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Detection of Genes *ermB*, *mecA*, bla *Z* and *msrA* in Uropathogenic *Staphylococcus aureus* Isolates between the Gram-Positive Bacteria that Cause Urinary Tract Infections

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ABSTRACT

Aims This study aimed to detect gram-positive bacteria that cause UTIs with multiple antibiotic resistance, as well as phenotype and genotype methods to determine some genes in S. aureus isolates.

Materials & Methods This study included 205 urine samples from outpatients with a urinary tract infection. The Polymerase Chain Reaction method was performed to evaluate the genotypic status of the 16S *rRNA*, *erm B*, *mecA*, bla *Z*, and *msrA* genes after DNA extraction.

Findings Only 51 (41.4%) were found to have gram-positive bacteria, isolated bacteria were divided into two groups that include 37 isolates (30.08%) were *Staphylococcus* spp. and 14 isolates (11.3%) diagnosed as *Enterococcus* spp. and *Staphylococcus* spp. divided into two groups that include 17 isolates (13.8%) diagnosed as *S. aureus* and 20 isolates (16.2%) diagnosed as coagulase-negative staphylococci, distributed as 7 isolates (5.6%) diagnosed as S. haemolyticus, 3 isolates (3.4%) diagnosed as *S. epidermidis*, 1 isolate (0.8%) diagnosed as S. vitulinus,4 isolates (3.2%) diagnosed as *S. sciuri*, 3 isolates (2.4%) diagnosed as S. homins, 1 isolate (0.8%) diagnosed as *S. warneri*, 1 isolate (0.8%) diagnosed as *S. lentus*.

Conclusion The most common gram-positive bacteria found to cause UTIs was *S. aureus*. A few pathogens of *S. aureus* were discovered to be resistant to Vancomycin and linezolid in this study. The prevalence of *blaZ* genes, which are responsible for resistance, was found to be high among pathogenic *S. aureus* isolates in Diyala province.

Keywords Uropathogenic *Escherichia coli*; *Staphylococcus aureus;* Urinary Tract Infections; Bacterial Proteins

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Introduction

A urinary tract infection (UTI) is characterized by *Escherichia coli* along with urinary symptoms, as well as the development of a large number of single-species organisms in the urine [1]. In both community and nosocomial settings, UTI is a prevalent health concern. UTI is one of the most frequent illnesses, especially among women [2]. The presence of microbial pathogens in the urinary tract, along with associated symptoms, is known as UTI. Cystitis is a lower UTI, and pyelonephritis is when it affects the upper urinary tract [3]. UTIs are more common in women; approximately one out of every three women will have had a UTI before the age of 24 [4]. *Enterococcus* spp., *Staphylococcus saprophyticus, and* Coagulase-negative Staphylococcus are a pathogen that causes UTIs [5].

Overuse and incomplete antibiotic courses, as well as empirical antimicrobial treatment, have all played a role in the emergence of Multi-Drug Resistant (MDR) bacteria [6]. Antibiotic resistance in uropathogens is becoming more of a problem. Drug resistance may have a significant clinical impact depending on the degree of resistance, the location of the infection, and the availability of safe, nontoxic treatment options [7]. Antibiotic resistance can occur through some mechanisms, including antibiotic degradation or modification, alteration of the antibiotic's bacterial target, target protection, and reduction of the antibiotic's intracellular concentration, either through decreased cell wall permeability or efflux of the antibiotic from the cell [8]. Efflux-mediated resistance has been eclipsed in contrast to other known routes. Many bacterial efflux pumps may expel multiple, unrelated groups of antimicrobial chemicals from the cell, supporting the establishment of multidrug resistance phenotypes [9].

The aim of this study was to detect gram-positive bacteria that cause UTIs with multiple antibiotic resistance, as well as phenotype and genotype methods to determine some genes in *S. aureus* isolates.

Materials and Methods

This study was conducted in Baquba teaching hospital from August 2020 to February 2021 and urine specimens which collected from both sexes in a sterile universal container and processed according to standard microbiological techniques [10]. All urine specimens were examined by microscope (Merseyside, UK) and only specimens have infection cultured on MacConkey agar and blood agar plate (Addgene, USA), identification of bacteria isolates was based on VITEK® 2 advice [10] and antibiotic susceptibility testing was performed also by the same advice. The ability of *S. aureus* bacteria to generate ESBLs was determined using the combined disc process. The bacterial suspension was prepared and separated on Muller Hinton agar-coated Petri dishes, then dried for 10 minutes. In the center of the inoculated plate, a disc containing a combination of Amoxicillin and Clavulanic acid (30mg/Disc) was affixed. The antibiotic discs of Tazobactam, cefuroxime and cefoxitin were then arranged at a distance of 3cm from the disc in the center, and after 24 hours of incubation at 37°C, the presence of an inhibition zone between the disc in the center and one or more antibiotic discs around indicated a positive result.

