Meticillin-resistant *Staphylococcus aureus* isolated from Iranian hospitals: virulence factors and antibiotic resistance properties

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ABSTRACT

Staphylococcus aureus is an important opportunistic pathogen responsible for a variety of diseases. Indiscriminate prescription of antibiotics caused severe antibiotic resistance especially against commonly used drugs. The present investigation was carried out to study the distribution of Panton-Valentine Leukocidin gene, SCC***mec*** types and antibiotic resistance properties of meticillin-resistant ***Staphylococcus aureus*** isolated from Iranian hospitals. A total of 132 clinical specimens were collected from two major Iranian hospitals. Samples were cultured and their positive results were subjected to several PCR methods. The patterns of antibiotic resistance were studied using the disk diffusion method. We found that 66 out of 132 samples (50%) were positive for ***Staphylococcus aureus***. The most commonly infected samples were superficial and surgical wounds (66.12%). The incidence of ***mecA***, ***tetK, ermA, ermC, tetM, aacA-D, linA, msrA, vatA, vatC*** and ***vatB*** antibiotic resistance genes were 80.30%, 34.84%, 30.30%, 25.75%, 24.24%, 19.69%, 7.57%, 7.57%, 6.06%, 3.03% and 1.51%, respectively. Totally, 40.90% of isolates harbored the Panton-Valentine Leukocidin gene. Of 53 ***mec*** positive strains, the distribution of ***SCCmec*** V, ***SCCmec*** III, ***SCCmec*** IVa, ***SCCmec*** IVc and ***SCCmec*** IVb were 28 (52.83%), 13 (24.52%), 6 (11.32%), 4 (7.54%) and 2 (3.77%), respectively. All isolates were resistant to penicillin, cephalothin, cefazoline and ceftriaxone. The high levels of ***Staphylococcus aureus*** resistance against commonly used antibiotics as well as high presence of ***SCCmec*** types of meticillin-resistant virulent strains of ***Staphylococcus aureus*** suggest that infections with these strains require more advanced hospital care with emerging demand for novel antibiotics.

KEY WORDS: ***Staphylococcus aureus***, ***SCCmec*** types, Panton-Valentine Leukocidin, antibiotic resistance properties, Iranian hospitals

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INTRODUCTION

The increasing prevalence of bacterial resistance to commonly used antibiotics, may result in an insufficient array of substances to combat some bacterial infections and it is more important in hospitals. ***Staphylococcus aureus*** (***S. aureus***) has long been recognized as a major pathogen of hospital acquired infections. The bacterium can colonize individuals both in the community and hospital settings [1]. Infections caused by this bacterium are treated mainly with meticillin but in recent years, increasing numbers of meticillin resistant ***S. aureus*** (MRSA) strains have been reported worldwide from patients with community-acquired infections [2-4]. Globally, fifteen to forty five percent of ***S. aureus*** strains isolated from hospital infections were meticillin resistant [5,6]. ***S. aureus*** is the most common cause of skin and soft-tissue infections (such as impetigo, furunculosis, superficial and surgical wounds and abscess), as well as systemic infections (such as pneumonia, urinary tract infections-UTIs and endocarditis) [7-13].

The pathogenicity of ***S. aureus*** depends on various bacterial surface components and extracellular proteins. The frequent recovery of staphylococcal isolates that produce leukocidal toxins from patients with deep skin soft tissue infections, particularly furunculosis, cutaneous abscesses, severe necrotizing pneumonia, and even UTIs, suggests that the Panton-Valentine leukocidin (PVL) is a virulence factor that has a major role in pathogenicity [7-14]. PVL-positive strains are associated with skin diseases, accounting for 96% of the cases [15,16]. PVL has also been associated with severe infections, including pneumonia [11], purpura fulminans [17] and osteomyelitis [18]. This toxigenic gene has also been isolated from the cases of Lemierre's syndrome [19], Fournier's

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gangrene [20], community-acquired necrotizing and hemorrhagic pulmonary infections affecting previously healthy children and young adults [14, 21].

SCC*mec* elements are currently classified into types I, II, III, IV and V according to the nature of the *mec* and *ccr* gene complexes, and are further classified into subtypes according to differences in their J region DNA [22-24]. Complete identification of bacterial genetic background and the SCC*mec* element is very important for molecular typing of MRSA [9,25].

