ORIGINAL ARTICLE

Prevalence and characterization of virulence genes among methicillin-resistant *Staphylococcus aureus* isolated from Sudanese patients in Khartoum state

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Abstract

Staphylococcus aureus is a versatile pathogen that can cause a variety of diseases, ranging from mild to fatal infection. This study aimed to detect the virulence genes (*cna, ica, hlg* and *sdr*E) in *S. aureus* isolated from different types of infections in Sudanese patients admitted to different hospital in Khartoum state. This is a descriptive cross-sectional study conducted over a period of 4 months from 1 April to 30 July 2017 in Khartoum. Overall, 65 *S. aureus* isolates were identified using standard biochemical and microbiologic tests. Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method. Nucleic acid was extracted using the guanidine hydrochloride method, and all the genes except for *sdr*E were detected by multiplex PCR. The *ica* gene was the predominant one, found in 73.85% of the isolates, with *sdr*E found in 38.46%, *cna* in 29.25% and *hlg* in 7.69%. The relationship between the virulence genes and resistance to antibiotics showed that the highest resistance was observed in isolates with *ica* and *sdr*E, followed by *cna* and *hlg*. There were significant relationships between methicillin resistance and the presence of *sdr*E and *ica* genes (p 0.01 for both) and between ciprofloxacin resistance and the presence of *sdr*E gene (p 0.03).

Keywords: *cna*, *hlg*, *ica*, MRSA, *sdr*E
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Introduction

The genus *Staphylococcus* is found as both commensals and pathogens in human and animals [1]. *Staphylococcus aureus* is one of the most common organisms; it causes mild to life-threatening infections by producing different virulence factors that help the microorganism adapt and survive in different tissues and environmental conditions [2,3]. Various genes encoding these factors are *sdr*E, *sdr*D and *sdr*C, which are found in different *S. aureus* strains [4,5]. The *sdr* proteins help bacteria to adhere to epithelial cells. Strains carrying the *sdr*E gene are reported to be 7.3% more prevalent among all *S. aureus* isolates. The *sdr*D gene is essential in abscess formation, and the *sdr*C gene has a low ability to infect bones [6].

Other virulence factors like *hlg* (γ-haemolysins *hlg*A and *hlg*C/*hlg*B) are common in *S. aureus* strains. This gene plays part in septic arthritis. It helps community-acquired methicillin-resistant *S. aureus* (MRSA) strains to persist in human through infection [7–9]. The *ica* A, C, B and D genes are associated with cell aggregation and biofilm formation. They encode for essential proteins to produce polysaccharide intercellular adhesion and capsule polysaccharide in *Staphylococcus* species. Various studies have reported the critical role that the *ica* gene

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plays as one of the staphylococcal infection's virulence factors [10,11] as well as in biofilm formation [12].

There is a global report of an increase in hypervirulent strains of MRSA [13]. *S. aureus* developed itself by acquiring antibiotic resistance genes; in addition, the presence of virulence genes complicates therapeutic interventions [14]. In Sudan, there are no published studies on the prevalence of virulent strains of *S. aureus*. Moreover, detection of *S. aureus* virulence genes is essential for epidemiologic reasons.

We therefore aimed to detect *Staphylococcus aureus* virulence genes. The genes *ica*, *cna*, *hlg* and *sdr*E were isolated from different clinical samples taken from people living in Khartoum state. We also assessed the frequency of these genes with MRSA as well as resistance to fluoroquinolones and aminoglycosides.

Materials and methods

Study area, design and population

We performed this descriptive cross-sectional study from 1 April to 30 July 2017 in Khartoum state, Sudan. Isolates were collected from Omdurman Military Hospital, Police Hospital, Soba Hospital and Bahary Hospital, representing different provinces in Khartoum state. Patient of both sexes admitted to hospitals for treatment of urinary tract infections, wound infections or sepsis and who were suspected to have *S. aureus* infection were included in this study.

