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# Role of Toll like receptor2 and 9 Polymorphism with urinary tract infection patients

**A Thesis**

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by

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﴿ بِسْمِ اللّهِ الرَّحْمَنِ الرَّحِيمِ ﴾

((وَيَسأَلونَكَ عَنِ الرّوحِ قُلِ الرّوحُ مِن أَمرِ رَبّي وَما أوتيتُم مِنَ العِلمِ إِلّا قَليلًا))

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## Dedication

**To …….**

**My Teachers….&……Mentors**

**The First Educator... My Father Soul**

**The Kind Heart….. My Mother**

**My Husband ...& …My children**

**The Supporters….My family**

**All Supporters…&...Assistants**

**I Dedicate this work.**

*Hieam*

*2022*

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**Summary:**

The current study aimed to study polymorphism TLR2 & TLR9 in Urinary tract infection . Blood and urine samples were collected form patients and healthy as control groups from Babylon province for the period between October 2020 to March 2021.

The study included three main axes: First isolation and identification of pathogenic, Second detection the SNPs of TLR2and TLR9 by using ARM-PCR techniques and PCR, Third axis included a estimation the concentration of TLR2 and TLR9 in Urine and serum to understand the effect of SNPs on the concentration of them. The present study showed that the highest incidence of urinary tract infection occurs in the age difference (28-38 years), while the lowest incidence of urinary tract disease occurred in the age between (38-48 years).

Bacteriology study included the identification of different bacteria from urine samples after culture and then determined by the Vitek two system and showed the percentage of pathogenic *for Escherichia coli* 80 (21.5%), followed by *Enterococcus faecalis* 50 (13.44%), while the *Klebsiella* was 40 (10.75%) and *Staphylococcus saprophyticus &Candida albicans* were 14(4.3% ) 46 (12.1) respectively.

The results of molecular study of gene polymorphism for TLR2 and TLR9 The results showed that no significant variations between study groups in the TLR 2(rs3804099) polymorphism (TT vs TC: OR=2.02, CI (95%) 0.0938 to 43.7262, P =0.6527). also no significant variations between study groups in the TLR2 rs3804099) polymorphism (TT vs CC :OR=5.2000, CI (95%): 0.6442 to 41.9722 , P =0.121.A high risk values have been detected in (the homo dominant , hetero codominant

model, as well as allele frequency (OR = 2.02 , 5.2 and11.01 respectively). The study showed a higher correlation between TLR2 (serum) level with TLR2 rs3804099 (P = 0.028) , TLR2 in serum level was 207±283,60.16±26 and 147±145 in CC,CT and TT ( respectively ).there are no significant correlation between age and TLR2 (urine) level with TLR2 rs 3804099, The sequencing results revealed there were six SNPs in the part of the exon 2 region of the *TLR9* gene while there were five SNPs in the promoter region site . some of these SNPs are previously SNPs in the part of the exon 2 region of the *TLR9* gene while there were five SNPs in the promoter region site . some of these SNPs are previously recorded in NCBI such as rs352140 in exon 2 and rs1228893923, rs1699624843 and rs1699624414 in promoter region Whereas, other SNPs are novel and not recorded previously like that located at the chromosomal site.

Immunolgic study the Concentration of TLR2 & TLR9 in urine and serum of patients was higher in comparison with healthy control. The important conclusion of this study that bacteria when infected urinary tract increased the nonspecific humoral immunity due to increase the expression of Pattern recognition receptors. And TLR 2 and TLR9 were significantly higher correlated with both Gram positive and Gram negative bacteria

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## List of Abbreviations

| Abbreviated Form | Meaning |
|---|---|
| **AMP** | **Adenosine Monophosphate** |
| **ABU** | **American Board of Urology** |
| **ARAM** | **Amplification-Refractory Mutation** |
| **Gr1** | **Anti-granulocyte receptor** |
| **ASB** | **Asymptomatic Bacteriuria** |
| **BC** | **Before Christ** |
| **BBD** | **Bladder and Bowel Dysfunction** |
| **CpG-ODN** | **CpG-containing Oligonucleotides** |
| **CLRs** | **C-type Lectin Receptors** |
| **CpG** | **Cytosine phosphate guanine** |
| **DCs** | **Dendritic Cells** |

| | |
|---|---|
| **ELISA** | **Enzyme –linked immunosorbent assay** |
| **FimH** | **Fimbriae D-mannose specific adhesin.** |
| **GPI** | **Glycosylphosphatidylinositol** |
| **HCC** | **Hepatocellular carcinoma** |
| **HEK** | **Human Embryonic Kidney** |
| **IgG** | **Immunoglobulin G** |
| **IL-1** | **Interleukin 1** |
| **LPSs** | **Lipopolysaccharides** |
| **MΦs** | **M1-like Macrophages** |
| **MMP9** | **Matrix metallopeptidase 9** |
| **MR-VP** | **Methyl red- Vogas Proskaur** |
| **MALT** | **Mucosa-Associated Lymphoid Tissue** |
| **MYD88** | **Myeloid Differentiation primary response 88** |
| **NLRs** | **Nod-Like Receptors** |
| **NFκB** | **Nuclear Factor kappa B** |
| **OR** | **Odds Ratio** |
| **PAMPs** | **Pathogen Association Molecular Patterns** |
| **PRRs** | **Pathogen Recognition Receptors** |
| **PCR** | **Polymerase Chain Reaction** |
| **PMNs** | **Polymorphonuclear leukocytes** |

| | |
|---|---|
| **Rs** | **Reference system** |
| **RR** | **Relative Risk** |
| **RIG-I** | **Retinoic acid Inducible gene** |
| **SNPs** | **Single-nucleotide polymorphisms** |
| **Th1** | **Thelper1** |
| **TcpC** | **TIR-containing protein** |
| **TRIF.** | **TIR-domain-containing adapter-inducing interferon-β** |
| **TIR** | **Toll/Interleukin-1 Receptor** |
| **TIRAP** | **Toll-Interleukin 1 Receptor (TIR) Domain Containing Adapto** |
| **TLR** | **Toll-like Receptor** |
| **TNF** | **Tumor Necrosis Factor** |
| **UTIs** | **Urinary Tract Infections** |
| **UPEC** | **Uropathogenic Escherichia coli** |

## Introduction

Urinary tract infections (UTIs) are one of the most common infectious diseases in millions of people every year. They are triggered when infecting bacteria gradually establish in the bladder and multiply in the urine ( Hashemi *et al*., 2019)**.**

UTIs are a significant cause of morbidity in infant boys, older men and females of all ages. Serious sequelae include frequent recurrences, pyelonephritis with sepsis, renal damage in young children, pre-term birth and complications caused by frequent antimicrobial use, such as high-level antibiotic resistance, UTIs are caused by both Gram-negative and Gram-positive bacteria, as well as by certain fungi causative agent for both uncomplicated and complicated . (Karishetti and Shaik, 2019).

Despite the presence of strong barriers made of urothelial cells in the human urinary tract, sometimes the uropathogenic microorganisms successfully enter the urinary tract. By the entrance of uropathogenic microorganisms into the urinary tract, the innate immune responses are going to be activated via expression of the related TLRs within the urothelial cells of bladder (cystitis) and kidneys (nephritis)( Jahandeh *et al*., 2015)**.**

The main role of the host immune system is to trigger both innate and adaptive immune responses. Innate immunity is activated with the participation of pattern recognition receptors (PRRs) on the dendritic cells, macrophages, polymorphonuclear leukocytes and epithelial cells. They recognize and distinguish pathogen-associated molecular patterns (PAMPs), related to the pathogenic potential of the bacterial cell walls. The most important distinguishing receptors are the Toll-like receptors

(TLRs) (Hug *et al*., 2018). TLRs bind and become activated by different ligands, located on different types of microorganisms or structures ,The TLR2 function by detecting different PAMPs and then mobilizing appropriate immune defences , The common TLRs encountered in urinary tract include TLR2 (recognizes bacterial lipoteichoic acid or lipoprotein ), TLR2 is stimulated by peptidoglycan,as might be presented by gram positive uropathogens including Enterococcus and Staphylococcus species as well as by lipoproteins and perhaps Omp A TLR9 (recognizes unmethylated CPG DNA of bacteria and viruses)( Song *et al*., 2008)**.**

The activation of TLRs leads to the induction of effector genes involving inflammatory cytokines and, as a result, provides links between innate and adaptive immunity (Qiu *et al*., 2019).

The SNPs correspond to the most frequent type of variation in the human genome, and due to the importance of TLR2 in the immune response, SNPs in the TLR gene are related to susceptibility or resistance to various diseases (Silva *et al.*,2021). Variations in the *TLR9* gene have been linked to viral and bacterial infections (Bochud .,2007).

However , the current study was aimed to find the correlation between Urinary tract infection and polymorphism of TLR2 and TLR9, to achieve that , the objective of study involved the following to:

1-Identify the types of bacteria associated with Urinary tract infection specially gram positive and negative bacteria .
2-Estimate the concentration of TLR2 and TLR9 in Urin and serum to understand the effect of SNPs on the concentration of them.

3-Detect the SNPs of TLR2and TLR9 by using ARM-PCR technique and PCR .

4-Sequence of for TLR2 and TLR9 new SNPs if found and for proving the type of SNPs.

## 2-1 Urinary Tract Infection

Urinary tract infections (UTIs; ranging from uncomplicated cystitis to severe pyelonephritis and nephrolithiasis) are the third most common types of infection in human medicine worldwide (after respiratory tract infections and infections of the alimentary tract), and the second most million cases/year( Wiedemann *et al*.,2014, Flores-Mireles *et al* .,2015 and Sobel and Kaya ,2015).

These infections affect outpatients and hospitalized patients to a significant extent (accounting for 25–50% of hospital-acquired infections overall), representing an important factor of morbidity, especially due to their recurring nature( Hooton *et al*.,2010 and Flores-Mireles *et al* .,2015). UTIs more commonly affect females, patients with immunosuppression or underlying diseases developmental abnormalities of the urinary system and they are associated with some lifestyle choices (sexual promiscuity, public baths( Gupta *et al*.,2011 and Flores-Mireles *et al* .,2015)

Up to 60% of women have at least one symptomatic UTI during their lifetime. Around 10%of women in the United States have one or more episodes of symptomatic UTIs each year. Young, sexually active women 18–24 years of age have the highest incidence of UTIs. About 25% of these women have spontaneous resolution of symptoms, and an equal number become infected (Sobel and Kaye 2015).

Predisposing factors to Asymptomatic Bacteriuria (ASB) are low socioeconomic status, increasing age, multiparty,sexual behavior, urinary tract anomalies, previous treatment for UTI, other medical conditions like diabetes, sickle cell disease and immune compromised states like

AIDS, spinal cord injuries and women in menopause age (Fareid,2012). Patients with asymptomatic urinary tract bacteriuria, 25%, will develop symptomatic urinary tract infection (cystitis, pyelonephritis) than those without (Nabbugodi *et al*., 2015).

Many factors such as sex, age, race, circumcision (Dias *et al*., 2010), urogenital tract abnormalities, urinary catheter (Mladenovic *et al.*,2015), infants, elderly, pregnancy and hospital (department) (Nelson and Good, 2015), are believed to increase the risk of recurrent UTIs.

In the other studies there has been increased appreciation that other factors,such as Bladder and Bowel Dysfunction (BBD) and defects in innate immunity, may also be important contributors to the recurrence of UTI (Keren *et al*., 2015) ,The risk of UTI in men rises with the increase in age. The occurrence of a urinary tractinfection will occur more during the first few months of life, Enlarged prostate gland hinders and slows the flow of urine escalatingthe risk of infection (Anuli 2016).

Urinary Tract Infection in young adult males of less than 50 is rare but rises thereafter. This is because of factors such as enlarged prostate, loss of prostatic fluid and kidney stones(Brusch,2017) .

UTI one of the most important causes of morbidity and also the second most common cause of hospital visit (Wagenlehner *et al*., 2016). It occurs in all populations and ages from the neonate to the geriatric age group (Thattil and Santhosh 2018) Patients diagnosed with type 2 diabetes mellitus are predisposed to UTI than the general population. It is usually more severe with a worse outcome than patients without. More often the UTI is triggered by resistant strains of pathogen. This is due to the immune system being impaired, poor metabolic control and bladder

dysfunction (Nielubowiz and Mobley ,2010). Kidney stones also lead to Urinary tract obstruction causing acute pyelonephritis. When urine outflow obstruction occurs, there is a possibility of incomplete emptying and urinary stasis which causes bacteria to multiply without being flushed out leading to UTI (Belyayeva ,2019).

also Numerous urinary tract infection studies have been performed on different age groups including preschool, school age, young adults, and the elderly (Thattil and Santhosh 2018). Among persons over age 65, bacteriuria affects men and women roughly equally, with the majority of infections being asymptomatic. Among persons between 1 and 65 years of age, UTI predominantly occurs in female patients, presumably because of the anatomy of the female urethra, which allows bacteria to access the urinary tract relatively easily. Routine screening and treatment has been found to decrease morbidity or mortality in this population. Among infants up to 6 months of age, UTI is more common in boys, who have a higher incidence of abnormalities of the urinary tract than girls (Litza and Brill, 2010).

Vesico-ureteral reflux a congenital, anatomical and functional disorder has been implicated to cause a high morbidity especially in young children. It occurs when there is a backward flow of urine from the bladder to the kidney. This facilitates movement of the bacteria from the lower urinary tract gain access to the upper urinary tract causing UTI (Duane, 2016).

Urinary tract infections usually become an opportunistic infection among people living with HIV because of the weakened immune system (schneede, 2003). Circumcision is a common practice worldwide and is one of the commonest surgical procedures practiced. As much as the

practice is carried out due to cultural and religious belief it has been documented that it protects men from acquiring UTI , Several studies conducted among uncircumcised boys and men swabbed before circumcision identified *E. coli*, *Proteus spp.* and *K. pneumonia* as the most frequent agent and *S. aureus* as the least prevalent. The Gram negatives are documented to be higher in occurrence than the gram positives (krieger and Morris, 2017).

Infections of the urinary tract are the most frequently reported bacterial infections in the community coming second to respiratory tract infections in humans (Thass *et al.,* 2019) Urinary tract infections may be nosocomial or community acquired. It is believed that most urinary tract infections develop and are acquired from the community. It is unclear how primary community-acquired infections occur or how they are spread, although most cases have been thought to arise sporadically (Djordjevic *et al.,* 2016).

UTIs are also commonly acquired in the hospital, often due to contaminated urinarycatheters.Hospital-acquiredinfections (known as nosocomial infections) tend to be more serious because the bacteria that cause them are often resistant to drug treatment and patients are often in poor general health (Lacovelli *et al*., 2014). UroPathogenic *E.coli* is the causative agent in 85% of uncomplicated UTIs, (Hooton *et al*.,2004) followed in frequency by other Gram-negative and Gram-positive uropathogens, such as *Klebsiella pneumonia* and *Staphylococcus saprophyticus* ,respectively (Ronald,2003).

FimH deficiency detrimentally affects bacterial colonization of the urinary tract, as the bacteria can no longer bind to the surface of the

bladder, rendering them susceptible to expulsion by urination. (Xie *et al*.,2006 and Rosen *et al*.,2008).

The enteric bacterium *E. coli* is documented as the reason for more than 80% of uncomplicated community-acquired UTI (UPEC) (Pabich, 2003).These bacteria normally inhabit the lower intestines as normal flora but when they enter the urinary tract, they form the basis for a UTI (Barber,2013).Other pathogens commonly associated include; *Staphylococcus saprophyticus, Klebsiella species, Proteus mirabilis*, and *Enterococcus faecalis.* Complicated UTIs caused by bacteria such as *Neisseria gonorrhea* and *Chlamydia trachomatis* are associated with compromise in the urinary tract or host defense including; urinary blockade, urinary retention caused by nervous disorders, low immunity, kidney failure, kidney transplantation, and gravidity (Flores-Mireles, 2015).

**2.1.1.Classification of UTI**

Urinary tract infection is a collective term that describes any infection involving any part of the urinary tract, namely the kidneys, ureters, bladder and urethra. The urinary tract can be divided into the upper (kidneys and ureters) and lower tract (bladder and urethra) (Tan and Chlebicki, 2016). Clinical entities encompassed by "UTI" include asymptomatic bacteriuria, acute uncomplicated cystitis, recurrent cystitis, complicated UTI, catheter-associated asymptomatic bacteriuria, catheter-associated UTI (CAUTI), prostatitis, and pyelonephritis. There are three common clinical manifestations of UTIs in pregnancy are: asymptomatic bacteriuria, acute cystitis and acute pyelonephritis (Karishetti and Shaik, 2019) . Appropriate classification of the UTI syndrome is crucial for optimal diagnosis and management (Gupta *et al*., 2011).

### 2.1.1.1.Acute Uncomplicated UTI

Acute uncomplicated urinary tract infection is a symptomatic bladder infection characterized by frequency, urgency, dysuria, or suprapubic pain in a woman with a normal genitourinary tract, and it is associated with both genetic and behavioral determinants (Gupta *et al*., 2011). Uncomplicated lower UTI remains one of the most commonly treated infections in primary care. Uncomplicated UTIs occur in otherwise healthy individuals who lack urinary anatomical abnormalities. Bacteria derived from fecal matter access the urinary tract through the urethra and subsequently colonize the bladder, resulting in inflammation and symptoms clinically characterized as cystitis. Left untreated, bacteria can ascend the ureters and colonize the kidneys, resulting in a more serious secondary infection termed acute pyelonephritis (Tan and Chlebicki 2016).

### 2.1.1.2.Complicated UTI

Complicated urinary tract infection, which may involve either the bladder or kidneys, is a symptomatic urinary infection in individuals with functional or structural abnormalities of the genitourinary tract or the presence of an underlying disease; this increases the risk of the outcome of a UTI being more serious than expected, as compared to its occurrence in individuals without any identified risk factors (Tan and Chlebicki 2016).

### 2.1.1.3.Asymptomatic Bacteriuria

Asymptomatic bacteriuria, is defined as the presence of greater than or equal to $10^5$ colony forming units (cfu) of bacteria per ml of the same single species in two consecutive cultures of clean-voided specimens of

midstream urine from an individual without symptoms of a urinary tract infection (UTI). The reason to repeat the culture is to discriminate between true bacteriuria and contamination. Asymptomatic bacteriuria (ABU) in pregnancy is a cause of serious maternal and perinatal morbidity. This morbidity can be reduced with the screening of pregnant mothers and giving appropriate treatment (Vasudevan, 2014)

In ABU, patients do not develop symptoms in spite of carrying high levels of uropathogens in the urinary tracts for months or years. It has been shown that E coli strains may downregulate the host cells, thus suppressing the host response. The host unresponsiveness could thus be partly caused by the ABU strains actively manipulating the host (Abraham and Miao, 2015). However, the outcomes of ABU vary; in some patients, ABU can predispose to kidney infection, while in other patients, ABU can prevent colonization by more virulent strains. The unnecessary treatment of ABU in some patients may be associated with the rapid spread of genes coding for antibiotic resistance, particularly in health care settings (Beatson *et al.*, 2015)..

**2-2 The causative agent for urinary tract infection (UTI)**

urinary tract infection is mostly caused by bacteria, through other microorganisms such as fungi and viruses that are rare etiologic agents (Olin and Bartges,2015) This type of infection can be classified as complicated or uncomplicated. Uncomplicated UTI is the most common type of infection and mainly occurs in the absence of functional or anatomical abnormalities within the urinary tract. The complicated one occurs in the presence of an abnormal urinary tract that increases susceptibility to infection( Sheerin and Glover,2019).

Among the uropathogen, *Escherichia coli* is the most common bacteria (75–90% of isolates) in both the community and hospital infections, whereas other pathogenic bacteria such as *Proteus mirabilis*, *Staphylococcus saprophyticus* (with particularly frequent isolation from younger female), *Enterococcus faecalis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* each are less important (Sheerin and Glover,2019,Bazaid, *et al*.,2021).

The uropathogenic bacteria express fimbrial adhesions that they attach to the glycolipids and glycoproteins on the epithelial surface. In this way, bacteria can overcome the flow of urine and maintain in the urinary tract. The bacteria also produce other substances such as toxins, hemolysin, and colony-necrotizing factors. These agents disrupt epithelial integrity, permit bacterial invasion, and, therefore, enhance the risk of infection (Behzadi,2020). Uropathogens also can internalize into host epithelial cells and divide inside there, so that it provides a reservoir for recurrent infection (Sheerin and Glover,2019).

In most cases, these uropathogens begin to colonize the surface of the perineum and periurethral and precede the development of infection. Colonization of bacteria could be inhibited by the normal microbiota, such as *Staphylococcus epidermidis*, *Lactobacillus spp*, and *Corynebacteria* (Matulay *et al*.,2016).Additionally,bacterial colonization and initial infection were eliminated by host defense mechanisms in the bladder. The foreign bodies such as urinary catheters or stone in the urinary tract provide an inert surface for bacterial colonization (Bazaid, *et al*.,2021).

### 2.2.1.*Escherichia coli*

Over eighty percent of all acute UTI's are caused by *E coli* infection (Alanazi *et al*.,2018) The recurrence rate of *E. coli* is cited to be 44% in all UTI infections (Totsika, 2012). The distinctive extra intestinal pathogenic *E. coli* (ExPEC) is usually the bacteria that is known to cause UTI but how it colonizes an individual is unknown. Although it is known that the immediate source is host's own fecal or vaginal flora (Pabich, 2003). These uropathogenic strains (UPEC) are different from those strains that are found in the gastro intestinal tract, because they can evade the immune system of the host better and have mechanisms to attach to the urinary tract (Valerie,2018). The bacteria cause uncomplicated urethritis, cystitis, symptomatic cystitis, urosepsis, prostatic abscess and pyelonephritis (Grabe,2013).

There are two possible ways that *E. coli* can infect an individual; *E. coli* can trigger an ascending infection by introducing the microorganism into the urethra from faecal matter causing an infection that can disseminate to infect the kidney and other urinary tract parts or it can cause a descending infection where the microorganisms enter the urinary tract system from blood (Najar, 2009).

### *2.2.2.Klebsiella pneumonia*

It is the second pathogen, after *E. coli* to cause urinary tract infection affecting persons with low immunity (Behzadi, 2020). It has challenged antibiotic therapy by producing carbapenemese producing bacteria which has a high resistance level to all beta lactams antibiotics (Totsika,2012) .Similarly to UPEC, *K. pneumoniae* uses biofilm formation and bladder colonization to cause UTI (Rosen *et al.*, 2008).This bacterium uses an

adhesin similar to the one used by UPEC called TYPE 1 fimbrial adhesin but has different binding specificities (Flores-Mireles, 2015).

### 2.2.3.*Proteus mirabilis*

It is among the bacteria that causes urinary tract infections but not as frequent as *E. coli.* After the first attachment, *P. mirabilis* produces mannose-resistant *Proteus*-like (MR/P) pili, which are Chaperon-Usher Pathway pili that aid in catheter-associated biofilm formation which precipitates colonization of the kidneys and bladder (Nielubowicz, 2018). It is thought that the majority of *P. mirabilis* urinary tract infections (UTI) result from ascension of bacteria. from the gastrointestinal tract while others are due to person-to-person transmission, particularly in healthcare settings (Sabih and Leslie, 2017).

### 2.2.4.*Staphylococcus saprophyticus*

*Staphylococcus saprophyticus*, causes community acquired UTI. Just like *E. coli* it has some virulence properties that inhibit it from detection by the immunity system of the host. The virulence properties include inhibition of growth of other bacteria by producing urease enzyme, haemagglutination and attachment to human uroepithelial cells (Viviane *et al*., 2017).

### 2.2.5.Fungal, Viral and Parasitic UTI

Urinary tract infection may be caused by viruses and Fungi. Fungi, such as *Candida*, is the second most common cause of nosocomial UTI in women, it can spread systemically and can be life threatening(Behzadi, *et al*., 2015). Fungal infections are seen in females who are on long-term antibiotics, patients who are Immunocompromised, or patients using

invasive devices like IVs, and catheters (Mayer *et al*., 2013). Treatment of Candiduria includes stopping antibiotics, removing or changing indwelling catheters, and starting antifungal therapy with antifungal agents like oral fluconazole, parental or intravesical amphotericin B (Fisher *et al*., 2015).Viral UTI can be caused by Adenoviruses, polyomavirus BK, and herpes simplex viruses (Iduoriyekemwen and Sadoh, 2012). Very few parasites can cause UTIs such as *Trichomonas vaginalis* which cause urethritis in both male and females, but most often considered as cause of vaginitis. *Onchocerca volvulus*, *Wuchereria bancrofti* and *Schistosoma haematobium* were also uncommon UTI agents (Cheesbrough, 2009).

## 2-3 Antibiotics for UTI infection

Antibiotic overuse and inappropriate use are associated with the emergence of resistant pathogens (Goossens *et al.*,2005). The majority of antibiotics are prescribed in ambulatory care (Shallcross and Davies.,2014) and urinary tract infections (UTI) are beside respiratory tract infections one of the most common reasons for prescribing antibiotics (Petersen and Hayward,2007) **.**as a result of inappropriate use of antibiotics among other associated factors such as poor adherence to infection prevention and control measures. Such practices have been cited to cause unnecessary use of antimicrobials (Seifu, 2018,WHO ,2015). These drugs exhibit a high antibacterial success rates at the same time possessing low resistance to common uropathogens (Betsy, 2010). Due to multidrug resistance concerns, treatment with broad spectrum antibiotics instead of narrow spectrum is now preferred (Brusch, 2017). The initial choice of treating UTI in areas where antibiotic resistance is a problem

are fluoroquinolone due to the high cure rates and low resistancerate(schneede, *et al.*, 2010).

Long-term low dose antibiotic use is currently the keystone of the preventive treatment forUTI recurrence.Indeed prophylactic antibiotics have been shown to decrease. UTI recurrence by 85% compared to patients with placebo (relative risk (RR) 0.15, 95% confidence interval (95%CI) 0.08 to 0.28) (Albert *et al.*, 2004). Moreover, with regard to urinary tract conditions such as neurogenic bladder, it has been suggested that weekly cycling of antibiotics could be the most optimal preventative strategy (Salomon *et al.*, 2006; Prabhakar, *et al.*, 2021). Antibiotic resistant UTI is becoming more common globally,( Edlin *et al.*,2013 , Bryce *et al.*,2016) .

increases morbidity and doubles healthcare costs. The usual m echanism is through acquisition of enhanced beta-lactamase enzyme properties. Some Gram negative uropathogens can inactivate beta-lactam ring antibiotics such as penicillin and early generation cephalosporins with inherent beta-lactamase enzymes. However, some have acquired further multidrug-resistant properties through evolution of these enzymes, including the ability to hydrolyse and inactivate extended spectrum cephalosporins and carbapenem antibiotics(Murray and Peaper,2015)

Antimicrobial resistance to drugs used for treating common infectious agents has markedly increased.Common antibiotics,such as fluoroquinolone, are now ineffective against a large proportion of Escherichia coli infections, the most common cause of community-onset urinary tract infections (UTIs) in many parts of the US and globally. Even carbapenem antimicrobials that are considered as “last-line” drugs to treat

highly resistant gram negative bacteria have become ineffective in more than half of the patients treated (World Health Organization, 2020).

An initial study done by Owusu-Ofori *et al.*, which characterized the usage of antimicrobials at Komfo Anoyke Teaching Hospital in Kumasi, Ghana, found the evidence of high rates of inappropriate usage of antibiotics in the Accident and Emergency (A&E) Department (Owusu-Ofori *et al*., 2017).

Fluoroquinolones and cefalexin were associated with lower antibiotic switch rates than the recommended UTI antibiotics (pivmecillinam,nitrofurantoin and trimethoprim). However, the rates of antibiotic switch following treatment of male patients with first-line empirical UTI antibiotics are relatively low, indicating that the current guidelines are safe(Marius *et al*.,2020).

**2-4 Pathogenesis of UTI**

Urinary tract infections is result of interaction between uropathogens and the host. Successful infection of urinary tract is determined by the virulence factors of the bacteria, the inoculums size and the inadequacy of host defense mechanism (McLellan and Hunstad 2016).

Adherence is a key event initiating each step in UTI pathogenesis. A UTI typically starts with periurethral contamination by a uropathogen residing in the gut, followed by colonization of the urethra and subsequent migration of the pathogen to the bladder, an event that requires appendages such as flagella and pili (figure(2- 1). In the bladder, the consequences of complex host–pathogen interactions ultimately determine whether uropathogens are successful in colonization or eliminated. Uropathogens have, in turn, evolved molecules to inhibit

these signaling pathways and the resulting bactericidal host effector functions. For example, TcpC is a TIR-domain-containing protein that impairs TLR and MYD88-dependent signaling.( Cirl *et al* .,2008).

Genetic alterations also occur in metabolic genes and pleiotropic regulators of bacterial gene expression. This evolutionary process is ongoing as ABU strains continue to evolve in the host environment, resulting in the establishment of new, host-specific strains.( Zdziarski *et al*.,2010).

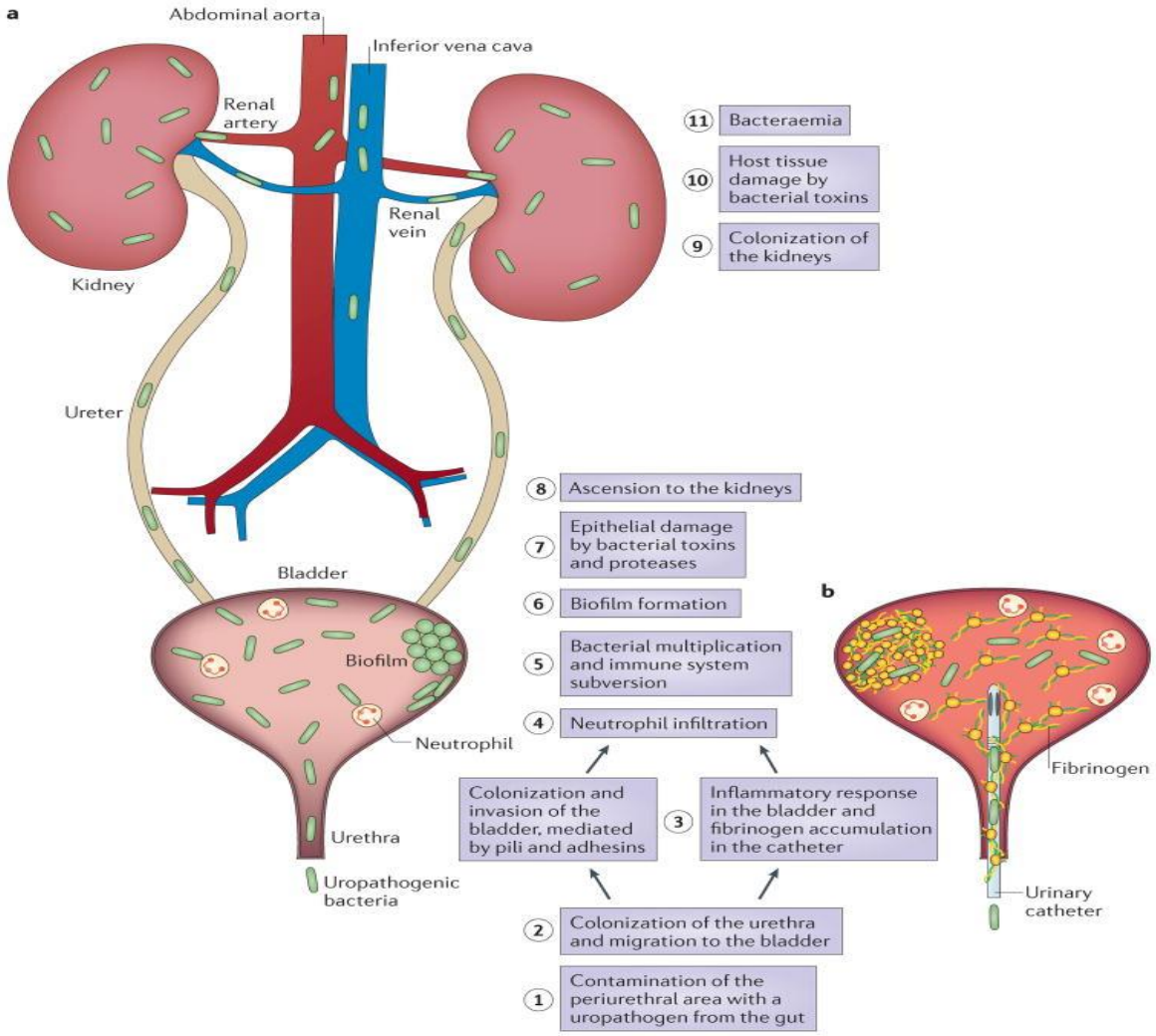


**Figure (2-1)Pathogenesis of urinary tract infections(Flores-Mireles, *et al*., 2015).**

Uncomplicated urinary tract infections (UTIs) begin when uropathogens that reside in the gut contaminate the periurethral area and are able to colonize the urethra. Subsequent migration to the bladder and expression of pili and adhesins results in colonization and invasion of the superficial umbrella cells ,Host inflammatory responses, including neutrophil infiltration and begin to clear extracellular bacteria. Some bacteria evade the immune system, either through host cell invasion or through morphological changes that result in resistance to neutrophils, and these bacteria undergo multiplication and biofilm formation ,

These bacteria produce toxins and proteases that induce host cell damage , releasing essential nutrients that promote bacterial survival and ascension to the kidneys , Kidney colonization results in bacterial toxin production and host tissue damage. If left untreated, UTIs can ultimately progress to bacteraemia if the pathogen crosses the tubular epithelial barrier in the kidneys Uropathogens that cause complicated UTIs follow the same initial steps as those described for uncomplicated infections, including periurethral colonization, progression to the urethra and migration to the bladder However, in order for the pathogens to cause infection, the bladder must be compromised.

