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Original Article

Prevalence of antibiotic resistance and virulence factors encoding genes in clinical *Staphylococcus aureus* isolates in Saudi Arabia

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ABSTRACT

Background: The present work intended to investigate the carriage of antibiotic resistance and virulence genes in multidrug-resistant *Staphylococcus aureus* isolated from various clinical specimens.

Methods: A total of fifty *S. aureus* isolates from blood cultures, wound swabs, urine sample, nasal swabs, and sputum sample were examined for their antibiotic resistance against 20 different antibiotics by means of E-test, M.I.C Evaluator Strips, and disk diffusion methods. Detection of resistance and virulence-encoding genes (*mecA*, *van*, fnBPA, and Pantone-Valentine Leucocidin (PVL)-encoding genes) was performed by PCR.

Results: In the current study, low number of MRSA isolates has been detected from five different clinical samples (22%, *n* = 50). In this study, we observed multiple drug resistance in *S. aureus* isolates from wound swabs; nasal swabs, blood cultures, and urine sample. No vancomycin-resistant genes were detected in all 50 isolates; similarly, no PVL and *van* genes were detected, while *mecA* and FnBP genes were detected in low number of isolates.

Conclusion: Although the number of MRSA and fnBPA-positive *S. aureus* reported in this study is generally low, and despite the absence of PVL and *van*-encoding genes, the results reported in this study may continue to shed some light on the prevalence of MRSA in Makkah, Saudi Arabia. Further epidemiological surveys are required together with additional infection control measures to limit the spread of MRSA particularly multidrug-resistant strains.

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1. Introduction

Staphylococcus aureus is one of the most common human pathogens and is capable of causing a wide range of infections such as cellulitis, impetigo, and soft tissue abscesses. The ability of *S. aureus* to cause a multitude of infections is probably due to the expression of a wide range of virulence factors, as the following: adhesion proteins, toxins and enzymes.¹ Methicillin-resistant *Staphylococcus aureus* (MRSA) is currently the most prevalent antibiotic-resistant pathogen causing nosocomial infections in hospitals (HA-MRSA) in many parts of the world with increasing prevalence in various community populations (CA-MRSA).^{2,3}

S. aureus expresses Fibronectin-Binding Protein A (fnBPA), a cell wall-anchored protein, which mediates the adhesion of the cells to fibrinogen, elastin, and fibronectin.⁴ The fnBPA protein is believed to be indispensable for adhesion to and internalization by nonprofessional phagocytic cells upon ingestion by inflammatory macrophages. FnBPA have been shown to be important in *in vitro* and *in vivo* infections by *S. aureus*; furthermore, the cooperation between fnBPA and fnBPB is essential for the induction of severe infections resulting in septic death.^{5–7} The Pantone-Valentine Leucocidin (PVL) is a bicomponent leucotoxin composed of S-related and F-related proteins that are secreted separately but act synergistically; the cytotoxin is found to cause leukocyte destruction and tissue necrosis.⁸

Methicillin resistance in *S. aureus* is conferred by the *mecA* gene, which codes for penicillin-binding protein 2a (PBP2a) causing decreased binding affinity for the β -lactam antibiotics, including the penicillinase-resistant penicillin. The *mecA* gene resides on a mobile genetic element, the staphylococcal cassette chromosome *mec* (SCC*mec*). The *mecA* gene complex contains insertion sites for other mobile genetic elements (e.g. plasmids and transposons) that facilitate the acquisition of resistance genes to other antibiotics.¹ Epidemiological studies have shown that hospital- and community-acquired MRSA infections are increasing in many parts of the world.²

Vancomycin (glycopeptides) has become an approved and highly recommended antibiotic worldwide for the treatment of *S. aureus* infections, particularly MRSA.⁹ Although vancomycin-resistant *S. aureus* is rare, the emergence of strains with decreased susceptibility and/or vancomycin intermediate resistance has been reported.^{10–12}

In Saudi Arabia, the incidence of community- and hospital-acquired MRSA infections is well documented.^{13–17} However, few published accounts have reported the prevalence of PVL-positive *S. aureus*,^{18,19} with no available reports regarding the fnBPA-positive *S. aureus* as well as vancomycin resistant and/or vancomycin intermediate *S. aureus* in Saudi Arabia. Therefore, this dearth of information about the incidence of *S. aureus* with PVL, fnBPA, and *van*-encoding genes in Saudi Arabia has led us to investigate the carriage of these genes in addition to *mecA* gene in a collection of *S. aureus* isolates from various clinical specimens in western Saudi Arabia.