Ethical approval to carry out this study was obtained from the institutional ethics committee, Deanship of Scientific Research, Sudan University of Science and Technology. Participants' privacy and confidentially were protected for all samples; personal information was not of great value in the current study and was thus not taken. Subsequently the ethical and scientific committee waived the need for patient consent.

Specimen collection

Bacterial isolates were collected from hospitals and subcultured onto blood agar and mannitol salt agar (Himedia Laboratories, Mumbai, India), then incubated aerobically for 24 hours at 37°C. Colonies were examined the next day. Identification of bacteria was made according to colony morphology, Gram stain and biochemical tests [15].

Antibiotic susceptibility patterns

Antibiotic susceptibility testing was carried out using the Kirby-Bauer disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) [16]. Antibiotic discs of methicillin (5 µg), vancomycin (30 µg), gentamicin (10 µg) and ciprofloxacin (5 µg) were placed on Müller-Hinton agar and incubated aerobically at 37°C for 24 hours (Himedia). The zone of inhibition was interpreted as sensitive or resistant using CLSI criteria.

Nucleic acid extraction and detection of virulence genes

DNA was extracted using the guanidine hydrochloride method described by Sabeel *et al.* [17]. Multiplex PCR reactions performed using 1 µL DNA solution (100–150 ng/L), 5 µL Qiagen Hot Star Taq Master Mix (Qiagen, Germantown, MD, USA) and 1 µL (10 pmol) of each gene-specific primer (*hlg*, *ica* and *cna*) in a final volume of 25 µL were used as recommended by Kumar *et al.* [18]. This mixture was introduced into a multiplex PCR protocol: 40 cycles of the following: 94°C for 30 seconds, 50°C for 30 seconds and 72°C for 30 seconds, 72°C for 40 seconds and a final extension at 72°C for 5 minutes. The origin and expected PCR amplicons size are listed in Table 1. For *sdr*E, the following single PCR protocol was conducted: 30 cycles of the following: 94°C for 5 minutes, 94°C for 40 seconds, 50°C for 40 seconds, 72°C for 40 seconds and final extension for 72°C for 5 minutes. Then visualization of PCR products was carried out by using 1% agarose on gel electrophoresis; the gel casting tray was flooded by 10 × Tris–borate–EDTA buffer near the gel cover surface and then 5 µL of amplified PCR products of each sample was put into each well. Then DAN ladder (100bp) and PCR products were loaded into gel. The gel electrophoresis apparatus was connected to a power supply (primer 125 V, 500 mA) (iNtRON Biotechnology Inc., Seoul, Korea). Electrophoresis was performed at 100 V/cm for 30 minutes; after that, the gel holder was removed and the gel visualized with a UV transilluminator (Transilluminator; Uvitec, UK).

TABLE 1. Primers and amplicon sizes

Gene	Sequence (5′–3′)	Product size (bp)	Reference
cna (collagen adhesin)	F: AGTGGTACTACTAATACTG R: CAGGATAGATTGGTTTA	740	[18]
hlg (haemolysin)	F: GCCAATCCGTTATTAGAAAATGC R: CCATAGACGTAGCAACGGAT	937	
ica (intracellular adhesin)	F: GATTATGTAATGTGCTTGGA R: ACTACTGCTGCGTTAATAAT	770	
*sdr*E (putative adhesin)	F: AGTAAAATGTGTCAAAAGA R: TTGACTACCAGGCTATATC	767	

Statistical analysis

Data were analysed by SPSS 20.0 software (IBM, Armonk, NY, USA). The chi-square test was used to find the relationship between virulence genes, antibiotic profiles and patient sex, with $p < 0.05$ considered statistically significant.

Results

Distribution of *Staphylococcus aureus* isolates according to sample source and sex

Of 65 isolates, 30 were from infected wounds (46.15%), 25 urine (38.5%) and ten blood samples (15.4%). Sex distribution among isolate donors was 36 women (55.6%) and 29 men (45.4%).

