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The Prevalence and Clinical Characteristics of Multidrug-resistant Hospital-acquired *Staphylococcus aureus* in Medina, Saudi Arabia

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Abstract

Hospital acquired-*Staphylococcus aureus* (HA-*Staphylococcus aureus*), particularly methicillin-resistant *Staphylococcus aureus* (MRSA), are an important source of nosocomial infections with high morbidity and mortality rates. Few reports showed that infections due to HA-*Staphylococcus aureus* in Saudi Arabia is increasing, particularly infections attributed to HA-MRSA. The study aimed to explore the prevalence and clinical characteristics of HA-*Staphylococcus aureus* for the first time in Medina, Saudi Arabia. A total of 1262 clinical samples of hospitalized patients were examined for the presence of *Staphylococcus aureus* through selective culturing on mannitol salt agar. Vitek Compact System and conventional methods were followed to confirm the isolates. Vitek Compact System tested the antimicrobial susceptibility of isolates whereas the standard PCR was employed to detect the genes encoding antimicrobial resistance (*mecA* and *vanA*) and virulence factors (*tst*, et, and *LukS-PV*). The overall HA-*Staphylococcus aureus* prevalence was low (6.58%, n = 1262) of which 84.34% (n = 83) were MRSA. Approximately, 57 samples of the 70 MRSA (81.5%) exhibited a multidrug-resistance (MDR) pattern. All the 83 HA-*Staphylococcus aureus* isolates were negative for the genes encoding toxic shock syndrome toxin, exfoliative toxin, and Panton-Valentine leukocidin. The study was conducted during the Covid-19 pandemic under partial lockdown, restricted hospitalization, and increased disinfection and infection control measures. Therefore, the low prevalence of HA-*Staphylococcus aureus* should be carefully interpreted and further multicenter investigations could reveal its true incidence in the city. The high prevalence of MDR HA-MRSA is alarming as it highlights inappropriate antibiotic prescriptions to counter staphylococcal infections. HA-*Staphylococcus aureus* investigated in this study might lack certain virulence factors. However, their MDR traits and invasive nature could worsen the situation if not properly handled.

Keywords: *Staphylococcus aureus*, MRSA, *mecA*, Hospital-acquired, Antimicrobial Resistance, Virulence Factors

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INTRODUCTION

Staphylococcus aureus is a Gram-positive coccus that commonly inhabits human skin and the nasopharyngeal cavity. *S. aureus* is a common part of human microbial flora that is present in the nares of 20 to 40% of adults and also colonizes the perineum, skin folds, vagina, and axillae.[1,2] Several infections such as self-limiting food poisoning, mild skin infections, and life-threatening diseases with high mortality and morbidity rates are associated with *S. aureus*.[2,3]

S. aureus causes various soft tissue infections (wound infections, boils, furuncles, cellulitis, impetigos, scalded skin syndrome, and carbuncles), life-threatening bloodstream infections (pulmonary, meningitis, and endocarditis), skeletal muscles infection (pyomyositis), bone infections, ear and eye infection, joint infections, and urinary tract infection. Staphylococcal toxic shock syndrome (STTS) is also a life-threatening multisystem infection that is characterized by skin rash, fever, diarrhea, hypotension, chills, renal failure, dizziness, sore throat, vomiting, conjunctivitis, and headache.[1-3]

S. aureus infections can be acquired from the community (CA-*Staphylococcus aureus*) and hospitals (HA-*Staphylococcus aureus*). CA and HA strains significantly vary in susceptibility to antimicrobial agents, virulence, and invasiveness. Both types of infections are widespread and rising globally.[2] HA-*Staphylococcus aureus* infection, especially related to methicillin-resistant *Staphylococcus aureus* (MRSA), might result in higher rates of mortality and morbidity.[1,3]

Several nosocomial infections are associated with HA-MRSA worldwide and multidrug-resistant (MDR) strains reduce the antibiotic efficacy leading to increased morbidity and mortality rates. HA-MRSA was categorized as the second most prevalent infection in the USA during the two nationwide studies.[4,5] HA-MRSA resistance to antibiotics, invasiveness, and enhanced morbidity and prevalence has been reported in various countries.[6-10] and different regions of Saudi Arabia as well.[11-14]

S. aureus infections (mild and invasive) could be due to the presence of enzymes, virulence factors, toxins, and different immune system response suppressing mechanisms.[3,15] A superantigen toxin (toxic shock syndrome toxin or TSST-1) encoded by the *tst* gene causes the fatal Toxic shock syndrome. TSST-1 is part of the mobile genetic element staphylococcal pathogenicity island and is classified among superantigens (SAgs). The three main characteristics of TSST-1 include (i) superantigenicity, (ii) pyrogenicity, and (iii) enhanced lethality in rabbits even at small amounts.[2,3,15] A serious skin infection known as scalded skin syndrome infection is caused by a staphylococcal exfoliative toxin (ET) (ET-A and ET-B). This infection peels the outer skin layer similar to dousing with hot liquid. *eta* gene encoded by transferable plasmid encodes the toxin of this infection that is carried by the bacteriophage.[2,15] In addition to the cytotoxicity of α-, β-, γ- and δ-hemolysins to red and white blood cells, a bi-component leukotoxin (Panton-Valentine leukocidin - PVL) causes the cell lysis of alveolar macrophage and neutrophils. Lysogenic phage transfers the *LukS-PV* and *LukF-PV* genes encoded toxins to staphylococci.[2,15]

There are limited reports regarding the HA-*Staphylococcus aureus* prevalence in Saudi Arabia. Only a few studies have investigated the HA-MRSA prevalence in the health care settings of Saudi Arabia.[16] Overall, they found the highest (40-60%) HA-MRSA prevalence in Assir and Riyadh provinces (southern and central Saudi Arabia) followed by Makkah province (western Saudi Arabia) (25-40%). Comparatively lower HA-MRSA infections were noted in the Eastern province (30%) and Al Jouf region (20%) (Northern Saudi Arabia). However, virulence factors (ET, TSST-1, and PVL) encoding genes and multidrug resistance patterns of HA-MRSA were not investigated during most of these studies.[11-22] HA-*Staphylococcus aureus* (MRSA) or methicillin-sensitive *Staphylococcus aureus* (MSSA) prevalence data of eight geographical Saudi Arabian regions are still not available, which include Medina (northwestern), Qassim and Hail (north central), Tabuk (north and northwestern), Northern Border (northeastern), Najran and Al-Baha (western), and Jazan (southwestern). This study first time aimed to investigate the prevalence, antimicrobial susceptibility patterns, virulence factors (TSST-1,

PVL, ET,) encoding genes, and multidrug-resistance of *S. aureus* in a health care setting of Medina city (Medina province), northwest of Saudi Arabia.

