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Detection of Antimicrobial Peptides in Pediatric Urinary Tract Infections, in Karbala Province

A thesis

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

قَالُوا۟ سُبْحَٰنَكَ لَا عِلْمَ لَنَآ إِلَّا مَا عَلَّمْتَنَآ ۖ إِنَّكَ أَنتَ ٱلْعَلِيمُ ٱلْحَكِيمُ ﴿٣٢﴾

صَدق الله العلي العَظيم

سورة البقرة- آية (٣٢)

Dedication

To….

My dearest and beloved man in my life who lighted my way, my father.

To….

The kindest woman in my life with the purest endless love in the universe, my mother.

To ….

those with whom I lived amongst them and between us are features and one pulse, my brothers & sisters

To….

My companions, my friends, and who are with me for better or worse, Israa and Boraq

To ….

The faces whose disease hides their smiles and to everyone who encouraged and helped me make this work see the light …

Tamani

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Summary

Urinary tract infections (UTI) are one of the most common bacterial infections in childhood. UTIs Dividing into three categories; upper UTI (acute pyelonephritis), lower UTI (cystitis), and urosepsis.

During the period extending between December 2020 to April 2021, Blood and urine samples were obtained from 109 suspected UTI patients and 40 healthy controls, their ages were ranged from 1 month to 12 years. Collection of samples carried out in two places (Karbala teaching hospital for children and private clinic). The Vitek2 system was the method that used for identification of bacterial species. The Human Neutrophil Peptide 1-3 (HNP 1-3) and Cathilcidin (LL-37) were measured by using the ELISA system, while C-Reactive Protein was measured by DIRUI CS-T180 auto chemistry analyzer and white blood cells by using Sysmex XP-300. The result showed that out of 109 urine samples, 14 (12.8%) had non-significant bacteria growth, 45(41.3%) had no growth, and 50 (45.9%) were found with significant bacterial growth, only them were included in the present work. Only 17/50 have pyelonephritis and 33/50 have cystitis.

The results of bacterial isolates were 30/50 (60%) *Escherichia coli*, 6/50 (12%) *Klebsiella pneumoniae*, 5/50 (10%) *Pseudomonas aeruginosa*, 4/50 (8%) *Proteus mirabilis*, 3/50 (6%) *Enterococcus facalies*, 1/50 (2%) *Achromobactin xylosoxidans*, and 1/50 (2%) *Streptococcus sanguinis*. The mean of urinary LL-37 levels showed significant differences ($P \leq 0.05$) between patients (2251.99+411.068) and control (627.4092$\pm$204.94316). Also, the difference was significant between pyelonephritis patients (2519.616$\pm$330.902) and cystitis (2114.12$\pm$382.554) patients. The highest urinary LL-37 levels were in

patients with *P. mirabilis*, *E. faecalis*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *A. xylosoxidans*, and *S. sanguinis*, respectively.

The comparison study revealed that there was a significant difference in urinary HNP (1-3) levels between patients (5.385±1.236) and control (0.904±0.523), meanwhile Mean of HNP (1-3) in pyelonephritis patients (5.8988±1.406) was much greater than cystitis (5.1203±1.066) patients. The highest urinary concentration of HNP (1-3) was in patients with *E. faecalis*, *P. aeruginosa*, *A. xylosoxidans*, *E. coli*, *K. pneumoniae*, *P. mirabilis*, and *S. sanguinis*, respectively. Statistical analysis indicated that was a strong positive correlation between WBCs and both Neutrophil count (0.843**) and CRP (0.598**) in blood. In addition, present study results showed no relationship between HNP (1-3) and Cathilciden (0.350) at level $P \leq 0.005$.

This study concluded that the elevated level of CRP and WBC in pyelonephritis patients is much more than cystitis, and urinary levels of HNP1–3 and LL-37 were much greater in patients in comparison with healthy control, and there is a positive correlation between urinary LL-37 and HNP (1-3) in UTI patients, and there is a positive strong correlation between blood WBC and CRP in UTI patients.

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Abbreviations

Acute Phase Reactants	APR
Asymptomatic Bacteriuria	ABU
Antimicrobial Peptides	AMPs
Acute Pyelonephritis	APN
Cathelicidin antimicrobial peptide	CAMP
Community Acquired Urinary Tract Infections	CAUTIs
Complete Blood Count	CBC
Chemokine Ligand 20	CCL20
C-reactive protein	CRP
Distilled Water	D.W.
Dendritic Cells	DCs
Ethylene-diamine-tetra-acetic acid	EDTA
Enzyme-Linked Immunosorbent Assay	ELISA
Functional Constipation	FC
Human Defensin	HDs
Human Neutrophil Peptide	HNP
High-sensitivity C-reactive protein	HS-CRP
Lipopolysaccharide	LPS
Natural Killer (NK) Cells	NKCs
Optical Density	OD
Red Blood Cell	RBC
Round Per Minute	RPM
Tamm-Horsfall protein	THP
Tumor necrosis factor	TNF
Triple Sugar Iron Agar	TSI
Uropathogenic *Escherichia coli*	UPEC
Urinary Tract Infections	UTIs
Vesicoureteral Reflex	VUR
White blood cell	WBC

Chapter One

Introduction and Literatures Review

1.1 Introduction:

Urinary tract infection (UTI) is one of the most prevalent types of bacterial infections in children where it affects 1% of boys and 1-3% of girls. The first UTI in a girl usually occurs before the age of five years, with peaks during infancy and toilet training, while boys being more susceptible in the first year of life, especially the uncircumcised (Roberts, 2017). The urinary system is constantly exposed to enormous amounts of bacteria, due to the proximity of the urethra to the rectum which makes it prone to infections. UTI also could occur secondary to hematogenous dissemination, where Group B *Streptococcus*, *Staphylococcus aureus*, *Candida*, and *Salmonella* could cause pyelonephritis through the hematogenous pathway, especially in infancy (Bell and Mattoo, 2009: Tewary and Narchi, 2015). Despite the unusual design, the majority occurrences of urinary tract infections in children are caused by anatomical or functional issues with the distal collecting system of the kidney and/or the bladder (Zasloff, 2007).

Infants often present late in the course of illness because of nonspecific signals of UTI, such as fever, the inability to express emotions or pinpoint where discomfort is felt. Early signs of UTI, such as dysuria or stomach pain, are usually localized in older children, and they emerge sooner in the clinical course. UTIs are caused by bacteria invading the urethra and ascending through the urinary tract to the bladder. When the infection and inflammatory reaction are limited to the bladder, cystitis develops. While Pyelonephritis, is a type of upper urinary tract infection (UTI), develops when bacteria and their inflammatory response spread to the ureters and kidneys (Bell and Mattoo, 2009; Flores-Mireles *et al*., 2015). Bacteria persist without generating symptoms or disease in children with asymptomatic bacteriuria (ABU) (Ragnarsdóttir and Svanborg, 2012).

The majority of pediatric UTIs are caused by Gram negative coliform bacteria that enter and ascend the urinary tract from fecal flora colonizing the perineum. The most frequent uropathogen is *Escherichia coli* (*E. coli*), Which causes around 80% of paediatric UTIs, because uropathogenic *E. coli* strains have special features, such as fimbriae that bind to the uroepithelial cell surface. *Klebsiella*, *Proteus*, *Enterobacter*, and *Enterococcus* species are among the most prevalent uropathogens (Edlin *et al*., 2013: Tullus, 2019).

The antibacterial defense of the urinary system is almost entirely dependent on innate immunity. Where innate immune response acts as the first line of defense against microbial attack and leads to adaptive immune system activation that plays a role in the kidneys', urinary tracts, and other organ systems' innate immune defense (Zasloff, 2007; Spencer *et al*., 2014). Tamm-Horsfall protein, lipocalin ,lactoferrin, and inducible bactericidal antimicrobial peptides such as α - and β - defensins and cathelicidin are released to prevent bacteria from attaching to the epithelia lining the urinary system (Babikir *et al*., 2018).White blood cells and/or epithelial cells produce AMPs, which are either constitutively expressed or increased when pathogens enter the urinary system (Spencer *et al*., 2014). Defensins and cathelicidin are the most well-studied AMPs in humans. Defensins are characterized by being compact peptides with a highly organized structure limited by three sets of disulfide links, and they are divided into classes based on how they fold (Zasloff, 2007) .Based on structural characteristics, two types of human defensins have been identified: Defensins α and β (Yang *et al*., 2004).

Human α-defensin molecules have been identified in six distinct forms. Because they are mostly expressed in neutrophils, human-defensin 1, 2, 3, and 4 are also known as human neutrophil peptide (HNP 1, 2, 3, and 4). The other two human α-defensins, human defensin HD-5 and HD-6, are expressed constitutively

in the Paneth cells of the small intestine epithelium, As a result, they're known as enteric defensins (Nagaoka *et al*., 2012).

Human Neutrophil Peptides (HNP1–3) is a group of three bactericidal α-defensins that are closely related so they are normally tested in aggregate (Watson *et al*., 2016). While Cathelicidins (also known as LL-37) are antimicrobial peptides produced by humans and animals in response to a variety of pathogenic organisms. In the case of inflammation, they serve as a major defense against bacteria and other pathogens. Bacteria and fungus are killed, and bacterial biofilms are inhibited and destroyed. Cathelicidins can destroy the membranes of many microorganisms and cancer cells directly and selectively, but they do not harm normal cells (Bandurska *et al*., 2015).

Aim of study:

The aim of the study was to find the correlation between the types of bacteria that cause UTI and some antimicrobial peptides in urine among Upper and Lower pediatric UTIs. The following steps are taken to achieve this aim:

1. Assessing the distribution of the bacterial pathogens by isolation and identification using the Vitek2 system.
2. Estimation of urinary HNP1-3 and LL-37 levels as an antimicrobial peptide in children with UTIs by using ELISA technique.
3. Investigate the difference in WBC and CRP in children with cystitis and pyelonephritis.

1.2 Literatures Review:

1.2.1 Urinary Tract Infection (UTI):

Urinary tract infection (UTI) is a frequent infection in children, and early identification and treatment are critical for reducing the morbidity associated with it. The likelihood of a first-time symptomatic UTI is highest in boys and girls during the first year of life, and then declines significantly after that (Morris and Wiswell, 2013; Simões and Oliveira, 2015; Leung *et al*., 2019). In comparison to boys, girls have a shorter distance to the bladder, that helps gut flora invade the bladder and live around the urethra, but they are usually rinsed out during micturition. Also, the urethral opening of girls is close to the rectum which makes urethra opening constantly exposed to gut bacteria. In addition, the antibacterial defenses supplied by the prostatic fluid, make UTIs in males is far less common (Bell and Mattoo; 2009; Geerlings, 2017; Tyagi *et al*., 2018).

UTIs have a wide range of symptoms, especially in infants and children. Fever is frequently the only symptom. However, all children with fever who are diagnosed with clinical findings such as pollakiuria, dysuria, flank pain, drop-by-drop urination, hematuria, flammable and cloudy urine should be considered to have a UTI (Başoğlu and İşlek, 2019; Simões and Oliveira, 2015). Confirmation of UTIs should be obtained with a urine culture, which must be conducted prior to the administration of any antimicrobial medication, although a clinical history and physical examination may imply UTI. Renal and bladder ultrasonography should be performed on febrile infants with UTIs (Simões and Oliveira, 2015).

The host's health determines how severe the infection is, in which Uropathogenic *Escherichia coli* is the most prevalent cause of uncomplicated infections, but *Proteus* mirabilis is more commonly the cause of severe infections and affects patients with underlying conditions including urinary tract abnormalities or catheterization (Nielubowicz and Mobley, 2010). UTI can be

caused by a variety of organisms, but *Escherichia coli* is the most prevalent, accounting for 80 to 90 percent of UTI in children. The other prevalent bacterial uropathogens in UTIs are *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, and *Enterobacter cloacae* (Foxman, 2013; Leung *et al*., 2019). Urine sampling through suprapubic aspiration or catheterization has a low contamination rate and confirms the presence of a UTI. A UTI can only be ruled out using a plastic bag and a test that is negative for both leukocyte esterase and nitrite, or microscopic investigation that is negative for both pyuria and bacteriuria. Based on clinical findings and a positive urinalysis, a clean voided midstream urine sample after cleaning the external genitalia provides good diagnostic accuracy in toilet-trained children with UTI to eradicate the infection and improve clinical result. Local data on antibiotic resistance patterns should be considered while selecting drugs (Stein *et al*., 2015; Leung *et al*., 2019).

Bacteriuria is defined as a presence of at least 10^5 bacteria per milliliter of fresh, unspun midstream urine. Stamm, who proposed a more sensitive meaning of at least 10^2 bacteria per milliliter of urine, has contested this concept. Both definitions are widely recognized at the moment. A pure culture of at least 10^2 bacteria per ml of urine in a symptomatic transplant patient with pyuria is diagnostic. Asymptomatic bacteriuria should be defined as at least 10^5 bacteria per ml of urine (Ness and Olsburgh, 2020).

Pyuria defined by the presence of ≥5 urinary white blood cells per high-powered field of unspun midstream urine. According to the most recent American Academy of Pediatrics guideline, pyuria should be present when diagnosing a UTI (Forster *et al*., 2018). Pyuria is a symptom of an inflammatory response in the urothelium, which usually occurs in response to pathogenic invasion. Pyuria can be caused by bacteriuria (bacterial urinary tract infection) or it can be asymptomatic (related to tuberculosis, malignancy, or adjacent non-

urinary-tract pathology) (Forster *et al*., 2018; Jones-Carson *et al*., 1999). Pyuria was unlikely to be caused by a UTI, and it's worth noting that the proportion of pyuria patients who had a UTI was likely overstated (Hooker *et al*., 2014)

1.2.2 Classification of UTI:

Urinary tract infections can be classed by their complexity into complicated and uncomplicated UTIs (Hooton, 2012) or by their symptoms into cystitis, pyelonephritis, and urosepsis, with urosepsis being the most severe kind and pyelonephritis being more severe than cystitis (Smelov *et al*., 2016)

1.2.2.1 Uncomplicated and Complicated UTIs:

Uncomplicated UTIs are more likely to develop in otherwise healthy individuals, like males between the ages of 15 and 50, particularly in those who are sexually active and uncircumcised, as long as they do not have any risk factors for complicated UTIs, such as urologic abnormalities, bladder outlet obstruction or recent urinary tract instrumentation. Both the lower urinary tract or the upper urinary tract can be affected by an uncomplicated UTI and it can be developed to cause bacteremia (Hannan *et al*.,2012, Hooton, 2012; Nielubowicz and Mobley, 2010). Complicated UTI, as contrast to uncomplicated UTI, is an infection that arises in a patient who has a structural or functional abnormalities, 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 (Flores-Mireles *et al.,* 2015; Kalra and Raizada, 2009).

1.2.2.2 Cystitis, Pyelonephritis and Urosepsis:

Cystitis is an inflammation of the bladder epithelium that is the most common site of a urinary tract infection (UTI), which is commonly develops as a result of bacteria from feces or vaginal flora that colonizing the periurethral mucosa and ascending to the urinary bladder. When bacteria enter the urinary

tract through the urethra and into the bladder, causing infection and inflammation. In the absence of vaginal discharge and vaginal discomfort, dysuria and frequency are significantly predictive of cystitis. As mentioned above, it can be divided into two categories: uncomplicated or complicated (Birder, 2019; Kolman,2019). Uncomplicated cystitis refers to lower urinary tract infection (UTI) in either non-pregnant women or men who are otherwise healthy but it linked to variables that raise the likelihood of infection or failure to respond to antibiotic treatment (Goldman and Julian, 2019; Duane *et al*., 2019). Uropathogens have virulence characteristics that allow them to bypass host defenses and infiltrate tissues of urinary tract. Due to the longer physical urethra and antibacterial defenses supplied by the prostatic fluid, UTI in males is far less common. (Tyagi *et al*., 2018).

Acute Pyelonephritis (APN) is a bacterial infection that causes kidney inflammation and is one of the most frequent kidney illnesses. Pyelonephritis is a consequence of a UTI that extends from the bladder to the kidneys and their collecting systems and is characterized by an infection of one or both of the renal parenchyma and renal pelvis, often producing localized flank or back pain combined with systemic symptoms such as fever, chills, and nausea (Morello *et al*.,2016; McCance *et al*., 2018). APN can be divided into uncomplicated and complicated. Complicated pyelonephritis includes pregnant women, people with uncontrolled diabetes, people who have had kidney transplants, people who have had urinary anatomical abnormalities, people who have had acute or chronic kidney failure, people who are immunocompromised, and people who have had hospital-acquired bacterial infections (Morello *et al*., 2016). APN typically manifests suddenly with signs and symptoms of both systemic and bladder inflammation (e.g., fever, chills, and malaise, urinary frequency, urgency, and dysuria), but up to 20% of individuals have no bladder symptoms, and some don't even have a temperature (Piccoli *et al*., 2006). Symptoms of

APN include fever, flank pain, nausea, vomiting, burning while urinating, increased frequency, and urgency, as well as isolation of the pathogen from urine (Bennett *et al*.,2015; Morello *et al*.,2016).

A urogenital tract infection causes urosepsis, which is a serious condition of sepsis accounts for around a quarter of all sepsis cases, and is usually caused by severe urinary tract infections. In about 10–30% of cases of severe sepsis or septic shock, the infection site is the urinary tract. It's mostly caused by ureterolithiasis, which is the most common cause of blocked uropathy of the upper urinary tract. Fever or hypothermia, tachycardia, tachypnoea, hypotension, oliguria, and leukopenia should all be recognized as early indicators of probable multi-organ failure. Proinflammatory cytokines are generated when pathogen or damage-associated molecular patterns are recognized by pattern recognition receptors in the host innate immune system, kicking off the intricate pathophysiology of sepsis. The adaptive immune system takes over until a TH2 antiinflammatory response takes over, resulting in immunosuppression (Wagenlehner *et al*.,2011; Wagenlehner *et al*., 2013; Grabe *et al*., 2015). Antimicrobial resistance is more likely when aggravating conditions are present, and treatment response is generally poor, even when medicines active against the primary microbial infections are used. Severe consequences are also common, leading to urosepsis, renal scarring, and possibly end-stage renal disease (Kalra and Raizada, 2009) .