PVL is mostly associated with community-acquired MRSA infections and distinguishable from nosocomial MRSA by no multi-drug resistance and carriage of the type IV staphylococcal chromosome cassette element (SCC*mec* type IV) [26,27]. The changing trend of MRSA epidemiology, showed the use of PVL locus detection as a marker of MRSA isolates, alongside with non multi resistant pattern and SCC*mec* type IV or V [28].

There were no previously published data about the distribution of PVL and SCC*mec* types among multi-drug resistant strains of ***S. aureus*** isolated from Iranian hospitals. According to the indiscriminate and excessive prescribing of antibiotics in Iran, it is necessary to study the distribution of PVL gene, SCC*mec* types and antibiotic resistance properties of MRSA isolated from various types of hospital's infections.

MATERIAL AND METHODS

Samples and *Staphylococcus* identification

From February to May 2013, a total of 132 clinical samples from individuals suffering from various types of infections including blood (n=13), UTIs (n=16), respiratory infections and lung abscesses (n=24), superficial and surgical wounds (n=62) and abscesses (n=17) were collected from 2 major hospitals of Iran (Baqiyatallah and Peyambaran Hospitals, Tehran, Iran). All samples were transported to the Microbiology and Infectious Diseases Research Center of the Islamic Azad University of Shahrekord in a cooler with ice-packs. All samples were directly cultured into 7% sheep blood agar (Merck, Darmstadt, Germany) and incubated aerobically at 37°C for 48 h. After incubation, suspicious colonies were examined by using techniques appropriate for diagnosing ***Staphylococcus spp***. (microscopical morphology, catalase and coagulase production). Studied colonies were cultured on Tryptic Soy Broth (TSB) (Merck, Darmstadt, Germany) and Tryptic Soy Agar (TSA) (Merck, Darmstadt, Germany). After growth, staphylococci were identified on the basis of colony characteristics, Gram staining, pigment production, hemolytic and the following biochemical reactions: catalyses activity, coagulated test (rabbit plasma), Oxidase test, glucose O/F test, resistance to bacitracin (0.04 U), mannitol fermentation on Mannitol Salt Agar (MSA) (Merck, Darmstadt, Germany), urease activity, nitrate reduction, novobiocin resistance, phosphatase, deoxyribonuclease (DNase) test and carbohydrate (xylose, sucrose, trehalose and maltose, fructose, lactose, mannose) fermentation tests [29].

Antibiotic susceptibility testing

S. aureus isolates were tested for susceptibility to penicillin (10 u/disk); imipenem (10 µg/disk); cefazoline (30 µg/disk); cefalotin (30 µg/disk); ceftriaxone (30 µg/disk); gentamicin (10 µg/disk); ciprofloxacin (5 µg/disk); clindamycin (2 µg/disk); azithromycin (15 µg/disk); erythromycin (15 µg/disk); mupirocin (30 µg/disk); rifampicin (5 µg/disk); tetracycline (30 µg/disk); trimethoprim (5 µg/disk); vancomycin (30 µg/disk) and nitrofurantoin (300 µg/disk) by the Kirby-Bauer disk diffusion method. The resistance break points were those recommended by National Committee for Clinical Laboratory Standards (2003) [30] and were reported as sensitive or resistant based on break points. ***Staphylococcus aureus*** ATCC 25923 and ***Escherichia coli*** ATCC 25922 were used as quality control organisms in antimicrobial susceptibility determination.

DNA extraction and Staphylococcus confirmation

A typical colony of the biochemically identified ***S. aureus*** was cultivated in 1 mL TSB for 24 h at 37°C. Chromosomal DNA was extracted from the typical colonies using the DNA Genomic Purification Kit (Fermentas, Germany) according to the manufacturer's instructions. Presence of ***S. aureus*** in each DNA samples was confirmed using the Banada et al. [31] method. The PCR reaction mix consisted of 1 X PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl and 0.001% (w/v) gelatin) with 4 mM MgCl2, 250 mM of each nucleotide (deoxynucleoside triphosphate), 0.5 mM of each primer (forward and reverse), 4 ng of the molecular beacon and 4 U of Taq DNA polymerase (Fermentas, Germany).