MATERIALS AND METHODS

Sample collection

A total of 1262 routine samples of hospitalized patients (males and females) in Ohoud Hospital were examined for the presence of *S. aureus* between October 2020 and February 2021. The samples were comprised of blood culture (15), wound swabs (38), nasal swabs (354), groin swabs (352), ear swabs (20), eye swabs (10), axial swabs (352), urine samples (46), sputum (65), and vaginal swabs (10). All samples were brought to the laboratory and examined within 6 hrs. Only the patient's gender was recorded, whereas the personal information, epidemiological data, and clinical history were not disclosed.

Detection of *Staphylococcus aureus*

Wound, nasal, eye, ear, groin, and axial swabs were streaked onto mannitol salt agar (MSA) (Oxoid, Basingstoke, UK), and sheep blood agar (Oxoid) plates.[23] Blood cultures with a positive growth index were subjected to Gram staining. Blood cultures containing clusters of Gram-positive cocci were considered positive for *Staphylococcus* sp. The standard method was followed to examine the blood cultures-containing blood agar plates.[2,24]

A calibrated loop (0.01 ml) of urine samples was cultured on cystine-lactose-electrolyte-deficient (CLED) (Oxoid) and blood agar plates.[25] Sputum samples were processed by adding sputasol (Oxoid), incubating for 30 min at 37°C, vortexing, and homogenizing in brain heart infusion broth (BHI) (Oxoid). Then, an aliquot (100 μl) was cultured onto sheep blood agar plates.[26] All plates (mannitol salt agar, CLED, and sheep blood agar) were incubated at 35±2°C for 24-48 hrs.[23]

Identification of presumptive *Staphylococcus aureus*

All presumptive *S. aureus* colonies on mannitol salt agar (white to pale yellow colonies surrounded with bright yellow zones), CLED (uniformly deep yellow colonies), and sheep blood agar plates (light to golden yellow pigment, usually with β-hemolytic activity),[27] were identified by Gram staining, catalase, and oxidase tests.[28] Mast®Staph latex agglutination kit (Mast Group Ltd., Liverpool, UK) was used to conduct Clumping factor tests.[23] Presumptive *S. aureus* isolates were further confirmed through Vitek® 2 Compact System (bioMerieux, Marcy, l'Etoile, France).[26]

Antimicrobial susceptibility testing

Antimicrobial susceptibility was tested by minimum inhibitory concentration (MIC) of confirmed *S. aureus* isolates using Vitek® 2 Compact System (bioMeriex).[23] *S. aureus*

Table 1. Primers sequence and product size for genes encoding virulence factors and antimicrobial resistance in hospital acquired *Staphylococcus aureus*

gene	sequence	Product size (bp)	Annealing Temp.	Ref.
	Sequence Genes encoding antimicrobial resistance			
mecA	F 5'-AAAATCGATGGTAAAGGTTGGC-3'	533	57°C	30
	R 5'-AGTTCTGGAGTACCGGATTTGC-3'	1029	57°C	30
van (vanA / vanA1)	F 5'-ATGAATAGAATAAAAGTTGCAATAC-3'			
	R 5'-CCCCTTTAACGCTAATACGAT-3'			
	Genes encoding virulence factors			
lukS-PV	F 5'-AGTGAACTTATCTTTCTATTGAAAAACACTC-3'	433	57°C	30
	R 5'-GCATCAASTGTATTGGATAGCAAAAGC-3'			
tst (TSST1/ TSST2)	F 5'-ATGGCAGCATCAGCTTGATA-3'	350	57°C	31
	R 5'-TTTCCAATAACCACCCGTTT-3'			
et (ETA1 / ETA2)	F 5'-CTAGTGCATTTGTTATTCAA-3'	119	57°C	31
	R 5'-TGCATTGACACCATAGTACT-3'			

susceptibility profiles were interpreted based on the recommendations of the Clinical Laboratory Standard Institute.[29] Antimicrobial agents, used in this study, belonged to twelve different classes and included benzylpenicillin [penicillin G], oxacillin [penicillins], moxifloxacin and levofloxacin, [fluoroquinolones], rifampicin [ansamycins], tobramycin and gentamicin, [aminoglycosides], teicoplanin and vancomycin, [glycopeptides], erythromycin [macrolides], clindamycin [lincosamides], linezolid [oxazolidinones], tigecycline and tetracycline [tetracyclines], nitrofurantoin [nitrofurans], fusidic acid [misc agent], and trimethoprim/sulfamethoxazole [folate pathway antagonists]. The Vitek® system was also used to screen the cefoxitin and clindamycin resistance of all isolates.

Molecular detection of genes encoding virulence factors and antimicrobial resistance

PCR was performed for the molecular detection of genes encoding virulence factors, toxic shock syndrome toxin (*tst*), exfoliative toxin (*et*), Panton-Valentine leukocidin (*lukS-PV*), and antimicrobial resistance (methicillin resistance (*mecA*) and vancomycin resistance (*vanA*). Table 1 presents the primer sequence, product size, and annealing temperature of each primer. PCR was carried out according to the previously described protocol.[30,31] Briefly, a total RNA extraction kit was used to extract staphylococcal genomic DNA (Geneaid Biotech Ltd, New Taipei City, Taiwan). PCR reaction mix consisted of 1 µl of primers (100 pM pH8) (*mec*A F, *mec*A R), (*van*A F, *van*A R), (*lukS-PV* F, *lukS-PV* R), (TSST1, TSST2), and (ETA1, ETA2)), 18 µl of dH$_2$O, 1 µl of template DNA, and 5 µl of Ultra-Pure *Taq* PCR master mix (Geneaid Biotech Ltd, New Taipei City, Taiwan). Thermal cycling was performed in Veriti 96-Well Thermal Cycler (Applied Biosystems, Massachusetts, USA) with an initial denaturation at 94°C for 2 min followed by 45 denaturation cycles at 94°C for 20 seconds, annealing at 57°C and 54°C for 30 seconds, and elongation for 1 minute at 72°C. A final elongation was carried out at 72°C for 7 min. PCR samples were subjected to gel electrophoresis (1.5% agarose) in an M12 Complete Electrophoresis Package (Edvotek Inc, Washington D.C., USA) for 40 min at 90 volts. PCR amplification bands were visualized under UV light

