Prevalence and characterization of virulence genes among methicillin-resistant \textit{Staphylococcus aureus} isolated from Sudanese patients in Khartoum state

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Abstract

\textit{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 sdrE) in \textit{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 \textit{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 sdrE were detected by multiplex PCR. The ica gene was the predominant one, found in 73.85% of the isolates, with sdrE 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 sdrE, followed by cna and hlg. There were significant relationships between methicillin resistance and the presence of sdrE and ica genes (p 0.01 for both) and between ciprofloxacin resistance and the presence of sdrE gene (p 0.03). © 2020 The Author(s). Published by Elsevier Ltd.

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Introduction

The genus \textit{Staphylococcus} is found as both commensals and pathogens in human and animals [1]. \textit{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 sdrE, sdrD and sdrC, which are found in different \textit{S. aureus} strains [4,5]. The sdr proteins help bacteria to adhere to epithelial cells. Strains carrying the sdrE gene are reported to be 7.3% more prevalent among all \textit{S. aureus} isolates. The sdrD gene is essential in abscess formation, and the sdrC gene has a low ability to infect bones [6].

Other virulence factors like hlg (\(\gamma\)-haemolysins hlgA and hlgC/hlgB) are common in \textit{S. aureus} strains. This gene plays part in septic arthritis. It helps community-acquired methicillin-resistant \textit{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 \textit{Staphylococcus} species. Various studies have reported the critical role that the ica gene
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 sdrE 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 confidentiality 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 Sable 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 sdrE, 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 |
|---------------------|---------------------------------------|-------------------|-----------|
| ica (collagen adhesin) | F: AGTGGTACTAATAACTACG                 | 740               | \[18\]    |
|                     | R: CAGATAGATGTGGTTTATA                |                   |           |
| hlg (haemolysin)    | F: GCCAATCCGTATTAGAAATGTC             | 937               |           |
|                     | R: CCATAGCAGTTAGCAGCGAT               |                   |           |
| ica (intracellular adhesin) | F: GATTATGAATTGTGGTTGGA     | 770               |           |
|                     | R: ACTACTGTGTGCTAAATAAT               |                   |           |
| sdrE (putative adhesin) | F: AGTAAAAAGTGTCAAGAAAAGA          | 767               |           |
|                     | R: TTGACTACGGCCTATATC                |                   |           |

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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 sdrE.

The present data showed that the sdrE 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 sdrE) 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 2. Sensitivity profile for all *Staphylococcus aureus* clinical isolates**

| Antibiotic  | Result | N (%) |
|-------------|--------|-------|
| Ciprofloxacin | Sensitive | 29 (44.6) |
| Gentamicin   | Resistant | 36 (55.4) |
| Vancomycin   | Sensitive | 25 (38.5) |
| Methicillin  | Resistant | 40 (61.5) |

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

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

| Gene | Finding (n) | Ciprofloxacin | Gentamicin | Vancomycin | Methicillin |
|------|------------|---------------|------------|------------|-------------|
|      |            | 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  | 0.31 | 10 | 7  | 0.32 | 9  | 8  | 0.31 | 9  | 8  | 0.36 |
| hlg  | Positive (3)  | 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 |      |
| sdrE | Positive (25) | 18 | 7  | 0.03 | 15 | 10 | 0.84 | 17 | 8  | 0.94 | 17 | 8  | 0.01 |
|      | Negative (40) | 18 | 22 |      | 23 | 17 |      | 23 | 17 |      | 12 | 28 |      |

$p$ values indicate significance at 95% confidence level. R, resistant; S, sensitive.
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 gene is responsible for the production of Y 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 sdrE genes. These strains are considered to have high virulence potential.

### Conclusions

Hypermurinal MRSA is predominant in Khartoum, Sudan. The sdrE 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.

### References

[1] Argudin MÁ, Mendoza MC, Rodicio MR. Food poisoning and Staphylococcus aureus enterotoxins. Toxins 2010;2:1751–73.
[2] Balasubramanian D, Harper L, Shopsin B, Torres VJ. Staphylococcus aureus pathogenesis in diverse host environments. Pathog Dis 2017;75: ftx005.
[3] Le Loir Y, Baron F, Gautier M. Staphylococcus aureus and food poisoning. Genet Mol Res 2003;2:63–76.
[4] Peacock SJ, Moore CE, Justice A, Kantzanou M, Story L, Mackie K, et al. Virulent combinations of adhesin and toxin genes in natural populations of Staphylococcus aureus. Infect Immun 2002;70:4987–96.
[5] Xiang H, Feng Y, Wang J, Liu B, Chen Y, Liu L, et al. Crystal structures reveal the multi-ligand binding mechanism of Staphylococcus aureus CjB. PLoS Pathog 2012;8:e1002751.
[6] Sabat A, Melles D, Martirosian G, Grundmann H, Belkum A, Hryniewicz W, et al. Distribution of the serine-aspartate repeat protein-encoding sdr genes among nasal-carriage and invasive Staphylococcus aureus strains. J Clin Microbiol 2006;44:4.