1.2.3 Epidemiology:

UTI is One of the most prevalent bacterial diseases in children,where identified in 1% of males and 1-3 % of girls (Roberts, 2017). In Specific, UTI rates vary according to age, gender, race, and circumcision status, where the highest baseline prevalence of UTI was found in uncircumcised male newborns under 3 months of age, 1 in 12 circumcised males get a UTI during their

lifetime, and female infants under 12 months of age (Shaikh *et al*., 2008; Morris and Wiswell, 2013; Leung *et al*., 2019).

At least one recurrence will occur in one-third to one-half of affected children, which is more typically due to unresolved bacteriuria or chronic bacteriuria than reinfection. Permanent renal cortical scarring can occur in 15% to 65% of children with recurrent UTI, and long-term effects can include hypertension and chronic renal insufficiency, which can lead to end stage renal disease (Paintsil, 2013; Zaffanello, 2015; Kavitha *et al*., 2017).

1.2.4 Risk factor:

1.2.4.1 Age and Sex:

The first symptomatic UTI is most common in children under the age of one year, with males being more impacted than females (Roberts, 2017). Boys and girls have the same chance of acquiring UTI throughout infancy, but girls have a higher risk after that.(RN Srivastava, 2011).

Males are more likely to be born with structural abnormalities of the urinary system, which makes UTI more common in the first six months of life. (Shaw *et al*., 1998). In the pre-school years, females are 10 to 20 times more likely than boys to get symptomatic infections (Schlager, 2017)

1.2.4.2 Circumcision:

In uncircumcised males, the periurethral area was shown to be more frequently and intensively colonized with uropathogens, particularly *Escherichia coli*, than in circumcised males, which tend them to have a higher risk of UTIs (Bettcher *et al*., 2021).That is because germs migrate up the urethra and colonize the bladder because uncircumcised males dwell in a warm, wet, and mucosal environment (Böhling *et al*., 2006). In uncircumcised infant male, urinary tract infection can progress to renal parenchymal disease of the still-developing pediatric kidney (Morris and Wiswell, 2013). One of the most prevalent reasons for circumcision of newborn boys is to reduce the risk of

urinary tract infection. UTI rates have been shown to be 10-20 times greater in uncircumcised boys than in circumcised boys in previous research (To *et al*., 1998).

1.2.4.3 Constipation:

Functional Constipation (FC) is a frequent pediatric condition, and one of the leading causes of recurrent urinary tract infections, because it causes stool to linger in the rectum for an extended period of time, allowing bacteria to proliferate in the perineum (Salvatore *et al*., 2011; Rowan-Legg *et al*., 2011, Sarvari *et al*., 2017). The rate of FC in children has ranged from 4% to 37% , and study observed that children with FC are more likely to get a urinary tract infection (UTI) than children who do not have FC (Van Summeren *et al*., 2018).

The prevalent incidence of bowel and bladder dysfunction is due to the fact that they share a similar route, where the pathophysiology of this common pathway is unknown, although it is assumed to have two basic processes (de Araújo Sant'Anna and Calçado, 1999; van der Wal *et al*., 2005) .First, because the bladder and colon are so close together, Significant amounts of feces in the rectum may exert direct pressure on the posterior bladder wall, resulting in bladder emptying and storage problems (Rajindrajith *et al*., 2016; Averbeck and Madersbacher, 2011). Second, there could be a neurogenic issue. In the hindgut, the genitourinary tract and the gastrointestinal system have the same embryologic origin. Because normal functioning of the pelvic organ systems requires neural pathway cross-sensitization, dysfunction of one of the two organ systems could lead to dysfunction of the other (Malykhina, 2007; Kaplan *et al*., 2013).

1.2.4.5 Anatomic or Physiological anomalies:

The urinary tract is a series of connected hollow organs whose major role is to collect, transport, store, and release urine on a regular and well-coordinated basis. Any anatomical or functional abnormalities of the urinary tract that

obstruct urine flow can make the host more vulnerable to UTI and the process of constant urine flow in the upper urinary tract and intermittent elimination from the lower urinary tract plays a crucially important part in cleansing the urinary tract, ridding it of microbes that might have already gained access (Kostakopoulos *et al*., 2021; Hickling *et al*., 2017).Short urethra in females, Vesico-ureteral reflux (VUR), urinary blockage, neurogenic bladder (Urine storage in the bladder is improper, and urine emptying from the bladder is incorrect), posterior urethral valves or bladder diverticulitis, and uncircumcised in boys are all anatomic anomalies. Functional voiding, infrequent voiding, inadequate bladder emptying, and constipation are all physiologic variables (Salvatore *et al*., 2011).

1.2.4.6 Diabetes mellitus:

Diabetes mellitus and infections are frequently linked, and diabetes patients have an increased risk of UTIs (Sewify *et al*., 2016; Calliari *et al*., 2020). Because high levels of glucose in the renal parenchyma promote the growth and proliferation of bacteria, which could be one of the causes of pyelonephritis and renal complications such as emphysematous pyelonephritis, where glucose provide carbon for bacteria to generate energy (Park *et al*., 2006; Schneeberger *et al*., 2014; Calliari *et al*., 2020). Furthermore, greater glucose levels in the urine may promote the growth of bacteria that increase the severity of infection (Fünfstück *et al*., 2012; Wang *et al*., 2013). Also, pediatric patients with diabetes have some immune system disorders that, when associated with high glycemia, increase the risk of infections and their severity (Calliari *et al*., 2020).

1.2.4.7 Catheterization:

UTIs acquired in the hospital are connected with a urinary catheter (indwelling urinary catheters) in around 75% of cases. In the United States, catheter-associated urinary tract infection accounts for about 35% to 40% of all

hospital-acquired infections, (Strouse, 2015). The majority of non-catheter-associated UTI in the community are caused by well-adapted uropathogenic *E. coli*, despite the fact that the diversity of species linked to community-acquired UTI (CAUTI) is greater, but in general Catheter-associated bacteria are assumed to be mostly derived from the patient's gut microbiota. For example, *Enterococci* are rarely associated with CAUTI but are among the most frequently isolated pathogens from polymicrobial communities on the surface of indwelling urinary catheters and biliary stents, and play a crucial role in the pathogenesis of CAUTI (Kline and Lewis, 2017) .

1.2.4.8 Stones:

UTIs were observed in 20% to 25% of children with nephrolithiasis. Where stones either develop in conjunction with an underlying metabolic or anatomical problem in the urinary tract, predisposes children to stasis and infection, which promote stones formation. Or infection could the primary cause of a stone, Because of bacterial ability to produce urease enzyme that closely linked to pediatric nephrolithiasis. And an example of bacteria that causes urinary tract stones are *Proteus, Providencia, Klebsiella, Pseudomonas*, and *Enterococci*. Because male is more likely to have obstructive uropathy, 80% of children with stones associated with infections are boys (Smith and Stapleton, 2015; Schwaderer *et al*.,2017)

1.2.4.9 Improper Toilet Training:

Improper toilet training is sufficient to cause UTIs, especially in girls, because toilet habits such as wiping from the back to the front or incompletely empty their bladder have a strong influence on the development of urinary tract infections due to fecal flora contamination of the urogenital system (Mazzola *et al*.,2003; Gebremariam *et al*.,2019). Girls are more susceptible to UTIs than boys, so proper toilet training for girls like wipe from front to back after bowel

movements gave ability that reduces the risk of infection (Nijman *et al*., 2002, ; Lorenzo *et al* .,2020; Zhu *et al*., 2020)

1.2.4.10 Inadequate Fluid Intake:

Studies approved a logical link between fluid intake and the incidence of UTI, where excessive fluid consumption will result in a larger urine volume. Larger urine volume which increases micturition process that in turn leads to the eradication of bacteria by prevents bacterial growth in the urinary tract through urine and mucus flow, which interfere with bacterial adhesion (Lean *et al*., 2019; Fasugba *et al*., 2020)

1.2.4.11 Incomplete Bladder Emptying:

In a normal situation, the bladder is completely empty each time it passes water. This was the most important defense mechanism against urinary tract infection which removes 99% of bacteria. Usually incomplete emptiness of the bladder is regarded as a factor that allows pathogenic bacteria to grow in urine as a result of not fully empty of the bladder (SHARIF, 2008). Patients with incomplete or absent bladder emptying present a particular challenge, as both their pathologic processes and bladder management strategies (Dray and Clemens, 2017).

1.2.5 Causative Agents of UTI:

Urine is being generally considered to be sterile and is believed to be free from any microorganisms but because of the close closeness of the urethra to the gut, uropathogenic *Escherichia coli* (UPEC) colonization is common, especially in catheterized patients. Ascending movement via the ureter can cause kidney damage and hematogenic spread by causing inflammation and protease release. *E. coli* was responsible for more than 80% of UTIS cases (Foxman, 2013; Flores-Mireles *et al*., 2015).

UPEC strains have a diverse set of virulence characteristics that allow them to adhere (fimbriae), defend and invade against immune cells

(endotoxins), travel and move up the urethral canal (flagella), feed (through iron receptors), and evade the immune system (capsule). UPEC works by disrupting urinary tract defenses and preventing neutrophils by forming biofilms. By sacrificing the exterior cells to protect the inner core, biofilms help to spread and colonize the urethra and bladder (Flores-Mireles *et al*., 2015). UPEC can evade TLR4-mediated expulsion, modify lysosomes to reduce their digesting capacity, And when released intracellularly, they stay within the autophagosome membrane to avoid phagocytosis(Abraham and Miao, 2015),As shown in figure (1-1)

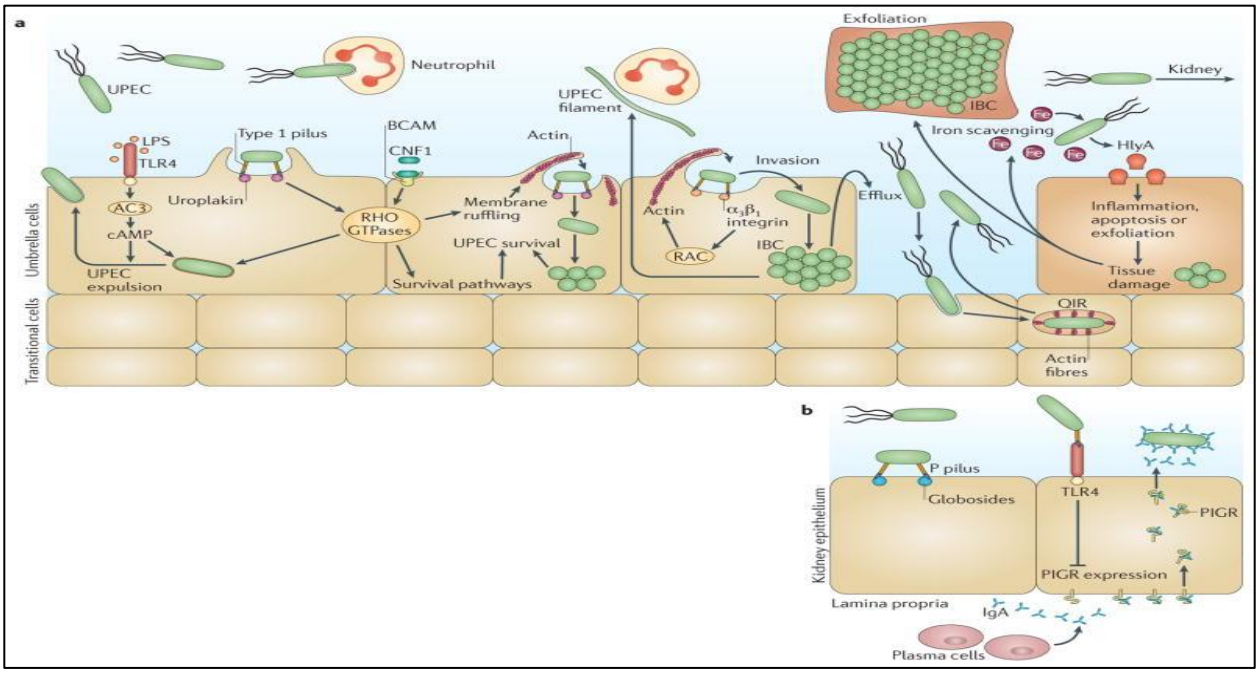

Figure (1-1) Virulence factors of Uropathogenic E.coli in UTIs(Flores-Mireles *et al*., 2015).

E. coli is the most common bacteria that causes acute pyelonephritis because of its ability to stick to and colonize the urinary tract and kidneys. P-fimbriae are sticky molecules found in *E. coli* that bind to receptors on the surface of uroepithelial cells. *E. coli* infection in the kidneys can produce an initial inflammatory response and scarring of the renal parenchyma. The exact renal scarring mechanism is unknown, it is assumed that bacterium attachment to renal cells disrupts the protective barriers, causing scarring. This resulted in

localized infection, hypoxia, ischemia, and clotting in an attempt to control the infection. Inflammatory cytokines, bacterial toxins, and other reactive processes induce complete pyelonephritis and, in many cases, systemic indications of sepsis and shock (Morello *et al.*,2016; Behzadi,2020).

The other uropathogens cause UTIs by expressing virulence factors that enhance adhesion (e.g., pili, adhesins), nutrition release (toxins), immune evasion (capsules, etc.), and iron acquisition (e.g., siderophores), 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. The occurrence of UTIs usually cause by bacteria and viral or fungal agents are a rare phenomenon (Kline *et al*., 2011; Parish and Holliday; 2012, Palou *et al*., 2013). *Staphylococcus aureus* infection is uncommon in children without indwelling catheters or other sources of infection, however coagulase-negative *staphylococci* and *Candida* spp. are linked to infections after urinary tract instrumentation (Schalger, 2001) .

1.2.6 Pathogenesis of Urinary Tract Infection:

The bacteria can enter and spread via the urinary tract through two primary pathways: ascending and hematogenous, but there is scant evidence that infection spreads to the urinary tract via lymphatics(Vasudevan, 2014). The hematogenous route, which is less common in newborns and children, and occurs mostly in individuals with ureteral blockages or those who are immunocompromised and weak. Through the hematogenous pathway, bacteria such as Group B *Streptococcus*, *Staphylococcus aureus*, *Candida*, and *Salmonella* can cause inflammation in the renal parenchyma and causeus pyelonephritis (Jones-Carson *et al*., 1999; Schwenger *et al.*, 2015).

In humans, blood-borne pathogens can cause UTI, however, this is less common than the ascending route. In patients with bacteremia or endocarditis

caused by gram-positive bacteria, such as *Staphylococcus aureus*, the kidney is commonly the site of abscesses, while infections of the kidney caused by Gram-negative bacteria are rare (Vasudevan, 2014). Except for the first 8 to 12 weeks of life, when infection of the urinary tract may be caused by a hematogenous source, UTI is thought to be caused by bacteria entering the urethra via the ascending route, and most patients will develop acute pyelonephritis this way (Schalger, 2001).

The ascending pathway is the more common mode of infection, which occurs in a series of steps. First fecal flora gains access to the urinary tract via colonization of the urethra and adheres to urethral mucosal epithelial cells, after that, they travel to the bladder. The ascending pathway of infection could be also caused through urinary instrumentation like catheter (Morello *et al*.,2016).

The microorganism's adhesion is determined by three major environmental factors: first, the bacteria's own adhesive properties, second, the urothelium's receptive features, and third, the fluid present between both surfaces, after adhering to the mucosal surface, bacteria will travel proximally and trigger an inflammatory reaction in the host (Manjula *et al*., 2013; Flores-Mireles *et al*., 2015). After the uropathogen adheres to the mucosal surface, the host trigger a TLR4-dependent, lipopolysaccharide (LPS)-stimulated inflammatory response from bladder epithelial cells, and promotes the expression of an inflammatory cytokines and neutrophil chemo attractants. Within hours of infection, epithelial cells exfoliate, and infected urothelial cells are shed during this process (Mirzaei *et al*.,2020). The progression of infection is determined by the type of organism, the amount of the inoculum, and the effectiveness of the host's defenses. When bacteria enter the bladder, they may grow before passing up the ureters and into the renal parenchyma, especially if vesicoureteral reflux is present (Vasudevan, 2014; Delcaru *et al*., 2017).

An important component in the development of urinary infection is anomalies of the urinary system that produce urine flow blockage. Incomplete bladder emptying owing to prostatic hyperplasia is more common than extrarenal blockage in infant boys or adult males due to posterior urethral valves or urethral strictures. The development of UTIs is also aided by bladder dysfunction caused by mechanical (prostate and pelvic floor relaxation) or neurological reasons (Vasudevan, 2014; Delcaru *et al*., 2017).

1.2.7 Host Immunity Against UTIs:

The urinary tract is constantly exposed to a range of microorganisms that colonized the gastrointestinal tract, and it is generally well prepared to resist these microorganisms' infections. This infection resistance is largely due to the urinary tract immune system's versatility, which is accomplished through the interaction of various molecules and cells involved in both innate and adaptive immune responses (Ortega Martell, 2020).

1.2.7.1 Innate Immunity:

The body's initial line of defense against pathogens entering the body is the innate immune system. It responds to all pathogens and other chemicals in the same way, which is why it's sometimes called the "nonspecific" immune system. Innate immune systems are important in protecting the urinary tract from uropathogens and pathogen susceptibility increases when these innate defenses are compromised or dysregulated(Ching *et al*., 2020). The urothelium is the primary barrier to infection in the urinary tract that have multiple layers of infection-resistant multinucleated umbrella cells and uroplakins, which are glycoprotein plates, are present, as are mucus glycosaminoglycans, which inhibit pathogen adherence. As mentioned above, exfoliation releases apoptotic infected epithelial cells into the bladder lumen, lowering the bacterial load and the inner basement stromal cells replace the dead cells and produce new urothelium. Uropathogenic *Escherichia coli* (UPEC) are prepared through

complex adhesive interactions to target the epithelial cell surface layer and then engage additional tissue compartments through a variety of toxic mucosal disturbances, often followed by invasion (Nielubowicz and Mobley, 2010; Nielubowicz and Mobley,2010; Ortega Martell,2020). Both the epithelium and the interstitial compartment contain immune cells. Dendritic cells, macrophages, neutrophils, and lymphocytes collaborate to fight microbes in the upper urinary tract. Mast cells, macrophages, neutrophils, and especially natural killer (NK) cells fight bacterial colonization in gastrointestinal and urinary tracts (Spencer *et al*., 2014; Abraham and Miao, 2015). Toll-like receptor 4 (TLR4) on superficial bladder epithelial cells triggers a varied innate immune response that aims to eliminate infection by Gram-negative microorganisms, as well as a possible lesser or different role for adaptive immunity (Ortega Martell, 2020). To successfully defend against uropathogens, antimicrobial peptides, pentraxin, uromodulin, and other local factors produced by the urothelium, as well as secretory IgA produced by specific B lymphocytes, can be used (Ortega Martell, 2020). As showed in figure (1-2).