Table 2. Prevalence of *Staphylococcus aureus* in clinical samples of hospitalized patients

Sample	Origin	N	P	% of positive
Blood culture	Male	10	7	70
	Female	5	5	100
Total		15	12	80
Wound swabs	Males	20	11	55
	Females	18	10	56
Total		38	21	55.3
Ear swabs	Males	5	1	20
	Females	15	3	20
Total		20	4	20
Eye swabs	Males	5	3	60
	Females	5	0	0
Total		10	3	30
Urine	Males	22	3	14
	Females	24	1	4.2
Total		46	4	9.1
Nasal swabs	Males	236	13	5.5
	Females	118	6	5.1
Total		354	19	5.4
Sputum	Males	44	7	16
	Females	21	2	9.6
Total		65	9	14
Groin swabs	Males	234	4	1.71
	Females	118	2	1.7
Total		352	6	2.4
Axillae swabs	Males	234	2	0.86
	Females	118	3	2.55
Total		352	5	1.42
Vaginal swabs	Males	N/A	N/A	N/A
	Females	10	0	0
Total	Males	810	51	6.3
	Females	452	32	7.1
	Grand total	1262	83	6.58

N = total number of samples, P = total number of positive samples for *S. aureus*

using a ChemiDoc-It2 Imaging System (Analytik Jena GmbH, Jena, Germany).

Control strains

S. aureus ATCC® 25923™, and *Escherichia coli* ATCC® 25922™ served as controls throughout the study.

RESULTS

The results demonstrated an overall low prevalence (6.58%, n =1262) of *S. aureus* in the clinical samples of individuals hospitalized at

Table 3. Antimicrobial resistance profiles of HA-*Staphylococcus aureus*

Samples	N/P	BP	OX	G	TO	LE	MO	E	CL	LI	TEI	VA	TET	TIG	NT	FA	RIF	TS
													Resistance profiles					
Blood culture	15/12	12	10	5	5	1	0	0	0	0	0	0	7	0	0	10	0	2
Wound swabs	38/21	21	18	8	8	5	3	3	2	0	0	0	4	0	0	16	1	1
Ear swabs	20/4	4	4	1	1	1	1	1	1	0	0	0	1	0	0	3	1	0
Eye swabs	10/3	3	1	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0
Urine	46/4	4	4	0	0	3	3	1	1	0	0	0	0	0	0	3	0	0
Nasal swabs	354/19	18	15	1	2	7	3	6	4	0	0	0	1	0	0	14	1	0
Sputum	65/9	8	7	0	1	5	0	2	2	0	0	0	1	0	0	5	0	1
Groin swabs	352/6	6	6	1	2	3	1	2	1	0	0	0	3	0	0	5	0	1
Axial swabs	352/5	5	5	0	0	3	3	2	1	0	0	0	0	0	0	3	0	1
Vaginal swabs	10/0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	1262/83	81	70	16	19	29	15	17	12	0	0	0	17	0	0	60	3	6

N = total number of samples, P = total number of positive samples for *Staphylococcus aureus,* BP = benzylpenicillin, OX = oxacillin, G = gentamicin, TO = tobramycin, LE = levofloxacin, MO = moxifloxacin, E = erythromycin, CL = clindamycin, LI = linzolid, TEI = teicoplanin, VA = vancomycin, TET = tetracycline, TIG = tigecyclin, NT = nitrofurantoin, FA = fusidic acid, RFI = rifampcin, TS = trimethoprim/sulfamethoxazole

Ohoud Hospital, Medina, Saudi Arabia (Table 2). A higher *S. aureus* prevalence was observed in blood cultures with 80% (n = 15) positive samples followed by 55.3% (n = 38) in wound swabs (Table 2). Sputum (14%, n = 65), urine (9.1%, n = 44), and nasal swab (5.4%, n = 354) samples presented a low *S. aureus* positivity rate (Table 2). *S. aureus* was detected from all types of samples at varying prevalence rates except vaginal swabs, which remained negative for *S. aureus* presence (Table 2). *S. aureus* prevalence was found to be slightly higher in females (7.1%) than in males (6.3%) (Table 2)

All the 83 HA-*Staphylococcus aureus* isolated from different clinical samples exhibited high resistance rates to benzylpenicIllin (penicillin G) (98%), oxacillin (85%), and fusidic acid (73%) followed by a comparatively low resistance against levofloxacin (35%). A low percentage of HA-*Staphylococcus aureus* was found resistant to tobramycin (23%), erythromycin (21%), tetracycline (21%), gentamicin (20%), moxifloxacin (18%), and clindamycin (14.5%) (Table 3). Only a very few isolates demonstrated resistance against trimethoprim/sulfamethoxazole (7.3%), and rifampicin (3.6%) (Table 3). HA-*Staphylococcus aureus* resistance was not observed against linezolid, teicoplanin, vancomycin, tigecycline, and nitrofurantoin (Table 3). 55 isolates (66%, n =83) exhibited Multidrug-resistance (MDR) (resistance to antibiotics of three different classes) mostly in males (64%, n = 55) (Table 4). Multidrug-resistant patterns were observed in 57 out of 70 (81.43%) oxacillin-resistant *S. aureus* isolates (Table 4).

Overall, the majority of the isolates (88%) did not demonstrate uniform multidrug-resistance patterns. However, one isolate from the ear swab (SErS4) and another from the nasal swab (SNAS7) exhibited significantly identical resistance patterns against eleven antimicrobial agents belonging to eight different classes (gentamicin, benzylpenicillin, rifampicin, oxacillin, moxifloxacin, tobramycin, fusidic acid, levofloxacin, erythromycin, clindamycin, and tetracycline) (Table 4, 5). Ten isolates (12%) originating from three blood culture samples notably displayed a similar multidrug-resistance pattern against tobramycin, benzylpenicillin, fusidic acid, oxacillin, gentamicin, and tetracycline. Three isolates from urine samples were resistant to oxacillin, benzylpenicillin, levofloxacin, fusidic acid, and moxifloxacin, whereas four isolates of wound swabs exhibited resistance to gentamicin, oxacillin, benzylpenicillin, fusidic acid, and tobramycin (Table 4, 5). In general, 25 (30%) isolates demonstrated the most frequent resistance pattern against fusidic acid, benzylpenicillin, levofloxacin, and oxacillin (Table 6). The most notable multidrug-resistance patterns by a single isolate were noted in the case of two isolates