PCR detection of mecA and PVL genes

Two pairs of primers were used for amplification of ***mecA*** and PVL genes of the ***S. aureus*** strains [32, 33]. The PCR reactions were performed in a total volume of 25 µL, including 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 200 µM dNTPs each (Fermentas, Germany), 2.5 µL PCR buffer (10X), 25 pmoL of each primer, 1.5 U of Taq DNA polymerase (Fermentas, Germany) and 5 µL (40-260 ng/µL) of the extracted DNA template of the ***Staphylococcus*** isolates. The two sets of primer pairs were used in each reaction mixture. The DNA thermo-cycler (Eppendorf Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) were used in all PCR reactions for DNA amplifications. The

thermal cycler was adjusted as follows: 94°C for 10 min, followed by 10 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min, and 25 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 1.5 min, followed by final extension at 72°C for 7 min; the PCR products were stored in the thermal cycler at 4°C until they were collected.

Antibiotic resistance genes amplification

The presences of ***tetK, tetM, ermA, ermC, aacA-D, linA, msrA, vatA, vatC*** and ***vatB*** genes were analyzed using the Kumar et al. [34] technique. List of primers are shown in Table 1. The PCR reactions were performed in a total volume of 25 µL, including 2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 150 µM dNTPs each (Fermentas, Germany), 2.5 µL PCR buffer (10X), 25 pmoL of each primers, 2 U of Taq DNA polymerase (Fermentas, Germany), and 4 µL (40-260 ng/µL) of the extracted DNA template of the Staphylococcus isolates. The four set of primer pairs were used in each reaction mixture. The thermal cycler was adjusted as follows: 94°C 5 min, 30 cycles of 1 min at 95°C for the denaturation step and 1 min at 55°C for the annealing-extension step and followed by final extension at 72°C for 90 seconds.

Detection of *SCCmec* types

The ***SCCmec*** types were detected using the Zhang et al. [12] method. Lists of primers are shown in Table 2. The PCR reactions were performed in a total volume of 25 µL, including 2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 250 µM dNTPs each (Fermentas, Germany), 2.5 µL PCR buffer (10X), 25 pmoL of each primer, 2 U of Taq DNA polymerase (Fermentas, Germany), and 5 µL (40-260 ng/µL) of the extracted DNA template of the Staphylococcus isolates. The thermal cycler was adjusted as follows: beginning with an initial denaturation step at 94°C for 5 min followed by 10 cycles of 94°C for 45 s, 65°C for 45 s, and 72°C for 1.5 min and another 25 cycles of 94°C for 45 s, 55°C for 45 s, and 72°C for 1.5 min, ending with a final extension step at 72°C for 10 min.

In order to confirm the PCR results, the sequencing method was used. For this reason, PCR products of some positive samples were purified with High pure PCR product purification kit (Roche Applied Science, Germany) according to manufacturer's recommendations. Single DNA strands were sequenced with ABI 3730 XL device and Sanger sequencing method (Macrogen, Korea). Result of the sequence of each gene was aligned with the gene sequences recorded in the GenBank database located at NCBI.

TABLE 1. Oligonucleotide primers for amplification of antibiotic resistance genes in *Staphylococcus aureus* strains isolated from Iranian hospitals.

Genes	Primer Sequence (5'-3')	Size of product (bp)
*aac*A-D	F : TAATCCAAGAGCAATAAGGGC R : GCCACACTATCATAACCACTA	227
tet K	F : GTAGCGACAATAGGTAATAGT R : GTAGTGACAATAAACCTCCTA	360
tet M	F : AGTGGAGCGATTACAGAA R : CATATGTCCTGGCGTGTCTA	158
*msr*A	F : GGCACAATAAGAGTGTTTAAAGG R : AAGTTATATCATGAATAGATTGTCCTGTT	940
*erm*A	F: AAGCGGTAAACCCCTCTGA R: TTCGCAAATCCCTTCTCAAC	190
*erm*C	F: AATCGTCAATTCCTGCATGT R: TAATCGTGGAATACGGGTTTG	299
*vat*A	F: TGGTCCCGGAACAACATTTAT R: TCCACCGACAATAGAATAGGG	268
*vat*B	F: GCTGCGAATTCAGTTGTTACA R: CTGACCAATCCCACCATTTTA	136
*vat*C	F: AAGGCCCCAATCCAGAAGAA R: TCAACGTTCTTTGTCACAACC	467
*lin*A	F: GGTGGCTGGGGGGTAGATGTATTAACTGG R: GCTTCTTTTGAAATACATGGTATTTTTCGA	323

Gel electrophoresis

Fifteen microliters of PCR products were resolved on a 1.5% agarose gel containing 0.5 mg/mL of ethidium bromide in Tris–borate–EDTA buffer at 90 V for 1 h, also using suitable molecular weight markers. The products were examined under ultraviolet illumination.

