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The Association between *TLR4* Gene Polymorphism (*TLR4*-Rs11536889 G/C) and Urinary Tract Infections Caused by Multidrug-Resistant Bacteria among Iraqi Patients

Akram Radhi Salim *[1a] and SEÇİL AKILLI ŞİMŞEK [2b]

[1]*Al-Rifae General Hospital, Thi-Qar province, Iraq*

[2]*College of Science , Çankırı karatekin university , Çankırı ,Turkey*

[b]E-mail:secilakilli@gmail.com

[a*]Corresponding author: akrmrady56@gmail.com

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***Abstract*— This current study investigated the association between functional gene polymorphisms Toll-like receptors 4 (*TLR4* rs11536889) and susceptibility to multidrug-resistant (MDR) bacterial urinary tract infections (UTIs). A total of 350 urine samples were collected from male and female patients aged 10 to 75 years at Al-Rifae General Hospital in Thi-Qar province, southern Iraq, between July 2022 and January 2023 .All bacterial isolates were identified biochemically and by the VITEK-2 system. All isolates were then confirmed with the 16S rRNA gene and resistance genes were detected using bacterial colonies in a PCR test. Tetra-ARMS-PCR was used to detect *TLR4* gene polymorphism (rs11536889). Of these, 150 samples (42.9%) showed positive bacterial growth, while 200 samples (57.1%) were culture-negative. The samples that were positive for bacterial growth were 150 samples, including 30 XDR samples, 20 sensitive to antibiotics, and 100 MDR samples Pathogens isolated from MDR samples included *Escherichia coli* (44%), *Staphylococcus aureus* (33%), *Klebsiella pneumoniae* (10%), and *Proteus mirabilis* (13%). Genotypic analysis of the *TLR4* rs11536889 single nucleotide polymorphism SNP (*TLR4* rs11536889) indicated that individuals carrying the homozygous CC and heterozygous GC genotypes exhibited a significantly increased risk of developing MDR UTIs, suggesting a potential role for this SNP (*TLR4* rs11536889) in host susceptibility to infection, therefore, this polymorphism may serve as a valuable genetic biomarker for the early diagnosis of UTI susceptibility and could contribute to personalized therapeutic approaches, helping to identify individuals at greater risk for resistant infections and to guide appropriate preventive or treatment strategies.**

Keywords—: Gene polymorphism, Multiple drug resistance, TLR4, UTI

I. INTRODUCTION

Urinary tract infections (UTIs) are one of the greatest prevalent bacterial diseases in people, both in the public and in hospitals, UTIs severe concern that affects millions of individuals every year the second most prevalent form of infection in the body is urinary tract infections [1] after respiratory infections UTIs are the most common human infections and they are also the main source of nosocomial (hospital-acquired) infections in hospitalized [2]. There are many urinary tract bacterial pathogens responsible for this infection.*Escherichia coli (UPEC*) is the predominant causative agent of UTIs, accounting for over 80% of community-acquired cases [3]. Other pathogens include (*Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus saprophyticus, Enterococcus faecalis*, Group B *Streptococcus, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Candida spp*), which are especially significant as agents of hospital-acquired and catheter-associated infections [4]. Multiple drug resistance (MDR), multidrug resistance , or multiresistance is antimicrobial resistance shown by a species of microorganism to at least one antimicrobial drug in three or more antimicrobial categories [5].

Animal cells depend on germline-encoded pattern recognition receptors (PRRs) to trigger protective innate immune responses. PRRs identify invading microbial pathogens through the combination of pathogen-associated molecular patterns (PAMPs) from the pathogens and danger-associated molecular patterns (DAMPs) generated by damaged or infected tissues [6]. PRRs can be categorized into 8 well-defined groups: Toll-like receptors (TLRs), retinoic acid-inducible gene-I (RIG)-I-like receptors (RLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), C-type lectin receptors (CLRs), opsonic receptors, AIM2-like receptors (ALRs), scavenger receptors (SRs), and stimulator of interferon genes (STING) [7].