Table 4. Multidrug resistance patterns of hospital-acquired *Staphylococcus aureus*

isolate	Sample/origin	Resistance pattern	No. of classes	Cefoxitin screen	Inducible clindamycin resistance
SBA1	Blood culture/male	BP, OX, G, TO, TET, FA	4	+	-
SBA2	Blood culture/female	BP, OX, G, TO, TET, FA	4	+	-
SBA3	Blood culture/female	BP, OX, G, TO, FA	3	+	-
SBA4	Blood culture/male	BP, OX, G, TO, TET, FA	4	+	-
SBA5	Blood culture/male	BP, OX, LE, FA	3	+	-
SBA6	Blood culture/male	BP, OX, TET, FA	3	+	-
SBA7	Blood culture/female	BP, OX, TET, FA	3	+	-
SBA8	Blood culture/male	BP, OX, LE, TET, FA, TS	5	+	-
SBA9	Blood culture/male	BP, TS	2	-	-
SBA10	Blood culture/female	BP, OX, G, TO, E, CL, TET, RIF	6	+	-
SBA11	Blood culture/female	BP	1	-	-
SBA12	Blood culture/male	BP, OX, FA	2	+	-
SWS1	Wound swabs/female	BP, OX, FA	2	+	-
SWS2	Wound swabs/female	BP, OX, FA	2	+	-
SWS3	Wound swabs/male	BP, OX, G, TO, TET, FA	4	+	-
SWS4	Wound swabs/male	BP, OX, G, TO, FA	3	+	-
SWS5	Wound swabs/male	BP, OX	1	+	-
SWS6	Wound swabs/female	BP, OX, LE, E, CL, FA	5	+	-
SWS7	Wound swabs/male	BP	1	-	-
SWS8	Wound swabs/female	BP, OX	1	+	-
SWS9	Wound swabs/female	BP, OX	1	+	-
SWS10	Wound swabs/male	BP, OX, G, TO, FA	3	+	-
SWS11	Wound swabs/male	BP, OX, G, TO, FA	3	+	-
SWS12	Wound swabs/male	BP, OX, G, TO, TET, FA	4	+	-
SWS13	Wound swabs/male	BP, OX, G, TO, FA	3	+	-
SWS14	Wound swabs/female	BP, LE, MOX, FA	3	-	-
SWS15	Wound swabs/male	BP, OX, FA	2	+	-
SWS16	Wound swabs/female	BP, LE, TS	3	-	-
SWS17	Wound swabs/female	BP, OX, LE, MOX, FA	3	+	-
SWS18	Wound swabs/female	BP, OX, G, TO, TET, FA	4	+	-
SWS19	Wound swabs/female	BP, OX, E, FA	3	+	-
SWS20	Wound swabs/male	BP, OX, G, TO, LE, MOX, CL, TET, FA, RIF	7	+	-
SWS21	Wound swabs/male	BP, OX, FA	2	+	-
SErS1	Ear swabs/female	BP, OX, FA	2	+	-
SErS2	Ear swabs/female	BP, OX	1	+	-
SErS3	Ear swabs/male	BP, OX, FA	2	+	-
SErS4	Ear swabs/female	BP, OX, G, TO, LE,MO, E, CL, TET, FA, RIF	8	+	-
SEyS1	Eye swabs/male	BP, OX, LE, MO, FA	3	+	-
SEyS2	Eye swabs/male	BP	1	-	-
SEyS3	Eye swabs/male	BP	1	-	-
SUR1	Urine/male	BP, OX, LE, MO, FA	3	+	-
SUR2	Urine/male	BP, OX, LE, MO, FA	3	+	-
SUR3	Urine/male	BP, OX, LE, MO, FA	3	+	-
SUR4	Urine/female	BP, OX, E, CL	3	+	+
SNAS1	Nasal swabs/male	BP, OX, LE, FA	3	+	-
SNAS2	Nasal swabs/male	BP, OX, E, CL	3	+	+
SNAS3	Nasal swabs/male	BP, OX, FA	2	+	-

Table 4. Cont...

Isolate	Sample/origin	Resistance pattern	No. of classes	Cefoxitin screen	Inducible clindamycin resistance
SNAS4	Nasal swabs/female	BP, OX, LE, FA	3	+	-
SNAS5	Nasal swabs/male	BP, OX, FA	2	+	-
SNAS6	Nasal swabs/male	BP. OX	1	+	-
SNAS7	Nasal swabs/male	BP, OX, G, TO, LE, MOX, E, CL, TET, FA, RIF	8	+	-
SNAS8	Nasal swabs/male	BP, OX, LE, FA	3	+	-
SNAS9	Nasal swabs/female	BP, E, CL	3	-	+
SNAS10	Nasal swabs/male	BP, OX, FA	2	+	-
SNAS11	Nasal swabs/female	BP, OX, FA	2	+	-
SNAS12	Nasal swabs/male	BP, OX. LE, MOX, FA	3	+	-
SNAS13	Nasal swabs/female	BP	1	-	-
SNAS14	Nasal swabs/female	BP, TO, E, FA	4	-	-
SNAS16	Nasal swabs/male	BP, OX, E, CL, FA	4	+	+
SNAS17	Nasal swabs/male	BP, OX, LE, MOX, FA	3	+	-
SNAS18	Nasal swabs/male	BP, OX, LE, FA	3	+	-
SNAS19	Nasal swabs/male	BP, OX, E, FA	3	+	-
SSPT1	Sputum/male	BP, OX, LE, E, CL	4	+	+
SSPT2	Sputum/male	BP, OX, LE, E, CL	4	+	+
SSPT3	Sputum/female	BP, OX, TO, TET, FA	4	+	-
SSPT4	Sputum/male	BP, OX, LE, TET, FA, TS	5	+	-
SSPT5	Sputum/male	BP, OX, FA	2	+	-
SSPT7	Sputum/male	BP	1	-	-
SSPT8	Sputum/male	BP, OX, LE, FA	3	+	-
SSPT9	Sputum/female	BP, OX, LE, FA	3	+	-
SGRS1	Groin swab/female	BP, OX, TO, TET	4	+	-
SGRS2	Groin swab/male	BP, OX, E, FA	3	+	-
SGRS3	Groin swab/male	BP, OX, G, TO, FA	3	+	-
SGRS4	Groin swab/female	BP, OX, LE, MO, E, CL, FA, TS	6	+	-
SGRS5	Groin swab/male	BP, OX, LE, TET, FA	4	+	-
SGRS6	Groin swab/male	BP, OX, LE, TET, FA	4	+	-
SAXS1	Axial swab/male	BP, OX, FA	2	+	-
SAXS2	Axial swab/female	BP, OX, LE, MO, FA	3	+	-
SAXS3	Axial swab/female	BP, OX, LE, MO, E, TS	4	+	-
SAXS4	Axial swab/female	BP, OX	1	+	-
SAXS5	Axial swab/male	BP, OX, LE, MO, E, CL, FA	5	+	+
Total MDR isolates (%)			55 (66)		
Total MDR-MRSA isolates (%)			57 (81.5)		
Total MDR in isolates of male origin (%)			35 (64)		
Total MDR in isolates of female origin (%)			20 (37)		

