

Staphylococcus aureus virulence genes and methicillin-resistant gene detection and antimicrobial resistance profiles isolated from different infection sites

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Background

One of the most significant pathogenic bacteria is *Staphylococcus aureus*, and both adults and children are susceptible to this bacterium from the front of the nose. In the United States, the Centers for Disease Control and Prevention estimate that 80,461 invasive methicillin-resistant *S. aureus* (MRSA) infections and 11 285 related deaths occurred in 2011. In the UK, around 190 people passed away from MRSA disease in 2021. Australia, Hong Kong, Singapore, Japan, and Greece also have MRSA infections, along with the whole world. MRSA caused less than 2% of bacterial diseases in the United States in 1974, while the percentage rate jumped to 64% in 2004 only 10 years to increase the infection rate by 300%.

Objective

This study aimed to detect medication susceptibility patterns, staphylococcal enterotoxins A to C, toxic shock syndrome toxin-1, and methicillin-resistant genes.

Materials and methods

Ninety-eight *S. aureus* strains were isolated from different infection sites from Salah Al-Din Teaching Hospital. There have only been a few studies conducted on the epidemiology and virulence genes of *S. aureus* in Salah Al-Din city, Iraq.

Results and conclusion

The rates of drug resistance among *S. aureus* strains to routinely used antibiotics were found to be extremely high. In this study, the expression of toxic shock syndrome toxin, *sec B*, and *sec C* genes in *S. aureus* strains was not detected, unlike in previous studies. While all the strains were *sec A* gene positive, another gene found in bacterial cells that enables them to be resistant to antibiotics like methicillin and other vancomycin drugs is *mecA*.

Keywords:

antibiotic resistance, antimicrobial susceptibility test, methicillin-resistant *Staphylococcus aureus*, *Staphylococcus aureus*, virulence genes

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Introduction

Gram-positive coccid bacterium *Staphylococcus aureus* is facultative, aerobic, and easily contaminates meat, food, and the environment [1]. Serious animal disorders such as sepsis, pneumonia, arthritis, and urinary tract infection are all brought on by *S. aureus* [2,3]. This bacterium is a significant contributor to nosocomial bacteremia, postoperative wound infections, pneumonia, and food poisoning in humans [1].

Skin and soft-tissue infections, as well as lower respiratory tract infections, are significantly attributed to *S. aureus* in all examined regions [4]. *S. aureus* is frequently responsible for toxin-mediated illnesses such as toxic shock syndrome and foodborne diseases. This bacterium has several MSCRAMMs, or microbial surface components that recognize adhesive matrix molecules, on its

surface that helps it connect to host tissues and begin colonization, which leads to infection [5]. These binding proteins include an A domain, which binds fibers such as fibrinogen and also interacts with histones, which causes the neutralization of antibacterial activity of these molecules. They also promote biofilm formation and platelet aggregation by enhancing cell-to-cell interaction [6].

Due to its high virulence and its outstanding resistance, *S. aureus* is considered to be of significant clinical value. Among the virulence components of *S. aureus* are surface proteins, enterotoxins, and enzymes. [7].

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In the case of life-threatening bacterial infections, the ability of *S. aureus* to withstand antibiotics may become more problematic. [8,9]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a member of the Micrococcaceae family and belongs to the genus *Staphylococcus*, which in turn has more than 30 species, including *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, and *Staphylococcus haemolyticus* [10]. *S. aureus* is the staphylococcal species that is most dangerous. However, different geographical areas have a variable prevalence of *S. aureus* nasal carriers [11].

Materials and methods

Sample collection and identification

Samples were collected from outpatients aged (from 6 to 50 years), who attended the Department of Salah Al-Din City General Hospital in salah al-Din, Iraq. The specimens were collected in the period between January and August 2022. The aim of this project was discussed and explained to the patients or the parents of children, and signed consent forms were obtained. A total of 98 *S. aureus* samples were collected for this study. Clinical specimens were isolated from the throat, ear, wound, and urine. Bacterial strains were identified using biochemical tests and conventional methods such as hemolysis and Gram staining, and it was determined that all strains belong to *S. aureus*.

Antimicrobial susceptibility test (AST)

Isolates were examined for antimicrobial susceptibility according to the recommendations of the CLSI, and AST was carried out using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar [12]. Rifampicin, Amoxicillin, Cefalexin, Teicoplanin, Cefotaxime, Ciprofloxacin, Tetracycline, Vancomycin, Meropenem, and Aztreonam were used as antibiotics. The sensitivity of the *S. aureus* samples for each antimicrobial agent was assessed after the incubation at 35°C overnight, and the results were interpreted in accordance with the NCCLS criteria [13].