2. Materials and methods

2.1. Isolates

A total of 50 *S. aureus* isolates were thankfully obtained from clinical laboratories in Makkah, western Saudi Arabia. These isolates were recovered from wound swabs (17), nasal swabs (24), urine (1), sputum (3), and blood cultures (5). The isolates were transported to the laboratory on blood agar plates, in an icebox.

2.2. Biochemical confirmation of the isolates

Once the isolates were received in the laboratory, they were given a code number, and each isolate was subcultured on blood agar and DNase agar plates (Oxoid, Basingstoke, UK); incubation was at 34 °C for 24 h for further identification and confirmation. All *S. aureus* isolates growing on blood agar plates were observed for their type of hemolysis, and were observed for their ability to produce deoxyribonuclease on DNase agar.²⁰ All isolates were examined for their ability to

Table 1 – Primers used for detection of *mecA*, *Van*, *PVL*, and *FnBPA*-encoding genes by PCR.

Gene	Primer sequence	Product size (bp)	Reference
<i>mecA</i>	F 5'-AAAATCGATGGTAAAGTTGGC-3' R 5'-AGTTCTGGAGTACCGGATTTCG-3'	533	27
<i>vanA/vanA1</i>	F 5'-ATGAATAGATAAAAGTTGCAATAC R 5'-CCCCTTTAACGCTAATACGAT	1029	28
<i>vanB/vanB1</i>	F 5'-CCCGAATTTCAAATGATTGAAAA R 5'-CGCCATCCTCCTGCAAAA	457	28
<i>vanC/vanC1</i>	F 5'-GCTGAAATATGAAGTAATGACCA R 5'-CGGCATGGTGTGATTTCGTT	811	28
<i>vanS/vanS1</i>	F 5'-AACGACTATTCCAACTAGAAC R 5'-GCTGGAAGCTCTACCTAAA	1094	28
<i>vanY/vanY1</i>	F 5'-ACTTAGGTTATGACTACGTTAAT R 5'-CCTCCTTGAATTAGTATGTGTT	866	28
PVL	LukS-PV 5'-AGTGAAGTATCTTTCTATTGAAAAACACTC-3' LukS-PV 5'-GCATCAASTGTATTGGATAGCAAAAGC-3'	433	29
fnBPA	F 5'-CACAAACCAGCAAATATAG-3' R 5'-CTGTGTGGTAATCAATGTC-3'	1362	29

produce clumping factor by using the Staphaurex latex agglutination test kit (Oxoid), and production of catalase.²¹

2.3. Antimicrobial susceptibility testing

The antimicrobial susceptibility testing of *S. aureus* isolates was carried out against twenty antibacterial agents. The MICs were determined by the E-test (Biomérieux, France) for the following antimicrobials: Oxacillin, Rifampin, Tetracycline, Vancomycin, Moxifloxacin, Linezolid, Levofloxacin, Imipenem, Gentamicin, Fosfomycin, Erythromycin, Daptomycin, Clindamycin, Ciprofloxacin, Azithromycin, Ampicillin, and Amoxycillin/Clavulanic acid, and by using M.I.C Evaluator Strips (Oxoid) for Penicillin, according to the manufacturer instructions. Testing for Fusidic acid ($10 \mu\text{g ml}^{-1}$)²² and Screening for Cefoxitin ($30 \mu\text{g ml}^{-1}$)²³ were determined by disk diffusion method (Kirby-Bauer technique). Fusidic acid and Cefoxitin disks were purchased from Oxoid. All antimicrobial susceptibility testing were performed by preparing a 0.5 McFarland standard suspension of each isolate and lawn culture was done on Mueller-Hinton agar (Oxoid); plates were incubated at 34°C for 18 h.²³ For the interpretation of susceptibility, the guideline of Clinical Laboratory Standard Institute²⁴ was followed for all antimicrobials except for fusidic acid, for which the European Committee on Antimicrobial Susceptibility Testing guidelines²⁵ were followed, because CLSI does not provide disk susceptibility breakpoints for fusidic acid.²²