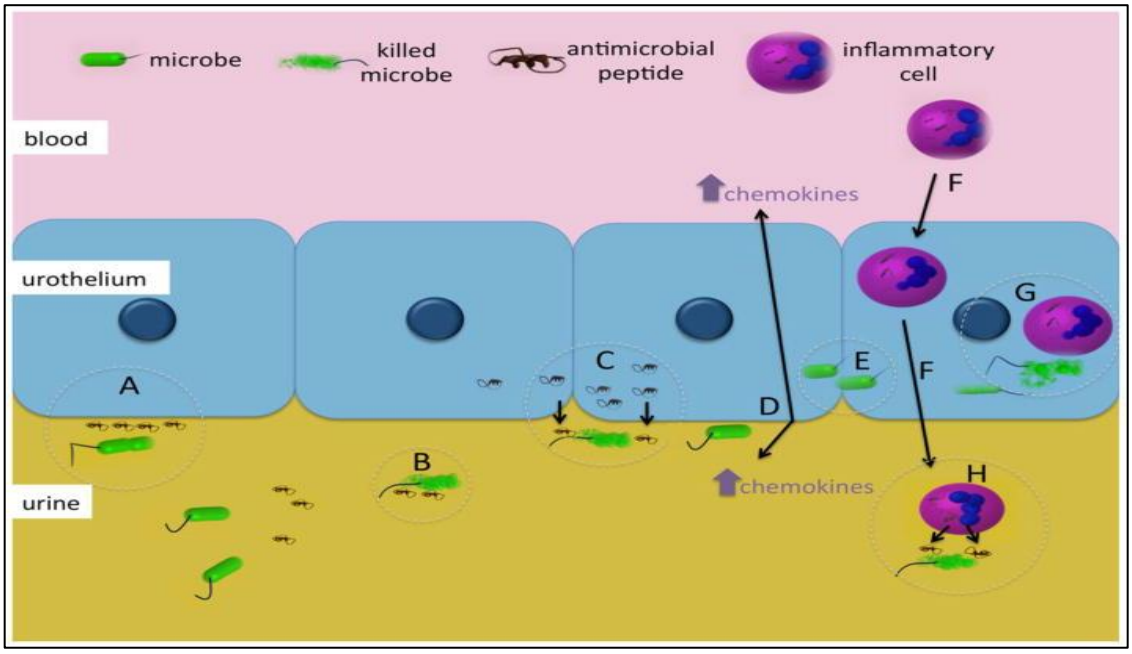

Figure (1-2) Innate immune mechanisms in the urinary tract (SPENCER *et al*., 2015).

Microbes enter the urinary system and come into contact with constitutively produced AMPs, which can prevent them from adhering to the urothelium. (A) or cause lysis of bacteria (B). When bacteria adhere to the urothelium, they can cause AMP synthesis, which causes adherent bacteria to die. (C) when bacteria connect to the urothelium and enter it, they can trigger the creation of chemokines (D/E) Inflammatory cells are drawn to the urothelium by these substances (F). Phagocytosis is the mechanism through which these cells control infection. (G) and intracellular AMP secretion (Zasloff, 2007; Weichhart *et al*., 2008; Ali *et al*., 2009)

The innate immune response, as well as physical parameters like urine flow, pH, and ionic composition, as well as the expression of endogenous antimicrobial peptides, all work together to protect against infection (AMPs) (Spencer *et al*., 2014, Ragnarsdóttir *et al*., 2011).

It is essential that the immune response react quickly to stop infection without causing more damage to the urothelium, and that the balance of pro-inflammatory and anti-inflammatory mechanisms is critical to avoid excessive damage to the urothelium and recurring infection (Ortega Martell, 2020).

1.2.7.1.1 Antimicrobial Peptides (AMPs):

Is a large family of compounds that are widely distributed across the organism, mainly in mucus layers. They are designed to prevent pathogens from colonization and increasing the permeability of bacterial cell membranes, resulting in bacterial cell suppression or death.Among these AMPs , defensins,cathelicidins , Tamm-horsfall protein (THP), Lactoferrin and lipocalin could be found (Hwang *et al*., 2014; Fabisiak *et al*., 2016; Babikir *et al*., 2018).

Human cathelicidin (LL-37):

A small soluble antimicrobial peptide (AMP) is the only member of the cathelicidins family discovered in humans so far. By targeting virulence factors,

it serves as a crucial component of the urinary tract's innate defense against infections, including UPEC (Nielsen *et al*., 2014; Abraham and Miao, 2015; Harcourt *et al*., 2016) . It was first discovered in bone marrow cells in 1995, and it was given the name LL-37 because the mature antimicrobial peptide comprises 37 amino acid residues, beginning with two leucines *(*Agerberth *et al*., 1995; Ramos *et al*., 2011). The amino acid sequence of although the physical characteristics of these effector molecules of the innate immune system of several vertebrates varies, they share physical characteristics such as positive charge and amphipathicity (Van Harten *et al*., 2018) . The peptide is specifically secreted in neutrophil secondary granules, but Macrophages, natural killer (NK) cells, and epithelial cells of the skin, airways, eyes, and gastrointestinal tract, also produce it (Esfandiyari *et al*., 2019). The secondary structure and cationic character of the LL-37 peptide, which are conferred by arginine and lysine residues, determine its bactericidal and immunomodulatory properties *(Koro et al*., 2016; Van Harten *et al*., 2018; Esfandiyari *et al*., 2019). LL-37 has many important functions, one of them is chemotaxis, which causes mast cells to migrate to the infection site (Niyonsaba *et al*., 2002). It's is also involved in mast cell degranulation, the release of inflammatory mediators, the enhancement of phagocytosis by opsonizing bacteria and upregulating bacterial recognition receptors, and the enhancement of DNA/RNA uptake, all of which contribute to increased intracellular toll-like receptor (TLR) signaling (Schiemann *et al*., 2009, Van Harten *et al*., 2018).

On the other hand, LL-37 is responsible for the synthesis of numerous cytokines and antibodies and has many immunomodulatory properties. For example, during monocyte-macrophage differentiation, 10 g/ml of LL-37 peptide causes a positive inflammatory response. This peptide is also important for macrophage growth and cytokine production, resulting in a drop in

interleukin 10 levels and an adjustment of 12p40 (Hwang *et al*., 2014, Esfandiyari *et al*., 2019, van der Does *et al*., 2010).

It's can also cause epithelial cells to migrate, influencing wound healing. Finally, cathelicidins can alter cell differentiation by polarizing macrophages toward an inflammatory (M1) phenotype. Cathelicidins also show a direct killing effect against Gram-positive and Gram-negative bacteria by binding and interaction with negatively charged bacterial cell membranes, causing electrochemical potential changes on the membranes, cell membrane degradation, and the penetration of bigger molecules like proteins, damaging cell shape and membranes, and eventually cell death (van der Does *et a*l., 2010; Van Harten *et al*., 2018; Esfandiyari *et al*., 2019; Lei *et al*.,2019). Cathelicidin functions summarized in figure (1-3).

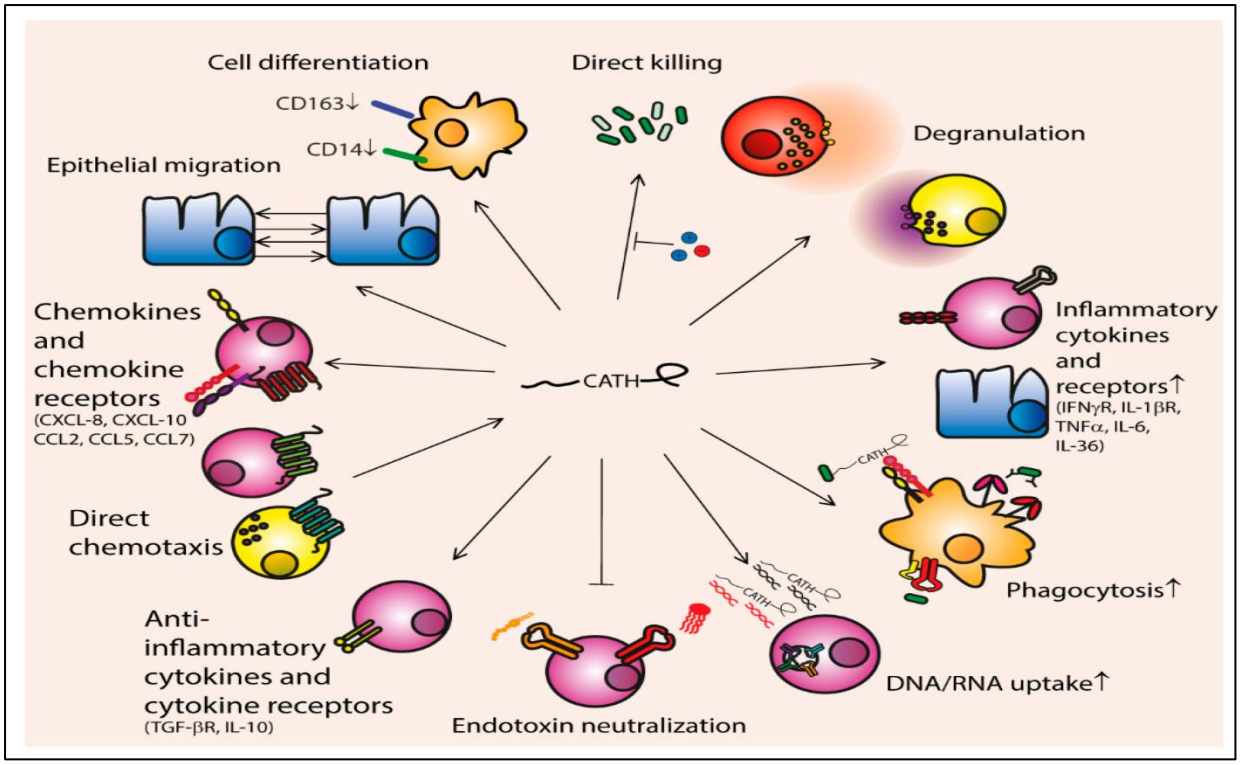

Figure (1-3) Summary of cathelicidin functions (Van Harten *et al*., 2018)

Defensin:

Defensins are amphipathic cationic peptides containing six cysteine residues linked by three disulfide bridges. They're classified into three groups :

α, β , and θ defensins .The structural differences between the three groups are reflected in antibacterial activity and serum stability (Falanga *et al*., 2017). Humans produce six α-defensins (29–35 aminoacids), which are classified as myeloid (HNP1-4) or enteric (HD5 and HD6) peptides based on their expression patterns, that are released after bacterial infection from promyelocytes, neutrophil precursor cells, and intestinal Paneth's cells (Sankaran-Walters *et al*., 2017).

HNP1-4 expression is usually seen in neutrophils; which account for more than 7% of the total protein composition in the azurophilic granules of neutrophils. However, it can also be found in monocytes and macrophages, T cells, B cells, and immature dendritic cells (DCs). Paneth cells in the intestine express HD5 and HD6, where its precursors are processed extracellularly in the Paneth cells of the intestinal crypt, and the process is mediated by a trypsin isoform. While epithelial cells in the male and female genitourinary tracts express HD5 (Falanga *et al*., 2017).

α-defensins precursors are removed by proteinase 3 and neutrophil elastase in neutrophils and are retained in their mature form in azurophilic granules. Pathogens are absorbed into phagocytic vacuoles containing defensins that causes killing to the pathogen. Defensin precursors are processed extracellularly in the Paneth cells of the intestinal crypt, and the process is mediated by a trypsin isoform (Falanga *et al*., 2017) .α-defensin has many functions that lead to killing a pathogen , main of them are represented by antimicrobial action , chemotactic activity and immune modulation :

1. Antimicrobial activity:

The ability of a substance to kill bacteria. The neutral nature of human cell membranes ensures that defensins interact selectively with host pathogens. When defensins interact with membranes, they accumulate in bacteria'

membranes and produce depolarization, which leads to pathogen death (Mattar *et al*., 2016; Falanga *et al*., 2017).

2. chemotactic activity:

α-defensins are also thought to be immune system-activating chemicals. In vitro investigations have shown that HNP1, HNP3, and HD-5 promote macrophage, T cell, and immature mast cell motility to the site of the infection (Fahlgren *et al*., 2003).

3. Modulation of the immune system:

Inflammation is induced and inflammatory responses are suppressed by acting on specific cells in different ways. Defensins can also modulate the immune response by forming a complex with cellular molecules including proteins, nucleic acids, and carbohydrates. Aside from their ability to recruit immune cells, α-defensins also play a role in the generation of pro-inflammatory cytokines. Interleukin-2, Interleukin -8 (IL-2, IL-8), Tumor Necrosis Factor-α (TNF- α), Chemokine (C–C motif) ligand 20 (CCL20), and Interferon-γ (IFN-γ) are all secreted by intestinal epithelial cells in response to HD-5 (Fahlgren *et al*., 2003; Fruitwala *et al*., 2019).

Lactoferrin and Lipocalin:

Lactoferrin, an iron-binding glycoprotein of the transferrin family with an 80-kDa molecular weight, is found in exocrine secretions including milk and saliva, as well as neutrophil granules (Wakabayashi *et al*., 2014)**.** Lactoferrin is a protein that plays a role in host defense and has a wide range of biological activities, including antibacterial, antiviral, antioxidant, immunomodulation, cell growth modulation, and binding of various bioactive chemicals (Wakabayashi *et al*., 2006).

Lipocalin proteins (lipocalins) are a large family of tiny proteins that have been found to play a key function in infections. Lipocalin binds to organic siderophores, which bacteria use to scavenge iron from the environment. It also

has bacteriostatic properties against organisms that produce siderophores in iron-deficient environments. Importantly, lipocalin-deficient mammilans are more susceptible to siderophores-producing microbes infecting them systemically (Du *et al*., 2015).

Tamm-Horsfall Protein (THP):

Tamm-Horsfall protein (THP), also known as uromodulin, is a high molecular-weight glycoprotein present in human urine. It is of renal origin, where it found in the thick ascending limb of the loop of Henle and the distal convoluted tubule and. THP is not antimicrobial in and of itself, but it works by sticking to certain mannosylated residues on uropathogenic bacterial fimbriae, primarily type 1 pili, to prevent bacteria from adhering to epithelia and thereby facilitating bacterial washout. THP can also activate dendritic cells through a TLR4-dependent mechanism, lowering the activation threshold against THP-bound uropathogenic bacteria (Wu *et al*., 2018).

1.2.7.1.2 Acute Phase Reactants (APR):

Are inflammation markers that exhibit significant changes in serum concentration during inflammation. Fever, chronic illness anemia, anorexia, somnolence, lethargy, amyloidosis, and cachexia are all side effects of acute phase reactants. Acute phase reactants are classified as positive or negative based on their serum concentrations during inflammation. Positive acute phase reactants are raised during inflammation, The concentrations of negative acute phase reactants are reduced as a result of downregulation. Procalcitonin, ferritin, C-reactive protein, fibrinogen, ferritin, hepcidin, and serum amyloid A are all positive acute phase reactants. Albumin, transferrin, prealbumin, antithrombin, and retinol-binding protein, are all negative acute phase reactants (Gulhar *et al*., 2020; Kushner, 2020).

C-reactive protein (CRP) is an acute pentameric inflammatory protein identified by Tillett and Francis in 1930. CRP was first discovered in the serum

of people with acute inflammation as a compound that responded with the pneumococcus capsule's "c" carbohydrate antibody. Whose level rises in response to inflammation, which is primarily produced by the activity of IL-6 on the gene responsible for CRP transcription during the acute phase of an inflammatory/infectious process. CRP is anti-inflammatory as well as pro-inflammatory. By attaching to phosphocholine, phospholipids, histone, chromatin, and fibronectin, it aids in the identification and clearance of invading infections and injured cells. It can activate the conventional complement pathway as well as Fc receptors on phagocytic cells, allowing it to remove cellular debris, injured or apoptotic cells, and foreign pathogens more quickly (Jungen *et al*., 2019; Kramer *et al*., 2019; Cleland and Eranki, 2020).

Native CRP (nCRP) is a homopentameric protein produced mostly by hepatocytes in the liver, but also by smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes. It can permanently dissociate into five monomers in areas of inflammation and infection (Sproston and Ashworth, 2018) .In children with fever, the qualitative CRP assay is more likely to be positive, and it can indicate considerable bacteriuria of gram negative bacteria, especially in young children (Mushi *et al*., 2019). Because C-reactive protein is significantly elevated in upper UTI and is a non-invasive diagnostic tool, it is a suitable tool for distinguishing upper UTI from lower UTI. Longer CRP monitoring could indicate high-risk patients who are more likely to develop chronic parenchymal renal disease (Mamatha *et al*., 2020).

CRP levels differ depending on age, gender, and race. Although CRP is a sensitive biomarker of inflammation, it can be elevated for a variety of reasons, including viral infections and noninfectious inflammatory conditions. Small changes in CRP levels, observed with extremely sensitive tests, can occur in the absence of acute or chronic inflammatory conditions, as they have historically been viewed (Kushner, 2015) .

