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Bacteriological and systemic immunity study of patients with urinary tract infection in Babylon province

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***Abstract*---**One hundred mid stream urine specimens and blood sample and fifty healthy specimen[urine and blood]as control for detection TLR-2 in serum have been collected From October to March 2021, urine specimen from urinary tract infection patients in Hospital Hilla were cultivated for isolation and identification in Uropathogenic bacteria can be found in a variety of places. The automated VITEK-2 compact system was used to perform the final identification, which was based on colony positive identification, morphology, microscopic examination, and biochemical tests, while final identification was done with the automated VITEK-2 compact system using Gram positive-identification morphology, microscopic examination, and biochemical tests [GP-ID] and Gram negative-identification [GP-ID] [GN-ID]. The current study's findings revealed that samples grew bacteria, which were classified into Gram negative bacteria [51.48%] and Gram positive bacteria [51.48%]. [48.52 percent]. E. coli was the culprit. 80 [21.5%] followed by *Klebsiella pneumoniae*40 [10.75%], and then *Staphylococcus aureus*36 [9.6%], 35[9.4%] for each *Staphylococcus epidermidis,* and *Staphylococcus saprophytics* 16[4.3%] while *Enterococcus* faecalis50[13.44%], while *Enterococcus facium 40[10.75%] Streptococcus agalactiae* 2[0.5%]and *Enterobacter cloacae* at a rate of 2[0.5%] and finally each of *Morganella morganii* 10[2.68%], *Pseudomonas aeruginosa* 40[10.75], *Proteus mirabilis* 20[5.3%].*Candida albicans 46.*

***Keywords*---**Urinary Tract Infection, bacteria, antibiotic, toll-like receptor 2 [TLR-2].

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Introduction

Urinary tract infections [UTIs] are the most prevalent bacterial disease that necessitates medical treatment, accounting for 8.6 million ambulatory care visits in 2007, with 23% of those visits occurring in the emergency department room. [1] In Iraq, urinary tract infectious are frequent, accounting for 23% of all illnesses [2] Between 2006 and 2009, In the United States, approximately 10.8 million patients sought treatment for UTIs in emergency rooms, with 1.8 million [16.7 percent] being admitted to acute care hospitals. *Coagulase-negative Staphylococci like Staphylococcus saprophyticus and S. epidermidis E. coli, Klebsiella, Proteus, Pseudomonas, Streptococci, Staphylococcus saprophyticus, S. epidermidis, and coagulase-negative enteric bacteria are among the staphylococci, Serratia, enterobacter, and Gramnegative enteric bacteria. staphyloccus* are among the most common pathogens that cause UTI.[3] The prevalence of urinary tract infections varies with age, gender, catheterization, hospitalization, and antibiotic use [4]. Although bacteria are the most common cause of urinary tract infections, viruses, fungi, and parasites may also play a role in the spread of the infection [5,6]. Gram-negative bacteria cause 90% of UTIs, with gram-positive bacteria accounting for the remaining 10%. According to a previous study, Escherichia coli [E. coli] is the most prevalent cause of UTIs, accounting for 65–90% of infections [7,8]. Enterococcus species, Klebsiella pneumoniae [K. pneumonia], Citrobacter species, Pseudomonas aeruginosa [P. aeruginosa), and Staphylococcus coagulase negative are some of the uropathogens that cause UTIs [CoNS] Laboratory tests as well as a review of the patient's clinical symptoms are used to diagnose UTIs. [9] Most UTI bacteria are becoming multidrug resistant [MDR] as a consequence of their greater resistance to routinely used medications [10]. Antibiotic use frequently results in an increase in resistant microorganisms [11]. Treatment failure in UTIs is, in fact, one of the key drivers of increasing resistance [12].

Antimicrobial drug resistance has increased dramatically in the treatment of common infectious pathogens. Antibiotics such as quinolones are no longer effective against E. coli infections, which are the most common cause of urinary tract infections. in society. infections [UTIs] in many parts of the United U.s. and around the globe. More than half of the patients treated with carbapenem antimicrobial drugs, which are considered "last-line" medications for treating highly resistant gram-negative bacteria, have been ineffective [13]. TLRs are elements of the bodies natural innate immune system. Pattern recognition receptors are a kind of receptor that recognizes pattern [PRRs].