Only a limited number of studies have conducted comparisons between multiplex PCR and urine culture in relation to the diagnosis of UTIs and acute cystitis [8]. The tetra-primer amplification refractory mutation system-polymerase chain (ARMS-PCR) can be employed to diagnose and categorize the specific mutations responsible

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for the condition this information proves indispensable for genetic counseling family planning. Early intervention ARMS-PCR finds application in forensic genetics, primarily for human identification in cases involving DNA profiling and paternity testing it plays a crucial role in distinguishing individuals by specific genetic markers, ensuring the reliability and accuracy of results. In the realm of research and epidemiology, ARMS-PCR serves as a tool for exploring genetic variations within populations. This data contributes to our understanding of allele or mutation prevalence and their connections to diseases, thus informing public health strategies and guiding research directions. Particularly in diseases with a genetic component, genotyping with ARMS-PCR can be instrumental in the early detection of such conditions [9].

The present study aims to evaluate the association between functional polymorphisms *TLR4*-rs11536889 and susceptibility to develop multidrug-resistant bacteria in urinary tract infections by the following objectives:

1. Association between *TLR4* gene polymorphism and urinary tract infections caused by multidrug-resistant bacteria in Iraqi patients in the southern regions.
Detection of *TLR4* receptor gene polymorphism *TLR4*-rs11536889 G/C in whole blood samples of patients with UTI and healthy controls by amplification refractory mutation system polymerase chain reactions (ARMS-PCR).

II. MATERIALS AND METHODS

In this study, 350 urine samples were collected from male and female patients between July 2022 and January 2023. For this purpose, face-to-face interviews were conducted with patients who applied to Al-Rifae General Hospital in Thi-Qar province in southern Iraq. During these interviews, patient age, Sex, signs and symptoms, and whether or not they had received antibiotic treatment in the week before sample collection were noted.

Urinary tract infection patients were diagnosed according to clinical and laboratory criteria. Clinical criteria were based on signs and symptoms, while laboratory criteria included general urine examination.

Microscopic analyses were performed for the presence of pus cells, bacteria, and positive urine culture containing <105 CFU/mL bacteria in the patient and control groups.

Sterile screw-capped containers were used to collect midstream urine samples for definitive diagnosis of urinary tract infections. All samples were then inoculated onto Blood Agar and MacConkey Agar. A sterile standard ring (0.001 mL) was inoculated at 37°C for 24 h. Cell morphology of pure bacterial colonies was examined using Gram staining reagents. All bacterial isolates were identified biochemically and by the VITEK-2 system.

All isolates were then confirmed with the 16S rRNA gene , and resistance genes were detected using bacterial colonies in a PCR test. Tetra-ARMS-PCR was used to detect *TLR4* gene polymorphism (rs11536889) in patient and healthy control

A. Blood samples collection

Blood samples were preserved in tubes containing K3-EDTA and stored at 4°C until DNA was extracted and purified. Amplification refractory mutation system polymerase chain reaction (ARMS-PCR) was used to detect *TLR4* gene polymorphism (rs11536889) in patient and healthy control blood samples. The extracted total DNA was checked by using Nanodrop (Thermo Scientific NanoDrop Lite UV Visible Spectrophotometer, USA) which measured DNA concentration (ng/µL) and checked the DNA purity at absorbance (260 /280 nm).

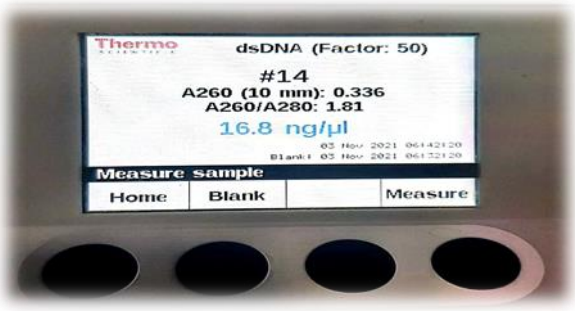

Figure 1: Thermo scientific nanodrop

The PCR products were analyzed by agarose gel electrophoresis method as follows

1. 1.5% agarose gel was prepared using 0.5X TBE and dissolved in a microwave for 5 minutes, and left to cool to 50°C.
2. Then 3µL ethidium bromide stain was added to the agarose gel solution.
3. Agarose gel solution was poured into the tray after fixing the comb in the proper position and left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray.
4. The gel tray was fixed in the electrophoresis chamber and filled with 0.5X TBE, these .
5. 10µ L PCR product was loaded into each well with 5µ L(DNA marker Ladder) added to the first well. Then electric current was performed at 100 volt and 80 AM for 1 hour.