The most common cause of a compromised bladder is catheterization. Owing to the robust immune response induced by catheterization, fibrinogen accumulates on the catheter, providing an ideal environment for the attachment of uropathogens that express fibrinogen-binding proteins. Infection induces neutrophil infiltration, but after their initial attachment to the fibrinogen-coated catheters, the bacteria multiply ,form biofilms, promote epithelial damage and can seed infection of the kidneys where toxin production induces tissue damage , If left untreated, uropathogens that cause complicated UTIs can also progress to

bacteraemia by crossing the tubular epithelial cell barrier (Gondim *et al*.,2018)

**2-5 Immunity of Urinary tract**

Immunity has two non-specific (innate immunity) and specific (adaptive immunity) components (Kucheria *et al*.,2005; and Becknel *et al*.,2015) A person is born with innate immunity as the natural resistances. The resistances are provided by innate immunity via various chemical, cellular and physical approaches. Thecolonization, entry and spread of microorganisms can be prevented by innate immunity (Schillinga *et al*.,2001 and Becknel *et al*.,2015 ) .

Two main sub-divided types of adaptive immunity are humeral and cellular. Naturally acquired immunity develops via non-deliberate contact with a disease causing agent, while artificially acquired immunity occurs just through deliberate measures like vaccination (Ulett *et al* .,2013 and Cheng *et al*.,2015).

The urinary tract is a sterile system under the normal circumstances. By age 6, 1%–2% of males and 3–7% of females suffer from a urinary tract infection (UTI). Long-term complications of UTI are scare, hypertension and chronic renal disease. Thus, it is necessary to find a greater understanding of UTI pathogenesis and how the body protects urinary tract and kidney from microbial pathogen (Rosen *et al*.,2007 and LüthjeP, Brauner ,2016)

Among several immune cells, macrophages (MΦs) actively combat with pathogenic microorganisms within diminished epithelial cells. By the entrance of microbial agents into the urinary tract epithelial (urothelial) cells, MΦs produce pro-inflammatory responses and molecules of pathogen recognition receptors (PRRs) at the same

time. The PRRs comprise a wide range of molecules such as Nod-like receptors (NLRs) (cytoplasmic proteins), retinoic acid inducible gene (RIG-I)-like receptors (RLRs) (cytoplasmic proteins), C-type lectin receptors (CLRs) (trans membrane proteins), and TLRs (trans membrane proteins). Among a wide range of innate immune responses against the penetrated pathogenic microorganisms into the host's urinary tract mucosal tissues in parallel with the breach of urothelial cells, TLRs are important parts of innate immune network responses which play a key role in association with the urinary tract defense system and the UTIs prevention. Indeed, TLRs have practically shown their unique potential for a rapid identification of infectious agents and launching signals for elimination of microbial pathogens or activation of adaptive immune responses (Takeuchi and Akira *et al*.,2010 ; Abraham and Miao,2015)

The nature of this human immune is unknown. The human defense mechanisms include numerous components of the immune system as well as genetic background and environmental exposures can change each component(Ulett *et al* ., 2013 and Lüthje and Brauner ,2016 ) .

**2-6 Innate Immunity versus Adaptive Immunity in Urinary System**

The urinary tract relies predominantly on innate immunity for its defense (Abraham and Miao, 2015). Specific immune responses with upregulation of T and B cells and secretion of immunoglobulins do occur in UTI, but they are not crucial for clearance of the acute infection (Koves and Wullt, 2016). The first step in the uropathogenesis is the attachment of bacteria to superficial bladder epithelial cells. The attachment is recognized by the cells and triggers intracellular signaling

proteins, transcription of target genes, and release of effector proteins (Godaly *et al.,* 2015). The most important cell-surface receptors for triggering an innate immune response are members of the toll-like receptor (TLR) family. The TLR4 recognizes pathogen-associated molecular patterns specifically on Gram-negative bacteria such as LPS, with the help of the coreceptor CD14 (Becknell *et al.,* 2015). Innate immune is composed of proteins and antimicrobial peptides (AMP) which speedily neutralize the chemokines, invader and cytokines that attract phagocytes to a threatened site as well as increase their microbicidal capacity and the phagocytes themselves (Lüthje and Brauner ,2016 ) .

The bacteria may remain in the urinary tract system in spite of the innate immune response. Hence, a more specific adaptive immune response including cellular and humoral responses is resulted , Phagocytic cells as a first line of defense are belonged to polymorphonuclear leukocytes (PMNs; neutrophils) and derived from the bloodstream directly to the site of bacterial invasion (Lüthje and Brauner ,2016 )

Adhesion of bacteria to epithelia lining the urinary tract can be prevented by release of constitutive and inducible bactericidal antimicrobial peptides such as alpha- and beta-defensins and cathelicidin, lactoferrin, Tamm-Horsfall protein and lipocalin. Cytokines (IL-1 and TNF alpha) and chemokines (IL-8) activate and attract large numbers of macrophages and neutrophils which damage tubulointerstitial parenchyma with persistence of growing numbers of microorganisms (Billips *et al*.,2008 and ; Khoury *et al*., 2016) .

Biochemical and anatomical defense consists of paraurethral glandular secretion urine stream, IgA secretory, urine inhibitors (PH, Osmolality and Urea) and bladder emptying (Khoury *et al*., 2016 ) Bacterial survival is decreased within the urinary tract via the presence of salts and urea, low pH, distance barrier of male urethra, other toxic metabolic end products, mucus in urine and hypertonic nature of kidney medulla (Mkaddem *et al*.,2010).

## 2-7 Innate immune responses, Inflammationand Pattern recognition receptors (PRRs)

Identification of the innate immune system goes back to 132-63 BC when the Parthian (Iranian) King, Mithridates VI (the King of Pontus) used snake venom to keep his immune system strong against toxins (he is known as the world's first immunologist).From that time until the present, a huge number of immunological mechanisms and cells have been discovered (Valle *et al*.,2012) . In general, the innate immune system is composed of (1) pattern recognition receptorslike Toll-like receptors (TLR); (2) plasma proteins,chemokines, and cytokines; (3) cellular components like epithelial cells, bone marrow-derived phagocytes, dendritic cells, and natural killer cells; (4) toxic molecules such as reactive oxygen and reactive nitrogen intermediates; and (5) antimicrobial peptides (AMPs) (Gong *et al* ., 2020). Additionally, normally present local microbiota in the urogenital system and intestinal tract serve as another source of innate immunity , altering the pH of the local environment and producing their own antimicrobial products to help control UTI, as well as simply acting ascompetitive inhibitors of more virulent bacterial strains such as UPEC (Baghbani *et al* ., 2020)**.**

The importance of TLRs has been recognized as a key regulator for innate and adaptive immune responses. The innate immunity is supported by a variety of natural hindrances including skin and mucosa, nonspecific molecules such as interferons and different types of cells comprising dendritic cells (DCs), MΦs (as the specific immune cells), fibroblasts, endothelial and epithelial cells (as the non-specific immune cells). Immune cells are able to produce and secrete all members of PRR families such as NLRs, RLRs, CLRs, and TLRs. The PRRs like TLRs are important immunologic biosensors for tracing pathogens within the host's cells and tissues by recognition of microbial conserved components, termed pathogen association molecular patterns (PAMPs) (Kagnoff and Eckmann,1997, Takeuchi and Akira ,2010 ,Reygaert , 2014 , Abraham and Miao,2015).

The innate immune response to UPEC infection is characterized by robust cytokine and chemokine expression, leading to rapid neutrophil and monocyte infiltration and subsequent bacterial clearance (Shahin *et al*.,1987, Engel *et al*.,2008).Depletion of both neutrophils and monocytes, by antigranulocyte receptor Gr1 antibody treatment (Daley *et al*.,2008). Epithelial cells make essential contributions to innate immunity by serving as physical barriers, communicating with hematopoietic cells, producing cytokines and chemokines, and secreting antimicrobial proteins and peptides that kill invading pathogens (Llorente *et al* .,2021) . Bacteria manipulate the innate immune response in the human urinary tract. A secreted TIR domain homologue inhibits MyD88 and inflammasome activation (Cirl, *et al*., 2008). ABU strains modulate host gene expression, by suppressing RNA polymerase II in patients with ABU (Lutay, *et al*., 2013). As a result of mutual adaptive strategies, bacteria adjust to individual hosts. During long-term ABU, *E. coli 83972*

reisolates showed host-specific reproducible genome alterations, suggesting that the evolution of fitness for the urinary tract is 'personal' (Zdziarski, *et al*., 2010).

Other study demonstrated that innate immune cell crosstalk is necessary for a coordinated innate response, whereby resident macrophages, responding to signals from infiltrating monocytes, induce MMP9 expression in neutrophils, in turn facilitating their trans-urothelial migration (Schiwon *et al*.,2014). This mechanism likely works in concert with cytokine and chemokine expression from infected urothelium that mediates neutrophil recruitment and trans-urothelial migration (Godaly *et al*.,2000). In summary, the innate immune cells in the urinary tract rapidly respond and evoke robust immune responses during UTIs. These responses are tightly controlled, but sometimes the innate responses of the bladder are prematurely terminated, which can have detrimental effects on the host (Abraham and Miao, 2015).

## 2-8 Role of the adaptive immune response in the urinary tract

Despite the innate immune response, bacteria still can persist in the urinary tract. Therefore, a more specific adaptive immune response ensues that protects the urinary tract.studies by Thumbikat *et al* demonstrated that both cell-mediated and humoral arms of the immune system are activated after UPEC infection that stimulates recruitment of activated T cells to the bladder and the production of specific IgG antibody in the serum and urine. As a result, their efforts demonstrated that mice were highly resistant to reinfection with a homologous UPEC strain (Song and Abraham , 2008).

Additionally, it has been postulated that the sensitized B lymphocyte cells migrate to the lamina propria from the lymphatics and differentiate into IgA secreting cells. However, it has been suggested that these antibodies inhibit bacterial colonization by lowering the bacterial adherence to the mucosa or assist in opsonization by WBCs. These results suggest that that urinary tract is able to mount an appreciable and protective adaptive immune response (Svanborg-Eden and Svennerholm ,1978,Thumbikat *et al*.,2006,Hashimoto *et al* .,2021).

**2-9 Toll-Like Receptors (TLRs)**

TLRs are a group of PRR molecules with evolutionary conserved expressed molecules of the human structures that act as the first The .system in the presence of the related target ligands innate immune the innate immune vital role of toll receptors in association with in the insect Drosophila. In parallel with system was first discovered discoveries, some homologs of toll receptors were progressive (TLR1 to TLR10) detected in mammals. Today, 10 members of TLRs are recognized in the human innate immune system. These trans- of I) protein molecules are recognized by the presence membrane (type two domains; the first one, an extracellular domain containing leucine rich repeat [LRR (consisting of repeated motifs of 24 amino is located in the N-terminal end and binds to its acids)] which proper ligands; and the second one, a conserved and homolog intracellular (cytoplasmic) signaling do-main of IL-1 receptor which is known as Toll/IL-1 receptor (TIR) and situated in the C-terminal end. Most of the TLRs adaptors are recognized in the cell membrane and some of them are expressed in sub-cellular endosomal structures.

TLRs are produced in a vast range of cells including non-hematopoietic endothelial cells, epithelial cells, parenchymal cells, synovial fibroblasts and hematopoietic originated cells of DCs, MΦs, mast cells, neutrophils, B cells, and T cells. Among 13 identified members of TLRs, 10 members of them comprising TLR1-10 are found in humans. The conserved members of TLR1-TLR9 are found in both humans and mice, while the TLR10 is detected only in humans and the TLR11-13 are identified only in mice (West *et al*.,2006 and ; Botos *et al*.,2011).

All TLRs utilize MyD88 except TLR3 that employs TRIF. TLRs 2 and 4 signaling requires TIRAP in conjunction with MyD88, while induction of antiviral interferon response and stimulation of nuclear factor kappa B (NFκB) by TLR 3 and 4 is dependent on TRIF, the TLR4-TRIF signaling pathway further utilizing TRAM(Schwabe *et al*.,2006).

**2-10 Toll like receptor 2 (TLR2)**

TLR2 is one of the most promiscuous members of the family, recognizing a large array of pathogen-associated molecular patterns (PAMPs), including peptidoglycan, lipoproteins, lipopeptides, phenol-soluble modulin, lipoteichoic acid, lipoarabinomannan, atypical lipopolysaccharides (LPSs), porins, glycoinositolphospholipids, glycolipids, and zymosan (Ozensky,A *et al* 2000 , Takeuchi O. 2001).

In recognizing this large panel of PAMPs, TLR2 forms either homodimers or heterodimers with TLR1 or TLR6. TLR2 is encoded by the gene bearing the same name, and more than 175 single-nucleotide polymorphisms (SNPs) have been reported for the gene located on chromosome 4q32 (Georgel *et al*.,2009 and Kleinnijenhuis *et al*.,2011).

TLR-2 is capable of detecting the widest PAMP repertoire within a range of pathogens, including Gram-positive and Gram-negative bacteria ,mycobacteria, fungi, viruses and parasites(Akira and Uematsu 2006) .

TLR-2, as a heterodimer with TLR-1 or TLR-6, recognizes alarge number of common bacterial motifs, including lipopeptides, peptidoglycan, glycosylphosphatidylinositol (GPI)-linked proteins and zymosan Two non-synonymous SNPs in TLR-2 have been linked to human diseases (Akira and Uematsu,2001) first, a C>T transition in nucleotide 2029 (rs121917864), which replaces Arg677with Trp (Arg677Trp), is common in African and Asian populations, but appears to be absent among white populations. In vitro, this SNP has been shown to inhibit both *Mycobacterium leprae* and *M. tuberculosis*-mediated NF-κB activation and production (Kang *et al*.,2002).

In a Korean and a Tunisian population, this SNP was associated, respectively, with leprosy (Kang and Chae ,2002) and susceptibility to tuberculosis (Ben-Ali *et al.*,2004). Another functional TLR-2 variant (rs5743708) consists of a G>A substitution at nucleotide 2251, which replaces Arg753 by Gln (Arg753Gln). This SNP maps in a region of highly conserved amino acids in the C-terminal end of TLR-2 is present in 3% of healthy white blood donor control subjects, is identified in patients with staphylococcal septic shock (Lorenz *et al*.,2000).

TLR2 is a key regulator of host immunity and plays a pivotal role in deciding the fate of several microbial and parasitic infections.( Pai *et al*.,2004) R677W is considered as the most common TLR2-SNP that affects pathogen recognition ability of TLR2 and dampens antipathogenic host responses. ( Pai *et al*.,2004, Ben-Ali *et al*.,2004) Population-based

studies have demonstrated that individuals inherited with R677W variant fail to recognize both *M leprae* and *M tb*.( Janeway and Medzhitov.,2002)**.**

**2-11 Toll like receptor 9 (TLR9)**

Toll-like receptor-9 (TLR9) is a tool of innate immunity responsible to detect unmethylated cytosine-phosphate-guanine (CpG) dinucleotide conserved structures of viruses and triggers the secretion of interferon-α (Arpaia and Barton, 2011). TLR9 is present on macrophages, dendritic cells including intestinal epithelium cells, along with respiratory epithelial and keratinocytes cells (Yusuf *et al*.,2016).

TLR9-encoding gene is emplaced on chromosome 3p21.3 and stretches about 5 kb (Yusuf *et al*., 2016). The coding region of the gene has 2 exons along with 1032 amino acids; the core area is present in the second exon (Tao *et al*., 2007). According to NCBI SNP database, twelve unique SNPs have been detected for TLR9 gene, among them few may be the leading SNPs related to the susceptibility of different (Papadimitraki *et al*., 2006; Sawhney & Visvanathan, 2011).

The expression level of TLR9 may deregulate due to genetic variations that lead to the production of auto-antibodies which may enhance the risk of disease (Christensen *et al*., 2005; Skevaki *et al*., 2015). researchers have reported that rs187084 C genotype is interlinked with decreased TLR9 transcription compared with T genotype (Wang *et al*., 2013; Yusuf *et al*., 2016).

TLR-9 SNPs were characterized in vitro in human embryonic kidney (HEK)293 cells and two relatively rare variants,Pro99Leu (rs5743844) and Met400Ile (rs41308230), were associated with altered receptor

function regarding NF-κB activation and cytokine induction. In the most impaired variant, P99L, the ability to respond to physiological and therapeutic TLR-9 ligands was severely compromised, while binding to CpG-containing oligonucleotides (CpG-ODN) remained normal, implying that their recognition by TLR-9 may involve two separate events, CpG-ODN binding and sensing and that residue Pro-99 is important for the latter process (Kubarenko *et al*.,2010).

**2-12 polymorphism of TLR**

Among the genetic markers that have been investigated are toll-like receptors (TLRs). They are a family of transmembrane receptors involved in recognizing conserved molecular patterns of a microbial origin. In addition to their role in maintaining tissue hemostasis due to inflammation, TLRs recruit leukocytes for microbial-infected tissues, and consequently, the innate and adaptive immune responses are induced (Rakoff and Medzhitov,2009).

These receptors are expressed by peripheral immune cells and urinary bladder epithelium, and their role in activating anti-UBC immune response has been introduced; especially, TLR-2, TLR-4, TLR-7, and TLR-9 (LaRue *et al*.,2013). In vitro and in vivo evaluations revealed their agonist effects in activating anti-tumor immunity and to determine the immunotherapy potential. The results are valuable, and TLR-7 agonists against UBC have been recognized; therefore, it is suggested that targeting TLR-7 is a promising strategy for both antiviral and anti-tumor therapies (Chi *et al*.,2017). Bacteria can still penetrate the innate immune defense, so defense of the urinary tract further involves adaptive immunity. Both cellular and humoral immunity are increased after UPEC infection. Activated T cells migrate to bladder and specific IgG antibodies are secreted in the serum and urine (Thumbikat,*etal*,2006)

TLRs act an instructive role in UTI. TLRs, expressed both by epithelial and non-epithelial cells, launch appropriate immune and inflammatory responses to get over microbial invasion and infection. Synthetic TLR agonists and TLR antagonists affect and manipulate the host defense systems. Some of these immunomodulating drugs may help to conquer intrinsic disturbances in the TLR mechanism, providing new therapeutic options for UTI.( Scherberich and Hartinger,2008).

With respect to genetic-association studies, four TLR single-nucleotide polymorphisms (SNPs), TLR2 (-196 to-174del), TLR3 (C1377T), TLR4 (Thr399Ile), and TLR9 (G2848A) genes, were investigated in North Indian UBC patients. It was demonstrated that TLR2 SNP is involved in UBC susceptibility, while the other SNPs were not associated with risk of disease (Singh *et al*.,2013).

In fact, mutations that affect the *tlr2* receptor expressions may impair the host response. The most commonly discussed polymorphisms in the *TLR2* (*4q31.3*), the R677W (rs5743704), and the R753Q (rs5743708) have been shown to be associated to several diseases (Mosconi *et al*., 2008; Zhang *et al*., 2019). The TLR2/G2477A/R753Q has been shown to lead to a decreased cellular activation in the presence of theTLR2 ligand lipopeptide (Lorenz, *et al*.,2000). Furthermore, the presence of a functional intronic polymorphism consisting of guanine-thymine repeats (approximately 100 bp upstream of the translational start site) in the TLR2 gene was reported as a risk factor in rheumatoid arthritis (Yim *et al*.,2008)

Toll-like receptors (TLR) are group of pattern recognition receptor molecules. It is a class of innate immune receptors that can be directly recognized. When combined with certain highly specific immune molecule, it can trigger a series of signal transductions that lead to

inflammatory transmitters. Many studies have found TLR's have the potential for identifying pathogens, transmitting a signal, and activating an immune response. Researchers have examined the association between single nucleotide polymorphisms (SNP) of TLRs and urinary tract infections; however, the results of these studies were inconsistent. Even though a previous meta-analysis had discussed the relationship between rs4986790 (TLR4 896 A > G) and urinary tract infections,( Ziakas *et al*.,2013).

TLR2 polymorphisms also possess significant impact in altering the human immune responses against major parasitic infections that include malaria and filariasis.( Hise *et al.*,2007, Junpee *et al*.,2010). TLR2 −196 to −173del, +597 T > C and +1350 T > C have been found as the key SNPs involved in inducing susceptibility to filarial (*Wuchereria bancrofti*) infection Individuals with these SNPs are mostly diagnosed with symptomatic bancroftian filariasis and are considered as genetic risk factors for *W bancrofti* infection. .( Junpee *et al*.,2010 ).

Therefore, providing improved precautionary measures to such individuals inherited with such higher risk SNPs could be a useful strategy in the lymphatic filariasis control programme. On the other side, TLR2 is known as the major innate immune receptor for malarial parasite(*Plasmodiumfalciparum*)-derived glycosylphosphatidylinositol, and therefore, polymorphic changes in TLR2 gene alter malarial pathogenesis Polymorphisms in 5' un-translated region (UTR) of TLR2 gene viz. a 22 base pair deletion in the first untranslated exon (Δ22) and a dinucleotide (GT) repeat in the second intron (GTn) were described for conferring protection against the immunopathological alterations of cerebral malaria. However, in the uncomplicated malaria cases, these

polymorphisms were diagnosed for elevating the levels of proinflammatory cytokines **(** Greene *et al*.,2012).

**2-13 Mucosal immunity**

the concept of the mucosal immune system has become increasingly important. This involves the immune function shared by, amongst other systems, the mucous membranes of the respiratory tract, the digestive tract, and the genitourinary tract. The genitourinary tract employs an innate and adaptive mucosal immune systemic response to fight against uropathogens. Since immunocytes pass through various mucosa-associated lymphoid tissue (MALT) sites, the activation of lymphocytes at a distant MALT site (Ho lmgren and Czerkinsky ,2005).

Some studies have found that stimulation of the sublingual mucosa is related to the activation of a broad-spectrum systemic and mucosal immune response in the genitourinary tract. In particular, the response at the site of the bladder mucosa is persistent and highly effective when the sublingual mucosa is stimulated (Cuburu *et al*.,2007).

Mucosal-associated invariant T cells (MAITs) have gained much attention as immune effectors. They constitute 1–8% of T cells in human blood, intestinal mucosa and mesenteric lymph nodes (Martin *et al*.,2009) In 2010, MAITs were shown to be activated by cells infected with bacteria or yeast. Moreover, patients with bacterial infections had lower numbers of MAITs in the blood, suggesting that these cells locate to the infected tissues (Le Bourhis *et al*.,2010).

Mucous epithelial surfaces where the host is confronted with a range of different microorganisms from the outside environment are suitable places for beginning of an infection with pathogens. Large surface areas

of mucosal membranes (200–400 $m^2$) are in constant contact with a highly diverse microbiota (ley, *et al.*,, 2005; Cash & Hooper, 2005) estimated to comprise 15,000–36,000 species and 1800 genera (Gill *et al*., 2006; Frank *et al*., 2007).

Secretory IgA (SIgA) is the principal immunoglobulin (Ig) on mucosal surfaces of humans and many other mammals. The intestinal tract contains IgA and some IgM but virtually no IgG, whereas the respiratory and urogenital tracts contain equivalent amounts of IgA and IgG in addition to some IgM (Brandtzaeg *et al*., 1999). The main function of IgA is the neutralization of pathogens and toxins without causing inflammation since it does not activate complement (Macpherson & Slack, 2007;Cerutti, 2008).

TLR2 (rs3804099) gene polymorphisms have been linked to sepsis. Gram-positive infections were linked to allelic variations in TLR2, while Gram-negative infections were linked to IL10 TLR2 allelic variants may help to lower preterm newborns' susceptibility to sepsis (Abu-Maziad *et al*., 2010).

Because of its ability to form heterodimers with TLR1, TLR6, and maybe TLR10, TLR2 is produced by a variety of lymphoid and non-lymphoid cells and binds to a variety of microbial-derived components (Nemati *et al.,* 2017). In addition, the rs3804099 polymorphism influences cancer susceptibility (Gao *et al.,* 2019). For the SNP rs3804099, the synonymous mutation did not result in asparagine (Asn) substitution at residue 199, resulting in a decreased macrophage response, lower TLR2 expression with attenuated host immune response and susceptibility to most of the diseases reported in this study: tuberculosis ,leprosy , acute pyelonephritis and acute lobar nephronia , infective

endocarditis , tuberculous meningitis , and filariasis . (Silva *et al*.,2021). In leprosy, it was demonstrated that this SNP caused increased expression of pro-inflammatory cytokines, with higher expression of TLR2 (Santana *et al.*,2017).

# 3. Materials and Methods

## 3.1: Materials

### 3.1.1 Equipment of Laboratory and Instruments

The laboratory equipment and instruments used in the present study were illustrated in Table (3-1).

**Table (3- 1):- Laboratory Equipment and instruments**

| Instruments | Company | Country origin |
|---|---|---|
| Autoclave | Hirayama | Japan |
| Bench centrifuge | Memmert | Germany |
| Burner | Amal | Turkey |
| Cooling centrifuge | Hettich | Germany |
| Disposable (Pteri Dish, Syringe, Plane and gel tube, plastic cap) | Citro | China |
| Distiller | Ogawa | Japan |
| Gel documentation system, UV source | Cleaver Scientific | UK |
| TRIO Thermal Cycler | Biometra | Germany |

| Instruments | Company | Country origin |
|---|---|---|
| ELISA system | Biotech | USA |
| Eppendorf tubes | Eppendorf | Germany |
| Hood | Bio LAB | Korea |
| Incubator | Selecta | Spain |
| Light microscope | Zeiss | (Germany) |
| Micropipette | Capp | Denmark |
| Oven | Memmert | Germany |
| Para film | BDH | England |
| Platinum Wire Loop | Himedia | Indian |
| Refrigerator | Kiriazi | Egypt |
| Slide | Sail Brand | China |
| Sterilize Swab | ATACO | Brand |
| Transfer swab | Al hanof factory | Jorden |
| Vitek 2 system | Biomerieux | France |

| Instruments | Company | Country origin |
|---|---|---|
| Vortex mixer | Griffin | Germany |
| Water bath | Gallen Kamp | Germany |

### 3.1.2: Chemical materials

Chemical materials, reagents, stains and solutions used in the present study illustrated in Table (3-2).

**Table (3- 2):- Chemical materials**

| Type of chemical | Company/origin |
|---|---|
| α- Kovac's reagent, Methyl red. | Sigma, USA |
| Ethanol 99% | Merck-England |
| Glycerol | Merck England |
| Gram stain solution | Syrbio |
| Tris-HCl, Tris-base | BDH, England |

### 3.1.3: Culture Media

According to (McFaddin, 2000) culture media used in this study are listed in table (3-5) . All media were prepared according to the manufacturers Himedia (India) except chromogenic UTI agar which from Condalab (Spain) and 5% fresh human blood was added to blood agar base after sterilization to prepare blood agar.

**Table (3- 3):- Cultures media and purpose of use**

| Culture media | Purpose of use |
|---|---|
| Blood Agar Base | Detection of hemolysin production |
| Brain Heart Infusion broth | It was used in the activation and preservation of bacteria. |
| Eosin Methylene Blue Agar | Selective and differential of members of Enterobacteriaceae. (*E. coli*) |
| MacConkey Agar | Isolation and identification of gram-negative bacteria based on lactose fermentation |
| Mannitol Salt Agar | Isolation of staphylococcus and differentiation of staphylococcus aureus |
| Mr -Vp Broth | Detect the partial and full glucose hydrolysis |
| Muller – Hinton Agar | Antibiotic susceptibility |
| Nutrient Agar, Nutrient | General purpose medium |

| | |
|---|---|
| Broth | |
| Peptone Water | Bacterial capacity demonstration to decompose the amino acid tryptophan to indole |
| Pseudomonas chromogenic agar | *Pseudomonas* isolation |
| Simmone Citrate Agar | Determining the bacteria capacity to use citrate as the carbon supply |
| UTI Chromogenic agar | Isolation and identification and selective media of bacteria |

### 3.1.4. Kits:

Kits were used in this study are listed intable (3-4) as follows

**Table (3-4): Kits used in this study**

| No. | Types of kits | Company/country |
|---|---|---|
| **1** | DNA extraction kit (contain Ethanol alcohol (96-100%), Proteinase K solution for precipitation proteins, Wash buffer, W1 buffer solution, FABG | G-spin/ Korea |

| | | |
|---|---|---|
| | buffer, ddH2O, Elution buffer, RNase solution). | |
| **2** | PCR Master ( mixDNA polymerase enzyme (Taq) dNTPs (400µm dATP, 400µm d GTP, 400µm dCTP, 400µm MgCl2 (3mM) Reaction buffer (pH 8.3) | Promega/USA |
| **3** | DNA ladder 100bp(Ladder consists of 11 double-stranded DNA with size 100-1500bp) | |
| **4** | Loading Dye has a composition (15% Ficoll, 0.03% bromophenol blue, 0.03% xylene cyanol, 0.4% orange G, 10mM Tris-HCl (pH 7.5) and 50mM EDTA) | |
| **5** | Primers of SNP( TLR2,TLR9) . | Macrogen/Korea |
| **6** | Human TLR 2 and TLR9 Elisa Kit | Elabscience China |

| | | |
|---|---|---|
| **7** | Gram Positive and Gram negative Card of Vitec 2 System | BioMerieux France |

**Table (3-5): Contents of Reaction Mixture**

| Contents of reaction mixture | Volume |
|---|---|
| Master Mix | 12.5 µl |
| Template DNA | 5 µl |
| Forward primer (10 pmol/µl) | 1.5 µl |
| Reverse primer (10 pmol/µl) | 1.5 µl |
| Nuclease free water | 4.5µl |
| **Total volume** | µl |

### 3.1.5.Antibiotics discs

All Antibiotics disc that used in this study based on Mast (UK) and bioanalyse (Turkey) are mentioned in table (3-6)**.**

**Table (3-6): Antibiotics disc that used in this study**

| Class | Sub class | antibiotic | symbol | Concentration / mg | Company |
|---|---|---|---|---|---|
| aminoglycoside | | amikacin | AK | 30 | Mast |
| cephems | Cephalosporin | ceftriaxone | CTR | 30 | Bioanalyse |
| Quinolones | fluoroquinolones | ciprofloxacin | CIP | 5 | Mast |
| penems | carbapeneme | imipenem | IMP | 10 | Mast |
| | | meropene | ME | | |
| pencillin | aminopenicillin | pipracillin | PRL | 100 | Bioanalyse |

## 3.2: Methods

### 3.2.1: Study design

The specimens were proceeded according to the study design shown in Figure (3-1).

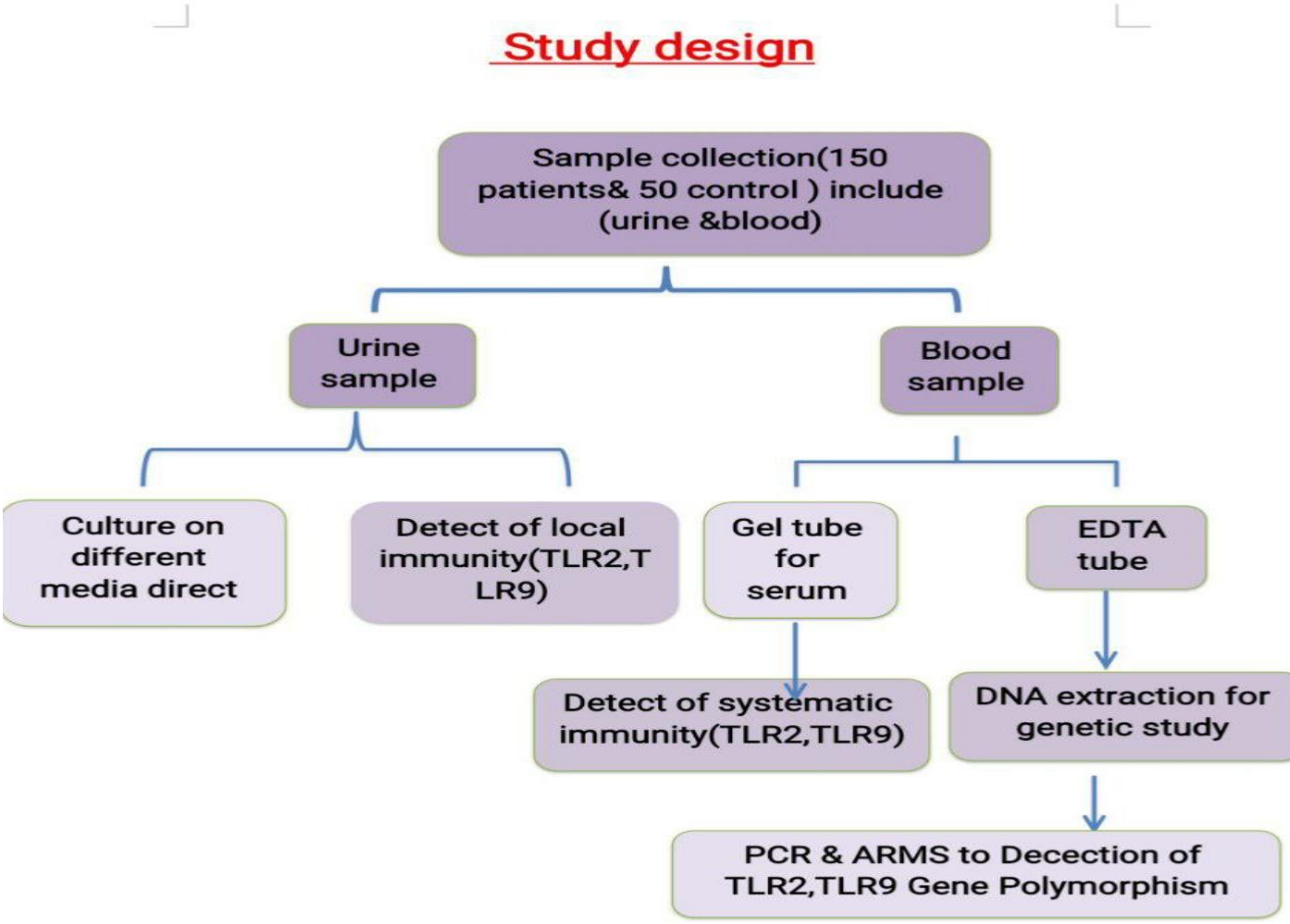


**Figure (3-1) The figure represent study design**

### 3.2.2: Patients of the Study:

Clinical specimens were collected from patients admitted to private clinics and two hospitals of Babylon Province: who attended to Al-Imam Al-Sadiq Hospital/ Babylon, and Al-Hilla General Teaching Hospital during the period from October to March (2020-2021) .

