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College of Science

Department of Biology

Antibacterial Activity of Arabic Gum Against Some Pathogenic Bacteria Isolated from UTI Patients

A Thesis

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By

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“يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ”

صدق الله العلي العظيم

سورة المجادلة, آية١١

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Dedication

To that great woman who has always been my right hand in everything I have reached (My mother)

To whoever Allah has chosen as my partner for this world, he was the best partner and supporter (My husband)..

To my brothers and sisters…..

Rabab

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Summary

The urinary tract infection is a widespread bacterial infection; it is one of the major problems suffered by humans, and responsible for many health problems. So many studies have sought to find the appropriate solutions to reduce the spread of pathogen in society.

During the period extending between May 2022 to July 2022, urine samples were obtained from 53 suspected UTI patients and 35 healthy controls, their ages were ranged from 3 to 69 years old. Collection of samples were carried out in two places (Al-Hussain Teaching Hospital and private clinic).

The bacterial growth was identified by using Vitek system. The Human Cathilcidin (LL-37) and Human lactoferrin were measured by using the ELISA system, The results showed that the percentage of infection in females was higher than in males where the infection rate in females was 50% ,but in males was 49% .In addition, the highest rate of infection was in the 20-29 age group while the lowest infection rate was in the 60-69 age group. Out of 88 urine samples, the results also showed that 2 (3.8%) had no bacterial growth and 51 (96.2%) had considerable bacterial growth.

The results of bacterial isolates were 16/51 (30.2%) *Escherichia coli*, 14/51 (26.4%) *Staphylococcus aureus*, 10/51 (18.9%) *Klebsiella pneumoniae*, 6/50 (11.3%) *Enterococcus faecalis*, 5/51(9.4%) *pseudomonas aeruginosa* .

The mean of urinary LL-37 levels showed significant differences ($P \leq 0.05$) between patients (1.96) and control (1.72). Also there was a significant difference ($P < 0.01$) between the levels of LTF and LL-37 with bacterial isolate. Where higher concentration was recorded in patients with *E.coli* (2.8), followed by *klebsiella pneumonia* (2.18), *Enterococcus faecalis* (1.77), *Staphylococcus aureus* (1.45) ,and *Pseudomonas aeruginosa* (0.88).

In Vitro effect of aqueous Arabic Gum solution against *S.aureus* and *E.coli* was estimated by well diffusion method at concentrations ranged from 100 mg/ml to 500 mg/ml ; the results revealed that the aqueous Arabic Gum solution had the antibacterial activity against the studied *E. coli* and *S. aureus* bacteria. The mean of inhibition zone diameter of *E.coli* isolates increased with concentration of aqueous Arabic gum solution increased to reach to 11.7 mm in the high concentration of aqueous solution 500 mg/ ml , but in low concentration 100 mg/ ml was there was no effect. While inhibition zone diameter against *S.aureus* bacteria reach to 10 mm in the high concentration of the aqueous solution 500 mg/ ml, but in low concentration 100 mg/ ml there was no inhibition zone. The aqueous solution of *Boswellia Serrata* was also used, but it did not give any antibacterial activity on the bacterial isolates on the bacterial isolates.

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List of abbreviations

UTI	Urinary tract infection
AMPs	Antimicrobial peptides
HDPs	Host defense peptides
PMNs	Polymorphonuclear leukocyte
UTIs	Urinary tract infections
ESBL	Extended- spectrum B-lactamase
NK	Natural killer cells
LTF	Lactoferrin
LL-37	Cathelicidin
TLR	Toll-like receptor
T-cell	T helper cell
LPS	Lipopolysaccharides
THP	Tamm-Horsfall protein
UPEC	Uropathogenic Escherichia coli
ELISA	**E**nzyme **L**inked Immune **S**orbent **A**ssay
GBS	Group B streptococcus
IBCs	Intracellular bacterial communities
ABR	Antibiotic resistance
AG	Arabic gum
GUE	General Urine Examination
GN-ID	Gram negative identifier
GP-ID	Gram positive identifier
HRP	Horseradish peroxides
OD	Optical density

PAMP	Pathogen -associated molecular pattern
NOD like receptor	Nucleotide- binding oligomerization domain

Chapter one

Introduction

Chapter one: Introduction

1.1. Introduction

Invasion of the urinary system by a pathogen that triggers an inflammatory response in the urothelium is what causes urinary tract infection (UTI). Chronic or acute infections are both possible. The intensity of the infection, the causative organisms, the location of the infection in the urinary tract, and the patient's immune system's capacity to respond to it all affect the clinical signs of UTI. Frequent or urgent urination, dysuria, and cloudy or odorous urine are only a few possible signs and symptoms. Urinary tract infection are ascending when they occur in urethra and contain up the urinary tract to the bladder and causing cystitis ,but when the infection spreads quickly to the ureters and kidney, it pyelonephritis develops (Schmiemann *et al*.,2009).

All kinds of life have an innate immune response, which is made up of tiny polycationic peptides called antimicrobial peptides (AMPs) often referred to as host defense peptides or HDPs. Numerous AMPs with activity against bacteria, fungi, protozoa, and even altered or cancer cells are eliminated by neutrophils (PMNs) and natural killer cells. Despite the differences in their lengths, sequences, and conformations, they share some characteristics. The capacity of AMPs to interact with bacterial membranes or cell walls determines how well they can kill bacteria. Because AMPs often have a net positive charge and a significant proportion of hydrophobic amino acids, they can bind specificity to negatively charged bacterial membranes . Non-enzymatic disruption results from AMP binding to the bacterial membrane. Due to variations in the membrane composition of various microorganisms and cell types, certain species are more selective than others (Ageitos *et al*.,2017).

Antibacterial peptides levels in healthy urine are relatively low because epithelial cells only release very little amounts of these peptide under normal circumstances. It is produced and released within minutes of contact with epithelial cells and any uropathogenic bacteria and appears to be a highly essential first line defensive strategy of the innate immune system of the urinary tract due to its extremely quick reaction, happening long before the generation of antibodies, and occurring prior to the inflow of neutrophils (Chromek., 2015). Pathogens are typically killed by AMPs by disrupting the integrity of bacterial cell membranes. However, some AMPs attach to cytoplasmic sites in bacteria and prevent them from synthesizing proteins or DNA (Becknell *et al*.,2015).

Lactoferrin (LTF) and cathelicidin (LL-37) have different biological function in human body such as immune modulatory ,anti tumor and antimicrobial. They also play a critical roles in innate immune system through its ability to interact with molecules of cell wall or cytoplasmic membranes for bacterial cells of altered cells that causes cell death (Tullus,2019) and LL-37 serving as chemo altered of mast cell to the site of infection in response to virulence factors of pathogen bacteria (Van Harten *et al* .,2018).

The extensive use of antibiotics in hospitals and communities, and agriculture, has been accelerated the apparent of multidrug resistance bacteria has arisen as a global health issue, antibiotics resistance is not just fatal problem that are difficult to treat ,but also key to develop different infections and cancer as well as having social and economic repercussions. The transmission of resistance genes between bacteria (through plasmids and transposons) causes resistance to spread among bacteria, people and geographical areas (Laxminarayan *et al*.,2013).

Therefor it was necessary to search and investigate to find natural alternative to these chemical compounds

Many different plant-based and natural products are utilized to cure a variety of diseases. Herbal remedies were utilized to treat a variety of infectious disorders in traditional medicine. According to Mehrotra *et al*. (2010), for primary healthcare 80% of the world's population used herbal products, drugs have been created to treat diseases based on the biological features of secondary metabolites found in plants, such as alkaloids, resins, steroids, tannis, oleosins, and terpenes.

Among these plant the aqueous solution of Arabic gum and *Boswellia serrata* have antibacterial peptides against both gram negative and gram positive bacteria such as *Staphylococcus. aureus*, *psudomonas .aeruginosa*, *proteus*, *Escherichia.coli*, *Klebsiella pneumonia* and *Enterobacter* (Bnuyan *et al*.,2015). That are responsible for pyelonephritis and cystitis

there for this study aim to evaluate the association between the virulence of pathogenic bacteria isolated from patients with urinary tract infection and levels of antimicrobial peptides in urine and determine the antimicrobial activity of some medical plants against most frequent isolate bacteria through the following objectives:

1. Collect the urine sample and collect information about the patients with urinary tract infection and the healthy individuals such as sex, age, urine profile (microscopic examination).
2. Culture the urine sample on general and selective media and the distribution of the bacterial pathogen was assessed by isolation and identification using the Vitek 2 compact system.

3. Estimating the level of an antimicrobial peptides in the urine of patients and control group by ELISA technique
4. Studying the relationship between the variation of antibacterial peptides with the risk factors suffered by the patient.
5. Studying the relationship between isolated bacterial species and variation in antibacterial peptides
6. Demonstrating the effect of an aqueous solution of Arabic gum and *Boswellia serrata* on the most common bacterial isolates in urinary tract infections isolated from patients.

Chapter two

Literature Review

Chapter two: Literature Review

2.1. Urinary tract:

The urinary system consists of a group of members that linked symmetrically and structurally to each other organs include kidneys ,ureters, bladder and urethra as show in Figure (2-1).

The urinary tract has three major function:

- Excretion
- Elimination
- Homoeostatic regulation of the solute concentration of the blood plasma.

Each member of those organs performed a specific function where the kidney perform the process of filtration blood (Choriyeva *et al*.,2022) .The kidneys are situated on either side of the vertebral column and they lie retroperitoneally between the 12 th thoracic and 3 rd lumbar vertebrae. The left kidney lies slightly superior to the right kidney and it is also slightly longer (Denic *et al*.,2022) . The kidney is bean-shaped, and approximately 10-12 cm in length, 5-7 cm wide and 2-5 cm thick. The blood supply, nerves and lymphatic vessels enter and exit at the hilum. The superior surface of the kidney is capped by the adrenal gland (Baranski, 2023). Each kidney is surrounded by three layers. The first one is a layer of collagen fibers that covers the outer surface of the entire organ called renal capsule which structure by fat capsule this keeps the kidney in it, in addition to those layers found renal fascia which is a dense fibrous outer layer that also secures the kidney to the posterior abdominal wall and to the surrounding structures

The kidney itself is made up of two layers, the cortex and the medulla. The cortex

is the outer layer and the medulla is the inner layer (De Grout *et al*.,2015). Within the medulla there are 8–18 distinct conical or triangular structures called the renal pyramids. The base of each pyramid is turned towards the cortex and the tip of the pyramid is directed towards the renal sinus. The tips of the pyramids are referred to as the renal papillae. The pyramids are separated from each other by bands of cortical tissue called the renal columns. The renal cortex and the pyramids together make up the parenchyma. The parenchyma consists of approximately 1.25 million nephrons, which are the functional units of the kidney as they (McGrath *et al*.,2005).

The nephron is the functional unit of the kidney. It is responsible for filtration of the blood and for the re-absorption of water salts and glucose. About 1.25 million nephrons can be found in the cortex. The nephron consists of a renal tubule and a renal corpuscle. The tubule is approximately 50 mm is length and consists of the convoluted tubule and the loop of Henle. The renal corpuscle is made up of the Bowmans capsule and the capillary network of the glomerulus (Theodorou *et al*.,2021). The filtered blood in the kidneys is transmitted to the ureters are muscular tubes that link the kidneys to the bladder. They are approximately 30 cm in length and 3mm in diameter. They consist of three layers the inner layer of transitional epithelium, a middle layer made up of longitudinal and circular bands of smooth muscle but the outer layer of connective tissue which is continuous with the renal capsule. There are slight differences in the ureters in men and women as they have to accommodate the position of the reproductive organs. Urine is forced along the ureter due to peristaltic action. The ureters enter the bladder on the posterior wall and pass into the bladder at an oblique angle. This prevents backflow when the bladder contract (Gupta and Das,2022). The bladder is a hollow, muscular organ that collect and stores urine.

It is situated in the lower part of the abdomen and is lined with a membrane called the urothelium. The cell of this membrane are called transitional cells or urothelial cells. The bladder wall has three layers: mucosa, submucosa and muscularis. The muscularis is made up of layers of longitudinal smooth muscle with circular layer sandwiched in between. This muscle layer is known as the detrusor muscle, and it is this muscle that contract to expel urine from the bladder and into the urethra. The bladder initially stores urine, however, afferent fibers in the pelvic nerves carry impulses to the spinal cord, which in turn sends massages to the thalamus and then along projection fibers to the cerebral cortex. At this point you become aware that your bladder requires emptying. The muscle of the bladder can then be contracted to force urine out of the body through a tube called the urethra (Hallgrímsson *et al* 2003). And the urethra extends from the neck of the bladder to the exterior of the body. In women, the urethra is a very short tube, in front of the vagina, approximately 4cm in length. In men, the tube is considerably longer, 18-20 cm long, it need to be longer as it has to pass through the prostate gland and the length of the penis. It is made up of stratified epithelium (Tritschler *et al.*,2013).

1.1. Immune system in urinary tract

The colonization, entry and spread of microorganisms in urinary tract can be prevented by innate immunity and two main sub-divided types of adaptive immunity (humeral and cellular). Naturally acquired immunity develops via direct contact with a disease causing agent, while artificially acquired immunity occurs usually through taking the vaccination (Medzhitov et al 2000).

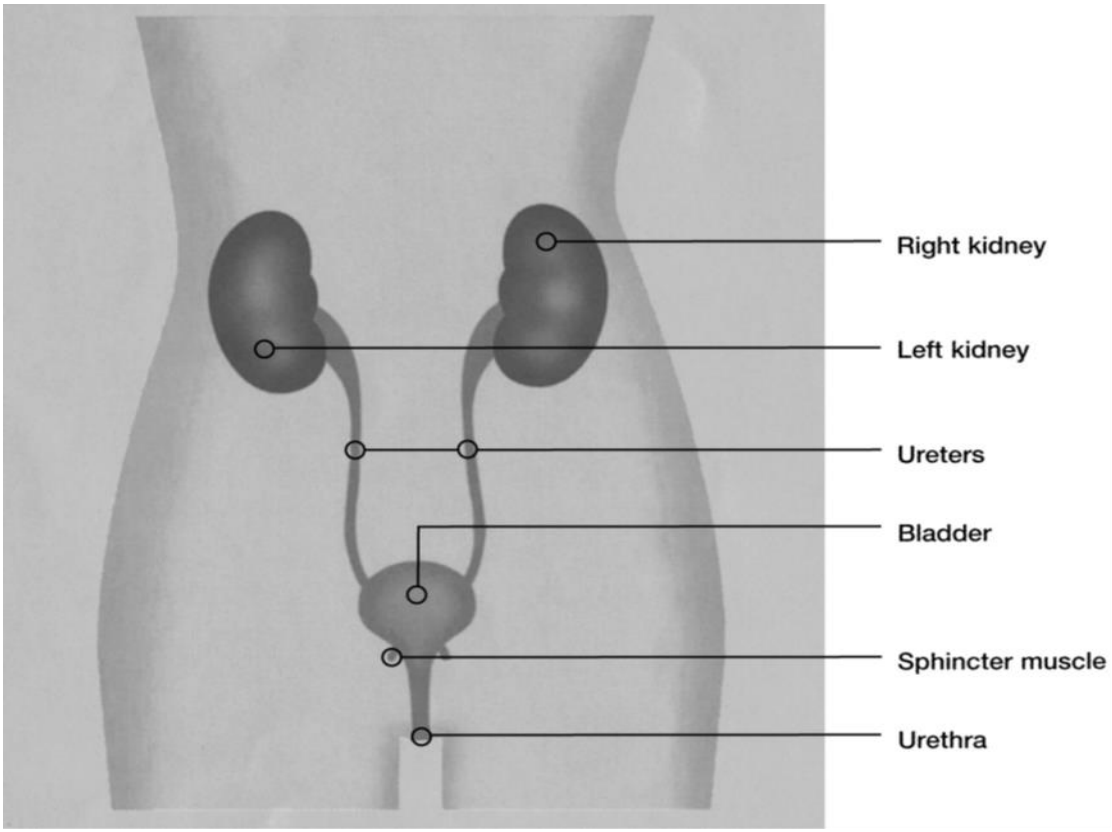


Figure 2-1 Urinary system (McGrath *et al* 2005)

The effector mechanisms of innate immunity which include antibacterial peptides phagocytes and the alternative complement pathway , are activated immediately after infection and rapidly control the replication of the infection pathogen as shown in Figure (2-2). One of the most important component of innate immunity in the human body which is responsible to contracting bacterial pathogen is antimicrobial peptide in urinary tract (Reygaert ,2014).