1.2.7.2 Adaptive Immunity:

Adaptive immunity is the second line of defense against the pathogen presented. This part of the immune system is activated when the innate immune response is insufficient to control an infection. In fact, without information from the innate immune system, the adaptive response could not be mobilized. There are two types of adaptive responses: the cell-mediated immune response, which is carried out by T cells, and the humoral immune response, which is controlled by activated B cells and antibodies. Adaptive immune responses are often restricted, especially in the bladder. Patients with UTIs that spread to the kidneys can create antibodies specific for the infecting agent, whereas those with UTIs that only affect the bladder fail to produce an antibody response for unexplained reasons. This apparent deficiency in the bladder's antibody response could be a major reason for high recurrence of UTIs, especially after bladder infection. Increased local IL-10 production has been linked to the bladder's failure to establish an adaptive response, as IL-10-deficient in mice demonstrated antibody responses to bladder infection that are substantial (Wiles *et al*., 2008). Following bacterial infection, mast cells are a primary generator of IL-10 in the bladder. Mast cells appear to switch to IL-10 production about 6 hours after infection, thereby shutting down this response, despite their relevance in initiating a strong immune response in the early stages of bladder infection. When mast cells produce IL-10, it prevents dendritic cells (DCs) from expressing co-stimulatory molecules, limiting their ability to act as effective antigen-presenting cells when they travel to draining lymph nodes. As a result, the bladder's inability to mount an antibody response to bacterial infection could be a side effect of its efforts to avoid damaging adaptive immunological responses to urine contents, as well as to facilitate rapid regeneration of its epithelium following infection-induced damage, which is consistent with the role of mast cell-derived IL-10 in attenuating innate immune response (Kurts *et*

al., 2013). The stimulus-dependent branches of the immune system in the urinary tract, like all immune responses, should strike a balance between potency and excessive inflammation. As a result of the imbalance, bacteria may survive, causing infection or inflammatory damage to the urothelium (Activation of TLR/PRR, for example, causes cell-specific inflammatory responses targeted at defense, but it's also linked to kidney illness) (Kurts *et al*., 2013).

Chapter Two

Materials and Methods

2. Materials and Methods

2.1 Materials

2.1.1 Laboratory Apparatuses and Instruments:

The main instruments with disposables material respectively that employed during the present study were listed down in table (2-1).

Table (2-1) Instruments and Apparatus used in this study.

Type of equipment	Company	Origin
Autoclave	Hirayama	Japan
Automatic chemistry analyzer	DIRUI CS-T180	China
Bench centrifuge	Hettich	Germany
Benson burner	Amal	Germany
Cell counter	Sysmex xp-300	Japan
Cover slide	Supertek	India
Deep freezer	Kirsh	Germany
Distillator (Water distiller)	GFL	Germany
ELISA instrument system	Combiwash	Germany
Incubator	Termaks	Denmark
Laminar flow safety cabinet	Labtec	UK
Micro titer plate reader Spectrophotometer	Humareader HS	Germany
Micropipette 0.5-10 µl	Shcheer	Germany
Micropipette 100-1000 µl	Shcheer	Germany
Microscope	Olympus	Japan
Microscope Slides	Supertek	India
Petri dish 9 cm	PLASTILAB	Lebanon
Refrigerator	Concord	Lebanon

Sensitive balance	Denver	Germany
Standard loop	Himedia	India
Urinalysis reagent strips	Acon	USA
Vacuum urine collection tube	Firatmer	Turkey
Vortex mixture	Stuart	UK

2.1.2 Biological and Chemical Materials:

The main biological and chemical materials which used in the present study were listed in table (2-2).

Table (2-2) Biological and Chemicals Materials used in this study.

Type of materials	Company	Origin
Glycerol ($C_3H_8O_3$)	Panreak	Spain
Gram stain	Himedia	India
Hydrogen peroxide (H_2O_2) 3%	Himedia	India
Iodine	Mast Diagnostic	UK
KOH	Himedia	India
Kovacs reagent	Himedia	India
Methyl red	BDH	UK
Sodium chloride (NaCl)	BDH	UK
Tetra-methyl–ρ-paraphenylene diamine dihydrochloride	Sigma	Germany

2.1.3 Culture media:

Culture media that used in this study were listed in table (2-3).

Table (2-3) Culture media that used in this study

Media	Company	Origin
Blood Agar	Neogen	UK
Brain Heart Infusion broth	Oxoid	UK
MacConkey Agar	Neogen	UK
MR-VP broth	Himedia	India
Muller-Hinton Agar	Oxoid	UK
Simmon citrate Agar	Oxoid	UK
Triple sugar Iron agar	Himedia	India
Urea agar base	Oxoid	UK
Pepton water	Oxoid	UK

2.1.4 Kits:

The kits used in this study with their sources are given in table (2-4).

Table (2-4) kits used in the present study

Kits	Company	Origin
Cathelicidin Antimicrobial Peptide (CAMP)	Mybiosource	America
C-Reactive Protein (CRP)	Dirui CS-T180	China
Human Neutrophil Peptide 1-3 (HNP1-3)	YH Biosearch Laboratory	China
VITEK identification (ID) card	BioMérieux	France

2.2 Methods:

2.2.1 Samples Collection:

Collection of samples carried out in two places (Karbala teaching hospital for children and private clinic) during the period extend between December 2020 to April 2021. Blood and urine samples were obtained from 109 suspected urinary tract infection (UTI) patients and 40 non hospitalized children as control group. Their ages were ranged from 1 month to 12 years. These patients were separated according to their residency, age, gender, infection site, and type of bacteria. Patients were considered as a positive UTI after general urine examination (G.U.E.) and urine cultivation for bacterial isolation, only patients with positive urine culture were involved in the present work and the negative urine culture were exclude. The present study excludes children who priorly take antibiotic and who are with chronic renal failure and immunocompromised.

2.2.1.1 Urine:

Approximately 10 ml clean-catch mid-stream or transurethral catheterization urine specimens were collected in sterile containers from patients with symptomatic UTIs as well as control and enrolled in the current investigation. In the medical laboratory, each urine sample was split into two halves; the first half was centrifuged and stored at -80 °C, the second half was directly inoculated on standard culture media (MacConkey and Blood agar) and incubated aerobically at 37 °C for 24-48 hours using conventional techniques. The remainder of the urine was centrifuged (1500 rpm for 5 minutes) to prepare urine debris for direct microscopic examination for pus cells, RBCs, epithelial cell count, casts, crystals, and parasite infection, if present. Normal urine sediment may contain a few counts of RBCs, pus cells, and epithelial cells.

Manual biochemical tests for bacterial isolate were used to identify the species, followed by a fully automated VITEK-2 compact system were processed to identify the bacterial species. Gram-Positive and gram-negative cards were utilized for bacterial identification, and the system was operated according to the manufacturer's instructions. Where Pure isolates of organisms to be tested were taken from MacConkey or Blood agar, then the polystyrene tube was filled with 3 ml of 0.45% sterile saline, and a homogeneous suspension of the organism in the saline was prepared. The density reading of bacterial suspension was 0.5 – 0.63 for gram-negative and gram-positive organisms. The identification cards are inoculated with microorganism suspensions. A test tube containing the microorganism suspension is placed into a special rack (cassette) and the identification card is placed in the neighboring slot while inserting the transfer tube into the corresponding suspension tube.

2.2.1.2 Blood:

Five ml of blood were collected from each participant, three ml of blood was extracted using a syringe method and placed in an anticoagulant tube containing Ethylene-diamine-tetraacetic acid (EDTA). The Sysmex XP-300 was used to test the blood samples directly for complete blood count (CBC) and measured total WBC and Neutrophil count.

In addition, two ml of blood was collected in a gel tube and sera were separated by centrifugation of blood for 5 minutes at 1000 rpm, then the serum was tested for measuring CRP by DIRUI CS-T180.

2.2.2 Ethical approval

In the present study, all specimens were taken from children subject after asking their parents. All of patient's parent showed their satisfaction in order to use their sample in this investigation. All ethical issues were considered and this research was performed with hospitals and clinics’ authorization.

2.2.3 Study Design

The study design of the present study is a case-control study, and the samples collection and investigations were descripted in figure (2-1).

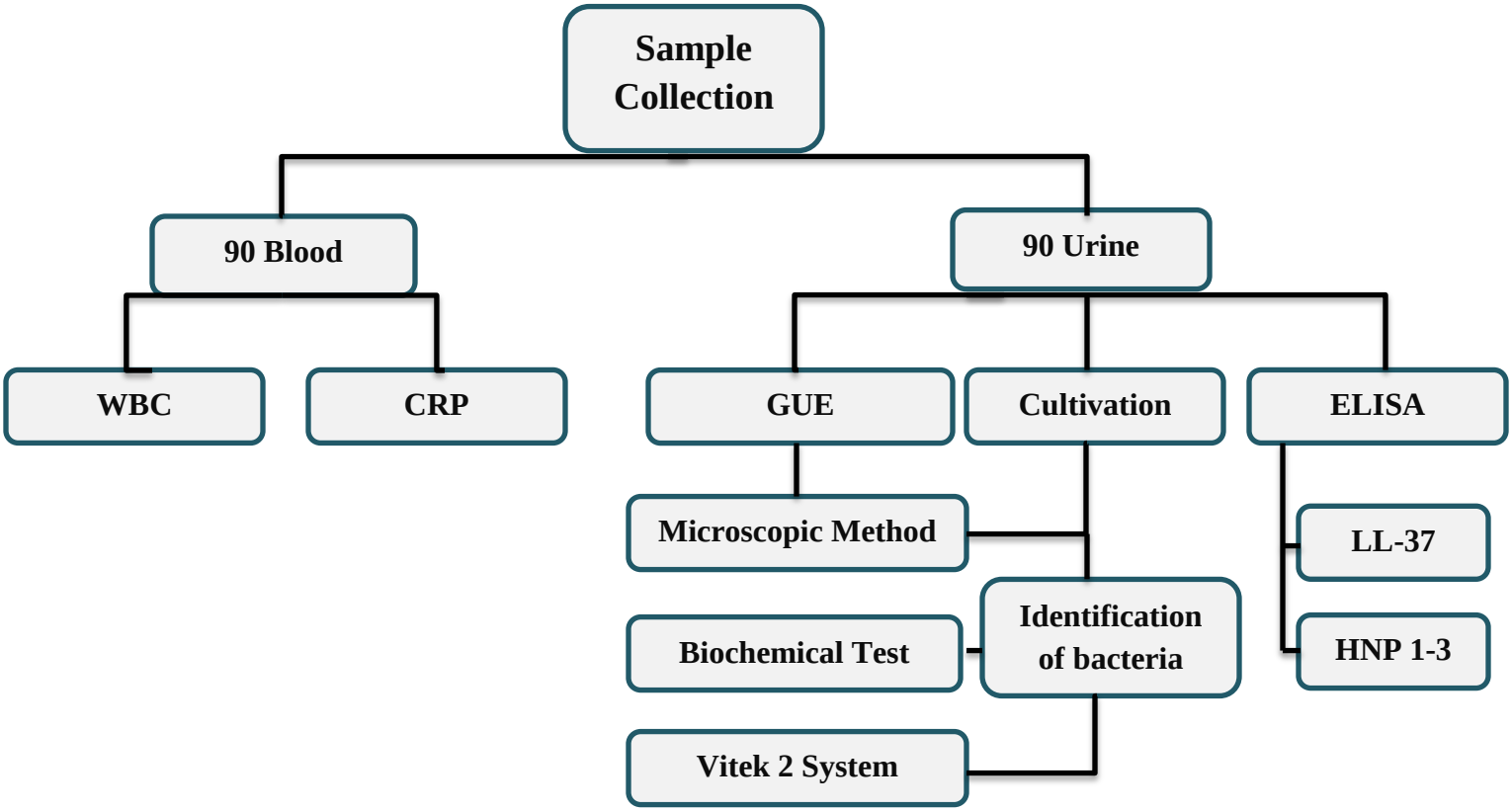

Figure (2-1) Study design and sample collection methods of the present study

2.2.4 Preparation of Solution and Reagents:

2.2.4.1 Catalase Reagent:

Commercially 3% hydrogen peroxide, this reagent help to identify the bacteria that capable of producing the enzyme catalase (Forbes *et al*., 2007).

2.2.4.2 Kovac´s Reagent (Barritt's Reagent):

Kovac's reagent is prepared by dissolving 10 gm of p-dimethyl aminobenzaldehyde in 150 ml of isoamyl alcohol and then slowly adding 50 ml of concentrated hydrochloric acid (MacFaddin, 2000).

2.2.4.3 Oxidase Reagent:

This reagent was made by dissolving 0.1 gm of tetramethyl p-phenyl diamine-dihydrochloride in 10 ml D.W. in a dark bottle. It was used to determine bacterial ability to produce oxidase (Forbes *et al*., 2007).

2.2.4.4 Normal Saline Solution:

To prepare normal saline, from NaCl were took 0.85 gm and dissolved in 90 ml D.W. to make this solution. Then autoclaved at 121 °C for 15 minutes for sterilization and use it for preparation of a bacterial suspension (Mackie *et al*., 1996).

2.2.5 Preparation of Culture Media:

Table (2-3) lists the media that were used in the previous study. All media were prepared according to the manufacturers fixed on their container.

2.2.5.1 Blood Agar:

Preparing of this agar was by suspending 40 gm of blood agar in one liter of distilled water, after heating to boiling and sterilizing in an autoclave, 5% new human blood was added after cooling to 45°C. It was utilized to demonstrate colonial morphology and the generation of haemolysin (Mackie *et al*., 1996).

2.2.5.2 Brain heart infusion broth-glycerol medium:

This medium was made by autoclaving 15 mL glycerol with 85 mL brain heart infusion broth at 121°C for 20 minutes. It was utilized for long-term preservation of bacterial isolates as a stock (Forbes *et al*., 2007) .

2.2.5.3 Methyl Red and Voges-Proskauer broth:

Dissolve 17 gm of in 1 liter of distilled water. Mix well, distribute into test tubes in amounts of 10 ml and sterilize by autoclaving at 121°C for 15 minutes. Inoculate two tubes of Methyl Red Voges Proskauer Broth per bacteria (MacFaddin, 2000).

2.2.5.4 Peptone Water Medium:

This medium was made by dissolving 8 gm of peptone in 1000 ml of distilled deionized water, mixing thoroughly, and autoclaving the mixture for 20 minutes, cooled and distributed into sterile test tubes. It was used to demonstrate bacterial ability to decompose the amino acid tryptophan into indole (MacFaddin, 2000).

2.2.5.5 Simmon citrate Agar:

Simmon citrate agar was made by dissolved 24.28 gm of medium in 1000 ml of distilled water to make this medium, after that, it was sterilized in an autoclave at 121°C for 20 minutes. Simmon's Citrate medium was used to test bacteria's capacity to use citrate as their only carbon source (MacFaddin, 2000).

2.2.5.6 Urea Agar Medium:

This medium was made by adding 5 ml of urea solution (40%) in a volume of 95 ml autoclaved urea agar base after cooling to 45 °C. The pH was adjusted to 7.1 and the medium was distributed into sterilized test tubes and allowed to solidify in a slant position. It was used to test the ability of bacteria to produce urease enzyme (MacFaddin, 2000).

2.2.5.7 Triple Sugar Iron Agar Medium:

This medium can be made by dissolving 40 gm of agar in 1000 ml of D.W and sterilizing it at 121°C for 20 minutes in an autoclave, the triple sugar iron agar (TSI) test can be performed to see if a gram-negative rod ferments glucose, lactose, or sucrose. The medium also tests for gas production during carbohydrate fermentation and hydrogen sulfide production, which are both important in identifying gram-negative rods from the Enterobacteriaceae family (MacFaddin, 2000).

2.2.6 Biochemical tests:

2.2.6.1 Catalase test:

A sterile inoculating loop or wooden applicator stick was used to introduce a little amount of organism onto a microscope slide from a well-isolated 18 to 24-hour colony. A drop of 3% H_2O_2 was applied to the organism on the microscope slide. Immediate effervescence indicates positive replies (bubble formation). The catalase test is used to detect the catalase enzyme in bacteria. It is necessary for distinguishing catalase positive *Staphylococcus* ssp. from catalase negative *Streptococcaceae* (Reiner, 2010).

2.2.6.2 Free Coagulase Test:

In a plain tube, was placed 0.2 ml of human plasma diluted with 1.8 ml of normal saline, and 0.1 ml of bacterial suspension was introduced to 0.4 ml of the diluted human plasma. The tube was incubated at 37 °C for 4 hours. In order to distinguish *Staphylococcus aureus* (positive), which produces the enzyme coagulase, from *S. epidermis* and *S. saprophyticus* (negative), which do not produce coagulase, the results were checked every 30 minutes (Forbes *et al*., 2007; Becker *et al*., 2014).

2.2.6.3 Indole Production Test:

The young testing bacteria were injected into peptone water and cultured at 37°C for 24-48 hours. In each tube, a few drops of Kovacs reagent were added and gently shaken. A positive test was indicated by the formation of a pink ring. (MacFadden, 2000).

2.2.6.4 Methyl Red-Voges Proskauer Test:

The fresh bacterial culture was added to Methyl red Voges Proskauer broth and cultured for 24 hours at 37°C. The result was read immediately after mixing five drops of methyl red solution. Bright red was the color of a positive test (MacFadden, 2000).

2.2.6.5 Novobiocin Susceptibility Test:

A Mueller-Hinton agar plate is heavily seeded with the test organism to produce a confluent growth on the agar surface. After the seeding, a novobiocin antibiotic disc is applied to the agar surface. Following incubation, the sensitivity of an organism to the antibiotic is determined by the Kirby-Bauer method. Novobiocin test is used to differentiate coagulase-negative staphylococci (CONS) and presumptively identify the isolate as *Staphylococcus* saprophyticus which is resistant to Novobiocin (Nicolle *et al*., 1983).

2.2.6.6 Oxidase Test:

A filter paper was soaked with the substrate tetramethyl-p-phenylenediamine dihydrochloride. The paper was moistened with sterile distilled water. The fresh colonies to be tested was picked with a wooden or platinum loop and smear in the filter paper. Oxidase positive represented by color changes to dark purple within 5 to 10 seconds. While delayed oxidase-positive by color changes to purple within 60 to 90 seconds and oxidase negative by color does not change (Tarrand and Gröschel, 1982).