**The characters of patients :**

**Inclusion criteria**

1-Both sex

2-Age (18-48) years

3- Urinary tract infection types.

**Exclusion criteria**

1- Pregnant women

2- Chronic diseases

3- Patients having antibiotic ( at least 3 days )

The study involved (150) patients with urinary tract infection were subjected for sampling which include both urine and venous blood sample from patients and (50) control. The age of patients & controls ranged from 18 to 48 years.General and medical informations were taken from all patients (Appendix).

**3.2.2.1 .a. Blood sample**

About five milliliters of venous blood were collected from each subject in the study. One part of the blood was used to separate the serum by centrifugation at 3000 rpm for 15 min, and then kept in Eppendorf tubes at -20 ºC until used , while other part of blood used to extract DNA to investigate genes polymorphisms in UTI patients and control.

**3.2.2.1.b.urine sample**

Urine samples were taken from patients and placed in a single-use container. Urine samples were planted on different culture media for bacteriological study .

**3.2.2.2. Ethical Approval:**

A valid consent was achieved from hospitals administration and from patients and controls before their inclusion in the study. the procedure had been informed before the samples were collected, making absolutely sure that they understood the procedure that was to be carried out. The subjects were sentient that they had the right to reject to be included in the study without any detrimental effects.

**3.2.3: Preparation of Reagents and Solutions**

**3.2.3.1: Catalase reagent**

The reagent was prepared by dissolving (3ml) of H2O2 in (100ml) of distilled water (3%) and stored in dark container. It was used to recognize bacterial potential to generate the enzyme catalase *(*Forbes,*et al.*, 2007*)*.

**3.2.3.2: Oxidase reagent**

It was prepared directly by dissolving (1gm) of tetramethyl-para phenylene diamine dihydrochloride in 10ml of distilled water and stored in a dark container. It was used to detect the ability of bacteria to produce oxidase enzymes (Forbes *et al*., 2007).

### 3.2.3.3: Methyl red reagent

A weight of (0.1gm) of methyl red was dissolved in (300ml) of (99) % ethanol and then, the volume was completed to (500ml) by distilled water. This reagent was used to identify the complete glucose hydrolysis (Forbes *et al.*, 2007**).**

### 3.2.3.4: Voges-Proskauer reagents (Barritt's reagent)

The reagent was prepared according to the method reported by (Winn *et al.*, 2006), as the follows: -

1-Five (g) of alpha naphthol dissolved in (100ml) of (99%) ethanol alcohol then stored in a dark bottle away from light in cool place.

**2-** Forty (g) of KOH was dissolved in (100ml) of distilled water. It was used to detect the acetone formation in the culture media so this test improves the partial fermentation that lead to butylene glycol formation.

### 3.2.3.5: Kovac´s reagent

It was prepared by dissolving 5g of (P-dimethyl amine benzaldehyde) in 75 ml of amyl alcohol and then 25 ml of concentrated HCl was added. The reagent used for the detection of indole (MacFaddin, 2000).

### 3.2.3.6: Gram stain solution

Gram stain solution was supplied from Syrbio company. These solutions included: four solutions Crystal violate, Iodine, Absolute alcohol, and safranine. It was used to study cells morphology and their arrangement, to

differentiate between Gram-negative and Gram-positive bacteria (Forbes *et al.*, 2007).

### 3.2.3.7: Normal saline solution

It was prepared by dissolving (8.5gm) of NaCl in a small volume of distilled water, and then completed to 1000ml, pH fixed at 7.2 and sterilized in an autoclave at 121°C for 15 minutes, then kept at 4°C. Sterile ready to use normal saline were used for all assays and experiments that need to use it (MacFaddin, 2000).

## 3.3: Preparation of culture media: -

The culture media were prepared according to the manufacture company and sterilized by autoclave at 121ċ for 15 min (Macfadden, 2000).

### 3.3.1: MacConkey agar medium

It was used for the primary isolation of most Gram-negative bacteria and to differentiate lactose fermenters from non-lactose fermenters (Winn *et al.*, 2006).

### 3.3.2: Nutrient agar medium

It was prepared according to the manufacture company and sterilized by autoclave at 121ċ for 15 min (MacFadden, 2000).

### 3.3.3: Nutrient broth

This medium was prepared according to the manufacture company and sterilized by autoclave at 121ċ and pressure 15 pounds for 15 min (MacFadden, 2000).

### 3.3.4: Muller-Hinton agar

Muller-Hinton agar medium was ready for conferring to the manufacturing company and it was used in antimicrobial susceptibility testing (Forbes *et al*., 2007).

### 3.3.5.: Brain heart infusion broth

It used for activation the bacterial isolates (Forbes *et al*.,2007).

### 3.3.6.: Blood agar medium

Blood agar medium was prepared according to manufacturer instructions by dissolving 40 g of blood agar base in 1000 ml D.W. The medium was autoclaved at 121 ºC for 15 min and pressure 15 pounds per square (psi), cooled to 50 ºC and 5% of fresh human blood was added. This medium was used as enrichment medium for the cultivation of the bacterial isolates and to determine their ability of blood hemolysis (Forbes *et al*., 2007).

### 3.3.7: Peptone water medium

This medium was prepared by dissolving 8 g peptone in 1000 ml of distilled water, the medium was autoclaved at 121 ºC for 15 min and pressure 15 pounds per square(psi), then distributed into test tubes. It was used for the demonstration of the bacterial ability to decompose the amino acid tryptophan to indole (MacFaddin, 2000).

### 3.3.8: Methyl red – vogas- proskauer medium (MR-VP)

MR-VP medium was prepared and used to detect the partial and complete hydrolysis of glucose (MacFaddin, 2000).

### 3.3.9: Brain heart infusion broth with 5% glycerol

This medium was prepared by adding 5 ml of glycerol to 95 ml of BHI broth before autoclaving at 121 ºC for 15 min and pressure 15 pounds per square (psi). The medium was used in preservation of bacteria (MacFadden, 2000).

### 3.3.10: Simmon's citrate medium

Simmon's citrate medium was used for determining the ability of bacteria to utilize citrate as the sole carbon source (MacFaddin, 2000).

### 3.3.11: Mannitol salt agar

This medium was prepared according to the manufacture company. It was used as a selective media for the isolation of Staphylococci and differentiation of *Staphylococcus aureus* (MacFaddin, 2000).

### 3.3.12: Motility medium (Semi solid)

It was prepared by dissolving (0.5gm) agar agar with (100ml) of nutrient broth, and then dispensed into sterile test tubes (5ml in each). This medium has been used to detect the motility of bacteria (Collee *et al.*, 1996).

### 3.3.13: Eosin methylene blue (EMB) agar

Culture media were prepared according to the instructions of the manufacturer for diagnostic lactose fermenting bacteria especially *E.coli* (Murray *et al.*, 2003).

### 3.3.14: UTI chromogenic agar

Suspend 47.5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in the autoclave at 15 lbs pressure (121° C) for 15 minutes. Cool to (45-50 ° C), mix well, and dispense into Petri sterile plates. (Abdullah *et al.*, 2009).

### 3.3.15: Pseudomonas chromogenic agar

Pseudomonas chromogenic agar medium was prepared according to the method recommended by the manufacturing company. (Abdullah *et al.*, 2009).

### 3.4: Laboratory Diagnosis

#### 3.4.1: Isolation and identification

After pure colonies were obtained, taken from each primary positive culture on Blood, MacConkey, chromogenic, and Mannitol salt agar medium and it was identified depending on its morphology (colony shape, size, color, borders, nature of pigments, elevation, and texture) and then it was examined by light microscope after being stained with Gram's stain. After staining, biochemical tests were done on each isolate to complete the final identification (Hemraj., *et al* 2013).

#### 3.4.2: Biochemical Test

##### 3.4.2.1: Catalase Test

Catalase is an enzyme that catalase the release of oxygen from hydrogen peroxide. A colony of organisms is transferred by sterile wooden stick to the surface of a clean dry glass slide, and one drop of (3%) H2O2 is added to it. The formation of gas bubbles indicated the positive result (Forbes *et al.*, 2007). It was prepared by dissolving 3 gm of $H_2O_2$ in 100 ml of distilled water and stored in dark container (MacFaddin, 2000).

##### 3.4.2.2: Oxidase test

The test depends on the existence of certain bacterial oxidases that would catalyze the transport of electrons between electron donors in the bacteria and a redox dye (tetramethyl-ρ-phenylenediamine dihydrochloride); the dye was reduced to a deep purple color. A piece of filter paper was saturated in a petri dish with oxidase reagent (freshly prepared); and a small portion of the

bacterial colonies was spread on the filter paper by a wooden stick. The turning of the color of the smear from rose to purple within 10 sec indicated a positive result (Forbes *et al*., 2007).

### 3.4.2.3: Coagulase test

Coagulase is an enzyme produced by *S. aureus* that converts fibrinogen into fibrin. It is an important method for differentiation between *S. aureus* and other species of *Staphylococcus*. There are two forms of coagulase enzyme, bound and free and they are detecting by slide and tube (MacFaddin, 2000).

Slide methods were used to detect bound coagulase by putting a drop of rabbit plasma and emulsify with isolated colonies gently, the slide was rocked for 5-10 seconds to see clumping (coagulation).

### 3.4.2.4: Indole production test

It is used for determination of organism's ability to produce indole from deamination of tryptophan by tryptophanase. Fresh colony was cultured on peptone water then incubated at 37ºC for 24-48 hrs. Some drops from Kovac's reagent were added. The presence of pink ring after adding of reagent considered a positive result of the test (Forbes *et al*., 2007).

### 3.4.2.5: Methyl red test

Young colony was cultured on methyl red –voges proskauer broth then incubated at 37ºC for 24 hrs., five drops of methyl red reagent added to a broth culture of bacteria. Immediately the result was read. The color changing to red conceded a positive result (Forbes *et al.*, 2007).

### 3.4.2.6: Voges-Proskauer test

Inoculate an MR / VP broth tube which has a pure research organism community Incubate at 35 ° C for 24 hours, taken 1 mL of broth to clean test tube at the end of this time. Add 0.6mL of 5% α-naphthol, followed by 0.2 mL of 40% KOH. (Note: adding reagents in this order is essential.) Gently shake the tube to expose the medium to atmospheric oxygen and allow the tube to remain undisturbed for 10 to 15 minutes (Devi *et al.*, 2015)

### 3.4.2.7: Citrate utilization test

Young colony was cultured on Simmons citrate slant then incubated at 37ºC for 48-72 hrs., the color changed from green to blue that considered a positive result (Forbes *et al.*, 2007).

### 3.4.2.8: Motility test

Tubes containing of semi solid (nutrient broth and 0.5 agar agar) were stabbed once at the center with on inoculating needle then, incubated at (35°C) for (24–48) hours. The motile bacteria spread out from the line of inoculating (MacFaddin, 2000).

### 3.4.2.9: Hemolysis Test

Hemolysis is determined by lysis blood cells by stretching isolates of bacteria on the blood agar plate and incubated them at (37°C) for (24-48 hrs.) an appearance of clear zone around the colonies referred to complete hemolysis (β-hemolysis) or the color of the media changes (greenish zone) around the colonies referred to partial hemolysis (α-hemolysis), while no changing referred to non-hemolysis (γ-hemolysis) (DeBoy *et al*., 1980).

### 3.4.3Antimicrobial Susceptibility Testing

The goal of antimicrobial susceptibility testing is to predict the vivo success or failure of antibiotic therapy. Tests are performed in vitro and measure the growth response of an isolated organism to a particular drug or drugs. It's done by a disc diffusion method (Mahon *et al.*, 2018).

### 3.4.3.1. Preparation of Bacterial Inoculum

Selected pure isolated fresh-growing colonies were suspended in 5 ml brain heart broth and incubated for 4-8 h at 37° C. The turbidity resulting from growth has been modified with sterile broth to give comparable optical density to the 0.5. Standards at McFarland (Mahon *et al.,* 2018).

### 3.4.3.3. Application of Antibiotic Discs

1-The antibiotic disks were discharged to the inoculated surface Plate agar. Every disk has been gently pressed to ensure it is complete Contact with the agar surface by assisting forceps with sterilization.

2-The plates were inverted and placed at temperature 37 ˚ C for 18-24 hours in an incubator.

3-The diameter of the growth inhibition zones on plates around the disks was determined by using a transparent ruler.

4-The tests obtained were compared with the CLSI (2021) tables.

**3.5.: Vitek 2 system**

The vitek 2 device was used to validate biochemical and antibiotic examination, which was carried out in compliance with the manufacturer's instructions. This machine consists of personal device, reader incubator, which consisted of several internal components including: card filling process, card cassette, c loading process, bar code scanner, card sealer, cassette carousel and incubator. Besides optical transmission, waste processing, electronic control instruments and firm ware. The system has been equipped with an extended identification database for all routine identification tests that provide an enhanced microbial diagnostic efficiency that reduces the need for additional testing to improve safety for both the test and the user. The following steps are planned according to the directions of the manufacturers. Three ml of normal saline are placed in the plane test tube and inoculated with an isolated colony full of loop. The colony must be 24 hours of age, the test tube inserted into the colony standardization adens testing system to Macfarlands standard solution ($1.5\times 10^8$ cell / ml). the standardization of colony inoculums is placed into. Cassette and specimen identification number by barcode in the device applications the vitek2 card is then attached to the specimen ID number. The cassette then positions all the following measures performed by the instrument in the filler box, the instrument manages the incubation temperature, the card's optical reading and constantly tracks and moves the test data to the device for review.

**1-Standardization**

The handling of the specimen inoculum preparation, standardization and dilution step is minimized after primary isolation. The standardization inoculum is placed in the cassete, and barcode is used to interfere with a specimen identification number in the computer software.

**2. Traceability**

The form of vitek2 card is then read from the barcode put on the card during fabrication, and the card is attached to the specimen ID. This is a simple barcode reading move for manufacturers to connect the card to patient details.

**3- load and go**

In the filler module position the tape. The cassette is transferred to the reader / incubator module when the card is filled in. The instrument shall handle all subsequent steps**.**

## 3.5 Moleculer Study

**3.5.1.a.DNA Extraction :**

Genomic DNA from white blood cells (WBCs) for both UTI patients and control group were extracted by using DNA extraction kit (Favorgen) According to the manufacturer's instructions is as follow (table 3-6)

**3.5.1.b.Kit Contents**

**Table (3-7): Components of DNA Extraction Kit.**

| Cat. No. / preps | FABGK (100)<br>( 100 preps) |
|---|---|
| 2 ml Collection Tube | 200 pcs |
| Elution Buffer | 30 ml |
| FABG Buffer | 30 ml |
| FABG Column | 100 pcs |
| FATG Buffer | 30 ml |
| RBC Lysis Buffer | 135 ml |
| W1 Buffer | 45 ml |
| Wash Buffer | 20 ml |

**3.5.1.c.Special Steps of DNA Extraction (for frozen blood)**

**Step 1- Sample preparation**

1-About 200µl blood was added to a 1.5ml microcentrifuge tube.

2-About 40µl proteinase k (10mg/ml) was added to the 1.5ml

3-microcentrifuge tube and briefly mixed. The mixture is incubated at 60 ºC for 15 minutes.

**Step 2 - Cell Lysis**

4-About 200µl of FABG Buffer was added to the 1.5 ml

5-microcentrifugetube and mixed by shaking vigorously.

6-The mixture was incubated in a 70 ºC water bath for 1minutes.

Duringincubation, the tube was inverted every 3 minutes.

7-At this time the required volume of Elution buffer was pre heated (100µl/sample) to 70 ºC (for Step 5 DNA Elution).

**Step 3 - DNA binding**

**8-** About 200 µl of absolute ethanol (96-100%) was added to the sample lysate and immediately mixed by shaking vigorously for 10 seconds. If precipitate appeared, it was broken up by pipetting.

**9-**The FABG Column was placed to a 2ml collection tube and the entire mixture (including any precipitate) was transferred carefully to FABG Column.

**10-**The samples were centrifuged at 14,000 × g for 5 minutes and 2 ml collection tube containing the flow-through was discarded and the FABG column placed in a new 2 ml collection tube.

**Step 4 – Wash**

11-About 400 µl of W1 Buffer was added to the FABG column and centrifuged at 14,000 × g for 30 seconds and the flow-through wasdiscarded and the FABG Column was placed back in the 2 ml collection tube.

12-About 600 µl of Wash Buffer was added to the FABG column and centrifuged at 14,000 × g for 30 seconds and the flow-through wasdiscarded and the FABG column was placed back in the 2 ml collection tube and centrifuged again at 14,000 × g for 3 minutes without anyaddition to dry the column matrix.

**Step 5 - DNA Elution**

13-The dried FABG column was transferred to a new 1.5 ml microcentrifugetube.

14-About 100 µl of pre-heated Elution buffer or TE buffer was added to the membrane center of the FABG column matrix. Stand FABG Column for 3-5 min or until the buffer is absorbed by the membrane.

15-The tube was centrifuged at 14,000 × g for 30 seconds to elute the purified DNA.

**Step Final -Pure DNA**

16-The DNA was stored at -20 °C.

### 3.5.2.Concentration and Purity Measurement of Extracted DNA

DNA quantity and concentration was determined using a spectrophotometer (Nano drop) as the following:

1-About 1µl of TE solution was added on the lens for empty- apparatus, be careful without touch the lens.

2-One µl of DNA sample was added to the lens and read the absorbance at 260/280 nm, then recorded the concentration of DNA at (65-198) ng/ml. and recorded the purity at (1.9) ng/ml.

### 3.5.3.Preparation of Solution

**A- Loading Dye**

Bromophenol blue 0.025 gm and sucrose 4 gm were dissolved in 10 ml $H_2O$ then were stored at 4°C (Sambrook and Rushell, 2001).

**B- TBE (10X) Stock Solution / Liter**

Prepared 500 ml of TBE (1X) by adding 50ml of TBE (10X) stock solution to a final volume of 500 ml d.dH2O..

**C- Red safe Staining Solution**

Red safe four drops of concentration 1.25 mg/ml in TBE buffer 100 ml 0.5X. The solution was stored in a dark bottle at room temperature (Robinson and Lafleche, 2000).

**3.6.Agarose Gel Electrophoresis**

Agarose gel electrophoresis was performed according to (Sambrook and Rushell, 2001)

1-The gel-casting tray was placed in plastic tray, checked that the teeth of the comb were approximately 0.5 mm above the gel bottom.

2-About 500 ml of TBE (1X) was prepared by added 50ml of TBE(10X) stock solution to a final volume of 500 ml of deionized water

3-About 100 ml of the TBE (1X) buffer was placed into a 500 ml flask and added 0.7 g of agarose. Agarose was melted by heated the solution on hot plate for approximately 10 min carefully was swirled. The agarose solution to ensure that the agarose was dissolved and no agarose particles were visible.

4-The agarose solution was cooled to approximately 60 ° C and added 1.5-2µl of red safe stock solution. The agarose was slowly poured into the gel casting tray. Air bubbles was removed by yellow tip.

5-The comb was position approximately 1.5 cm from the edge of the gel . The agarose was solidified for approximately 20-30 minutes. After the agarose has solidified the comb was removed with a gentle back and forth motion.

6-The gel-casting tray was removed and placed the tray on the central

platform of the gel box.Electrophoresis buffer was added to the buffer chamber until it reached a level of 0.5-1cm above the surface of the gel.About 3 µl of loading dye was mixed with 5µl of DNA specimen and then or PCR-proudct loaded in the wells.

7-The samples were loaded into the wells using a yellow tips under the surface of the electrophoresis buffer just above the well. The sample was expelled slowly, allowing it to sink to the bottom of the well. Samples must be loaded in sequential sample wells.

8-The lid was placed on the gel box and connect the electrodes. DNA was travel towards the positive (red) electrode positioned away from the well. The power supply was Turn on.

9-The electrophoresis continued until the loading dye reached 75% of the gel.

### 3.7. U.V Documentation

Agarose gel was visualized in a UV transilluminator provided with gel documentation unit. Agarose gel was placed above the UV transilluminator device and the photos were captured .

### 3.8.Primer Dilution

Marcogene primers were commonly shipped in a lyophilized state. The units of a lyophilized primer are given as a mass, in Picomoles .To create a stock of primers, reconstituted was the primer in sterile 1X TE (1mM Tris, mM EDTA, Ph 8.0) .The company supplies the amount of TE or sterile nuclease-free $H_2O$ to be added to each primer to obtain master stock that would be used again to obtain working stock.

1-The following steps are followed for reconstituting and diluting the primers

2-The tube was spin down before opening the cap.

3-The desired amount of water (300µl) was added according to the oligos manufacturer to obtain a master Stock. Vortex properly for re-suspend the primers evenly.

4-About 10µl was transferred of the master stock to a 0.2ml Eppendorf tube that contains 90µl of sterile, nuclease-free $H_2O$ (Working Stock).

5-The master stock and working stock was stored at -20 °C.

The working stock was thawed on ice and vortex before using in PCR and then stored at -20 °C. Once the primers are reconstituted and/or diluted, it is recommended that the primers should be distributed into single-use aliquots. Making single-use aliquots limits the freeze-thawing of primers and therefore will extend their life. It is recommended to store both primers at -20 °C. Table (3-7) which explained the sequences of four primers that used for PCR amplification.

**Table (3-8): Sequences of primers used for PCR- amplification refractory mutation system (ARMS) of *TLR2* gene (Ehsan *et al*., 2018).**

| Gene | | Prime sequence (5′-3′) | Product Size /bp | Reference |
|---|---|---|---|---|
| *TLR2* *rs3804099* | F | ATTGCAAATCCTGAGAGTGGGAA | 349 bp TT | |
| | R | CAAACTTTCATCGGTGATTTTCACA | 228bp CC | |
| | F | CCAAAAAGTTTGAAGTCAATTCAGAAT | 173 bp CT | |
| | R | TCATATGAAGGATCAGATGACTTCCG | 228 bp and 173 bp | Mirkamandar, *et al*., 2018 |

**3-9 :PCR- Amplification Refractory Mutation System (ARMS) of *TLR 2* Gene**

PCR- ARMS was done as a first step using a gradient temperature ranging from 51°C to 53°C. The reaction was performed 25µl volume containing ( premix mastermix 12 µl, deionizing distal water 5 µl, DNA 4µl and 1 µl for each forward and reverse primer. After determination of the optimum annealing temperature (53°C), the following program was set in the thermocycler to amplify the target DNA fragments (*TLR2*gene) as shown in Table (3-9).

**Table (3-9): The program used for *TLR2* gene amplification.**

| Stage | Temp. C° | Time(min) | Cycles |
|---|---|---|---|
| Initial denaturation | 94.0 | 5:00 | |
| Denaturation | 95.0 | 00:30 | 35 |
| Primer annealing | 53.0 | 00:30 | |
| Template elongation | 72.0 | 1:00 | |
| Final elongation | 72.0 | 10:00 | |
| Incubation | 4.0 | 10:00 | Hold |

Primers sets used in this study are listed in table (3-10).

**Table (3-10): List of primers sets**

| Gene | Prime sequence (5′-3′) | | product Size /bp | Reference |
|---|---|---|---|---|
| TLR2 (rs5743708) | F | GTGGTGCAAGTATGAACTGGAC | 221 | Tenhu *et al.*, 2020 |
| | R | AGGACTTTATCGCAGCTCTC AG | | |
| TLR9 (rs5743836) | F | CCCTGTTGAGAGGGTGACAT | 154 | Tenhu *et al.*, 2020 |
| | R | CCTGCTTGCAGTTGACTGTG | | |
| TLR9 (rs352140) | F | TTGGCTGTGGATGTTGTTGT | 177 | Tenhu *et al.*, 2020 |
| | R | AAGCTGGACCTCTACCACGA | | |

### 3.10.Polymerase Chain Reaction (PCR)

Conventional PCR was used to amplify a target DNA using specific primer pairs. PCR typically consists of three consecutive steps (denaturation, annealing,and elongation) of repeated cycles to get PCR product (amplicon). The PCR thermal cycling conditions are mentioned in the table (2-5).The size of PCR products (5 µl) were analyzed in 1.5% (w/v) agarose gel by electrophoresisusing 1× TBE buffer and visualized by staining with simply safe dye. Product size was determined by comparison with Gene

Ruler 100 bp DNA ladder (Intronbio, Korea).

**Table (3-11): Condition program of PCR**

| SNPS | Steps | Temperature (C°) | Time (mint) | No. of cycles |
|---|---|---|---|---|
| **TLR2 (rs5743708)** | Initial denaturation | 95 | 2 | 1 |
| | Denaturation | 95 | 30 sec | 40 |
| | Annealing | 61 | 30 sec | |
| | Extension | 72 | 40 sec | |
| | Final extension | 72 | 5 min | 1 |
| **TLR9 (rs5743836)** | Initial denaturation | 95 | 5min | 1 |
| | Denaturation | 95 | 50 sec | 35 |
| | Annealing | 62 | 50 sec | |
| | Extension | 72 | 30 sec | |
| | Final extension | 72 | 3 min | 1 |
| **TLR9 (rs352140)** | Initial denaturation | 95 | 2min | 1 |
| | Denaturation | 95 | 40 sec | 35 |
| | Annealing | 56 | 40 sec | |
| | Extension | 72 | 60 sec | |
| | Final extension | 72 | 2 min | 1 |

All PCR products of target regions were electrophoresed on 1% agarose at 70 V for 1h and visualized by red safe. Photos were taken using gel documentation system.

### 3.11.Immunological assay

### 3.11.1.Estimation of Human Toll Like Receptor 2 (TLR2) concentration by ELISA kit

This kit is used to assay the *TLR2 i*n the specimen of human serum and urine

#### 3.11.1.1.Assay principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human TLR2 antibody. TLR2 present in the specimens added and binds to antibodies coated on the wells. And then biotinylated human TLR2 Antibody is added and binds to TLR2 in the specimens. Then Streptavidin-HRP is added and binds to the Biotinylated TLR2 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human TLR2. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

#### 3.11.1.2.Assay Procedure

1-All reagents, standard solutions, and specimens were prepared as instructed, and all reagents were brought to room temperature before use. The assay is performed at room temperature.

2- Fifty microliter of the standard was added to the standard well.

3- Forty microliter of the specimen was added to specimen wells and then was added 10µl anti-TLR2 antibody to specimen wells, then was added 50µl streptavidin-HRP to specimen wells and standard wells (Not blank control well). Mixed well, and cover the plate with a sealer and incubated for 60 minutes at 37°C.

4.The sealer removed and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash.

5- Fifty microliter substrate solution A was added to each well and then was added 50µl substrate solution B to each well. Incubation plate covered with a new sealer for 10 minutes at 37°C in the dark.

6- Fifty microliter of Stop Solution was added to each well, the blue color will change into yellow immediately.

7- The optical density (OD value) of each well determined immediately by used a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

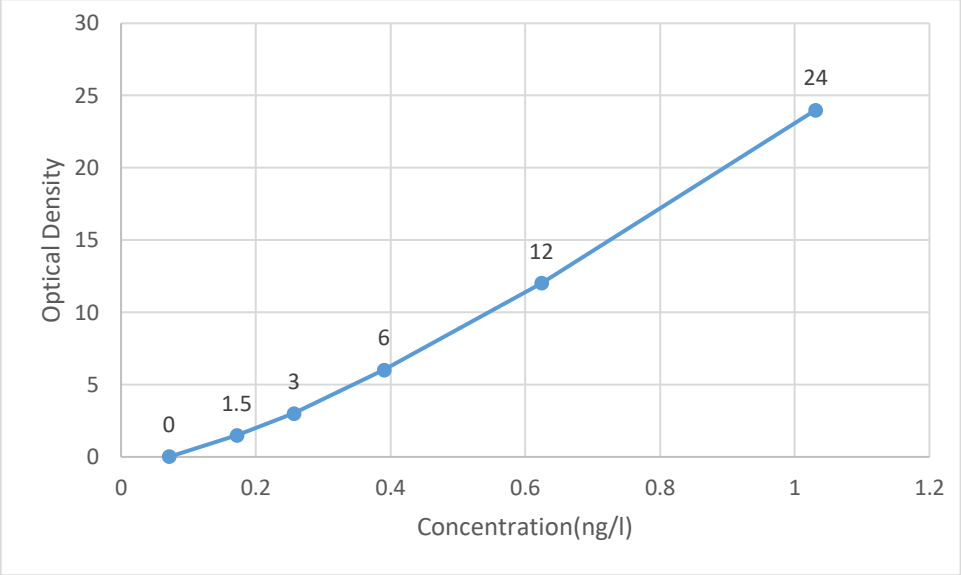


**Figure (3-1):- Standard curve of (TLR2) concentration**

### 3.11.2. Estimation of Human Toll Like Receptor 9 (TLR9) concentration by ELISA kit

#### 3.11.2.1 Assay principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human TLR9 antibody. TLR9 present in the specimens added and binds to antibodies coated on the wells. And then biotinylated human TLR9 Antibody is added and binds to TLR9 in the specimen. Then Streptavidin-HRP is added and binds to the Biotinylated TLR9 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human TLR9. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

### 3.11.2.2.Assay Procedure

1- Prepare all reagents, standard solutions and specimens as instructed. all reagents were brought to room temperature before use. The assay is performed at room temperature**.**

2- Fifty microliter of the standard was added to standard well. Note: antibody Don't add to standard well because the standard solution contains biotinylated antibody.

3-Forty microliter of the specimen was added to specimen wells and then was added 10µl anti-TLR9 antibody to specimen wells, then was added 50µl streptavidin-HRP to specimen wells and standard wells (Not blank control well). Mixed well and cover the plate with a sealer. Incubated for 60 minutes at 37°C.

4-The sealer removed and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash.

5-Fifty microliter of the substrate solution A was added to each well and then was added 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

6-Fifty microliter of Stop Solution was added to each well, the blue color will change into yellow immediately.

7-The optical density (OD value) of each well determine immediately used a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

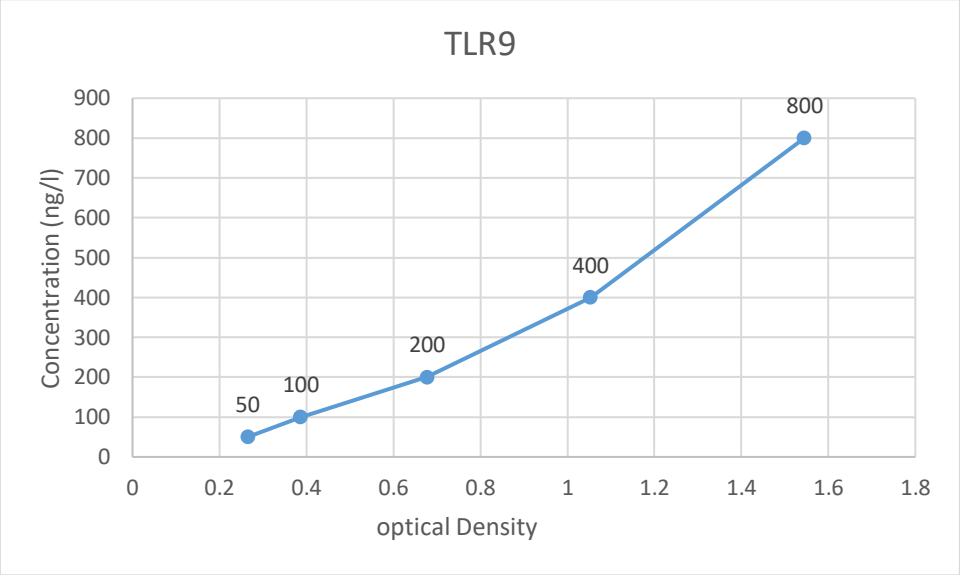


**Figure (3- 3):- Standard curve of (TLR9) concentration**

## 3.12. Statistical analysis

Data was analyzed using SPSS (version 20, SPSS Inc. Chicago, Illinois, USA). Descriptive statistics (mean, standard error), and differences were compared by student –t test at $p \leq 0.05$. Hardy-Weinberg equation: $p^2+ 2pq+ q^2 = 1$ to estimate the frequency of the alleles . The odds ratios (ORs) and 95% confidence intervals (95% CIs) were used to evaluate the potential associations between genetic variants with patients

## 3.13. Biosafety and Hazard Material Disposing:

Biosafety aspects followed during the work include disposing of all swabs, petri dishes and all contaminated supplies by autoclaving and then incineration. All benches cleaned with alcohol before and after the work.