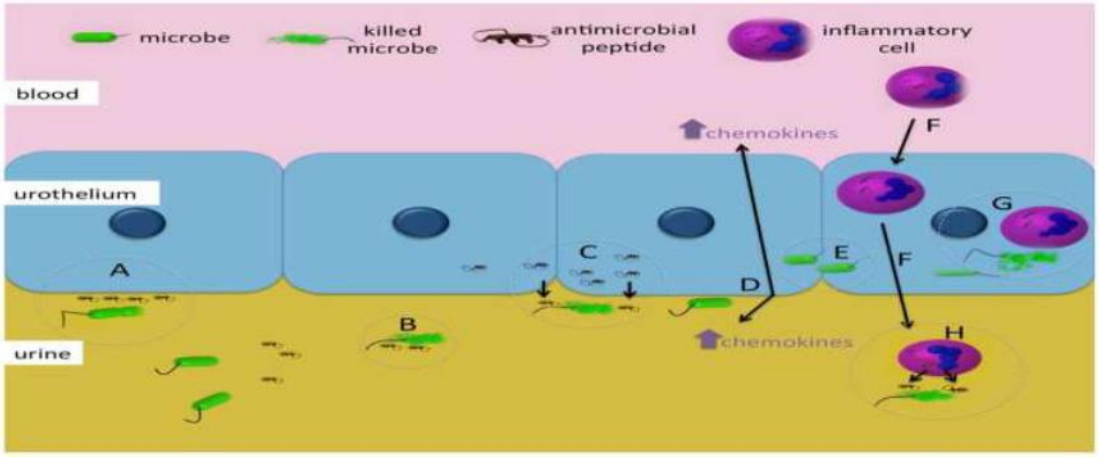


Figure 2-2 Mechanisms of innate immune in urinary tract ((Ortega Martell, 2020)

2.1.1.Antimicrobial peptide

Antimicrobial peptides are small, between 20 to 60 amino acids, positively charged because of the presence of lysine and/or arginine residue, and amphipathic, thus, permits them to achieve high concentrations in both an aqueous environment or within a membrane. AMPs act on a microbe's membrane and do so rapidly (within seconds to minutes) and lethally. AMPs have many different structures, and so long as the peptides retain their cationic charge and amphipathicity, they retain activity. Among these AMPs, defensin ,cathelicidin, lipocalin, and lactoferrin, etc. (Zasloff,.2007). Immediately after contact with bacteria, epithelial cells substantially increased the synthesis and secretion of antimicrobial peptides protecting the urinary tract from bacterial adherence and they significantly effect on bacteria pathogen which causes UTIs (Chromek *et al*.,2006). a high level of AMPs in UTI infection illness as a result of harmful bacteria being killed by these peptides produced by immunological and epithelial cells, resulting in lower bacterial loads and tissue damage (Ali and AL- Dujaili, 2022).

2.1.1.1. Cathelicidin (LL-37)

Cathelicidins are antimicrobial peptides produced by humans and animals in response to variety pathogenic microbes as well as against viruses. By targeting virulence factors, it act as a important element of the urinary tract innate defense against infections (Nielsen *et al*., 2014; Bandurska,et al.,2015). The mature antimicrobial peptide, which starts with a pair of leucines, contains 37 amino acid residues, which is why it was given the designation LL-37(Ramos *et al*.,2011). Although the physical features of these effector different for several vertebrates, the amino acid sequences share physical characteristic such as amphipathicity and positive charge (Van Harten *et al*.,2018). The peptide is secreted specifically by neutrophil secondary granules, but it is also produce by macrophages, natural killer (NK) cells, and epithelial cells of the skin, airways, eyes, and gastrointestinal tract (Esfandiyari *et al*.,2019). The LL-37 peptide's secondary structure and cationic character, which are conferred by arginine and lysin residues, determine its bactericidal and immunomodulatory properties (Koro *et al*., 2016; Van Harten *et al*., 2018; Esfandiyari *et al*., 2019). LL-37 preforms a variety of important function, one of which chemotaxis, which causes mast cells to migrate to the site of infection (Niyonsaba *et al*.,2002). Additionally, it contributes to mast cell degranulation, inflammatory mediator release, phagocytosis enhancement microorganisms by opsonizing them and increasing their bacterial recognition receptors , and DNA/RNA uptake enhancement, all of which contribute to increase intracellular (TLR) signaling (Schiemann *et al*.,2010,Van Harten *et al*.,2018). Cathelicidins also have a direct killing effect on Gram-positive and Gram-negative bacteria by binding and interacting with negatively charged on the membranes, cell membrane degradation, and the penetration of larger molecules such as proteins, causing cell

shape and membrane damage, and eventually cell death as shown in figure (2-3) (Van der dose et al.,2010 ;Van Harten *et al*., 2018;Esfandiyari *et al*.,2019). Under healthy conditions, epithelial cells release very small amounts of this peptide so the levels of cathelicidin in healthy urine are very low. When epithelial cells contact with any uropathogenic bacteria, the cathelicidin is synthesized and released within minutes. Because it so rapid response and preceding the influx of neutrophils and occurring long before the production of antibodies, cathelicidin seems to be a very important first line defense strategy of innate immune system of urinary tract (Chromek.,2015)

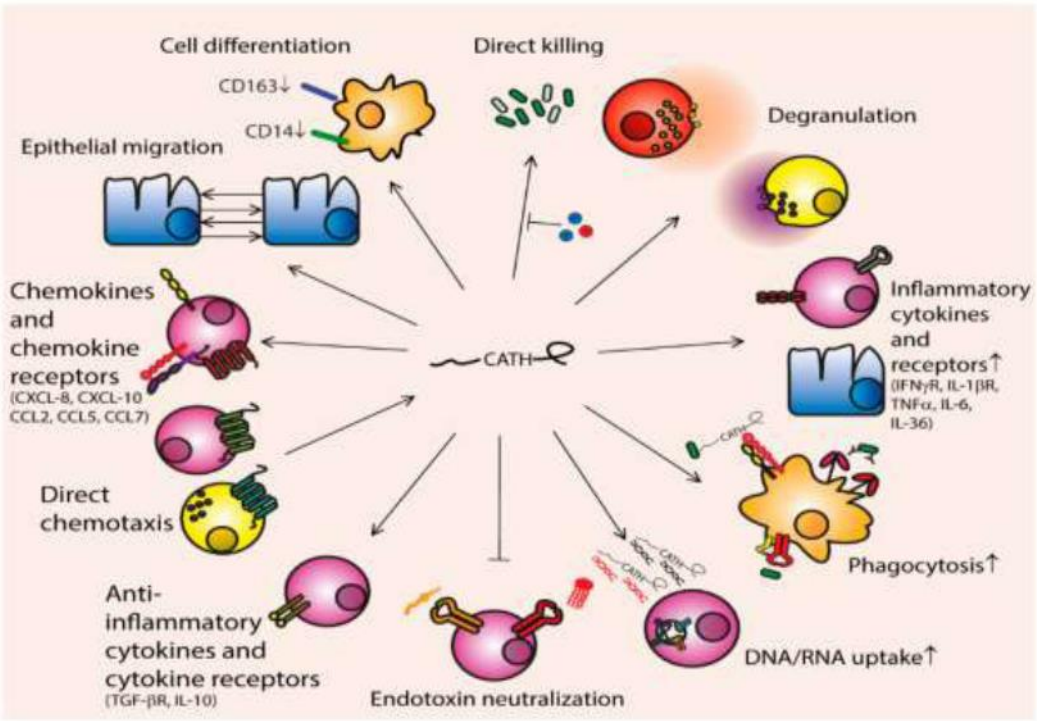


Figure 2-3 Function of cathelicidin (Van Harten *et al*.,2018)

2.1.1.2. Lactoferrin:

Lactoferrin (LTF) is an iron-binding protein which have similar structure to transferrin, lactoferrin was isolated for the first time by Sorensen from bovine milk in 1939 (Baker and Baker,2012) . In 1960 it was concurrently determined to be the main iron binding protein in human milk by three independent laboratories (Superti, 2020). Subsequent research identified lactoferrin in secretion from exocrine glands and in specific granules of neutrophils and in other organs such as pancreas, kidney, prostate, seminal vesicle, etc (Adlerova *et al*.,2008). The protein which is about 80 Kda consist of two homologous lobes, each lobe have one iron-binding site. Lactoferrin has varies biological functions, including antimicrobial, antioxidant, anti-tumor and immunomodulatory effects (Ching *et al*.,2020). Partial degradation of LF by pepsin in stomach, can result to peptides called lactoferricin which have more antimicrobial activity. LF has bactericidal effect on pathogenic bacteria, either by sequestering free iron which is one of fundamental elements for growth of bacteria or by the effect of lactoferricin (Hassan *et al*.,2023). Lactoferrin also has profound modulatory action on the adaptive immune system by promoting the maturation of T-cell precursors into competent helper cells and by differentiation of immature B cells into efficient antigen presenting cells (Jeffrey *et al*.,2009) . Three different isoforms of lactoferrin have been isolated Lactoferrin-α is the iron binding form, but has no ribonuclease activity. The other one was lactoferrin-β and lactoferrin –γ demonstrate ribonuclease activity but they are not able to bind iron (Alderova *et al* .,2008).

2.1.1.3. Defensins

Cysteine rich cationic proteins with amino acid sequences ranging from 18 to 145 are found in vertebrates, invertebrates, and plants. Defensins were discovered for the first time in 1980, defensins are divided into three major groups: alpha, beta, and theta, each of them have their genetic symbol and produced by different cells (Esfandiyari *et al*.,2019). The natural nature of cell membrane of human ensures that defensins interact selectively with host pathogens. When defensins react to membranes, they accumulate in bacteria membranes and produce depolarization, which guide to pathogen death (Mattar *et al*., 2016; Falanga *et al*.,2017). In immature individuals, defensins (present in breast milk) play a very important role in protecting the disease due to the weak immune system. Antimicrobial effect on Gram-positives is different from Gram-negative bacteria. The reason is that, first, the cell wall structure of Gram-positive bacteria is different from Gram-negatives (the cell wall of Gram-negative bacteria is rich in lipopolysaccharide (LPS), which has a negative charge, while in Gram-positive bacteria the cell wall have teichoic acids (TA) with less negative charge. Secondly, the peptidoglycan layer is thick in Gram-positive bacteria and the formation of pores by defensin on the surface of the Gram-positive cells requires more time. Therefore, defensin has weaker anti-microbial activity in Gram-positive bacteria (Esfandiyari *et al*.,2019)

2.1.1.4. Tamm-Horsfall protein:

THP, also referred to as uromodulin, is a glycoprotein with a large molecular weight that is present in human urine. Both the distal convoluted tubule of the kidney and the thick ascending limb of the Loop of Henle contain it. THP is not inherently antibacterial, but by binding to specific mannosylated residues on uropathogenic bacterial fimbriae, especially type 1 pili, it inhibits bacterial adherence to epithelia. According to Wu *et al*., (2018), THP can also activate dendritic cells through a TLR4-dependent mechanism, which lowers the activation threshold for uropathogenic bacteria coupled to THP.

2.2. Adaptive immune:

Whereas the urinary tract's wide-ranging innate immune responses are highly responsive to infections, adaptive immune responses, especially in the bladder, tend to be limited. UTIs that progress to the kidneys can result in the production of antibodies specific for the infecting agent, but patients with infections limited to the bladder fail to elicit an antibody response for unknown reasons. This apparent defect in the bladder's antibody response could be a major reason for the high recurrence of UTIs (Martell, 2020). The adaptive immune system is capable of recognizing almost any antigenic structure; however, because antigen receptors are generated at random, they bind to antigens of any origin — bacterial, environmental, or self. In contrast, innate immune receptors are specific for structures found only in microbial pathogens (pathogen-associated molecular patterns), which is why they function to signal the presence of infection. The signals produced by the innate immune system, in turn, control the activation of adaptive immune responses; the adaptive immune system responds to a pathogen only after the innate immune system has recognized it (Medzhitov and Janeway,2000).

2.3. Infection of urinary tract:

Urinary tract one of the most common frequented locations of bacterial infection caused by bacterial genus that colonize and invade one part of the urinary tract (Gupta *et al*.,2017).

Urinary tract infections are among the most bacterial infection disease in human which classified to community-acquired infections and health-related infection, consequently, when there was infection in this site of the body the urine did not become sterile (Smelov *et al*.,2016). UTI is ascending infection, when lower UTI (pathogenic bacteria grow in the urethra and colonize Urethritis) was left untreated this infection could improve and reaching the upper urinary tract. Clinically, UTI are considerably suspected with symptoms, it may caused pain, urgency, voiding frequency. It is worth mentioning that could develop urinary symptoms without positive culture of urine (Ferry *et al*.,2007).

Anatomically there are two types of UTI ,Lower UTI- this infection occurs in the bladder (cystitis) or urethra (urethritis) (Hickling *et al*.,2017) . Cystitis begins to develop when uropathogens come up by urethra to the bladder from periurethral mucosa or intestine and start infection in bladder. Cystitis occurs without complications in healthy people who do not get pregnant and do not have latent flaws in the urinary tract apparatus such as catheters or other dysfunction. Upper urinary tract infection is found in the upper urinary tract, also known as pyelonephritis; a disease far more serious than lower UTI and frequently results in sepsis (Urosepsis) (Sobel and Kaya.,2014). Pyelonephritis is brought on by a UTI that spreads from the bladder to the kidneys and their collecting systems, and it is characterized by an infection of one or both of the

renal parenchyma and renal pelvis, resulting in localized flank or back pain as well as systemic symptoms such as fever, chills, and nausea (Morello *et al.*, 2016; McCance *et al*., 2018). By their complexity, urinary tract infection can classification into complicated and un complicated (Hooton,2012). Patients with uncomplicated UTI have a normal, unobstructed genitourinary tract, no history of recent instrumentation, and symptoms confined to the lower urinary tract. Uncomplicated UTIs are most common in sexually active young women. Patients typically complain of dysuria, urinary frequency, urinary urgency, and/or suprapubic pain. Upper urinary tract involvement is indicated by fever or costovertebral angle tenderness (Mehnert-Kay and S.A, 2005). Complicated urinary tract infection referred is an infection that develop in a patients who has a functional or structural abnormalities in urinary tract, , obstructing urine flow, in a host with weakened defenses, or in people with metabolic diseases such as diabetes or azotemia, individuals who are not healthy due to an illness or immunosuppression, are on long-term catheterization, or have had a kidney transplant (Kalra and Raizada 2009; Flores-Mireles *et al*., 2015).

2.4. Causative agents of urinary tract infections

Because of the urethra's near proximity to the gut, uropathogenic *Escherichia coli* (UPEC) colonization is widespread, especially in catheterized patients, despite the general belief that urine is sterile and free of bacteria. Ascending migration via the ureter can harm the kidneys and disseminate hematogones by triggering inflammation and protease release. According to Foxman (2014) and Flores-Mireles *et al*., (2015), *E. coli* bacteria are to blame for more than 80% of instances of urinary tract infections. Due to its virulence characteristics, which allow it to adhere to and colonize in the urine system and kidneys, *E. coli* is the most prevalent bacterium to infect the urinary system. P-

fimbriae, a sticky molecule present in this type of bacteria, is one of these elements and aids in the binding of the bacteria to its target. Infection with *E. coli* in the kidneys can lead to initial inflammatory response as well as scarring of the renal parenchyma. Although the exact process causing renal scarring is unknown, it is believed that when bacteria attach to renal cells, the protective barriers are disrupted, leading to scarring. As a result, there was an attempt to manage the infection, which led to localized infection, hypoxia, ischemia, and coagulation. Inflammatory cytokines, bacterial toxins, and other reactive processes induce complete pyelonephritis and, in many status , systemic indications of sepsis and shock (Morello *et al*.,2016; Behzadi *et al*.,2023). The other uropathogens produce virulence factors which enhance adhesion (e.g., pili, adhesins), nutrition release (toxins), immune avoidance (capsules, etc.), and iron gaining (e.g., siderophores), which result in UTIs , are *Klebsiella pneumoniae* (about 7%), *Proteus mirabilis* (about 5%) *Enterococcus faecalis* (about 2%), *Staphylococcus saprophyticus*, group *B Streptococcus* (GBS), *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida* spp. caused by bacteria, viruses, or fungi are an uncommon occurrence (Kline *et al*., 2011; Parish and Holliday, 2012; Palou *et al*., 2013).

