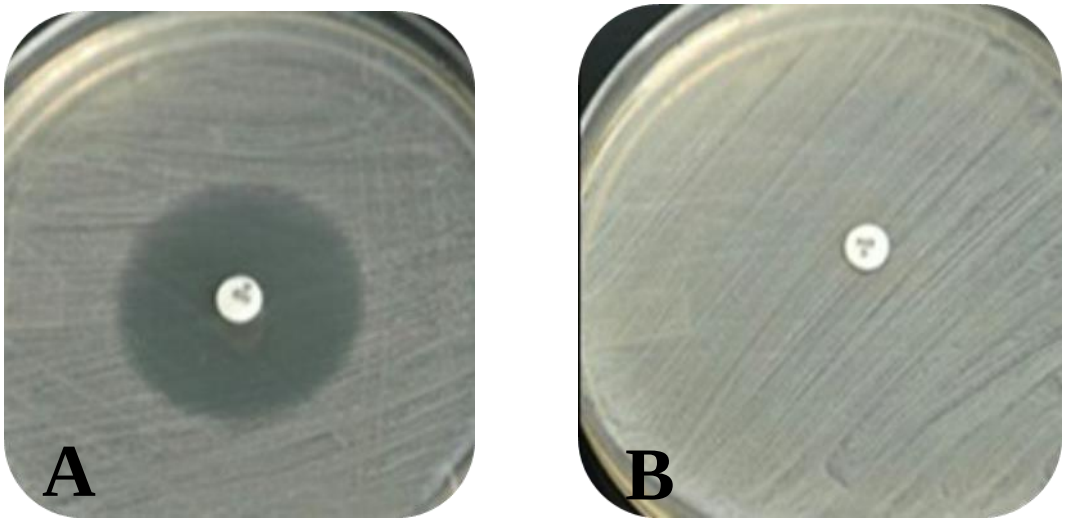

Figure 4.6 Resistance to novobiocin, A: *S. saprophyticus*, B: *S. aureus*

4.6 Antibiotics Sensitivity Test

Multi-resistant bacteria pose significant medical challenges, causing difficulty in selecting appropriate treatments due to indiscriminate antibiotic use without testing sensitivity (Rivera-Tapia 2003). The antibiotic susceptibility of the isolated bacteria was determined using the method of diffusion on Muller Hinton medium. The results were interpreted in accordance with the CLSI (2014) as sensitive, intermediate susceptibility, or resistance. The resistance patterns exhibited by the bacterial isolates towards the antibiotics that were tested varied.

The findings of this research demonstrated that a significant proportion of the E. coli isolates exhibited resistance to antibiotics, with resistance percentages of 84% for Cefalexin, 100% for Oxacilin, 96.7% for Vancomycin, and 87% for Tobramycin. The compounds with the least resistance were imipenem (0%), nitrofurantoin (13%), ciprofloxacin 40%, and sulfamethoxaole 44% (Figure 4.7). The results of this study are almost consistent with the results of Herr *et al.* (2004), where the percentage of Nitrofurantoin resistance was 34%, and it does not agree with Handayani (2018), where the percentage was 43% does not agree with Nasciment (2000), where the percentage was 0%. Also, agrees with Handayani (2018) relatively, where the percentage of resistance towards Imipenem was 72% as well. It agrees with Nasciment (2000), where the percentage of Vancomycin resistance was 91.6%. Chemotherapy drugs, including

antibiotics, can remove sensitive bacteria in the urinary tract. High concentrations in urine allow resistant bacteria to survive, causing an increase in bacterial strains. Treatment of antibiotic-resistant infections is a major issue in urinary tract infections. (Colin *et al.* 1981), Individuals exhibit high resistance to beta-lactam antibiotics, particularly Ampicillin, Amoxicillin, and Cephalosporins, due to their acquired resistance enzymes. (Bradford 2001).