## 4- Results and Discussion

### 4.1- Specimen collection

A total of 150 specimen (blood and urine) were collected from patients, for both male and female patients who were suffering from urinary tract infection in Al- Hilla province .During October to March (2020-2021), for age ranging (18-48) years old .and 50 specimens ( blood and urine )were collected as control which apparently healthy. There were many Demographic data included in the present study includes; age, sex distribution, exposure to a previous infection of UTI, personal hygiene and residens (Appendix).

### 4.1.1 Age and sex distribution of UTI patients

There was 48 (32%) males and 102(68%) females of patients as shown in figure (4-1). These results were in the same line with the results of the study to ( Ganesh, *et al* ,2019 ), mentioned that It was found that the rate of females with UTI were higher 55.23% in comparison with males 44.79%, also Which was 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). Women are more susceptible than men, due to several clinical factors including anatomic difference, hormonal effects and behavioral pattern (Srinath *et al*., 2018).

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 quickaccess 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 (Saeed *et al*., 2015).

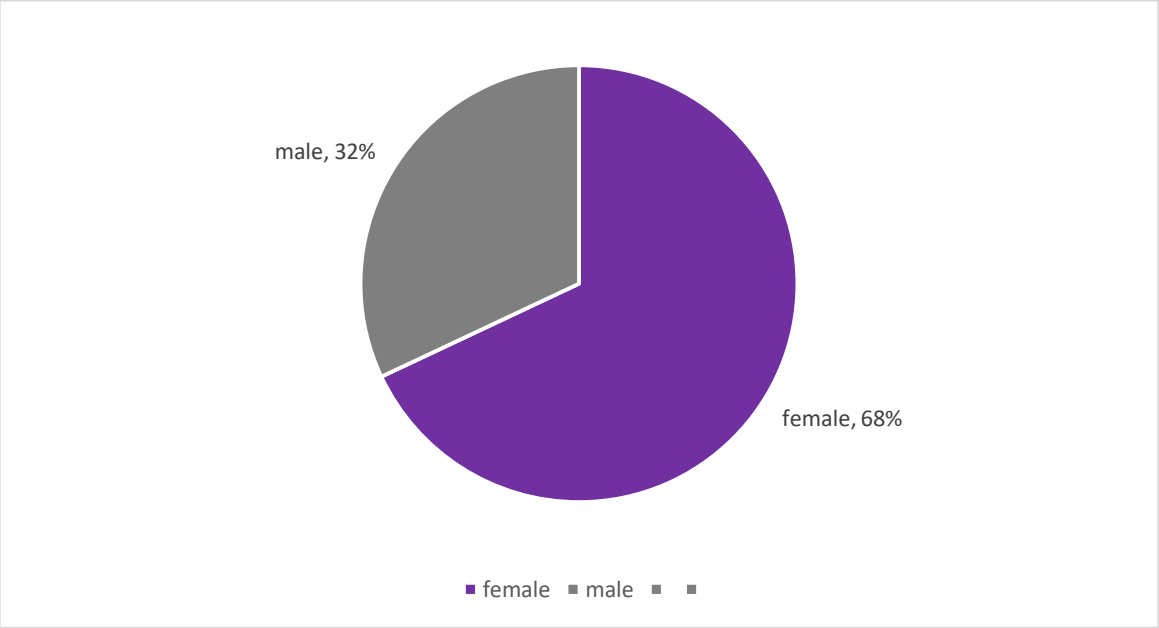


Figure (4-1) Distribution of patients with UTI according sex

However, the infection percentage increased in the age groups of (28-38) to reach 44% of the total number of UTI patients. While the number of cases were decreased in the age groups of (18-28) years 28.6% and (38-48) years 27.3% as shown in table (4-1)

**Table (4-1) Distribution of age in UTI patients**

| Age group | No | Percentage |
|---|---|---|
| **(18-28)years** | **43** | **28.6%** |
| **(29-38) years** | **66** | **44%** |
| **(39-48) years** | **41** | **27.3%** |
| **Total** | **150** | **100** |

The results that were expressed in table (4-1) revealed the wide age range of UTI Patients. The rate of infection varies depending on the age. Concordant results were obtained from other studies in Ethiopia by(Gachui, 2017) (Derese *et al.*, 2016). Similarly, research done at Teerthanker Mahaveer Medical College and Research Centre India corresponds with the results of this study which depicted UTI to be highest in age group (20-29) years (Mahak, 2015) male patients between the age of 25-34 years had the highest number of UTI 125 (32.4 %) (Gachui, 2017). Similarly, This large occurrence of participants between ages 20-29 years can probably be due to them being at their topmost reproductive years, Individuals in this age group have a high probability of self-diagnosis and treatment due to availability of information that can be read online. Inappropriate use of antibiotics without proper prescription and may lead to bigger chance of wrong, unwarranted treatment, wrong diagnosis, delays in proper treatment and increased disease (Bennadi, 2014**).**

This result was Disagreement with what have been reported by (Muthulakshmi ,2017) that the infection percentage increased in the age groups of approximately 44% , there are many factors which lead to increase

the risk of infection among elderly persons such as: poor nutrition, immobility leading to poor hygiene, chronic illnesses and increased stress on the body and strain on the body‘s defense mechanisms against that disease, this makes the person more susceptible to other infections, such as: prostatic hypertrophy and degeneration of nerves to bladder that cause urine stasis in bladder as a result of incomplete emptying, stasis predisposes to urinary tract infection (Dewit ,2005 ).additional Female anatomy, age, sexual activity, certain types of birth control, and menopause are the most risk factors for UTI.

Other risk factors are urinary tract abnormalities, blockages in the urinary tract, suppressed immune system, catheter use, and a recent urinary procedure (Storme *et al*.,2019).

### 4.1.2 Clinical History of Urinary Tract Infections:

Figure (4-2) showed that 88 (58.66%) of patients had a previous attack with UTI, while 62 (41.33%) no history of UTI.

This study is agreement with Derese *et al.,* (2016) and several other studies ( Ochei *et al.,* 2018 and Tazebew *et al* ,2013) they reported that pregnant women with the past history of UTI were highly affected by UTIs. This finding could be attributed to the presence of antibiotics resistant strains from those who had the previous history of UTI (Sekharan *et al.*, 2017).

On the other hand, anther studies were given reverse results of the present study and mentioned that showed that after a first episode of cystitis caused

by *E. coli* in young women, 24% had a second infection within 6 months (Sobel and Kaye,2015).

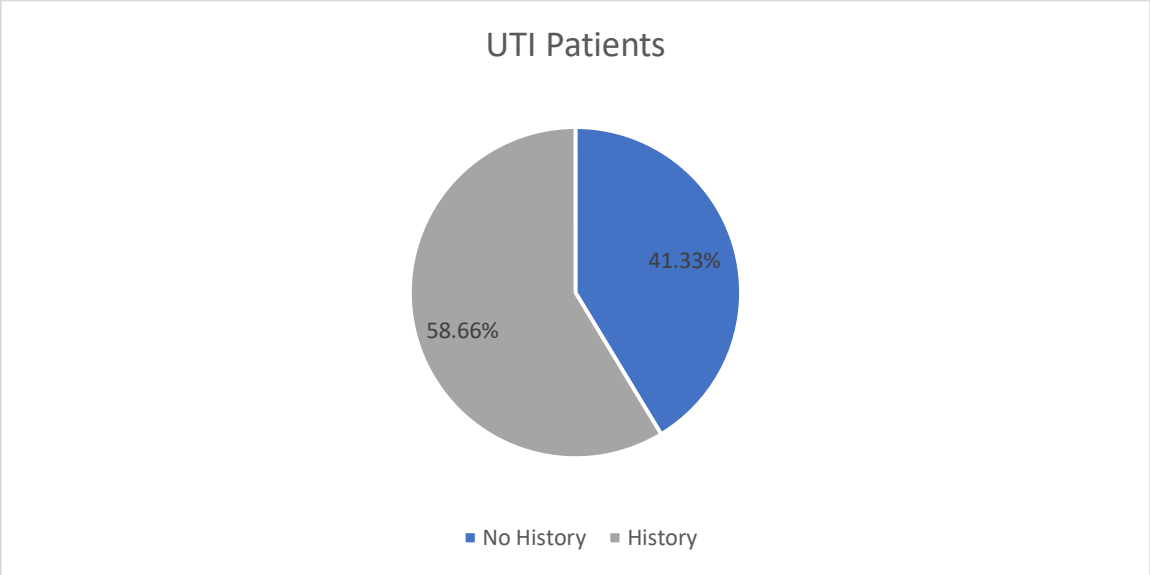


**Figure (4-2) Percentage of urinary tract infections in patients according to history of UTI**

### 4.1.3 Residence

This study has showed that 93 (62%) of UTI patients are urban residents, and 57 (38%) are residents of countryside This result is in agreement with Entesar *et al.*,(2018) and Derese *et al.*,(2016) who showed that patients who live in the city have a higher Percentage of UTI (70.7% and 92.3% respectively) than those are living in the rural areas. However, this finding may be explained by that samples collected in this study were taken from a hospital in the center of the city and no samples were taken from hospitals in the rural ares.

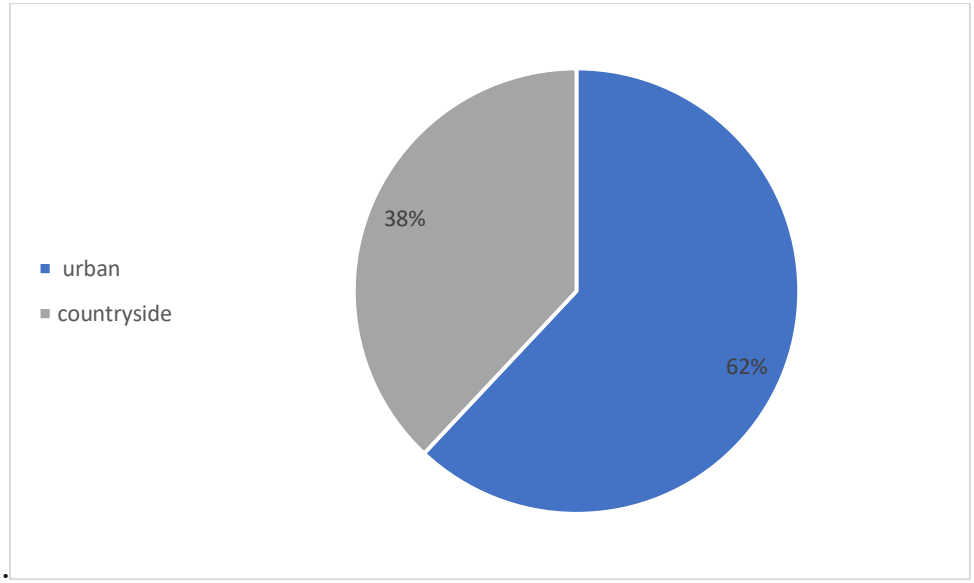


**Figure (4-3) Percentage of urinary tract infections in Patients in relation to residence**

### 4.1.4 .personal hygiene

In current study showed that (67 %) of patients of UTI have used reapted washing genitals after urination while (33%) have normal washing genitals after urination or defecation that agree with .(Obiora *etal* .,2014) mentioned that UTI had a significant relationship with the direction of washing genitals after urination or defecation. Washing of genitals from back to front is more likely to lead to the spread of anal or vaginal flora into the urethra. Education on the direction of washing and advice to micturate shortly after sexual activity can reduce the percentage of ABU and UTI (Elzayat *et al.*, 2017).

Screening for asymptomatic bacteriuria has been shown to be cost effective when compared with treating urinary tract infection and pyelonephritis without screening.( Abdullahi & Thairo 2015) have suggested

that urine should be cultured in each trimester to improve the detection of bacteriuria in those who were not bacteriuric at initial screening.

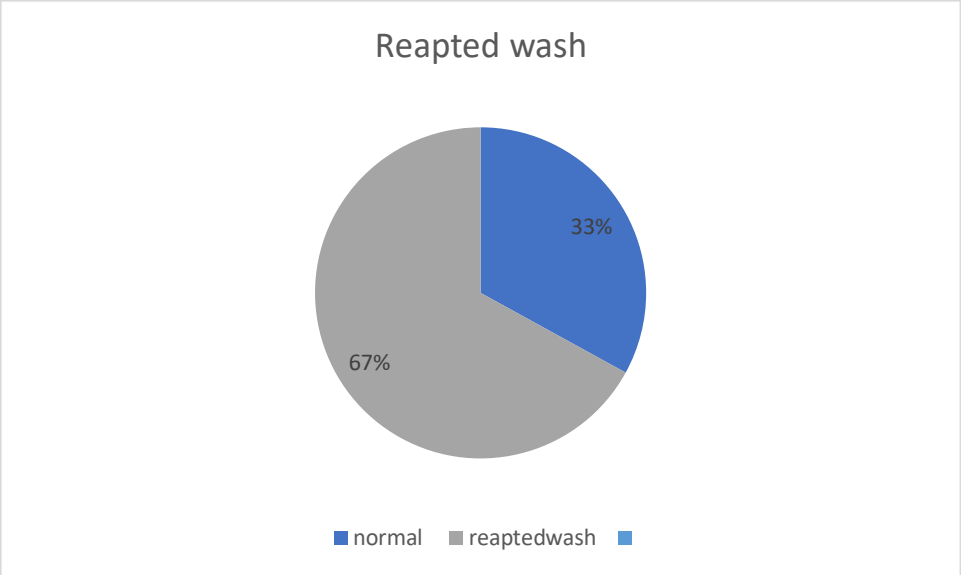


**Figure (4-4): Percentage of patients are suffering UTI according to personal hygiene**

## 4.2. Bacteriological Study

### 4.2.1 The Percentage bacterial isolated from UTI

Urine culture was done for all of the 200 samples (150 patients and 50 control), The current study's found the isolation and diagnostic of microorganism from patients were suffered from UTI involved growths of bacteria are divided to Gve-(51.48%) and Gve+(48.52%).

The result of this study was found approximately similar with 44.5% reported by Al-Khafaf and Shareeef (2013) from Iraq,and it was in agreement with other two studies conducted by Onoh *et al*., (2013) 46.5% However, this result is lower than many findings reported by Shah *et al*.,

(2015), Oladeinde *et al*., (2015) Nwachukwu *et al*., (2018) and AL-Kadassy *et al*.,(2016) .

In similar study in Sudan, researchers stated that the no–growth culture is either a viral infection or an acute urethral syndrome which is a condition in which there are complaints suggesting UTI, but when cultured, organism is below the number that could be effectively cultured (Hamdan *et al*., 2011). Moreover, symptomatic patients whose urine culture didn't show appreciable growth might be due to other less frequent UTI causing microorganisms, such as parasites, fungi and viruses (Tsegay, 2014).this study accord with the findings of another study that the frequency of Gram-positive microorganisms bacteria was not very high in when compared with prevalence of Gram negative bacilli (Ibrahim,2017 )and . Okonko *et al* (2018) 47.5% from Nigeria.and 44% observed by Valentina and Srirangaraj from India (2021)However this study is disagree with other reports in Iraq Al-Wazni and Hadi (2015) who found that the most predominant isolated pathogens was Gram positive bacteria (83%) where *Staphylococcus aureus* isolates comprise (39%). This work is also concordant with another two studies conducted by Almukhtar (2018) in Iraq and Tadesse *et al.,* (2014) in Ethiopia, who both reported that the percentage of Gram positive bacteria was higher than Gram negative bacteria.

The similarities and differences between this study and other studies from one geographical region to another could be attributed to differences in UTI recognition, methods of screening, and confounding risk factors, different environmental conditions and practices such as socio economic standards,

education programs, health care, and hygiene practices. (Ghaima *et al.*, 2018).

### 4.2.2 Bacterial Pathogens Isolated from Urine

The isolation and diagnostic of microorganism from patients were suffered from UTI involved Growth of *E. coli, S.aureus* ,S. *Saprophyticus*, *K. pneumonia, .P. aeruginosa, Enterococcus faecalia*s, *Enterococcus facium* and *Proteus mirabilis*were identified using enriched and differential media (Blood agar and MacConkey agar ,Eosin methylene blue , mannitol salt agar. UTI-chromogenic agar base that different colors colonies .

Colonies on MacConkey of *E. coli* were seen as non-mucoid pink colonies, on Eosin methylene blue were seen metallic sheen and on UTI chromogenic agar were seen pink colonies while *K. pneumonia* were seen as large, pink and mucoid colonies on MacConkey agar, and dark blue or purple on UTI chromogenic agar *p. aeruginosa* were seen as large green _brown colonies on MacConkey and appear non pigmented on UTI chromogenic After isolation of the organisms and culture on different agar used, the organisms were Gram stained such as *S. agalactiae* were seen as Gram positive cocci some appearing in chains ,*S.aureus* & *Saprophyticus* were also seen as Gram positive cocci appearing in clusters. *E. coli*, *K. pneumonia*, and *P. aeruginosa* were Gram negative rods, under the light microscope .The catalase and oxidase test done indicated that *S. agalactiae* were a negative catalase and oxidase while *E. coli, K. pneumonia,* and *P. aeruginosa* were positive catalase activity and *E.coli* was negative oxidase. *P. aeruginosa* were positive oxidase and *K. pneumonia* was negative oxidase.

*Staphylococcus* were positive catalase and negative oxidase. Coagulase test done indicated that *S. aureus* were positive coagulase test but *S. epidermis* & *Saprophyticus* were negative for this test. *saprophyticus* were also seen as on UTI chromogenic agar white with pink halo while *S. aureus* were seen non pigmented white, while *Enterococcus faecalis* were seen on UTI chromogenic agar blue green colony while *Enterococcus faecium* were seen purple on UTI chromogenic agar Gram negative bacteria were diagnostic by biochemical test such as indole, methyl red, Voges-Proskauer, *Simmons' citrate test* and Kligler iron agar test the result Positive. Result of indole formation of red ring after added Kovacs reagent, Simmons citrate positive result convert of color media after incubation 24 hr from blue color to green color negative result remain the blue color. Voges-Proskauer test positive result formation of red ring after added of reagent Table in appendix showed biochemical test for gram negative bacteria.

**Table (4-2)** Biochemical test for gram bacteria that isolated from UTI

| Bacteria | Indole | methyl red | Voges Proskauer | Simmos citrate | Catalase | Oxidase |
|---|---|---|---|---|---|---|
| *Pseudomonas aeruginosa* | - | + | - | + | + | + |
| *Klebsiella pneumoniae* | - | + | - | + | + | - |
| *Proteus mirabilis* | - | - | - | + | + | - |
| *Escherichia coli* | + | + | - | - | + | - |

*E.coli* was the most common 80 (21.5%) followed by *Klebsiella pneumoniae 40*(10.75%), and then *Staphylococcus aureus* 36 (9.6%), 35(9.4%) for each *Staphylococcus saprophytics 16(4.3%)* while *Enterococcus faecalis50(13.44%),* while *Enterococcus facium 40(10.4%) Streptococcus agalactiae 2(0.5%)*and *Enterobacter cloacae* at a rate of *Staphylococcus epidermidis,* 2(0.5%) and finally each of 10( 2.68%), *Pseudomonas aeruginosa 40(10.4), Proteus mirabilis 20(5.3%).Candida albicans 46* This finding was in agreement with previous study conducted in Iraq and different countries which found Gram negative bacteria particularly *E.coli* are the most frequent isolates causing UTI in pregnant women (Abdulla and Oleiwi 2016; Samuel *et al.,* 2016;Ghaima *et al.,* 2018).

Although the UTI etiology are diverse showing the geographical variability of causative agents, the Gram negative bacteria, usually *Enterobacteriaceae* and specifically *E. coli* remains invariably predominant in most regions of the world (Lema and lema 2018).

It is clearly mentioned that in 85% of UTI cases Gram-negative bacteria are the dominant etiologic agents and undoubtedly they are also the normal flora of the intestinal tract and rectum lying in very near to urethral orifice (Flores-Mireles *et al.*, 2015; Abbo and Hotoon, 2014).

The high rate of isolation of Gram-negative uropathogens could be due to the presence of unique structure in Gram-negative bacteria which help for attachment to the uro-epithelial cells and prevent bacteria from urinary lavage, allowing for multiplication and tissue invasion (Gessese *et al.*, 2017).

*E.coli* was considered as the most prominent uro-pathogenic bacteria due to a number of virulence factors specific for colonization and invasion of the

urinary epithelium (Tazebew *et al.*, 2012). It is also associated with microorganisms ascending from the peri-urethral areas contaminated by fecal flora due to the close proximity between anus and due to warm, moist environment of the vagina (Ramos *et al.*, 2012).

In this study appear that the most commonly isolated germs from patients with *E. coli* is the source of UTI. this study similar with the results of Al-Jebouri (Al-Jebouri, 2013) who found the most isolated was *E.coli* bacteria and differ from the result of Ibrahim (Ibrahim,2017)The most isolated pathogen was *E.coli*, *Pseudomonas aeruginosa*,*Staphylococcus aureus* and *Enterococcus faecalis* it's the first isolated microorganism in the current study with a rate of (48.52%).of total UTI positive cultures. Some of studies had previously linked the increasing cause of UTIs by *Staphylococcus* to increased use of instrumentation such as bladder catheterization (Kline and Lewis 2016; Dougnon *et al.*, 2016). The presence of *Staphylococcus* species and *Streptococcus* species indicated that the gram-positive cocci are also causative agents of urinary tract infections. Their presence in low number comparing to Gram negative isolates may due to the fact that they are normal flora of the skin and hence form a lesser proportion of the microflora of the gastrointestinal tract (Samuel *et al.*, 2016).

Although, *Staphylococcus* species was known for years as a rare urinary isolate, One of the studies showed the high percentage of *Staphylococcus* and was the main cause of UTI in Nigeria (Top *et al.*, 2012). Some of studies had previously linked the increasing cause of UTIs by *Staphylococcus* to increased use of instrumentation such as bladder catheterization (Kline and Lewis 2016; Dougnon *et al.*, 2016).

**Table (4-3)** Frequency and percentages of culturing from urine for UTI patient

| Bacteria | No. | Percentage % |
|---|---|---|
| *E.coli* | 80 | 21.5% |
| *Enterococcus faecalis* | 50 | 13.44% |
| *Candida albicans* | 46 | 12.2% |
| *Klebsiella pneumonia* | 40 | 10. 4% |
| *Enterococcus faecium* | 40 | 10.4% |
| *Pseudomonas aeruginosa* | 40 | 10.4% |
| *Staphylococcus aureus* | 36 | 9.6% |
| *Proteus mirabilis* | 20 | 5.3% |
| *Staphylococcus saprophyticus* | 14 | 3.2% |
| *Staphylococcus epidermidis* | 10 | 2.68% |
| *Streptococcus agalactiae* | 4 | 1% |
| *Total* | 380 | 100% |

The Gram positive & Gram negative bacteria isolated from urine *S. aureus* & *S.saprophytics* and other of microorganism and diagnostic by different test such as macroscopic and microscopic ,biochemical test , and Vitec 2 compact system results were mentioned in Table(4-5) , probability of

bacteria diagnostic by Vitec 2 compact system between (91-99%) as in table (4-4).

**Table (4-4)** The probability of diagnostic bacterial isolates from UTI by vitec2compact system

| Bacteria | Probability% |
|---|---|
| *Staphylococcus saprophytics* | 99 |
| *Pseudomonas aeruginosa* | 91 |
| *Klebsiella pneumoniae* | 99 |
| *Proteus mirabilis* | 91 |
| *Eschericha.coli* | 98 |
| *Enterococcus faecalis* | 99 |
| *Staphylococcus aureus* | 99 |
| *Enterococcus faecium* | 98 |
| *Candida albicans* | 85 |

### 4.2.2.Patterns of sensitivity and antibiotic resistance to bacterial isolates

The sensitivity test of all bacterial isolates of antimicrobial agents were performed on Muller Hinton agar by modified Kirby Bauer disc diffusion method. All Gram-negative bacteria were screened against Six antimicrobial

agents belonging to various groups showed that the isolates differed in their antibiotic resistance. The isolates were interpreted as susceptible, intermediate or resistant to particular antibiotics by comparison with standard inhibition zones according to Clinical Laboratories Standards Institute CLSI (Humphries *al.*, 2021) .

It also reveals that these isolates have a high degree of resistance to commonly used antibiotics. Overall, all Gram. negative bacteria isolated in this study were susceptible to carbapenems such as Meropenem (88 %) and Imipenem was (84 %) the highest rate of antibiotic susceptibility was from Carbapenems class for both Meropenem and Imipenem. Respectively the rate of sensitivity to amino glycoside amikacin was (72%).The overall rates sensitivity for the remaining antimicrobial agents were as followed, for the third generation of cephalosporin such as Ceftriaxone (54%). The Quinolones class show sensitivity to Ciprofloxacin was (60%). The penicillin agents such as Pipracillin (6 %), as shown in table (4-5).

As a result of the increased tolerance of Enterobacteriaceae to many antibiotics, the use of Carbapenem has increased. By interfering with essential bacterial cell wall synthesis, it exerts its bactericidal action. The strong bactericidal action of Meropenem against a wide variety of aerobic and anaerobic bacteria is demonstrated by the ease with which it penetrates bacterial cell walls, (Shah and Narang, 2005). And were confirmed by using Vitek2 system compact.

**Table (4-5)** Antibiotic sensitivity for some gram negative bacterial isolate from UTI

| **Antibiotic** | **Symbol** | **Resistance** | **%** |
|---|---|---|---|
| Imipenem | IMP | 3 | 6 % |
| Meropenem | MEM | 3 | 6 % |
| amikacin | AK | 9 | 18 % |
| ceftriaxone | CTR | 15 | 30 % |
| ciprofloxacin | CIP | 18 | 36 % |
| Piperacillin | PRL | 39 | 78 % |

R= Resistant

study reveals that the most susceptible antibiotic was Meropenem and Imipenem followed by Amikacin. The present study was nearly from a study done in Basrah showing that most of the isolated bacteria were susceptible mainly to Meropenem, ciprofloxacin, gentamicin, followed by Kanamycin and Lincomycin, then Piperacillin ( Sahm,2001) India (Nurullaev,2004) and Iraq (Al-Jebouri, and Salih , 2013) Resistance to basic antibiotics used in therapy is extremely prevalent. of UTI such as cephotaxime, Ampicillin, Gentamicin has pointed to a real problem (Karananou,*etal* ,2016). agree with study ( Ibrahim,2017) who found that

the most of bacterial isolated from study resistances to gentamycin and agree with previous studies in USA.

While in Al-Hilla the result shows the Gram-negative bacteria demonstrated strong resistance to Amikacin and Imipenem (Alatoom*et al.*, 2017), In Iran , susceptibility results identified Meropenem antibiotic as a more effective agent against *E. coli* (Rahim*i et al.*, 2020).

### 4.2.3Types of Antibiotic Resistance in Gram Negative Bacteria

In all isolates of Gram-negative bacteria, the incidence of multi-drug resistance (MDR), severe drug resistance (XDR) and pan-drug resistant (PDR) has been investigated. MDR isolates were defined are organisms which have acquired resistance to multiple unrelated classes of antibiotic, While XDR extensive drug resistance bacteria are a type of multidrug resistant organisms that are resistant to almost all or all approved antimicrobial agents, and finally, PDR pan drug resistance are the non-susceptibility of bacteria to all antimicrobial agents in all antimicrobial categories **(**Vatan *et al.*, 2018).

Based on the result of this study, the MDR 15 (55%) isolates ,distributed according to the type of of bacteria such as *Escherichia coli* were *10 (25%),Proteus mirabilis* 1 (10%) , *Klebsiella pneumoniae* 4 (20%) and that were resistant to three or four classes antibiotics of the total isolates. While, XDR were 7 (25 %) isolates, distributed as *Escherichia coli 2 (5%),* and *Pseudomonas aeruginosa* 6 (20%) that isolated resistant because it was resistant to most of the antibiotics used in this study that resistant to 5 or. 6 classes of antibiotic. The presence or absence of PDR to antibiotics were not

achieved in this study, because it needs a more detailed study and a statistical procedure for all drugs used by UTI in Iraq.

**Table (4-6) Distribution of the Gram-negative isolates according to Multidrug resistance (MDR),extensive drug resistance (XDR) and pan drug resistance (PDR) of Gram-negative isolate**

| **Type of Bacteria** | **No.** | MDR | % | XDR |
|---|---|---|---|---|
| | | | | |
| *Escherichia coli* | 40 | 10 | 25 | 2 |
| *Proteus mirabilis* | 10 | 1 | 10 | 0 |
| *Klebsiella pneumoniae* | 20 | 4 | 20 | 0 |
| *Pseudomonas aeruginosa* | 30 | 0 | 0 | 6 |
| Total & Percentage | 100 | 15 | 55 | 7 |

this study was similar to study of Ghajiri etal (Ghajiri,*etal* ,2020) who found that the antibiotic susceptibility patterns of the isolated bacteria indicated a wide range of variations. High amounts of the test organism were found, as shown by the results. (*E.coli)* was sensitive to(meropenem, gentamicin, amikacin). Amikacin is an aminoglycoside antibiotic which can be used to treat a variety of bacterial infections.

Additionallys, Gram-negative bacteria can have a number of pathways for antibiotic resistance Extended-Spectrum Beta Lactamases (ESBLs) are a type of beta lactamase that has a wide range of (ESBL) is one of these processes, which makes bacteria resistant to beta-lactam antibiotics and also makes them susceptible to developing resistance to other antibiotic families

like quinolones, aminoglycosides, and cotrimoxazole. (Pitout and Laupland ,2008)

*Proteus mirabilis* also showed a sensitivity to meropeneme , amikacine gentamycin and ciprofloxacin this study differ from the study of Ghajiri *etal* (Ghajiri,*etal* ,2020) who found that meropenem may be a safe option for now

**4.3 molecular study**

The genomic DNA was extracted from the blood samples as a first step to amplify the target region of TLR2 and TLR9 genes as shown in figure (4.5)

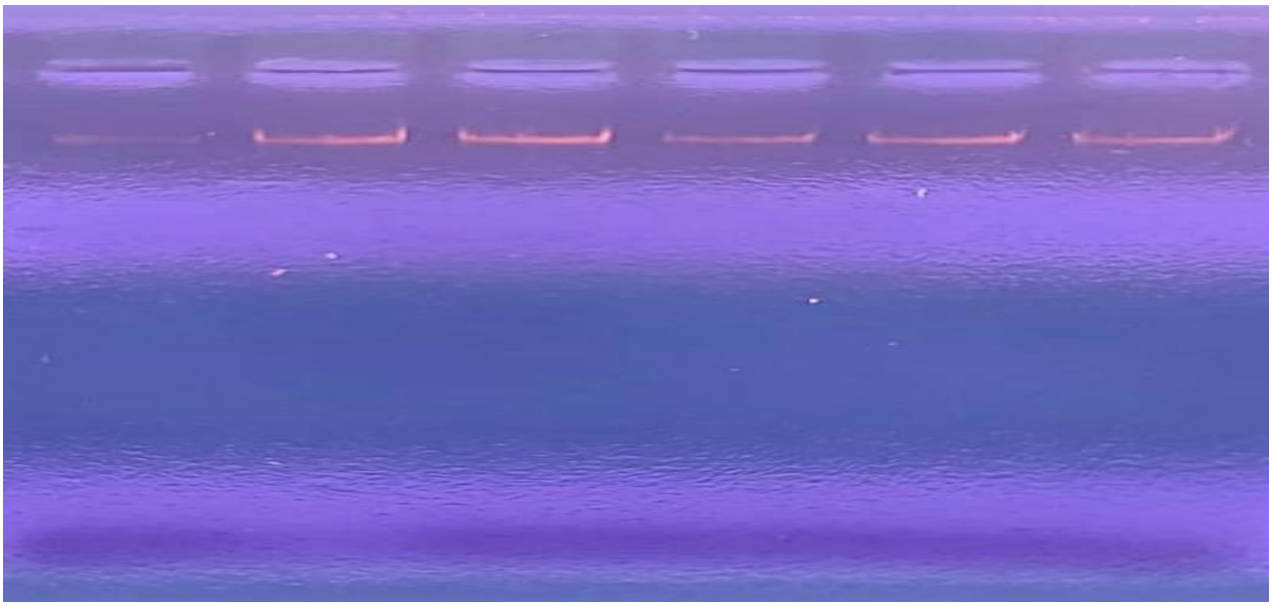

**Figure (4-5)**The electrophoresis pattern of gnomic DNA extracted from blood samples of UTI patients and healthy control groups.

lanes 1 – 3 patients and lanes 4 - 6 healthy control groups refer to genomic DNA from blood samples; Electrophoresis conditions, 1% agarose, 75 V, 20 mA for 1h , stained with red safe dye

### 4.3.1 Genetic polymorphisms of TLR2 gene

#### 4.3.1.1 Genotyping of *TLR2 rs3804099* Gene Polymorphisms

The amplified of TLR-2 (rs3804099) target sequences of studied groups were detected by ARMS technique are summarized in (table 4-7) and figure (4-6) . by electrophoresis on a 2% agarose gel , the product sizes of the PCR reaction was determined. Respectively, the allele-specific products were 228 bp and 173 bp for the presence of T and C alleles and the common PCR product was 349 bp, as shown in figure (4-6).