2.5. Pathogenesis of urinary tract infection:

Urinary tract infection occur in two pathway: ascending and hematogenous, bacteria can enter and spread via urinary . blood –borne pathogens can cause UTIs in patients with bacteremia or endocarditis, Although it is less common than ascending route. Kidney is commonly site of abscesses which caused by gram positive bacteria such as *S. aureus* (Vasudevan, 2014). While in the scending route UPEC can causes the infection in urinary tract infection when it reach to the bladder lumen and adhere to the surface of superficial umbrella cells by using their sticky filament called type 1 pili such as

E.coli . After attachment, bacteria inter to the epithelial cells, where they multiply within this protected intracellular niche to form large biofilm like intracellular bacterial communities (IBCs). Here bacteria can evade Toll-like receptor 4(TLR4)-mediated bacteria can evade ejection and replicate in the urothelial cell cytoplasm before filamentation and re-entry into the bladder lumen and adhering to nearby cells to causes infections (Klein and Hultgren,2020).). This bacteria infects the urinary system is due to its possession of many virulence factors that enable it to adhere (fimbriae), defend against immune cells (endotoxins), (flagella) which use by bacteria to travel through the urethral canal , feed (by iron receptors), and sidestep the immune system through (capsule). uropathogenic bacteria works by damage urinary tract defenses and preventing neutrophils by forming biofilms. Biofilms assist in the spread and colonization of the urethra and bladder by sacrificing the outer cells to protect the inner core (Flores-Mireles *et al*., 2015). UPEC can avoid TLR4-mediated expulsion, modify lysosomes to decrease their digesting ability , and stay within the autophagosome membrane to evade phagocytosis when released intracellularly (Abraham and Miao, 2015). In previous studies *E. coli* was the most common bacteria that cause urinary tract infection and followed by other species. *E.coli* constitutes (34.5%), *Klebsiella* spp. (18.2%), *Staphylococci* (20.0%), *Pseudomonas* (9.1%), and various Gram negative Bacilli (18.2%) in a study conducted by (Akter *et al*.,2013) and reported that *E. coli*, *Klebsiella*, *Pseudomonas* and *Staphylococci* were the most common organisms associated with UTI for both community acquired and hospitalized cases. In a local study conducted in Karbala find that *E. coli* (60%) was the most prevalence bacteria that identified in urinary tract infections patients, followed by *Klebsiella pneumonia* (12%) , *Pseudomonas*

aeruginosa (10%) , *Proteus mirabilis* (8%) , *Enterococcus faecalis* (6%) (Tamani et al.,2021)

2.6. Antibiotic resistance:

antibiotic resistance (ABR) is a natural part of the evolutionary process. In situations where antibiotics are prevalent and hence a significant selection pressure, mutations that result in resistance give a competitive advantage to the bacteria carrying it and thus preferred in natural selection. The widespread use of antibiotics in hospitals and communities, as well as in agriculture, has expedited this process to the point where ABR has arisen as a worldwide health hazard. Antibiotics resistance is not only causing difficult-to-treat diseases with significant mortality, but it is also important advances in contemporary medicine, such as major surgery and cancer treatment, as well as having social and economic consequences (Laxminarayan *et al.*,2013). Resistance spreads across bacteria, humans, and areas through the transmission of resistance genes between bacteria (thought plasmids and transposons),inadequate sanitation and hygiene in hospitals and communities, and worldwide travel, commerce, and migration (Sjölund *et al*., 2008). Bacteria from the Enterobacteriaceae family have emerged as significant cause of UTI, developing extended-spectrum- lactamases (ESBL)-enzymes that provide resistance to practically all lactam antibiotics (such as penicillin and cephalosporins) except carbapenems. They have now expanded, including resistance to co-trimoxazole, aminoglycosides, and ciprofloxacin, all of which are key antibiotics in the empirical therapy of UTI (Pitout and Laupland ,2008). The Delhi metallo-lactamase 1(NDM-1) gene was found in *E-coli* and *K. Pneumonia*, and it was coupled with plasmids encoding a large number of genes

causing resistance to almost all antibiotics (Nordmann *et al*.,2011). The selection of resistance genes and the spread of germs with ABR is mostly a local process, with particular hospital and community behaviors having a crucial influence. Resistance genes may be transmitted to bacterial strains capable of causing illnesses other than the original. Successful genes may subsequently be picked and transmitted to other hosts, where they may be amplified and established as major resistance genes, especially if antibiotic selection pressure persists. They can then spread almost everywhere thought the world's interconnected commensal, environmental, and pathogenic bacterial communities for example, sulfonamide resistance has been identified everywhere over the world encoded by only two resistance genes(Van Boeckel *et al*.,2014).

2.7. Medical plants:

The unrestricted use of antibiotics and the occurrence of various antibiotic resistances in human pathogens represented significant health problems and are one of the most significant causes of morbidity and mortality globally, which contributes to the search for new drugs from natural sources, and it is critical to search for fresh agents that are better and can be used against resistance pathogens without side effects (Kahkashan *et al*.,2012).A wide variety of plant/natural products are used in the treatment of various infections . In traditional medicine, herbal products were used to treat various infectious diseases. Approximately 80% of the global population used herbal products for primary health care (Mehrotra *et al*.,2010). Plants have secondary metabolites (alkaloids, resins, steroids, tannis, oleosins, and terpenes, for example) that have biological properties, and drugs have been developed to treat diseases based on these properties. As a result, new compounds with antimicrobial potential must be discovered and developed on a regular basis, as well as the search for

medicinal plants with novel action mechanisms to treat infectious diseases (Egwaikhaid *et al*., 2009).

2.7.1.*Acacia senegal*:

A natural polysaccharide compound derived from the secretions of Asenegal and A.seval trees,it is a complex of glycoprotein and polysaccharide , called galactans composed of D-galactose units and side chain of D-glucoronic acide with L-rhamnose or L-arabinose terminal, it is about 200-600 Kda in molecular weight (Montenegro *et al* , 2012). The term gum has been used because the substance has gummy characteristics as shown in figure (2-4).

Figure 2-4 pre- and post-harvest Acacia senegal (Alarifi,2017)

It is the source of arabinose and ribose sugars, so it has been used as a stabilizer in the food industry and the techniques of encapsulation not just in food preparation, yet also in the medical cosmetic and pharmaceutical industries (A Mohamed *et al*,2014). It is one of the hydrocolloids in the food sector that is most frequently utilized. Arabic gum functions in foods and cosmetics that have oil-water interfaces as a potent antifungal and long-term stabilizer. It is a naturally occurring substance made of a hydrophilic carbohydrate component that

prevents the flocculation and coalescence of molecules and the voiding in food additives and a hydrophobic protein component that is adsorbed on the surface of oil droplets. (Lelon *et al*., 2010). Gum Arabic is known by many researchers that consists mainly of three parts (Dror *et al*., 2006). The main part is a highly branched polysaccharide and consists of a galactose backbone with associated branches of arabose and rhamnose, which ends with gluuronic acid found in nature as its magnesium, potassium and calcium salt. The second, smaller fragment is a higher molecular weight arabinogalactan protein complex in which the arabinogalactan chains are covalently attached to a protein chain through serine and hydroxyproline groups. The attached arabino-galactanin complex contains glucuronic acid. The third smallest fraction with the highest protein content is a glycoprotein that differs in its amino acid composition. Many studies have reported many favorable kidney effects such as a reduced concentration of plasma phosphate, blood pressure, proteinuria and additional effects on the kidneys such as slowing the transport of intestinal glucose which can be of benefit for obesity and diabetes prevention and treatment(Nasir *et al* , 2012 ; Nasir,2013 ; Rashed *et al*.,2018).

2.7.1.1. Antibacterial effects of Arabic gum:

Infectious infections are a leading source of morbidity and mortality in the general population, particularly in the poor countries. Nosocomial and community-acquired illnesses are influenced by microorganisms' capacity to produce and disseminate antibiotic resistance. Finding new antimicrobials has become necessary due to the development of antibiotic resistance in already recognized microbes (Mattana *et al*., 2012). According to Seigler (2003), Alkaloids, cynogenic glycosides, fluoroacetate, gums, and terpenes are just a few of the secondary metabolites that are thought to be abundant in the various

components of Arabic gum. *S. aureus, S. epidermidis, St. pneumonia, P. aeruginosa, Proteus merabilis, Acinetobacter, Enterobacter, Klebsiella pneumonia, Serratia s*pp., *E. coli, Salmonella typhi,* and *C. albican* were the twelve Gram positive and Gram negative bacteria tested for antimicrobial activity by Acacia seyal as a prebiotic extract (Bnuyan *et al.*,2015). Gram-positive bacteria have more complex cell walls that act as barriers, making them more resistant to antibacterial agents than Gram-negative bacteria, which can affect the effectiveness of the Arabic gum solution. The concentration of the Arabic gum solution also increases the inhibition zone of *E. coli and* other bacteria. (Al-Behadily *et al* ., 2020). Arabic gum has been shown to reduce urinary nitrogen excretion by increasing urea disposal in the cecum and lowers serum urea concentration in normal rat and human (Almajed *et al*., 2002). It is found that Ga has potential effect on reducing serum urea level due to high excretion of bacterial nitrogen in feces in patients with chronic renal failure. Another evidence has proved that consumption of 50g/day of Gum Arabic in patients undergoing regular hemodialysis will affect positively on their biochemical and blood pressure profiles, Arabic gum has bifidogenic potential in healthy humans. It also has immune-stimulant effect, anti inflammatory effect and decreasing pro-inflammatory cytokines (Al-Alawi *et al*.,2018).

2.7.2.*Boswellia serrata* frankincense

Frankincense, also known as olibanum or al-luban, is an aromatic resin as shown in figure (2-5) isolated from the shrub-like of the genus Boswellia in the family Burseraceae. While there are a large number of members of the Boswellia genus, the majority of frankincense has been isolated from *Boswellia carterii* in East Africa and China, It has been widely used as an herbal medicine in the

medical traditions of the Arabian Peninsula, India, China, and Northeast Africa in the treatment of both acute and chronic conditions. Frankincense consists of three main components: essential oils, an ethanol-soluble gum and water-soluble polymeric fraction. Although the specific composition of frankincense varies species depending on species growth condition ,and harvesting condition, in general the essential oils comprise 5-9% of frankincense, while 65-85% are an ethanol soluble gum, with the remainder being water –soluble polymeric compounds (Hosain *et al*.,2019). Studies conducted prior to this study have proven that the frankincense have significant antimicrobial activity against several species of bacteria that causes disease such as *E.coli*, *S.aureus*, and *K. Pneumonia* (Ismail *et al*.,2014).

Figure 2-5: Boswellia serrata tree frankincense

Chapter three

Materials and Methods

Chapter Three: Materials and methods

3.1. Materials:

3.1.1.apparatuses and instruments

Table 3-1:Apparatus and instrument used in the study

Apparatus and instrument	Company name (origin)
Autocleave	Hirayama (Japan)
Plane tube	Afco-dispo (Jordan)
Eppendorf tubes	Sartorius (Germany)
Centrifuge	Hettich (Germany)
Loop	Writeg (Germany)
Tips	Jippo (Japan)
Micropippte	Shcheer (Germany)
Rack tube	Bioneer (Korea)
Vitek compact	Biomatrix (Germany)
ELISA washer and incubator	Human COMBI wash (Germany)
ELISA reader	Human humareder HS (Germany)
Microscope	Olympus (Japan)
Petri dishes	PLASTILAB (Lebanon)
Microscope slides	Supertek (India)
Cover slides	Sail Brand (China)
Refrigerator	Kenwood (Japan)
Urine container	Firatmer (Turkey)
Urinalysis reagent strips	Acon (USA)

Incubator	Gallenhamp (England)
Sensitive balance	Denver (Germany)
Medical cotton	HAD (china)
Latex gloves	Broche (Malaysia)
Biological Safety Hood	Labconco (USA)
Cylinders	HBG (Germany)
Water bath	Tafesa (Germany)
Water distilling	GFL (Germany)
Vortex mixture	Stuart (UK)

3.1.2.Chemical and biological materials:

Table 3-2 :Chemical and biological materials used in the study

No.	chemicals	Company/ origin
1	Glycerol	Panreak/ Spain
2	Alcohol (ethanol) 70%	Aljoud /Iraq
3	Gram stain kit	Himedia/ India
4	Hydrogen peroxide (H_2O_2)	Himedia /India
5	Methyl red dye	BDH / England
6	KOH solution	Himedia / India
7	Alpha naphthol	BDH/USA
8	Tetra-methyl–ρ-paraphenylene diamine	Sigma / Germany

	dihydrochloride	

3.1.3.Culture media

Table 3-3:Culture media used in this study

No.	Culture media	Company	Origin
1	Nutrient agar	Oxoid	U.K
2	Nutrient broth	Hi-media	India
3	Blood agar	Oxoid	U.K
4	MacConkey agar	Oxoid	U.K
5	Muller Hinton agar	Oxoid	U.K
6	Urea agar	Himedia	India
7	Cetrimide agar	Himedia	India

3.1.4.Kits:

Table 3-4:Kits used in the study:

Kit	Company	Origin
Human LTF /LF (lactoferrin) ELISA kit	Elabscience	USA
Human LL-37(Antibacterial Protein LL-37) ELISA Kit	Elabscience	USA

3.2. Method:

3.2.1.Collection of urine samples

Fifty three samples collected from patients with urinary tract infections and visitors to Al-Hussein Teaching Hospital and outpatient clinic,and 35 samples were collected from control group (People who are considered healthy from UTIs) ranging in age from 3 to 69 years, of both sexes, male and female for the period of time from May 2022 to July 2022. Depending on the medical diagnosis by the specialist doctors, a special form was made for each patient that included some important information According to the questionnaire sheet in Appendix No. 1., which included the patient's name, age, gender, residence, chronic diseases (Diabetes and blood pressure) . A sterile containers with a capacity of 10 ml were used for collecting a midstream urine samples from the patients and control groups. In the laboratory, each urine sample was divided into two parts in the lab; the first part was centrifuged and placed in a deep freezer ar 80 C, and the second part was immediately inoculated on general and selective culture media MacConkey and Blood agar and incubated aerobically at 37 °C for 24-48 hours using an incubator. The remainder of the urine (5ml) was centrifuged to make direct microscopic examination for pus cells, RBCs, epithelial cell count, casts, crystals, and parasite infection, if exist. Normal urine sediment may contain a few counts of RBCs, pus cells, and epithelial cells.