S. aureus antimicrobial susceptibility testing

Of 65 isolates, 36 were sensitive to methicillin (55.4%), 25 to vancomycin (38.5%), 27 to gentamicin (41.5%) and 29 to ciprofloxacin (44.6%) (Table 2).

Detection of virulence genes

The *ica* gene was predominant as it was detected in 48 isolates (73.85%). The *cna* gene was detected in 19 isolates (29.2%). Five isolates (7.6%) were positive for *hlg* and 25 isolates (38.46%) for *sdr*E.

TABLE 2. Sensitivity profile for all *Staphylococcus aureus* clinical isolates

Antibiotic	Result	*N* (%)
Ciprofloxacin	Sensitive	29 (44.6)
	Resistant	36 (55.4)
Gentamicin	Sensitive	27 (41.5)
	Resistant	38 (58.5)
Vancomycin	Sensitive	25 (38.5)
	Resistant	40 (61.5)
Methicillin	Sensitive	36 (55.4)
	Resistant	29 (44.6)

According to CLSI 2017 criteria: Zone of inhibition interpreted as sensitive and resistant.

The present data showed that the *sdr*E gene is more prevalent in ciprofloxacin and methicillin resistance strains (p 0.03 and 0.01 respectively) (Table 3).

Tables 4 and 5 provide information regarding the distribution of sample sources of *S. aureus* as well as sex and its association with virulence genes.

Discussion

The current study determined the incidence of some of the various virulence markers of *S. aureus* in different clinical specimens. We specifically targeted a small portion of genes associated with *S. aureus* virulence. These genes (*cna*, *hlg*, *ica* and *sdr*E) were chosen because they predominate among aggressive isolates. PCR amplification of these targeted genes showed that they were distributed among different isolates. In addition, all the isolates showed diverse combinations of genes, among which all the tested genes were found in four isolates, thus indicating a high level of genetic diversity among the study population. This finding was in agreement with Stotts *et al.* [19], who found that eight isolates from their study population had these four genes in combination. They reported a high rate of resistance in MRSA and vancomycin-resistant *S. aureus* (VRSA), which were 44.6% and 61.5% respectively. This finding of resistance was similar to that of other studies, including one from Egypt (which shares a north border with Sudan) which reported that the prevalence of VRSA and MRSA were (27% and 14.5%) in dromedary camels and (54% and 55%) in humans, respectively, [20]. Such findings are similar to those of a study from Ethiopia (which shares an east border with Sudan) which reported that of 31 *S. aureus*, the percentages of VRSA and MRSA were 65.1% and 55.8% respectively [21]. However, these results are unlike those of a study from Tehran, which reported that out of 1789 *S. aureus*, only four VRSA were detected [22]. This reports indicate the presence of an alarming situation in Sudan, which is a neighbouring country, regarding the spread of

TABLE 3. Correlation between *Staphylococcus aureus* virulence genes and antibiotic resistance

		Ciprofloxacin			Gentamicin			Vancomycin			Methicillin		
Gene	Finding (*n*)	R	S	p	R	S	p	R	S	p	R	S	p
cna	Positive (19)	9	10	0.95	11	8	0.32	13	6	0.94	7	12	0.11
	Negative (46)	27	19		27	19		27	19		22	24	
ica	Positive (48)	25	23	0.69	28	20	0.95	31	17	0.21	20	28	0.01
	Negative (17)	11	6		10	7		9	8		9	8	
hlg	Positive (5)	2	3	0.31	4	1	0.32	4	1	0.31	2	3	0.12
	Negative (60)	34	26		34	26		36	24		27	33	
*sdr*E	Positive (25)	1 8	7	0.03	15	10	0.84	17	8	0.94	17	8	0.01
	Negative (40)	1 8	22		23	17		23	17		12	28	

p values indicate significance at 95% confidence level. R, resistant; S, sensitive.