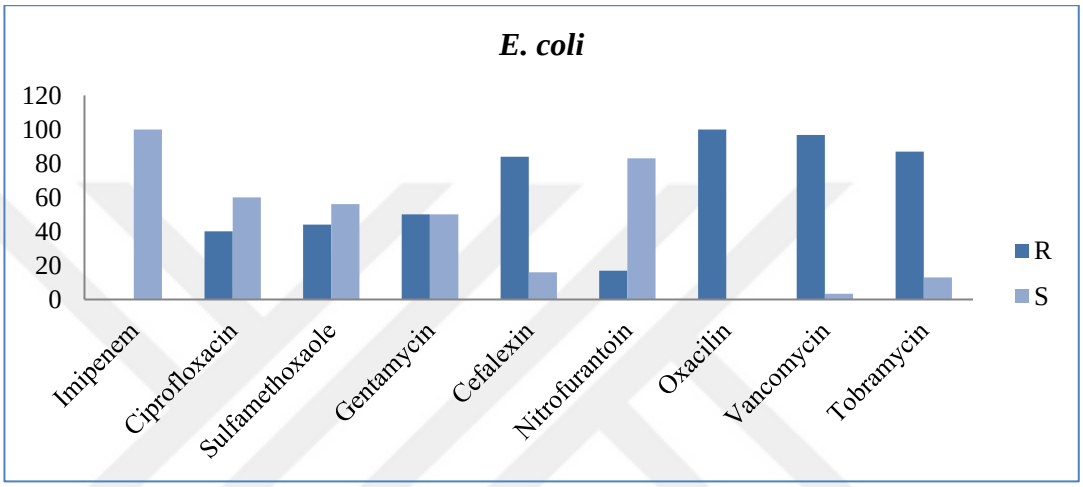

Figure 4.7 Patterns of *E.coli* resistance to antibiotics

The study revealed that the highest levels of resistance were observed for Oxacillin (100%), Vancomycin (100%), Nitrofurantoin (80%), Cefalexin (73.4%), and Tobramycin (66.7%). Conversely, the lowest levels of resistance were observed for Imipenem (0%), Ciprofloxacin (0%), Sulfamethoxazole (40%), and Gentamycin (7%). These findings are depicted in Figure 4.8. It agrees and differs with the results of previous studies, as it agreed with Kandela (2011), where the percentage of resistance to Imipenem was 0%, and agreed with Inabo and Obanib (2006), as the percentage of resistance to Vancomycin was 100%. Also, agreed with Rupinder (2012), where the percentage of resistance to Nitrofurantoin was 83%. While differed With Nasciment (2000), where the rate of resistance to Gentamycin was 40% and Ciprofloxacin 60%.