The ethidium-bromide-agar cartwheel (EtBrCW) procedure was used to identify enhanced efflux activity in large groups of clinical isolates from a variety of bacteria. This procedure uses to compare the ability of different isolates to extrude EtBr. Bacterial isolates were grown in a shaker overnight with Nutrient broth before being changed the next day to 0.5 of a McFarland standard [11]. Radial lines were used to separate the nutrient agar plates into a cartwheel pattern. The bacteria were then swabbed onto nutrient agar plates having an increased concentration of EtBr and cultured for 16 hours at 37°C. An ultraviolet (UV) transilluminator (D-4642; Sigma, St. Louis, MO, USA) was used to study the culture on the plates. Isolates that emit fluorescence at higher ethidium bromide concentrations were thought to have a more active efflux mechanism than isolates that emit fluorescence at lower ethidium bromide concentrations.

Most gram-positive bacterial isolates are resistant to most antibiotics, including tetracycline and oxacillin. The etiology of bacteria that cause UTIs, as well as their resistance to antimicrobials, has changed over time and differs between countries [12]. The genetic study enhances traditional investigation and detection methods by using PCR, an effective tool in clinical microbiology studies that has been commonly used to identify genes of interest [13]. DNA extraction kit (Zymo Research, Irvine, CA, USA) method was successful and efficient for extraction. The extracted DNA was electrophoresed (Microamp; Applied Biosystems, Foster City, CA, USA) on a 1% agarose gel (Addgene, USA), stained with ethidium bromide (Addgene, USA), electrophoresed at 70 volts for 1 hour, and photographed with the UV transilluminator. The sequences of the *16S rRNA, erm B, mecA, bla Z, and msrA* were amplified using specific primers (Table 1). As indicated in Table 2, the PCR used a total volume of 50µl of a mixture. The PCR schedule program for the *16S rRNA, erm B, mecA, bla Z,* and *msrA* genes was shown in Table 3. The PCR results were stained with ethidium bromide and photographed after electrophoresis on a 2% agarose gel.

Table 1) Primers used in the current study

No.	Gene		Sequence Primer (5′→3′)	Product (bp)
1	*16S RNA*	F	GATGACGTCAAATCATCATGC	355
		R	AGGAGGTGATCCAGCCGCA	
2	*ermB*	F	CTATCTGATTGTTGAAGAAGGATT	142
		R	GTTTACTCTTGGTTTAGGATGAAA	
3	*mecA*	F	TGCTATCCACCCTCAAACAG	286
		R	ACGTTGTAACCACCCCAAGA	
4	*msrA*	F	TCCAATCATTGCACAAAATC	163
		R	AATTCCCTCTATTTGGTGGT	
5	*bla Z*	F	ACTTCAACACCTGCTGCTTTC	173
		R	TGACCACTTTTATCAGCAACC	

Table 2) Polymerase Chain Reaction materials used in the current study

Components and Concentration	Volume (50 µl)
2X PCR *Taq* Master Mix (1X)	25
Forward primer (10 µM/µl)	4
Reverse primer (10 µM/µl)	4
ddH_2O	13
DNA (40 ng)	4

Table 3) Programs of PCR thermo-cycling (Agilent, Palo Alto, CA, USA) conditions used in the current study

Phase	Temperature (°C)	Time (sec)	N of Cycles
Initial denaturation	94	300	1
Denaturation	94	30	35
Annealing	56	30	
Extension	7	60	
Final extension	72	300	1

Findings

Gram-negative bacteria were the most prevalent bacteria, with 72 isolates (58.5%), followed by gram-positive bacteria with 51 isolates (40%). Biochemical tests revealed that gram-negative bacteria were the most prevalent bacteria, with 123 (60%) having bacterial positive culture and 82 (40%) having bacterial negative culture (41.4%).