TABLE 4. Relationship between *Staphylococcus aureus* virulence genes and sample source

Gene	Reaction	Sample source, *n* (%)				
		Urine (*n* = 25)	Wound swab (*n* = 30)	Blood (*n* = 10)	Total	p
cna	Positive	7 (36.8)	8 (42.1)	4 (21.1)	19 (34)	0.71
	Negative	18 (39.1)	22 (47.8)	6 (13.0)	46 (71)	
ica	Positive	19 (39.6)	21 (43.8)	8 (16.7)	48 (74)	0.78
	Negative	6 (35.3)	9 (52.9)	2 (11.8)	17 (26)	
hlg	Positive	0	1 (20.0)	4 (80.0)	5 (8)	0.01
	Negative	25 (41.7)	29 (48.3)	6 (10.0)	60 (92)	
*sdr*E	Positive	9 (36.0)	13 (52.0)	3 (12.0)	25 (38)	0.71
	Negative	16 (40.0)	17 (42.5)	7 (17.5)	40 (62)	

TABLE 5. Incidence of *Staphylococcus aureus* virulence genes with patient sex

Gene	Reaction	Sex, *n* (%)		p
		Male	Female	
cna	Positive	8 (42.1)	11 (57.9)	0.79
	Negative	21 (45.7)	25 (54.3)	
ica	Positive	22 (45.8)	26 (54.2)	0.74
	Negative	7 (41.2)	10 (58.8)	
hlg	Positive	1 (20.0)	4 (80.0)	0.24
	Negative	28 (46.7)	32 (53.3)	
*sdr*E	Positive	11 (44.0)	14 (56.0)	0.94
	Negative	18 (45.0)	22 (55.0)	

MRSA and VRSA. More comprehensive molecular epidemiologic monitoring studies on MRSA and VRSA are urgently needed.

In the current research, notable differences were observed in antimicrobial sensitivity between those isolates that harbour all four virulence factors and those that contain varying or no virulence factors. The acquisition of virulence genes among *S. aureus* species was noted more in methicillin-sensitive strains. This finding may be due to the adjacent position of the resistance gene to the virulence gene [19,23]. In this research, we observed that the presence of the *ica* gene in methicillin-resistant strains was more common (p 0.01). This finding entirely disagreed with the study of Almeida *et al.* [24], which concluded that there was no prevalence of either *cna* or *ica* genes among *S. aureus* isolates. Moreover, the presence of the *cna* gene, which encodes the collagen-binding protein, was not found in the *S. aureus* isolates of Smeltzer *et al.* [25].

The *hlg* (γ-haemolysin) gene, which was present in 7.69% of the isolates in this study, was not been reported by Almeida *et al.* [24]. The *sdr*E gene was found to have a prevalence of 38.46% among our study isolates. They may play a role in microbial surface component–recognizing adhesive matrix molecules. The *S. aureus* proteins are one of this family that is formed by the tandem arranged *sdr*C, *sdr*D and *sdr*E genes [4]. Although the exact role of *sdr*E adhesins in staphylococcal infection has not yet been identified, a strong association between the *sdr*E genes of *S. aureus* and some diseases has been described, with several studies reporting a significant growth incidence of *sdr*E genes in invasive *S. aureus* strains, mainly in *S. aureus* strains causing osteomyelitis and bone infections [4,26].

Interestingly, a statistically significant association (p 0.01) was detected between the presence of the *hlg* gene and blood samples. This gene is responsible for the production of γ toxins, which could help both community-acquired and hospital-acquired MRSA to survive in human blood during infection [7–9]. Of 29 MRSA isolates, 20 MRSA strains possessed *ica* genes, two *hlg* genes, seven *cna* genes and 17 *sdr*F genes. These strains are considered to have high virulence potential.

Conclusions

Hypervirulent MRSA is predominant in Khartoum, Sudan. The *sdr*E and *ica* genes are highly prevalent in MRSA. Further, there are a high number of *Staphylococcus aureus* isolates with different levels of virulence-determining genes in Sudanese patients. The results provide evidence of a high frequency of MRSA in Sudanese patients.

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Conflict of Interest

None declared.

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