Statistical analysis

The results were transferred to a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA) for analysis. Statistical analysis was performed using SPSS/16.0 software (SPSS Inc., Chicago, IL) for significant relationship between incidences of PVL gene and SCC***mec*** types of ***S. aureus*** isolated from clinical samples. A χ^2 test and Fisher's exact 2-tailed test analysis

TABLE 2. Oligonucleotide primers for amplification of SCC***mec*** types in *Staphylococcus aureus* strains isolated from Iranian hospitals.

Types	Primer Sequence (5'-3')	Size of product (bp)
SCCmec I	F: GCTTTAAAGAGTGTCGTTACAGG R: GTTCTCTCATAGTATGACGTCC	613
SCCmec II	F: CGTTGAAGATGATGAAGCG R: CGAAATCAATGGTTAATGGACC	398
SCCmec III	F: CCATATTGTGTACGATGCG R: CCTTAGTTGTCGTAACAGATCG	280
SCCmec IVa	F: GCCTTATTCGAAGAAACCG R: CTACTCTTCTGAAAAGCGTCG	776
SCCmec IVb	F: TCTGGAATTACTTCAGCTGC R: AAACAATATTGCTCTCCCTC	493
SCCmec IVc	F: ACAATATTTGTATTATCGGAGAGC R: TTGGTATGAGGTATTGCTGG	200
SCCmec IVd	F: CTCAAAATACGGACCCCAATACA R: TGCTCCAGTAATTGCTAAAG	881
SCCmec V	F: GAACATTGTTACTTAAATGAGCG R: TGAAAGTTGTACCCTTGACACC	325

were performed for study the differences between patterns of antibiotic resistances. Statistical significance was set at a p value < 0.05.

Ethical considerations

The present study was accepted by the ethical committees of the Baqiyatallah and Peyambaran Hospitals, Tehran, Iran and Microbiology and Infectious Diseases Center of the Islamic Azad University of Shahrekord Branch, Iran. Written informed consent was obtained from all of the study patients or their parents.

RESULTS

Of 132 clinical samples, 66 were positive for ***S. aureus***. Superficial and surgical wounds had the highest incidence of ***S. aureus*** (66.12%), while blood samples had the lowest incidence (15.38%) (Table 3).

mecA (80.30%) were the most commonly detected antibiotic resistance genes followed by ***tetK*** (34.84%) and ***ermA*** (30.30%) (Table 4).

S. aureus isolates of superficial and post surgical wounds and respiratory infections had the highest incidence of antibiotic resistance genes. ***vatB*** (1.51%) and ***vatC*** (3.03%) had the lowest incidence in ***S. aureus*** strains. Of 66 ***S. aureus*** isolates, 27 strains harbored the PVL gene (40.90%) (Table 5).

There were no positive results for the PVL gene in the ***S. aureus*** strains of blood and urinary system. V phonotype (52.83%) and III phonotype (24.52%) had the highest incidence of ***SCCmec***types (Table 6).

The incidence of IVa, IVc and IVb ***SCCmec*** types were 11.32%, 7.54% and 3.77%, respectively. There were no positive results for I, II and IVd types. All of the ***S. aureus*** isolates were resistant to penicillin, cefalotin, cefazoline and ceftriaxone (Table 7).

S. aureus isolates harbored the highest levels of antibiotic resistance against azithromycin (62.12%), tetracycline (57.57%) and erythromycin (54.54%). ***S. aureus*** isolates harbored no antibiotic resistance against nitrofurantoin and vancomycin.

TABLE 3. Distribution of *Staphylococcus aureus* in various types of hospital infections in Iran.