3.2.1.1. Control group

urine samples were collected from 35 healthy individuals without any chronic and acute infection and immunological diseases after the physical and clinical examination people were used as a control group

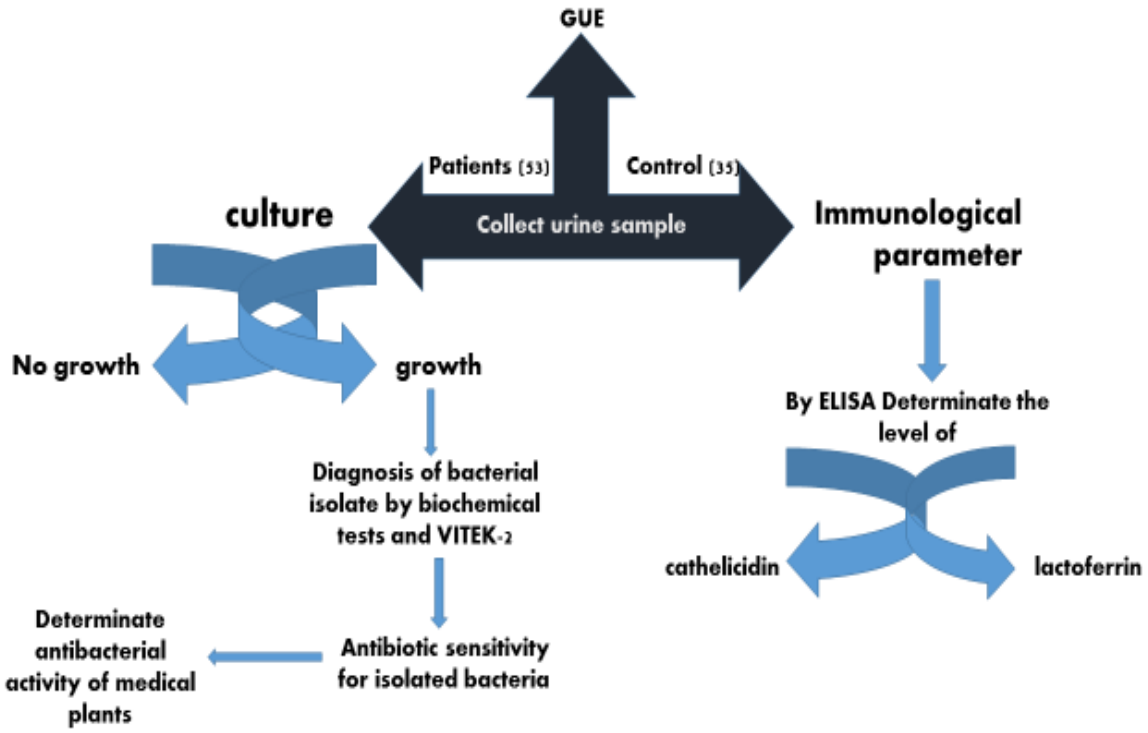
3.2.1.2. Ethical Approval:

Samples were collected from adults after their consent to use the samples for the purpose scientific research in this study, which was conducted according to the official instructions for the approval of a committee research ethics.

3.2.1.3. Exclusion Criteria:

All patients with one of the chronic diseases (such as high blood pressure and diabetes) ,autoimmune diseases and patients who receive antibiotic treatment were excluded from this study.

3.2.2.Study design



3.2.3.Preparation of Reagents and solution:

3.2.3.1. MacFarland solution 0.5

A ready-made McFarland solution (0.5) was obtained from the Public Health Laboratory at the Holy Karbala health department and this solution was used to arrive at an approximate number of bacterial cells estimated (0.5X 10) one cell per milliliter (Esther and Fatima,2020).

3.2.3.2. Catalase reagent

the reagent was prepared by mixing 3 mL of hydrogen peroxide H2O2 in 100 ml of distilled water and using it to detect the ability of bacteria to produce catalase enzyme (Tille,2015).

3.2.3.3. Gram stain

This ready-made dye consisting of (crystal violet, iodine solution and sufranine) was used to study the phenotypic properties of isolated bacteria and for the purpose of differentiating bacteria into Gram-negative or positive dye.

3.2.3.4. Methyl red reagent

Prepared by dissolving 0.1 of methyl red dye in 300 ml of 95% ethyl alcohol, then the volume was completed to 500 ml using distilled water and was used to detect the total decomposition of glucose sugar (MacFaddin,2010)

3.2.3.5. Vogue's- Proskauer reagent

It consists of two solutions:

- Solution (A) KOH: It was Prepared by dissolving 40 g of the KOH in 90 ml of distilled water and then complete the volume to 100 ml

- Solution (B) Alpha-naphthol: It was Prepared by dissolving 5 g of the Alpha-naphthol in 90 ml of ethyl alcohol at a concentration of 99% and then complete the volume to 100 ml using the same alcohol.

This reagent was used to investigate the susceptibility of bacteria to the fermentation of glucose sugar and produced Acetyl Methyl Carbinol in Voges-Proskauer test (MacFaddin,2000)

3.2.3.6. Oxidase reagent

It was prepared freshly by dissolving 1 g of tetraphenylene para Amine dihydrochloride in 100 ml of distilled water in clean, dark, sterile vial . it used to investigate the ability of bacteria to produce the oxidase enzyme (Tille,2015).

3.2.3.7. Normal saline solution:

To prepare normal saline, 0.85 grams of sodium chloride were taken and dissolved in 90 ml of deionized water. After that, it was sterilized in an autoclave at 121 °C for 15 minutes and used to prepare a bacterial suspension (Tille,2015).

3.2.4.Preparation of Culture Media:

All solution culture and diagnostic media were prepared according to the manufacture instructions and were sterilized by using autocleave at 121C for 15 minutes, after adjusting the ph for (2-7). The solutions that are destroyed by heat were sterilized using 0.22 Mm Millipore filter paper.

Nutrient agar medium:

It was Prepared by dissolving 28 g of the media in 1 liter of distilled water, then outoclaved and left to cool to 45-50 C and poured into petri-dishes and then kept in refrigerator until use. This media is used for conserving of bacterial isolates (MacFaddin,2000).

3.2.4.1. Blood Agar Medium:

Fourty gram of the medium powder was dissolved in a liter of distilled water, then it was sterilized in the autocleave and let it cool 40- 45C. then 5% of human blood was added, this enrichment medium was used to isolate bacteria

and to detect the ability of bacteria to hemolysis red blood cell (Niederstebruch et al.,2017).

3.2.4.2. Cetrimide agar:

Cetrimide agar was prepared by dissolving of 46.7 g of the medium in a liter of distilled water, 10 milliliters of glycerol were added to it, then autoclaved, and left to cool to 45-50 C and poured into sterile dishes, used as a selective medium to isolate and diagnose *Pseudomonas aeruginosa* bacteria (McFadden, 2000).

3.2.4.3. Urea agar

This medium was prepared by dissolving 24 g of basic urea agar in a liter of distilled water and autoclaved, then left to 45C and then 50 ml of filter sterile urea solution (40%) were added and poured into sterile tubes. This medium was used to detect the susceptibility of bacteria to produce the urease enzyme, which decomposes urea into ammonia and carbon dioxide (McFadden, 2000).

3.2.4.4. MacConkey agar medium:

It was prepared by dissolving 51.5 g of the medium in 1 liter of distilled water, boiling it until completely dissolved, autoclaving it, and left it to cool to 40-45C then pouring it into Petri dishes. It was used to isolate gram negative bacteria and in differenation of lactose fermenters from non-lactose fermenters gram-negative bacteria (McFadden,2000).

3.2.4.5. Nutrient broth:

It was prepared by dissolving 13 g of media in 1 liter distilled water, poured into tubes(5 ml/tube), then autoclaved ,left cool down and kept in refrigerator until use. the purpose of used this media was activation of bacteria (MacFaddin,2000).

3.2.4.6. Muller Hinton agar:

It was prepared by dissolving 38 g of the medium in 1 liter of distilled water, boiling it until completely dissolved, and autoclaving it, then pouring into Petri dishes. It was used to test the biological activity of bacteria isolates (Uwizeyimana *et al*.,2020)

3.2.4.7. Motility medium:

It was prepared by adding 0.5 g of agar into 100 ml of Nutrient broth , pour into tubes (5 ml/tube), autoclave and left to solidifies vertically . This medium used for detection of bacterial mobility.

3.2.5.Identification the isolated bacteria:

3.2.5.1. Morphologic and microscopic identification:

The properties of bacterial isolated had been studied by observing the shape, texture, and size of bacterial colonies . A single pure colonies were picked up and stained with Gram stain for microscopic examination to observed the shape, length under oil immersion and distinguish between Gram positive and Gram negative bacteria (Collee *et al*., 2006).

3.2.5.2. Biochemical tests

3.2.5.2.1 Catalase test

This test was performed by transforming a bacterial colony aged 18-24 to a glass slide and then a drop of 3% hydrogen peroxide reagent was placed on top of it. The appearance of gaseous bubbles an indication of a positive test and bacteria have the ability to produce the enzyme catalase, which decomposes the H_2O_2 reagent into oxygen and water (Reiner,2010).

3.2.5.2.2 Oxidase test :

Filter paper was saturated with oxidase reagent. A bacterial colony was transferred by a wooden stick and rubbed on the filter paper .The development of violate color within 10-20 seconds indicates that the isolate was able to produce oxidase enzyme (Tille,2014).

3.2.5.2.3 Indole test:

A fresh testing bacterial culture were administered into peptone water and incubated for 24-48 hours at 37°C. A few drops of Kovacs reagent were added to each tube and gently shaken. The formation of a pink ring indicated a positive result(MacFadden 2000).

3.2.5.2.4 Coagulase test

This test was performed by adding 0.5 ml of human blood plasma in sterile tubes. A fresh bacterial colony 18-24 hours was transferred by a sterile wooden stick to the tubes containing the plasma, and after mixing them well, incubated at 37 C temperature for 4 hours, plasma coagulation is evidence of a positive test (Becker *et al*., 2014).

3.2.5.2.5 Methyl red test

The tube of MR-VP liquid medium was inoculated with a tested bacteria , after incubated for 24 hours at 37 C, 5 drops of methyl red reagent were added. The positive test is a bright red that indicate the bacteria have the ability to ferment glucose sugar and produce acids (MacFadden 2000).

3.2.5.2.6 Voges –Proskauer test

The tube of MR-VP liquid medium was inoculated with the bacteria and incubated for 24 hours at 37 C ,then 0.6 ml of alpha-naphthol reagent solution

and 0.2 ml of potassium hydroxide solution were added and mixed together in the test tube . The transformation of the color of the medium from yellow to pink is evidence of a positive test (MacFadden, 2000).

3.2.5.2.7 Simmons citrate test:

The surface of Simmons citrate slant was inoculated with a fresh bacterial culture, and the medium was incubated at 37 °C for 48-72 hours. The appearance of a blue color indicate the positive result (MacFadden,2000)

3.2.5.2.8 Urease test:

After being injected onto the urea agar slant, the bacteria were left to grow for 48 hours at 37 C, after 24 hours, the appearance of pink color slant indicate, the urease test was positive (Collee et al.,2006)

3.2.5.2.9 Hemolysis production test:

The bacteria were cultured on the blood agar by the planning method and incubated for 24 hours at 37 C in aerobic conditions to assess an isolated bacterial capcity to hemolyze red blood cells and to determine the type of hemolysin present around the bacteria colonies (Collee *et al*.,2006).

3.2.6. Identification of bacteria Using VITEK-2 Compact System

Bacterial isolates were biochemically diagnosed according to the results report of the VITEK 2 Compact automated system according to the manufacturer's instructions (Biomatrix Company / France) . The bacterial suspension was prepared by transferring a quantity of bacterial colonies that has been purified advance age (18-24) hours to 3 ml of sterile saline sodium chloride solution at a concentration of (0.45%), and the turbidity was adjusted using 0.5 McFarland tube for Gram-negative bacteria and 0.63,McFarland tube for Gram-positive bacteria, as negative bacteria are diagnosed and used Gram-staining (Gram Negative Identifier) (GN-ID) and Gram-positive bacteria Gram Positive Identifier

(GP-ID) is a completely enclosed system and no addition is required any auto reagents. The card is placed on a cassette designed for use with the VITEK-2 system, Place it in the machine, fill it automatically in a vacuum chamber, seal it, and incubate it at 35.5.°C, and is automatically subjected to a colorimetric measurement (with a new reading) every 15 minutes for a maximum incubation period of 8 hours. Data were analyzed using the VITEK-2 database, which allows identification of the organism actively starting 180 min after incubation initiation (Karagöz et al., 2015).

3.2.7.Preservation of Bacterial Isolate:

3.2.7.1. Short term storage:

The diagnosed bacterial isolates were kept in the nutrient medium after being inoculated with bacterial isolates by stabbing method and incubated at 37 C for 18 hours, then transferred to the refrigerator at 4 C and the preservation process was repeated in order to maintain the vitality of the isolates and avoid contamination every 3-4 weeks (Zhung *et al*.,2020).

3.2.7.2. Long term storage

For the purpose of preserving bacterial isolates for a long time without any damage or change in genetic traits, A sterile brain - heart infusion broth with 15-20% glycerol was used .After inoculation with bacterial isolates, incubated for 4 hours, then sealed tightly with a parafilm and kept at a temperature of -20 for (4-6) months (Gulliksson,2003).

3.2.8.Measurement of the lactoferrin and cathelicidin levels in the urine of the studied groups:

3.2.8.1. Test principle:

This ELISA kit uses the sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human LTF/ LL-37. Samples (or standards) are added to the micro ELISA plate wells and combined with specific antibody. Then a biotinylated detection antibody specific for Human LTF/ LL-37 and a Avidin-Horseradish Peroxides (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human LTF / LL-37 , biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2nm. The concentration of LTF/LL-37 in the samples by comparing absorbance the OD sample to the standard curve.

3.2.8.2. ELISA Kit component of LTF/ LL-37:

Table 3-5: ELISA Kit component

Items	Specifications
Micro ELISA plate (dismountable)	8 wells ×12 strips
Reference standard	2 vials
Concentrated biotinylated	1 vial, 120 Ml

detection Ab (100x)	
Concentrated HRP conjugate (100x)	1 vial, 120 Ml
Reference standard& sample diluent	1 vial, 20 Ml
Biotinylated detection Ab diluent	1 vial, 14 Ml
HRP conjugate diluent	1 vial, 14 Ml
Concentrated wash buffer (25x)	1 vial, 30 Ml
Substrate reagent	1 vial, 10 Ml
Stop solution	1 vial, 10 Ml
Plate sealer	5 pieces
Product description	1 copy
Certificate of analysis	1 copy

3.2.8.3. Reagent preparation of LTF/LL-37:

Reagent were prepared according to the manfucturing indtructions.All reagent brought to room temperature (18-25C) before use. if the kit will not be used up in one assay, it was just taken out the necessary strips and reagent for present experiment, and store the remaining strips and reagent at required condition.Wash buffer: 30 ml of concentrated wash buffer was diluted with 720 ml of deionized or distilled water to prepare 750ml of wash buffer., warm it in a

40C water bath and mix it gently until the crystals have completely dissolved. Standard working solution: the standard was centrifuged at 10,000xg for 1 min. add 1 ml of reference standard &sample diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with pipette. This reconstitution produces a working solution of 20 ng/ml (or add 1ml of reference standard &sample diluent, let it stand for 1-2 min and then mix it thoroughly with a vortex meter of low speed. Bubbles generation during vortex could be removed by centrifuging at a relatively low speed. Then make serial dilutions as needed. The recommended dilution gradient is as follow: 20 , 10 , 5 ,2.5 ,1.25 , 0.63 , 0.31 , 0 ng/ml. Biotinylated detection Ab working solution: the required amount was calculated before the experiment (100 ml/well). In preparation, slightly more than calculated should be prepared. Centrifuge the concentrated Biotinylated detection Ab to 1x working solution with biotinylated detection Ab diluent = 1:99).

5- HRP conjugate working solution: the required amount was calculated before the experiment (100ml/well). In preparation, slightly more than calculated should be prepared. Centrifuge the concentrated HRP conjugate at 800 x g for 1 min, then dilute the 100 x concentrated HRP conjugate to 1 x working solution wit HRP conjugate diluent (concentrated HRP conjugate to 1x working solution with HRP conjugate diluent (concentrated HRP conjugate: HRP conjugate diluent = 1:99).