2.2.6.7 Simmons Citrate Test:

A fresh bacterial culture was injected into a Simmons citrate slant, which was then incubated at 37°C for 48-72 hours. A positive test is indicated by a blue tint and a streak of growth. (McFadden, 2000).

2.2.6.8 Triple Sugar Iron (TSI) Test:

A dense growth of tested bacteria was streaked across the slope's surface and pierced into the bottom, then incubated for 24 hours at 37°C. To test an organism's ability to ferment glucose, lactose, and sucrose, as well as its ability to create hydrogen sulfide (MacFaddin, 2000).

2.2.6.9 Urease Production Test:

The bacteria were injected onto the entire slop of urea agar and let to grow for 48 hours at 37°C. The results were read after 24 hours. The urease test was positive if the indicator changed color from medium to purple-pink (MacFaddin, 2000).

2.2.7 Preservation of Bacterial Isolate:

The organisms were suspended in a 15% glycerol brain heart infusion broth. The suspension was frozen and kept at a temperature of -15 to -30°C.

2.2.8 Isolation and Identification of Microorganisms:

Well-mixed urine specimens were seeded on MacConkey agar, blood agar, and orientation plates independently for quantitative culture and presumptive identification from each urine specimen. They were incubated in bacteriological incubators overnight at 37°C under aerobic conditions. Bacterial identification was based on morphological characteristics with Gram's stain that providing primary identification of bacteria. Pure cultures were generated for biochemical testing to determine the bacteria's species (MacFaddin,2000). Also, conclusively identification for bacteria species was by using the Vitek-2 method (bioMerieux) based on the typical culture and biochemical properties of isolates, where it used according to the manufacturer's instructions,

2.2.9 Measurement of the Hematological Parameter:

About 3 ml of blood was withdrawn in Ethylene-diamine-tetra-acetic acid (EDTA) anticoagulant tube and directly tested for WBC count by using sysmex xp-300.

2.2.10 Measurement of the immunological parameter:

2.2.10.1 Concentration of C-reactive protein test:

To determine CRP, two ml of venous blood was taken into a gel tube and centrifuged at 1500 rpm for 15 minutes to separate serum. Automatic biochemical analyzers DIRUI CS-T180 were used to test CRP concentration in the serum.

2.2.10.2 Determination of urinary human Cathelicidin LL-37 level by ELISA:

The levels of Cathelicidin LL-37 in urine were measured by using one competitive inhibition enzyme immunoassay technique ELISA kit.

Principle of assay:

This assay employs the competitive inhibition enzyme immunoassay technique. A monoclonal antibody specific to Cathelicidin antimicrobial peptide (CAMP) has been pre-coated onto a microplate. A competitive inhibition reaction is launched between biotin-labeled CAMP and unlabeled CAMP (Standards or samples) with the pre-coated antibody specific to CAMP. After incubation, the unbound conjugate is washed off. Next, avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of CAMP in the sample. After the addition of the substrate solution, the intensity of color developed is reverse proportional to the concentration of CAMP in the sample, the kit compounds were listed in table (2-5).

Table (2-5) Components of LL-37 kit.

Reagents	Quantity	Reagents	Quantity
96-well strip plate that has been pre-coated and is ready to use.	1	Plate sealer for 96 wells	4
Standard	2	Standard Diluent	1×20mL
Detection Reagent A	1×120μL	Assay Diluent A	1×12mL
Detection Reagent B	1×120μL	Assay Diluent B	1×12mL
TMB Substrate	1×90 mL	Stop Solution	1×6mL
Wash Buffer (30 × concentrate)	1×20mL	Instruction manual	1

Preparation of Reagents:

The reagents of kits were prepared according to the manufacturing instructions as below:

Wash buffer:

Concentrated wash buffer was diluted into 580 ml of distilled water to prepare 600 ml of Wash Solution (1×).

LL-37 standard solution:

1. Standard solution was reconstituted with 0.5 ml of Standard Diluent, then held at room temperature for 10 minutes and properly mixed. The standard concentration in the stock solution is 10,000 ng/ml.
2. Next, we made a triple dilution series using 5 Eppendorf tubes with 0.6mL Standard Diluent, as illustrated in the diagram below. Before moving on to the next transfer, properly mix each tube. Set up 5 points of diluted standard at 10,000ng/ml, 3,333.3ng/ml, 1,111.1ng/ml, 370.4 ng/ml, and 123.5ng/ml, with the blank at 0ng/ml in the last EP tube with Standard Diluent. The Sample/Standard dilution buffer 0.3 ml was added into each tube 4.
3. Then, into the first tube, 0.3 ml of the 10,000 ng/ml standard solution was introduced and mixed as shown in figure (2-2).

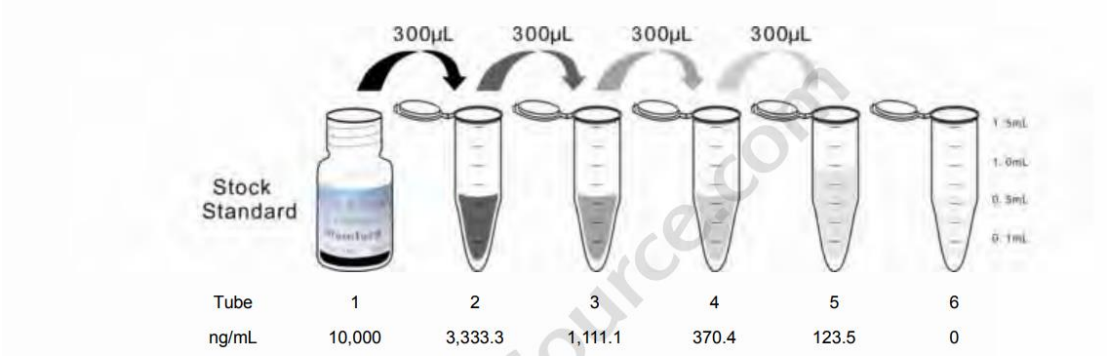

Figure (2-2) LL-37 standard solution

4. 0.3 ml was transferred and blended from the first to the second tube.
5. From the second to the third tube. 0.3 mL was transferred and well mixed, and so forth.

Detection Reagent A and Detection Reagent B:

Before using, we centrifuged the stock Detection A and Detection B and diluted them to the working concentration 100-fold with Assay Diluent A and B, respectively.

Assay procedure:

1. We Determine wells for diluted standard, blank, and sample. Prepared 5 wells for standard points, 1 well for blank. Add 50 µl each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells, respectively, and then we added 50 µl of Detection Reagent A to each well immediately. The plate was Shaked gently. The plate was sealed with a cover and incubated for 1 hour at 37 ^{o}C.
2. The solution was aspirated and washed with 350 µL of 1X Wash Solution to each well using a squirt bottle, multichannel pipette, manifold dispenser, or auto washer, and let it sit for 1-2 minutes. The remaining liquid was Remove from all wells completely by snapping the plate onto absorbent paper. Repeat 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
3. Each well-received 100 µl of detection Reagent B working solution. After covering it with the Plate sealer, it was incubated for 30 minutes at 37°C.
4. The aspiration/washing procedure was repeated five times in total, as described in step 2.

5. Each well received 90 µl of Substrate Solution. Cover with a new Plate sealer. Incubate for 10 - 20 minutes at 37 °C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue with the addition of a Substrate Solution.
6. Each well received 50 µl of Stop Solution. When you add the Stop solution to the liquid, it turns yellow. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
7. On the bottom of the plate, any drop of water and fingerprints were eliminated. Then, immediately use the microplate reader to perform a 450nm measurement. As shown in figure (2-3) :

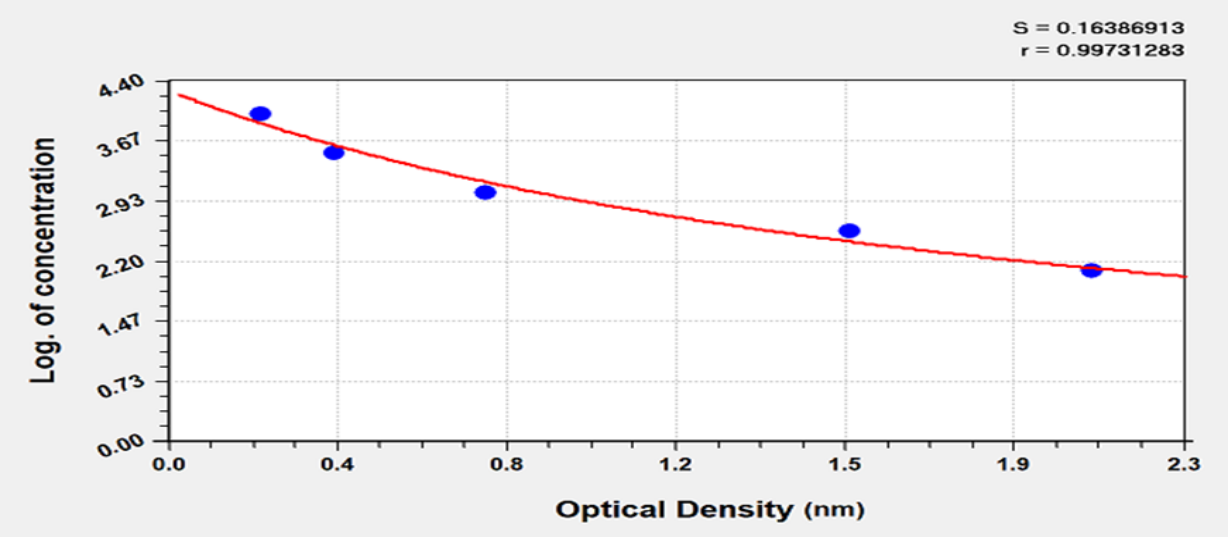

Figure (2-3) Standard curve of LL-37 in urine

2.2.10.3 Determination of Urinary Human Neutrophil Peptide (1-3) level by ELISA:

HNP (1-3) in urine was measured by using one sandwich enzyme-linked immune-sorbent assay technology kit.

Principle:

This kit assays Human Neutrophil Peptide 1-3 using an enzyme-linked immunosorbent assay (ELISA) based on biotin double antibody sandwich technology (HNP1-3). Incubate Neutrophil peptide 1-3(HNP1-3) in wells that have been previously coated with Neutrophil peptide 1-3 (HNP1-3) monoclonal antibody. After incubation, add biotin-labeled anti-HNP1-3 antibodies to form the immunological complex with streptavidin-HRP. After incubation and washing, remove any unbound enzymes before adding substrates A and B. With the addition of acid, the solution will turn blue and then yellow. The concentration of Human Neutrophil Peptide 1-3 (HNP1-3) is positively associated with the color of the solution.As shown in table (2-6)

Table (2-6) Components of HNP (1-3) kit.

Reagents	Quantity	Preservation
Coated ELISA plate	1(96 well)	2-8℃
Standard solution(64ng/ml)	0.5ml×1	2-8℃
Streptavidin-HRP	6ml×1	2-8℃
Stop Solution	6ml×1	2-8℃
chromogenic reagent A	6ml×1	2-8℃
chromogenic reagent B	6ml×1	2-8℃
Anti HNP1-3 antibodies labeled with biotin	1mlx1	2-8℃
Standard dilution	3ml×1	2-8℃
Washing concentrate	(20ml×30) ×1	2-8℃

Preparation of Reagents:

The reagents of kits were prepared as the manufacturing instructions:

Wash buffer:

The washing concentration (30X) were diluted into 580mL of distilled water to prepare 600mL of Wash Solution (1×). As shown in table (2-7) and figure (2-4).

Table (2-7) HNP (1-3) standard solution

Standard Numbers	Concentration	Solutions
Standard No. 5	32ng/ml	120µl Original Standard + 120µl Standard diluents
Standard No. 4	16ng/ml	120µl Standard No.5 + 120µl Standard diluents
Standard No. 3	8ng/ml	120µl Standard No.4 + 120µl Standard diluent
Standard No. 2	4ng/ml	120µl Standard No.3 + 120µl Standard diluent
Standard No. 1	2ng/ml	120µl Standard No.2 + 120µl Standard diluent

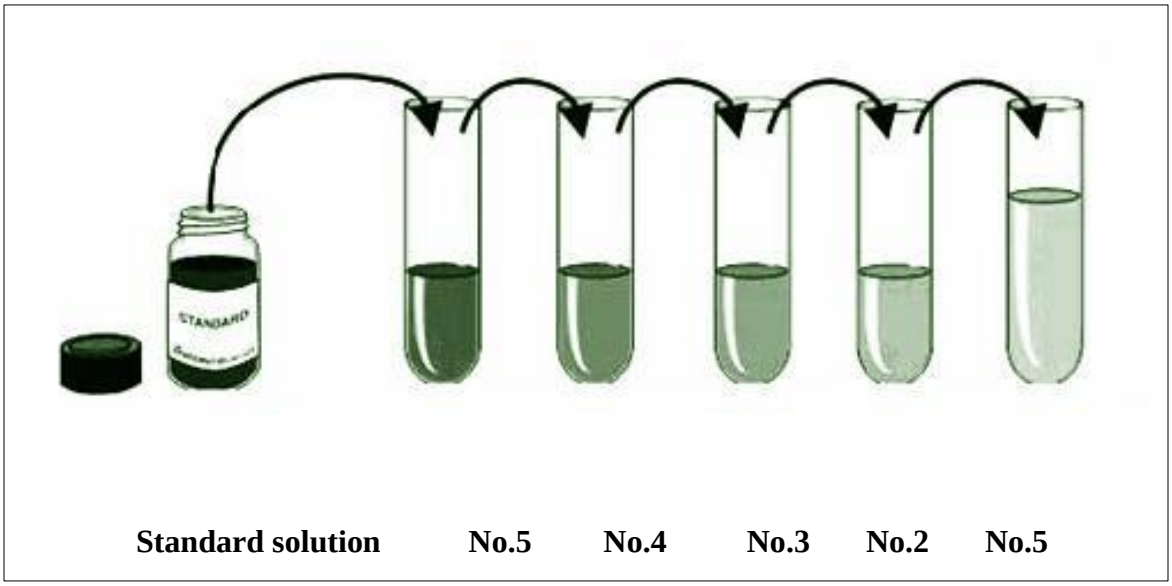

Figure (2-4) HNP (1-3) standard solution

Assay procedure:

1. Blank well: The chromogen reagents A and B, as well as the stop solution, were added; the rest of the steps are the same.
2. Standard solution well: 50 μl of standard were added, as well as 50 μl of streptomycin-HRP (biotin antibodies have united in advance in the standard so no biotin antibodies are added).
3. 40 μl of material were added, followed by 10 μl of HNP1-3 antibodies and 50 μl of streptavidin-HRP. The plate was then covered with seal plate membrane and incubated for 60 minutes at 37°C.
4. Washing: The seal plate membrane was carefully removed, the liquid was emptied, and the rest was shaken off. Each well was filled with washing solution, then drained after 30 seconds. This operation was carried out five times before the plate was blotted.
5. Color development: First, 50 μl of chromogen reagent A was added to each well, followed by 50 μl of chromogen reagent B, which was gently mixed before being incubated for 10 minutes at 37°C away from light for color development.
6. Stop: To stop the reaction, 50 μl Stop Solution was applied to each well.
7. Assay: Each well's absorbance (OD) was measured one by one at a wavelength of 450 nm.
8. Each well's absorbance (OD) was measured one by one at a wavelength of 450 nm.

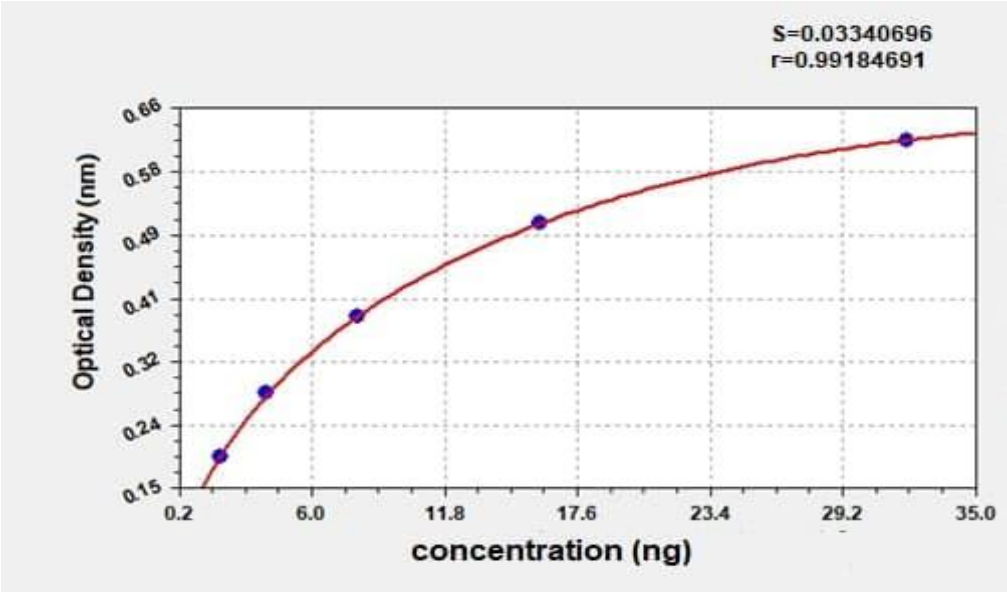

Figure (2-5) Standard curve of HNP (1-3) in urine

2.2.11 Statistical analysis

For data entry and statistical analysis, the Statistical Package for Social Sciences (SPSS version 21) was utilized. For the comparison of two independent groups, the independent t-test was utilized. The Pearson's $\chi 2$ test was used to examine categorical data. The one-way ANOVA test was used to compare more than two groups at the same time in order to see if there was a relationship between them. The kind and degree of the association between variables was explained using Pearson correlation (R). The tests were conducted using a significance level of $\alpha = 0.05$.