Staphylococcus aureus DNA extraction

After 24 h of cultivation on blood agar, the isolates were resuspended in 100 ml of DW and then heated for 15 min at 99°C in a thermomixer. The mixture was then centrifuged at 1000 g at 14°C for 5 min. The supernatant and bacterial crude DNA extract were transferred to another tube after removing the precipitant [14].

PCR gene detection

The PCR amplification reaction mixture was prepared according to Wang *et al.* [15]. The toxic shock

syndrome toxin (TSST-1), (SEa), (SEb), (SEc), and *mecA* genes were amplified using PCR. The reaction products were then electrophoresed in a 2% agarose gel with tris-acetate electrophoresis buffer, and a 100-bp DNA ladder was used as a molecular marker to determine the presence of PCR products [16]. A multiplex PCR test was used to investigate the genes.

Preparation of primers

Multiplex PCR was performed to detect the presence of virulence genes (SEA, SEB, SEC, *mecA*, and *tsst*) [16]. To amplify each of the target genes, appropriate primers were used. This experiment was carried out using primers used in earlier investigations.

Table 1 displays the primer sequence and the size of the amplified product. Multiplex PCR is a fast and reliable technique for identifying and amplifying multiple genes at the same time using specific primers, and so allowed to save money, time, and energy. To carry out the multiplex PCR reaction, specific primers related to virulence genes with annealing temperatures in the same range were grouped together.

The reaction mixtures for each group of virulence genes were done in accordance to AubaisAljelehawy *et al.* [17]. To do this, the reaction mixture of each group of virulence genes was put into sterile microtubes, and PCR was performed after changing the temperature and time of the reaction. Following PCR amplification, PCR products were electrophoresed for 60 min on a 1% (w/v) agarose gel in ×1 TBE solution (Tables 1 and 2).

Results

In this study, 98 strains of *S. aureus* were isolated from clinical samples in Salah Al-Din Teaching Hospital, Iraq. Identification of clinical specimens using biochemical tests and conventional methods such as hemolysis and Gram staining showed that all strains

Table 1 Sequence of primers with related sizes

Primer	Oligopeptide sequence	Product size
<i>mecA</i> ^F	TGTCCGGGGAAATAACTGAA	305
<i>mecA</i> ^R	TGGCATGATTTCTTCTGCAA	
Se A ^F	TCGATTGACCGAAGAGAAAAA	203
Se A ^R	CGATTAATCCCCCTGAACC	
Se B ^F	CCTAAACCAGATGAGTTGCACA	404
Se B ^R	CCATCTCAAATACCCGAACA	
Se C ^F	AAACATGAAGGAAACCACTTTGA	126
Se C ^R	TTCTTGAGCTGTTGCACTTTTC	
Tsst-1 ^F	CTTGCGACAATCGCTACAGA	537
Tsst-1 ^R	TTTCCAATAACCACCGTTT	

Tsst, toxic shock syndrome toxin.

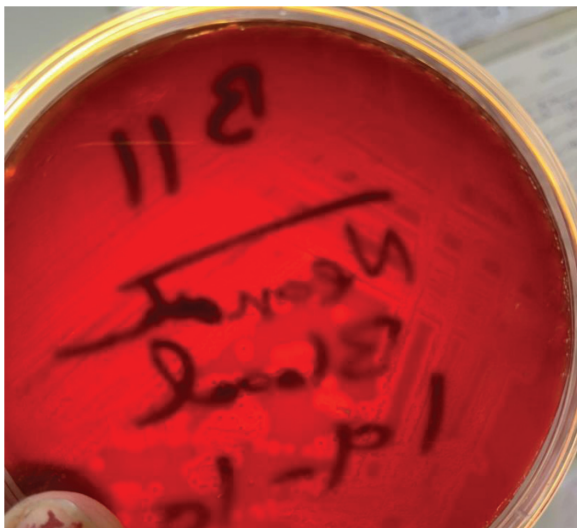
belonged to *S. aureus* as shown in Fig. 1. The strains were isolated from the wound, urine, burns, otitis media infections, and throat, which accounted for 58, 9, 16, 8, 8, and 8% of the samples in each group, respectively, as shown in Fig. 2.