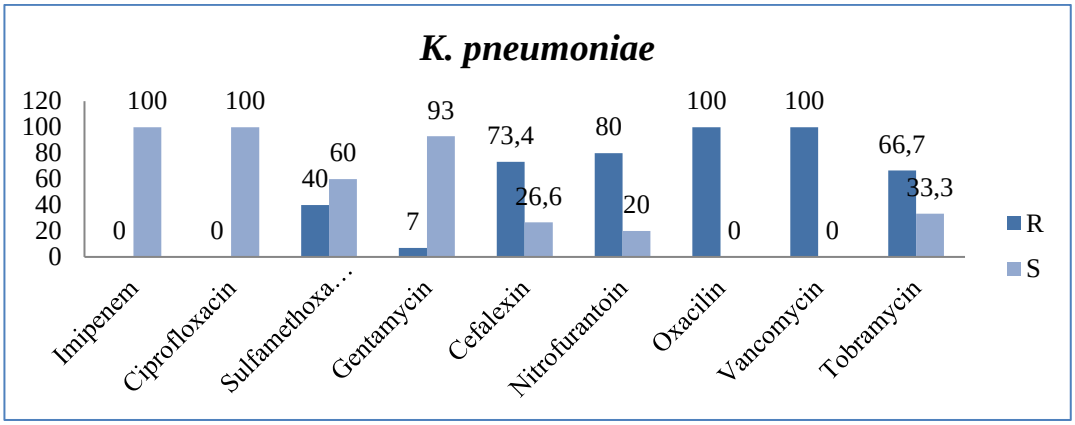

Figure 4.8 *K. pneumoniae* resistance patterns to antibiotics

The results presented in Figure 4.9 indicate that the maximum percentage of resistance was observed towards Oxacilin 100%, Vancomycin 100%, and Cefalexin 75%. Conversely, the minimum percentage of resistance was detected towards Imipenem 7%, Sulfamethoxaole 12%, Ciprofloxacin 25%, and Tobramycin 37%. The results of this study differ and agree with the results of previous studies. In agreement with Rupinder (2012), the study found a 95% resistance rate to Vancomycin, and consistent with Herr et al. (2004), a 100% resistance rate to Oxacillin was observed. However, it differs from Egbuna and Ifemeje (2016), who reported a 50% resistance rate to Gentamicin. The possible reason for this disparity could be attributed to the emergence of P. mirabilis isolates resistant to multiple antibiotics, potentially due to mutational changes or the presence of plasmids carrying multiple resistance traits. Such factors significantly contribute to the dissemination of multi-antibiotic resistance among bacteria. Additionally, this bacterial strain demonstrates the ability to form biofilms, which further contributes to antibiotic resistance, as highlighted by Kwiecinska et al. (2013).

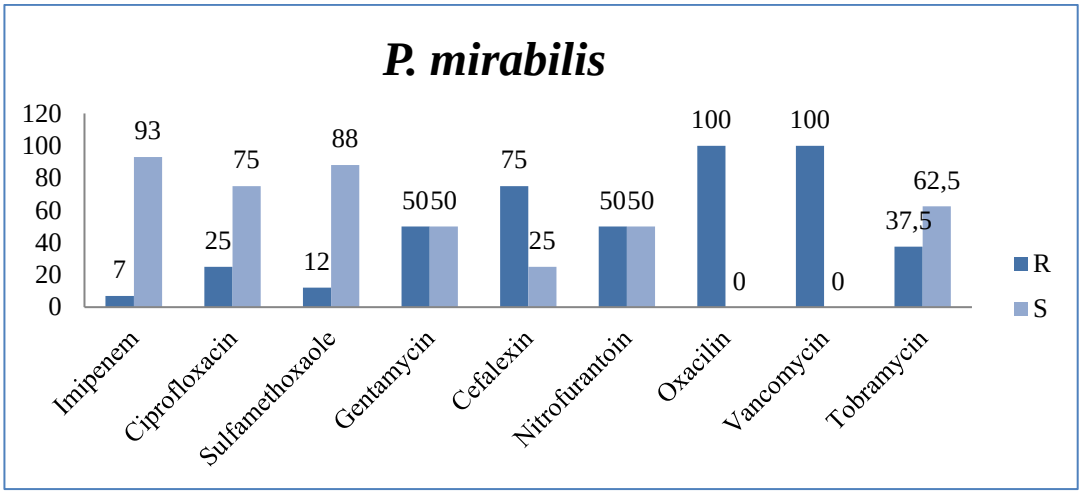

Figure 4.9 Patterns of *P.mirabilis* resistance to antibiotics

This study reveals 40% resistance to Oxacilin, 40% to Vancomycin, 0% to Imipenem, 9% to Nitrofurantoin, and 20% to Gentamicin, Ciprofloxacin, Tobramycin, and Cefalexin (Figure 4.10). The results of this study agree with Egbuna and Ifemeje (2016) ,where the percentage of Vancomycin resistance was 50% and Gentamicin 20%, but differ with Najee (2011), where the percentage of Vancomycin resistance was 77.7% and Gentamicin 100%. It differs with the results of Herr *et al.* (2004) , where it was The percentage of resistance to Oxacilin is 100%. It does not agree with Handayani (2018), where the rates of Sulfamethoxaole resistance were 100% and 33%, respectively, for Gentamicin and Nitrofurantoin. Anti-aminoglycosides can be resisted by bacteria, particularly type S. aureus, and this resistance is thought to be mostly mediated by cellular enzyme inhibition.