2.4. Control strains

S. aureus ATCC 29213 was used as control strain for biochemical identification and for antimicrobial susceptibility testing by Etest and disk diffusion methods. *S. aureus* ATCC 49775 was used as a control strain for PVL-encoding gene. *S. aureus* ATCC was used as a control strain for fnBPA-encoding gene and *S. aureus* ATCC 33591 was used as a control strain for *mecA* gene.

2.5. PCR detection of *mecA*, *van*, *fnBPA*, and PVL-encoding genes

Isolation of genomic DNA from *S. aureus* isolates grown overnight on blood agar plates was performed according to the procedure described by Sambrook and Russell.²⁶ A volume of $1.0 \mu\text{l}$ extracted DNA (approximately $0.5 \mu\text{g}$) was added to a final volume of $25 \mu\text{l}$ of PCR mixture containing 1.25 IU of Taq polymerase (ABgene, Surrey, UK), $1.5 \text{ mmol l}^{-1} \text{ MgCl}_2$, $0.15 \text{ mmol l}^{-1} \text{ dNTPs}$, $0.7 \mu\text{l}$ of $0.8 \mu\text{mol l}^{-1}$ of each primer, and $12.6 \mu\text{l}$ of sterile deionized water. The thermal cycling for the detection of *mecA* gene was performed as described by Purnajat et al.²⁷; thermal cycling for the detection of *vanA*, *vanB*, *vanC*, *vanS*, and *vanY* genes was carried out as described by Abulreesh²⁸ and for the detection of PVL and fnBPA-encoding genes, thermal cycling was performed according to the Netsvyetayeva et al.²⁹ procedure (Table 1). A total of $20 \mu\text{l}$ of PCR reaction mix was analyzed by gel electrophoresis on a 0.8% agarose gel (Bioline, London, UK) in Tris-borate buffer, and made visible by ethidium bromide ($0.5 \mu\text{g ml}^{-1}$) staining and UV transillumination.

Table 2 – Antimicrobial profiles of *Staphylococcus aureus* isolates.

Isolate origin	No. of <i>S. aureus</i>	No. of resistant isolates (%)															
		Amox	Amp	Azi	Cefo	Cip	Clin	Dapt	Eryt	Fos	Fus	Gen	Imi	Lev	Lin	Mox	Ox
Wound swab	17	8.0 (47)	17 (100)	2.0 (12)	8.0 (47)	0.0	0.0	0.0	2.0 (12)	0.0	6.0 (35)	2.0 (12)	8.0 (47)	0.0	0.0	0.0	8.0 (47)
Nasal swab	24	0.0	24 (100)	4.0 (17)	0.0	0.0	4.0 (17)	0.0	4.0 (17)	0.0	2.0 (8.0)	0.0	0.0	0.0	0.0	0.0	0.0
Urine	1.0	0.0	1.0 (100)	0.0	1.0 (100)	0.0	0.0	0.0	0.0	0.0	0.0	1.0 (100)	0.0	0.0	0.0	0.0	1.0 (100)
Sputum	3.0	0.0	3.0 (100)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Blood cultures	5.0	0.0	5 (100)	0.0	2.0 (40)	0.0	0.0	0.0	0.0	0.0	1.0 (20)	0.0	1.0 (20)	0.0	0.0	0.0	2.0 (40)
Total	50	8.0 (16)	50 (100)	6.0 (12)	11 (22)	0.0	5.0 (10)	0.0	5.0 (10)	0.0	9.0 (18)	3.0 (6.0)	9.0 (18)	0.0	0.0	0.0	11 (22)

Amox, amoxicillin/clavulanate; Amp, ampicillin; Azi, azithromycin; Cefo, cefoxitin; Cip, ciprofloxacin; Clin, clindamycin; Dapt, daptomycin; Ery, erythromycin; Fos, fosfomycin; Fus, fusidic acid; Gen, gentamicin; Imi, imipenem; Lev, levofloxacin; Lin, linezolid; Mox, moxifloxacin; Ox, oxacillin; P, penicillin; Rif, rifampin; Tet, tetracycline; Van, vancomycin.