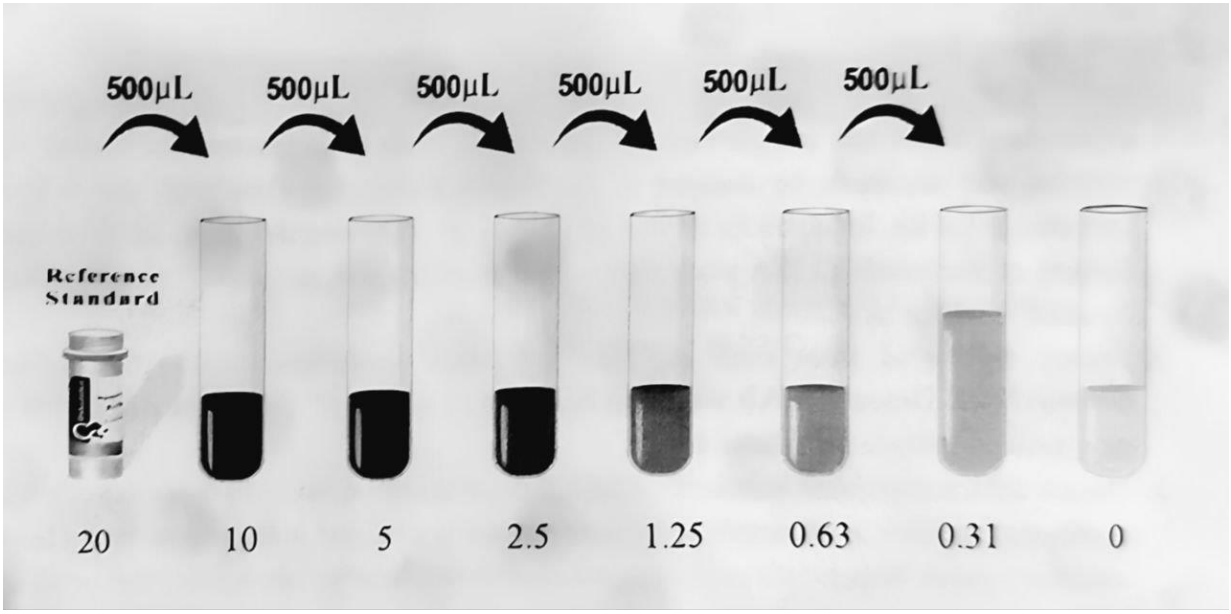


Figure 3-1 standard solution

3.2.8.4. Assay procedure

1- wells for diluted standard, blank and sample were determined . 100ml each dilution of standard, blank and sample was added into appropriate wells (it is recommended that all samples and standards be assayed in duplicate). Cover the plate with sealer provided in the kit. It was incubate for 90 min at 37 C.

2- liquid was decanted from each well, do not wash. Immediately 100 ml of biotinylated detection Ab working solution added to each well. Cover the plate with a new sealer. It was Incubated for 1 hour at 37 C.

3- the solution was decanted from each well, 350ml of wash buffer added to each well. Soak for 1 min and aspirate or decant the solution from each wall and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.

4-100 ml of HRP conjugate working solution was added to each well. Cover the plate with a new sealer. Incubate for 30 min at 37 C.

5- the solution was decanted from each well, the wash process was repeated for 5 times as conducted in step 3.

6- 90 ml of substrate reagent added to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37 C. protect the plate from light

7- 50 ml of stop solution added to each well.

8- The optical density (OD) value was determined of each well at once with micro-plate reader set up 450 nm

3.2.8.5. Calculation of results of LTF:

Average readings are extracted for each standard and samples, then subtract the average zero standard optical density. Plot a four parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis as shown in figure (3-2)

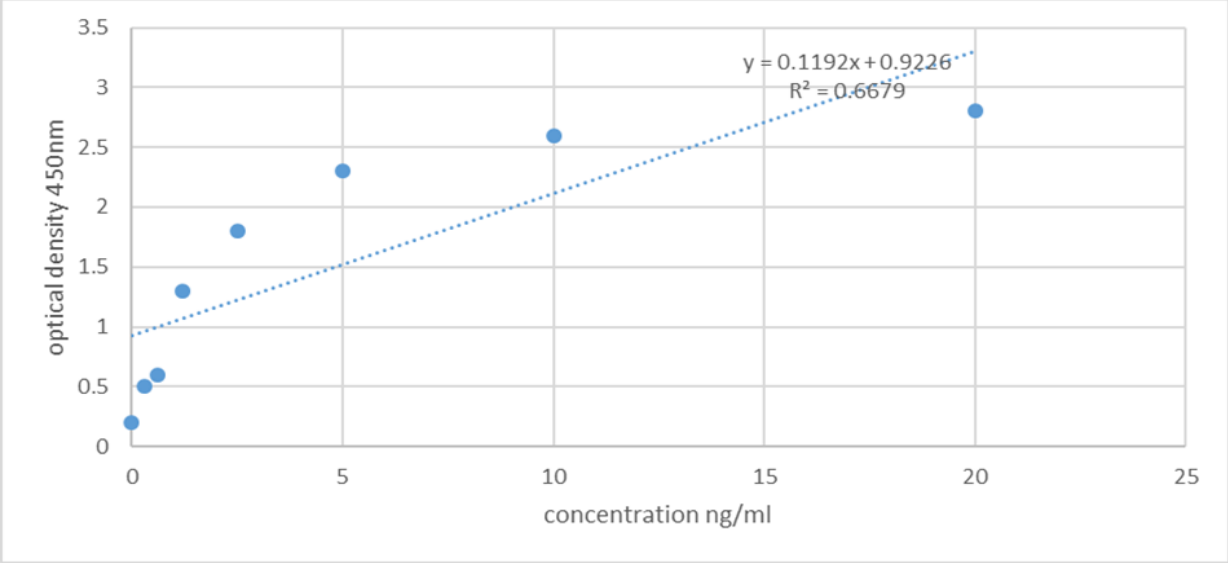


Figure 3-2: Human LTF standard curve

3.2.8.6. Calculation of results of LL-37:

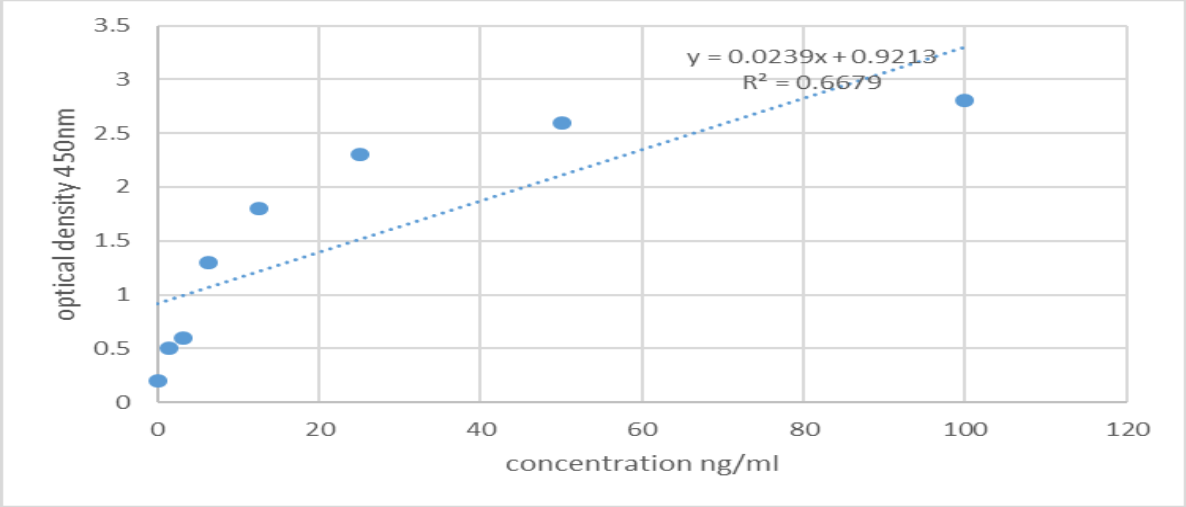


Figure 3-3: Human LL-37 standard curve

3.2.9.Biological activity of medical plants

3.2.9.1. Biological activity of *Acacia Senegal:*

3.2.9.1.1 Preparation of Aqueous solution of *Acacia Senegal* :

The aqueous solution of *Acacia Senegal* was prepared according to (Al-Behadliy *et al*.,2020), an aqueous solution of *Acacia Senegal* was prepared by freshly dissolving 50 g of Yemeni Arabic gum in 100 ml of sterile distilled water to achieve a final concentration of 500 mg/ml. The conical flask was covered and left for 24 hours to complete the melting process before sterilizing the solution via filtration . From this stock solution, the concentrations (400,300,200,100) mg/ml were prepared. The solutions were stored in sterile bottles in refrigerator until using in biological activity .

3.2.9.1.2 Identification of bacterial isolates for biological studying:

Twelve bacterial isolates of *E.coli* and *S. aureus* were selected for determing the antibacterial activity of *Acacia senegal* aqueous solution, which were characterized by being multidrug resistance, three of the patients had high levels antibacterial peptides and the other had low level of antibacterial peptides, as shown in appendix.

3.2.9.1.3 Antibacterial activity of aqueous solution of *Acacia senegal* in vitro:

Agar well diffusion method was used to determin the antibacterial activity of *Acacia senegal* aqueous solution, bacterial suspensions were prepared by diluting the bacteria colonies in normal saline to obtain an turbidity similar to that of the standard McFarland tube (0.5) which is equivalent to (1.5 × 108 CFU\ml) one hundred microleters of bacterial suspension were spread using sterile cotton swab on the surface of Muller- Hinton agar. The plates were set for 15 min to absorb the bacterial suspension. Aseptically six holes were made in culture Muller-Hinton agar by cork borer, then 0.5 ml from each prepared dilutions (100-500 mg/ ml) of AG aqueous solution was placed in each hole by using micropipette with added distilled water . only to one hole to be as negative control used Ciprofloxacin disk (5 mg./0.1ml) as positive control.

3.2.9.2. Antibacterial activity of *Acacia senegal* aqueous solution on the ability of bacteria isolate to form a bio film:

The ability of the bacterial isolates (*E.coli* and *S. aureus)* for biofilm formation was investigated by tube method:

3.2.9.2.1 Tube method:

This method described by (Mathur *et al*.,2006; Bose *et al* .,2009;Coffey and Anderson., 2014) to investigate the ability of bacterial isolates to form a biofilm in a qualitative manner:The heart brain infusion broth medium was prepared and distributed in test tubes of 5 mm for each tube. The tubes were then inoculated with young bacterial isolates and then incubated at a temperature of 37 °C for 24 hours. After that, the culture media was poured out, and the tubes were washed 3 times with saline phosphate buffer and left to dry. The tubes were filled with crystal violet dye and left for ten minutes, then washed with distilled water free of ions, and the tubes were inverted to dry. The appearance of a violet layer on the inner walls and at the bottom of the tubes is an indication of the bacteria's ability to form a biofilm. The intensity of the color formed on the tube walls determines the bacteria's ability to form a biofilm compared to empty tubes as a negative control. These tubes were considered as a positive control. Steps 1-4 were completely repeated after adding the aqueous solution of *Acacia senegal* to the tubes prepared in paragraph (1) and according to the concentrations (100,200,300,400 mg/ml) of each tube before inoculating it with the bacterial suspension.

3.2.9.3. Preparation of Aqueous solution of *Boswellia Carterii*:

In this study, the *Boswellia Carterii* was used to determine the biological activity of its aqueous solution against pathogenic bacteria and to demonstrate its in *vitro* protective use. According to (Patel and Patel 2015), an aqueous solution of Boswellia Serrata was prepared by freshly dissolved 50 g of dried *Boswellia Carterii* in 100 ml of sterile distilled water to achieve a final concentration of 500 mg/ml. The Conical flask was covered and left for 24 hours to complete the melting process before sterilizing the solution via filtration. From this stock

solution, other concentrations (500,400,300,200,100) mg/ml were prepared. The solution was stored in sterile bottles and refrigerated until further testing for antibacterial activity.

3.2.9.4. Antibacterial activity of Aqueous solution of *Boswellia Carterii*:

Agar well diffusion method was used to determined the antibacterial activity of *Boswellia Carterii* aqueous solution , bacterial suspensions were prepared by diluting the bacteria colonies in normal saline to obtain an turbidity similar to that of the standard McFarland tube (0.5) which is equivalent to (1.5 × 108 CFU\ml). 0.1 ml of bacterial suspension were spread by sterile cotton swab on the surface of Muller- Hinton agar for 15 minutes to absorb the bacterial suspension. Six holes were made in culture Muller-Hinton agar by corky bore, then 0.1 ml from each prepared dilutions (100-500 mg/ ml) of *Boswellia Carterii* aqueous solution was placed in each hole by using micropipette with added D.W. only to one hole to be as negative control and added 0.1 ml of Ciprofloxacin disk (5 mg./0.1ml) as positive control.

3.2.10. Statistical Analysis

Data of studied specimens were entered and analyzed using the statistical package for social sciences (S.P.S.S.) version 25. The outcomes were expressed as mean ± Standard deviation (Mean ±S.D.). Statistical analysis for the significance of differences of the quantitative data was conducted by using independent-sample T test.

The following statistical tests were used:

1- The square-Chi: test to evaluate the association between any two categorical variables, provided that the number of cells is less of 20% is expected to be less than 5.

2- One way analysis of variance (ANOVA) to evaluate the difference in the mean of numerical variables between more than two groups, provided that these numerical variables are normally distributed, and one way ANOVA was followed by the LSD post hoc test to assess individual differences in values The mean between any two groups among the groups that were primarily tested using One way ANOVA.
3- Fisher exact test: One of the statistical tests that are used to test hypotheses, in order to ascertain whether the differences between two samples or two groups are equal or unequal.

Significance was determined at a P-value equal to or less than 0.05. The level of significance was considered high when the P-value is equal to or less than 0.01

Chapter Four

Results and Discussion

Chapter four: Results and discussion

4.1. Distribution of UTI according sex and age:

The current study showed that there is no significant difference between patients depending on sex a ($p>0.05$) as shown in Table (4-1). Where the number of females infected with urinary tract diseases 27 (50.9%) while the number of males 26(49.1%). Although there were no statistical indications of the difference between males and females, this study was close to the results of many studies that proved that the number of females is more than males in many countries**.** Such as the study of Baghdad Teaching Hospital which showed that the frequency of females (62.2%) was higher than males (37.8%) (AL-Khikani *et al*.,2019). Also the study of (Saeed., 2015) showed that the percentage of females (53.2%) is more than that of males (30.2%). Numerous variables contribute to the higher proportion of females, one of which is the anatomical factors which lead to easily access of microbes to the bladder.Proximity of the genitals to the urinary tract, and the shortness of the urethra, which facilitates the entry of bacteria, and The method of cleaning from the anus towards the vagina, (John *et al*., 2016, Saeed.,2015). The distribution according to age was heterogeneous and there was a non-significant difference as shown in same table. Where there was predominance in the years between 20-29, with a percentage of 30.18% , then the years were between 30-59,While the lowest percentage was in the years between 60-65. This result was in the same line with study which showed that the highest percentage was in the age groups between 20-29 years (46.8%), and the lowest frequent age with urinary tract infection was aged 50 years and above (3.2%) (Ondari,2020).also the study carried out in Nakuru level 5 Hospital was corresponds with the result of this study, which showed that

patient with age group 25-34 years had the largest frequent of UTI (32.4%) (Gachui,2017).

Table 4-1:Distribution of patients and control subjects according to age and sex

Characteristic	Control *n* = 35	Patient *n* = 53	*p-value*
Age (years)			
Mean ± SD	34.77 ± 13.64	36.60 ± 15.47	0.571 T NS
Range	10 - 64	3 - 62	
< 20, *n* (%)	2 (5.71)	5 (5.66)	
20-29, *n* (%)	11 (31.42)	16 (30.18)	
30-39, *n* (%)	11 (31.42)	10 (18.86)	
40-49, *n* (%)	5 (13.88)	10 (18.86)	
50-59, *n* (%)	3 (14.28)	10 (18.86)	
60-69, *n* (%)	3 (8.57)	4 (7.54)	
Sex			
Male, *n* (%)	14 (40.00)	26 (49.1)	0.038 C *
Female, *n* (%)	21 (60.00)	27 (50.9)	

n: number of cases; **SD**: standard deviation; **T**: Independent sample T test; C: Chi-square test; **NS**: not significant at $p > 0.05$;*: significant at p-value less than 0.05

Similarly, research that done at Teerthanker Mahaveer Medical College and Research Centre India agree with the results of this study which depicted UTI to be highest in age group (20-29) years (Mashkoor *et al*., 2017).The higher frequency of these age groups (20-29) was due to them begins at their top reproductive years , being sexually active and persons in this age are more likely to self-diagnose and treat themselves through to the availability of information that can be read online thus use inappropriate antibiotic without a proper

prescription may increase the likelihood of incorrect treatment (Bennadi,2013). The result of this study differ from result of (Fahimzad *et al*.,,2010), who found that the urinary tract infections among patients with age group 0-9 years could be caused by factors for example being uncircumcised and use disposable diapers for an extended period of time thus the lower frequency of diaper change among infant increased the likelihood of bacterial buildup from urine and feces colonizing the peri-urethral area and ascending up the urethra to the bladder causing urinary tract infection.