According to the results of the current study's cultural, microscopical, and biochemical tests, 51 different gram-positive bacterial isolates from 205 urine samples of patients with UTI were divided into two groups: 37 isolates (30.08%) were *Staphylococcus* spp. and 14 isolates (11.3%) were *Enterococcus* spp. *Staphylococcus* spp. divided into two groups that include 17 isolates (13.8%) diagnosed as *S. aureus* and 20 isolates (16.2%) diagnosed as Coagulase-negative staphylococci (CONS), distributed as 7 isolates (5.6%) diagnosed as *S. haemolyticus*, 3 isolates (3.4%) diagnosed as *S. epidermidis*, 1 isolate (0.8%) diagnosed as *S. vitulinus*,4 isolates (3.2%) diagnosed as *S. sciuri*, 3 isolates (2.4%) diagnosed *as S. homins,* 1 isolates (0.8%) diagnosed as *S. warneri*, 1 isolates (0.8%) diagnosed as *S. lentus*. *Enterococcus* spp. represents (14 isolates,11.3%) from the total isolates, 7 isolates (5.6%) diagnosed as *E. faecalis*, 5 isolates (4.06%) diagnosed as *E.faecium* and 2 isolates (1.6%) diagnosed as *E.avium*. The multidrug resistance phenotype of gram-positive bacteria was 29.1% (Figure 1).

The multidrug resistance phenotype of gram=positive bacteria was shown by the *S. aureus* 41.1%, CONS 25%, and E*nterococcus* spp. 21.4%. Most *S. aureus* isolates were highly resistant to most antibiotics, especially Oxacillin, according to the results of antibiotic susceptibility tests. The antibacterial effects of erythromycin and vancomycin were the best against the majority of isolates (Table 4).

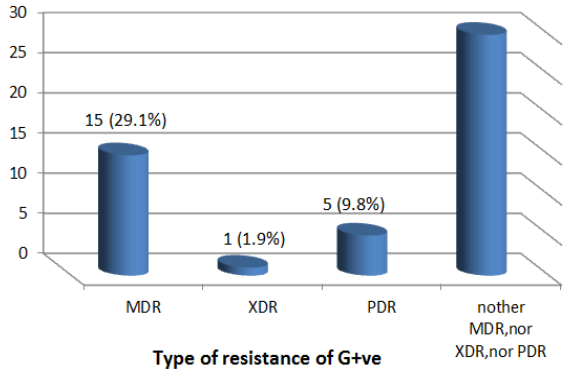

Figure 1) Incidence of MDR, XDR, and PDR Gram-Positive bacteria isolated from UTIs patients. MDR: Multidrug resistance; XDR: Extensive drug resistance; PDR: Pandrug resistance.

Table 4) Antimicrobials susceptibility test of isolates *S. aureus* and test of isolates CONS and test of isolates *Enterococcus* spp. from the urine of outpatients infected with UTI

Antibiotic		S (100%)	I (100%)	R (100%)
***S. aureus* 17 (100)**				
Benzylpenicillin	P	11 (64.7)	0 (0)	6 (35.2)
Ciproflaxcin	CIP	14 (82.3)	0 (0)	3 (17.6)
Clindamycin	CM	10 (58.8)	0 (0)	7 (41.1)
Erythromycin	E	15 (88.2)	0 (0)	2 (11.7)
Fosfomycin	FOS	10 (58.8)	0 (0)	7 (41.1)
Fusidic acid	FA	11 (64.7)	1 (5.8)	5 (29.4)
Gentamycin	GM	9 (52.9)	1 (5.8)	7 (41.1)
Imipenem	IMP	12 (70.5)	2 (11.7)	3 (17.6)
Linezolid	LNZ	13 (76.4)	1 (5.8)	3 (17.6)
Moxifloxacin	MXF	12 (70.5)	1 (5.8)	4 (23.5)
Oxacillin	OX1	7 (41.1)	1 (5.8)	9 (52.9)
Rifampicin	RA	11 (64.7)	1 (5.8)	5 (29.4)
Teicoplanin	TEC	13 (76.4)	0 (0)	4 (23.5)
Tetracycline	TE	10 (58.8)	0 (0)	7 (41.1)
Tigecycline	TGC	14 (82.3)	0 (0)	3 (17.6)
Trimethoprim/sulfamethoazole	SXT	10 (58.8)	0 (0)	7 (41.1)
Vancomycin	VA	15 (88.2)	0 (0)	2 (11.7)
CONS 20 (100)				
Benzylpenicillin	P	12 (60)	0 (0)	8 (40)
Ciproflaxcin	CIP	14 (70)	1 (5)	5 (25)
Clindamycin	CM	13 (65)	2 (10)	5 (25)
Erythromycin	E	14 (70)	0 (0)	6 (30)
Fosfomycin	FOS	14 (70)	2 (10)	4 (20)
Fusidic acid	FA	13 (65)	1 (5)	6 (30)
Gentamycin	GM	14 (70)	0 (0)	6 (30)
Imipenem	IMP	14 (70)	1 (5)	5 (25)
Linezolid	LNZ	18 (90)	0 (0)	2 (10)
Moxifloxacin	MXF	14 (70)	0 (0)	6 (30)
Oxacillin	OX1	12 (60)	0 (0)	8 (40)
Rifampicin	RA	14 (70)	0 (0)	6 (30)
Teicoplanin	TEC	16 (80)	1 (5)	3 (15)
Tetracycline	TE	19 (95)	1 (5)	0 (0)
Tigecycline	TGC	15 (75)	0 (0)	5 (25)
Trimethoprim/sulfamethoazole	SXT	19 (95)	0 (0)	1 (5)
Vancomycin	VA	19 (95)	0 (0)	1 (5)