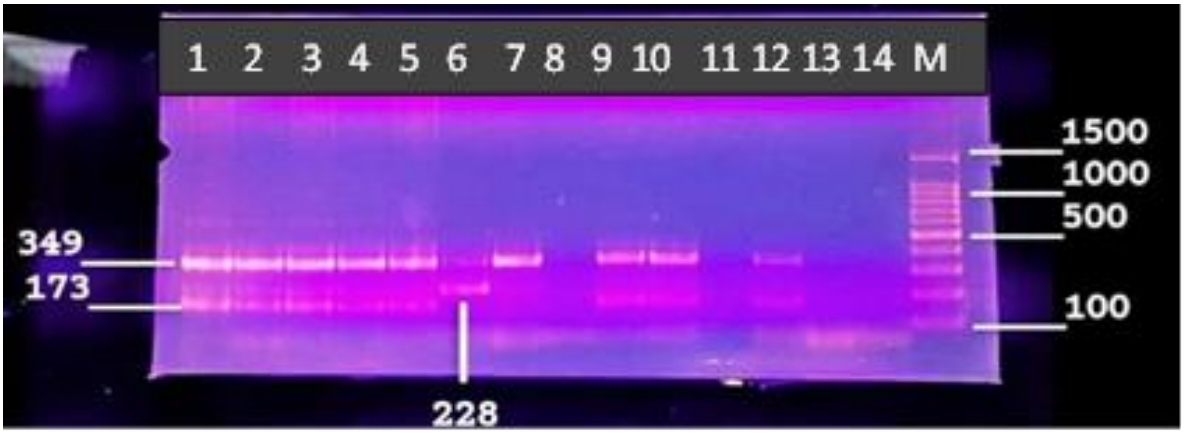


**Figure (4-6):** Determination of TLR2 rs3804099gene polymorphism by ARMS-PCR method; The M column : a ladder pattern (100bp); the columns (8,11,13and 14) are blank; the columns (1,2, 5,9,10 and 12) represent the TT genotype, the columns (3 and 4 ) represent the TC genotype , the column (6) represent the CC genotype. Electrophoresis conditions, 1% agarose, 75 V, 20 mA for 1h , stained with red safe dye

It can be seen that the frequency of TT genotypes in patients and control groups (which reached 80% and 76%, respectively) it was significantly increased in patients than control. While ,the frequency of TC genotypes in

patients and control groups which reached 2% and 0%, respectively. It was significantly increased in patients than control. On the other hand, the frequency of CC genotype in patients and control groups was 17.4% and 24%, respectively, that decreased in patients compared with control group. Analyses showed no significant variations between study groups in the TLR 2 (rs3804099) polymorphism (TT vs TC: OR=2.02, CI (95%) 0.0938 to 43.7262, P =0.6527). also no significant variations between study groups in the TLR 2 (rs3804099) polymorphism (TT vs CC :OR=5.2000, CI (95%): 0.6442 to 41.9722 , P =0.121. However high risk values have been detected in the homo dominant , hetero codominant model, as well as allele frequency (OR = 2.02 , 5.2 and 11.01 respectively).

**Table (4-7):** Genotype distribution and odd ratio of ARMS of TLR2 gene rs3804099 polymorphisms between the patients and healthy control

| **Genotype rs3804099** | **Patients No.(%)** | **Control No.(%)** | **P value** | **O.R** | **CI (95%)** |
|---|---|---|---|---|---|
| TT[a] | 60(80%) | 24 (76%) | | | |
| TC | 2 (2.6%) | 0 (0%) | 0.6527 | 2.0248 | 0.0938 to 43.7262 |
| CC | 13(17.4%) | 1 (24%) | 0.1218 | 5.2000 | 0.6442 to 41.9722 |
| Total No. | 75 (100%) | 25 (100%) | | | |
| **Allele** | **Frequency** | **Frequency** | | | |
| T | 0.81 | 0.96 | | | |
| C | 0.19 | 0.04 | 0.0201 | 11.0164 | 1.4578 to 83.2492 |

P≤0.05;OR=(95%CI);[a] reference

TLRs (Toll-like Receptors) are a group of transmembrane proteins that recognize pathogen-associated molecular sequences. It's an important part of the body's innate immune system. TLRs activate a similar signaling pathway that results in the activation of a wide range of inflammatory genes. TLRs use different intracellular adaptor proteins to induce diverse cellular responses to infections. Recent research have demonstrated the role of TLR subcellular localization in pathogen identification and signaling (Kumar *et al.*,2009). A number of biological proteins that reduce inflammation adversely influence TLR signaling pathways. TLR2 is primarily responsible for identifying Gram-positive bacteria's cell wall features (Lorenz *et al.*, 2000). TLR2 mutations, according to detectives, are linked to a decreased response to Gram-positive lipoproteins, putting them at an increased risk of Gram-positive infections (Takeda and Akira , 2005). SNPs, epigenetic alterations, and microRNA variations can all cause TLR2 to be downregulated. Previous research has connected genetic differences in TLR2, particularly those resulting from polymorphisms, to a variety of infectious illnesses (Junjie *et al.,* 2012).

One of the links between infection and auto inflammatory or autoimmune disease are Toll-like receptors (TLRs), together with nucleotide oligomerization domain–like receptors (NLRs) and retinoic acid-inducible gene 1–like receptors (RLRs), are members of the family of pattern recognition receptors (PRRs), which represent the main innate immune sensors.(Medzhitov *et al.,* 1997). Toll-like receptors (TLRs) are involved in the induction of an adequate immune response on infection. the genetic

variation in *TLR4* and *TLR2* genes could influence this response and lead to variability in cytokine production and survival (May *et al.*,2010 ) .

The proinflammatory response induced by Toll-Like receptors (TLR) is considered the host's first defense line. The SNPs correspond to the most frequent type of variation in the human genome, and due to the importance of TLR2 in the immune response, SNPs in the TLR gene are related to susceptibility or resistance to various diseases (Silva *et al.*,2021).

The SNP rs3804099 is found in the exonic region of the TLR2 gene. TLR2 gene expression regulation is influenced by variations in these areas. As a result, SNPs may impact TLR2 expression and activity by affecting transcription factor binding and mRNA stability (Thomas *et al.,* 2006). The TLR2 gene (rs3804099) has been related to hepatocellular carcinoma (HCC) (Junjie *et al.,* 2012) and gastric cancer (Zeng *et al.,* 2011).

The synonymous polymorphism +597T>C (rs3804099) is found in the third exon of the TLR2 gene on chromosome 4q32 (Uno *et al*.,2014). Previous research has linked the SNP rs3804099 to a variety of infectious diseases, including bacterial meningitis and pulmonary tuberculosis (Zhang *et al.,*2017). Because of its ability to form heterodimers with TLR1, TLR6, and maybe TLR10, TLR2 is produced by a variety of lymphoid and non-lymphoid cells and binds to a variety of microbial-derived components (Nemati *et al.*, 2017).

TLR2 (rs3804099) gene polymorphisms have been linked to sepsis. Gram-positive infections were linked to allelic variations in TLR2, while Gram-negative infections were linked to IL10 (p 0.05). TLR2 allelic variants may

help to lower preterm newborns' susceptibility to sepsis (Abu-Maziad *et al.*, 2010).

Another study found that the TLR2 gene mutation rs3804099 was substantially linked to the development of proteinuria following kidney donation. Their findings can help anticipate proteinuria and could lead to more personalized treatment for people who have had a kidney transplant. After kidney transplantation, this result suggested that the risk of proteinuria was toughly associated with the rs3804099 (Zheng *et al.*, 2022). Other authors discovered that the rs3804099 SNP has been associated to a number of illnesses, including polycystic ovarian syndrome and H.pylori infection and peptic ulcer, when compared to non-significant SNPs . (Kuliczkowska-Paksej *et al.*, 2021). In addition, the rs3804099 polymorphism influences cancer susceptibility (Gao *et al.*, 2019).

For the SNP rs3804099, the synonymous mutation did not result in asparagine (Asn) substitution at residue 199, resulting in a decreased macrophage response, lower TLR2 expression with attenuated host immune response and susceptibility to most of the diseases reported in this study: tuberculosis ,leprosy , acute pyelonephritis and acute lobar nephronia , infective endocarditis , tuberculous meningitis , and filariasis (Silva *et al.*,2021). In leprosy, it was demonstrated that this SNP caused increased expression of pro-inflammatory cytokines, with higher expression of TLR2 (Santana *et al.*,2017).

For the SNP rs3804099, the allele (T) variant was associated with the susceptibility of APN (acute pyelonephritis) and ALN (acute lobar nephronia) (Cheng *et al.*,2013). the variant (C) allele was associated with

susceptibility to infectious endocarditis (Golovkin *et al.,* 2015). After 24 h of *L. pneumophila* stimulation, the mRNA expression level of MyD88 was significantly lower with TLR2 (C597T) CT/TT (p = 0.0482), The (T) variant allele provided higher protection against infection by *Legionella pneumophila,* with the level of MyD88 mRNA expression (myeloid differentiation protein) significantly lower (Zhang *et al.,*2013).

The (C) wild allele was associated with the protection against bacterial vaginosis by *Gardnerella vaginallis* among HIV-infected women (Mackelprang *et al.,* 2015). TLR2 genotype rs3804099 was associated with susceptibility to Tuberculous meningitis TBM.The association was found with meningeal rather than pulmonary TB and was strongest when miliary TB was found on chest radiography. Furthermore, the association increased with the severity of neurologic symptoms . These results demonstrate a strong association of TLR2 SNP T597C with the development of TBM and miliary TB and indicate that TLR2 influences the dissemination of M. tuberculosis. The allele (C) variant was associated with a risk of 3 to 6 times greater for the individual to contract tuberculous meningitis (Thuong *et al.,*2007 ).

Table (4-8) shows the comparison of clinical characteristics according to TLR2 rs3804099 genotypes in patients, The study showed a higher correlation between TLR2 (serum) level with TLR2 rs3804099 (P = 0.028) , TLR2 in serum level was 207±283, 60.16±26 and 147±145 in CC,CT and TT ( respectively ). there are no significant correlation between age and TLR2 (urine ) level with TLR2 rs3804099.

**Table (4-8):** Comparison between local and systemic TLR2 concentration rs3804099 genotypes in patients**.**

| **Parameters** | **TLR2 rs3804099 genotypes** | | | ***P*** **value** |
|---|---|---|---|---|
| | **CC: 13(17.4%)** | **TC: 2 (2.6%)** | **TT: 60(80%)** | |
| TLR2 in serum, mean±SD | 207±83 | 60.16±26 | 147±45 | 0.028 |
| TLR2 in urine, mean±SD | 133.04±63 | 173±0.9 | 151.89±78.08 | 0.731 |
| Age mean±SD | 37±10.4 | 29±12.7 | 34.9±8 | 0.440 |

TLR2 expression may be influenced by the SNP rs3804099.After stimulation with peptidoglycan, PBMCs from individuals with Behcet's disease who held the rs3804099 TT genotype produced larger quantities of TLR2 mRNA than those who carried the CC or CT genotypes (Fang *et al.*,2013). rs3804099 (genotypes CC, CT, and TT) carriers of the T allele produced higher serum levels of IL-17, and this difference was kept significant by pooling CT and TT individuals and comparing against CC individuals, the carriers of the T allele are higher producers of IL-6, especially when they combine CT and CC with TT genotype (Santana *et al.*,2017). TLR2 was found to be strongly expressed in epithelial colon cancer cells, with TLR2 mRNA and protein levels much lower in tumor tissues than in normal tissues. In silico analysis of the TLR2 nucleotide

substitution in SNP rs3804100 and rs3804099 revealed that these single nucleotide variants have a 67 percent and 70 percent chance of altering splicing phenotypes, respectively. Patients had a 44 percent TT genotype, while controls had a 21 percent TT genotype. In the Saudi Arabian population, this genotype increases the risk of colon cancer by more than fivefold (Semlali *et al.*,2018). In active hepatitis and cirrhotic groups, the TT genotype was substantially related to lower IL-6 levels (P = 0.005 and P = 0.001, respectively), indicating that TLR mutations are associated with milder hepatitis activity and a decreased risk of disease progression (Elbrolosy *et al.*,2022).

### 4.3.1.2.Genotyping of TLR2 (exon 3) Gene Polymorphisms

For TLR2 (exon 3) genotyping, the genomic DNA was amplified using specific primers and accomplished by the Thermo-cycler apparatus under the optimal conditions The results revealed that the presence a one bands (221bp) of the target sequence of TLR2 (exon 3) gene in agarose gel (Fig. 4-7).

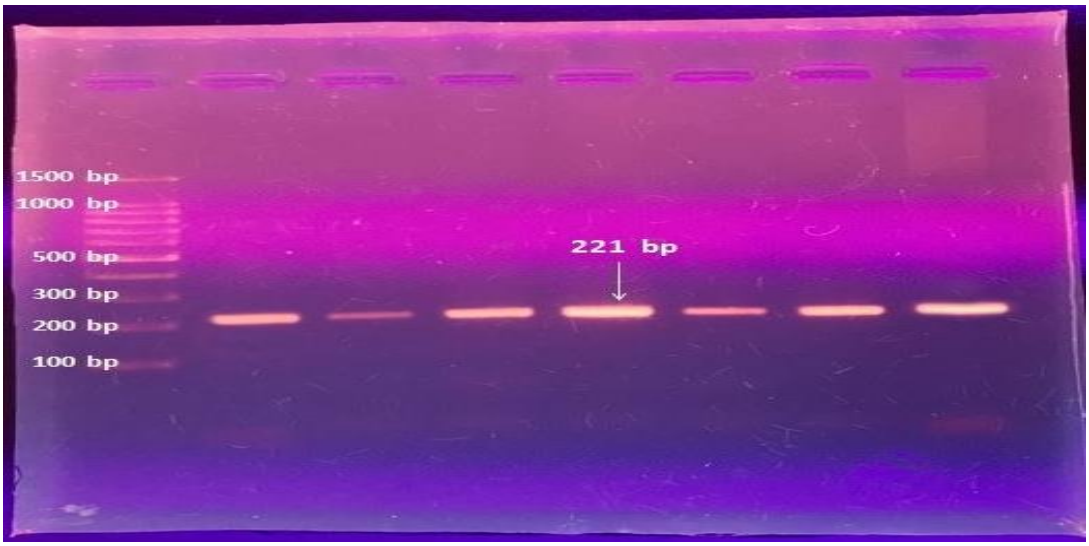

**Figure (4-7) Agarose gel electrophoresis of an amplified product patterns of TLR2 (exon 3) with specific primer. Electrophoresis conditions, 1% agarose, 75 V, 20 mA for 1h , stained with red safe dye**

Many SNPs were found between the one resolved haplotypes and the *TLR2* gene (exon 3) for reference sequences, according to the sequencing data. According to the reference sequence alignment of the human *TLR2* gene (NCBI Reference Sequence: NG 016229.1), the findings revealed the existence of several SNPs (Fig. 1 and Table 1). (https://www.ncbi.nlm.nih.gov/nuccore/NG 016229.1/).

Four SNPs were detected in the *TLR2* gene's exon 3 region, according to the sequencing data. As shown in table 1, one of these SNPs, rs1317290627 (exon 3), was previously documented in NCBI. Other SNPs, such as those found at the chromosomal sites 153705147 (exon 3), 153705148 (exon 3), and 153705211 (exon 3) as listed in table (4-9), are new and haven't been identified before.

When using the Bio Edit program version 7.2.5 to translate the DNA sequence according to the reference sequence alignment of the human *TLR2* gene ID: NG 016229.1. As shown in fig. (4-8), the results seemed to change three amino acid residues in the TLR2 protein. These amino acid substitutions were Asn (N) 17→ Thr (T) 17 in Samples 2 and 5 (Fig.4-9), Gln(Q) 36 → Leu (L) 36 in sample 3 (as a result of nucleotide substitution A and T leading to change in the code from CAG to CTA) , and Glu(E) 57

→ Asp (D) 57 in sample 3. These changes were thought to affect the *TLR2* protein's expression and/or function.

**Table (4-9):** All SNPs in the target sequence of the *TLR*2 gene , as identified from sequencing information

| Location | SNPs | References seq. | | Variation | | Seq. BASE/AA | Previously Recorded SNP |
|---|---|---|---|---|---|---|---|
| | | Code | Amino acid(AA) | Code | Amino acid(AA) | | |
| 153705090 (exon 3) | A/C | AAC | N | ACC | T | 51 / 17 | **Rs1317290627** |
| 153705147 (exon 3) | A/T | CAG | Q | CTG | Q | 108 /36 | - |
| 153705148 (exon 3) | G/A | CAG | Q | CAA | Q | 109 /36 | - |
| 153705211 (exon 3) | G/C | GAG | E | GAC | D | 172/57 | - |

Seq.:Sequences ;SNPs: Single nucleotide polymorphisms; N: asparagine ; Q :glutamine; E: glutamate ; D:aspartate.

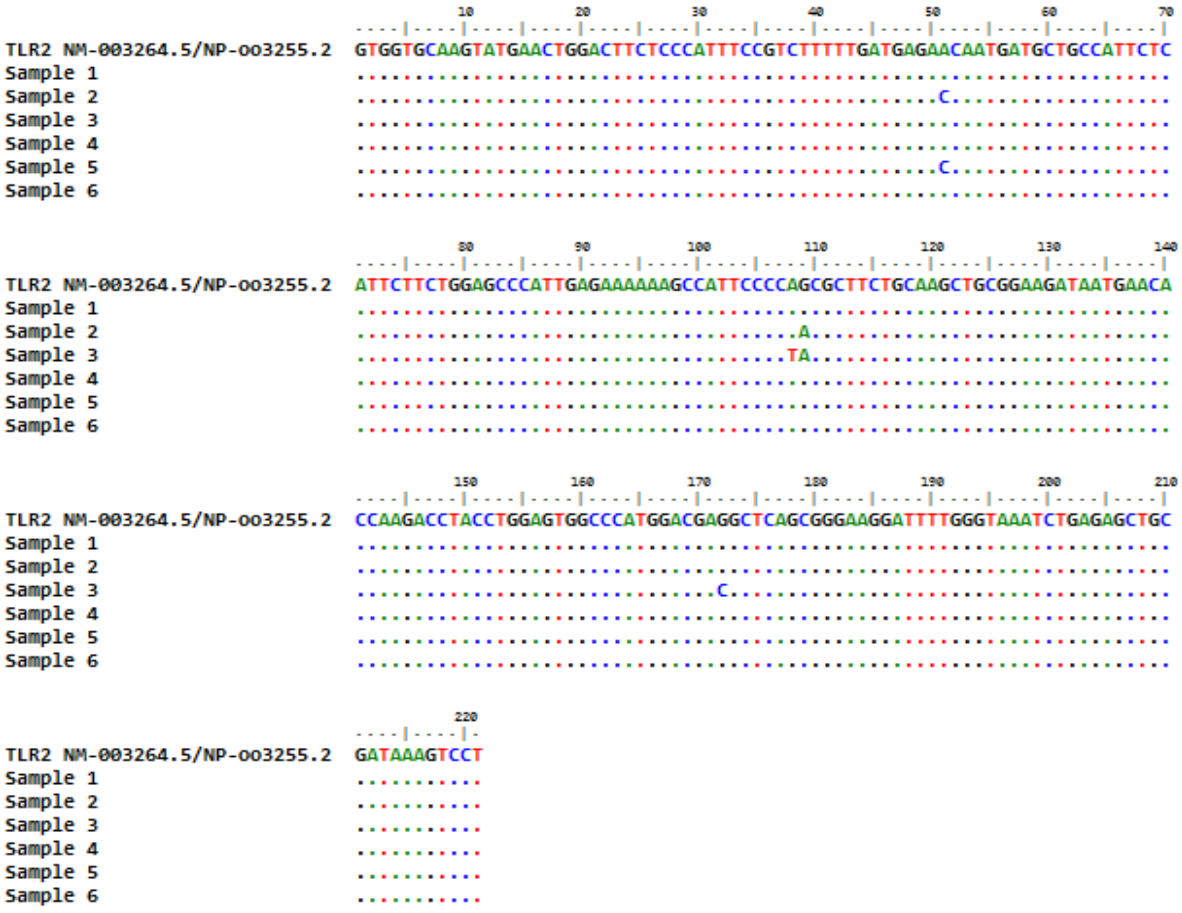


**Fig. (4-8):** Sequences alignment results for *Homo sapiens TLR2* gene (Exon 3 ) fragment by Bio Edit program version 7.2.5. Sample 1-6 refer to a patient with UTI diseases.

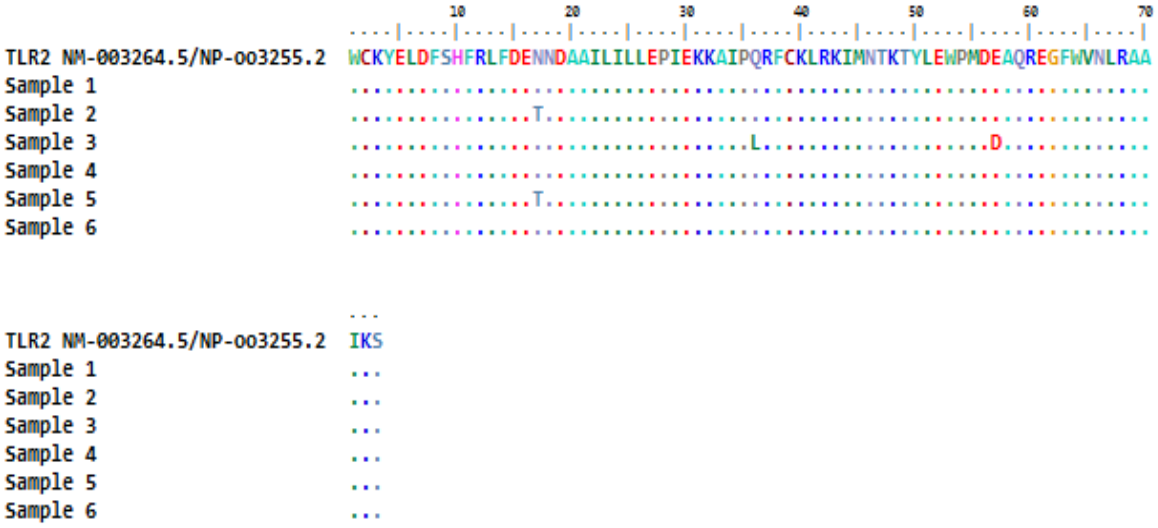


**Fig. (4-9):** Pair sequence alignment of the amino acid of TLR2 draw based on the alignment of Bio Edit program version 7.2.5.

N: asparagine ; Q :glutamine; E: glutamate ; D:aspartate; L:leucine

Different types of microbial agents, such as uropathogenic *Escherichia coli* (UPEC) and *Candida albicans*, cause urinary tract infections (UTIs). Pathogens may not be able to enter the urinary system if substantial physical barriers are present. However, harmful bacteria can sometimes get past the barriers and trigger innate and adaptive responses. Toll-Like Receptors (TLRs) are one of the most unusual and fascinating molecules in the context of UTIs, among a range of innate immune responses (Behzadi and Behzadim 2016) .

TLRs are remarkable molecules with high sensitivity and specificity for detecting and recognizing a variety of microbial components. They are among a wide variety of innate immune responses that operate quickly and can manifest in many parts of the human body. TLRs are multipurpose

molecules that can be expressed by many types of pathogens in different types of infectious illnesses, which is incredible. The most capable molecules in the urinary tract defense mechanism are TLR2, TLR4, and TLR5 (Spencer *et al*.,2014).

Because the TLR1–TLR2 and TLR2–TLR6 dimers are known to be involved in detecting gram-positive bacteria, *Mycoplasma*, and *mycobacteria*, numerous studies have looked at the effect of TLR1, TLR2, and TLR6 SNPs on susceptibility to infections with these pathogens (Doyle and O'Neill , 2006).

suggest that *TLR2* gene could be the predisposing factor for urinary tract infection. Additionally, they observed that subjects carrying the *TLR2* Arg753Gln allele had higher risk of urinary tract infection with gram-positive pathogens, history of more than two attacks of UTI and asymptomatic UTI( Tabel *et al*.,2007).

The *TLR2* G2258A polymorphism, which causes reduced lipopeptide-induced signaling, has been linked to an increased incidence of asymptomatic bacteriuria in women (Hawn *et al.*, 2009). These findings imply that *TLR2* SNPs may have a role in early in vivo human bladder immune responses before symptomatic urinary tract infections develop (UTIs).

The R688W and R753Q *TLR2* polymorphisms have been investigated the most, resulting in both strong relationships with infectious illnesses and a lack thereof. The R753Q polymorphism has been linked to cytomegalovirus infection after liver transplantation (Kang *et al.*, 2012), nasopharyngeal

bacterial colonization in infants, and higher rates of infection recurrence and initial septic shock in liver-transplant recipients with gram-positive infections(Lee *et al.*, 2011).

### 4.3. 2Genetic polymorphisms of TLR9 gene

#### 4.3.2.1 Genotyping of *TLR9* (exon 2 and promoter) Gene Polymorphisms

For TLR9 (exon 2) genotyping, the genomic DNA was amplified using specific primers and accomplished by the Thermo-cycler apparatus under the optimal conditions The results revealed that the presence a one bands (221bp) of the target sequence of TLR9 (exon 2) gene in agarose gel (Fig. 4-10).

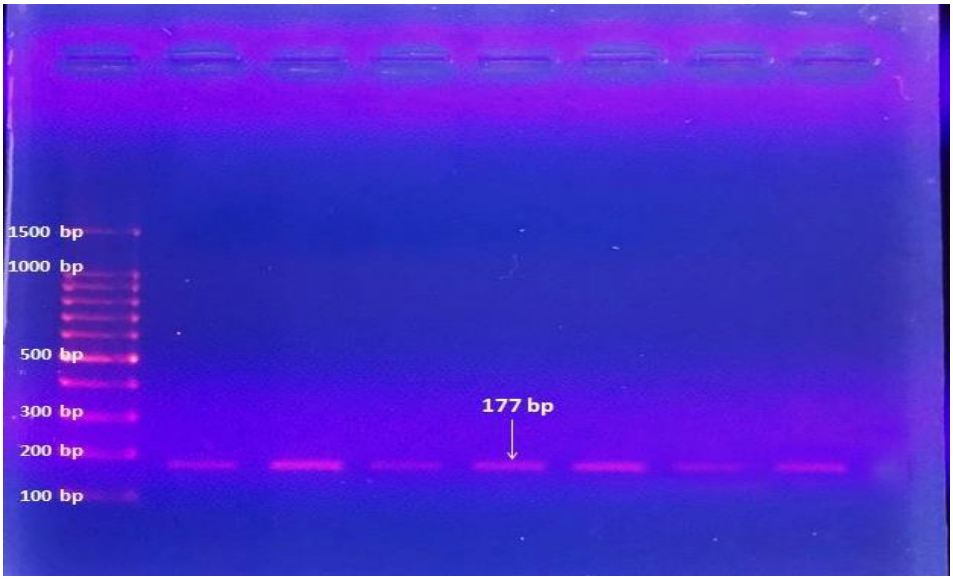


**Figure (4-10) Agarose gel electrophoresis of an amplified product patterns of TLR9 (exon 2) with specific primer. Electrophoresis conditions, 1% agarose, 75 V, 20 mA for 1h , stained with red safe dye**

For TLR9 (Promoter3) genotyping, the genomic DNA was amplified using specific primers and accomplished by the Thermo-cycler apparatus under the optimal conditions The results revealed that the presence a one bands (221bp) of the target sequence of TLR9 (Promoter ) gene in agarose gel (Fig. 4-11).

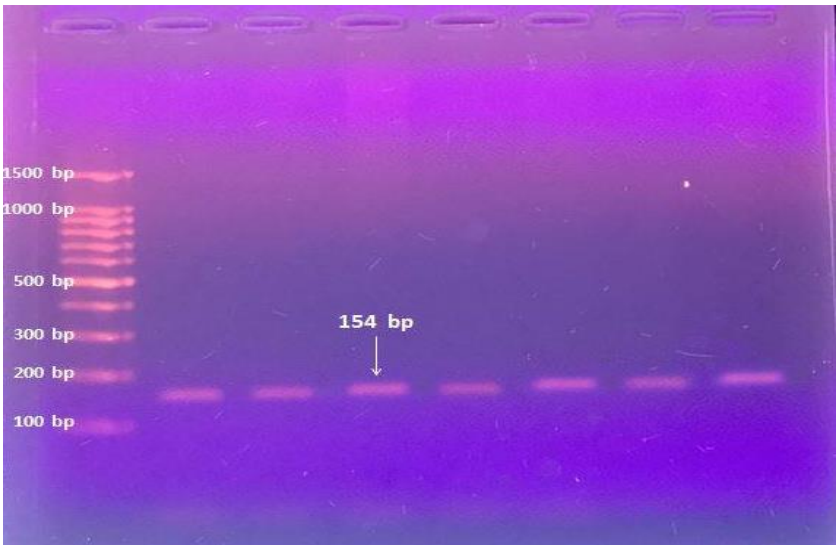


**Figure (4-11) Agarose gel electrophoresis of an amplified product patterns of TLR9 (Promoter) with specific primer. Electrophoresis conditions, 1% agarose, 75 V, 20 mA for 1h , stained with red safe dye.**

The sequencing results observed that many SNPs between the one resolved haplotypes and between the *TLR9* gene (promoter region and exon 2) for reference sequences. The results appeared in the presence of many SNPs (Fig.3 , 4 and Table 2) according to the reference sequence alignment of the human *TLR9* gene(https://www.ncbi.nlm.nih.gov/nuccore/NG_033933.1/). (NCBI Reference Sequence: NG_033933.1).

The sequencing results revealed there were six SNPs in the part of the exon 2 region of the *TLR9* gene while there were five SNPs in the promoter region site . some of these SNPs are previously recorded in NCBI such as rs352140 in exon 2 and rs1228893923, rs1699624843 and rs1699624414 in promoter region as showed in the table 2. Whereas, other SNPs are novel and not recorded previously like that located at the chromosomal site 522222632 (Exon 2), 522222631 (Exon 2), 522222613 (Exon 2), 522222628(Promoter region), and others as shown in table 2.

SNPs in the coding area (exons) are thought to affect the gene's coding sequence, protein expression, and, eventually, protein function. Variants outside of exons, on the other hand, have been found to influence gene function by increasing or suppressing protein expression and activity (Willie, 2014) .

When translating the DNA sequence using by Bio Edit program version 7.2.5 according to the reference sequence alignment of the human *TLR9* gene ID: NG_033933.1. The results appeared to change two amino acid residues of TLR9 protein as shown in fig. 5. These amino acid substitutions were Gln 31→ Ala 31 in all Samples (Fig.5), Phe 37 → Tyr 37 in sample 4, and Phe 39 → Tyr 39 in sample 5. The TLR9 protein's expression and/or function were hypothesized to be affected by these alterations.