4.2. Distribution of UTI according to risk factors:

The results given in the Table (4-2) show non-significant differences between urinary tract infections and place of residence. Where the number of people with urinary tract infections in rural areas 30(56.6%) was relatively higher than in urban areas 23(43.4%). This result was in line with result of study conducted by De Lusignan *et al*.,(2018), which showed that there is no significant difference in the incidence of urinary tract infection. Also, this study agreed with a study conducted in Basrah, where the highest percentage of infections was found in rural areas (53%) than urban areas (Alhamedy and shani .,2020**).**According to Hilwana's findings, 14.28% of UTI patients were from rural regions, the majority (85.71%) of UTI patients were from urban areas (Lutifta Hilwana, 2017). The outcome was interpreted by the scientists as tracking community social customs, environmental changes, and insufficient sanitary standards that increase their exposure to various organisms (Saeed et al., 2015). The current results also showed that there were significant difference between urinary tract infections and infection recurrence ($p<0.001$). Aging may reduce the urinary system's defense mechanism against bacterial invasion, which increases infection and its recurrence in people over fifty (Sepúlveda-Loyola et al.,2020).

Table 4-2:Distribution of patients and control subjects according to risk factors

Risk factor	Control *n* = 35	Patient *n* = 53	*p-value*
Residence			
Urban, *n* (%) **Rural, *n* (%)**	15 (42.9) 20 (57.1)	23 (43.4) 30 (56.6)	1.000 C NS
Recurrent infection			
Positive, *n* (%) **Negative, *n* (%)**	0 (0.0) 35 (100.0)	30 (56.6) 23 (43..4)	< 0.001 C ***
Family History			
Positive, *n* (%) **Negative, *n* (%)**	0 (0.0) 35 (100.0)	30 (56.6) 23 (43.4)	< 0.001 C ***
Renal stones			
Positive, *n* (%) **Negative, *n* (%)**	0 (0.0) 35 (100.0)	28 (52.8) 25 (47.2)	< 0.001 C ***

n: number of cases; **C**: Chi-square test; **NS**: not significant at $p > 0.05$; ***: significant at p-value less than 0.001

Also twenty-five percent of women will have recurrent UTI after their primary infection although they have no apparent anatomical anomalies in the urinary tract, Such recurrences are often caused by the same bacterial strain, which indicates insufficient primary infection resolution rather than a new infection and this may explain why, following antibiotic treatment, some patients develop UTI

very frequently (Finer and Landau,2004). The strongest predicates were characteristic of bacteria causing infection itself (Foxman ,2002). The reason of recurrence of urinary tract infection may be due to sexual intercourse and use spermicides that may alter vaginal ph. and this effect on flora and thus lead to recurrence of UTI.

The results of this study also showed that there are significant differences between urinary tract infection and the history of family. Where strome explained that there is a relation between family history and UTIs infections through genetic predisposition. Where his studies showed that there is a great genetic predisposition for vaginal colonization by uropathogens appear to run in families. Potentially due to the increase ability of bacteria to adhere to the epithelium due to an increase expression of *E.coli* receptors on vaginal epithelial cell(Strome *et al*.,2019). This predisposition may be attributed to versatility Genetic abnormalities in cytokines and other components of the immune system increase the chance of infection in patients from the same family (Scholes *et al*., 2000).

Based on the results of this study, there were significant differences between the incidence of UTIs infections and the presence of kidney stones ($p<0.001$). Yongzhi *et al* 2018 found in their study that there patients with renal stone have high rate to have UTIs infection ($p=0.001$). Where this result justified that there renal stone can not pass smoothly and inflamed narrowing of the ureter or injuries made by stone when moving down the ureter could easily cause infection. While other study showed that the renal stone may cause damage to the microstructure of the kidney as well as infection, which may be a contributing factor to kidney failure in these patients (Alelign,2018).

4.3. Morphological and Biochemical Identification of isolated bacteria:

The initial diagnosis of the isolated bacterial species was based on some basic criteria, including the shape of the colonies and their color and appearance characteristics on the general culture media such as Nutrient agar medium, blood agar medium and some differentiation media as MacConkey agar media, which helps in the growth and isolation of Gram-negative bacteria only, as well as the use of many biochemical tests for the purpose of initial diagnosis of the isolated bacterial species according to the methods described by (Collee *et al*., 2006) and as shown in Tables (4-3),(4-4).

Table 4-3 :biochemical tests of gram-negative bacteria

Biochemical tests	*E. coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumonia*
Catalase test	+	+	+
Oxidase test	-	+	-
Indole test	+	-	-
Citrate test	-	+	-
Urease test	-	_	+
Motility test	+	+	-
Glucose test	+	-	+
Gram stain	-	-	-
Methyl red	+	-	-
Voges Proskauer	-	-	+

Table 4-4: biochemical tests of gram-positive bacteria

Biochemical test	*S. aureus*	*Enterococcus spp.*
Catalase test	+	-
Oxidase test	+	-
Motility test	-	-
Hemolysis	B	β & α
Glucose	+	+
Gram stain	+	+
Coagulase	+	-
Voges Proskauer	+	+

4.4. Percentage of isolated bacteria

Fifty-three urine samples were collected from the patients with urinary tract infection. Two of these samples showed negative bacterial culture, and the rest of samples were positive. The highest percentage were *Escherichia coli* (30.2%), *Klebsiella Pneumonia* (18.9%), *Pseudomonas aeruginosa* (9.4%) while Gram

positive bacteria were *Staphylococcus aureus* (26.4%) and *Enterococcus faecalis* (11.3%). As shown in Table (4-5)

Table 4-5: percentage of isolated bacteria

Bacteria	Patient (*n* = 53)	
	N	**%**
Escherichia coli	16	30.2
Staphylococcus aureus	14	26.4
Klebsiella pneumonia	10	18.9
Enterococcus faecalis	6	11.3
Pseudomonas aeruginosa	5	9.4
No Growth	**2**	**3.8**

The result of the study was similer to the results of a study conducted in Erbil showed that the most prevalent uropathogen bacteria was *E.coli* (33.9%),followed by *Klebsiella Pneumonia (*10.1%), *pseudomonas aeruginosa* (6.8%), respectively (Saeed *et al*.,2015). Another study showed that *E.coli* was the dominant bacteria which causes UTIs, followed by *staphylococcus aureus*, (Odoki *et al*.,2019).). According to the results, Gram-negative coliform bacteria that enter and ascend the urinary tract from fecal flora populating the perineum are the main cause of pediatric UTIs. Due to unique characteristics that enable them to penetrate the uroepithelial cell surface, such as fimbriae that cling to the uroepithelial cell surface, uropathogenic E. coli strains are the primary cause of pediatric UTIs (80–90%) (Edlin *et al.*, 2013).These bacteria were common

microorganisms that caused male and female urinary tract infections at any age because of the ability of these bacteria species to produce many virulence factors especially those that improve these bacteria's ability to adhere to and invade surface epithelial cells like pili and form biofilm (Boye *et al*.,2012). When the same pathogenic bacteria strain was have ability to produce virulence factors interacts with their infectivity (AL-baidy and AL-badi, 2010). *Staph. aureus* can adhere to surface epithelial cells, and this adherence is usually due to the protein-associated cell surface that mediates adherence to the host's extracellular matrix called MSCRAMMs (Microbial Surface Components Recognizing Adhesive Matrix Molecule), which allows these bacteria to infect the host, (Corrigan *et al*., 2007).

4.5. Distrubtion of bacterial isolates according to age and sex:

In the current study 53 urine sample cultured and 51 sample were positive culture and 2 have no growth, results showed that there was no significant associations between age and sex with the type of bacterial isolate from urine. The *Klebsiella pneumonia* was the first one (41.64) that cause UTI in ranged ages between 25-54, followed *Pseudomonas aeruginosa* (38) with age ranged between 26-62, *E.coli* which showed that it can be at almost all ages (38.5) *Enterococcus faecalis* and *Staphylococcus aureus* did not vary much with age . As shown in table (4_6)

Table 4-6 : Distribution of bacterial isolates according to age and sex

Characteristic	***Escherichia coli* *n = 16***	***Staphylococcus aureus* *n = 14***	***Klebsiella pneumonia* *n = 10***	***Enterococcus faecalis* *n = 6***	***Pseudomonas aeruginosa* *n = 5***	***No growth* *n* = 2**	***p-value***
Age (years)							
Mean ± SD	38.59 ±16.66	30.71 ± 14.62	41.64 ±10.66	35.83 ±22.30	38.00 ± 16.31	31.00 ± 5.65	0.600 A NS
Range	4 – 64	8 - 55	25 - 54	3 – 62	26 - 62	27 - 35	
Sex							

Male, *n* (%)	7 (43.2)	10 (71.4)	5 (45.5)	2 (33.3)	1 (20.0)	1 (50.0)	0.379 FE NS
Female, *n* (%)	9(56.8)	4 (28.6)	5 (50.5)	4 (66.7)	4 (80.0)	1 (50.0)	

n: number of cases; **SD**: standard deviation; **A**: One-way ANOVA; FE: Fisher exact test; **NS**: not significant at $p > 0.05$

The reason of the non significant difference between bacterial isolate for all species with age and sex may be due to defensive factors such as virulence factors of bacteria which can be the cause of the infections regardless of gender and age. An examples of such virulence factors is membrane proteins play roles in adhesion, colonization, and invasion, promote adherence to host cell surfaces, are responsible for antibiotic resistance, and facilitate intercellular communication, Polysaccharide capsules that surround the bacterial cell and have antiphagocytic properties and Secretory proteins, such as toxin, which can modify the host cell environment and are responsible for some host cell–bacteria interactions, also some bacteria form biofilm as a virulence factor such as such as *Pseudomonas aeruginosa* and *staphylococcus aureus*, Biofilm formation confers a selective advantage for persistence under environmental conditions and for resistance to antimicrobial agents and also facilitates colonization in the host by the bacteria (Wu *et al*.,2019). The abundance of *E. coli* and *S. aureus* may be attributed to the numerous virulent factors they possess that enable them to cause urinary tract infection, such as hemolysin, which is capable of forming holes in the host cell membrane, resulting in the release of cellular materials such as iron, hemoglobin, and other bacterial growth components that support bacterial growth within the living body; thus, hemolysin acts as a cytotoxic agent in addition to its ability to analyze red blood cells, *S. aureus* and *E. coli* bacteria can produce ß lactamase enzymes that are resistant to most antibiotics and play an important role in the virulence of these bacteria (Husien *et al*.2017).

4.6. Distrubtion of bacterial isolates according to risk factors

The results in Table (4-7) explain that there is no significant relationship between residence and type of bacterial isolate (p>0.05). Almost all types of bacteria appear more in rural areas than in urban areas.

Table 4-7 :Distribution of bacterial isolates according to risk factors

Characteristic	*Escherichia coli* *n* = 17	*Staphylococcus aureus* *n* = 14	*Klebsiella pneumonia* *n* = 11	*Enterococcus faecalis* *n* = 6	*Pseudomonas aeruginosa* *n* = 5	*No growth* *n* = 2	*p-value*
Residence							
Urban, *n* (%)	10 (58.8)	6 (42.9)	5 (45.5)	2 (33.3)	1 (20.0)	0 (0.0)	0.570 FE NS
Rural, *n* (%)	7 (41.2)	8 (57.1)	6 (54.5)	4 (66.7)	4 (80.0)	2 (100.0)	
Recurrent infections							
Positive, *n* (%)	12 (70.6)	6 (42.9)	7 (63.6)	2 (33.3)	3 (60.0)	2 (100.0)	0.401 FE NS
Negative, *n* (%)	5 (29.4)	8 (57.1)	4 (36.4)	4 (66.7)	2 (40.0)	0 (0.0)	
Family history							
Positive, *n* (%)	7 (41.2)	5 (35.7)	6 (54.5)	4 (66.7)	2 (40.0)	1 (50.0)	0.845 T NS
Negative, *n* (%)	10 (58.8)	9 (64.3)	5 (45.5)	2 (33.3)	3 (60.0)	1 (50.0)	
Renal stones							
Positive, *n* (%)	11 (64.7)	8 (57.1)	5 (45.5)	2 (33.3)	2 (40.0)	2 (100.0)	0.589 FE NS
Negative, *n* (%)	6 (35.3)	6 (42.9)	6 (54.5)	4 (66.7)	3 (60.0)	0 (0.0)	

n: number of cases; **SD**: standard deviation; FE: Fisher exact test; **NS**: not significant at $p > 0.05$

This study was similar study which explain many risk factors that make rural areas more exposure to such uropathogenic bacteria such as low household wealth, maternal undernutrition, primiparity and low paternal education (Lee *et al* .,2020). Also, there was no significant association between recurrent infections (p>0.05). there were a relationship between recurrent infection and type of

bacterial isolate , recurrence was more common in patients which had a history of UTI. According to the results of a study conducted in 1996 showed that recurrences were more common in patients who had a history of UTI; 47.4% of patients with a history of UTI had at least one recurrence during the follow-up period. *E.coli* accounts for (65%) of all isolates, and antimicrobial susceptibility tests on these strains revealed multidrug and extensive drug resistance. All patients had recurrent UTIs (some for many years) and a poor response to antibiotics (Flores-Mireles *et al*., 2015). As for renal stone and their relationship with type of bacteria isolate, there was no significant difference between them (p>0.05). The most dominant bacteria was *E.coli* (62.5%), followed by other uropathogenic bacteria. This result was closed to other study which found that the most common pathogenic bacteria was *E.coli* and urinary tract stone can obstruction and provide a favorable environment for the growth of pathogenic bacteria, and the stone are a favorable place for bacterial colonization, at the same time bacteria can further promote the growth of stone (Yong *et al* .,2022).According to the current study result there was no significant difference between bacteria isolate from urinary tract infection patients and history of family (P>0.05). Through genetic predisposition showed there is a link between family history and bacteria that causes UTI, colonization by uropathogens appear to run in families. Because of increased expression of *E.coli* or other uropathogenic bacteria receptors on urinary tract epithelial cells, bacteria may have a greater ability to adhere to the epithelium (Strome *et al*.,2020).

4.7. level of cathelicidin and lactoferrin in the studied groups:

The current results listed in Table (4-8) showed that there were highly significant difference (p>0.05) in LL-37 urine level in paients group (1.96)compared to control group (1.72). This result agreed with what Awadallah *et al* (2019) . Who

confirmed that significant deference in LTF and LL-37 urine level among patients with UTI compared to control ,(P<0.001). Another study showed that the LL-37 concentration in urine sample from patients with UTIs when measured during and after infection and found that the LL-37 was higher in all patients during infection than after infection (Nielsen et al.,2014). While the results of (Hacihamdiglu et al,.2016) disagree with current results, where they found no significant difference (p=0.05) in the LL-37 urinary levels were high in children with UTI compared to control groups

Table 4-8 : LTF and LL-37 in the patients and control group

Factor	**Control** ***n* = 36**	**Patient** ***n* = 55**	***p-value***
LTF			
Mean ± SD	3.21 ± 2.43	4.87 ± 4.08	0.031 T *
Range	0.46 - 9.66	0.11 - 15.46	
LL-37			
Mean ± SD	1.72 ± 1.46	1.96 ± 1.51	0.043 T *
Range	0.12 - 5.65	0.00 – 7.14	

n: number of cases; **SD**: standard deviation; **T**: Independent-sample T test;, *: significant at p-value less than 0.05

According to (Chromek *et al*.,2006) who found that direct contact with microbes causes urinary epithelium to produce more LL-37 subsequently shielding the urinary tract from adherence . The current result also showed significant difference (P<0.01) in lactoferrin levels among the studied groups where (4.87) for patients while (3.21) for control group. The regression model implied that this difference related to presence of inflammation (Brenton *et al*.,2012). LTF serves as antibacterial, antiviral and antifungal through the ability of lactoferrin

to limit iron availability to microbes, most bacteria require iron for growth, and because of their iron sequestering properties, the iron free (apo) forms of lactoferrin and transferrin arev able to impede iron utilization by bacteria. A large number of studies have demonstrated a bacteriostatic effect and in some cases a bactericidal effect of lactoferrin in vitro on a wide range of microorganisms, including Gram positive and Gram negative bacteria, aerobes, anaerobes, and yeasts. However, mechanisms other than simple iron withholding may be involved in the antibacterial action of lactoferrin, such as blockade of microbial carbohydrate metabolism'" or destabilization of the bacterial cell wall, perhaps through binding of calcium and magnesium (Sanchez *et al*.,1992). In the urinary tract, lactoferrin serves as a biomarker for UTI with a 100-fold increase compared to healthy controls but the source of lactoferrin during infection has not been fully characterized. Infiltrating neutrophils , as well as renal collecting tubules may be sources of urinary lactoferrin (Patars *et al*.,2018).