Adenoaltransferase (ANT), acetyltransferase (AAC), and phosphotransferase (APH) have been modified to the antagonist. Some of them lose the antagonist by changing it at the amine group, like AAC, while others lose it by changing it at the hydroxyl group, like ANT and APH. the capacity to attach to the ribosome's inhibitory site (Paulsen *et al.* 1997). The bifunctional enzymatic activity of AAC (6') and APH (2'), which are encoded by the aac (6)-Ie and aph genes, inhibits gentamicin. On psk1 family plasmids, conjugative plasmids, and other chromosomal loci, (2") and those harboring the jumping gene Tn4001 have been discovered (Firth and Skurray 1998).

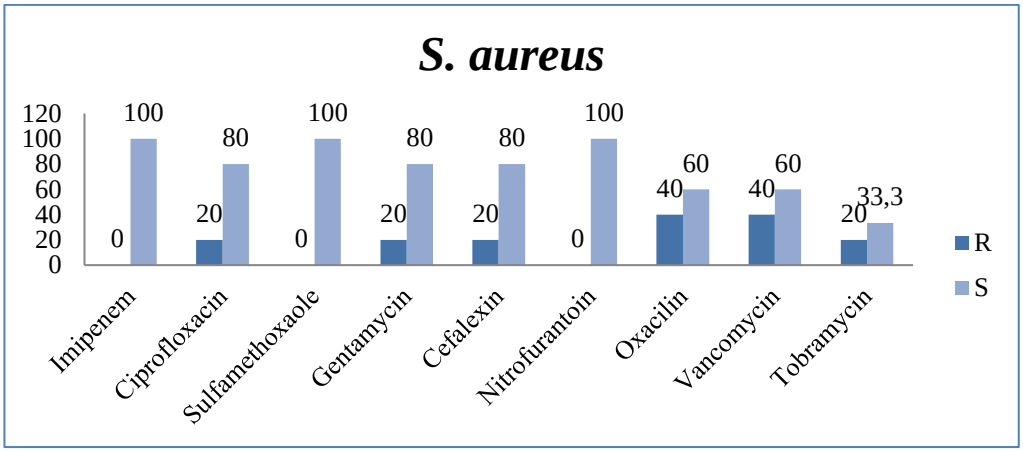

Figure 4.10 *S.aureus* resistance patterns to antibiotics

4.7 Effect of Aqueous and Alcoholic Plant Extracts on Bacterial Isolates

The results of the qualitative statements of the plant extracts included in the study showed that they contain many chemical compounds represented by glycosides, tannins, flavonoids, phenols, and others, as shown in Table 4.8 and Table 4.9. Their use in folk medicine treatments.

Table 4.8 Chemical qualitative disclosures of ginger

Detection	soaps	alkaloids	turbines	phenols	flavonoids	tannins	glycosides
results	+	+	+	+	+	+	+

Table 4.9 Chemical qualitative disclosures of fenugreek plants

Detection	soaps	alkaloids	turbines	phenols	flavonoids	tannins	glycosides
results	+	+	+	+	+	+	+

4.8 Effect of Plant Extracts on *E. coli*

Table 4.10 showed that the two extracts had equal effectiveness in inhibiting the growth of E.coli bacteria, as the results showed that both alcoholic extracts showed inhibition at

an average of 8.3 mm, while the two aqueous extracts did not show any inhibition against bacteria, meaning that there was no significant difference between them.

Table 4.10 Effect of plant extracts on *E.coli* bacteria

Plant type	The concentration is mg/mL	25%	50%	75%
	Extract type	The diameter of the inhibition zone		
Ginger root	alcoholic extract	5	10	10
	aqueous extract	0	0	0
Fenugreek bead	alcoholic extract	5	10	10
	aqueous extract	0	10	0

4.9 Effect of Plant Extracts on *K. pneumonia*

According to Table 4.11, it was evident that the most potent extract for inhibiting the growth of K. pneumoniae was the alcoholic ginger root extract, averaging 11.5 ml, followed by the alcoholic fenugreek extract with an average of 10 ml. The ginger and fenugreek aqueous extract exhibited no inhibitory effect, averaging 0 mL.