Types of infections	No. samples	Positive results (%)
Blood	13	2 (15.38)
Urinary tract infection	16	4 (25)
Pneumonia and lung abscess	24	12 (50)
Superficial and surgical wound infection	62	41 (66.12)
Abscess	17	7 (41.17)
Total	132	66 (50)

DISCUSSION

In our research we found high incidence of multi-drug resistant ***S. aureus*** strains in patients' clinical samples. All of the examined blood, urine, respiratory secretions, superficial and surgical wounds and abscesses of the patients were infected with these strains. Unfortunately, these results showed that the Iranian hospitals' environments were so infected. Also, our results showed that antibiotics were used in a highly irregular manner in Iranian hospitals. These two findings may lead to the emergence of resistant staphylococcal diseases which can infect patients and even healthy people in hospitals. Similar results have been reported previously by some authors [8-13].

The results of our study showed that 80.30% of all ***Staphylococcus*** strains had the gene coding resistance against meticillin. In addition to meticillin, the Staphylococcus strains had the genes coding resistance against macrolides (7.57%), erythromycin (56.06%), lincosamides (7.57%), aminoglycosides (19.69%) and tetracycline (59.09%). Staphylococcus strains of our investigation had the highest levels of antibiotic resistance against penicillin (100%), cefalothin (100%), cefazoline (100%), ceftriaxone (100%), azithromycin (62.12%) and tetracycline (57.57%). Statistical analysis showed significant differences about p<0.05 between the incidence of ***mecA*** and other antibiotic resistance genes. We also found statistically significant (p<0.05) association between the levels of resistances to penicillin, cefalotin, cefazoline, and ceftriaxone with mupirocin, rifampicin, trimethoprim, imipenem and gentamicin antibiotics. Tokajian et al. [2] reported that 72% of ***S. aureus*** isolates of Lebanon's hospitals were meticillin-resistant and 18% of them were resistant to 10-18 antibiotics. Study of Udo et al. [4] showed that 1,765 (95.6%) inpatients and 81 (4.4%) outpatients of Kuwait hospitals were positive for ***S. aureus*** with 32% incidence rate of the meticillin resistant strains. Similar incidence rate of MRSA

TABLE 4. Distribution of antibiotic resistance genes in *Staphylococcus aureus* strains isolated from hospital infections in Iran.

Types of infections (*S. aureus* positive)	Antibiotic resistance genes (%)										
	*mec*A	*aac*A-D	*tet*K	*tet*M	*msr*A	*vat*A	*vat*B	*vat*C	*erm*A	*erm*C	*lin*A
Blood (2)	1	-	1	-	-	-	-	-	1	1	1
Urinary tract infection (4)	2	1	1	1	-	-	-	-	1	1	-
Pneumonia and lung abscess (12)	10	5	8	4	2	2	-	1	9	7	2
Superficial and surgical wound infection (41)	37	6	10	8	3	2	1	1	7	5	1
Abscess (7)	3	1	3	3	-	-	-	-	2	3	1
Total (66)	53 (80.30)	13 (19.69)	23 (34.84)	16 (24.24)	5 (7.57)	4 (6.06)	1 (1.51)	2 (3.03)	20 (30.30)	17 (25.75)	5 (7.57)

were reported previously by Alghaithy et al. [35] (61% in Saudi-Arabia), Młynarczyk et al. [36] (40% in Warszawie) and Rijal et al. [37] (56.1% in Pokhara). In a study of Virdis et al. [3], 56% of ***S. aureus*** isolates were resistant to one or more antimicrobial agents including kanamycin (28%), oxytetracycline (16%), and ampicillin (12%). The most commonly used antibiotics included oxacillin, nafcillin, cefathiamidine and vancomycin had the highest resistance to ***S. aureus*** strains of the Deng et al. [38] report. Nishijima and Kurokawa [39] reported thatthe incidence of resistance to penicillin, cephalosporins and clindamycin were 20 to 30% and the occurrence of gentamicin, erythromycin, roxithromycin and meticillin resistance were 55.2, 39.6, 39.1% and 21%, respectively. The ***S. aureus*** isolates of Kumar et al. [40] were highly resistant to antibiotics, i.e. 36.4% were resistant to streptomycin, 33.6% to oxytetracycline, 29.9% to gentamicin and 26.2% each to chloramphenicol, pristinamycin and ciprofloxacin which was similar to our results. It seems that meticillin would not be effective antibiotic in treatment of severe ***S. aureus*** infections.