Chapter Three

Results and Discussion

3. Results and Discussion

3.1 Characterization of UTI Patients and Clinical Isolates:

In current study, blood and urine sample were collected from 109 child suspected with UTI. Out of 109 urine sample, 14 (12.8%) had non-significant growth and 45(41.3%) had no growth of bacteria and they were excluded. The remaining was 50 (45.9%) had significant bacterial growth and they were involved in the study. Also, urine and blood sample were collected from forty healthy child, whom representing the control group. The presence of gram-ve among significant bacteriuria in this study was (46/50) 92%, while (4/50) 8% was gram +ve bacteria with highly P≤0.05 , as shown in figure (3-1).

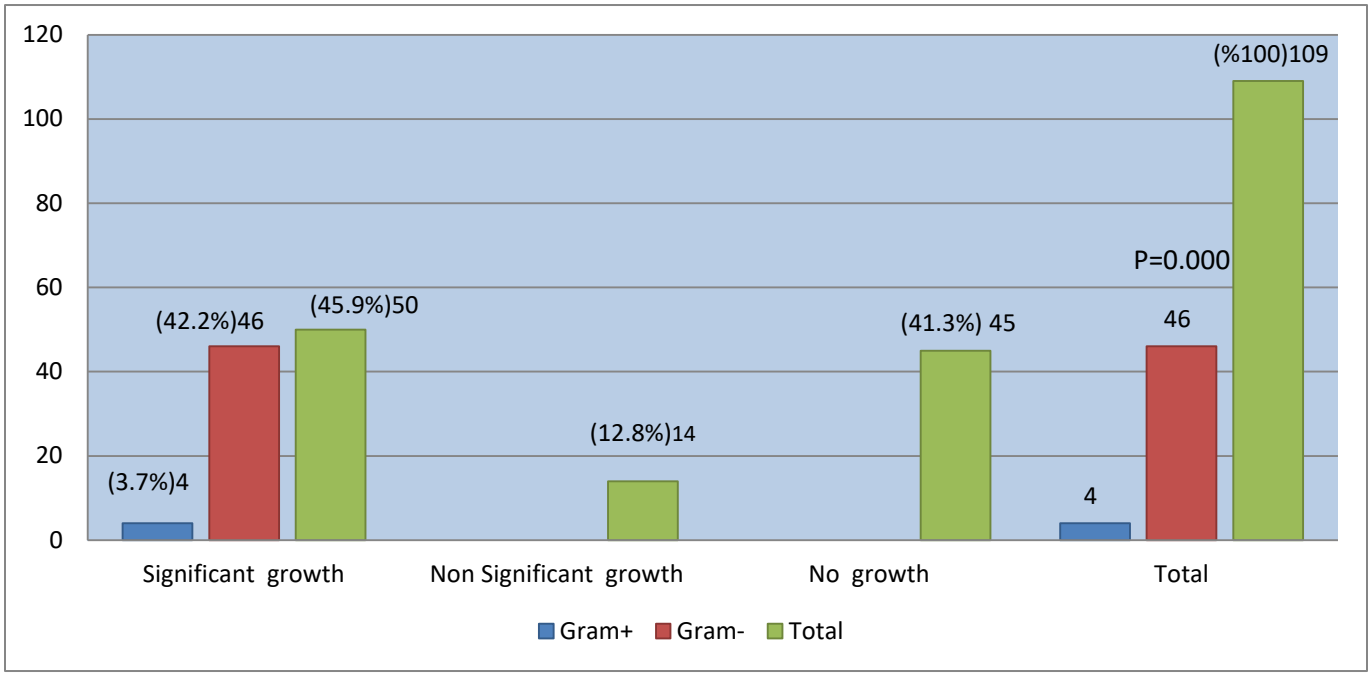

Figure (3-1) Distribution of patients according to the bacterial growth.

Although UTI may be caused by different pathogens that colonize the urinary tract, most causative agents are gram-negative bacteria because of the continued exposition to the enteric origin gram-negative bacteria (Svanborg, 2013; Shaker and Al-Hadrawi, 2021).

3.1.1 Distribution of patients according to gender:

In the present research, an attempt was made to evaluate the demographic distribution of UTI patients according to gender. Where it was observed that the number of patients with significant bacteriuria was higher (62%) were female, meanwhile, (38%) were male. without any statistical deference P>0.05. As shown in figure (3-2).

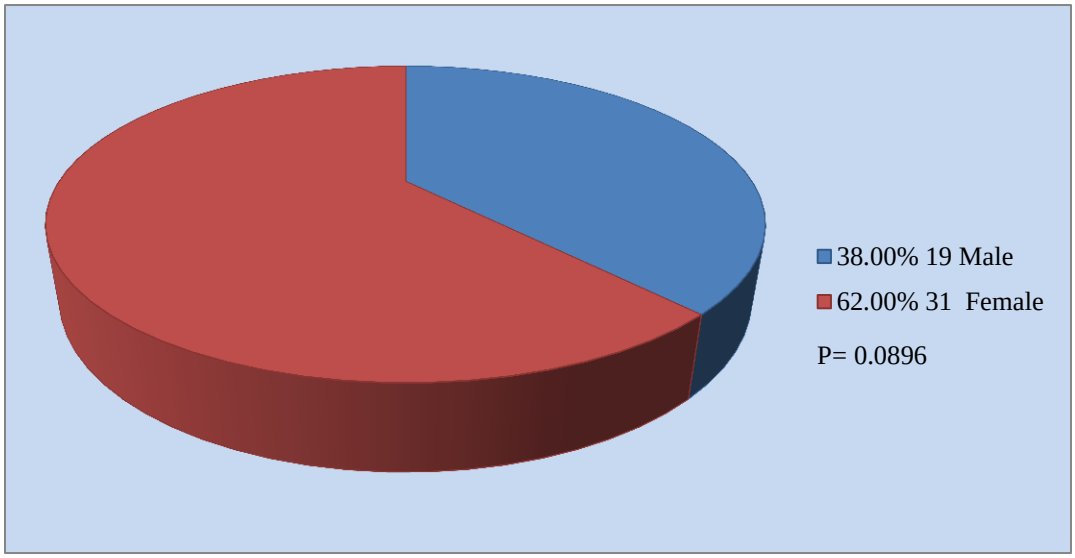

Figure (3-2) Distribution of patients according to gender

These results were in the same line with the results of the study in Rapareen Pediatric Hospital in Erbil city who referred that most of UTI patients were female (Saeed *et al*., 2015). Which was also in agreement with the study in Egypt that show the prevalence of UTI in female 54.8% while the prevalence of UTI in male 45.2% (Amin *et al*., 2020).

Two other studies were conducted in Iran and proved similar results to the present study, where they also found that UTI is more common in females than males (Pouladfar *et al*., 2017, Sherkatolabbasieh *et al*., 2020).The high UTI incidence in

females can be attributed to many reasons such as anatomical factors that allow quick access of bacteria to the bladder (John *et al*., 2016).

Furthermore, other reasons like poor perineal hygiene, self-management errors, and management errors performed by females include cleaning the perineum forwards from the anus to the valuva and micturating infrequently. Micturating infrequently causes bladder distension with subsequent partial emptying of leftover urine then interferes with bladder defenses (Saeed *et al*., 2015).

3.1.2 Distribution of patients according to residence:

Present data revealed that the distribution of UTI patients were highest in rural regions 27 (54%), then 23 (46%) urban, without any statistical difference P≥0.05. As shown in figure (3-3).

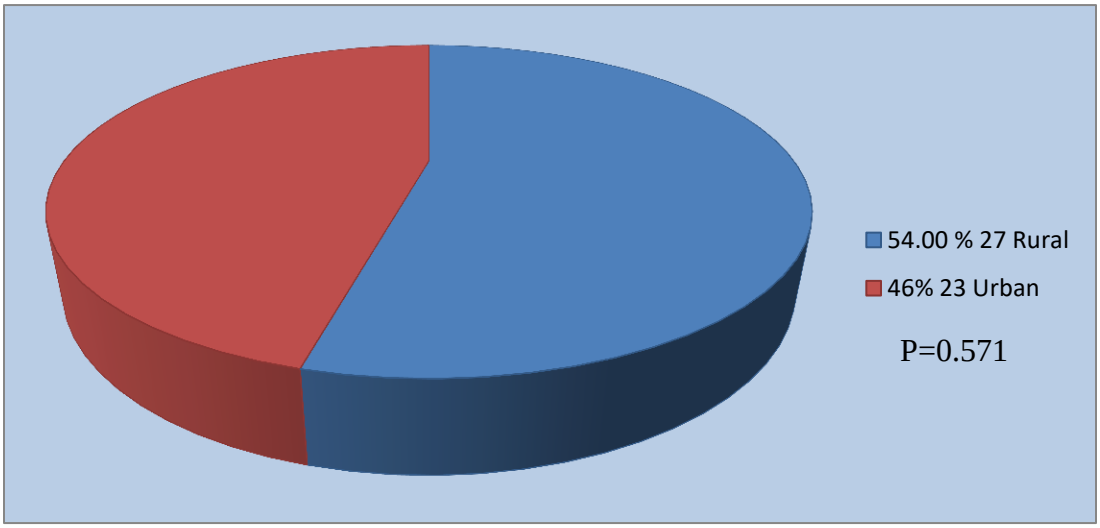

Figure (3-3) Distribution of patients according to residence

That was in agreement with study in Basra showed that the distribution of UTI patients were highest in rural regions 53%, while 47% were in urban (Alhamedy and Shani, 2020). The same result was observed by a study in Shashemene referral

hospital, in Ethiopia where its results present (62.9%) from UTIs patients were from rural regions and (37.1%) were from urban (Seifu and Gebissa, 2018).

Disagreement with present work was noticed by Al-Jawadi in Mosul, who showed that 71% of schooling UTI patients were urban and 29% were rural (Al-Jawadi, 2012) , and also same results noticed by Hilwana who showed that (85.71%) of UTI patient were urban and (14.28%) were rural (Lutifta Hilwana, 2017).

Researchers interpret the majority of patients with UTIs were from rural areas because they had inadequate sanitary standards that make them more exposed to different microorganism that cause infections (Saeed *et al*., 2015).

3.1.3 Distribution of samples according to age group:

Recent work documented that the highest percentage of UTIs were in patients with age <5 (46.0%), followed by age 5-10 years (36.05%), whereas the lowest percentage with UTI patients ages is >10(18%) with no significant differences P> 0.05. As shown in table (3-1).

Table (3-1) Distribution of samples according to age group

Variables		Cases		control		Total		P Value
		No	%	No	%	No	%	
Age groups	<5Y	23	46.00%	19	45.0%	42	46.7%	0.773
	5-10Y	18	36.00%	15	40.0%	33	36.7%	
	>10Y	9	18.00%	6	15.0%	15	16.7%	
Total		50	100.%	40	100%.	90	100%	

*Y=Years

The current study proved that younger children are more likely to have UTIs than older children, Which is in agreement with a study in Baghdad that showed a high rate of UTIs within the age group (0-3), while a low rate was observed within 10-12 age groups (Al-Rawazq *et al*., 2012).

In similar Egyptian study was noted that the risk of renal damage from UTI is the greatest in children younger than five years in comparison with older children (Amin *et al*., 2020). Also, study in Ethiopia were highest percentage (57.4%) of UTIs patients were below the age of 3 years (Merga Duffa *et al*., 2018) .

But a study was conducted in Erbil disagree with the present study, where it found highest percentage of UTIs (39.2%) occurred at age group 6-10 years. followed by (31.5%) at age <5, then (17.7%) at age 1<, and the lowest percentage (11.5%) was at age 10-15 (Saeed *et al*., 2015).

Perhaps the reason for the high incidence of urinary tract infection in infants and young children is due to the frequent exposure of the urethra and bladder to bacteria resulting from feces, and their consideration of mothers' concern for the internal hygiene of children. This helps the colonic bacteria to pass through the urethra and stick to the bladder and cause internal inflammation.

3.1.4 Distribution of patients according to the bacteria isolates:

Figure (3-4) show the percentage of each identified uropathogens across all patient groups; Where *Escherichia coli* (60%) was the most prevalence bacteria that identified in UTI patients, followed by *Klebsiella pneumonia* (12%) , *Pseudomonas aeruginosa* (10%) , *Proteus mirabilis* (8%) , *Enterococcus faecalis* (6%) and *Achromobacter xylosoxidans* (1%) and *Streptococcus sanguinis* (1%) .

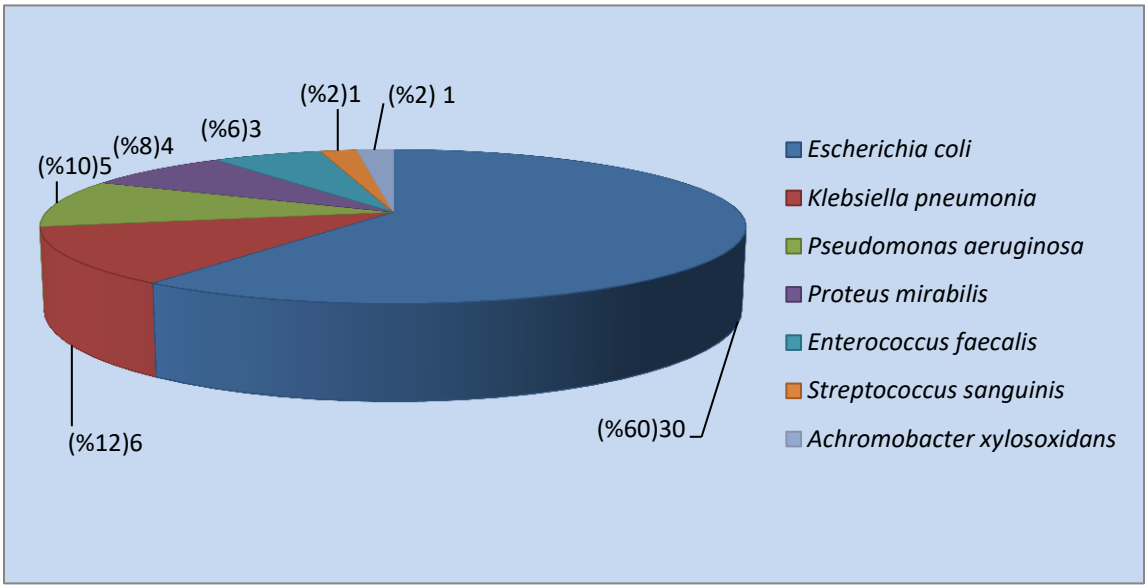

Figure (3-4) Types of bacterial isolates from UTI patients

The pattern and the frequency of bacterial isolates obtained in this study were comparable with different study findings done in different countries. For example, A study conducted in Al-Najaf City found that *E. coli* was the most prevalent bacteria (38.9%) cause UTIs followed by *K. pneumonia* (27.8%), *P. aeruginosa* (14.3%), *C. freundii* (12.9%), *E. aerogenes* (6.3%) and *P. mirabilis* (3.2%) (Majeed and Aljanaby, 2019).

Another study in Erbil city showed that the most frequently recorded microorganism also was *E.coli* (33.9%), *Klebsiella* spp. (10.1%), *P. mirabilis* (9.3%) *P. aeruginosa* (6.8%) and others 48.9% , respectively (Saeed *et al*., 2015). In addition, a study on Egyptian school-going children found that the common isolate was *E.coli* (58%) , *S. aureus* (22%), *Enterobacter spp*. (10%) , *K. pneumoniae* (5%) *P. Vulgaris* (5%),respectively (Mohammed *et al*., 2016).

A similar study was done by Amin and his team mention that the most common infecting organism was *E.coli* that represents (76.2%) followed by *K.*

pneumonia (9.5%), *E. fecalis* (7.1%), *P. mirabilis* (4.8%), and coagulase-negative *Staphylococcus spp.* (2.4%) (Amin *et al*., 2020). The same result was observed in other studies in Ethiopia and Iran (Merga Duffa *et al*., 2018, Pouladfar *et al*., 2017).

These results led the researchers to the truth that most pediatric UTIs are caused by Gram-negative coliform bacteria that enter and ascend the urinary tract from fecal flora colonizing the perineum. Uropathogenic *E.coli* strains cause (80-90%) of pediatric UTIs because they have specific properties, such as fimbriae that attach to the uroepithelial cell surface, to allow them to overcome the uroepithelial cell surface (Edlin *et al*., 2013, Tullus, 2019) .

3.1.5 Distribution of patients according to the site of infection:

The present study indicated that among 50 patients with UTI divided into 31 female and 19 males, about 83.9% of patients with lower urinary tract infection (cystitis) were females and 16.1% were males. In contrast, about 63.2% of patients with upper urinary tract infection (pyelonephritis) were males and 36.8% were females and the association between gender and site of bacterial infection was significant (P=0.001), where females were more likely to have cystitis, on contrary, males were more likely to have pyelonephritis.

Regarding the age of the children with UTI, about 14 (60.9%) of the patients aged less than 5 years had pyelonephritis while about 9 (39.1%) had cystitis. On the other hand, only 2 (11.1%) of the patients aged 5-10 years had pyelonephritis and about 16 (88.9%) had cystitis. As for patients aged more than 10 years, only 1(11.1%) had pyelonephritis while about 8 (88.9%) had cystitis, and the association was significant (P=0.001). As a result, younger children are more likely to had pyelonephritis than cystitis.

As for the relationship between congenital anomalies and the site of infection, the study found that of 9 patients who had congenital anomalies, 8 patients had pyelonephritis and another one had cystitis with a highly significant difference (P=0.000), which makes patients with congenital anomalies more likely to had pyelonephritis.

The statistical analysis showed that only 7(25.9%) of patients were from rural regions and 10 (43.5%) from urban regions had pyelonephritis. While about 20(74.1%) of patients were from rural regions and 13(56.5%) from urban regions had cystitis, that difference was statistically non-significant (P=0.192).