BP = benzylpenicillin, OX = oxacillin, G = gentamicin, TO = tobramycin, LE = levofloxacin, MO = moxifloxacin, E = erythromycin, CL = clindamycin, TET = tetracycline, FA = fusidic acid, RFI = rifampcin, TS = trimethoprim/sulfamethoxazole

including SNAS7 from nasal swab and SErS4 from ear swab. Both isolates were resistant to 11 antimicrobial agents belonging to 8 different classes (rifampicin, oxacillin, benzylpenicillin, gentamicin, fusidic acid, tobramycin, clindamycin, levofloxacin, tetracycline, moxifloxacin, and erythromycin). Similarly, one isolate from

Figure. Agarose gel electrophoresis for *mecA* gene amplified 533 bp as compared with 1kbp ladder (lane 1), negative control *S. aureus* ATCC® 25923™ (lane 2), isolate SBA5 positive for mecA from blood culture (lane 3), isolate SWS20 positive for mecA from wound swabs (lane 4), Isolate SSPT6 not resistant to any antibiotic from sputum, negative for mecA (lane 5), isolate SNSA9 susceptible to oxacillin, negative for mecA (lane 6) isolate lane SNSA12 positive for mecA from nasal swabs (lane 7), isolate SSPT4 positive for mecA from sputum (lane 8) and isolate SUR1 positive for mecA from urine (lane 9)

wound swabs (SWS20) also expressed multidrug resistance to 10 antimicrobial agents belonging to seven different classes including moxifloxacin, benzylpenicillin, tobramycin, oxacillin, tetracycline, gentamicin, rifampicin, levofloxacin, clindamycin, and fusidic acid (Table 4).

PCR results revealed the absence of genes encoding virulence factors (toxic shock syndrome toxin (*tst*), exfoliative toxin (*et*), and Panton-Valentine leukocidin (*LukS-PV*)) in all the 83 HA-*Staphylococcus aureus* isolates (Table 7). Similarly, the *vanA* gene (encoding resistance to vancomycin) was also not detected in any isolate, whereas the *mecA* gene (encoding resistance to methicillin) was detected in 70 HA-*Staphylococcus aureus* isolates (85%, n = 83) originating from all types of samples (Table 7, Figure).
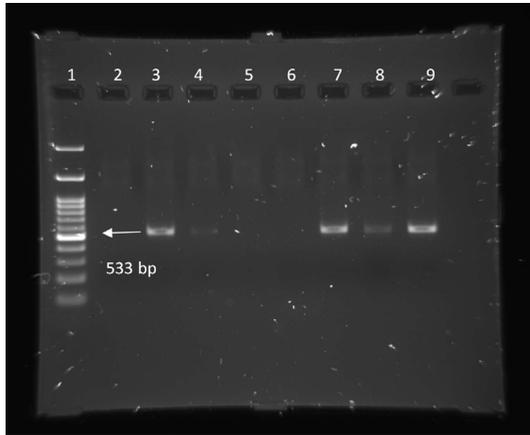
DISCUSSION

S. aureus, especially the MRSA strains, are a major global source of hospital-acquired infections.[1] Recently, two epidemiological point prevalence surveys have been conducted across the United States. One of the surveys involved 11,282 patients from 183 hospitals,[4] and revealed that *S. aureus* infections (10.7%) were second to *Clostridiodis difficile* (12.1%) among hospital-acquired infections.[4] The second survey involved the data of 12,299 patients from 199 hospitals nationwide and *S. aureus* infections (10%) were again found to be second among hospital-acquired

Table 5. HA-*Staphylococcus aureus* isolates with uniformed multidrug-resistance patterns

Isolate	Sample/origin	Resistance pattern
SNAS7	Nasal swabs/male	BP, OX, G, TO, LE, MOX, E, CL, TET, FA, RIF
SErS4	Ear swabs/female	BP, OX, G, TO, LE, MOX, E, CL, TET, FA, RIF
SBA1	Blood culture/male	BP, OX, G, TO, TET, FA
SBA2	Blood culture/female	BP, OX, G, TO, TET, FA
SBA4	Blood culture/male	BP, OX, G, TO, TET, FA
SUR1	Urine/male	BP, OX, LE, MO, FA
SUR2	Urine/male	BP, OX, LE, MO, FA
SUR3	Urine/male	BP, OX, LE, MO, FA
SWS4	Wound swabs/male	BP, OX, G, TO, FA
SWS10	Wound swabs/male	BP, OX, G, TO, FA
SWS11	Wound swabs/male	BP, OX, G, TO, FA
SWS13	Wound swabs/male	BP, OX, G, TO, FA

BP = benzylpenicillin, OX = oxacillin, G = gentamicin, TO = tobramycin, LE = levofloxacin, MO = moxifloxacin, E = erythromycin, CL = clindamycin, TET = tetracycline, FA = fusidic acid, RFI = rifampcin

infections in the United States.[5] The current study depicts a low *S. aureus* prevalence (6.8%, n = 1262) in the clinical samples of hospitalized patients in Medina, northwestern Saudi Arabia. Contrarily, a previous study in the hospitals of Makkah (western Saudi Arabia), reported a higher prevalence (53%) of HA-*Staphylococcus aureus,* especially MRSA strains.[11] Similarly, a high (22%) HA-*Staphylococcus aureus* prevalence was noted in the hospitals of Riyadh in central Saudi Arabia.[14] The low prevalence of HA-*Staphylococcus aureus* during this study could be attributed to the Covid-19 pandemic when the hospitalization was restricted to only Covid-19-patients and critically ill non-Covid-19 patients. The intensive sanitation and disinfection protocols during the Covid-19 pandemic might have reduced the HA-*Staphylococcus aureus* prevalence by decreasing the bacterial shedding in the hospital environment. The bacterial shedding from the skin or respiratory tract of health care workers and contaminated fomites are considered major sources of HA-*Staphylococcus aureus.*[1]