[7] Viquez-Molina G, Aragón-Sánchez J, Pérez-Corrales C, Murillo-Vargas C, López-Valverde ME, Lipsky BA. Virulence factor genes in Staphylococcus aureus isolated from diabetic foot soft tissue and bone infections. Int J Low Extrem Wounds 2018;17:36–41.

[8] Ventura CL, Malachowa N, Hammer CH, Nardone GA, Robinson MA, Kobayashi SD, et al. Identification of a novel Staphylococcus aureus two-component leukotoxin using cell surface proteomics. PLoS One 2010;5:e11634.

[9] Dumont AL, Nygaard TK, Watkins RL, Smith A, Kozhaya L, Kreiswirth BN, et al. Characterization of a new cytotoxin that contributes to Staphylococcus aureus pathogenesis. Mol Microbiol 2011;79:814–25.

[10] Frank KL, Patel R. Poly-N-acetylglucosamine is not a major component of the extracellular matrix in biofilms formed byicaADBC-positive Staphylococcus lugdunensis isolates. Infect Immun 2007;75:4728–42.

[11] Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol 2004;2:95–108.

[12] Chen Q, Xie S, Lou X, Cheng S, Liu X, Zheng W, et al. Biofilm formation and prevalence of adhesion genes among Staphylococcus aureus isolates from different food sources. Microbiologica 2020;9:e00946.

[13] Kateete DP, Namazzi S, Okee M, Okeng A, Baluku H, Musisi NL, et al. High prevalence of methicillin-resistant Staphylococcus aureus in the surgical units of Mulago hospital in Kampala, Uganda. BMC Res Notes 2011;4:326.

[14] Abdeen EE, Mousa W, Hussein H. PCR for detection of virulence and antibiotic resistance genes of coagulase-positive Staphylococcus aureus from clinical mastitis in Egypt. Int J Basic Appl Sci 2015;4:315.

[15] Manza S, Gedam Y, Mshelia G. Morphological and biochemical characterization of staphylococci isolated from food-producing animals in northern Nigeria. Direct Res J Vet Med Anim Sci 2016;1:1–8.

[16] Clinical Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Twenty-seventh informational supplement. Document M100-S27. Wayne, PA: CLSI; 2017.

[17] Sabeel SM, Salih MA, Ali M, El-Zaki SE, Abuzeid N, Elgadi ZA, et al. Phenotypic and genotypic analysis of multidrug-resistant Mycobacterium tuberculosis isolates from Sudanese patients. Tuberc Res Treat 2017;2017:8340746.

[18] Kumar JD, Negi YK, Gaur A, Khanna D. Detection of virulence genes in Staphylococcus aureus isolated from paper currency. Int J Infect Dis 2009;13:e450–5.

[19] Scotts SN, Negro OD, Fowler TL, Fukushima RS, Steward GF. Virulence and antibiotic resistance gene combinations among Staphylococcus aureus isolates from coastal waters of Oahu, Hawaii. J Young Invest 2004;12:8.

[20] Al-Amery K, Elhariri M, Elsayed A, El-Moghazy G, Elhelw R, El-Mahallawy H, et al. Vancomycin-resistant Staphylococcus aureus isolated from camel meat and slaughterhouse workers in Egypt. Antimicrob Resist Infect Control 2019;8:129.

[21] Beyene T, Hayishe H, Gitaw F, Beyi AF, Abunna F, Mammo B, et al. Prevalence and antimicrobial resistance profile of Staphylococcus in dairy farms, abattoir and humans in Addis Ababa, Ethiopia. BMC Res Notes 2017;10:171.

[22] Shekarabi M, Hajikhani B, Salimi Chirani A, Fazeli M, Goudarzi M. Molecular characterization of vancomycin-resistant Staphylococcus aureus strains isolated from clinical samples: a three year study in Tehran, Iran. PLoS One 2017;12:e0183607.

[23] Campbell TL, Henderson J, Heinrichs DE, Brown ED. The yjeQ gene is required for virulence of Staphylococcus aureus. Infect Immun 2006;74:4918–21.

[24] Almeida LMD, Almeida MZPRBD, Mendonça CLD, Mamizuka E. Prevalence and antimicrobial resistance gene combinations among Staphylococcus aureus and Staphylococcus lugdunensis isolated from paper currency in Brazil. J Microbiol 2013;44:493–8.

[25] Smeltzer MS, Gillaspy AF, Pratt FL, Thames MD, Iandolo JJ. Prevalence and chromosomal map location of Staphylococcus aureus and Staphylococcus lugdunensis isolates from coastal waters of Oahu, Hawaii. J Young Invest 2006;74:4918–21.

[26] Trad S, Allignet J, Frangeul L, Davi M, Vergassola M, Couve E, et al. DNA macroarray for identification and typing of Staphylococcus aureus isolates. J Clin Microbiol 2004;42:2054–64.