6. PCR products were visualized using a UV transilluminator

TABLE 1: PCR PRIMERS WITH NUCLEOTIDE SEQUENCES AND PRODUCT SIZES OF BACTERIA ISOLATED FROM URINE . THE PCR PRIMERS FOR MOLECULAR DETECTION OF SOME UTI BACTERIAL BASED *16S RIBOSOMAL RNA* GENE AND SOME .THE RESISTANCE GENES WERE DESIGNED IN THIS STUDY USING NCBI GENBANK DATABASE AND PRIMER 3 PLUS , THESE PRIMERS WERE PROVIDED BY SCIENTIFIC RESERCH.CO.LTD IN IRAQ , AS IN THE FOLLOWING TABLE 1

Primary		Sequence (5'-3')	Product size	Genbank ID number
16S rRNA gene *Escherichia coli*	F	TCCCCATCTTTGTCCATCTC	453bp	LR739012.1
	R	AGAATACCGGTGACGAATGC		
16S rRNA gene *Staphylococcus aureus*	F	GAGGGTCATTGGAAACTGGA	611bp	L37597.1
	R	TAGCACGTGTGTAGCCCAAA		
16S rRNA gene *Proteus mirabilis*	F	CAGAAGAAGCACCGGCTAAC	368bp	LR739004.1
	R	CGGTTTCAAGACCACCAACCT		
16S rRNA gene *Klebsiella pneumoniae*	F	ACCTTGGCGATTGACGTTAC	562bp	HG416956.1
	R	AAGGGCACCAATCCATCTCTG		

B. *Tetra-ARMS-PCR Primers*

This study utilized Tetra-ARMS-PCR primers, the NCBI-SNP *TLR4* -rs11536889 database, and Primer1 ARMS-PCR online design for the *TLR4* rs11536889 gene polymorphism. As indicated in Table 4, these primers were sourced from the Scientific Researcher Co. Ltd. in Iraq.

TABLE 2:PCR PRIMERS, NUCLEOTIDE SEQUENCES , AND PRODUCT SIZES OF BETA-LACTAM ANTIBIOTIC RESISTANCE GENES ASSOCIATED WITH GRAM-NEGATIVE BACTERIA

Primary		Sequence (5'-3')	Product size	Genbank ID number
BlaCTX-M	F	CAGACTGGGTGTGGCATTGA	241bp	OM326869.1
	R	GCTAAGCTCAGCCAGTGACA		
blaSHV	F	TATCGGCCCTCACTCAAGGA	412bp	OL906381.1
	R	ATCGCTCATGGTAATGGCGG		
blaTEM	F	GAGAGTTTTCGCCCCGAAGA	522bp	ON221404.1
	R	AATAAACCAGCCAGCCGGAA		
blaAMPC	F	ACATCTCGCAACCTACACCG	655bp	LC455576.1
	R	ATAGCTACCAAATCCGCCGG		

Table 3: PCR PRIMERS, NUCLEOTIDE SEQUENCES , AND PRODUCT SIZES OF BETA-LACTAM ANTIBIOTIC RESISTANCE GENES ASSOCIATED WITH GRAM-POSITIVE BACTERIA.

Primary		Sequence (5'-3')	Product size	Genbank ID number
*Staphylococcus aureus*mecA	F	ACCTCTGCTCAACAAGTTCCA	683bp	KC243783.1
	R	ACCACCCAATTTGTCTGCCA		
*Staphylococcus aureus*ErmA	F	AGCGGTAAACCCCTCTGAGA	306bp	KT803896.1
	R	ACAGAGTCTACACTTGGCTTAGG		
*Staphylococcus aureus*ermC	F	TGGCTCAGGAAAAAGGGCATT	558bp	JF968539.1
	R	TCGTCAATTCCCGCATGTTT		