Table (3-2) Correlation between the site of infection and demographic factors

Variables		Pyelonephritis		Cystitis		Total		P value
		No.	%	No.	%	No.	%	
Gender	Female	5	16.1%	26	83.9%	31	100%	0.001
	Male	12	63.2%	7	36.8%	19	100%	
Age	<5Y	14	60.9%	9	39.1%	23	100%	0.001
	5-10Y	2	11.1%	16	88.9%	18	100%	
	>10Y	1	11.1%	8	88.9%	9	100%	
Residence	Rural	7	25.9%	20	74.1%	27	100%	0.192
	Urban	10	43.5%	13	56.5%	23	100%	
Congenital anomalies	No	9	22.0%	32	78.0%	41	100%	0.000
	Yes	8	88.9%	1	11.1%	9	100%	

*Y=Years

Figure (3-5) revealed, *P. aeruginosa* was the most frequent pathogen detected in pyelonephritis, where it was found in 5(29.44%) pyelonephritis patients, while *E. coli, E. fecalis*, *P. mirabilis,* and *K. pneumonia*, each was found in 3(17.64%) from pyelonephritis patients.

In comparison, *E. coli* was the predominant isolated bacteria in patients with cystitis and it was found in 27 (81%) of them, followed by *K.Pneumonia* 3(9.09%), *P.mirabilis* 1(3.33%), *S.Sanguinis 1*(3.33%) and *A.xylosoxidans also* 1(3.33%) .

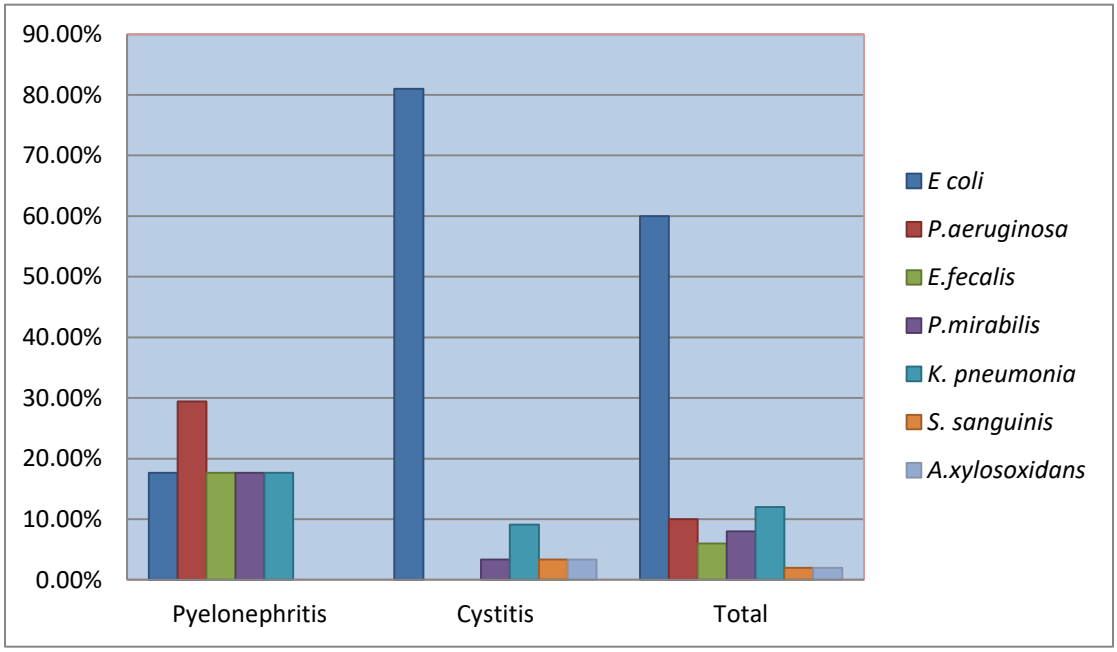

Figure (3-5) Distribution of bacteria according to the site of infection.

These result disagree with Hilwana study which showed that major cause of pyelonephritis is *E.coli* (40%) and major cause of cystitis is also *E.coli* (40,7%) (Lutifta Hilwana, 2017). Also, it's disagreed with another study conducted on 225 patients, where *Enterococci* represent (10.7%), *Staphylococcus* species (8.0%), *Proteus mirabilis* (6.7%), *Enterobacter species* (5.3%), and *Pseudomonas aeruginosa* accounted for the remainder of the cases (5.3%) (Efstathiou *et al*., 2003).

3.2 Hematological Study:

3.2.1 Determination of White blood cell count in patients:

Documented data showed significant elevation ($P \leq 0.05$) of total WBC in UTI patients (14.8100 $\pm$ 10.25780) in compare with control (7.4275 $\pm$ 1.48358), as shown in table (3-3).

Table (3-3) White blood cell count in patients with UTIs

Variables		N	Mean	Std. Deviation	P Value
WBC (x 10^3 / µl)	Cases	50	14.8100	10.25780	0.000
	Control	40	7.4275	1.48358	

*N=Number, STD.=Standard deviation

Also, there was significant elevation ($P \leq 0.05$) in neutrophils count (8.2320$\pm$6.92677) in cases in comparison with control (3.8975 $\pm$ 1.26764), As shown in table (3-4).

Table (3-4) Neutrophils count in patients with UTIs

Variables		N	Mean	Std. Deviation	P Value
Neutrophil's count (10^3/ µl)	Cases	50	8.2320	6.92677	0.000
	Control	40	3.8975	1.26764	

Consistent with our findings, A study in Kerbala done by Khalil Alaaraji *et al*., 2020 on 105 children with UTI and 105 non-UTI as a control, their age ranging from fifteen days to fourteen years old, was observed that the mean of WBC in cases was (12.99 $\pm$ 6.08) and Neutrophile count was (0.57 $\pm$ 0.18), While WBC in control was (9.29 $\pm$ 2.52) and Neutrophile count was (0.47 $\pm$ 0.14).

This is in agreement with a study on children with UTI in Korea, where there was a significant difference between cases (13.664 $\pm$ 5.693) and control (11.020 $\pm$ 6.573) (Moon *et al*., 2020). Which is relatively similar to the result obtained by Sim who also found elevated in total WBC count ($P<0.001$) in children with UTIs (Sim *et al*., 2015). In addition, Significant elevation at level in plasma leukocyte and plasma Neutrophil mean have been found in patients with acute uncomplicated lower urinary tract infections with pyuria (Gülünay *et al*., 2019).

The ascending entry of UPEC or other pathogenic bacteria into the mammalian urinary system triggers an inflammatory response, which is triggered by Toll-like receptor activation (TLRs). TLR5 activation in response to bacterial flagellin can also cause acute inflammation during a UTI (Andersen-Nissen *et al*., 2007, Smith *et al*., 2011).

The neutrophil response may be sustained by cytokines like IL-17, which is a modulator of the innate response during experimental UTI and has an emerging function in bridging innate and adaptive immunity (Smith *et al*., 2011). The recruitment of neutrophils to the bladder is a key outcome of the soluble inflammatory response, which makes their presence in the urine is a diagnostic feature of a human UTI (Ingersoll *et al*., 2008, Svensson *et al*., 2008, Svensson *et al*., 2011).

3.2.2 Association of WBC count with site of infection among UTI patients:

The current study also previously reported highly significant difference (P<0.05) in total WBC between pyelonephritis (23.5647 ± 10.85305) and cystitis (10.3000 ± 6.36666) and it was found the counts more in pyelonephritis patients as shown in table (3-5).

Table (3-5) Association of total WBC count and site of infection among UTI patients.

Variables		N (50)	Mean	Std. Deviation	P Value
Total WBC (x 10^3 µl)	pyelonephritis	17	23.5647	10.85305	0.000
	cystitis	33	10.3000	6.36666	

Also in this investigation, the result revealed that the difference in neutrophils count between pyelonephritis (23.5647 ± 10.85305) and cystitis (10.3000 ± 6.36666) was significantly high, As shown in table (3-6).

Table (3-6) Association of neutrophils counts and site of infection among UTI patients.

Variables		N (50)	Mean	Std. Deviation	P Value
Neutrophil count (10^3\ µl)	pyelonephritis	17	23.564	10.853	0.000
	cystitis	33	10.300	6.366	

A research in Iran done on children their mean age with a range of two months to 13 years agreed with these results, where WBC count was significantly (P=0.001) higher in the pyelonephritis group compared to the cystitis group (Razavi *et al*., 2021). Also, consistent with our findings, among 43 patients with APN and 51 patients with cystitis were investigated prospectively by Tekin *et al*., where they

observed that WBC count was higher in the APN group than in the lower UTI group (Tekin *et al*., 2015).

In addition, case-control study was conducted in China found that Infants with acute pyelonephritis (APN) had a higher WBC count than those with cystitis, with significant difference (P=0.002) (Yang *et al*., 2016). Although, study in Iran showed that the amount of WBC and Neutrophil count, in acute pyelonephritis group was higher than that in the lower UTI group (Mahyar *et al*., 2013). The same result was observed by (Han *et al*., 2016).

While a study with opposite results done by Moon *et al*., showed that there wasn't a significant difference (P=0.213) between pyelonephritis and cystitis in WBC among febrile children (Moon *et al*., 2020).

3.2.3 Relationship of total WBC count with the type of bacterial Isolates:

The present study showed a strong association between the WBC count and the types of bacteria (P = 0.026). Where WBC count in children with UTI caused by, *P.aeruginosa, P. mirabilis, , E.faecalis, K. pneumonia, E.coli, Streptococcus sanguinis, and Aachromobacter xylosoxidans* were (24.680 $\pm$ 13.293), (22.900 $\pm$ 10.659), (21.766 ± 11.476), (17.733 $\pm$ 14.987), (11.226 $\pm$ 6.722), (9.200 $\pm$ 0.00), and (7.8000 $\pm$ 0.00) respectively. As shown in table (4-7).

Table (3-7) Relationship of WBC count with the type of bacterial infections:

Type of bacteria		WBC (x 10^3 /µl)			P value
		N (50)	Mean	Std. Deviation	
Escherichia coli	30	11.2267	6.72242		0.026
Pseudomonas aeruginosa	5	24.6800	13.29368		
Enterococcus faecalis	3	21.7667	11.47621		
Proteus mirabilis	4	22.9000	10.65927		
Klebsiella pneumoniae	6	17.7333	14.98702		
Streptococcus sanguinis	1	9.2000	0.00		
Aachromobacter xylosoxidans	1	7.8000	0.00		

These findings might be explained by the fact that gram +ve and gram -ve bacteria produce cytokine patterns in human mononuclear cells in distinct ways. Gram-positive bacteria preferentially increase monocyte interleukin 12 productions, but gram-negative bacteria are potent inducers of IL10.Which is produced in response to bacterial products from monocytes/macrophages and has major immune system consequences.IL12 activates cytotoxicity and gamma interferon release by NK cells and T cells, whereas IL-1 inhibits these actions (Hessle *et al*., 2000).

However, other studies do not agree with these results where total WBC count showed no differences between the patients with UTI caused by *E. coli, Klebsiella spp.* or *Enterococcus spp.* (Falup-Pecurariu *et al*., 2020). And other have reported that the difference in both total WBC (P=0.637) and Neutrophils (P= 0.525) count was not statistically significant between the gram +ve and gram –ve (Akya *et al*., 2019).

3.3 Immunological study:

3.3.1 Determination of C-Reactive protein concentration:

Documented data showed highly significant elevation ($P \leq 0.05$) of CRP in UTI patients (26.646 ± 27.560) in compare with control (1.360 ± 1.088), as shown in table (3-8).

Table (3-8) C-Reactive protein concentration in serum of patients and control groups

Variables		N (90)	Mean	Std. Deviation	P Value
CRP(mg/l)	Cases	50	26.646	22.560	0.0000
	Control	40	1.360	1.088	

These results agreed with a study in Basra that reported a statistically significant increase in CRP between UTI patients and control (Alhamedy and Shani, 2020). Where C-reactive protein is a good diagnostic tool in differentiating upper UTI and lower UTI as it is significantly raised in upper UTI (Mamatha *et al*.,2020).

The presence of pro-inflammatory cytokines such as interleukin-1 and interleukin-6, which prompt the liver to create a high level of CRP, explains this (Lacour *et al*., 2001).

3.3.1.1 Relationship between CRP concentration and site of infection among UTI patients:

In the present study, significant difference ($P \leq 0.000$) was observed between the mean levels of CRP in patients with pyelonephritis (56.107 ± 28.911) and patients with cystitis (11.470 ± 6.824), as shown in table (3-9).

Table (3-9) Relationship between CRP concentration and site of infection

Variables		N (50)	Mean	Std. Deviation	P Value
CRP(mg/l)	pyelonephritis	17	56.1071	28.911	0.000
	cystitis	33	11.4700	6.824	

These results agreed with the Iranian study was done on children with UTI, which showed a significant difference (P=0.000) between acute pyelonephritis patients where CRP mean was (53) mg/l in comparison with that in lower UTI patients where it was (4.05) mg/l (Mahyar *et al*., 2013).

Elevated serum levels of C-reactive protein in acute pyelonephritis patients also was observed by a study where CRP mean (84.1 ± 62.1) mg/l and cystitis patients were (36.7 ± 25.8) mg/l (Lee *et al*., 2015). The same result was shown in other studies where they found that CRP was a good predictive marker for the presence of APN in patients with UTIs (Mithaq *et al*., 2011; Sim *et al*., 2015, Moon *et al*., 2020).

3.3.1.2 Relationship between CRP level and gender among patients with UTI:

The present study showed that there was no significant gender difference in CRP level among the patients in the sample ($P > 0.05$), where CRP mean in males was (36.027 ± 29.504) and in females, it was (20.897 ± 15.069), as shown in table (3-10).

Table (3-10) Relationship between CRP level and gender among children with UTIs

Gender		CRP (mg/l)			P value
		N	Mean	Std. Deviation	
Male	19	36.027		29.504	0.059
Female	31	20.897		15.069	

These results disagree with a study in Baghdad were found a significant difference (P=0.000) between males and females in CRP levels in patients with UTIs (AL-Khikani and Ayit, 2019).

3.3.1.3 Relationship of CRP level concentration with bacterial isolates:

This study indicated that a significant association was found between the CRP level and type of bacterial isolate (P<0.05). Although, the patients infected with P. mirabilis recorded a high level of CRP (52.65 ± 36.77) Followed by *P.aeruginosa*. (45.502 ± 25.693), *K. pneumoniae*. (41.616 ± 43.756), *E. coli*. (17.560 ± 20.091), *S.sanguinis* (11.900±0.000) and *A.xylosoxidans* (5.1000±0.000), respectively. No standard deviation was measured in patients who were infected by *S.sanguinis* and *A.xylosoxidans* because standard deviation measures the amount of variation or dispersion of a set of values, while the present study found only one sample infected with each one from these bacteria, as shown in table (3-11).

Table (3-11) Relationship of CRP level with type of bacterial infections

Variables		CRP (mg/l)			P value
		N (50)	Mean	Std. Deviation	
bacterial Isolates	*Escherichia coli*	30	14.64	12.43	0.049
	Pseudomonas aeruginosa	5	45.5022	25.69396	
	Enterococcus faecalis	3	33.5667	7.51953	
	Proteus mirabilis	4	52.6500	36.77957	
	Klebsiella pneumoniae	6	14.05	12.29	
	Streptococcus sanguinis	1	11.9000	0.00	
	Aachromobacter xylosoxidans	1	5.1000	0.00	

Similar results have been reported with other authors were they discovered a strong link between CRP and bacterial isolation in the urinary tract infection (AL-Khikani and Ayit, 2019).

In a study done by Gao and his team , CRP in the Gram-negative bacterial infection group was significantly higher than in the Gram-positive group (Gao *et al*., 2017) , which is contrast with study in Basra which indicated that there was no correlation between the high-sensitivity C-reactive Protein (hs-CRP) and the type of bacteria in UTI patients although the patients infected with *E. coli* recorded a high level of hs-CRP in comparison with other recorded bacterial type with no significant difference (Alhamedy and Shani, 2020).

3.3.2 Determination of LL-37 level in urine of patients with UTIs:

The present study results presented the LL-37 urine concentration of studied groups was (2251.995 ± 411.068) for patients and (627.409 ± 204.943) control, with highly significant differences P≤0.05 between the two groups, As shown in table (3-12):

Table (3-12) Determination of LL-37 level in urine of patients with UTIs

Variables		N	Mean (ng/ml)	Std. Deviation	P Value
LL-37	Cases	50	2251.995	411.068	0.000
	Control	40	627.409	204.943	

Also, a significant difference (P≤0.05) was observed between Pyelonephritis and cystitis in urinary LL-37 concentration as shown in table (3-13).

Table (3-13) Determination of LL-37 in urine of patient according to the site of infection

Variables		N (50)	Mean (ng/ml)	Std. Deviation	P Value
LL-37	Pyelonephritis	17	2519.616	330.902	0.001
	Cystitis	33	2114.129	382.554	

Nielsen *et al.*, 2014 observed in their study that patients with UTIs had much higher LL-37 in their urine during infection than thereafter. And their post-infection LL-37 levels were significantly lower than those of controls, LL-37 concentrations in urine samples from UTI patients were measured during and after

infection, The concentration of LL-37 in urine was considerably higher during infection than after infection for all UTI patients except one and for urine samples from healthy controls who had never had a UTI. Furthermore, baseline LL-37 levels in controls were considerably greater than levels in UTI patients following infection.

A case-control study was conducted at Omdurman Hospital, Sudan where LL-37 is notably increased among patients with UTI compared with normal control subjects. The severity of UTI increases the levels of LL-37. The increased level was not only in the patient's urine but also it was observed in patients serum *(Babikir et al*., 2018).