4.8. Level of LTF and LL-37 according to age and sex:

The results showed that there was significant association between LTF and LL-37 with age (P=0.05). As shown in figure (4-1), levels of these antibacterial peptides increase with age, Where the numbers were high at ages (50-59), while the years between 60-65 have low numbers because It was so difficult to obtain samples from patients without any chronic diseases at these ages group. These results were in the same line with results of study conducted in Najaf which reveals a significant increase in cathelicidin level in urine at advanced age 60-69 years old (7.595 ± 0.543) in comparison with other ages 50-59 years (6.2 ± 0.302), 40-49 years (5.08 ± 0.624), and 0-39 (3.3 ±0.516) (Ali H. and AL- Dujaili A,2022). the role of older ages in urinary tract infection patients and their association with high cathelicidin levels and diseases as a result of LL-37 in severity of urinary

tract infection and decline in production and secretion of antioxidants such as catalase, peroxidase, and superoxide dismutase with low concentration of all antioxidant defense system lead to greater survivability (Chu *et al*.,2018).

Also these results showed there was significant difference between levels of these antibacterial peptides and sex. As shown in Table (4-9), there was significant difference between these antibacterial peptides and sex. these result may related with hormonal and physiological changes in both sex.

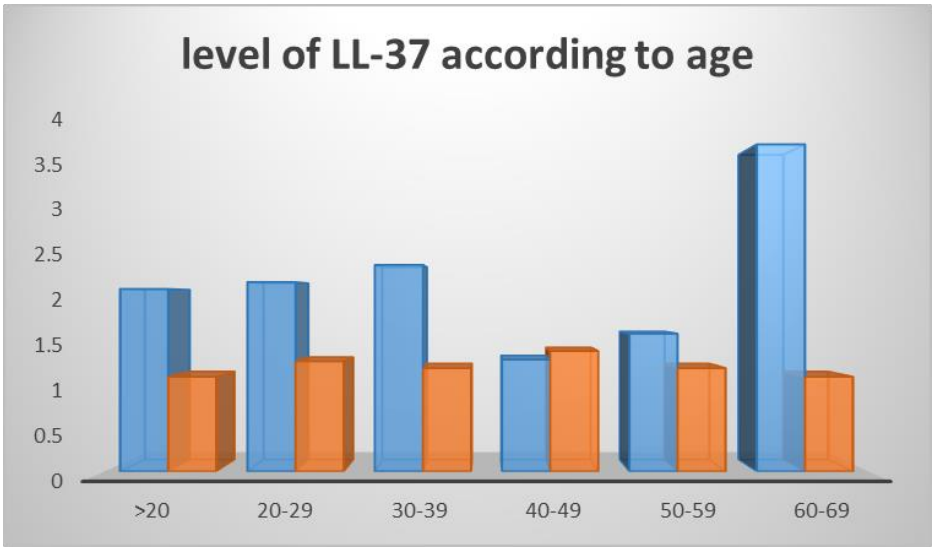


Figure 4 Level of LL-37 according to age

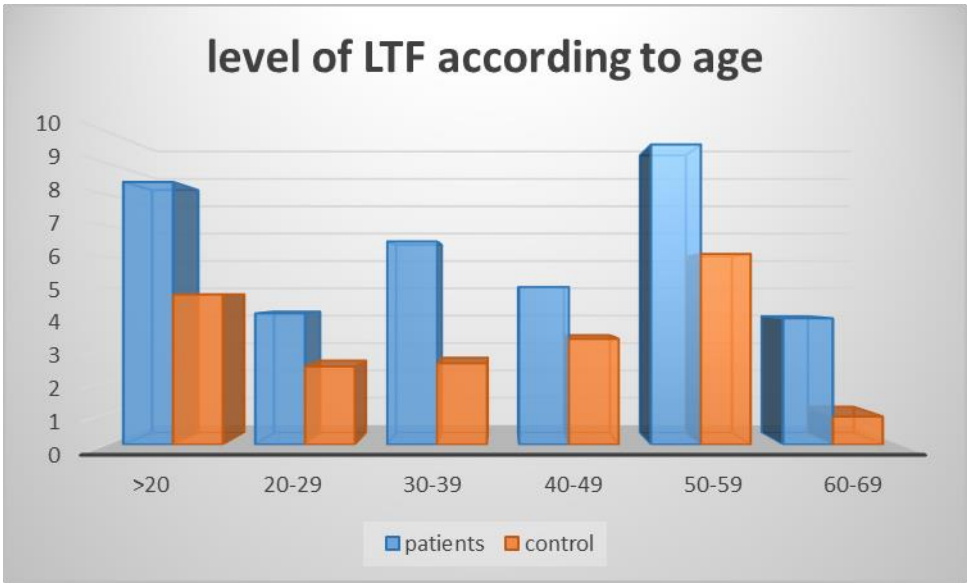


Figure 5 Level of LTF according to age

Table 4-9 : Level of LTF and LL-37 according to sex

Characteristic	**LTF**	***p-value***	**LL-37**	***p-value***
Sex				
Male, *Mean* ± SD	4.80 ± 3.13	0.015 T *	1.74 ±1.43	0.038 T *
Female,*Mean*±SD	4.14 ± 3.29		1.56±1.32	

SD: standard deviation; **T**: Independent-sample T test; *: significant at p-value less than

Males are more susceptible than females to many infectious pathogens, and hormones are hypothesized to contribute to male-biases in infection. sex steroid hormones are one proximate mechanism that underlie sex differences in infection. Hormones in general, and sex steroids specifically, may affect the genes, immune responses, and behaviors that influence susceptibility and resistance to infection. Males generally exhibit lower immune responses than female conspecifics Humoral immune responses (i.e. antibody production by B-cells) are typically elevated in females as compared to males, This explains the higher levels of peptides in men compared to women (Klein ,2000). Estrogen may effect continence by increase urethral resistance, raising the sensory threshold of the bladder or increasing an adrenoreceptor sensitivity in urethral smooth muscle (Konapala *et al*.,2018). In women, changes in the vaginal flora due to estrogen depletion lead to colonization with gram negative bacteria which in addition cause local irrative symptoms, specially in elderly women which have a reduced flow rate, increased urine residuals, higher filling pressures, reduced bladder capacity, and lower maximum voiding pressures, these all changes in urinary tract cumulatively increase the chance of urinary tract infection, thus, increase in antibacterial peptides (Robinson *et al* .,2013).

4.9. Levels of LTF and LL-37 according to bacterial isolate:

Current study records a significant association (P>0.001) between levels of LL-37 and bacteria isolate. Where higher concentration was recorded in patients with *E.coli* (2.8), followed by *Klebsiella pneumonia* (2.18), *Enterococcus faecalis* (1.77), *Staphylococcus aureus* (1.45), and *pseudomonas aeruginosa* (0.88), respectively, as shown in table (4-10).

These results contradicted other study in Basrah, which showed that there is no significant association between the type of bacterial isolate from urine of patients with UTI and levels of LL-37 (Alhamedy and Shani.,2020). (Awadallah *et al*.,2019). The lactoferrin, it have significant association with bacterial isolate from urine ($p>0.001$), where the *Enterococcus faecalis* was the most concentrated (7.76), followed by *pseudomonas aeruginosa* (6.40), *klebsiella pneumonia* (4.80), *E.coli* (4.42) ,and *Staphylococcus aureus (*2.92), respectively. This association may be due to the fact that bacteria have certain mechanisms or factors that enable them to survive and cause infection in the host and thus increase such peptides as a result of stimulating the immune response such as LPS , H-antigen,and O-antigen, etc. One of these factor is Lipopolysaccharide that constitutes the outer leaflet of the outer membrane of most Gram-negative bacteria is referred to as an endotoxin. It is comprised of a hydrophilic polysaccharide and a hydrophobic component referred to as lipid A. Lipid A is responsible for the major bioactivity of endotoxin, and is recognized by immune cells as a pathogen-associated molecule. Lipopolysaccharide also composed from exposed part called O-antigen, which seem to play an important role at several stages of the infection process, including the colonization (adherence) step and ability to by pass or over come host defiance mechanisms. Another antigen, the bacterial flagellum or H-antigen, which is a complex structure made up of more than 20 different proteins and is important for immune protection in mammals, is capable of inducing an immune response: Flagellin is recognized by the immune system, which causes an innate and adaptive immunological response. In eukaryotic cells, cytoplasmic NOD-like receptors engage with the conserved N- and C-termini of monomeric flagellin involved in flagella assembly to cause the creation of an inflammasome, which results in pyroptosis. Toll-like receptor 5 (TLR5) detects molecules with a pathogen-associated molecular pattern (PAMP),

including flagellin. TLR5 binds to the conserved termini of flagellin, which is mostly expressed on the basolateral surface of intestinal epithelial cells as well as by monocytes and fibroblasts and binds the conserved termini of flagellin, which leads to the activation of cytokine secretion in host (Haiko and Wikström.,2013)

Table 4-10: LTF and LL-37 according to bacterial isolates

Characteristic	*Escherichia coli* *n* = 17	*Staphylococcus aureus* *n* = 14	*Klebsiella pneumonia* *n* = 11	*Enterococcus faecalis* *n* = 6	*Pseudomonas aeruginosa* *n* = 5	*No growth* *n* = 2	*p-value*
LTF							
Mean ± SD	4.42 ± 3.67	2.92 ± 2.30	5.74 ± 3.98	7.76 ± 6.92	6.40 ± 3.75	3.16 ± 2.08	0.004 A **
Range	0.11 – 9.78	0.47 – 8.85	0.86 – 13.11	2.27 – 15.46	0.96 – 9.65	1.08 – 5.24	
LL-37							
Mean ± SD	2.80 ± 2.24	1.45 ± 1.27	2.18 ± 1.84	1.77 ± 1.36	0.88 ± 0.65	0.37 ± 0.15	0.008 A **
Range	0.14 – 4.27	0.01 – 4.30	0.04 – 7.14	0.24 – 3.77	0.21 – 1.84	0.27 – 0.49	

n: number of cases; **SD**: standard deviation; **A**: One-way ANOVA; **: significant at p-value less than 0.01

4.10. levels of cathelicidin and lactoferrin according to risk factors:

In the of current study, the results show that there was significant difference between level of lactoferrin and residence of patients ($p< 0.05$) ,while there was no significant difference between level of cathelicidin and residence in rural or urban areas ($p>0.05$). it supposed that the increase in levels of these peptides may related with the increase of urinary tract infection in rural regions than in urban regions. Also these results show that there was no significant difference between both antibacterial peptide (LL-37, LTF) with recurrent infection. As we mentioned earlier, urinary tract infections increase in patients who have recurrent

infections more than others, after invasion, pathogens reach a transient protective environment in which they can replicate, re-emerge from the host cell, invade the urinary tract, the existence of such reservoirs with urinary tract may help to explain the recurrent nature of UTI, thus, may increase in level of these antibacterial peptides as a response of these infection (Patars *et al.*,2018).

Family history and genetic predisposition It could be a greater predisposition for vaginal colonization by uropathogens appears to run in families, potentially due to the increased ability of bacteria to adhere to the epithelium (Strome *et al* 2019), This adhere of bacteria also increase the chance of infection thus increase the level of antibacterial peptide in patient in compared with control group. Finally, the results show that there was a significant difference between level of antibacterial peptide and renal stone. As mentioned earlier, there is an association between urinary tract infections and the presence of kidney stones, beacause stones can not pass smoothly, and may moving down the ureter and causing infections (Yongzhi.,2018) which can trigger an immune response that increases the levels of antibacterial peptides.

Table 4-11: LTF and LL-37 according to risk factors

Characteristic	**LTF**	***p-value***	**LL-37**	***p-value***
Residence				
Urban, *Mean* ± SD	4.02 ± 3.37	0.026 T *	1.89 ± 1.33	0.521 T NS
Rural, *Mean* ± SD	4.36 ± 3.79		1.84 ± 1.02	
Recurrent Infections				

Positive, *Mean* ± SD	4.79 ± 3.77	0.265 T NS	2.08 ± 1.10	0.492 T NS
Negative, *Mean* ± SD	3.90 ± 3.49		1.75 ± 1.42	
Family history				
Positive, *Mean* ± SD	4.30 ± 3.70	0.884 T NS	2.05 ± 3.06	0.621 T NS
Negative, *Mean* ± SD	4.18 ± 3.58		1.80 ± 1.22	
Renal stones				
Positive, *Mean* ± SD	4.92 ± 3.68	0.016 T *	2.47 ± 1.42	0.003 T **
Negative, *Mean* ± SD	3.87 ± 3.52		1.57 ± 1.09	

SD: standard deviation; **T**: Independent-sample T test; *: significant at p-value less than 0.05; **: significant at p-value less than 0.01; **NS**: not significant at $p > 0.05$

4.11. Antibacterial Activity of Aqueous Arabic Gum solution:

The antibacterial activity of Arabic Gum aqueous solution against *E. coli* and *S.aureus* isolates (these isolate are most common in patients) were determined by using the well diffusion method . The results of the statistical analysis that shown in the Table (4-12) showed there wer ahighly significant differences (p=0.001) between the isolates of bacteria with concentration, and the interaction between them. The highest inhibition activity of the aqueous solution of Arabic gum at a concentration 500 against the E.coli6 isolate with an inhibition zone (11.7mm) , while there was no inhibition zone at low concentration against isolates E.coli3 and E.coli5.The high rate of inhibition activity of the same solution on *S. aureus* isolates was also at a concentration 500, the S.aureus2 with an inhibition zone (10mm), while the lowest activity was on the S.aureus 4 without any inhibition

zone. The current study relied on aqueous solution of plants rather than other chemical extracts because they are safe for human consumption. In this study, Arabic gum aqueous solution demonstrated varying degrees of antibacterial activity against various microorganisms, and nearly all isolates were sensitive to it. the current results are supported by earlier studies on an aqueous solution that exhibited highly activity against isolates bacteria (AL-behadliy *et al* .,2020). The majority of Arabic gum's antimicrobial properties are due to secondary metabolites or saponin, saponin glycosiodes, volatile oil, hydrolysable tannin, triterpenoid, phenol, and alkaloids (Chaubal and Tambe, 2006). In general, the cell walls of Gram-positive bacteria are more complex and serves as barrier making it more resistant to the antibacterial agents compared to the Gram negative bacteria and effect on work of the Arabic gum solution . Pharmacological studies on this plant have revealed that Arabic gum is used to clean the urinary bladder (Ljubuncic *et al*., 2005) and to mitigate the adverse effects of chronic kidney disease (Ali *et al*., 2009). Arabic Gum contains a variety of chemical compounds such as neutral sugars arabinose, galactose acids such as 4-methoxyglucuronic acid and glucuronic acid, magnesium, calcium, sodium, and potassium (Al Alawi *et al*., 2018). Arabic gum contains numerous primary and secondary metabolic compounds such as flavone, polyphenols, catechin, , calcons, tannins, flavonoids, and alkaloids, and these components are already used as medicines to treat a variety of chronic diseases (Marwah *et al*., 2007). Because of its high terpene content, Arabic gum has antibacterial and antifungal properties. Terpenes are bioactive molecules that are thought to be part of plant defense mechanisms (Wisdom and Shittu, 2010).The high salt content of Mg+2 , Ca +2 in Arabic gum contributes to its antibacterial activity and K+1 of polysaccharides, as well as the effect of Arabic gum on the metabolism of Ca+2 and phosphate, AG contains several antimicrobial enzyme forms such as

peroxidases, oxidases, and pectinases (Saini *et al*., 2008). This results was also supported by the results of Bnuyan *et al*., (2015), who stated that Arabic gum aqueous extract inhibited the growth of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumonia*, *Salmonella typhi*, *Ps. aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Enterobacter* spp and *Acinetobacter*.