In our study, microorganisms showed lowest resistance to imipenem. Totally, 6.06% of the ***S. aureus*** isolates of our study were resistant to imipenem. Fifty seven percent of MRSA strains of the Boyce et al. [41] were imipenem resistant which was entirely higher than our results. Similar studies have been reported by Totsuka et al. [42] and Yamazaki et al. [43]. We have observed no bacterial resistance to vancomycin and nitrofurantoin antibiotics in our study. Resistance against vancomycin have been showed previously [44-46]. Current recommendations showed that antibiotics may trigger release of PVL and progression to bad clinical complications. Furthermore, due to the inappropriate prescription, it was not surprising that our study found that resistance to penicillin, cefalotin, cefazoline, ceftriaxone, azithromycin and tetracycline were 100%, 100%, 100%, 100%, 62.12% and 57.57%, respectively.

The Staphylococcus strains of our study had the highest incidence in superficial and surgical wounds (66.12%). Significant differences ($p<0.05$) were observed for the incidence of bacteria between the superficial and surgical wounds and blood and urine samples. Similar results have been reported previously [47-49]. The main reason for this finding is the fact that the ***S. aureus*** strains represent the major micro flora of a healthy skin. Also, these strains are ubiquitous

TABLE 5. Distribution of PVL gene in *Staphylococcus aureus* strains isolated from hospital infections in Iran.

Types of infections (***S. aureus*** positive)	PVL positive (%)
Blood (2)	-
Urinary tract infection (4)	-
Pneumonia and lung abscess (12)	5 (41.66)
Superficial and surgical wound infection (41)	19 (46.34)
Abscess (7)	3 (42.85)
Total (66)	27 (40.90)

TABLE 6. Distribution of SCC***mec*** types in *Staphylococcus aureus* strains isolated from hospital infections in Iran.

Types of infections (***mec*** positive ***S. aureus***)	*SCCmec* types (%)							
	I	II	III	IVa	IVb	IVc	IVd	V
Blood (1)	-	-	-	1 (100)	-	-	-	1 (100)
Urinary tract infection (2)	-	-	1 (50)	-	1 (50)	-	-	1 (50)
Pneumonia and lung abscess (10)	-	-	4 (40)	-	-	4 (40)	-	6 (60)
Superficial and surgical wound infection (37)	-	-	8 (21.62)	4 (10.81)	1 (2.70)	-	-	18 (48.64)
Abscess (3)	-	-	-	1 (33.33)	-	-	-	2 (66.66)
Total (53)	-	-	13 (24.52)	6 (11.32)	2 (3.77)	4 (7.54)	-	28 (52.83)

TABLE 7. Distribution of antibiotic resistance pattern in *Staphylococcus aureus* strains isolated from hospital infections in Iran.

Types of infections (no. positive)	Resistance to antibiotics (%)																
	P 10*	Met 5	IMP 10	CZ 30	CF 30	CRO 30	GM 10	CIP 5	CC 2	AZM 15	E 15	MPR 30	RA 5	TE 30	TMP 5	V 30	F/M 300
Blood (2)	2	2	-	2	2	2	-	1	-	1	-	-	-	2	-	-	-
Urinary tract infection (4)	4	4	-	4	4	4	1	2	1	-	3	1	-	3	2	-	-
Pneumonia and lung abscess (12)	12	12	1	12	12	12	9	3	6	8	10	1	2	8	2	-	-
Superficial and post-surgical wound infection (41)	41	41	3	41	41	41	8	18	14	32	23	8	10	23	10	-	-
Abscess (7)	7	7	-	7	7	7	1	2	2	-	-	1	-	2	2	-	-
Total (66)	66 (100)	66 (100)	4 (6.06)	66 (100)	66 (100)	66 (100)	19 (28.78)	26 (39.39)	23 (34.84)	41 (62.12)	36 (54.54)	11 (16.66)	12 (18.18)	38 (57.57)	16 (24.24)	-	-

* P10= penicillin (10 u/disk); Met5= meticillin (5 µg/disk); IMP10= imipenem (10 µg/disk); CZ30= cefazoline (30 µg/disk); CF30= cephalothin (30 µg/disk); CRO30= ceftriaxone (30 µg/disk); GM10= gentamicin (10 µg/disk); CIP5= ciprofloxacin (5 µg/disk); CC2= clindamycin (2 µg/disk); AZM15= azithromycin (15 µg/disk); E15= erythromycin (15 µg/disk); MPR30= mupirocin (30 µg/disk); RA5= revamping (5 µg/disk); TE30= tetracycline (30 µg/disk); TMP5= trimethoprim (5 µg/disk); V30= vancomycin (30 µg/disk); F/M300= nitrofurantoin (300 µg/disk)

bacteria that are found anywhere, in hospital environment as well. Respiratory swabs had also high incidence of ***S. aureus*** bacteria (50%).