Alhamedy and Shani found that the mean concentration of anti-microbial peptide cathelicidin in the urine of UTI patients was much more than healthy subjects, With highly significant difference al level ($P \leq 0.001$) (Alhamedy and Shani, 2020)

While Hachamdiglu *et al*. in 2016 disagree with these results, where they found that the LL-37 urinary levels were (151.9 ± 59) in children with UTI, while it was (164.5 ± 27.8) in control groups, with no significant differences (Hacıhamdioğlu *et al*.,2016). Also other study done by Caterino *et al*., showed that LL-37 was not significantly higher in patients with positive urine culture in comparison with control (Caterino *et al*., 2015)

3.3.2.1 Association between type of bacteria and LL-37 level of the patients:

The present research records a significant association between the LL-37 level and the type of bacteria isolate ($P < 0.05$), where the higher concentration was recorded in patients with *P. mirabilis* (2641.421 ± 264.985), followed by E. *faecalis* (2613.233 ± 222.558), *P. aeruginosa* (2418.470 ± 377.246), *E. coli*

(2243.433±389.901), *K. pneumoniae* (1894.401±311.934), *A.xylosoxidans* (1829.633 ±0.000) and *S.sanguinis* (1602.973±0.000) ,respectively .As shown in table (3-14) :

Table (3-14) Association between bacterial isolates and LL-37 level of the patient

bacterial Isolates	LL-37 (ng/ml)			P value
	N (50)	Mean	Std. Deviation	
Escherichia coli	30	2243.433	389.901	0.013
Pseudomonas aeruginosa	5	2418.470	377.246	
Enterococcus faecalis	3	2613.233	222.558	
Proteus mirabilis	4	2641.421	264.985	
Klebsiella pneumoniae	6	1894.401	311.934	
Streptococcus sanguinis	1	1602.973	0.00	
Aachromobacter xylosoxidans	1	1829.633	0.00	

These results disagree with Awadallah study who noticed nonsignificant (P=0.54) relation between LL-37 levels and type of bacteria that isolated from urine of patients with UTI (Awadallah *et al*.,2019).

Also, contradicted another study conducted in Basra, which proved that there is no significant difference correlation between the type of bacterial infection and concentration of LL-37 in urine (Alhamedy and Shani, 2020).

The simple explanation is that antimicrobial peptides is widely present in nature and they have antimicrobial activity against many pathogens that include Gram-positive and Gram-negative bacteria, fungi, parasites, and even coated viruses(Babikir *et al*., 2018).

3.3.3 Determination of HNP 1-3 level in urine:

Present result showed that there is a significant difference at level (P<0.05) in urinary HNP (1-3) concentration between UTIs patients (5.3850+1.23619) and control (0.9047±0.52382) group, As showed in table (3-15).

Table (3-15) Determination of HNP 1-3 level in urine of UTI patients

Variables		N (90)	Mean (ng/ml)	Std. Deviation	P Value
HNP (1-3)	Cases	50	5.3850	1.23619	0.000
	Control	40	0.9047	0.52382	

These results were similar to Watson and his team results, where they agreed that urine HD5 and HNP (1–3) concentrations were considerably greater in culture-positive urine samples compared to culture-negative urine samples and that both HD5 and HNP1–3 functioned well as diagnostic tests for predicting positive urine culture in children. (Watson *et al*., 2016).

In addition, a study published by Caterino *et al*., 2015 agreed with these findings, were HNP (1-3) significantly higher in those with positive than negative urine cultures.

In the present study the levels of HNP (1-3) in the urine of patients with pyelonephritis (5.898±1.406) which is much higher than patients with cystitis (5.120+1.066) as seen in table (3-16).

Table (3-16) Determination of HNP 1-3 in urine of patient according to the site of infection

Variables		N (50)	Mean	Std. Deviation	P Value
HNP (1-3)	pyelonephritis	17	5.8988	1.40645	0.033
	cystitis	33	5.1203	1.06697	

This is agreed with another study that displayed HNP(1–3) levels in the urine increased eightfold in chronic pyelonephritis patients compared to controls and glomerulonephritis patients in one research (Spencer *et al*., 2014).

The interpretation of these results is that (HNP1–3) are bactericidal expressed in neutrophils and the kidney, its expression increase in the setting of pyelonephritis as a defense mechanism against infection (Spencer *et al*., 2014).

3.3.3.1 Association between type of bacterial isolates and HNP (1-3) level:

The present research showed that there was no significant differences between the type of bacteria and concentration of HNP (1-3) at level P $\geq$0.05, where the higher HNP (1-3) concentration were observed in patients with *E.faecalis*(7.3467+1.93725) ,Followed by *P.aeruginosa* (5.7160 $\pm$ 1.260), *A.xylosoxidans* (5.520 $\pm$ 0.00), *E.coli* (5.367 $\pm$ 1.1649), *K. pneumoniae* (4.905 $\pm$ 0.787), *P. mirabrlis* (4.71 $\pm$ 0.503) and *S.sanguinis* (3.800 $\pm$ 0.00). As shown in table (3-17).

Table (3-17) Association between type of bacterial isolates and HNP 1-3 level

Bacterial Isolates	HNP (1-3)			P value
	N (50)	Mean	Std. Deviation	
Escherichia coli	30	5.3670	1.164	0.058
Pseudomonas aeruginosa	5	5.7160	1.260	
Enterococcus faecalis	3	7.3467	1.937	
Proteus mirabilis	4	4.7175	0.503	
Klebsiella pneumoniae	6	4.9050	0.787	
Streptococcus sanguinis	1	3.8000	0.00	
Aachromobacter xylosoxidans	1	5.5200	0.00	

Chemokines are released during UTIs, and leukocytes are drawn to the infection site. Bacterial adhesion to epithelial lining of the urinary tract can be inhibited by the release of bactericidal antimicrobial peptides such as α and β defensins, cathelicidin, lactoferrin, and others, Tamm-Horsfall protein and lipocalin. Large numbers of macrophages and neutrophils are activated and attracted by cytokines (IL-1 and TNF alpha) and chemokines (IL-8) that damage tubulointerstitial parenchyma and allow bacteria to persist (Akhavan Sepahi *et al*., 2018).

Antimicrobial peptides (AMPs) are key effectors of innate immunity in the urinary tract that have antimicrobial activity through several mechanisms, including inhibition of bacterial binding, cell lysis, and induction of other immune components, Preliminary studies have documented increased urinary levels of several AMPs in response to infection (Zasloff, 2007).

A model antimicrobial peptide is demonstrated to have multiple hits on bacteria, including surface protein delocalization. While cell surface modification to

decrease cationic peptide binding is a recognized resistance mechanism for pathogenic bacteria, it is also used as a survival strategy for commensal bacteria.(Wang *et al*., 2015) .

Both Gram-positive and Gram-negative bacteria are able to decorate their cellular surfaces to make them less attractive to cationic antimicrobial peptides. Interestingly, a recent study reveals that gut bacteria can use a similar mechanism by removing a phosphate group from LPS(Cullen *et al*., 2015).

3.3.4 Correlation between urinary and blood markers in patient with UTIs:

Statistical analysis indicated that was strong positive correlation between WBCs and both of Neutrophil count (0.843**) and CRP (0.598**) in blood. In addition, present study results showed no relationship between HNP (1-3) and Cathilciden (0.350) in urine, as shown in table (3-18).

Table (3-18) Correlation of LL-37 with HNP1-3 level in patient with UTIs

		Neutrophils	WBC	CRP	HNP (1-3)
WBC	r	0.843**			
CRP		0.575**	0.598**		
HNP (1-3)		0.059*	0.201	0.171	
LL-37		0.211	0.341	0.400	0.350

*Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed)

The strong relationship between WBC and (CRP) could be because both of them are a clinical marker of inflammation and play central part of the immune system by help to protect the body against foreign substances, microbes, and infectious diseases so their synthesis increased during inflammation (Ansar, and Ghosh,2016)

Antimicrobial peptides are stored in a nonfunctional form in neutrophils and macrophage secretory granules and become activated after being processed and released upon leukocyte activation. Because they are one of the key components of innate immunity is the antimicrobial peptides and their execration increase during inflammation. Also, they play a critical role in warding off invading microbial pathogens, apoptosis, wound healing, and immune modulation (Wang, 2014)

Because this is a time-limited study, the sample size is less. To increase authenticity, a large number of UTI cases will be investigated. The current study did not follow up on CRP, WBC, LL-37, or HNP (1-3) after antibiotic therapy due to cost constraints.

Conclusions and Recommendations

Conclusions

1. The present study observed that frequency of UTIs in children were caused by *E. coli*, *K. Pneumonia*, *P. areginosa*, *P. Mirabils* and *E.faecalis* respectively and other uncommon bacteria.
2. Elevated level of CRP and WBC in pyelonephritis patients much more than cystitis.
3. Urinary levels of HNP1–3 and LL-37 were much greater in patients in comparison with healthy control.
4. The present study demonstrates urine levels of LL-37 and HNP1–3 is significantly greater in pyelonephritis patients in comparison with cystitis patients.
5. There is no relationship between urinary HNP (1-3) and LL-37 in UTI patients
6. There is a positive correlation between blood WBC and CRP (1-3) in UTI patients.

Recommendations:

1. Determination of genetic expression of cathelicidin and HNP 1-3 in UTI patients.
2. More studies are headed for Screening of cathelicidin and HNP 1-3 in UTI patients as a marker for silent UTI in children.
3. Study the relationship between UTIs in children and other antimicrobial peptide.
4. Further studies related to HNP 1-3 and cathelicidin in pyelonephritis should be done on adults.

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Appendix

Appendix

Appendix I: Questionnaire Form

Assessment Questionnaire

Personal information

Name :	
Gender :	
Weight :	
Age :	
Date :	

Have you ever had any of the following ?

diabetes mellitus		**urinary catheter for a time**	
renal calculi		**take antibiotic before**	
immunocompromised state		**acute kidney injury and chronic renal failure**	
congenital anomaly as reflux nephropathy		**toilet training**	
Male circumcised		**webbing from back to front**	
worm infestation		**Constipation**	

Urinalysis **Microscopic Examination**

Color		**Glucose**		**Pyuria**	
Turbidity		**Sp.Gr**		**Bacteriuria**	
UBG		**Nitrite**		**RBCs**	
Ery's		**Leukocyte**		**Crystals**	
Ketone		**: Glucose**		**EPth**	

The diagnosis that related to symptoms?

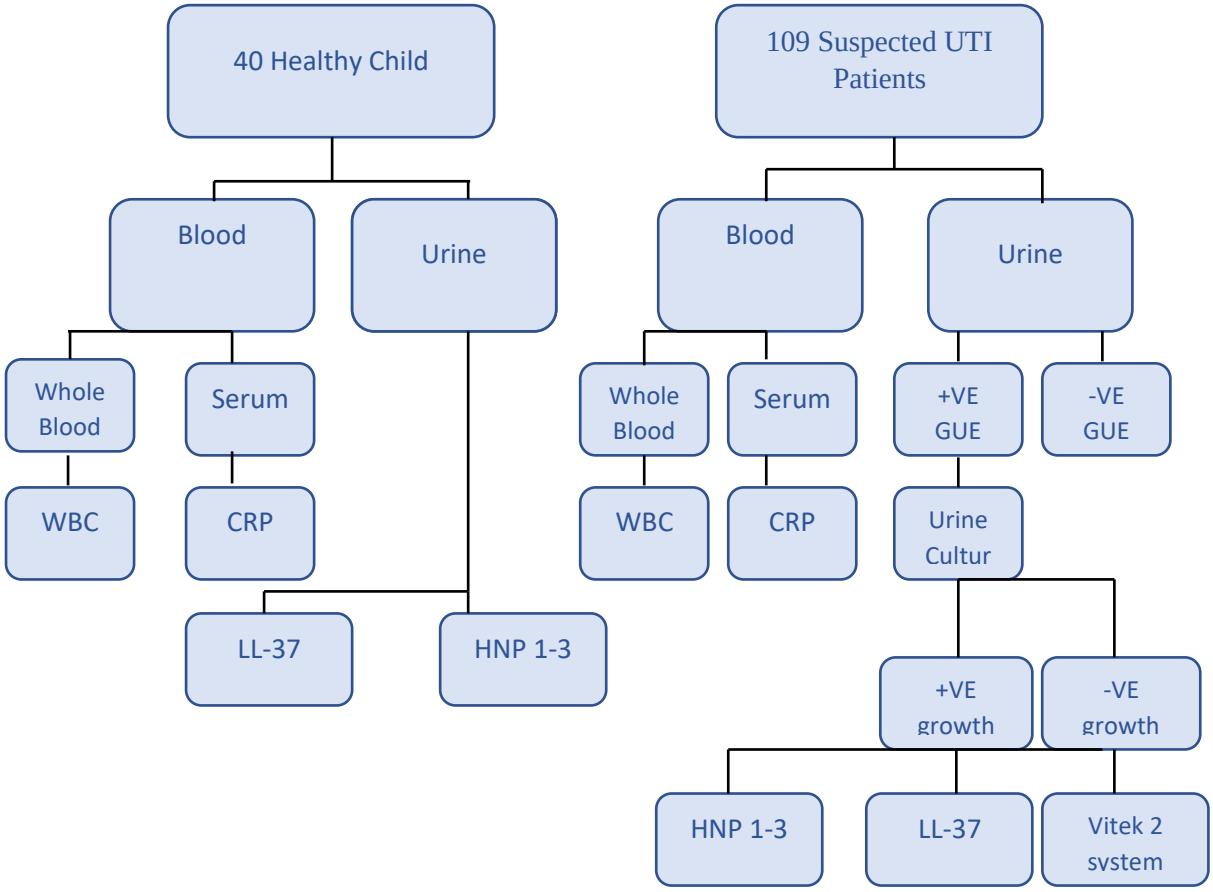
Cystitis		**Pyelonephritis**		**Urosepsis**	

Tests result

CRP	
Urine cathicidin	
Urine HNP 1-3	

Appendix II: Schematic diagram of present study

Only 109 patient were diagnosed by physician in urinary tract infection , only positive urine culture were included in the present study



الخلاصة

تعد التهابات المسالك البولية (UTI) واحدة من أكثر أنواع العدوى البكتيرية شيوعًا في مرحلة الطفولة. عدوى المسالك البولية تنقسم إلى ثلاث فئات؛ التهاب المسالك البولية العلوي (التهاب حويض الكلية الحاد)، التهاب المسالك البولية السفلي (التهاب المثانة)، وتسمم البول.

خلال الفترة الممتدة من ديسمبر 2020 إلى أبريل 2021، تم الحصول على عينات من الدم والبول من 109 من مرضى المسالك البولية المشتبه بهم و40 من الأصحاء، تراوحت أعمارهم من شهر واحد إلى 12 عامًا. تم جمع العينات في مكانين (مستشفى كربلاء التعليمي للأطفال والعيادة الخاصة). كان نظام Vitek2 الطريقة المستخدمة للتعرف على الأنواع البكتيرية. تم قياس ببتيد العدلات البشرية 1-3 (HNP 1-3) و(LL-37) Cathelicidin باستخدام نظام ELISA، بينما تم قياس C-Reactive Protein بواسطة محلل الكيمياء التلقائي DIRUI CS-T180 وخلايا الدم البيضاء باستخدام Sysmex XP-300. أظهرت النتائج أنه من بين 109 عينة بول، 14 (12.8٪) لديها نمو غير معنوي للبكتيريا، 45 (41.3٪) ليس لديها نمو، و50 (45.9٪) وجدت بنمو بكتيري معنوي، فقط تم تضمينهم في العمل الحالي. يعاني 50/17 فقط من التهاب الحويضة والكلية بينما يعاني 50/33 من التهاب المثانة.

كانت نتائج العزلات البكتيرية 50/30 (60٪) *Escherichia coli*، 50/6 (12٪) *Klebsiella pneumoniae*، 50/5 *Pseudomonas aeruginosa* (10٪)، 50/4 (8٪) *Proteus mirabilis*، 50/3 *Enterococcus facalies* (6٪)، 50/1 (2٪) *Achromobactin xylosoxidans*، و50/1 (2٪) *Streptococcus sanguinis*. أظهر متوسط مستويات LL-37 البولية فروق معنوية ($P \leq 0.05$) بين المرضى (2251.9951 $\pm$ 411.06814) والمجموعة الضابطة (627.4092 $\pm$ 204.94316). كما كان الفرق معنويا بين مرضى التهاب حويض الكلية الحاد (2519.6169 $\pm$ 330.90265) والتهاب المثانة (2114.1294 $\pm$ 382.55454). كانت أعلى مستويات LL-37 في البول في مرضى *P. mirabilis* و *E. faecalis* و *P. aeruginosa* و *E. coli* و *K. pneumoniae* و *A. xylosoxidans* و *S. sanguinis* على التوالي.

كشفت دراسة المقارنة عن وجود فرق معنوي في مستويات HNP البولي (1-3) بين المرضى (5.3850 $\pm$ 1.23619) ومجموعة الاصحاء (0.9047 $\pm$ 0.52382)، بينما متوسط HNP (1-3) في مرضى التهاب الحويضة والكلية (5.8988 $\pm$ 1.40645) كان أكبر بكثير من

مرضى التهاب المثانة (5.1203 ± 1.06697). كان أعلى تركيز في البول (1-3) في مرضى *E. faecalis* و *P. aeruginosa* و *A. xylosoxidans* و *E. coli* و *K. pneumoniae* و *P. mirabilis* و *S. sanguinis* على التوالي. أشار التحليل الإحصائي إلى وجود علاقة إيجابية قوية بين كرات الدم البيضاء وعدد العدلات (0.843 **) وCRP (0.598**) في الدم. بالإضافة إلى ذلك، أظهرت نتائج الدراسة الحالية عدم وجود علاقة بين HNP (1-3) وCatheliciden عند مستوى P≤0.005.

لخصت هذه الدراسة إلى أن المستوى المرتفع لـ CRP وWBC في مرضى التهاب حويض الكلية الحاد هو أكثر بكثير من التهاب المثانة، وأن المستويات البولية من HNP1-3 وLL-37 كانت أعلى بكثير في المرضى بالمقارنة مع الاصحاء، وليس هناك علاقة إيجابية بين LL-37 وHNP (1-3) في مرضى المسالك البولية، وهناك علاقة إيجابية بين WBC في الدم وCRP في مرضى المسالك البولية.

وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية العلوم الطبية التطبيقية
قسم التحليلات المرضية

الكشف عن الببتيدات المضادة للميكروبات في التهابات المسالك البولية عند الأطفال، في محافظة كربلاء

رسالة مقدمة

الى مجلس كلية العلوم الطبية التطبيقية - جامعة كربلاء

كجزء من متطلبات نيل شهادة الماجستير في التحليلات المرضية

مقدمة من قبل

تمني ماجد حميد محمد رضا

بكلوريوس تحليلات مرضية ٢٠١٨ - جامعة الكوفة

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