Most Coagulase-Negative *Staphylococcus* isolates were highly resistant to most antibiotics, especially

Oxacillin and Benzylpenicillin, according to the results of antibiotic susceptibility tests. Vancomycin and Trimethoprim were the most effective antibacterial agents against the majority of isolates. The results of antibiotic susceptibility tests showed that most CONS isolates were highly resistant to most antibiotics, according to the results of antibiotic susceptibility tests. The antibacterial effect of linezolid was the best against the majority of isolates.

Extended-spectrum β-lactamase enzyme production was used to detect *S. aureus* isolates ability to produce ESBLs enzyme. 9 (52.9%) of *S. aureus* isolates are ESBLs enzyme producers. Efflux pump activity, The results showed that 15 (88.2%) isolates have the efflux pump. Efflux pump activity occurs in uropathogenic *S. aureus* significantly (p=0.002).

The most ten isolates of *S. aureus* resistant to antibiotics were diagnosed based on the *16S rRNA* gene and the results showed that all isolates contained this gene (Figure 2).

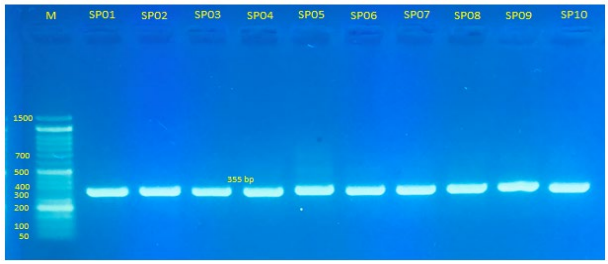

Figure 2) Agarose gel electrophoretoGram detect *16S rRNA* gene (355 bp) products of *S. aureus*, the gel was 1.5% and the DNA dye is RedSafe, V: 70, Time: 42 minutes. M: DNA ladder

Ten isolates were investigated for the presence of genes encoding macrolide, methicillin, and penicillin resistance. The study demonstrated that *S. aureus* isolates *which* positive for each of *ermB* (60%), *mecA* (60%), *blaZ* (100%), and *msrA* (20%).

Discussion

According to the results, the number of isolates that produced a positive result for bacterial culture was 123 (60.0%), whereas the number of isolates that gave a negative result was 82 (40.0%), these results are different from the results of [14] as the percentage was (32.6%). *S. aureus* was shown to be the most prevalent gram-positive bacteria responsible for UTIs, with a percentage of isolation reaching 13.8. Gram-positive bacteria account for (41.4%) of all bacteria, mostly Enterococci, and Staphylococci, The high frequency of *S. aureus* in UTI is not unique to this study. Earlier studies [15-20] reported high rates of *S. aureus* (43.6%, 22.5%, 28%, 31.4%, 40.4%, and 39.15%), respectively.

CONS represents (20 isolates,16.2%) of the total isolates, and the high prevalence diagnosed for *S. haemolyticus* followed by *S. epidermidis*. This study agreement with an earlier study (21,22) reported a low rate of *S. epidermidis* one isolate (0.25%) and 5 isolates (7.14%) respectively, and this study disagreement with an earlier study [21] reported high rates of CONS 58 isolates, (38.1%).

An earlier study [22] found the most common clinical CONS species to be *S. haemolyticus* (35%), *S. epidermidis* (15%), *S. hominis* (15%) and *S. warneri* (2.5%). Previously De *et.al*., reported high rates of CONS (72 isolates,36% from urine), *S*. e*pidermidis* recorded the highest percentage of 26 isolates 13% [23]. This result in agreement with the previous study [24] reported low rates of *S. epidermidis*. This study disagreement with an earlier study [25] in the Diyala governorate showed that *S. epidermidis* reported high rates of *S. epidermidis,* 29 isolates) 27.35%).