Table 4-12: Antibacterial activity of Arabic gum aqueous solution on *E.coli*

Isolate / Concentration	*E.coli1*	*E.coli2*	*E.coli3*	*E.coli4*	*E.coli5*	*E.coli6*	Con. average
500	9.5	8.7	7.7	10	6.5	11.7	9.04
400	8.5	8.7	6.5	9.5	5.2	9.7	8.04
300	7.7	7.5	5.2	3.7	4.1	8.7	6.18
200	5.5	7.7	3.5	6.2	2.5	7.2	5.45
100	2.2	4.7	0	3.5	0	4.2	2.45
Isolate average	6.07	6.35	3.28	6.14	3.55	7.53	
D.W	0	0	0	0	0	0	
Ciprofloxacin	9	7	0	10	6.5	11	7.25
p- value	For bacteria isolate <0.001		For concentration <0.001		For interaction <0.001		
LSD	For bacteria isolate = 0.494		For concentration=0.533		For interaction= 1.307		

Table 4-13: Antibacterial activity of Arabic gum aqueous solution on *s. aureus*

Inhibition of isolates / Concentration	*S.aureus 1*	*S.aureus 2*	*S.aureus 3*	*S.aureus 4*	*S.aureus 5*	*S.aureus 6*	Con. average
500	7.8	10	7.7	6.2	4.5	3.7	6.6

400	**5.7**	**8**	**7.2**	**3.5**	**5.2**	**5.5**	**5.8**
300	**3.7**	**7.5**	**5**	**4.7**	**0.5**	**3.6**	**4.9**
200	**6.2**	**4.5**	**3.7**	**4.5**	**4.7**	**2.7**	**4.6**
100	**5.9**	**1.5**	**2.7**	**0**	**4.2**	**2**	**1.7**
Isolate average	**6.5**	**5.9**	**4.7**	**4.8**	**3.1**	**3.7**	
D.W	**0**	**0**	**0**	**0**	**0**	**0**	
ciprofloxacin	**10**	**9.5**	**8.5**	**10**	**7**	**7.5**	**8.7**
p- value	**For bacteria isolate <0.001**		**For concentration <0.001**		**For interaction <0.001**		
LSD	**For bacteria isolate = 0.363**		**For concentration=0.392**		**For interaction= 0.960**		

4.12. Antibacterial activity of Aqueous solution of Boswellia Serrata:

The antibacterial activity of Boswellia serrata against *E. coli* and *S.aureus* isolates was determined using the well diffusion method. After the incubation period, the current results show that the aqueous solution of Boswellia Serrata have no inhibition zone at different concentration used in this study.

Conclusions

1. The number of female infected with UTIs are more than males and UTIs was predominant in the years between 20-29
2. The urinary levels of LL-37 and LTF were elevated in patients in compare to healthy control and there was significant associated between levels of these parameter with age, sex, and renal stone
3. *E.coli* and *S.aureus* were the most common pathogens isolated from patients with UTIs
4. There is no relationship between levels of antibacterial peptides and levels of bacteria most commonly associated with urinary tract infections.
5. Aqueous solution of Arabic gum has antibacterial activity against most common isolated bacteria from patients with urinary tract infections and rate of inhibition zone were variable with change of concentration without association with the rate of antimicrobial peptide in patients urine.
6. Aqueous solution of Bosweilla Serrata frankincense hasn't any antibacterial activity against most common isolated bacteria from patients with urinat tract infections.

Recommendations

1. Separation and purification of the active compounds present in the aqueous solution of Arabic gum and determine the antibacterial activity for each one.

2. Studying the effect of the solution on the ability of pathogenic bacteria to produce virulence factors qualitatively and quantitative

3. Study of variations in the genes responsible for the production of antimicrobial proteins and their relationship to infection frequency

4. A study of the relationship between the variation in the rates of antimicrobial peptides with some immunological parameters (interleukins and chemokines)in patients with urinary tract infections.

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Appendixes……..……

Appendix I:

Karbala University
College of Science
Department of Biology

Questionnaire for the patients with UTIs & the control

1. Name: No. The date:

2. Gender: Male Female

3. Age:

4. Urine profile:

Microscopical examination	Culture (register types of bacteria and other pathogens)	Discharges associated with the infection (describe color and texture)

5. Status of cigarette-smoking Yes No

6. Alcohol consuming Yes No

7. Recurrent UTI Yes No
   - ❖ Time duration between the recurrent UTIs

8. Relatives having recurrent UTI Father Mother Sister

9. Types of antibiotics have been taken as a medicine:

Appendixes……..……

10. Does there are stones in the urethra & kidney: Yes No

11. Other infections around the body associated with renal disease :

12. Macro albuminuria (U200 μg/min).

13. Hyper glycaemia

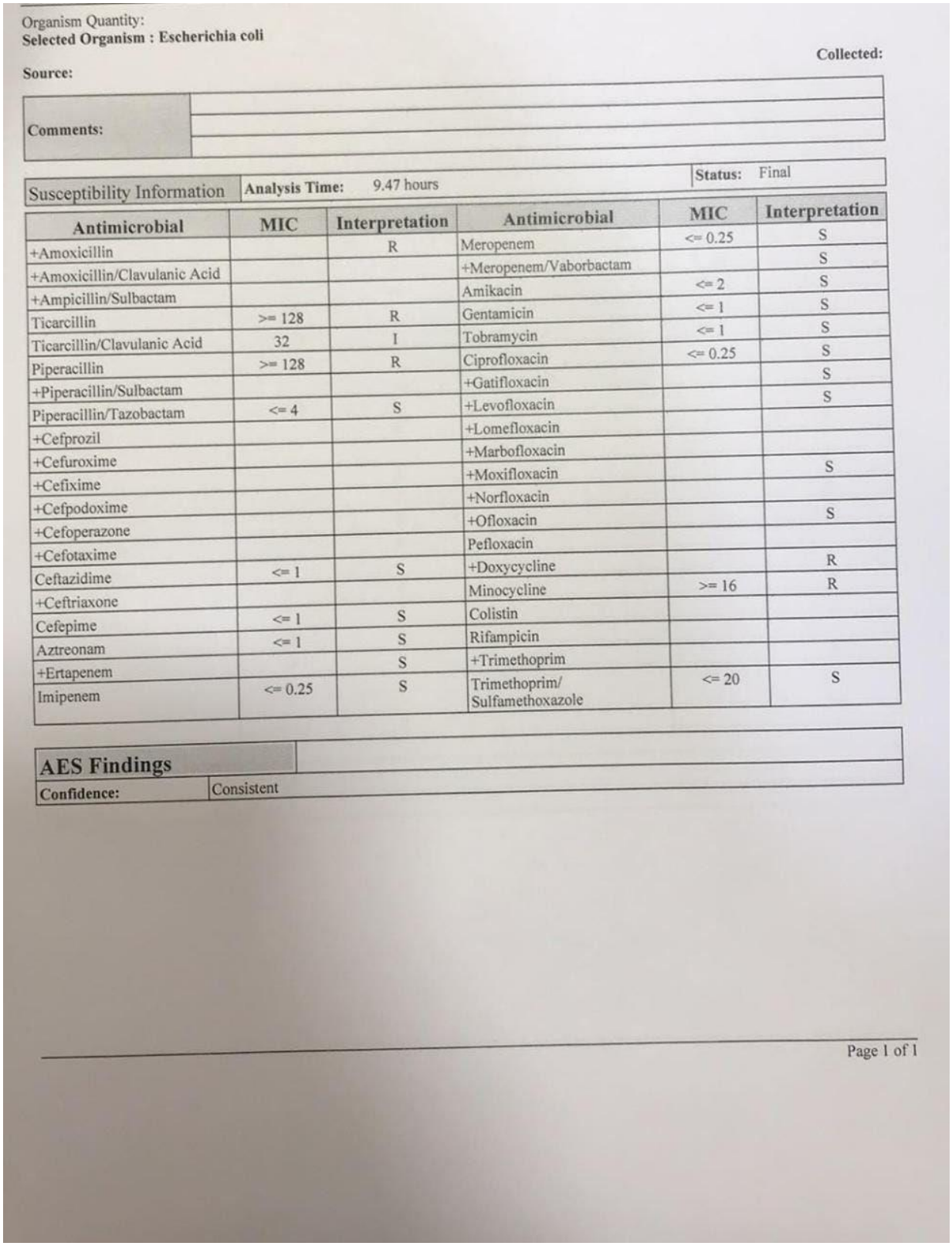
14. Increased blood pressure levels

15. Elevated serum lipids

16. Dietary protein

Appendixes……..……

Appendix II: susceptibility information for *E.coli* strain:



Organism Quantity:
Selected Organism : Escherichia coli

Source: **Collected:**

Comments:	

Susceptibility Information	**Analysis Time:** 9.47 hours	**Status:** Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
+Amoxicillin		R	Meropenem	<= 0.25	S
+Amoxicillin/Clavulanic Acid			+Meropenem/Vaborbactam		S
+Ampicillin/Sulbactam			Amikacin	<= 2	S
Ticarcillin	>= 128	R	Gentamicin	<= 1	S
Ticarcillin/Clavulanic Acid	32	I	Tobramycin	<= 1	S
Piperacillin	>= 128	R	Ciprofloxacin	<= 0.25	S
+Piperacillin/Sulbactam			+Gatifloxacin		S
Piperacillin/Tazobactam	<= 4	S	+Levofloxacin		S
+Cefprozil			+Lomefloxacin		
+Cefuroxime			+Marbofloxacin		
+Cefixime			+Moxifloxacin		S
+Cefpodoxime			+Norfloxacin		
+Cefoperazone			+Ofloxacin		S
+Cefotaxime			Pefloxacin		
Ceftazidime	<= 1	S	+Doxycycline		R
+Ceftriaxone			Minocycline	>= 16	R
Cefepime	<= 1	S	Colistin		
Aztreonam	<= 1	S	Rifampicin		
+Ertapenem		S	+Trimethoprim		
Imipenem	<= 0.25	S	Trimethoprim/ Sulfamethoxazole	<= 20	S

AES Findings	
Confidence:	Consistent

Page 1 of 1

Appendixes……..……

Susceptibility information of *S. aureus* strain

Organism Quantity:
Selected Organism : Staphylococcus aureus

Source: **Collected:**

Comments:	

Susceptibility Information	**Analysis Time:** 14.77 hours	**Status:** Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Cefoxitin Screen	NEG	-	+Norfloxacin		S
Benzylpenicillin	>= 0.5	R	+Ofloxacin		S
+Amoxicillin		R	Inducible Clindamycin Resistance	NEG	-
Ampicillin			+Azithromycin		
+Ampicillin/Sulbactam		S	+Clarithromycin		
+Carbenicillin		R	Erythromycin	<= 0.25	S
Oxacillin	<= 0.25	S	+Roxithromycine		S
+Cefixime		R	Clindamycin	<= 0.25	S
+Ceftazidime		R	+Lincomycin		
Imipenem			Linezolid	<= 0.5	S
+Meropenem		S	Teicoplanin	<= 0.5	S
Gentamicin High Level (synergy)			Vancomycin	<= 0.5	S
Streptomycin High Level (synergy)			+Doxycycline		S
+Amikacin			Tetracycline	<= 1	S
Gentamicin	<= 0.5	S	Tigecycline	<= 0.12	S
+Tobramycin			Fosfomycin		
Ciprofloxacin	<= 0.5	S	Fusidic Acid	<= 0.5	S
+Gatifloxacin		S	Rifampicin	<= 0.5	S
+Levofloxacin		S	+Trimethoprim		
+Lomefloxacin			Trimethoprim/ Sulfamethoxazole	<= 10	S
Moxifloxacin	<= 0.25	S			

AES Findings	
Confidence:	Consistent

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عدوى المسالك البولية هي عدوى بكتيرية منتشرة, وهي من المشاكل الرئيسية التي يعاني منها الانسان, ومسؤولة عن العديد من المشاكل الصحية , لذلك سعت العديد من الدراسات الى ايجاد الحلول المناسبة للحد من انتشار مسببات الامراض المسؤولة عنها في المجتمعز

خلال الفترة الممتدة من أيار 2022 الى تموز 2022, تم الحصول على عينات ادرار من 53 مريضاً يشتبه في اصابتهم بالتهاب المسالك البولية و 35 شخصاً , تراوحت اعمارهم بين 3 و 69 عاما تم جمع العينات من مكانين (مستشفى الحسين التعليمي وعيادة خاصة).

تم تشخيص البكتريا المعزولة بأستخدام نظام الVitek . تم قياس الكاثيليسيدن واللاكتوفيرن البشري بأستخدام نظام ال ELISA , واظهرت النتائج ان نسبة الاصابة عند الاناث كانت اعلى منها في الذكور حيث كانت نسبة الاصابة عند الاناث 51% بينما عند الذكور 49% . بالاضافة الى ذلك , كانت اعلى نسبة اصابة في الفئة العمرية 20-29 سنة بينما كانت اقل نسبة اصابة في الفئة العمرية 60-69 سنة . كما اظهرت النتائج ان من بين 88 عينة ادرار كانت هنالك 2 فقط من العينات لم يظهر فيها نمو بكتيري بينما اظهرت بقية العينات 51 نمو بكتيري واضح.

وكانت نتائج العزلات البكتيرية 51/16 (30.2%) الإشريكية القولونية، 51/14 (26.4%) المكورات العنقودية الذهبية، 51/10 (18.9%) الكلبسيلة الرئوية، 50/6 (11.3%) المكورات المعوية البرازية، 51/5. 9.4%) الزائفة الزنجارية .

اظهر متوسط مستويات LL-37 في الادرار اختلافات معنوية ($P<0.05$) بين المرضى (1.96) ومجموعة الاصحاء (1.72) كما ان هنالك فرق معنوي بين اللاكتوفيرن والكاثيليسدن مع العزلة البكتيرية. حيث تم تسجيل اعلى تركيز في مرضى الاشيريكية القولونية (2.8) تليها بكتريا الكلبسيلا (2.18), المكورات المعوية البرازية (1.77), والمكورات العنقودية الذهبية (1.45) والزائفة الزنجارية (0.88)

اما فيما يخص تأثير المحلول المائي للصمغ العربي فقد تم اختبار تأثير المحلول ضد بكتريا المكورة العنقودية الذهبية والاشريكية القولونية بطريقة الانتشار بتركيزات متفاوتة من 100مجم/مل الى 500 مجم/مل,اظهرت النتائج ان محلول الصمغ العربي المائي له فعالية مضادة للجراثيم ضد البكتريا المذكورة اعلاه. حيث زاد قطر منطقة التثبيط للعزلات مع زيادة تركيز المحلول المائي للصمغ العربي

الخلاصة

وصلت منطقة التثبيط لبكتريا الاشريكية القولونية في اعلى تركيز 500 ملغم/مل الى 11.7ملم ,ولكن عند التركيز المنخفض 100مجم/مل لم تظهر اي منطقة تثبيط

بينما وصل قطر التثبيط ضد بكتريا المكورات العنقودية الذهبية في اعلى تركيز الى 10 ملم وفي التركيز المنحفض ايضا لم يظهر اي قطر تثبيط.

أيضا تم استخدام المستخلص المائي للبان الذكر لاختبار فعاليته على العزلات البكتيرية لكنه لم يظهر أي قطر تثبيط عند استخدامه.

جامعة كربلاء

كليـة العلـوم

قســم علــوم الحيـاة

الفعالية المضادة للصمغ العربي في نمو البكتريا المرضية المعزولة من المرضى المصابين بألتهابات المسالك البولية

رســالة

مقدمــة إلـــى مجلـس كليـة العلـوم ــ جامعـة كربلاء

وهـي جـزء مـن متطلبـات نيـل درجـة الماجستيـر علــوم فــي علـوم

الحيــاة

من قبل

رباب كاظم جابر الأسدي

(بكالوريوس علوم / علوم الحياة –2019)

بإشراف:

الاستاذ الدكتور

وفاء صادق الوزني

الاستاذ المساعد الدكتور

علي احمد حسين الميالي

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