TLRs could be present TLR1, TLR2, TLR4, macrophages, immature dendritic cells, natural killer cells, T- and B-cells [TLR7, TLR9, TLR10], and tissue cells [TLR7, TLR9, TLR10] are all involved in TLR activation [enterocytes, epithelial cells of the kidney, mesangial cells] TLRs use homophilic and heterophilic interactions to detect and identify pathogen-associated molecular patterns, allowing immune cells and pro-inflammatory cytokines to protect mucosal barriers.... There really are approximately 12.5 members of the TLR system, which has evolved to be evolutionary conserved across various microorganism species. In humans, up to 11 TLRs have indeed been identified. TLRs bind to a large variety of ligands, both natural and synthetic. TLR signals bind to LPS [TLR4], peptidoglycans [TLR2], viral [double-stranded] RNA or DNA motifs [TLR3], extracellular matrix

components, heat shock proteins, synthetic lipopeptides, and oligodeoxynucleotides, and activate the nuclear transcription factor NF-B, causing cells to secrete cytokines like TNF [TNF] Pathogen identification and host defense are both aided by TLRs. in urinary tract infections. [14,15] as patient-related immunomodulation variables.

In the engagement and reaction, both the adaptive and innate immune systems are involved. In UTIs, it's necessary for adequate host defense against bacterial virulence factors [Complement factors, TLR, etc.] TLR4 [the human TLR4 gene, which may be found on chromosome 9q32q33] was the first PRR to recognize a specific ligand: for example, bacterial endotoxin [LPS] is recognized by a receptor complex that includes CD14, LPS-binding protein [LBP], TLR4, and the MD-2 component. LBP carries LPS to membrane-bound CD14 [mCD14], which distributes it to the TLR4–MD-2 receptor complex. MD-2 is an anchorless protein that is linked to MD-1, a secretory protein found in B lymphocytes. TLR4 has been able to function thanks to MD2. mice lacking the MD2 gene do not respond to LPS. TLR4 signaling can, however, be triggered even when CD14 and MD-2 are not present.in some circumstances [16]. The goal of this study was to isolate bacteria that cause UTIs, perform antibiotic sensitivity testing, and evaluate TLR2 serum levels between patients and healthy individuals.

Materials and Methods

Sample collection

One hundred mid stream urine specimens Patients' information was gathered who are having to deal with urinary tract infection in Hila During the months of October to March, I worked at a hospital. 2021 for both genera with age ranging from [18-50] years and 50 as healthy [control].

Bacterial culturing and identification

To make a diagnosis and determine the bacterium that causes a urinary tract infection, you'll need to only individuals who seemed to have a positive urine culture were tested. Each urine sample was inoculated onto MacConkey Agar, Blood Agar Base, and Muller-Hinton Agar plates, as well as other culturing methods, such as Simmons Citrate Agar, are used to find unknown microorganisms [this differential medium used to identify bacteria based on citrate utilization, especially for the Enterobacteriaceae spp.]. All inoculation plates were kept at 37°C for 18–24 hours in an aerobic environment. The plate was cultivated with a sufficient population of bacteria, as well as bacteria isolated and subcultured on selective plates containing, and the cultures were examined microscopically and biochemically. Scientists have used a variety of approaches to identify bacteria species, including studying bacterial growth features [color, texture, growth pattern, and so on] and Gram staining [27].

Serum level of TLR-2

The concentration of TLR-2 was determined by sandwich ELISA test were calculated by using stander curve this test achieved according to the company [Elabscience].

Statistical method

The study was using Version 23 of The data was analyzed using the Statistical Package for Social Science [SPSS].the different biomarkers. To evaluate percentage, Chi-square analysis has been used, and analysis of variance [ANOVA], least significant difference, and Duncan test or t-test have been used to compare the means.

Results

Urine culture was done for all of the 100 samples of urine The current study's findings showed that samples gave bacterial growth, bacterial are divided to Gve-[51.48%] and Gve+[48.52%]. *E.coli* was the most common 80 [21.5%] followed by *Klebsiella pneumoniae40* (10.75%), and then *Staphylococcus aureus*36 [9.6%], 35[9.4%] for each *Staphylococcus epidermidis,* and *Staphylococcus saprophytics 16[4.3%]* while *Enterococcus faecalis50[13.44%],* while *Enterococcus facium 40[10.75%] Streptococcus agalactiae 2[0.5%]*and *Enterobacter cloacae* at a rate of 2[0.5%] and finally each of *Morganella morganii* 10[2.68%], *Pseudomonas aeruginosa 40[10.75], Proteus mirabilis 20[5.3%].Candida albicans 46*.show in table [1]