Enterococcus spp. represents (14 isolates,11.3%) from the total isolate, *Enterococcus* species are more often associated with UTI in hospitalized patients [26, 27]. this study agreement with an earlier study [19] reported low rates of *Enterococcus* spp. (7%). E*nterococcus faecalis* represents 9 isolates (7.3%) from the total isolates, this study agreement with an earlier study [28] reported low rates of *E.faecalis* (9%). This study disagreed with an earlier study [29] that reported *E. faecalis* was the most common Gram-positive coccus isolated from community-acquired UTI (4.0%, and 54.11%, respectively). *Enterococcus faecium* represents 5 isolates (4.06%) from the total isolates, this study disagrees with an earlier study [30] that reported high rates of *E. faecium* (58.3%).

Antibiotics can disrupt the peri-urethral flora, allowing uropathogens to proliferate and penetrate the urinary system, leaving doctors with few alternatives for UTI treatment. Additionally, this situation permits bacteria to exchange genetic material via horizontal gene transfer, resulting in antibiotic-resistant genes [31]. Inadequate and imprecise antimicrobial agent delivery as empirical therapy, as well as a lack of appropriate infection management strategies, can lead to a rise in the incidence of resistant organisms in the population, leading to MDR [32].

S. aureus has developed resistance to both modern and conventional antibiotics in recent years. As a result, treating antibiotic-resistant bacteria is a clinical challenge, and investigating the susceptibility form which is useful to determine the future challenges of effective therapy [33].

According to a previous study, MDR is increased around the world, which is a worrying sign because we are losing our therapeutic options for treating simple bacterial infections as time goes on, MDR bacteria have also been shown to cause significant clinical issues in outpatient departments, according to the study [2].

Infections caused by multidrug-resistant (MDR) bacteria are associated with a higher mortality rate than infections caused by susceptible bacteria, and they impose significant economic costs [34]. The high rate of multidrug resistance observed in this study

may be due to a combination of microbial characteristics such as selective pressure on antimicrobial use, as well as a rise in irrational antibiotic intake, the transmission of resistant isolates between people, self-medication and non-compliance with medication, and sales of substandard antibiotics. It is also clear that regional habits affect the exponential growth of resistance, as most people in the community depend on patent medication stores without the proper prescription, shelf life, or dosage [35].

The production of β-lactamases is one of the first mechanisms of antibiotic resistance, and it has become an issue that affects the healthcare sector and public health in many parts of the world [36].

The *ermB* gene was determined in 60% of the *S. aureus* isolates included in this study. This result disagreed with the previous study [37] showed that 8.5% of isolates have *the ermB* gene.

The *mecA* gene encoding methicillin resistance was determined in 60% of the *S. aureus* isolates included in this study. This result agreed with the previous study [38] that showed that 63.6% of isolates have *mecA* gene encoding methicillin resistance. In this study, the coexistence rate of *mecA* was 60%, which is lower than those from Japan (100%) and slightly higher than similar reports from Europe (57.1%) [39]. This result disagreed with the previous study [38] that showed that 25.9% of isolates have *mecA* gene encoding methicillin resistance.

The *blaZ* gene was determined in 100% of the *S. aureus* isolates included in this study. This result agreed with the previous study [37] showed that 93.5% of isolates have *blaZ* gene.

The *msrA* gene was determined in 20% of the *S. aureus* isolates included in this study. This result agreed with the previous study [40] in Iran showed that 17.3% of isolates have *msrA* gene. In contrast to this finding, Goudarzi *et al*. found that among 51 *S. aureus* isolates, only 3.9% had *msr*A [40], and three studies in Iran did not detect any *msr*A [41-43]. In this study, the rate of *msrA* was 20%, which is higher than similar reports (9.4%) [37].

Conclusion

The most common gram-positive bacteria found to cause UTIs was *S. aureus.* A few pathogens of *S. aureus* were discovered to be resistant to Vancomycin and linezolid in this study. The prevalence of *blaZ* genes, which are responsible for resistance, was found to be high among pathogenic *S. aureus* isolates in Diyala province.

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Ethical Permissions: Considering ethical issues, data were provided to the researcher under the supervision of the Baquba teaching hospital without the name of the patients.

Conflicts of Interests: No conflict of interest was reported.

Authors' Contribution: Ahmed Khalaf Sh (First Author), Introduction Writer (40%); AL-Tameemi HK (Second Author), Statistical Analyst/Discussion Writer/Main Researcher (30%); Jasem Abdullah Y (Third Author), Methodologist (30%)

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