Significant differences were observed among the concentrations used, with 75 mg/ml identified as the most effective concentration. However, no significant difference was found between the 50 mg/ml and 25 mg/ml concentrations. Furthermore, there was no significant distinction between the various concentrations of the aqueous extracts of ginger and fenugreek.

Table 4.11 Effect of plant extracts on *K. pneumoniae*

Plant type	The concentration is mg/mL	25%	50%	75%
	Extract type	The diameter of the inhibition zone		
Ginger root	alcoholic extract	10	10	15
	aqueous extract	0	0	0
Fenugreek bead	alcoholic extract	5	10	15
	aqueous extract	0	0	0

4.10 Effect of Plant Extracts on *S.aureus*

Table 4.12 showed that the most effective extract in inhibiting the growth of S. aureus was the alcoholic extract of fenugreek at an average of 15 ml, followed by the aqueous extract of fenugreek at an average of 11.6 mg/ml, then the alcoholic extract of ginger at an average of 10 mg/ml, then the extract aqueous content of ginger at an average of 6.6 mg/ml.

Furthermore, significant differences were observed between the concentrations used, with the most effective concentration identified as 75 mg/ml. Additionally, there were significant differences at the 50 mg/ml and 25 mg/ml concentrations.

Table 4.12 Inhibitory efficacy of plant extracts on *S. aureus*

Plant type	The concentration is mg/mL	25%	50%	75%
	Extract type	The diameter of the inhibition zone		
Ginger root	alcoholic extract	5	10	15
	aqueous extract	5	5	10
Fenugreek bead	alcoholic extract	10	20	15
	aqueous extract	10	10	15

4.11 Effect of plant extracts on *P. aeruginosa*

According to Table 4.13, it was evident that the most effective extract for inhibiting the growth of P. aeruginosa was the alcoholic extract of fenugreek seed, with an average of 10.3 mm. This was followed by the aqueous extract of fenugreek at an average of 9 mm, and the least effective was the aqueous extract of ginger root, averaging 6.6 mm. While the alcoholic ginger root extract did not show any effect on bacteria. Furthermore, a significant difference was discovered between the aqueous and alcoholic extracts of fenugreek seeds, with the alcoholic extract averaging 10.3 ml and the aqueous extract averaging 9 ml. Moreover, there were significant differences observed between the concentrations used, with the most effective concentration noted at 75 mg/ml, followed by 50 mg/ml, while the least effective was at a concentration of 25 mg/ml.

Table 4.13 Inhibitory efficacy of plant extracts on *P. aeruginosa*

Plant type	The concentration is mg/mL	25%	50%	75%
	Extract type	The diameter of the inhibition zone		
Ginger root	alcoholic extract	0	0	0
	aqueous extract	10	10	0
Fenugreek bead	alcoholic extract	3	13	15
	aqueous extract	5	7	15

In general, after analyzing the results we obtained and comparing them with previous studies regarding plant extracts and their effect on bacteria, they were as follows: With regard to the results of ginger root extract, our current study agreed with many studies. The bacteria that between the rates of the diameters of the areas of inhibition for the growth of different types of pathological bacteria, if an increase in the diameter of the inhibition zone is observed with an increase in the concentration of the extract..and it came in agreement with many studies, including the results of the researcher (Mohammed and Al Dabbagh 2017), who showed that the high effectiveness of the aqueous extract of the ginger plant on different types of Bacteria, especially E. coli, P. aeruginosa, as well as S. aureus, and the researcher Faruq and his group showed in 2011 that the diameter of the inhibition zone of ginger plant extract reaches (12-18) mm for E. coli bacteria). Whereas, Kadnur and Goyal (2005) that all Gram-negative bacteria were more sensitive to the alcoholic extract of ginger root than Gram-positive bacteria.