The susceptibility testing against *S. aureus* is demonstrated in Table 3 and Fig. 2, which clearly

Table 2 PCR program for genes (*sea*, *seB*, *seC*, *mecA*, toxic shock syndrome toxin-1)

Stages	Time (s)	Temperature (°C)	Cycle
Denaturation	300	94	1
Denaturation	30	95	35
Annealing	45	56	
Elongation	60	72	
Final elongation	350	72	1

Figure 1

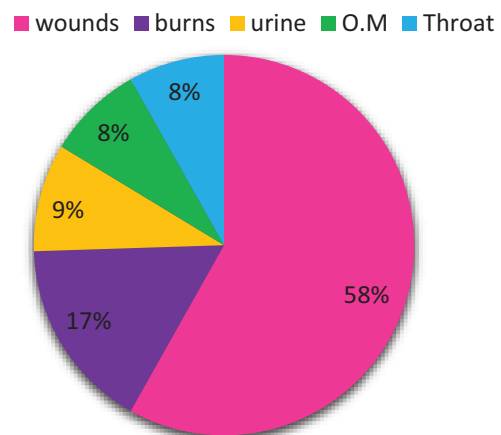


Identification of *Staphylococcus aureus* isolates using the hemolysis method.

show that all isolates were vancomycin and amoxicillin resistant, with 100% resistance for all isolates, while the prevalence of *S. aureus* showed full resistance to the antibiotic Cefotaxime in the wound, burn, and urine samples, while otitis media samples showed the least resistance with 69%. Cephalexin is effective against 88% of *S. aureus* in urine samples, followed by Rifampicin for throat samples with only 13% resistance and 6% resistance to Teicoplanin.

All 98 *S. aureus* isolates were MRSA, and these entire collections tested positive for the *mecA* gene. The presence of toxin genes in the strains isolated from wound, burn, urinary, throat, and otitis media infections is shown in Table 4. All specimens showed 100% for the presence of the SeA gene and tested negative for SeB, SeC, and TSST-1 genes (Fig. 3). Multiplex PCR was performed to detect the specific genes, which is shown in Fig. 4.

Figure 2



Frequency of *Staphylococcus aureus* isolates in a clinical specimen.

Table 3 Antibiotic resistance frequency among clinical strains isolated from different parts of the body

	Wounds		Burns		Urine		Otitis media		Throat	
	R* %	S* %	R %	S %	R %	S %	R %	S %	R %	S %
Rifampicin	57	43	48	52	100		81	19	13	87
Amoxicillin	100		100		100		100		100	
Cephalexin	85	15	70	30	12	88	64	38	81	19
Teicoplanin	72	28	82	18	71	29	94	6	6	94
Cefotaxime	100		100		100		69	31	94	6
Ciprofloxacin	43	57	49	51	100		25	75	100	
Tetracycline	72	28	52	48	94	6	44	56	100	
Vancomycin	100		100		100		100		100	
Meropenem	44	56	64	36	82	18	94	6	19	81
Aztreonam	86	14	79	21	65	35	12	88	81	19

R, resistance; S, sensitive.

Table 4 The frequency of virulence genes and *mecA* gene among clinical strains isolated from different parts of the body

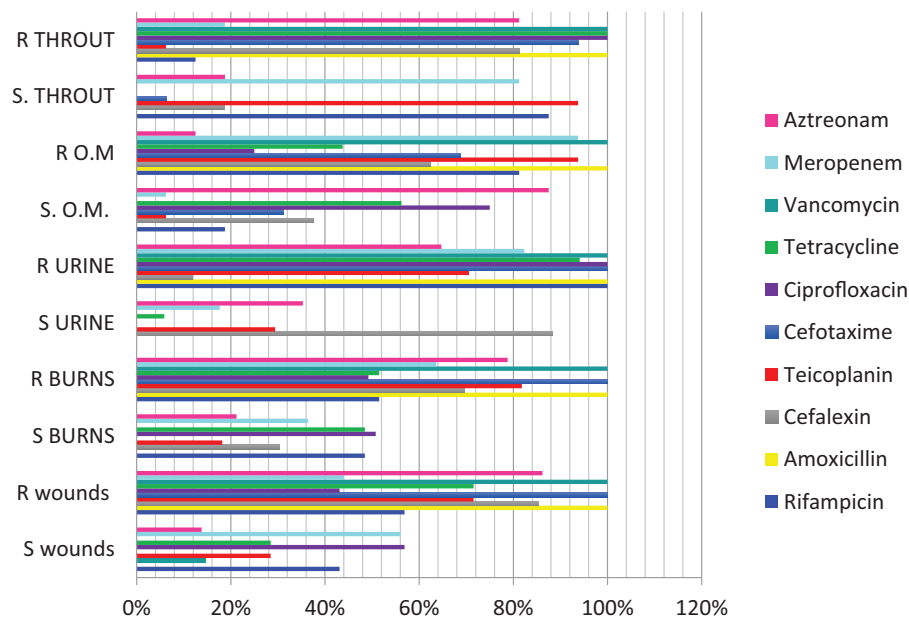
Samples	Sea (%)	SEB (%)	SEC (%)	TSST-1 (%)	mecA (%)
Wounds	100	0	0	0	100
Burns	100	0	0	0	100
Urine	100	0	0	0	100
Otitis media	100	0	0	0	100
Throat	100	0	0	0	100