Table 3 – Detection of antimicrobial resistance and virulence factors encoding genes in *Staphylococcus aureus*.

Isolate origin	No. of <i>S. aureus</i>	<i>mecA</i>	<i>vanA</i>	<i>vanB</i>	<i>vanC</i>	<i>vanS</i>	<i>vanY</i>	PVL	fnBPA
Wound swab	17	8.0 (47%)	0.0	0.0	0.0	0.0	0.0	0.0	4.0 (24%)
Nasal swab	24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Urine	1.0	1.0 (100%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sputum	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Blood culture	5.0	2.0 (40%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total	50	11 (22%)	0.0	0.0	0.0	0.0	0.0	0.0	4.0 (8.0%)

Fig. 1 – Agarose gel electrophoresis (1% agarose) for *mecA* gene amplified size 533 bp as compared with 1 kbp ladder. Positive results in lanes 1 (SW3), 2 (SW9), and 3 (SABC4).

3. Results

A collection of 50 *S. aureus* isolates derived from various clinical specimens were investigated for their carriage of antimicrobial-resistance and virulence genes. The antimicrobial-resistance profiles of the 50 isolates are shown in Table 2. Out of the 50 isolates, only eleven (22%) were resistant to oxacillin and cefoxitin, followed by fusidic acid (18%). Resistance to tetracycline, erythromycin, and clindamycin was exhibited by (16%), (10%), and (10%) of the isolates respectively. No vancomycin and rifampin resistances were detected among these 50 isolates (Table 2). It is worth mentioning that

all eleven isolates that were resistant to oxacillin were susceptible to cefoxitin (Table 2).

None of the 50 isolates were positive for *van* genes (*vanA*, *vanB*, *vanC*, *vanS*, and *vanY*) and PVL gene (Table 3). The same isolates that showed resistance to oxacillin and cefoxitin were positive for *mecA* gene (Fig. 1); these isolates were derived from wound swab (47%, $n = 17$) and blood cultures (40%, $n = 5$). The carriage of fnBPA gene was exhibited in 8% ($n = 50$) of the isolates, this gene was carried by isolates recovered from wound swabs (Table 3).

S. aureus isolates with multiple resistance profiles were observed in this study (Table 4). The most notable isolate is (SAW2), derived from a wound swab that exhibited resistance

Table 4 – Resistance levels (MIC) and multidrug-resistance profiles of selected *Staphylococcus aureus* isolates.

Isolate	Isolate origin	MIC ($\mu\text{g ml}^{-1}$)								Disk breakpoints (mm)				
		Azi	Ery	Amp	Cli	Amox	Ox	Tet	Imi	Gen	Cef	Fus	<i>mecA</i>	FnBPA
SAW2	Wound	S	S	>8	S	≤ 4	>2	>8	≤ 4	>8	≤ 19	<22	+	+
SAW3	Wound	S	S	S	S	≤ 4	>2	>8	≤ 4	S	≤ 19	S	+	+
SAW4	Wound	S	S	S	S	≤ 4	>2	>8	≤ 4	S	≤ 19	S	+	–
SAW8	Wound	S	S	S	S	≤ 4	>2	>8	≤ 4	S	≤ 19	<22	+	+
SAW9	Wound	S	S	S	S	≤ 4	>2	S	≤ 4	S	≤ 19	<22	+	–
SAW15	Wound	>4	>4	S	S	S	S	S	S	S	S	S	–	+
SANS3	Nasal	>4	>4	S	≤ 0.25	≤ 4	S	S	S	S	S	<22	–	–
SANS12	Nasal	>4	>4	S	≤ 0.25	≤ 4	S	>8	S	S	S	<22	–	–
SANS16	Nasal	>4	>4	S	≤ 0.25	≤ 4	S	>8	S	S	S	S	–	–
SANS22	Nasal	>4	>4	S	≤ 0.25	≤ 4	S	S	S	S	S	S	–	–
SABC4	Blood culture	S	S	>8	S	S	>2	S	≤ 4	S	≤ 19	<22	+	–
SAUR1	Urine	S	S	>8	S	S	>2	S	S	>8	S	S	+	–