**Table (4-9):**All SNPs in the target sequence of the *TLR9* gene , as identified from sequencing information

| Location | SNPs | References seq. | Variation | Seq. BASE/A | Previously Recorded |
|---|---|---|---|---|---|

| | | Code | Amino acid(AA) | Code | Amino acid(AA) | A | SNP |
|---|---|---|---|---|---|---|---|
| 522222681 (Exon 2) | G/A | CCG | P | CCA | P | 42/14 | **Rs352140** |
| 522222632 (Exon 2) | C/G | CAG | Q | GCA | A | 91 /31 | - |
| 522222631 (Exon 2) | A/C | CAG | Q | GCA | A | 92 /31 | - |
| 522222630 (Exon 2) | G/A | CAG | Q | GCA | A | 93/31 | - |
| 522222613 (Exon 2) | T/A | TTC | F | TAC | Y | 110/ 37 | - |
| 522222607 (Exon 2) | T/A | TTC | F | TAC | Y | 116/ 39 | - |
| 52226789 Promoter | T/G | - | - | - | - | 54/ - | Rs1228893923 |
| 5222674 | C/A | - | - | - | - | 101/ - | Rs16996248 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 2 Promoter | | | | | | | 43 |
| 52222262 8 Promoter | A/T | - | - | - | - | 115/ - | - |
| 52222261 2 Promoter | G/T/ A | - | - | - | - | 131/- | Rs1699624414 |
| 52222260 7 Promoter | A/T | - | - | - | - | 136/- | Rs1699624368 |

Seq.:Sequences ;SNPs: Single nucleotide polymorphisms; P: Proline ; Q :glutamine; A:Alanine ; F: Phenylalanine ; Y:Tyrosine

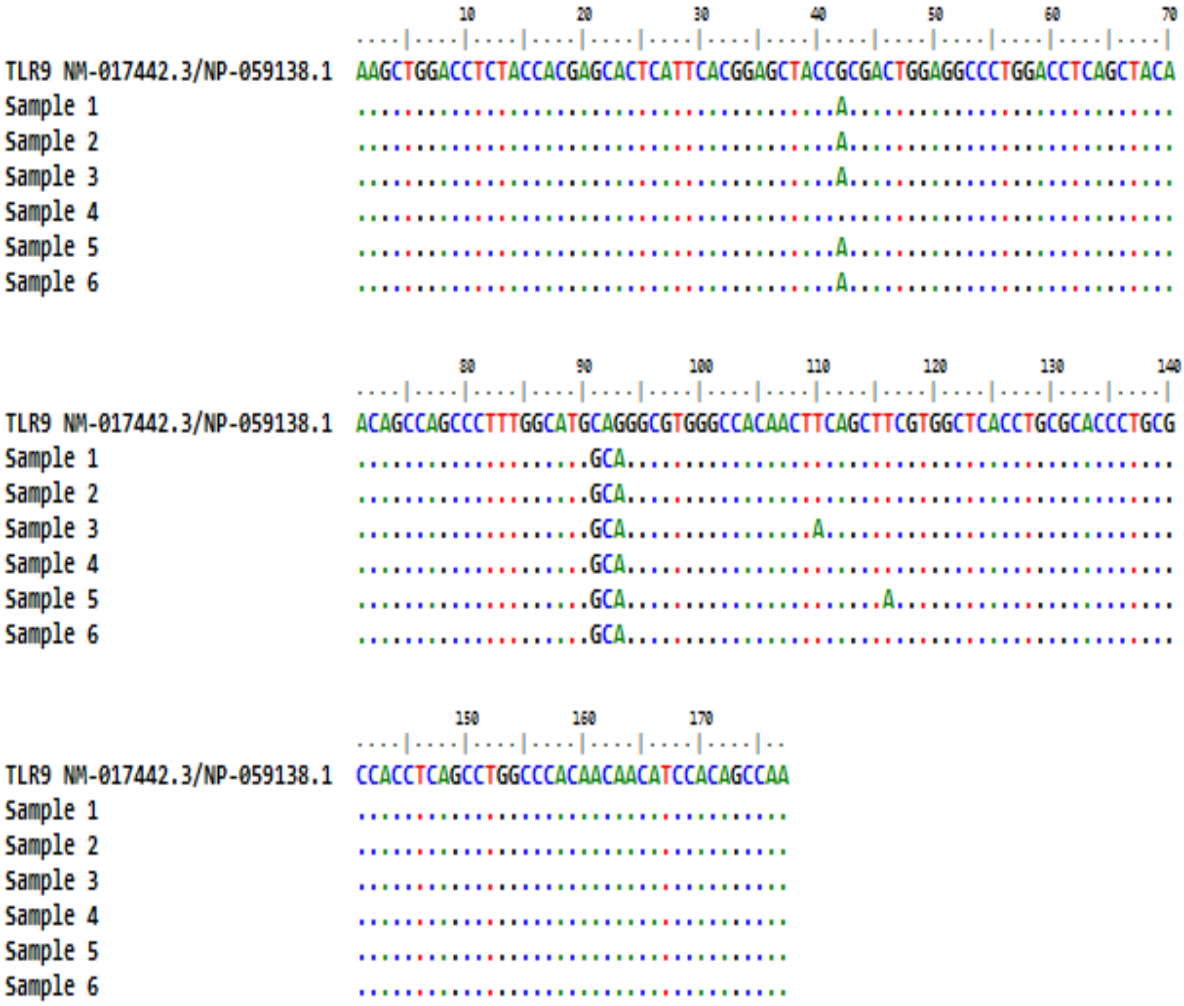


**Fig. (4-12):** Sequences alignment results for *Homo sapiens TLR9* gene (Exon 2 ) fragment by Bio Edit program version 7.2.5. Sample 1-6 refer to a patient with UTI diseases**.**

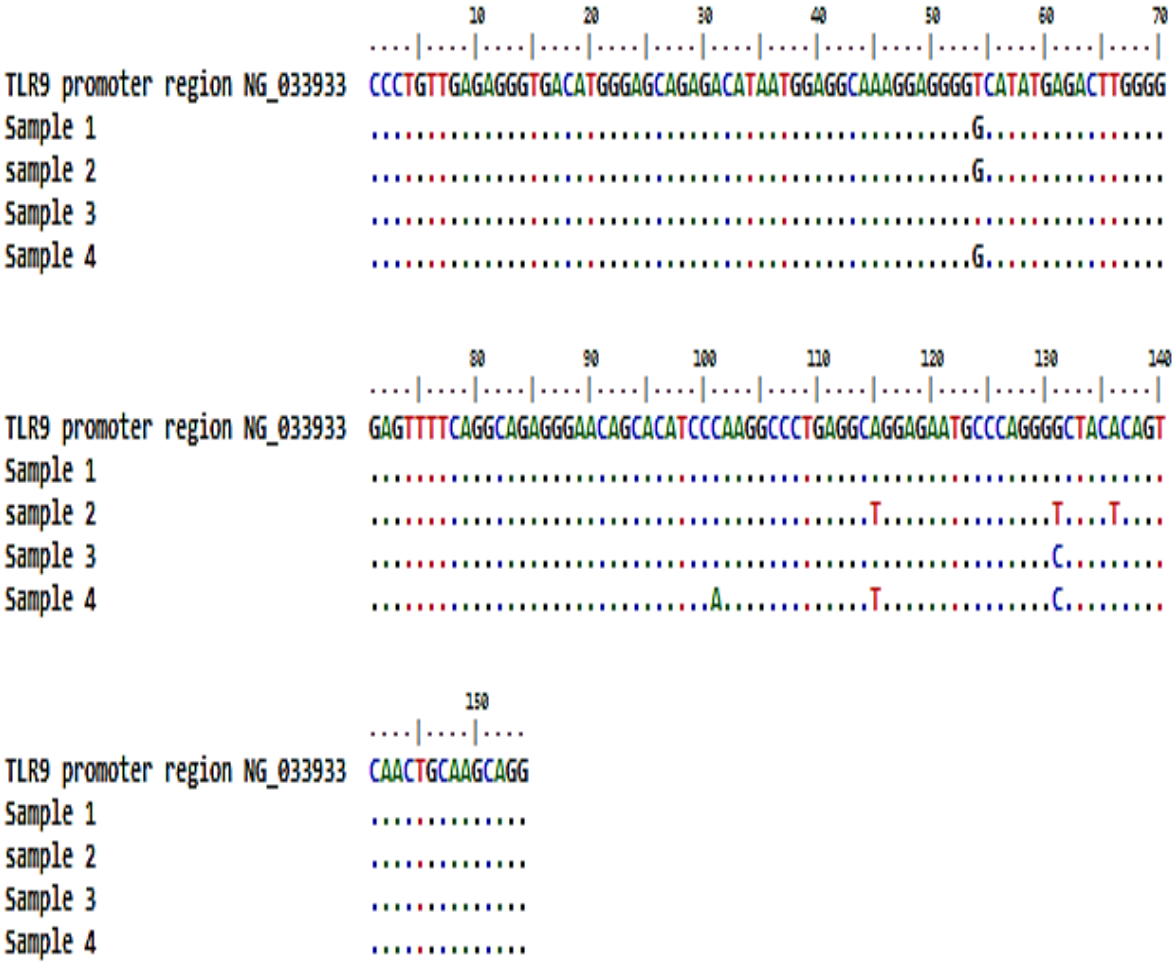


**Fig. (4-13): Sequences alignment results for *Homo sapiens TLR9* gene (Promoter region ) fragment by Bio Edit program version 7.2.5. Sample 1-4 refer to a patient with UTI diseases**.


**Fig. (4-14)**: **Pair sequence alignment of the amino acid of TLR9 draw based on the alignment of Bio Edit program version 7.2.5.**

**Q :glutamine; A:Alanine ; F: Phenylalanine ; Y:Tyrosine** .

Toll-like receptors (TLRs) are germ-line-encoded innate immune sensors that identify conserved microbial structures and host alarmins and signal macrophages, neutrophils, dendritic cells, and other cell types to produce MHC proteins, costimulatory molecules, and inflammatory mediators. These pathways begin and organize adaptive immune responses, as well as trigger immediate and early systems of innate host defense. Several TLR single-nucleotide polymorphisms (SNPs) have been linked to increased susceptibility to infectious, inflammatory, and allergy illnesses, as well as being implicated in carcinogenesis (Medvedev, 2013)

TLRs help in the eradication of bacteria and virus infections and play a critical role in innate immunity against microbial pathogens in addition to initiating immune responses.

TLR9 is employed to regulate the synthesis of pro-inflammatory and anti-inflammatory cytokines by regulating unmethylated cytosine-phosphate-guanine movement. Multiple disorders, including pulmonary TB, duodenal ulcers, and altered cytokines in the gastric mucosa, as well as systemic lupus erythematous and chronic obstructive pulmonary disease, have been linked to TLR9 gene variants (Bharti *et al.,* 2014; Berenson *et al*.,2015).

Because of its clinical relevance in identifying and eradicating a wide spectrum of invading pathogenic microorganisms, TLR has gotten greater attention in clinical research investigations than other receptors. There is a growing body of evidence linking TLR gene SNPs to greater risk or protection against bacterial, viral, and fungal infections, as well as inflammatory and autoimmune illnesses (Medzhitov and Janeway 2000) .

Variations in the *TLR9* gene have been linked to viral and bacterial infections, as well as alterations in patient responses to treatment interventions. For instance, the 1635A/G and +1174G/A TLR9 SNPs in the linkage disequilibrium were related with fast progression of HIV infection in HIV-positive individuals from the Swiss HIV cohort research, indicating their potential usefulness for therapeutic targeting and vaccine development (Bochud .,2007).

Tao *et al* .,(2007) show that the GG genotype of the synonymous coding SNP rs352140 (G + 2848A) was related to protection from symptomatic

malaria, but no similar protective effect for parasitemia was identified in our cohort population. in the same manner, Xue *et al*.,(2020) revealed in contrast to some studies, the TLR9 rs352140 polymorphism is associated with a decreased risk for bacterial meningitis.

### 4.4. Immunological study

#### 4.4.1.Evaluation TLR2 concentration in serum & urine by ELISA Assay

Results demonstrated that there was an increasing in the serum concentrations & urine concentrations of TLR2 in patients with febrile UTI patients in comparation with control group by using ELISA as a result in table (4-11), There is simple differential between mean for the febrile UTI patients and the healthy control group of TLR2 in urine

**(4-11)Comparison of the TLR2 between patient and control in urine**

| ParameterTLR-2in patients and control | M±SD ng/l | P value(sig) |
|---|---|---|
| Mucosal for patient | 3.877 ±1.497 | 0.045* |
| Mucosal for control | 3.318 ± 0.4733 | |
| Systemic for patient | 5.023 ± 1.792 | 0.043* |
| Systemic For control | 2.759 ±0.658 | |

***P ≤ 0.05 *Correlation is significant at the 0.05 level.**

This study found that there is a significant difference in urine TLR-2 concentration between patients infected with UTI and control group when p value 0.045 less than 0.05 show in table above , also in this study found that there is a significant

difference in serum TLR-2 concentration between patients infected with UTI and control group when p value 0.043 less than 0.05 show in table above ,also noticed a considerable differential between mean for the febrile UTI patients and the healthy control group of TLR2 in serum.

The present study the expression of TLR2 correlated with activation by the TLR2 ligand peptidoglycan agreed with (Zhu *etal*.,2007)who found that TLR2 receptor concentration increased in patients with febrile UTI than control Regardless of whether TLR2 is released in a small quantity during bacterial infection , that the cascade of cytokine production during bacterial infection differs from the classical pro-inflammatory cytokine cascade that occurs during acute inflammation.

in the different study, Clinical manifestations of febrile UTI depend on the production of different cytokines, and measurement of urinary ILs seems to be useful for the rapid diagnosis and localization of inflammation within the urinary tract system. TLR2 is critical for identifying significant microbial causal agents of urinary tract infections, including as *C.albicans, Staphylococcus* spp., *Streptococcus* spp., *Mycoplasma spp.,* and *Ureaplasma spp.* TLR2 recognizes and interacted with a wide range of microorganisms in Gram-positive bacteria, including lipopeptides, peptidoglycan, and lipoteichoic acid, as well as *Mycoplasma* and *mycobacteria* possess lipoproteins. ( Di Gioia,

& Zanoni, 2015.). There are link between TLR-2 and the streptococcal infection by (Fieber and Ovarik,2014) which showed that TLRs recognize specific pathogen-associated molecular patterns (PAMPs) which are common to different pathogen species. *Streptococcus pyogenes*, also called Group A Streptococcus, is an important Gram-positive human pathogen yet its recognition by innate immune cells remains unknown, also the study by (Joosten *etal.*,2009) that examine the role of TLR ligands in experimental arthritis, streptococcal cell wall induced arthritis was examined.

The study agreed with (Mukherjee *etal.*,2016) who found increased TLR2 concentration due to their extreme ability of identifying distinct molecular patterns from invading pathogens. These pattern recognition receptors (PRRs) not only act as innate sensor but also shape and bridge innate and adaptive immune responses. (Alvarez,2008). TLR2 identifies and interacts with a variety of microbial components such as lipopeptides, peptidoglycan, and lipoteichoic acid in Gram positive bacteria and lipoproteins in mycoplasmas and mycobacteria. (Drage,2009).

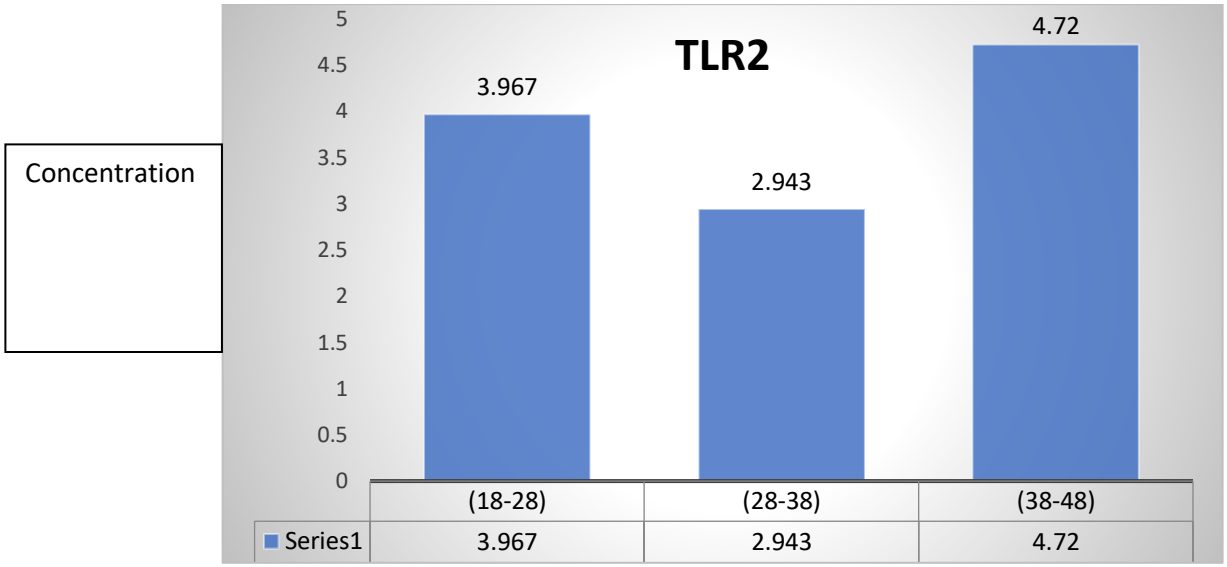


**Figure (4-15) Comparison of the mean to TLR2 between age group for patient in urine**

Many study exhibited The TLR2 is able to detect and identify the PAMP molecules of triacylated lipoproteins in Gram –ve bacteria including *E.coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* which are known as important microbial causative agents of UTIs. Moreover, the other members of Enterobacteriaceae, which possess triacylated lipoproteins in their outer membrane, can be recognized by TLR2 heterodimers.

the present study agreed with (Behzadi and Behzadi ,2016) which found that increased concentration of TLR2 during different infection because they act rapidly and are able to be expressed in different parts of the human body. It is unbelievable that multifunctional molecules of TLRs are able to be expressed by different types of pathogens in different types of infectious diseases. These fabulous biosensors do their best try to protect the host's

body against microbial infections. However, sometimes they have to be supported by the adaptive immune system. that linked with study TLRs that are expressed on the mucosal surfaces of the urinary tract and in the kidney sense bacteria and provoke an inflammatory response that leads to bacterial clearance, In conclusion, Our results were revealed might be that there is a difference mean in TLR-2 between age group for patients infected with UTI figure (4-15), the TLR2 increased in age group (38-48) years compared other groups . in fact, as per our previous data with progressive age, increased damage ,TLR2 has an alert sentinel function and is the main recruitment/phagocytic receptor for innate cells in case of bacterial infection and it is also involved in promoting angiogenesis during wound healing (Mulfaul *etal.*,2020).

### 4.4.2.Evaluation of TLR9 concentration in serum & urine

In the current study, concentration of TLR9 was measured in serum & urine of febrile UTI patients and control group as a result in table (4-12)**.**The mean of urine level of TLR9 in all patients of UTI was higher (146.526 ±74.423 pg/ml) than the control individuals (117.135 ± 17.871 pg/ml). This study reveals a no significant difference between the serum level of TLR9 for UTI patients and control when p value was 0.07 more than 0.05 show in table below.

**Table (4-12)Comparison of the TLR9 between patient and control in urine**

| ParameterTLR-9in patients and control | M±SD ng/l | P value(sig) |
|---|---|---|

| | | |
|---|---|---|
| mucosal for patient | 146.526 ±74.423 | 0.07 |
| mucosal for control | 117.135 ± 17.871 | |
| systemically for patient | 158.565 ± 67.316 | 0.24 |
| systemically for control | 131.840 ± 76.242 | |

The mean of serum concentration of TLR9 among patients with UTI was significantly higher in the UTI group than in the control group, suggesting a relationship of high serum levels of these receptors with active UTI . in this study found that there is no significant difference in serum for TLR-9 concentration between patients infected with UTI and control group when p value was 0.24 more than 0.05 ($p<0.05$) show in table above ,also noticed a considerable differential between mean for the febrile UTI patients and the healthy control group of TLR9 in serum and this study showed simple differential between mean for the febrile UTI patients and the healthy control group of TLR9 when compare between urine & serum . the study agreed with (Yang *et al*.,2019) who found that TLR9 receptor concentration increased in patients with Fatal Bacterial Sepsis than control.

TLR9 is expressed intracellularly by different cells of the immune system and has a key role in activation of the innate immune system against mycobacterium infection (Torres-Garcia *et al.,* 2013). These studies suggest a possible association of TLR9 variants with susceptibility to UTI. Our study is agreement with these studies. TLRs have a critical role in pathogen recognition and activation of innate immunity and act in multiple cellular processes such as cytokine secretion (Kawai and Akira, 2011), modulation of the adaptive immune response and apoptosis (Thada *et al.*, 2013).

Moreover, it is now known that the contribution of genes to the incidence of or predisposition to diseases can be determined by comparing inter-individual genetic differences. Each individual, to a greater or lesser degree than others, is genetically prone to develop certain diseases. Evidence suggests that polymorphisms within TLRs may cause an impairment of the immune response against UTI.

When divided the patients to three age groups table(4-13) the results were appeared concentration of systemic TLR2 was not significantly different among groups while there were significantly different in mucosal TLR2 .this may be that mucosal immunity don't effect by age factor this also present with TLR9 . when compared each group of patients with matched group of control. The results appeared concentrations of TLR9 (systemic) was not significantly different among groups while there were significantly different in mucosal TLR9 .this may be that mucosal immunity don't effect by age factor

**Table (4-13 )Comparison of the TLR2 & TLR9 between urine&serum in patient**

| Age/group / Parameters | Mean ±SD | | | |
|---|---|---|---|---|
| | (18-28)years | (28-38) years | (38-48) years | P value |
| TLR2 urine ng/l | 3.967 ± 0.682 ab | 2.943 ± 1.353 a | 4.720 ± 1.776 b | 0.02* |

| | | | | |
|---|---|---|---|---|
| TLR2 serum ng/l | 3.953 ± 1.754<br>a | 6.032 ± 0.643<br>A | 5.084 ± 3.794<br>a | 0.69 |
| TLR9 urine ng/l | 132.352 ± 61.798<br>A | 110.028 ± 52.005<br>a | 197.198 ± 82.263<br>b | 0.02* |
| TLR9 serum ng/l | 96.732 ± 77.501<br>a | 195.783 ± 20.092<br>a | 183.180 ± 89.428<br>a | 0.3 |

**Different letters in the same raw refer to significant different ($P \leq 0.05$) stander deviation S.D**

Toll like receptors at mucosal and systemically changed with the age. Increasing TLR2 and TLR9 was strictly correlated with increasing age-related expression of ICAM-1, then inflammation. Such "ageing patterns" may cause alteration of local immunity ,Age-related alteration of TLRs local expression may be a preclinical and/or subclinical condition that participates to the failure of the para-inflammatory compensatory mechanisms (Di Zazzo *et al.,* 2019)

The present study agreed with (Bonini *et al.,* 2005) that expresses high levels of TLR9, compared with the average expression of TLR-2, TLRs are primary components of the innate immune system involved in signal transduction and are expressed allowing a prompt innate response to pathogenic strains that might trigger local inflammation

Reygaert ,2014 indicated to the importance of TLRs has been recognized as a key regulator for innate and adaptive immune responses. The innate

immunity is sup- ported by a variety of natural hindrances including skin and mucosa, nonspecific molecules such as interferons and different types of cells comprising dendritic cells (DCs), MΦs (as the specific immune cells), fibroblasts, endothelial and epithelial cells (as the non-specific immune cells). Immune cells are able to produce and secrete all members of PRR families such as NLRs, RLRs, CLRs, and TLRs. The PRRs like TLRs are important immunologic biosensors for tracing pathogens within the host's cells and tissues by recognition of microbial conserved components, termed pathogen association molecular patterns (PAMPs) ( Abraham & Miao,2015). Particularly TLR1, TLR2, TLR4, TLR5, TLR6 and TLR11, which are expressed on cell surfaces, recognize mainly microbial membrane components such as lipids, lipoproteins and proteins, while TLR3, TLR7, TLR8 and TLR9, which are expressed exclusively in intracellular vesicles, such as the endoplasmic reticulum (ER), endosomes, lysosomes and endo-lysosomes, mainly link nucleic acids ( Micera *et al.*, 2014)

TLR2 and TLR9 likely play different roles in the immune response to bacterial infection. the others studies in gram negative found that both TLR2 and TLR9 promote NK cell cytolytic function against bacteria-infected macrophages, TLR9 deficiency leads to macrophage dysfunction, where TLR2 deficiency augments the resistance to bacteria by enhancing the bactericidal capability of macrophages,( Scherberich,*etal*,2008).

**4.5 Correlation between mucosal and systemically for TLR2 & TLR9 in UTI patients**

Correlation between mucosal and systemically for TLR2 in UTI patients that was weak negative correlation and p value was no significant at $p > 0.05$ Show in figure(4-16)

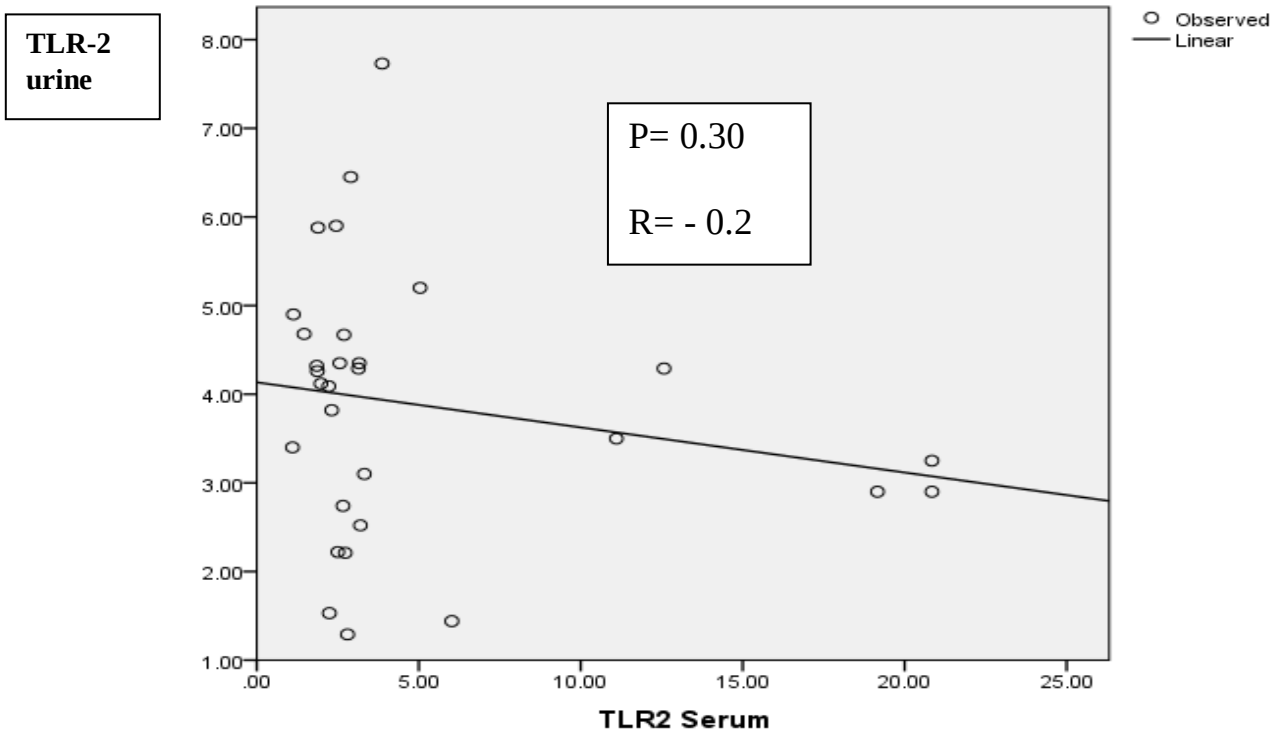


**Figure (4-16) correlation between TLR2 mucosal and systemic**

Correlation between mucosal and systemically for TLR9in UTI patients that was weak negative correlation and p value was no significant at $p > 0.05$ Show in figure(4-17)

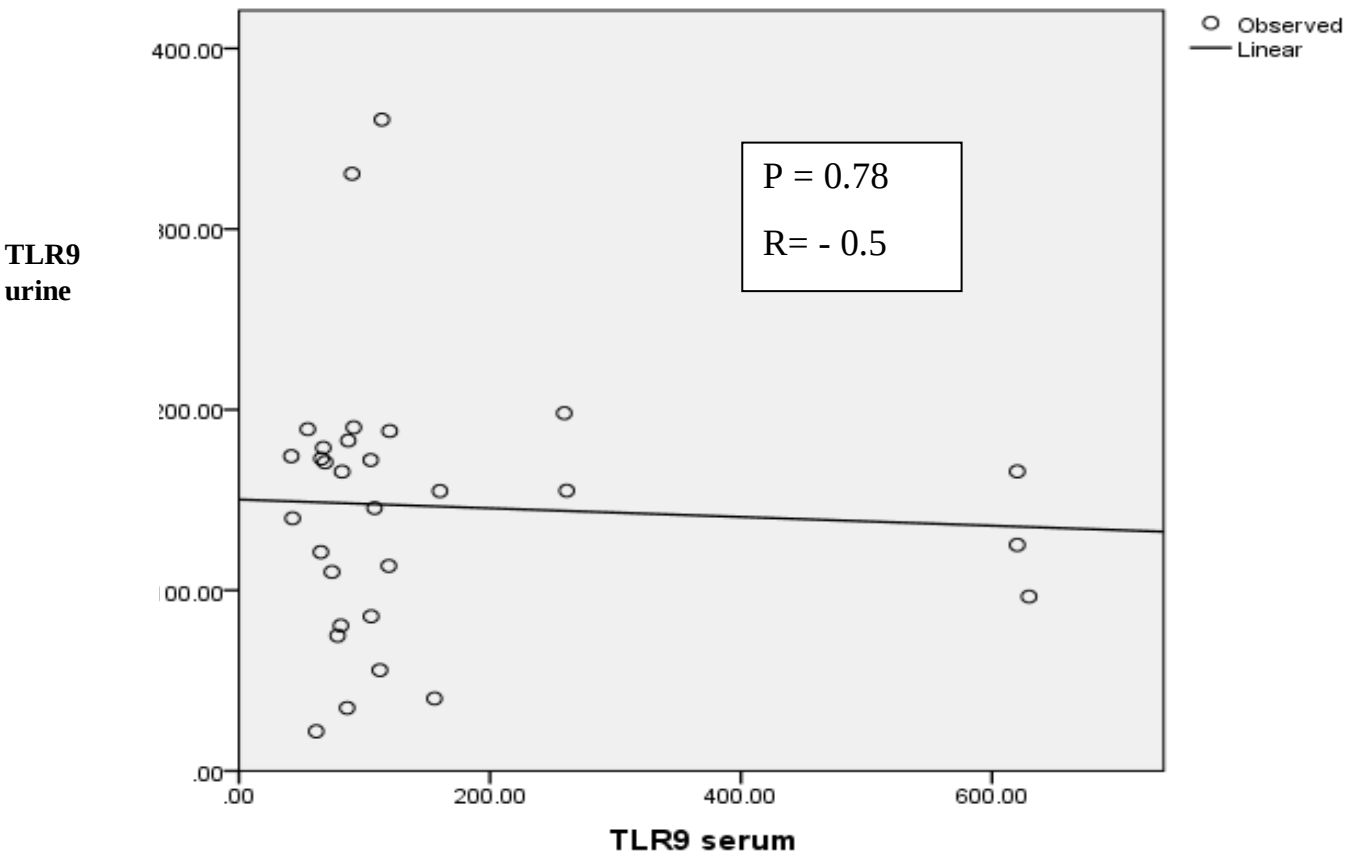


**Figure (4-17) correlation between TLR9 mucosal and systemic**

Correlation between mucosal for TLR9 and TLR2 in UTI patients that was strong positive correlation and p value was significant at $p < 0.05$ Show in figure(4-18)

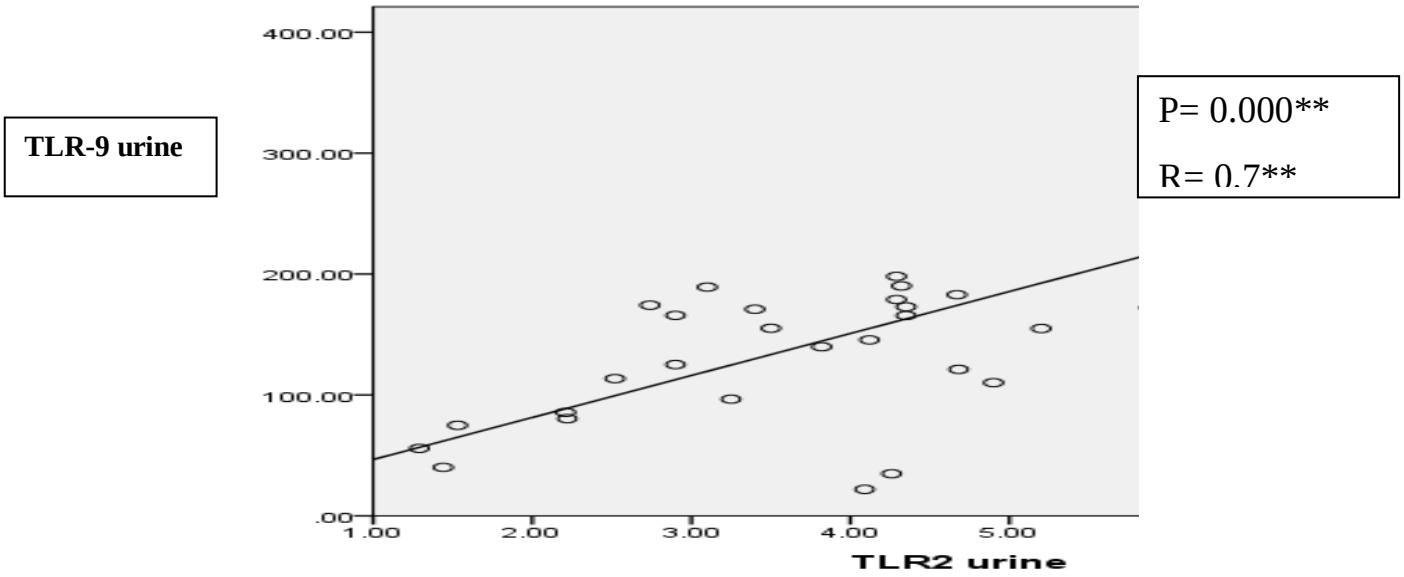


**Figure (4-18) correlation between mucosal TLR9 and mucosal TLR2**

Correlation between systemically for TLR9 and TLR2 in UTI patients that was strong positive correlation seem perfect correlation and p value was significant at $p < 0.05$ Show in figure (4-19 )

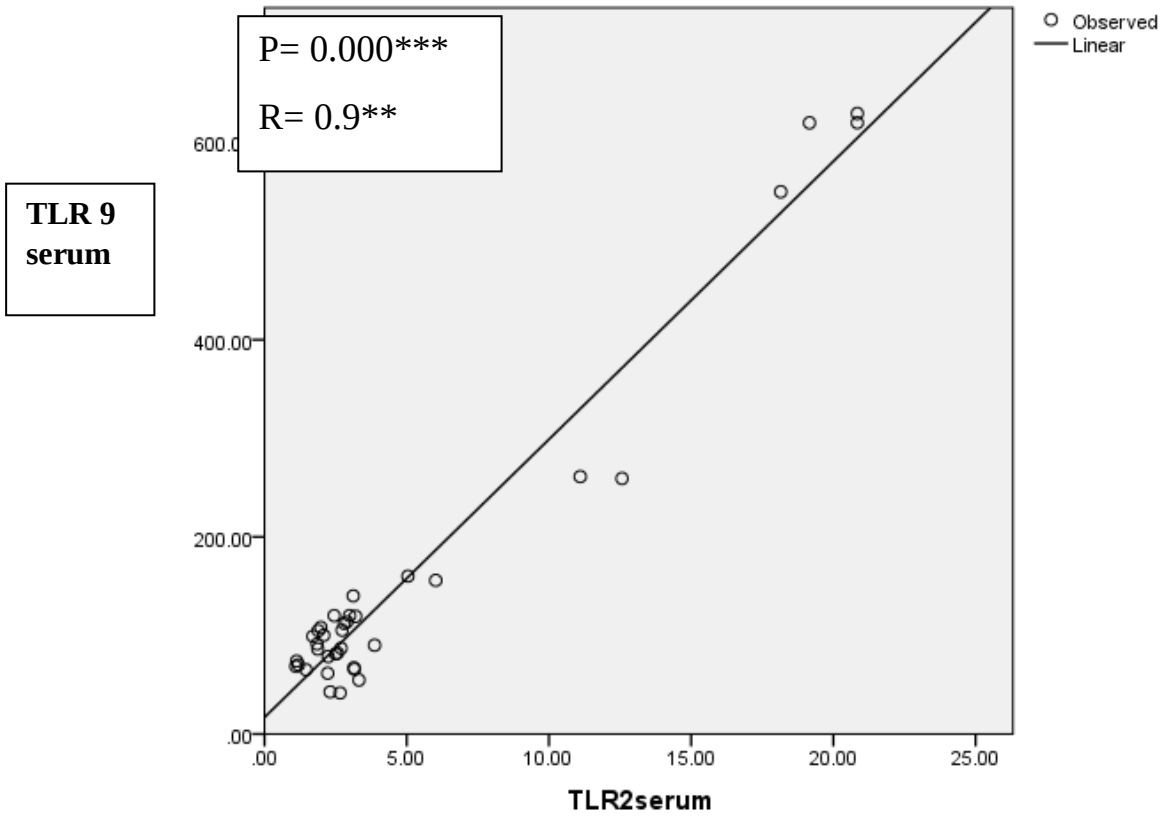


**Figure (4-19) correlation between systemic TLR9 and systemic TLR2**

Regulation of TLR expression, cellular localization, and functions in mucosal epithelial cells provides one of the mechanisms by which bacteria , host cell interactions are directed to avoid onset of persistent local inflammation Mucosal pathogens use diverse and highly specific molecular mechanisms to activate mucosal inflammation. It may even be argued that their virulence depends on the inflammatory response that they induce. Some bacteria target epithelial cells and trigger them to produce inflammatory mediators but others cross the mucosa and activate macrophages or dendritic cells. Although systemic release of inflammatory

mediators causes many symptoms and signs of infection, local chemokine production leads to the recruitment of inflammatory cells and lymphocytes that participate directly in the clearance of bacteria from mucosal sites. In this way, mucosal inflammation is a two-edged sword responsible for disease associated tissue destruction and crucial for the antimicrobial defence. Understanding of these pathways should create tools to enhance the defence and interfere with disease.( Catharina .*et al* ,1999)

TLR2 were capable of responding to stimulation by all the bacteria tested, including Gram-positive organisms and gram negative , all appear to activate cells through the same receptor system.( Enersen, .*et al*,2013) TLR-9 is a receptor for bacterial and viral CpG-DNA and has an important role in host defenses against infection (Patten, *etal* ,2013) that lead to strong positive correlation between systemic TLR2 and systemic TLR9 & strong positive correlation between mucosal TLR2 and mucosal TLR9

**Conclusions:**

1-The most commoni bacteria caused urinary tract infection were *E.coli*& *Enterococcus faecalis*

2- Polymorphism of TLR2 rs3804099genie polymorphism by ARMS-PCR method are considered as a risk factor for UTI .