TSST, toxic shock syndrome toxin.

Discussion

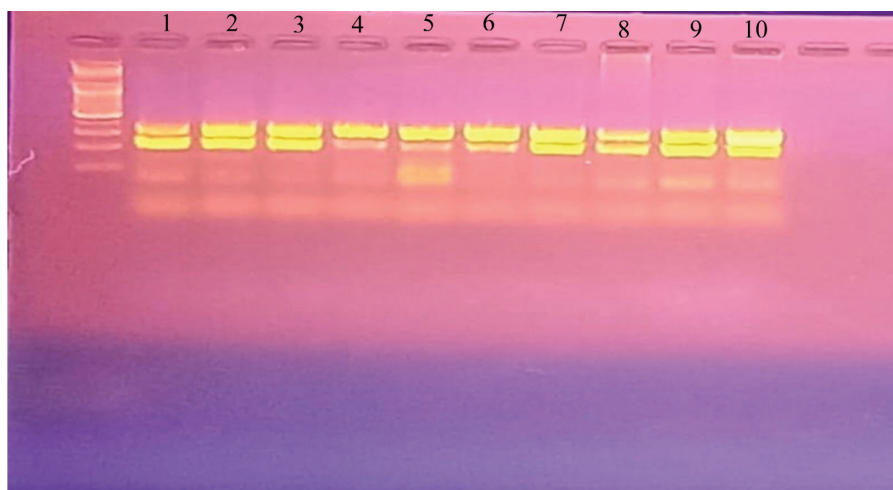
MRSA is most common in newborns, the elderly, and persons with chronic diseases such as burn survivors, organ transplant recipients, chemotherapy-treated cancer patients, steroid users, diabetics, intravenous drug users, and HIV patients [18–21]. Accumulation of microorganisms in the human body causes increased contamination levels. It can happen in the skin, perineum, throat, and gastrointestinal system, as well as the vagina [22].

Figure 3



Frequency of virulence genes among *Staphylococcus aureus* strains isolated from Salah Al-Din Teaching Hospital. Among the strains, *sea* and *mecA* genes with 100% frequency showed the highest prevalence among pathogenic genes. *Seb*, *Sec*, *tsst-1* genes with 0%. *tsst*, toxic shock syndrome toxin.

Figure 4



Multiplex PCR of virulence and resistance genes among *Staphylococcus aureus* strains. 1, 2 are from wounds, 3, 4 are burns, 5, 6 are urine samples, 7, 8 from otitis media, and 9, 10 from the throat

The location of infection in the body influences treatment options and antimicrobials used for people with MRSA [23]. Only four medications demonstrated activity *in vivo* against MRSA, Quinupristin, dalfopristin, minocycline, daptomycin, linezolid, and vancomycin. Rifampicin is an efficient antibiotic against staphylococci, although its effectiveness in MRSA infections has yet to be demonstrated. MRSA's antibacterial activity changes in response to trimethoprim-sulfamethoxazole [24–29].

We reported 100% resistance in urine samples to cefotaxime, compared with the Salman *et al.* [30] report, which showed no resistance that formed 37.5% of all urine samples. Ansari *et al.* [31] observed a 36.7% prevalence of ciprofloxacin resistance. Previous research in Nepal found no resistance to vancomycin or linezolid; our findings revealed 100% resistance [32]. However, among commonly used antibiotics, vancomycin and amoxicillin had the highest rate of resistance to *S. aureus*, and the highest rate of susceptibility to Cephalexin (88%) in urine samples, followed by Rifampicin (87%) and Teicoplanin (94%) in throat samples, and Aztreonam (88%) in OM samples, implying the possibility of using these drugs for preliminary treatment of *S. aureus* infections in our settings. *S. aureus* isolates were resistant to penicillin, ampicillin, and amoxicillin in 77.2%, but susceptible to co-amoxiclav in 81.8% and cephradine in 86.3%. [33]. In developing countries like Iraq, the low cost of these drugs will be an extra benefit.