TABLE 4: THE TETRA-ARMS-PCR PRIMERS FOR *TLR4* RS11536889 GENE POLYMORPHISM WITH THEIR SEQUENCE AND AMPLICON SIZE

Primer	Sequence (5'-3')	Product size
Forward inner primer (C allele)	TCCTTGACCACATTTTGGGTAC	303bp
Reverse inner primer (G allele)	TGTTTTCTCAATGATAACATCCAGTC	236bp
Forward outer primer	CTCATGAAATGAGTTGCAGCAG	491bp
Reverse outer primer	TAACGGCTACACCATTTTCCAT	

C. *STATISTICAL ANALYSIS*

Data were collected, summarized, analyzed, and presented using the statistical package for the social sciences (SPSS) version 26 and Microsoft Office Excel 2010. Numeric data were presented as mean, standard deviation, after performance of the Kolmogorov-Smirnov normality test and making a decision about normally and non-normally distributed variables. An Independent sample t-test was used to study the difference in mean between any two groups, provided that the variable is normally distributed. One-way Anova test is used to study the difference in means between more than two groups provided that the variable is normally distributed. Chi-square test was used to study association between any two categorical variables. Odds ratio and 95% confidence interval were estimated to measure risk. The level of significance was considered at a P-value of less than 0.05 and a highly significant level at 0.01 or less [10].

III. RESULTS

A. Bacterial Isolation

A total of 350 individuals, aged between 10 and 75 years, participated in the study. Of these, 150 (42.9%) showed positive bacterial growth in their urine samples, while 200 (57.1%) had negative bacterial growth. Among the 150 positive samples, 100 were identified as multidrug-resistant (MDR), 30 as extensively drug-resistant (XDR), and 20 were sensitive to antibiotics

TABLE 5:TYPES OF DRUG RESISTANCE IN BACTERIAL SAMPLES

Bacterial samples	Sensitive n%	MDR n%	XDR n%	PDR n%	*P*
Staphylococcus aureus	5 (%3,3)	33 (%22)	13 (%8,7)	0(%0)	
Escherichia coli	9 (%6)	44 (%29,3)	9 (%6)	0(%0)	0,635 ¥: NS
Klebsiella pneumonia	2 (%1,3)	10 (%6,7)	5 (%3,3)	0(%0)	
Proteus mirabilis	4 (%2,7)	13 (%8,7)	3 (%2)	0(%0)	
Total	20 (%13,3)	100 (%66,7)	30 (%20)	0(%0)	

n: number of cases; SD: standard deviation; ¥: Chi-square test; NS: significant at *P* >0.05.

Percentages of MDR bacteria according to PCR diagnosis of urinary tract infection

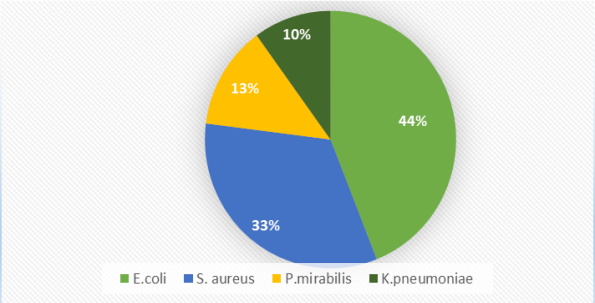

Figure 2: percentages of mdr bacteria according to pcr diagnosis of urinary tract infection

TABLE 6:PERCENTAGES OF MDR BACTERIA ACCORDING TO PCR DIAGNOSIS THE UTI DETECTION OF MECA, ERMA AND ERMC GENES OF S. AUREUS.

Results	Antibiotic resistance genes		
	mecA	***ErmA***	***ermC***
	Frequency	**Frequency**	**Frequency**
Positive, n (%)	33 (100.0%)	33 (100.0%)	33 (100.0%)
Negative, n (%)	0	0	0

SD: standard deviation; n: number of cases; ¥: chi-square test; S: significant at p<0.05