3- Many SNPs were found between the one resolved haplotypes and the *TLR2* gene (exon 3) for reference sequences, according to the sequencing data,

4- The change two amino acid of TLR9 protein were Gln to Ala and Phe to Tyr effected TLR9 protein's expression in Exon 2.

5- Concentration of TLR2 & TLR9 in urine and serum of patients was higher in comparison with healthy control.

6- TLR2 andTLR9 were significantly higher correlated with both Gram positive and Gram negative bacteria.

**Recommendations:**

The following points for future work are recommended.

1- Application of SNPs-geniotypes comibination principals on other gene SNPs that may be associated with UTI

2- Detect the role of baicteria biofilm in UTI that may be more associated with gene polymorphism of TLR2 & TLR9.

- Abbo, L. and Hooton, T. (2014). Antimicrobial Stewardship and Urinary Tract Infections. Antibiotics. 3:174-192.
- Abdulla, M. and Oleiwi, I. (2016). Prevalence, Associated Factors, Bacterial and Antimicrobial Susceptibility Patterns of Urinary Tract Infection in a Sample of Iraqi Pregnant Women in Al-Najaf. European Journal of Biology and Medical Science Research. 4(6): 7-29.
- Abdullah, A. M., Abdullah, R. M., and Salman, S. L. (2009). Use of chromogenic agar in detection of urinary tract pathogens and antimicrobial Susceptibility. *Journal of the Faculty of Medicine Baghdad*, *51*(1), 39-42.
- Abdullahi, H. and Thairu, Y. (2015). Asymptomatic Bacteriuria among Pregnant Women Attending Antenatal: Evaluation of Screening Test. IOSR Journal of Pharmacy. 5(8):41-47.
- Abraham, S. and Miao, Y. (2015). The nature of immune responses to urinary tract infections. Nature Reviews Immunology. 15(10): 655-663.
- Abu-Maziad, A., Schaa, K., Bell, E. F., Dagle, J. M., Cooper, M., Marazita, M. L., and Murray, J. C. (2010). Role of polymorphic variants as genetic modulators of infection in neonatal sepsis. Pediatric research, 68(4), 323–329.
- Akira, S. and Uematsu, S. (2006). Toll-like receptors and innate immunity. Journal of Molecular Medicine, 84(9), 712-725.
- Akira, S., Takeda, K., and Kaisho, T. (2001). Toll-like receptors: critical proteins linking innate and acquired immunity. Nature immunology, 2(8), 675-680.
- Alanazi, M. Q., Alqahtani, F. Y. and Aleanizy, F. S. (2018). An evaluation of E. coli in urinary tract infection in emergency

department at KAMC in Riyadh, Saudi Arabia: retrospective study. Ann Clin Microbiol Antimicrob. Feb 9;17(1):3. doi: 10.1186/s12941-018-0255-z. PMID: 29422058; PMCID: PMC5806437.

- Alatoom, A., Elsayed, H., Lawlor, K., AbdelWareth, L., El-Lababidi, R., Cardona, L., Mooty, M., Bonilla, M. F., Nusair, A. and Mirza, I. (2017). Comparison of antimicrobial activity between ceftolozane-tazobactam and ceftazidime-avibactam against multidrug-resistant isolates of Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa. Int J Infect Dis. Sep;62: 39-43. doi: 10.1016/j.ijid.2017.06.007. Epub 2017 Jun 10. PMID: 28610832.
- Albert, X., Huertas, I., Pereiro, I., Sanfélix, J., Gosalbes, V., and Perrotta, C. (2004). Antibiotics for preventing recurrent urinary tract infection in non-pregnant women. *Cochrane Database of Systematic Reviews*, (3).
- Al-Jebouri, M. M and Salih, M. H. (2013). Antibiotic Resistance Pattern of Bacteria Isolated from Patients of Urinary Tract Infections in Iraq, "Open Journal of Urology,3,124-131.
- AL-Kadassy, A., Baraheem, O. and Ogaili, M. (2016). The Prevalence of Urinary Tract Infection (UTI) Among Pregnant Women In Hodeidah City. IOSR Journal of Dental and Medical Sciences. 15(12): 105-109.
- Alvarez-Pellitero, P. (2008). Fish immunity and parasite infections: from innate immunity to immunoprophylactic prospects. Veterinary immunology and immunopathology, 126 (3-4), 171-198.

- Al-Wazni, W. and Hadi, B. (2015). Antivirulence effects of pomegranate peel extracts on most common urinary tract infection pathogens in pregnant women. Journal of Contemporary Medical Sciences. 1(4): 7–12.
- Amin, E. K., Zaid, A. M. A., Abd El Rahman, I. K. and El-Gamasy, M. A. (2020). Incidence, risk factors and causative bacteria of urinary tract infections and their antimicrobial sensitivity patterns in toddlers and children: A report from two tertiary care hospitals. Saudi Journal of Kidney Diseases and Transplantation, 31, 200.
- Anuli, J., Mboto, C. and Bassaye, A. (2016). A review of the prevalence and predisposing factors responsible for urinary tract infections among adults. European journal
- Arpaia, N., and Barton, G. M. (2011). Toll-like receptors: key players in antiviral immunity. *Current opinion in virology*, *1*(6), 447-454.
- Baghbani, T., Nikzad, H., Azadbakht, J., Izadpanah, F., and Haddad, K. H. (2020). Dual and mutual interaction between microbiota and viral infections: a possible treat for COVID-19. *Microbial Cell Factories*, *19*(1), 1-25
- Barber, A., Orton, J., Spivak, A. and Mulvey, M. (2013). Urinary tract infection; current and emerging managment strategies. Clinical infectious diseases: 57(5),719-724.
- Bazaid, A. S., Saeed, A., Alrashidi, A., Alrashidi, A., Alshaghdali, K., Hammam, S. A., and Aldarhami, A. (2021). Antimicrobial Surveillance for Bacterial Uropathogens in Ha'il, Saudi Arabia: A Five-Year Multicenter Retrospective Study. *Infection and Drug Resistance*, *14*, 1455.

- Beatson, S., Zakour, N., Totsika, M., Forde, B. and Watts, R. (2015). Molecular Analysis of Asymptomatic Bacteriuria *Escherichia coli* Strain VR50 Reveals Adaptation to the Urinary Tract by Gene Acquisition. Infection and Immunity. 83(5):1749 – 1764.
- Becknell, B., Schwaderer, A., Hains, D. S., and Spencer, J. D. (2015). Amplifying renal immunity: the role of antimicrobial peptides in pyelonephritis. *Nature Reviews Nephrology*, *11*(11), 642-655.
- Behzadi, E. and Behzadi, P. The role of toll-like receptors (TLRs) in urinary tract infections (UTIs). Cent European J Urol. 2016;69(4):404-410. doi: 10.5173/ceju.2016.871. Epub 2016 Nov 30. PMID: 28127459; PMCID: PMC5260452.
- Behzadi, P. (2020). Classical chaperone-usher (CU) adhesive fimbriome: uropathogenic Escherichia coli (UPEC) and urinary tract infections (UTIs). *Folia microbiologica*, *65*(1), 45-65.
- Belyayeva, M. and Jordan, M. (2019). Acute phylonephritis. Treasure Island: Stat Pearls publishers,
- Ben-Ali, M., Barbouche, M. R., Bousnina, S., Chabbou, A., and Dellagi, K. (2004). Toll-like receptor 2 Arg677Trp polymorphism is associated with susceptibility to tuberculosis in Tunisian patients. *Clinical and Vaccine Immunology*, *11*(3), 625-626.
- Bennadi, D. (2014). Self-medication: A current challenge. Journal of Basic and Clinical Pharmacy: 19-23.
- Betsy, F. (2010). The epidemiology of urinary tract infection. Nature review urology: 7(12);63.
- Billips, B. K., Schaeffer, A. J., and Klumpp, D. J. (2008). Molecular basis of uropathogenic Escherichia coli evasion of the

innate immune response in the bladder. *Infection and immunity*, *76*(9), 3891-3900.

- Bonini, S., Micera, A., Iovieno, A., Lambiase, A. and Bonini, S. (2005). Expression of toll-like receptors in healthy and allergic conjunctiva. Ophthalmology. 112: 1528.e11528.e8. https://doi.org/10.1016/j.ophtha.2005.04.009.
- Botos, I., Segal, D. M., and Davies, D. R. (2011). The structural biology of Toll-like receptors. *Structure*, *19*(4), 447-459.
- Brandtzaeg, P., Farstad, I. N., Johansen, F. E., Morton, H. C., Norderhaug, I. N., Yamanaka, T. and Yamanaka, T. (1999). The B-cell system of human mucosae and exocrine glands. *Immunological reviews*, *171*(1), 45-87.
- Brusch, J. L. ( 2017). Pathophysiology of Complicated Urinary Tract Infections. medscape: 2039975.
- Bryce, A., Hay, A. D., Lane, I. F., Thornton, H. V., Wootton, M., and Costelloe, C. (2016). Global prevalence of antibiotic resistance in paediatric urinary tract infections caused by Escherichia coli and association with routine use of antibiotics in primary care: systematic review and meta-analysis. *bmj*, *352*.
- Cash, H. L., and Hooper, L. V. (2005). Commensal bacteria shape intestinal immune system development. *ASM news*, *71*(2), 77-83.
- Cerutti, A. (2008). The regulation of IgA class switching. *Nature reviews immunology*, *8*(6), 421-434.
- Cheesbrough, M. (2009). District laboratory practice in tropical countries. 2nd Ed. Cambridge University Press. New York, USA. 132-304.
- Cheng, C. H., Lee, Y. S., Chang, C. J. and Lin, T. Y. (2013). Genetic polymorphisms in Toll-like receptors among pediatric

patients with renal parenchymal infections of different clinical severities. PLoS One.;8(3): e58687.

- Cheng, C. H., Lee, Y. S., Chang, C. J., Lin, J. C., and Lin, T. Y. (2015). Genetic polymorphisms in inflammasome-dependent innate immunity among pediatric patients with severe renal parenchymal infections. *PloS one*, *10*(10), e0140128.
- Chi, H., Li, C., Zhao, F. S., Zhang, L., Ng, T. B., Jin, G., and Sha, O. (2017). Anti-tumor activity of toll-like receptor 7 agonists. *Frontiers in pharmacology*, *8*, 304.
- Christensen, S. R., Kashgarian, M., Alexopoulou, L., Flavell, R. A., Akira, S., and Shlomchik, M. J. (2005). Toll-like receptor 9 controls anti-DNA autoantibody production in murine lupus. *The Journal of experimental medicine*, 202(2), 321-331.
- Cirl, C., Wieser, A., Yadav, M., Duerr, S. and Schubert, S. (2008). Subversion of Toll-like receptor signaling by a unique family of bacterial Toll/interleukin-1 receptor domain-containing proteins. Nature Medicine. 14(4): 399–406.
- Cirl, C., Wieser, A., Yadav, M., Duerr, S., Schubert, S., Fischer, H. and Miethke, T. (2008). Subversion of Toll-like receptor signaling by a unique family of bacterial Toll/interleukin-1 receptor domain–containing proteins. *Nature medicine*, *14*(4), 399-406.
- Collee, J. G., Duguid, J. P., Fraser, A. G., Marmion, B. P., and Simmons, A. (1996). Laboratory strategy in the diagnosis of infective syndromes. *Mackie and McCartney practical medical microbiology*, *14*, 53-94.
- Çuburu, N., Kweon, M. N., Song, J. H., Hervouet, C., Luci, C., Sun, J. B. and Czerkinsky, C. (2007). Sublingual immunization

induces broad-based systemic and mucosal immune responses in mice. *Vaccine*, *25*(51), 8598-8610.

- Cui, Y., Franciszkiewicz, K., Mburu, Y. K., Mondot, S., Le Bourhis, L., Premel, V. and Lantz, O. (2015). Mucosal-associated invariant T cell–rich congenic mouse strain allows functional evaluation. *The Journal of clinical investigation*, *125*(11), 4171-4185.
- Daley, J. M., Thomay, A. A., Connolly, M. D., Reichner, J. S., and Albina, J. E. (2008). Use of Ly6G-specific monoclonal antibody to deplete neutrophils in mice. *Journal of leukocyte biology*, 83(1), 64-70.
- DeBoy, J. M., Wachsmuth, I. K., and Davis, B. R. (1980). Hemolytic activity in enterotoxigenic and non-enterotoxigenic strains of Escherichia coli. *Journal of Clinical Microbiology*, 12(2), 193-198.
- Derese, B., Kidir, H., Teklemariam, Z., Weldegebrial, F. and Balakrishnan, S. (2016). Bacterial profile of urinary tract infection and antimicrobial susceptibility pattern among pregnant women attending at Antenatal Clinic in Dil Chora Referral Hospita. Therapeutics and Clinical Risk Management. 12: 251-60.
- Dewit, C. (2005) Fundamental Concepts and Skills for Nursing, Infection Control in the Hospital and Home, Elsevier Saunders, Second Edition, USA, PP. 201,203,207,222, 225.
- Di Gioia, M. and Zanoni, I. (2015). Toll-like receptor co-receptors as master regulators of the immune response. *Molecular immunology*, 63(2), 143-152.
- Di Zazzo, A., Micera, A., Coassin, M., Varacalli, G., Foulsham, W. and De Piano, M. (2019). InflammAging at Ocular Surface:

Clinical and Biomolecular Analyses in Healthy Volunteers. Invest Ophthalmol Vis Sci; 60:1769–75.

- Dias, C. S., Silva, J. M. P., Diniz, J. S. S., Lima, E. M., Marciano, R. C., Lana, L. G. and Oliveira, E. A. (2010). Risk factors for recurrent urinary tract infections in a cohort of patients with primary vesicoureteral reflux. *The Pediatric infectious disease journal*, 29(2), 139-144.
- Djordjevic, Z., Folic, M., and Jankovic, S. (2016). Community-acquired urinary tract infections: causative agents and their resistance to antimicrobial drugs. *Vojnosanitetski pregled*. 73: 1109-1115.
- Dougnon, T., Bankole, H., Johnson, R., Hounmanou, G. and Toure, I. (2016). Catheter-Associated Urinary Tract Infections at a Hospital in Zinvie, Benin (West Africa). International Journal of Infection. 3(2): 34-41.
- Drage, M. G. (2009). Toll-like receptor 2-mediated recognition of mycobacterial lipoproteins and glycolipids. Case Western Reserve University.
- Duane, R., xue-Ru, W. and Tung-tien, S. (2016). Vesico-ureteral reflux a congenital, anatomical and functional disorder has been implicated to cause a high morbidity especially in young children. It occurs when there is a backward flow of urine from the bladder to the kidney. This facilitates movement. microbiological Spectrum: 3(4);0016.
- Edlin, R. S., Shapiro, D. J., Hersh, A. L. and Copp, H. L. (2013). Antibiotic resistance patterns of outpatient pediatric urinary tract infections. *The Journal of urology*, *190*(1), 222-227.

- Elbrolosy, A. M., Elabd, N. S., ElGedawy, G. A. (2022). Toll- like receptor 2polymorphism and IL-6 profile in relation to disease progression in chronic HBV infection: a case control study in Egyptian patients. Clin Exp Med.
- Elzayat, M., Barnett-Vanes, A., Dabour, M. and Cheng, F. (2017). Prevalence of undiagnosed asymptomatic bacteriuria and associated risk factors during pregnancy: a cross-sectional study at two tertiary centres in Cairo, Egypt. BMJ Open. 7: 131-98.
- Enersen, M., Nakano, K., and Amano, A. (2013). Porphyromonas gingivalis fimbriae. *Journal of oral microbiology*, *5*(1), 20265.
- Engel, D. R., Maurer, J., Tittel, A. P., Weisheit, C., Cavlar, T., Schumak, B. and Kurts, C. (2008). CCR2 mediates homeostatic and inflammatory release of Gr1high monocytes from the bone marrow, but is dispensable for bladder infiltration in bacterial urinary tract infection. *The Journal of Immunology*, *181*(8), 5579-5586.
- Entesar, M., Makhlouf, A. and Abdelhafez, A. (2018). Prospective Study on The Prevalence of Urinary Tract Infections In Pregnancy: Al Dawadmi, Riyadh, Saudi Arabia. IOSR Journal of Nursing and Health Science. 7(1): 48-52.
- Fang, J., Hu, R., Hou, S., Ye, Z., Xiang, Q. and Qi, J. (2013). Association of TLR2 gene polymorphisms with ocular Behcet's disease in a Chinese Han population. Invest Ophthalmol Vis Sci.; 54:8384–92.
- Fareid, M. A. (2012). Frequency and susceptibility profiles of bacteria causing urinary tract infection among women. New York Sci. J., 5: 284-298.

- Fieber, C. K. and ovarik, P. (2014). Responses of innate immune cells to group A Streptococcus. Front CellInfect Microbiol.; 4:140.
- Fischer, R. and Maier, O. (2015). Interrelation of oxidative stress and inflammation in neurodegenerative disease: role of TNF. Oxidative Medicine and Cellular Longevity. Vol: 2015. ID:610813: 1-18.
- Flores-Mireles, A. L., Walker, J. N., Caparon, M. and Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature reviews microbiology*, *13*(5), 269-284.
- Flores-Mireles, A., Jennifer, N., Michael, C. and Scott, J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature Reviews Microbiology. 13(5): 269–284.
- Forbes, B. A., Daniel, F. S. and Alice, S. W. (2007). Bailey and Scott's Diagnostic Microbiology. 12th ed., Mosby Elsevier company. USA. 62-465.
- Frank, D. N., Amand, A. L., Feldman, R. A., Boedeker, E. C., Harpaz, N. and Pace, N. R. (2007). Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proceedings of the national academy of sciences*, *104*(34), 13780-13785.
- Gachuhi, G. T. (2017). Antibiotic Susceptibility Pattern of Bacterial Uropathogens Isolated from Patients in Nakuru Level 5 Hospital, Kenya. Master's Thesis, Nairobi: Kenyatta University.
- Ganesh, R., Shrestha, D., Bhattachan, B. and Rai, G. (2019). Epidemiology of urinary tract infection and antimicrobial

resistance in a pediatric hospital in Nepal. BMC Infectious Diseases, 19(1), 1-5.

- Gao, S. L., Chen, Y. D., Yue, C., Chen, J., Zhang, L. F. and Wang, S. M. (2019). -196 to -174del, rs4696480, rs3804099 Polymorphisms of Toll-like Receptor 2 Gene Impact the Susceptibility of Cancers: Evidence from 37053 Subjects. Biosci. Rep. 39, BSR20191698.
- Georgel, P., Macquin, C. and Bahram, S. (2009). The heterogeneous allelic repertoire of human toll-like receptor (TLR) genes. PLoS One, 4(11), e7803.
- Gessese, Y., Damessa, D., Amare, M., Bahta, Y. and Shifera, A. (2017). Urinary pathogenic bacterial profile, antibiogram of isolates and associated risk factors among pregnant women in Ambo town, Central Ethiopia: a cross-sectional study. Antimicrobial Resistance and Infection Control. 6:132-142.
- Ghaima, K., Khalaf, Z., Abdulhassan, A. and Salman, N. (2018). Prevalence and Antibiotic Resistance of Bacteria Isolated from Urinary Tract Infections of Pregnant Women in Baghdad Hospitals. Biomedical and Pharmacology Journal. 11(4):1989-1994.
- Ghajiri, H. A., Abdullah, R., Nasser, M. M., Roomi, A. B. (2020). Isolation and Diagnosis of Some Bacteria That Cause Urinary Tract Infection and The Effect of Some Antibiotics on Them in Nasiriyah In the Shattera General Hospital. International Journal of Pharmaceutical Research:12(2).
- Gill, S. R., Pop, M., DeBoy, R. T., Eckburg, P. B., Turnbaugh, P. J., Samuel, B. S. and Nelson, K. E. (2006). Metagenomic analysis

of the human distal gut microbiome. *science*, *312*(5778), 1355-1359.

- Godaly, G., Ambite, I. and Svanborg, C. (2015). Innate immunity and genetic determinants of urinary tract infection susceptibility. Current Opinion in Infectious Diseases. 28:88–96.
- Gondim, R., Azevedo, R., Braga, A. A. N. M., Veiga, M. L., & Barroso Jr, U. (2018). Risk factors for urinary tract infection in children with urinary urgency. *International braz j urol*, *44*, 378-383.
- Golovkin, A. S, Ponasenko, A. V. and Yuzhalin, A. E. (2015). An association between single nucleotide polymorphisms within TLR and TREM-1 genes and infective endocarditis. Cytokine.;71(1):16-21.
- Gong, P., Zhou, Y., Wang, J., Bai, Y., Chen, B. and Zhu, Z. (2020). Mapping global urban boundaries from the global artificial impervious area (GAIA) data. *Environmental Research Letters*, *15*(9), 094044.
- Goossens, H., Ferech, M., Vander Stichele, R. and Elseviers, M. (2005). Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet (London, England), 365(9459):579–87.
- Grabe, M., Bjerklund-Johansen, T., Botto, H., Cek, M., Naber, R., Pickard, P., Tenke, P., Wagenheiler, F. and Wullt, B. (2013). Guideline on urologic infections. European association of urology: 5(2):435-440.
- Greene, J. A., Sam-Agudu, N., John, C. C., Opoka, R. O., Zimmerman, P. A. and Kazura, J. W. (2012). Toll-like receptor polymorphisms and cerebral malaria: TLR2 Δ22 polymorphism is

associated with protection from cerebral malaria in a case control study. Malar J,11:47.

- Gupta, K. (2011). International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin. Infect. Dis. 52, e103–e120
- Gupta, K., Hooton, T., Naber, K., Wullt, B. and Colgan, R. (2011). International Clinical Practice Guidelines for the Treatment of Acute Uncomplicated Cystitis and Pyelonephritis in Women: A 2010 Update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clinical Infectious Diseases. 52(5):103–120.
- Hamdan, H., Ziad, A., Ali, S. and Adam, I. (2011). Epidemiology of urinary tract infectionsand antibiotics sensitivity among pregnant women at Khartoum North hospital Ann. Clinical Microbiology and Antimicrobials. 10(1): 2.
- Hashemi, N., Khani, M. and Bavarsad, H. (2019). Evaluation and Management of Urinary Tract Infections as a Medical Emergency. Emergency Medicine and Critical Care. 3:(3)109.
- Hashimoto, M., Ma, Y. F., Wang, S. T., Chen, C. S. and Teng, C. H. (2021). Iron Acquisition of Urinary Tract Infection Escherichia coli Involves Pathogenicity in Caenorhabditis elegans. Microorganisms, 9(2), 310.
- Hemraj, V., Diksha, S. and Avneet, G. (2013). A review on commonly used biochemical test for bacteria. Innovare Journal of Life Science, Vol. 1, pp. 1–7.

- Hise, A. G., Daehnel, K. and Gillette-Ferguson, I. (2007). Innate immune responses to endosymbiotic Wolbachia bacteria in Brugia malayi and Onchocerca volvulus are dependent on TLR2, TLR6, MyD88, and Mal, but not TLR4, TRIF, or TRAM. J Immunol,178:1068-1076.
- Hofstetter, P., Naber, A., Vahlensieck K., ludwig, W., Bach, M. and Leitinien, D. (2003). Guideline on urinary and male genital tract infections." European association of urology: 42(1),104-112.
- Holmgren, J., Czerkinsky, C. (2005). Mucosal immunity and vaccines. Nat Med. ,11(4): S45–53
- Hong, W., Zeng, J. and Xie, J. (2014). Antibiotic drugs targeting bacterial RNAs. Acta Pharm. Sin B. 4(4):258-265.
- Hooton, T. M. (2010). Diagnosis, prevention, and treatment of catheter-associated urinary tract infection in adults: 2009 international clinical practice guidelines from the Infectious Diseases Society of America. Clin. Infect. Dis. 50, 625–663
- Hooton, T. M., Besser, R., Foxman, B., Fritsche, T. R. and Nicolle, L. E. (2004). Acute uncomplicated cystitis in an era of increasing antibiotic resistance: a proposed approach to empirical therapy. Clin. Infect. Dis. 39, 75–80.
- Hug, H., Mohajeri, M. and La Fata, G. (2018). Toll-Like Receptors: Regulators of the Immune Response in the Human Gut. Nutrients. 10(2):203.
- Humphries, R., Bobenchik, A. M., Hindler, J. A., & Schuetz, A. N. (2021). Overview of changes to the clinical and laboratory standards institute performance standards for antimicrobial susceptibility testing, M100. *Journal of clinical microbiology*, *59*(12), e00213-21.

- Ibrahim, I. I. and Abdulkareem, R. S. (2017). Study of bacteria isolated from urinary tract infections of patient attending Tikrit Teaching Hospital for the year 2014 and its sensitivity to commonly used antibiotics. Tikrit Medical Journal 2017; (23) (1) :67-74.
- Iduoriyekemwen, N. and Sadoh, A. (2012). Asymptomatic bacteriuria in HIV positive Nigerian children. The Journal of Medicine and Biomedical Research. 11(1):88-94.
- Jahandeh, N., Ranjbar, R., Behzadi, P. and Behzadi, E. (2015). Uropathogenic Escherichia coli virulence genes: invaluable approaches for designing DNA microarray probes. *Central Eur. J. Urol.* 68(4):452. [PMC free article] [PubMed] [Google Scholar].
- Janeway, C. A. and Medzhitov, R. (2002). Innate immune recognition. Annu Rev Immunol,20:197-216
- John, A. S., Mboto, C. I. and Agbo, B. (2016). A review on the prevalence and predisposing factors responsible for urinary tract infection among adults. Euro J ExpBio, 6, 7-11.
- Joosten, L. A., Oppers-Walgreen, de Mos, M., B., van Schie, J. T., Jahr, H., van Osch, G. J., & Verhaar, J. A. (2009). Tendon degeneration is not mediated by regulation of Toll-like receptors 2 and 4 in human tenocytes. *Journal of Orthopaedic Research, 27*(8), 1043-1047.
- Junjie, X., Songyao, J. and Minmin, S. (2012). The association between toll-like receptor 2 single-nucleotide polymorphisms and hepatocellular carcinoma susceptibility. BMC Cancer.;12:57.
- Junpee, A., Tencomnao, T., Sanprasert, V. and Nuchprayoon, S. (2010). Association between Toll-like receptor 2 (TLR2)

polymorphisms and asymptomatic bancroftian filariasis. Parasitol Res,107:807-816

- Kagnoff, M. F. and Eckmann, L. (1997). Epithelial cells as sensors for microbial infection. J Clin Invest,100: 6-10
- Kang, T. J. and Chae, G. T. (2002). Detection of Toll-like receptor 2 (TLR2) mutation in the lepromatous leprosy patients. FEMS Immunol Med Microbiol , 31:53–8.
- Karananou, P., Fleva, A., Tramma, D., Alataki, A. and Pavlitou-Tsiontsi, A .,Emporiadou-Peticopoulou,M and Papadopoulou-Alataki E. (2016). Altered Expression of TLR2 and TLR4 on Peripheral CD14+ Blood Monocytes in Children with Urinary Tract Infection. Biomed Res Int. 2016: 6052891.
- Karishetti, M. and Shaik, H. (2019). Clinicomicrobial assessment of urinary tract infections in a tertiary care hospital. Indian Journal of Health Sciences and Biomedical Researches. 12:69-74.
- Kawai, T. and Akira S. (2011). Toll-like receptors and their crosstalk with other innate receptors in infection and im Micera A.
- Keren, R., Shaikh, N., Pohl, H., Gravens-Mueller, L., Ivanova, A., Zaoutis, L. and Hoberman, A. (2015). Risk factors for recurrent urinary tract infection and renal scarring. Pediatrics, 136(1), e13-e21.
- Khoury, A. E., Bägli, D. J., McDougal, W. S., Wein, A. J. and Kavoussi, L. R. (2016). Vesicoureteral Reflux. Campbell-Walsh Urology, 11 ed. Elsevier, 3134-55
- Kleinnijenhuis, J., Oosting, M., Joosten, L.A., Netea, M.G., and Van Crevel, R. (2011). Innate immune recognition of Mycobacterium tuberculosis. *Clin. Dev. Immunol*:405310

- Kline, K. and Lewis, A. (2016). Gram-Positive Uropathogens, Polymicrobial Urinary Tract Infection, and the Emerging Microbiota of the Urinary Tract. Microbiology Spectrum. 4(2):1-54.
- Koves, B. and Wullt, B. (2016). The Roles of the Host and the Pathogens in Urinary Tract Infections. European Urology Supplements. 15:88– 94.
- Krieger, J. and Morris, B. (2017). Penile inflammatory skin disoder and the prweventive role of circumcision." *international journal of preventive medicine*: 8(32).
- Kubarenko, A. V., Ranjan, S. and Rautanen, A. (2010). A naturally occurring variant in human TLR9, P99L, is associated with loss of CpG oli- gonucleotide responsiveness. J Biol Chem, 285:36486–94.
- Kucheria, R., Dasgupta, P. and Sacks, S. H. (2005). Urinary tract infections: new insights into a common problem. Postgrad Med J, 81(952): 83-6.
- Kuliczkowska-Płaksej, J., Jończyk, M., Jawiarczyk-Przybyłowska, A., Stachowska, B., Zembska, A. and Grzegrzółka, J. (2021). The Frequency of TLR2 (Rs3804099, Rs3804100, and Rs5743708) and TLR4 (Rs4986790 and Rs4986791) Polymorphisms in Women with Polycystic Ovary Syndrome – Preliminary Study. Gynecol. Endocrinol. 37, 1027–1034.
- Kumar, H., Kawai, T. and Akira, S. (2009). Toll-like receptors and innate immunity. Biochem Biophys Res Commun.;30;388(4):621–5.

- Lacovelli, V., Gaziev, G., Topazio, L., Bove, P. and Vespasiani, G. (2014). Nosocomial urinary tract infections: A review. Urologia, 81: 222-227.
- LaRue, H., Ayari, C., Bergeron, A. and Fradet, Y. (2013) Toll-like receptors in urothelial cells—targets for cancer immunotherapy. Nat Rev Urol 10:537–545
- LeBourhis, L., Martin, E., Péguillet, I. (2010). Antimicrobial activity of mucosal-associated invariant T cells Nat Immunol, ,11:701–708
- Lema, V. and Lema, A. (2018). Sexual Activity and the Risk of Acute Uncomplicated Urinary Tract Infection in Premenopausal Women: Implications for Reproductive Health Programming. Obstetrics and Gynecology International Journal. 9(1): 55-62.
- Ley, R. E., Bäckhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D. and Gordon, J. I. (2005). Obesity alters gut microbial ecology. Proceedings of the national academy of sciences, 102(31), 11070-11075.
- Litza, J. and Brill, J. (2010). Urinary tract infections. Primary Care. 37(3): 491-507.
- Llorente, C., Bruellman, R., Cabré, N., Brea, R., Pell, N., Maccioni, L. and Schnabl, B. (2021). Il6st-Induced Muscarinic Receptor Opens Goblet Cell Associated Antigen Passages To Suppress Alcoholic Liver Disease. In *Hepatology* (Vol. 74, pp. 76A-77A). 111 River St, Hoboken 07030-5774, Nj Usa: Wiley.
- Lorenz, E, Cornish, K. L., Arbour, N. C. and Schwartz, D. A. (2000). A novel polymorphism in the toll-like receptor 2 gene and its potential association with staphylococcal infection. Infect Immun.; 68:6398–6401.