By PCR methods, all of the isolates in our investigation were identified as MRSA, which is considered the highest when compared with Kshetry *et al.* [34] findings (37.6%) and Sanjana *et al.* [32] (39.6%). However, Subedi and Brahmadathan [35] (15.4%) MRSA isolates and in 2011, Baral *et al.* [36] (26%) reported lower prevalence, while in 2017, another study by Baral and Khanal [37] reported MRSA isolates to be 41%, whereas Jha [38] reported MRSA incidence to be 68% and Tiwari *et al.* [39] reported a higher prevalence (69.1%). The difference in isolation rates for MRSA between studies can be due to differences in study regions and time periods, differences in hygienic conditions maintained in different hospitals, healthcare facilities provided by the hospital, infection control program implementation, and rational use of antibiotics, which may vary from hospital to hospital [40].

Enterotoxins (SEs) belong to a large superantigen family of acidic toxins that cause vomiting. The

classic SEs (sea, seb, sec, sed, and see) were found in studies of *S. aureus* species involved in *S. aureus* botulism transmission (SFP) and were classified into different serotypes. In the current study, the most prevalent gene for virulence was associated with the *seA* gene, which was present in 100% of bacterial samples isolated from patients. In a report by Iran Kamarehei *et al.* [41], the frequency of this gene was determined to be around 50.4% in *S. aureus* strains.

In the above report, the prevalence of the *tsst-1* gene was relatively high; however, its frequency was not detectable in the results obtained in our study. The product of this gene is the main cause of *tsst-1*, which is produced in the second type of *S. aureus* isolates (MRSA) [42]. Thus, the production of this toxin is a variable genetic trait. It is known as an acute disease that is associated with symptoms such as fever, skin rash, high blood pressure, and dysfunction of multiple systems [43]. In the present study, the frequency of the *tsst-1* gene among *S. aureus* strains was about 0%. Nagao *et al.* [44] (Japan) found that the prevalence of this gene among 152 MRSA strains was about 75%. Xie *et al.* [45] estimated the frequency of this gene among the 108 *S. aureus* strains isolated from 16 different hospitals in 14 provinces of China to be about 48.1%, which was the most common virulence gene among the studied genes. In the study of Pakbaz *et al.* [46], the frequency of the mentioned gene among 98 samples was obtained from Tehran University of Medical Sciences, with a total of 21 strains containing the *tsst* gene.

In most of the above reports, the expression of the *tsst-1* gene in *S. aureus* strains was higher than in the present study. *MecA* is a gene discovered in bacteria that permits them to withstand medications like methicillin and other penicillin-like drugs. *MecA* is involved in the coding of the PBP2A protein (a transpeptidase that promotes bacterial cell wall construction) as well as antibiotic resistance [47]. In this study, the frequency of the *mecA* gene among samples was about 65%. The frequency of this gene in the studies of Galdiero *et al.* [48] (Italy-2003) among 43 MRSA strains was estimated to be about 46.5%.

The frequency of this gene among coagulase-negative *S. aureus* and MRSA was described in a report published by Choi *et al.* [49] and determined to be 98% and 81%, respectively. In the study of Askari *et al.* [50], the prevalence of this gene among 2690 strains was estimated to be about 52.7%. The prevalence of the *mecA* gene in the above reports was different from the results of the present study.

Conclusions

Substantial rates of drug resistance among *S. aureus* strains to routinely used medicines were found in this study. MRSA isolation from community-acquired disease was shown to be common in Iraq. In the present study, the expression of *tsst*, *sec B*, and *sec C* genes in *S. aureus* strains were not detected, unlike in previous studies. While all the strains were *sec A* gene positive, another gene found in bacterial cells that enables them to be resistant to antibiotics like methicillin and other vancomycin drugs is *mecA*. *mecA* is involved in the coding of PBP2A protein (a transpeptidase that promotes bacterial cell wall formation) and has the ability to resist some antibiotics. This study was limited to Salah Al-Din Teaching Hospital and therefore more investigations are required to evaluate virulence factors of isolated *S. aureus* strains from other antibiotic-resistant patients. Also, new efficient techniques should be applied to determine the presence of virulence genes among antibiotic-resistant strains.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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