TABLE 7: DETECTION OF *BLACTX-M, BLASHV, BLATEM* , AND *BLAAMPC* GENES IN GRAM- NEGATIVE BACTERIAL SPECIES

Antibiotic resistance genes	Bacterial samples			*p*
	E. coli	***K. pneumonia***	***P. mirabilis***	
BlaCTX-M gene				
Positive, n (%)	36 (81.8%)	10 (100.0%)	4 (30.8%)	0.001 ¥ S
Negative, n (%)	8 (18.2%)	0	9 (69.2%)	
BlaSHV gene				
Positive, n (%)	13 (29.5%)	10 (100.0%)	13 (100.0%)	0.001
Negative, n (%)	31 (70.5%)	0	0	¥ S
BlaTEM gene				
Positive, n (%)	44 (100.0%)	10 (100.0%)	13 (100.0%)	
Negative, n (%)	0	0	0	
BlaAMPC gene				
Positive, n (%)	40 (90.9%)	10 (100.0%)	4 (30.8%)	0.001
Negative, n (%)	4 (9.1%)	0	9 (69.2%)	¥ S

B. Detection of TLR4-rs11536889 Polymorphism

TLR4-rs11536889 polymorphism distribution was detected using the ARMS-PCR technique for100 healthy individuals as controls and 100 MDR patients with UTI. At this locus, three genotypes are present: GG, GC, and CC. The wild-type homozygote genotype exhibited amplification of only the G allele at a product size of 236 bp, while the mutant-type homozygote genotype showed amplification of only the C allele at a product size of 303 bp. The heterozygote genotype exhibited amplification of the G and C alleles at a product size of 491 bp, as shown in Figure 2.

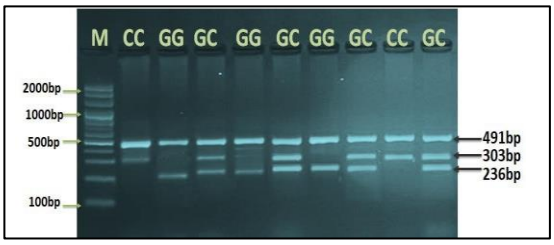

Figure 3: Agarose gel electrophoresis image that showed the T-ARMS-PCR product analysis of the *TLR4*rs11536889 G/C gene polymorphism,

IV. HARDY-WEINBERG EQUATION

TLR4-rs11536889 genotypes (GG, GC, and CC) distribution within the control group was analyzed using the Hardy-Weinberg equation The homozygous wild genotype GG was found in 77 of the 100 control subjects; the heterozygous GC genotype appeared in 13 of the 100 control subjects, while the homozygous mutant CC

genotype was observed in 10 of the 100 control subjects, as shown in Table 8. The distribution of control subjects based on *TLR4*-rs11536889 genotypes showed a highly significant difference from the expected distribution (P = 0.001).

Table 8: Hardy-Weinberg equation genotyped of *TLR4* gene

Genotypes	Observed	Expected	χ^2	*P*
Homozygote reference GG	77	69.7	27.92	0.001 ¥HS
Heterozygote GC	13	27.6		
Homozygote variant CC	10	2.7		

¥: Chi-square test; HS: Highly significant at $P > 0.05$

A. Genotypic and Allele Analysis for the Studied Gene in Patients and Healthy Controls

Table 9 presents the comparison of genotypes and allele frequencies of the *TLR4*-rs11536889 SNP *TLR4*-rs11536889 between patients and healthy controls. In terms of the co-dominant mode, the frequency distribution of genotypes differed significantly between patients and control groups (p = 0.019). The risk analysis showed that the homozygous CC genotype was a considerable risk factor (OR= 1.82), and the heterozygous G/C genotype was a non-significant risk factor with an OR = 2.7 This means that patients with the homozygous CC genotype are about twice more liable to develop disease in comparison with patients with other genotypes. Regarding the dominant mode analysis, there was a significant difference between patients and control groups (p> 0.05). Also, regarding the allele analysis, there was a significant difference between the patients and the control groups (P=0.007). But regarding the recessive mode, there was a non-significant difference between patients and control groups (p> 0.05).

TABLE 9:TLR4-RS11536889 POLY GENOTYPE FREQUENCY IN PATIENTS AND HEALTHY CONTROLS .