Amox, amoxicillin/calvulanate; Amp, ampicillin; Azi, azithromycin; Cefo, cefoxitin; Clin, clindamycin; Ery, erythromycin; Fus, fusidic acid; Imi, imipenem; Ox, oxacillin; Tet, tetracycline.

to ampicillin, amoxicillin/calvulanate, oxacillin, tetracycline, imipenem, gentamicin, ceftioxin, and fusidic acid, and also carried *mecA* and *fnBPA*-encoding genes. Similarly, isolate (SAW8) from wound swab exhibited multiple resistance and carried *mecA* and *fnBPA*-encoding genes. Multiple resistance profiles were also noted among nasal swab isolates (e.g. SAN12) that showed resistance to azithromycin, erythromycin, clindamycin, amoxicillin/calvulanate, tetracycline and fusidic acid. Among isolates recovered from blood cultures, (SABC4) exhibited resistance to ampicillin, tetracycline, imipenem, oxacillin, ceftioxin, and fusidic acid and carried *mecA* gene (Table 4).

4. Discussion

In the current study, low number of MRSA isolates has been detected from five different clinical samples (22%, $n = 50$). Infections due to MRSA remain a major problem worldwide.^{30,31} In Saudi Arabia, the prevalence of MRSA in clinical specimens is well documented, with various degrees of detection rates.^{15–17} The abundance of MRSA isolates varies within different regions in the same country and across countries, where there are different epidemiological factors such as different geographical regions and health system capability in running infection control program. These factors play a role in the variability of prevalence of MRSA isolates.³² Infections due to MRSA have been associated with a variety of clinical manifestations, including mild skin infection, pneumonia, and sepsis. Furthermore, there have been reports of increasing mortality associated with MRSA bacteremia and ventilator-associated pneumonia associated with MRSA. Generally, the main prognostic factor in patients with MRSA infections is inadequate antibiotic therapy.²

Infections due to multidrug-resistant *S. aureus* are a major problem, due to the difficulty of its treatment. In this study, we observed multiple drug resistance in *S. aureus* isolates from wound swabs, nasal swabs, blood cultures, and urine sample. Wounds can be the source of Staphylococcal infection, where it can be contaminated by nasal *S. aureus* (both MRSA and non-MRSA).³³ The observed multiple-resistance among some of the wound isolates in this study may suggest that these isolates were from hospitalized patients, because multiple-resistant *S. aureus* strains from wounds are more common in the hospital community.³⁴

S. aureus is part of the natural microbiota found in the nasal cavities, including MRSA.³⁵ In the current study, multiple resistance was observed in only four out of 24 nasal swab isolates. Similar results were reported by other researchers in Saudi Arabia in hospitalized patients and healthcare personnel.^{36–38} Nasal cavity is the natural ecological niche of staphylococci, and the nasal carriage of multidrug-resistant *S. aureus* is thought to be a major risk factor of staphylococcal transmission among humans.^{35,39,40}

In the current study, resistance to fusidic acid was observed (18%, $n = 50$) in wound swab and nasal swab isolates. Similarly, low number of isolates resistant to fusidic acid was reported by Alsterholm et al.⁴¹; however, a high rate of fusidic acid resistant *S. aureus* was reported by Katopodis et al.²² Fusidic acid has been used as a topical treatment for skin and soft

tissue infections caused by *S. aureus*.⁴¹ Due to unrestricted use of fusidic acid, a number of outbreaks of impetigo and other skin and soft tissue infections have been related to fusidic acid resistant *S. aureus* and therefore the use of fusidic acid should be restricted to prevent the spread of fusidic acid resistant *S. aureus*.^{22,41}