S. aureus* is a common community or hospital-acquired bacteremia that causes 20 to 30 bloodstream infection cases per 100,000 individuals/annum worldwide.[2,6] During this study, blood cultures demonstrated the highest *S. aureus* prevalence (80%, n = 15), which is in line with previous reports in Saudi Arabia and other countries.[6,8,14,32] HA-*Staphylococcus aureus* especially the infection of HA-MRSA strains could result in higher patient mortality (20–30%).[2,33] The wound swabs presented the second-highest rate of HA-*Staphylococcus aureus* prevalence (55.3%, n = 38). These results were well anticipated as *S. aureus* is a common nosocomial pathogen in postsurgical settings.[9] The rising rates of wound-associated *S. aureus* infections, particularly MRSA, have been reported in various hospitals in Saudi Arabia and other regions.[9-11,16,34]

S. aureus* commonly causes eye infections such as keratitis conjunctivitis, postoperative endophthalmitis, and septal cellulitis.[35] The results of this study revealed a moderate prevalence rate of HA-*Staphylococcus aureus* (30%, n = 10) only in the eye swabs of males. Das *et al.*[36] have also reported frequent *S. aureus*-related nosocomial ocular infections in 29 patients. Another study conducted in Dallas, Texas, involved 3460 patients with ocular infections, which were mostly (1088

patients) caused by HA- *Staphylococcus aureus*.[37] Recently, increased HA-MRSA ocular infections have been reported in Taiwan and the patients with healthcare exposure suffered from MRSA more than the patients with CA-*Staphylococcus aureus* ocular infections.[38] Previous epidemiological studies of eye infections have reported a total absence of HA-*Staphylococcus aureus* in the eye swab cultures.[11] Similarly, previous epidemiological studies of ear infections have suggested a rare involvement of *S. aureus* but recently an increasing trend of HA-MRSA and CA-MRSA-based ear infections has been noticed.[39] The current study found *Staphylococcus*-positive cultures (20%) from the ear swabs (20) of hospitalized patients. Duarte *et al.*[40] studied 173 patients of acute otitis externa and revealed an approximately similar HA-*Staphylococcus aureus* prevalence rate (30%).

S. aureus* is commonly found in the normal upper respiratory flora of about 30% of humans. This type of *S. aureus* colonization could result in invasive infections such as ventilator-associated pneumonia and hospital-acquired pneumonia. However, respiratory infections of *S. aureus* are less frequent than skin and soft-tissue infections.[41] Therefore, only 9 sputum cultures (14%, n = 65) were found HA-*Staphylococcus aureus* positive during this study. Multiple studies in Saudi Arabia have reported a low prevalence of HA-*Staphylococcus aureus*-associated pneumonia.[11,14] Contrarily, various Asian countries are facing a rapid rise in HA-MRSA-associated nosocomial pneumonia.[42] Likewise, a low prevalence of HA-*Staphylococcus aureus* was noted in urine samples (9%, n =44). *S. aureus* role in catheter-associated urinary tract infections (UTI) is common but less frequent.[43] Several studies of HA-MRSA epidemiology in Saudi Arabia and other countries have reported a low frequency of HA-*Staphylococcus aureus* involvement in catheter-related UTI.[11,14,44,45]

S. aureus* mainly inhabits the epithelium of anterior nares and skin in humans.[46] Multiple factors such as gender, geographical location, body niche, and age determine the *S. aureus* carriage. Generally, the percentage of *S. aureus* carriage in humans remains as 4%-64% (nasal and skin), 15% (chest), 17%-31% (intestine), 8% (axillae), 22% perineum, and 5% (vagina).[46] *S. aureus* carriage percentage in this study was on

Table 6. Frequency of resistance patterns to benzylpenicillin, oxacillin, levofloxacin and fusidic acid among HA-MDR-*Staphylococcus aureus*

Isolate	Sample/origin	Resistance pattern
SBA5	Blood culture/male	BP, OX, LE, FA
SBA8	Blood culture/male	BP, OX, LE, TET, FA, TS
SWS6	Wound swabs/female	BP, OX, LE, E, CL, FA
SWS17	Wound swabs/female	BP, OX, LE, MOX, FA
SWS20	Wound swabs/male	BP, OX, G, TO, LE, MOX, CL, TET, FA, RIF
SErS4	Ear swabs/female	BP, OX, G, TO, LE,MO, E, CL, TET, FA, RIF
SEyS1	Eye swabs/male	BP, OX, LE, MO, FA
SUR1	Urine/male	BP, OX, LE, MO, FA
SUR2	Urine/male	BP, OX, LE, MO, FA
SUR3	Urine/male	BP, OX, LE, MO, FA
SNAS1	Nasal swabs/male	BP, OX, LE, FA
SNAS4	Nasal swabs/female	BP, OX, LE, FA
SNAS7	Nasal swabs/male	BP, OX, G, TO, LE, MOX, E, CL, TET, FA, RIF
SNAS8	Nasal swabs/male	BP, OX, LE, FA
SNAS12	Nasal swabs/male	BP, OX. LE, MOX, FA
SNAS17	Nasal swabs/male	BP, OX, LE, MOX, FA
SNAS18	Nasal swabs/male	BP, OX, LE, FA
SSPT4	Sputum/male	BP, OX, LE, TET, FA, TS
SSPT8	Sputum/male	BP, OX, LE, FA
SSPT9	Sputum/female	BP, OX, LE, FA
SGRS4	Groin swab/female	BP, OX, LE, MO, E, CL, FA, TS
SGRS5	Groin swab/male	BP, OX, LE, TET, FA
SGRS6	Groin swab/male	BP, OX, LE, TET, FA
SAXS2	Axial swab/female	BP, OX, LE, MO, FA
SAXS5	Axial swab/male	BP, OX, LE, MO, E, CL, FA

BP = benzylpenicillin, OX = oxacillin, G = gentamicin, TO = tobramycin, LE = levofloxacin, MO = moxifloxacin, E = erythromycin, CL = clindamycin, TET = tetracycline, FA = fusidic acid, RFI = rifampcin

the lower side with a nasal carriage of 5.4% (n = 345) followed by 2.4% and 1.42% (n = 352) in groins and axillae respectively, whereas vaginal swabs were *S. aureus* -negative. The increased *S. aureus* colonization in patients could enhance the risk of acquiring nosocomial infection, and hospital- and community-acquired invasive *S. aureus* infections.[47]