Totally, 40.9% of the ***S. aureus*** isolates of our study carried the PVL gene. This gene had the highest incidence in the superficial and surgical wounds in our study (46.34%). The main factor contributing to its high incidence in the wounds is the fact that the PVL gene is a bicomponent cytotoxin that is preferentially linked to furuncles, cutaneous abscesses and severe necrotic skin infections. We found no significant statistical differences for distribution of the PVL gene between the various types of infections. The PVL gene was found in 100% of cases with pneumonia, 80% of cutaneous abscesses, 100% of cases with furunculosis and 33.33% of finger pulp infections in a study of Ezzat et al. [50]. Many other investigations showed the presence of PVL genes in the staphylococcal infections including Esan et al. [51](Nigeria) (18%), Khosravi et al. [52] (Iran) (7.23%) and Shallcross et al. [53] (United Kingdom) (11.3%). Also, the prevalence of PVL gene is estimated to be some 2–35% among MRSA strains in hospital infections as previously reported [54, 55]. The PVL toxin's ability to cause the death of polymorphonuclear cells including neutrophils, basophils and eosinophils has been known since its very discovery.

The V phenotype (52.83%) and III phenotype (24.52%) were the most commonly detected ***SCCmec*** types among the ***mec*** positive strains of ***S. aureus*** of our study. We found significant differences ($p<0.05$) between the incidence of ***SCCmec*** V and ***SCCmec*** IVc, ***SCCmec*** IVb and ***SCCmec*** Iva types. Similar studies have been reported previously [23, 25, 56, 57]. D'Souza et al. [56] showed that a total of 97 ***mecA***-positive strains were SCC***mec*** III (25%), 136 were ***SCCmec*** IV (34%), and 162 were ***SCCmec*** V (41%) which was concordant to our results. They also showed that all of the ***SCCmec*** III strains, 73% of ***SCCmec*** IV and V strains and 72% of ***SCCmec*** IV and ***SCCmec*** V strains were multidrug resistant. Moussa et al. [8] reported that the most predominant ***SCCmec*** type among the examined isolates in Saudi Arabia were type V (42.5%) followed by, ***SCCmec*** type III 39 (38.6%) which was similar to our findings.

The above mentioned data highlight huge differences in the prevalence of ***SCCmec*** types in different studies, as well as differences in PVL gene, antibiotic resistance genes and antibiotic resistance patterns in the clinical samples. This could be related to differences in the type of sample (stool, blood, urine, meat, milk, respiratory swabs, wound materials and other clinical samples) tested, number of samples, method of sampling, experimental methodology, geographical area, antibiotic prescription preference among clinicians, antibiotic availability, and climate differences in the areas where the samples were collected, which would have differed between each study.

CONCLUSION

In conclusion, we identified a large number of MRSA, PVL virulence genes and antibiotic resistance properties in the ***S. aureus*** strains of hospitalized infections. Our data indicated that MRSA strains are predominant in Iran. High PVL gene distribution was also found. Our data revealed that ***tetK, tetM, ermA, ermC, aacA-D, linA, msrA, vatA, vatC*** and ***vatB*** genes and resistance to penicillin, cefalotin, cefazoline, ceftriaxone, azithromycin and tetracycline were the most commonly detected characteristics of the MRSA strains isolated from hospitals infections. The high levels of ***Staphylococcus aureus*** resistance against commonly used antibiotics as well as high presence of ***SCCmec*** types of meticillin-resistant in virulent strains of ***Staphylococcus aureus*** represent that infections with these strains require higher levels of hospital cares with emerging demand for novel antibiotics. Hence, the clinicians' role in judicious usage of antibiotics is pivotal.

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DECLARATION OF INTEREST

The authors declare no conflict of interest.

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