Vancomycin has been successfully used for the past 50 years for the treatment of staphylococcal infections, and particularly the cases involved MRSA. However, its reliability has been questioned by the emergence of *S. aureus* strains that exhibit complete or intermediate resistance.⁴² Using the disk diffusion methods, the detection of vancomycin-resistant *S. aureus* was reported from clinical as well as from food and water specimens.^{28,35,43,44} However, in the United States, the Clinical and Laboratory Standard Institute (CLSI) only approves methods that determine MIC values for the detection of vancomycin resistance in staphylococci due to its accuracy; this is also standardized in other countries such as Japan and European Union.⁴⁵ In this study, resistance to vancomycin was not detected in any of the 50 isolates, neither by using E-test method, not by PCR for the detection of *van* genes. Despite the sporadic occurrence of *S. aureus* that carry vancomycin-resistant genes, it is generally accepted that these strains are rare.^{42,45–47}

PVL-encoding genes are frequently detected in *S. aureus* from skin and soft tissue infections, and yet none of the 17 isolates from wound swab gave negative PCR for PVL-encoding genes. This might be explained by the fact that PVL toxin is associated with community-associated *S. aureus* and not all CA-*S. aureus* isolates produce PVL; additionally, both PVL-positive and negative staphylococci belong to various clonal complexes,⁴⁸ and possibly the success of PVL-positive staphylococci to cause infections is associated with particular clones.⁴⁹ In Saudi Arabia, Ahmed and Asghar¹⁹ reported the presence of PVL genes in 21% ($n = 206$) of CA-*S. aureus* in Makkah city, and the results reported in this study showed no PVL-positive isolates, while in other parts of the world there has been an increase of the PVL-positive staphylococci; this might be attributed to social, host, and environmental factors, which include travel-associated spread of these isolates.⁵⁰ Another reason that could explain the absence of PVL-positive isolates reported in this study is the fact that the production of PVL toxin has been preferentially linked to furuncles, cutaneous abscesses, and severe necrotic skin infection,⁸ which means that the PVL gene could be more frequent in staphylococcal strains causing disease by direct invasion and tissue destruction (e.g. primary skin infection) than strains causing secondary infections after skin injury, urinary tract infection, infective endocarditis, toxic shock syndrome and mediastinitis.⁸

The presence *fnBPA*-encoding gene (adhesion factor) among clinical isolates in this study was low (8.0%, $n = 50$). To the authors' best knowledge, this is the first report of *fnBPA*-positive *S. aureus* in Makkah, Saudi Arabia. This gene is more prevalent in *S. aureus* isolates colonizing the human nasal epithelium²⁰; however, none of our nasal swab isolates were positive for *fnBPA*-encoding genes. It was reported that *fnBPA*-encoding genes are more associated with methicillin-sensitive *Staphylococcus aureus* (MSSA) rather than MRSA^{20,29}; however, in this study, *fnBPA*-encoding genes were detected in three

MRSA isolates and one MSSA isolate recovered from wound swabs. FnBPA protein is an important adhesion factor that helps the internalization by cells and the induction of severe infections resulting in septic death.⁶ The cooperation between fnBPA and fnBPB helps the progress of staphylococci infections by establishing bacterial colonization, followed by bacterial multiplication and abscess formation on various organs, furthermore fnBPA and fnBPB together are indispensable factors in the establishment of staphylococcal septicemia.⁶

5. Conclusions

Although the number of MRSA and fnBPA-positive *S. aureus* reported in this study is generally low, and despite the absence of PVL and *van*-encoding genes, the results reported in this study may continue to shed some light on the prevalence of MRSA in Makkah, Saudi Arabia. Further epidemiological surveys are required together with additional infection control measures to limit the spread of MRSA, particularly multidrug-resistant strains.

Conflicts of interest

The authors have none to declare.

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