Due to high mortality and morbidity rates and difficulty in treatment, the Methicillin-resistant *Staphylococcus aureus* (MRSA), especially HA-MRSA, has attracted considerable global attention.[48] Antimicrobial susceptibility testing and detection of methicillin resistance gene (*mecA)* during this study exhibited an overall high prevalence of HA-MRSA (85%, n = 83). High detection rates of HA-MRSA in most of the tested samples of hospitalized patients (nasal, blood cultures, sputum, wound swabs, groin,

urine, eye, axillae swabs, and ear) represent its higher prevalence in the western regions of Saudi Arabia.[49] Wide-spread HA-MRSA infections have been documented in Saudi Arabia,[12,16,30] Asia,[8] Europe,[7] Africa,[10] and the United States.[50] HA-MRSA prevalence might differ in various Saudi Arabian regions but a significant rise in MRSA infections has been reported.[51] Therefore, HA-MRSA infections with high morbidity and mortality rates are emerging as an alarming clinical threat.[48] The enhancing HA-MRSA infection risks could be attributed to inappropriate antibiotics prescriptions, prolonged hospital stays, and invasive clinical procedures with medical devices.[19,50,52] The rising trends of HA-MRSA infections could enhance the burden on the healthcare system. A recent study in Japan estimated MRSA infections related total financial burden of 2 billion US dollars on the healthcare system with 14.3 thousand annual

Table 7. Prevalence of genes encoding virulence factors and antimicrobial resistance in HA-*Staphylococcus aureus*

Samples	Antimicrobial resistance			Virulence factors		
	N/P	*mecA*	*vanA*	*et*	*tst*	*LukS-PV*
Blood culture	15/12	10	0	0	0	0
Wound swabs	38/21	18	0	0	0	0
Ear swabs	20/4	4	0	0	0	0
Eye swabs	10/3	1	0	0	0	0
Urine	46/4	4	0	0	0	0
Nasal swabs	354/19	15	0	0	0	0
Sputum	65/9	7	0	0	0	0
Groin swabs	352/6	6	0	0	0	0
Axial swabs	352/5	5	0	0	0	0
Vaginal swabs	10/0	-	-	-	-	-
Total	1262/83	70	0	0	0	0

deaths.[53] Despite the various previous studies, this is the first report on the true HA-MRSA prevalence in Medina province, northwest Saudi Arabia.

HA-*Staphylococcus aureus* resistance to antimicrobial agents other than oxacillin should not be ignored as it could hinder the treatment strategies. The results of this study depicted a high HA- *Staphylococcus aureus* resistance (98%, n = 83) to penicillin that has also been reported worldwide.[54,55] CA- and HA- *Staphylococcus aureus* resistance to penicillin G is known since the 1940s that has steadily increased with time. *blaZ* gene on the *S. aureus* chromosome encoding the secretion of beta-lactamase mediates the resistance to penicillin G. *blaZ* gene could also be acquired via transferable plasmid to explain the *S. aureus* resistance to penicillin G.[48] In this study, HA-*Staphylococcus aureus* presented a high resistance (73%, n = 83) to fusidic acid that has also been reported from the hospitals in Makkah. These results contradict the findings of Abulreesh *et al.*[30] who reported a low *S. aureus* resistance to fusidic acid (18%, n = 50). The increased resistance could be associated with the excessive use and unrestricted fusidic acid (topical cream) availability in Saudi Arabia for treating *S. aureus*-related skin infections. *S. aureus*, particularly MRSA, associated with fusidic acid resistance has been reported on a global scale.[56]

S. aureus, especially MRSA, resistance to fluoroquinolones (moxifloxacin, levofloxacin, and ciprofloxacin) has enhanced alarmingly, which reduces the choice of drugs for treating MRSA infections.[57] The results revealed a high HA-*Staphylococcus aureus* resistance to levofloxacin (35%, n = 83) and moxifloxacin (18%, n = 83). The studies have also reported higher HA-MRSA resistance to fluoroquinolones in Saudi Arabia and other parts of the world.[11,58] Aminoglycoside antibiotics (tobramycin and gentamicin are also important for treating *S. aureus,* particularly MRSA infections.[59] In the current study, 16 (20%, n = 83) and 19 (23%, n = 83) isolated cultures of HA-*Staphylococcus aureus* demonstrated resistance against gentamicin and tobramycin, respectively. Multiple studies have reported the resistance of *S. aureus* clinical isolates to aminoglycosides in Saudi Arabia and worldwide.[11,60,61] The increased resistance of MRSA to fluoroquinolones and aminoglycosides complicates the treatment of *S. aureus* infections.

The choice of treatment for clinical *S. aureus*, especially MRSA, depends upon the type of infection. Vancomycin (glycopeptides) is prescribed for treating the bloodstream respiratory infections of *S. aureus*.[48] *S. aureus* resistance to vancomycin has been reported in various countries.[62] However, the current and previous studies in Saudi Arabia have noticed complete susceptibility of clinical *S. aureus* including MRSA to vancomycin. These results were deduced based on the absence of vancomycin resistance encoding *van* genes and standard antimicrobial susceptibility testing.[11,30] Linezolid (oxazolidinones) or clindamycin (lincosamides) are recommended for the treatment of *Staphylococcus*-associated

pneumonia.[48] The results of this study depicted the susceptibility of clinical *S. aureus* populations including MRSA to linezolid, which has also been reported in previous studies in Saudi Arabia.[30] In contrast, the resistance of clinical *S. aureus* to clindamycin is rising in Saudi Arabia as also noted in this study (4.5%, n = 83). Several studies have revealed the enhanced HA-*Staphylococcus aureus* resistance to clindamycin.[11,30]

Different antimicrobial agents are recommended to treat mild *S. aureus* skin infections including tetracycline (tetracyclines),[63] erythromycin (macrolides),[64] and trimethoprim/ sulfamethoxazole (folate pathway antagonists).[65] Due to the emergence of resistance, these agents are generally recommended against *S. aureus* (particularly MRSA) invasive infections. The data of the current study exhibits 21% (n = 83) *S. aureus* resistance to tetracycline and erythromycin, which is in line with previous local and global reports.[11,30,63-65] We noticed 7.3% (n = 83) *S. aureus* resistance to trimethoprim/sulfamethoxazole, which is significantly lower than the 41% (n = 39) observed by El Amin and Faidah.[11] Thus, tetracycline, erythromycin, and trimethoprim/ sulfamethoxazole are becoming unsuitable choices to counter *S. aureus* infections because of emerging resistance.