Table [1]
Frequencies and percentages of bacterial types

Bacteria	No.	%
E.coli	80	21.5%
Staphylococcus aureus	36	9.6%
Klebsiella pneumonia	40	10.75%
Staphylococcus epidermidis	14	4.3%
Staphylococcus saprophytics		
Enterococcus faecalis	50	13.44%
Enterococcus facium	40	10.75
Streptococcus agalactiae	2	0.5%
Enterobacter cloacae	2	0.5%
Morganella morganii	10	2.68
Pseudomonas aeruginosa	40	10.75
Proteus mirabilis	20	5.3%

Antibiotics sensitivity test

The antibacterial This susceptibility testing was conducted out through using the diffusion disc method for the isolated bacteria to the common antibiotics that used in the treatment of UTI. The isolated bacteria high sensibility to amikacin, tobramycin and resistances to other antibiotic such as trimethoprim. A large

number of bacterial isolated were used in this study was the erythromycin resistance to this antibiotic, trimethoprim and gentamycin
The result of antibiotic sensitivity test of E.coil show that sensitive meropeneme, amikacine and gentamycin Proteus mirabilis also showed a sensitivity to and meropeneme, amikacine gentamycin and ciprofloxacin.

Serum level of TLR-2 concentration

TLR2-TLR6 heterodimers are 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 Mycoplasmas and mycobacteria possess lipoproteins. TLR4 is a signaling receptor, on the other hand for lipopolysaccharide [LPS]. [20] [21]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 less than 0.045 show in table [2] this study agree with study show that A In patients, The expression of CD14/TLR2 was found to be higher [90,07%] than in controls [85,48 percent] Children who have had one or more bouts of acute UTI have increased TLR2 and TLR4 expression on CD14 positive monocytes than children who have not. I've never had a severe UTI. This could indicate the activation of a signaling systems that will aid in the eradication of germs from the urinary tract. This study could TLRs may have a role in the genesis of UTI in children, according to an increasing body of evidence. [22]

Table [2]
Mean TLR-2 serum concentration in UTI patients and control

Parameter TLR-2 in patients and control	M±SD	P value[sig]
patient	3.877 ±1.497	
Control	3.318 ± .4733	0.045*

The age groups of the patients and the controls are shown in the table below [3].

Table [3]
Effect of age group between patients and control

Age /years	M±SD of TLR-2 Patients	control	p- value
[18-28]	3.953 ± 3.754	1.560 ± 0.877	0.22
[29-39]	6.031 ± 6.643	3.140 ± 2.070	0.27

Discussion

In our study found the Gve-more than Gram positive bacteria 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 [17]and this is similar to some of other studies in variable regions [18].

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 THE Al-Jebouri [19] who found the most isolated was *E.coli* bacteria and differ from the result of Ibrahim [17] The most isolated pathogen was *Pseudomonas aeruginosa*. in Antibiotics sensitivity test this study agree with study [17] who found that the most of bacterial isolated from study resistances to gentamycin and agree with previous studies in USA. [23] India [24], and Iraq [19] Resistance to basic antibiotics used in therapy is extremely prevalent of UTI such as cephotaxime, Ampicillin, Gentamicin has pointed to a real problem [22], The result of antibiotic sensitivity test of E.coil show that sensitive meropeneme ,amikacine and gentamycin this study was similar to study of Ghajiri etal [25] 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. It works by forming a bond with the target bacterial 30s subunitof ribosomal, causing mRNA misreading, preventing the bacterium from generating essential proteins for growth.

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. [26]

These bacteria *Proteus mirabilis* also showed a sensitivity to and meropeneme, amikacine gentamycin and ciprofloxacin this study differ from the study of Ghajiri *etal* [25] who found that meropenem may be a safe option for now and showed All antibiotics tested in this study [including amoxicillin] were found to be resistant, and all antibiotics tested in this study were found to be resistant. [including amoxicillin] [including amoxicillin]. [amoxicillin ciprofloxacin, gentamicin, amikacin].

Serum level of TLR-2 concentration

TLR2-TLR6 heterodimers are 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 Mycoplasmas and mycobacteria possess lipoproteins. TLR4 is a signaling receptor, on the other hand. For lipopolysaccharide [LPS] in this study also included effect of age group along with many individuals that have a urinary tract infection by measurement TLR2 levels were measured in ill and healthy persons as part of a study. And, according to the findings of this study, there is no statistically significant difference between the two groups.

Conclusions

The results of the present study indicate that the patients with febrile Urinary tract infection males and females causes gram positive than gram negative bacteria. The present study indicate that the patients with Febrile urinary tract infection had significantly higher Toll-like receptor2 than control group.

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The authors declare no conflict of interest."

Author Contributions:
Exhaustive analysis in working and writing by Haem Adnan Ali and supervised by Frial Gemeel Abd in proof reading and analysis.

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