The inhibition mechanisms of plant extracts can be attributed to the hindrance of microorganism cell wall formation or the suppression of fundamental protein synthesis within the cell. It can also involve the creation of complexes with the cell wall, thus impeding permeability regulation, as well as the inhibition of crucial enzymes responsible for growth and reproduction. Additionally, it may involve the disruption or alteration of cellular membranes and their functions (Mohammed and Al Dabbagh 2017). Its effectiveness may also be due to the fact that it contains gingerol, zingerone, and other bactericidal substances (Kumar and Mohammad 2011), and thus these results

support the use of ginger to treat bacterial infections in this study. As for the alcoholic and aqueous fenugreek extracts, the effectiveness of Punica Grantum against urinary tract pathogens may be due to the fact that it contains effective antioxidant chemical compounds, which are flavonoids and phenols. It also contains alkaloids, the most important of which are granatin, Gallotannin, Pelletierine, and a bitter substance called Punicine, as well Tanning materials Kawabata *et al.* (2011), thus fenugreek seed extract is used to treat more than 26 pathological bacterial infections and is considered as one broad spectrum antibiotic.

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

1. The incidence of urinary tract infections in women was higher in females than in males for anatomical and physiological reasons.
2. The most isolated and dominant types of bacteria were Escherichia coli with an isolation rate of 43.5%, and the least isolated bacteria were Micrococcus luteus and Citrobacter freundii with a percentage of 1.4% each.
3. The antibiotics used in our study showed a clear variation in their effect on the bacteria under study.
4. Tests showed alcoholic and aqueous extracts of ginger root and fenugreek were more effective against isolated bacteria than aqueous extracts, indicating significant differences between the two plant extracts. (aqueous and alcoholic).

5.2 Recommendation

Periodic examinations are crucial for detecting urinary tract infections to prevent complications like renal diseases. Antibiotics should be used with medical advice to prevent resistant strains. Modern methods, such as genetic engineering and PCR, can help diagnose difficult cases.

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APPENDICES

APPENDIX 1. Equipments and instruments

APPENDIX 2. Culture media used in this study

APPENDIX 3. Chemicals and Biological materials used in the laboratory work

APPENDIX 4. Ethics approval certificate

APPENDIX 1. Equipments and instruments

Name of Item	Company and Origin
Incubator	Memmert (Germany)
Autoclave	Universal (Germany)
Electric oven	Memmert (Germany)
Centrifuge	Universal (Germany)
Sensitive electrical balance	Sartorious , (Germany)
Compound light microscope	Olympus (Japan)
Distillator	Gallenkamp (England)
Refrigerator	Concord (Lebanon)
ELISA(Human reader,comBI wash	Biotek instruments(Korea)
vitek 2 compact	(France) Biomerieux

APPENDIX 2. Culture media used in this study

Name of Item	Company and Origin
England	Blood Agar BA
India	MaCconkey Agar MA
India	Nutrient Agar NA
England	Muller Hinton Agar MHA
England	Mannitol Salt Agar (MSA)
England	Eosin Methylen Blu EMB
England	Peptone Water PW
England	Methyl Red& VogesProskauer Broth
India	Simmon Citrate Agar SC A
England	Triple Suger Iron Agar TSI A
India	Nutrient broth Medium

APPENDIX 3. Chemicals and Biological materials used in the laboratory work

Company and Origin	reagent
England BDH	Ethyl alcohol
BDH (England)	Oxidase reagent
BDH (England)	Catalase reagent
Difco(USA)	Methyl red
Bioassay	Interleukin-23 ELISA Kit Human
India Hi Media	Voges – Proskauer VP1 VP2
India Hi Media	Kovacs reagent
(S.A.R) Syrbio	Gram Stain Solution
USA DensiCHEK	McF standar

APPENDIX 4. Ethics approval certificate

CURRICULUM VITAE

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