Global epidemics of multidrug-resistance (MDR) related infections have raised serious concerns.[66] Epidemiological studies of MDR *S. aureus* have revealed MDR-MRSA as a major source of antibiotic-resistant infections in hospitalized patients.[67] 55 HA-*Staphylococcus aureus* isolates (66%) out of a total 83 exhibited MDR patterns during this study, whereas 57 HA-MRSA isolates (81.5%) out of a total 70 were MDR. Only a few studies have elaborated HA-MRSA susceptibility profiles against other antimicrobial agents. El Amin and Faidah.[11] have reported that 29.1% of *S. aureus* especially HA-MRSA were MDR in Makkah city. Abulreesh *et al.*[30] reported a lower MDR-MRSA prevalence (24%, n = 50) among clinical *S. aureus* samples in Makkah city. To the best of our knowledge, this study first time reports MDR-HA-MRSA from Medina, which is the highest in Saudi Arabia to date. Higher MDR HA-MRSA prevalence has been observed in various countries including Nepal,[68] Egypt,[69] Poland,[54] Vietnam,[70] and Eritrea.[71] However, an overall decreasing trend of HA-

MRSA, particularly MDR strains, has been noted in western European countries and the United States.[72] MDR HA-MRSA high incidence among hospitalized patients during this study is alarming and demands strict continuous monitoring of antibiotic use and the application of efficient strategies for infection control.

The results revealed four distinct MDR phenotypic patterns among HA-MRSA of which three MDR phenotypes were associated with the same infection site. The first MDR phenotypes were observed in bloodstream infection against tetracycline, benzylpenicillin (penicillin G), tobramycin, oxacillin, fusidic acid, and gentamicin. The second MDR pattern in UTI MRSA was noted against moxifloxacin, benzylpenicillin (penicillin G), levofloxacin, oxacillin, and fusidic acid. The third MDR phenotype was observed in MRSA isolated from wound swabs against fusidic acid, benzylpenicillin (penicillin G), gentamicin, oxacillin, and tobramycin. The fourth MDR pattern was noted in the isolates from nasal and ear swabs against rifampicin, benzylpenicillin (penicillin G), oxacillin, tetracycline, moxifloxacin, gentamicin, clindamycin, tobramycin, fusidic acid, levofloxacin, and erythromycin. The fourth pattern might have been from the same patient, whereas different MDR HA-MRSA phenotypes from similar samples could be related to the common regime of infection treatment in the anatomical sites. HA-MRSA MDR phenotypes reported in this study are different and diverse than the previous reports in Saudi Arabia and worldwide.[11,30,54,68-71] A varying diversity of HA-MRSA clonal populations in different geographical locations could explain this phenomenon. MDR HA-MRSA clonal genotypes diversity could be further confirmed through molecular typing of resistance genes.

The current study also investigated the TSST (toxic shock syndrome toxin) encoding *tst* gene in HA-*Staphylococcus aureus* for the first time in Saudi Arabia. This virulence factor has never been explored and reported in Saudi Arabia. The results demonstrated a total absence of the *tst* gene in all the 83 HA-*Staphylococcus aureus* isolates including MRSA strains depicting that TSST is not produced by the majority of the clinical *S. aureus*. This is in agreement with the literature suggesting that only 20% of *S. aureus* isolated from the samples of infected patients

and asymptomatic carriers produced the toxin.[2] DeVries *et al.*[73] reported the presence of TSST-1 in only 61 (0.82%) out of 7491 hospitalized patients in Minnesota from 2000 to 2006. Similarly, the presence of staphylococcal exfoliative toxin (ET) in HA- or CA-*Staphylococcus aureus* has never been investigated in Saudi Arabia. Therefore, this was the first attempt for detecting the *eta* gene in HA-*Staphylococcus aureus*. However, the *eta* gene was absent in all the 83 isolates of hospitalized patients. The absence of exfoliative toxin encoding gene could be due to the overall low carriage (1-2%) of *eta* and *etb* genes in *S. aureus*.[2,15] Previous epidemiological studies revealing a low annual prevalence of Staphylococcal scalded skin syndrome (7.76% per 1 million patients) in the United States support the results of our study.[74] The incidence of Panton-Valentine leukocidin (PVL) among clinical *S. aureus* isolates has been investigated during a few studies in Saudi Arabia. The results of these studies varied from the total absence of the *LukS-PV* gene in HA- *Staphylococcus aureus* of Makkah[30] to a surprisingly high prevalence (54.2%, n = 107) in Riyadh.[75] The overall PVL carriage among HA-MRSA isolates remained low, whereas the *PVL* genes were present in almost every CA-MRSA.[2,15]

CONCLUSION

HA-*Staphylococcus aureus*, especially MDR HA-MRSA, is a leading cause of nosocomial infections. This study first time explored HA-*Staphylococcus aureus* prevalence in one healthcare setting in Medina (northwest Saudi Arabia) during the Covid-19 pandemic. The low *S. aureus* prevalence could be due to the partial lockdown, restricted hospitalization, and increased measures of disinfection and infection control during the pandemic. Further multicenter investigations are required to assess the true incidence of HA-*Staphylococcus aureus* in Medina city. Despite the overall low HA-*Staphylococcus aureus* prevalence, the majority of the isolates were MRSA and alarmingly more than 80% of MRSA isolates exhibited MDR patterns. These results highlight incorrect prescription of antimicrobial agents for treating staphylococcal infections. HA-*Staphylococcus aureus* isolated during this study lacked important virulence factors such as

an exfoliative toxin, toxic shock syndrome toxin, and Panton-Valentine leukocidin. However, their invasiveness coupled with MDR traits could not be ruled out, which might ultimately lead to serious outcomes and difficulty in treatments.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

HHA and TFHA conceptualized the study. HHA and TFHA applied methodology. THFA, and ZZA performed Investigation. HHA, LAN and KE performed data curation. HHA, and TFHA performed formal analysis. KE and LAN collected resources. HHA performed supervision. TFHA and HHA wrote the original draft. HHA and IA wrote, reviewed and edited the manuscript. All authors read and approved the final manuscript for publication.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Department of Biology postgraduate and Research Ethics Committee and Faculty of Applied Science postgraduate and Research Ethics Committee, approval number (3421209144114).

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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