- Lutay, N., Ambite, I., Gronberg, H., Rydström, G. and Ragnarsdóttir, B. (2013). Bacterial control of host gene expression through RNA polymerase II. Journal of Clinical Investigation. 123:2366–79.
- Lüthje, P., Brauner, A. (2016). Novel Strategies in the Prevention and Treatment of Urinary Tract Infections. Pathogens,5(1): 13.
- MacFaddin, J. F. (2000). Biochemical Tests for Identification of Medical Bacteria. 3rd. Lippincott Williams and Wilkins, USA.
- Mackelprang, R.D, Scoville, C. W. and Cohen, C. R. (2015). Toll-like receptor gene variants and bacterial vaginosis among HIV-1 infected and uninfected African women. Genes Immun.;16(5):362-365.
- Macpherson, A. J. and Slack, E. (2007). The functional interactions of commensal bacteria with intestinal secretory IgA. Current Opinion in Gastroenterology, 23, 673–678.
- Mahak, J., Umar, F., Rehena, B., Vibhor, T., Anil, V. and Jyoti, G. (2015). Bacteriological study of Urinary Tract Infection in Antenatal patients in Teerthanker Mahareer University,Moradabad. International Journal of scientific studies: 3(3);32-36.
- Mahon, C. R., Lehman, D. C. and Manuselis, G. (2018). Textbook of diagnostic microbiology-e-book. Elsevier Health Sciences.
- Marius, A., Skow, H. and Sigurd, H. (2020). Antibiotic switch after treatment with UTI antibiotics in male patients. Infectous Diseases,52, (6): 405-412.
- Martin, E., Treiner, E. and Duban, L. (2009). Stepwise development of MAIT cells in mouse and human PLoS Biol ,7, e1000054

- Matulay, J. T., Mlynarczyk, C. M. and Cooper, K. L. (2016). Urinary tract infections in women: pathogenesis, diagnosis, and management. Curr Bladder Dysfunct Rep 11:53–60.
- May, L., van Bodegom, D. and Frölich, M. (2010). Polymorphisms in *TLR4* and *TLR2* genes, cytokine production and survival in rural Ghana. *Eur J Hum Genet* 18, 490–495
- Mayer, F., Wilson, D. and Hube, B. (2013) *Candida albicans* pathogenicity mechanisms. Virulence. 4: 119–128.
- McLellan, L. and Hunstad, D. (2016). Urinary Tract Infection: Pathogenesis and Outlook. Trends in Molecular Medicine. 22(11): 946–957.
- Medzhitov, R., Preston-Hurlburt, P. and Janeway, C. A. (1997). A human homologue of the Drosophila toll protein signals activation of adaptive immunity. *Nature*; 388: 394–397.
- Mirkamandar, E., Nemati, M., Hayatbakhsh, M. M., Bassagh, A., Khosravimashizi, A., & Jafarzadeh, A. (2018). Association of a single nucleotide polymorphism in the TLR2 gene (rs3804099), but not in the TLR4 gene (rs4986790), with Helicobacter pylori infection and peptic ulcer. *The Turkish Journal of Gastroenterology*, *29*(3), 283.
- Mkaddem, S. B., Chassin, C., Vandewalle, A. (2010). Contribution of renal tubule epithelial cells in the innate immune response during renal bacterial infections and ischemia reperfusion injury. Chang Gung Med J, 33(3): 225-8.
- Mladenovic, J., Veljovic, M., Udovicic, I., Srdjan Lazic, S. and Jadranin, Z. (2015). Catheter associated urinary tract infection in a surgical intensive care unit. Vojnosanitetski Pregled, 72: 883-888.

- Mosconi, L., Tsui, W. H., Herholz, K., Pupi, A., Drzezga, A., Lucignani, G. and De Leon, M. J. (2008). Multicenter standardized 18F-FDG PET diagnosis of mild cognitive impairment, Alzheimer's disease, and other dementias. Journal of nuclear medicine, 49(3), 390-398.
- Mukherjee, S., Karmakar, S., and Babu, S. P. S. (2016). TLR2 and TLR4 mediated host immune responses in major infectious diseases: a review. Brazilian Journal of Infectious Diseases, *20*, 193-204.
- Mulfaul, K., Ozaki, E., Fernando, N., Brennan, K., Chirco, K. R. and Connolly. (2020). Toll-like Receptor 2 Facilitates Oxidative Damage-Induced Retinal Degeneration. Cell Rep.; 30:22092224. e5. https://doi.org/10.1016/j.celrep.2020.01.064.
- Murray, P. R., Jorgensen, J. H., Pfaller, M. A. and Yolen, R. H. (2003). Manual of Clinical Microbiology. 8 th Edition. Washington. D.C.
- Murray, T. S., Peaper, D. R. (2015). The contribution of extended-spectrum β-lactamases to multidrug-resistant infections in children. Curr Opin Pediatr ,27:124–31.
- Muthulakshmi, M. and Gopalakrishnan, S. (2017). Study on urinary tract infection among females of reproductive age group in a rural area of Kancheepuram district, Tamil Nadu. Int J Community Med Public Health; 4:3915-21.
- Nabbugodi, W.F., J. Wanyoike Gichuhi and N.W. Mugo, (2015). Prevalence of urinary tract infection, microbial aetiology and antibiotic sensitivity pattern among antenatal women presenting with lower abdominal pains at Kenyatta national hospital, Nairobi, Kenya. J. Sci. Technol., 3: 1-6.

- Najar, S., Saldahna, L. and Banday K. (2009). Approach to urinary tract infections." *Indian journalof nephrology*: 19(4): 129–139.
- Nelson, J.M. and E. Good, (2015). Urinary tract infections and asymptomatic bacteriuria in older adults. Nurse Practitioner, 40: 43-48.
- Nemati, M., Larussa, T., Khorramdelazad, H., Mahmoodi, M., Jafarzadeh, A. (2017). Toll-like receptor 2: An important immunomodulatory molecule during Helicobacter pylori infection. Life Sci;178:17–29.
- Nielubowiz, R. and Mobley, H. (2010). Host–pathogen interactions in urinary tract infection. Nature Review of Urology: 7; 430–441.
- Nurullaev, R. B. (2004). The role of Asymptomatic bacteriuria in epidemiologic study of the urinary tract infection (UTI). Lik Aprava: 7:23-5.
- Nwachukwu, E., Onyebuchi, O. and Michael, O. (2018). Prevalence of urinary tract infections in pregnant women in Onitsha, Nigeria. Journal of Bacteriology and Mycology Open Access. 6(5):284–285.
- Obiora, C., Dim, C., Ezegwui, H., Nwogu-Ikojo, E. and Okeudo, C. (2014). Asymptomatic bacteriuria among pregnant women with sickle cell trait in Enugu, South Eastern Nigeria. Nigerian Journal of Clinical Practice. 17(1):95–9.
- Ochei, J., Enitan, S., Adejumo, E., Faloye, G. and Oniyide, F. (2018). Prevalence of Urinary Tract Infection among Pregnant Women in Ogun State, Nigeria. Microbiology Research Journal International. 24(6): 1-14.

  of experimental Biology: 69(4),57-6.

- Okonko, I., Ijandipe, L., Ilusanya, O., Donbraye-Emmanuel, O. and Ejembi, J. (2018). Incidence of urinary tract infection (UTI) among pregnant women in Ibadan, South-Western Nigeria. African Journal of Biotechnology. 8:(23) 6649-6657.
- Oladeinde, B., Omoregie, R. and Oladeinde, O. (2015). Asymptomatic Urinary Tract Infection Among Pregnant Women Receiving Ante-Natal Care in a Traditional Birth Home in Benin City, Nigeria. Ethiopian Journal of Health Sciences. 25(1):3-9.
- Olin, S. J., Bartges, J. W. (2015) Urinary tract infections: treatment/comparative therapeutics. Vet Clin North Am Small Anim Pract 45:721–746
- Onoh, R., Umeora, O., Egwuatu, V., Ezeonu, P. and Onoh, T. (2013). Antibiotic Sensitivity Pattern of uropathogens from pregnant women with urinary tract infections in Abakaliki, Nigeria. Infection and Drug Resistance. 6:225-233
- Owusu-Ofori, A., Biney, E. and Oppong, C. (2017). Antibiotic use in an accident and emergency department in a teaching hospital in Ghana. Open Forum Infect Dis ,4: S247-S247
- Ozensky, A. (2000). The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between Toll-like receptors. Proc. Natl. Acad. Sci. U. S. A. 97:13766–13771.
- Pabich, W., Fihn, S., Stamm, W., Scholes, D. and Boyko, E. (2003). Prevalance and determinants of vaginal flora alterations on post menosomal women. journal of infectious diseases: 37(1),57.
- Pai, R. K., Pennini, M. E., Tobian, A., Canaday, D. H., Boom, W. H., Harding, C. V. (2004). Prolonged toll like receptor signaling by

Mycobacterium tuberculosis and its 19-kilodalton lipoprotein inhibits gamma interferon-induced regulation of selected genes in macrophages. Infect Immun,72:6603-6614.

- Papadimitraki, E. D., Choulaki, C., Koutala, E., Bertsias, G., Tsatsanis, C., Gergianaki, I. and Boumpas, D. T. (2006). Expansion of toll-like receptor 9-expressing B cells in active systemic lupus erythematosus: Implications for the induction and maintenance of the autoimmune process. Arthritis Rheumatol., 54(11): 3601-3611.
- Patten, D. A., and Collett, A. (2013). Exploring the immunomodulatory potential of microbial-associated molecular patterns derived from the enteric bacterial microbiota. *Microbiology*, *159*(Pt_8), 1535-1544.
- Petersen, I. and Hayward, A. C. (2007). Antibacterial prescribing in primary care. J Antimicrob Chemother,60(1):43–7.
- Pitout, J. D., Laupland, K. B. (2008). Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public-health concern. The Lancet infectious diseases. 1;8(3):159-66.
- Pouladfar, G., Basiratnia, M., Anvarinejad, M., Abbasi, P., Amirmoezi, F. and Zare, S. (2017). The antibiotic susceptibility patterns of uropathogens among children with urinary tract infection in Shiraz. Medicine, 96.
- Prabhakar, N. K., Mian, M., and Elliott, C. S. (2021). Weekly Sequential Antibioprophylaxis for Recurrent UTI Among Patients with Neurogenic Bladder: A Randomized Controlled Trial. Clinical Infectious Diseases: an Official Publication of the Infectious Diseases Society of America.

- Qiu, Y., Zheng, J., Yang, J., Li, F. and Zhou, X. (2019). The predictive role of toll-like receptor-4 genetic polymorphisms in susceptibility to and prognosis of prostatic hyperplasia. Iranian Journal of Basic Medical Sciences. 22:86-92.
- Rakoff-Nahoum, S. and Medzhitov, R. (2009) Toll-like receptors and cancer. Nat Rev Cancer 9:57–63
- Ramos, N., Sekikubo, M., Dzung, D., Kosnopfel, C. and Kironde, F. (2012). Uropathogenic *E. coli* isolates from pregnant women in different countries. Journal of Clinical Microbiology. 50(11):3569–74.
- Reygaert, W. C. (2014). Innate immune response to urinary tract infections involving Escherichia coli. *J Clin Cell Immunol*, *5*, 280.
- Robinson, D. and Lafleche, G. (2000). Nucleic and electrophoeresis in agarose gels. Essential molecular biology, Chapter five, Volume one, 2nd edition.
- Ronald, A. (2003). The etiology of urinary tract infection: traditional and emerging pathogens. Dis. Mon. 49, 71–82.
- Rosen, D. A., Jerome, S., Pinkner, J. M., Jones, J. N., Walker, S., Clegg, S. and Hultgren, J. (2008). Molecular variations in Klebsiella pneumoniae and Escherichia coli FimH affect function and pathogenesis in the urinary tract. Infect. Immun. 76, 3346–3356.
- Rosen, D. A., Hooton, T. M. and Stamm, W. E. (2007). Detection of Intracellular Bacterial Communities in Human Urinary Tract Infection. PLoS Med, 4(12): e329.
- Sabih, A. and Leslie, S. (2017). Urinary Tract Infections, Complicated. StatPearls. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/28613784.

- Saeed, C. H., Al-Otraqchi, K. I. and Mansoor, I. Y. (2015). Prevalence of urinary tract infections and antibiotics susceptibility pattern among infants and young children in Erbil city. Zanco Journal of Medical Sciences (Zanco J Med Sci), 19, 915_922-915_922.
- Sahm, D. F., Thornsberry, D. C., Jones, M. E., Karlowsky, J. A. (2001). Multidrug resistance urinary tract isolates of *Escherichia coli* :prevalence and patient demographics in the united states in 2000.Antimicrob Agents Chemother:45:(5):1402-6.
- Salomon, J., Denys, P., Merle, C., Chartier-Kastler, E., Perronne, C. and Gaillard, J.-L. (2006). Prevention of urinary tract infection in spinal cord-injured patients: safety and efficacy of a weekly oral cyclic antibiotic (WOCA) programme with a 2-year follow-up—an observational prospective study. *J. Antimicrob. Chemother.* 57, 784–788.
- Sambrook, J. and Russell, D. W. (2001). Molecular cloning. A Labroratory Manual.3rd ed, Cold Spring Harbor, New York.
- Samuel, O., Victoria, O. and Ifeanyi, O. (2016). Prevalence of Asymptomatic Bacteriuria among the Pregnant Women Receiving Antenatal Care at Federal MedicalCentre Owerri, Nigeria. Universal Journal of Clinical Medicine. 4(1): 1-5.
- Santana, N. L, Rêgo, J. L. and Oliveira, J. M. (2017). Polymorphisms in genes TLR1, 2 and 4 are associated with differential cytokine and chemokine serum production in patients with leprosy. Mem Inst Oswaldo Cruz.;112(4):260-268.
- Sawhney, R. and Visvanathan, K. (2011). Polymorphisms of toll-like receptors and their pathways in viral hepatitis. Antivir Ther, 16(4): 443-458.

- Scherberich, J. E. and Hartinger, A. (2008). Impact of Toll-like receptor signalling on urinary tract infection. Int J Antimicrob Agents. Feb;31 Suppl 1: S9-14. doi: 10.1016/j.ijantimicag.2007.07.032. Epub 2007 Oct 17. PMID: 17942281.
- Schillinga, J. D., Mulveya, M. A. and Hultgrena, S. J. (2001). Dynamic interactions between host and pathogen during acute urinary tract infections. Urol , 57(6): 56-61.
- Schiwon, M., Weisheit, C., Franken, L., Gutweiler, S. and Dixit, A. (2014). Crosstalk between sentinel and helper macrophages permits neutrophil migration into infected uroepithelium. Cell 156: 456–468.
- Schneede, P., & Schlenker, B. (2010). Sexually transmitted infectious diseases (STDs): updated synopsis for practicing urologists. *Urogenital Infections*, 765.
- Schwabe, R. F., Seki, E. and Brenner, D. A. (2006). Toll-like receptor signaling in the liver. Gastroenterology, 130:1886–1900
- Seifu, W. and Gebissa, A. (2018). Prevalence and antibiotic susceptibility of uropathogens from cases of urinary tract infections in Shashemene referal hospital,Ethiopia. BMC infectious diseases: 18(1),1-9.
- Sekharan, C., Kumar, D., Kumari, K. and Joachim, C. (2017). Determination of Prevalence of Urinary Tract Infection Among the Pregnant Women with Lower Abdominal Pain. United Kingdom Journal of Pharmacology and Bioscience. 5(2): 51-57.
- Semlali, A., Parine, N. R., Al-Numair, N. S., Almutairi, M., Hawsawi, Y. M., Amri, A. A., Aljebreen, A. M., Arafah, M., Almadi, M. A., Azzam, N. A., Alharbi, O., and Alanazi, M. S.

(2018). Potential role of Toll-like receptor 2 expression and polymorphisms in colon cancer susceptibility in the Saudi Arabian population. Onco Targets and therapy, 11, 8127–8141.

- Shahin, R. D., Engberg, I., Hagberg, L., Svanborg Eden, C. (1987). Neutrophil recruitment and bacterial clearance correlated with LPS responsiveness in local gram-negative infection. Journal of immunology 138: 3475–3480.
- Shallcross, L.J. and Davies, D. S. C. (2014). Antibiotic overuse: a key driver of antimicrobial resistance. Brit J Gen Pract,64(629):604–5.
- Sherkatolabbasieh, H., Firouzi, M., Shafizadeh, S. and Nekohid, M. (2020). Evaluationof the relationship between vitamin D levels and prevalence of urinary tract infections in children. New Microbes and New Infections, 37, 100728.
- Silva, M. J. A., Lima, M. B. M., Lima, K. V. B., and Lima, L. N. G. C. (2021). The Relationship of TLR2 Polymorphisms with Infectious Diseases. Annual Research and Review in Biology, 57-73.
- Singh, V., Srivastava, N., Kapoor, R., Mittal, R. D. (2013). Single-nucleotide polymorphisms in genes encoding toll-like receptor -2, -3, -4, and-9 in a case–control study with bladder cancer susceptibility in a North Indian population. Arch Med Res 44:54–6.
- Skevaki, C., Pararas, M., Kostelidou, K., Tsakris, A. and Routsias, J. G. (2015). Single nucleotide polymorphisms of Toll-like receptors and susceptibility to infectious diseases. Clin. Exp. Immunol., 180(2): 165-177.

- Sobel, J. D. and Kaye, D. (2014). Urinary tract infections. In: Mandell GL, Bennett JE, eds. Principles and Practice of Infectious Diseases, 8th ed. Philadelphia: Elsevier Saunders :886-913.
- Sobel, J. D. and Kaye, D. (2015). Urinary tract infections. In Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases 8th ed (eds Bennett, J. E.) 886-913.e3.
- Song, J. and Abraham, S. N. (2008). Innate and adaptive immune responses in the urinary tract. Eur J Clin Invest;38(2):21–28.
- Srinath, P., Shajina, M. and Fasalu, M. (2018). Etiology and prevalence of urinary tract infections among pregnant women in Kerala. International Journal of Community Medicine and Public Health. 5(8):3298-3303.
- Storme, O., Tiran Saucedo, J., Garcia-Mora, A., Dehesa-Davila, M. and Naber, K. G. (2019). Risk factors and predisposing condition s for urinary tract infection. Ther Adv Urol 11:1756287218814382.
- Svanborg-Eden, C. and Svennerholm, A. M. (1978). Secretory immunoglobulin A and G antibodies prevent adhesion of Escherichia coli to human urinary tract epithelial cells. Infect Immun , 22:790–797.
- Szablewski, L. (2011). Glucose Homeostasis – Mechanism and Defects. Diabetes - Damages and Treatments, doi: 10.5772/22905.
- Tadesse, E., Teshome, M., Merid, Y., Kibert, B. and Shimelis, T. (2014). Asymptomatic urinary tract infection among pregnant women attending antenatal clinic of Hawassa Referral Hospital, Southern Ethiopia. BMC Research Notes. 7:155.
- Takeuchi, O. (2001). Discrimination of bacterial lipoproteins by Toll-like receptor 6. *Int. Immunol.* 13:933–940.

- Takeuchi, O. and Akira, S. (2010). Pattern recognition receptors and inflammation. Cell,140: 805-820.
- Tan, C. and Chlebicki, M. (2016). Urinary tract infections in adults. Singapore Medical Journal. 57(9): 485-490.
- Tao, K., Fujii, M., Tsukumo, S. I., Maekawa, Y., Kishihara, K., Kimoto, Y. and Yasutomo, K. (2007). Genetic variations of Toll-like receptor 9 predispose to systemic lupus erythematosus in Japanese population. Ann. Rheum. Dis., 66(7): 905-909
- Tazebew, D.; Getenet, B.; Selabat, M. and Wondewosen, T. (2012). Urinary bacterial profile and antibiotic susceptibility pattern among pregnant women in north West Ethiopia. Ethiopian Journal of Health Sciences. 22(2):121–8.
- Tazebew, E.; Getenet, B.; Wondewosen, T. and Silabat, M. (2013). Associated risk factors of urinary tract infection among pregnant women at Felege Hiwot referral hospital, Bahir Dar, Northwest Ethiopia. BMC Research Notes. 6:292.
- Tenhu, E., Teräsjärvi, J., Cruzeiro, M. L., Savonius, O., Rugemalira, E., Roine, I., ... & Pelkonen, T. (2020). Gene Polymorphisms of TLR4 and TLR9 and Haemophilus influenzae meningitis in Angolan children. *Genes*, *11*(9), 1099.
- Thada, S., Valluri, V. and Gaddam, S. (2013). Influence of Toll-like receptor gene polymorphisms to tuberculosis susceptibility in humans. Scand. J. Immunol.,78(3):221–9.
- Thass, N., M. Kumar and R. Kaur, (2019). Prevalence and antibiogram of bacterial pathogens causing urinary tract infection in a tertiary care hospital. Int. J. Med. Sci. Public Health, 8: 53-57.

- Thattil, S. and Santhosh, S. (2018). Prevalence of UTI in Different Age Groups in a Tertiary Care Hospital and their Antibiogram. International Journal of Contemporary Medical Research. 5(1):3-6.
- Thomas, K. H, Meyn, P. and Suttorp, N. (2007). Single nucleotide polymorphism in 5′-flanking region reduces transcription of surfactant protein B gene in H441 cells. Am J Physiol Lung Cell Mol Physiol. 2006;291(3): L386–L390.
- Thumbikat, P., Waltenbaugh, C., Schaeffer, A. J., and Klumpp, D. J. (2006). Antigen-specific responses accelerate bacterial clearance in the bladder. The Journal of Immunology, 176(5), 3080-3086.
- Thuong, N. T, Hawn, T. R. and Thwaites, G. E. (2007). A polymorphism in human TLR2 is associated with increased susceptibility to tuberculous meningitis. *Genes Immun*.;8(5):422-428.
- Top, K., Buet, A., Whittier, S., Ratner, A. and Saiman L. (2012). Predictors of *Staphylococcus aureus* Rectovaginal Colonization in Pregnant Women and Risk for Maternal and Neonatal Infections. Journal of the Pediatric Infectious Diseases Society. 1(1): 7–15.
- Torres-Garcia, D., Cruz-Lagunas, A., Garcia-Sancho Figueroa, M., Fernandez-Plata, R., Baez-Saldana, R., Mendoza-Milla, C., Barquera, R., Carrera-Eusebio, A., Ramírez-Bravo, S., Campos, L., Angeles, J., Vargas-Alarcón, G., Granados, J. and Gopal, R. (2013). Variants in toll-like receptor 9 gene influence susceptibility to tuberculosis in a Mexican population. J. Transl. Med.,11:220.
- Totsika, M., Moriel, G., Roger, A., Wurpel, J., Phan, D., Paterson, D., Schembri, A. (2012). Uropathogenic E*scherichia Coli* mediated urinary tract infections. *microbioline*: 132(2):376.

- Tsegay, E. (2014). Bacterial Profile and Drug Susceptibility Pattern of Urinary Tract Infection in Pregnant Women Attending Antenatal Care at Mekelle Hospital, Mekelle, Northern Ethiopia. MSc thesis. School of Graduate Studies. Addis Ababa university. Ethiopia.
- Ulett, G., Totsika, C. and Schaale, M., K. (2013). Uropathogenic *Escherichia coli* virulence and innate immune responses during urinary tract infection. Curr Opin Microbiol., 16(1): 100-7.
- Uno, K, Kato K, Shimosegawa T. (2014). Novel role of toll-like receptors in *Helicobacter pylori* - induced gastric malignancy. World J Gastroenterol.;20:5244–51.
- Valerie, S., Chelsie, E., Evans, S., Harry, L., Sara, N., Stephanie, D., Cody, A., Ali, P., Mathew, S., Greta, N. (2018). Rapid Growth of Uropathogenic *Escherichia col*i during Human Urinary Tract Infection. American society of microbiology: 10:00186- 18.
- Valle, G., Carmignani, M., Stanislao, M., Facciorusso, A., Volpe, A. (2012). Mithridates VI Eupator of Pontus and mithridatism. Allergy, 67: 138-139.
- Vasudevan, R. (2014). Urinary tract infection: an overview of the infection and the associated risk factors. Journal of Microbiology and Experimentation. 1(2):42–54.
- Vatan, A., Saltoglu, N., Yemisen, M., Balkan, I. I., Surme, S., Demiray, T. (2018). Diabetic Foot Association between biofilm and multi/extensive drug resistance in diabetic foot infection. International journal of clinical practice, 72(3), e13060.
- Viviane, S. S., Ana Paula, Souza, S., Leif S., Raphael P. P., Renata, F. R., Eloiza, H. C., Márcia, S. P., Lyssa, O. F., Santos, N. M., Ana Carolina, N. B., Renata, C. P., Sérgio, E. L., Fracalanzza, L. W.,

George, S. and Beatriz, M. M. *Staphylococcus saprophyticus* Recovered from Humans, Food, and Recreational Waters in Rio de Janeiro, Brazil. (2017). International Journal of Microbiology, vol. Article ID 4287547, 11 pages,. https://doi.org/10.1155/2017/4287547.

- Wagenlehner, F., Tandogdu, Z., Bartoletti, R., Cai, T. and Cek, M. (2016). The Global Prevalence of Infections in Urology Study: A Long-Term, World wide Surveillance Study on Urological Infections. Pathogens. 5(1):10.
- Wang, Y. L., Tan, M. S., Yu, J. T., Zhang, W., Hu, N., Wang, H. F. and Tan, L. (2013). Toll-like receptor 9 promoter polymorphism is associated with decreased risk of Alzheimer's disease in Han Chinese. J. Neuroinflammation, 10(1): 101.
- West, A. P., Koblansky, A. A., Ghosh, S. (2006). Recognition and signaling by toll-like receptors. Annu Rev Cell Dev Biol, 22: 409-437.
- Wiedemann, B., Heisig, A. and Heisig, P. (2014). Uncomplicated urinary tract infections and antibiotic resistance-epidemiological and mechanistic aspects. Antibiotics 3, 341–352 .
- Winn, W. C.; Allen, S. D.; Janda, W. M.; Koneman, E. W.; Procop, G. W.; Schreckenberger, P. C. and Woods, G. L. (2006). Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Lippincott Williams and Wilkins, USA:234-241.
- World Health Organization. Antimicrobial Resistance. (2000). World Health Organization, Available at: World Health Organization www.who.int/mediacentre/factsheets/ fs194/en/.
- Xie, B. Zhang, H., Peixiang, C., Rongliang, Q. and Xiong, Y. (2006). Distinct glycan structures of uroplakins Ia and Ib: structural

basis for the selective binding of FimH adhesin to uroplakin Ia. J. Biol. Chem. 281, 14644–14653.

- Yang, Z., Wang, L., Yu, H., Wang, R., Gou, Y., Zhang, M., Kang, C., Liu, T., Lan, Y., Wang, X., Liu, J., Cooper, M. A., Li, X., Yue, K., Yu, Y., Wang, L., Kim, B. Y. S., Jiang, W. and Sun, W. (2019). Membrane TLR9 Positive Neutrophil Mediated MPLA Protects Against Fatal Bacterial Sepsis. Theranostics. 14;9(21):6269-6283. doi: 10.7150/thno.37139. PMID: 31534550; PMCID: PMC6735515.
- Yim, J., Kim, H. J., Kwon, O. J. and Koh, W. (2008). Association between microsatellite polymorphisms in intron II of the human Toll-like receptor 2 gene and nontuberculous mycobacterial lung disease in a Korean population. Human Immunology, 69, (9): 572–576.
- Yusuf, J. H., Kaliyaperumal, D., Jayaraman, M., Ramanathan, G. and Devaraju, P. (2016). Genetic selection pressure in TLR9 gene may enforce risk for SLE in Indian Tamils. Lupus, 26(3) 307-310
- Zdziarski, J., Brzuszkiewicz, E. and Wullt, B. (2010). Host imprints on bacterial genomes--rapid, divergent evolution in individual patients. PLoS Pathog. 6, e1001078.
- Zhang, F., Gao, X. D., Wu, W. W., Gao, Y., Zhang, Y. W. and Wang, S. P. (2013). Polymorphisms in toll-like receptors 2, 4 and 5 are associated with Legionella pneumophila infection. *Infection*.;41(5):941-948.
- Zhang, P., Zhang, N., Liu, L., Zheng, K., Zhu, L. and Zhu, J. (2017). Polymorphisms of toll-like receptors 2 and 9 and severity and prognosis of bacterial meningitis in Chinese children. Sci Rep; 7:42796.

- Zhu, J., Martinez, J., Huang, X., & Yang, Y. (2007). Innate immunity against vaccinia virus is mediated by TLR2 and requires TLR-independent production of IFN-β. *Blood*, *109*(2), 619-625.
- Zheng, M., Chen, H., Sun, L., Tao, J., Han, Z., Ju, X., Gu, M., Tan, R. and Li, X. (2022). Association Between a TLR2 Gene Polymorphism (rs3804099) and Proteinuria in Kidney Transplantation Recipients. Front. Genet. 12:798001.
- Ziakas, P. D., Prodromou, M. L., El Khoury, J., Zintzaras, E. and Mylonakis, E. (2013). The role of TLR4 896 A> G and 1196 C> T in susceptibility to infections: A review and meta-analysis of genetic association studies. PLoS ONE,8(11): e81047.

# الخلاصة


هدفت الدراسة الحالية إلى دراسة التغاير الوراثي للمستقبل Toll like 2 ، و Toll like reseptor 9 للمرضى التهاب المسالك البولية. تم جمع عينات الدم والبول للمرضى والمرضى الصحيين كمجموعات ضابطة من محافظة للفترة ما بين تشرين الأول 2020 الى أذار 2021. اشتمل البحث على دراسة لكلا الجنسين للأعمار ما بين 48-18 سنة.

تضمنت الدراسة ثلاثة محاور رئيسية: الأول يشمل عزل وتحديد البكتيريا ، ، بينما المحور الثاني تضمن دراسه جزيئية للكشف عن تعدد الأشكال عن طريق تقنية (ARM-PCR) للبعض المناطق الجينيه ومستقبل Toll likereseptor 2 و Toll like reseptor 9 والثالث هو قياس مستوى تركيز مستقبل Toll like reseptor 2 وكذلك قياس تركيز Toll like reseptor 9 باستخدام ELISA في المصل والادرار

أظهرت الدراسة الحالية أن أعلى نسبة إصابة بالتهاب المسالك البولية تحدث في فارق العمر28-38 سنة بينما أقل نسبة إصابة بأمراض المسالك البولية كانت في الفئة العمرية38 -48 سنة كما اشتملت الدراسة على تحديد بكتيريا مختلفة من عينات البول بعد الزرع ثم تحديدها بواسطة نظام Vitek 2 ، وأظهرت أعلى نسبة بكتريا (21,5٪ *Escherichia coli*.80) ، تليها بكتريا *Enterococcus faecalis* بواقع 50عزله وبنسبه 13.44% ، كانت نسبة الكلبسيلا( 10.75%) بواقع 40 عزله ونسبه *Candida albicans* ونسبه *Staphylococcus saprophyticus* (4.3%)14 (12.1%) 46 على التوالي

تضمنت الدراسة الجزيئية تعدد الأشكال للمستقبلات TLR2 و TLR9 لل TLR2 (rs3804099) عدم وجود فروق معنويه ذات دلالة إحصائية بين الأنماط الجينية TT) TC CC ) (rs3804099 لجينTLR2 أظهر التركيز أن توزيع الأنماط الجينية في كل من المرضى والأصحاء لم يختلف بشكل كبير فيTLR2 ، لكنه أظهر فروقًا معنوية بين أليل T وأليل C لتركيز TLR2 كما تبين انه تشكل عامل خطوره من قيمه نسبه

الارجحيه كانت 5.2, 2.02 ل TC,CC على التوالي كما يظهر اليل C عامل خطر للاصابه

كما أظهرت هناك علاقه كبيره بين تركيز TLR2 في المصل والأنماط الوراثيه TC,CC

لجين ( rs3804099) لجينTLR2 وكانت قيمة المعنويه 0,28ولايوجد فرق معنوي بين تركيز TLR2 في الادرار و الأنماط الوراثيه TC,CC ( rs3804099) لجينTLR2

كما أظهرت نتائج السكونس وجود سته من تعدد الاشكال النقطي في منطقه الثانيه المشفره اكسون 2 لل *TLR9* وخمسه من تعدد الاشكال النقطيه في منطقه المشغل لTLR9 غير مسجله مسبقا

الاستنتاج المهم لهذه الدراسة أن البكتيريا عندما تصيب القناة البولية زادت من المناعة الخلطيةغير النوعية بسبب زيادة التعبير عن مستقبلات التعرف على الأنماط. ووجد ان تراكيز TLR2 وTLR9 ترتفع في المرضى في السيرم والادرار عن الاصحاء .

**جمهورية العراق**

**وزارة التعليم العالي والبحث العلمي**

**جامعة بابل / كلية العلوم**

**قسم علوم الحياة**

# دورتعدد اشكال جين TLR2و TLR9 للمصابين بالتهاب المجاري البولية

**أطروحة مقدمة**

**الى مجلس كلية العلوم / جامعة بابل وهي جزء من متطلبات نيل درجة الدكتوراه فلسفة في العلوم/علوم الحياة**

**من قبل**

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**بإشراف**

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1444ه 2022 م