Mode	*TLR4-rs11536889*	Patients *n* = 100	control *n* = 100	*P*	OR	95% CI
Co-dominant	CC	14 (14.0%)	10 (10.0 %)	0.019 ¥S	1.82	0.75-4.40
	G/C	27 (27.0%)	13 (13.0 %)		2.7	1.2 -5.70
	GG	59 (59.0 %)	77 (77.0 %)		Reference	
Dominant	CC+G/C	41 (41.0 %)	23 (23.0 %)	0.006 ¥S	Reference	
	GG	59 (59.0 %)	77 (77.0 %)		0.429	0.23-0.79
Recessive	CC	14 (14.0%)	10 (10.0 %)	0.384 ¥S	1.46	0.61-3.47
	G/C+GG	86 (86.0%)	90 (90.0 %)		Reference	
Alleles	C	55 (27.5%)	33 (16.5 %)	0.007 ¥S	1.92	1.18-3.11
	G	145 (72.5%)	167 (83.5%)		Reference	

¥: Chi-square test; NS: not significant at $P > 0.05$; S: significant at $P \leq 0.05$.

TABLE 10:COMPARISON OF FREQUENCY DISTRIBUTION OF GENOTYPE ACCORDING TO SPECIES OF BACTERIA. THE FREQUENCY DISTRIBUTION OF GENOTYPE ACCORDING TO SPECIES OF BACTERIA WAS SHOWN IN TABLE (10). THE PRESENT RESULTS SHOW THERE WAS A SIGNIFICANT ASSOCIATION BETWEEN BACTERIAL SPECIES AND RESULTS OF ARMS-PCR (P = 0.001).

Characteristic	Bacterial species				*p*
	E. coli	*S. aureus*	*K. pneumonia*	*P. mirabilis*	
Genotype					
GG, *n* (%)	28 (63.6 %)	12 (36.4%)	10 (100%)	9 (69.2%)	0.001 ¥
GC, *n* (%)	13 (29.5%)	14 (42.4%)	0	0	
CC, *n* (%)	3 (6.8 %)	7 (21.2 %)	0	4 (30.8%)	S

SD: standard deviation; *n*: number of cases; ¥: chi-square test; S: significant at $p > 0.05$

V. DISCUSSION

Antibiotic resistance is a global health problem due to the increased rates of deaths and healthcare costs associated with resistant bacteria. Antibiotic-resistant bacteria have become a serious threat globally, with antibiotic-resistant bacteria causing more than 700,000 deaths each year, and the number of deaths is expected to rise to 10 million annually by 2050 [11]. A total of 350 individuals aged 10 to 75 years, participated in the study. Of these, 150 (42.9%) showed positive bacterial growth in their urine samples, while 200 (57.1%) had negative bacterial growth. Among the 150 positive samples, 100 were identified as multidrug- resistant (MDR), 30 as extensively drug-resistant (XDR), and 20 were sensitive to antibiotics .The present study agree with [12] who found (64.7%) had an MDR in UTI, and among them, 81.5% were Gram-negative, and 18.5% were Gram-positive isolates . Also, this study agree with [13] who observed a higher MDR (92%) in Gram- negative bacterial isolates and a lower MDR (46.2%) in Gram-positive bacterial the reason for the rise in antibiotic-resistant bacteria can be attributed to several factors: the irrational use of antibiotics is on the rise; antimicrobials are readily available in non-controlled pharmacies; resistance genes are transmitted among individuals and between people; and over-prescription contributes to these issues. These elements promote the development of resistance and lead to extremely high levels of bacterial resistance in the general population, resulting in the excessive use of high-potency antibiotics for UTIs [14].

In the current study, *E. coli* was the most common bacterium isolated from 44 (44%) UTI samples. This result agree with study in Iraq by [15] who showed bacterial infection in UTI was *Escherichia coli* (36%) followed by *S. aurous* (24%) and agree with study in Iraq by [16] who showed bacterial infection in UTI was

Escherichia coli (90.85%) and agree with study by [17] , where 20 (32.8%) *E. coli* were culture positive in a total sample of 61 UTI patients, . In comparison, 9 (14.8%) *S. aureus* were culture positive in a total sample of 61 UTI patients. This study is also consistent with [18] who showed that the most common bacterial isolate was *E. coli* (44.2%), followed by *S. aureus* (21.1%) from UTIs .This study differed from the study conducted in Karbala Governorate by researcher [19] who recorded 6 (11.5%) of *E. coli* bacteria from the total sample, which was 52 positive bacterial growth cultures in patients with urinary tract infection, and 20 (38.5%) of *S. aurous* bacteria from the total sample, which was 52 positive bacterial growth cultures in patients with urinary tract infection the high infectivity of is attributed to its possession of multiple virulence factors, most notably enzymes and adhesion factors, which enable it to overcome the host's mechanical defenses. These properties contribute to enhanced infection severity and the stimulation of acute inflammatory responses, increasing the likelihood of developing urinary tract infections (UTIs) [20].

In this study, we obtained 100% positive results for mecA. This result agreed with the study by [21] .The mecA gene encodes the protein PBP2a, which is responsible for resistance, to methicillin and other drugs in the beta-lactam group. Bacteria carrying this gene encoding mecA can survive in the presence of beta-lactam antibiotics, creating a weak link between PBP2a and these antibiotics .PBP2a can also exhibit cross-resistance to additional antibiotics within the beta-lactam class, making infections caused by bacteria carrying the mecA gene difficult to treat [22] .The resistance of *Staphylococcus aureus* to erythromycin is mostly attributed to a common mechanism that confers resistance to the entire macrolide group Methyl transferase enzymes play a key role in this process, and these enzymes are encoded by three genes known as the *erm* gene [23].

Our findings agree with those of the research by [24] in China, which involved collecting 130 *Escherichia coli* samples from five hospitals across various Chinese cities In this study, bla CTX-M genes were amplified in 126 (96.9%) of the ESBL-producing isolates This also agree with the findings of [25] who isolated 99 samples of *K. pneumonia* This finding aligns with the research by [26] in Tanzania which discovered that 98.9% of *E.coli* isolates were infected with ESBLs (blaCTX-M, blaTEM or blaSHV), while only 1% were negative. Moreover, it aligns with the findings of [27] , which indicated that 1% of the isolates were negative for the tested ESBL alleles,while bla ampC was identified in clinical isolation in 80% of cases. However, it does not align with the research carried out by [28] in Benin City, Nigeria, which demonstrated that 29.7% of 350 samples contained uropathogenic *E. coli* Additionally, this the current study disagree with [29] in Thi-Qar, Iraq which indicated that the rate of gram-negative bacteria was 90 (14.3%) among 630 samples from pregnant women The reported frequency of ESBL in this study can be attributed to the uncontrolled, prescription and sale of antibiotics in Iraq, as well as insufficient, attention to laboratory screening for ESBL production in clinical isolates [27].

Statistical analysis results indicated a clear association between the rs11536889 polymorphism and an increased risk of prostate cancer in a sample of the Korean population [31]. This polymorphism along with other genetic polymorphisms within the *TLR4* gene, was also observed to be associated with an increased risk of chemotherapy-induced neutropenia in children with leukemia [32]. On the other hand, the ability of *E. coli* to form biofilms is a prominent biological characteristic These bacterial aggregates are organized within a complex protective matrix, enabling them to adhere to the surface of the lining of the bladder or urethra These membranes provide a protected environment for the bacteria, making it difficult for both the immune system and antibiotic therapy to effectively access them, thus enhancing resistance to infection. Some strains exhibit a unique pattern in the formation of these membranes , adding additional complexity to the treatment process [33].

We began exploring the genetic factors that may contribute to susceptibility to UTIs, focusing on two SNP *TLR4* rs11536889 , that to our knowledge, have not previously been analyzed in the context of this infection .This novel approach adds an additional dimension to the study's findings, enhancing our understanding of the genetic aspects associated with the occurrence of UTIs.

VI. Conclusions

In summary, the risk analysis indicated that the homozygous CC genotype was a genetic factor that may significantly contribute to the increased risk of developing the disease, with an odds ratio (OR) of 1.82. In contrast, the heterozygous G/C genotype showed a statistically insignificant association , although the OR was relatively high (2.7). These results suggest that individuals carrying the CC genotype are approximately twice as likely to develop the disease as those carrying different genotypes.

Conflict of interest

Authors declare that they have no conflict of interest.

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