The distribution of Pathogenic and toxigenic genes among MRSA and MSSA clinical isolates.

ARTICLE *in* MICROBIAL PATHOGENESIS · MARCH 2015

Impact Factor: 2 · DOI: 10.1016/j.micpath.2015.03.013 · Source: PubMed

10 AUTHORS, INCLUDING:

Shafie Ghorbani
Zanjan University of Medical Sciences
4 PUBLICATIONS **0** CITATIONS
SEE PROFILE

Mohsen Amin
University of Toronto
15 PUBLICATIONS **32** CITATIONS
SEE PROFILE

Ali Choopani
Payame Noor University
11 PUBLICATIONS **2** CITATIONS
SEE PROFILE

Abbas Ali Imani Fooladi
130 PUBLICATIONS **284** CITATIONS
SEE PROFILE

Microbial Pathogenesis 81 (2015) 60–66

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

The distribution of pathogenic and toxigenic genes among MRSA and MSSA clinical isolates

Abbas Ali Imani Fooladi [a], Elnaz Ashrafi [b], Shafie Gorbani Tazandareh [c], Roohollah Zarei Koosha [d], Hamid Sedighian Rad [a], Mohsen Amin [e], Mina Soori [c], Reza Abbasi Larki [f], Ali Choopani [g], Hamideh Mahmoodzadeh Hosseini [a, *]

[a] Applied Microbiology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
[b] Microbiology Department, Islamic Azad University of Arak Branch, Arak, Iran
[c] Microbiology Department, Islamic Azad University of Zanjan Branch, Zanjan, Iran
[d] Biology Department, Islamic Azad University of Jahrom Branch, Jahrom, Iran
[e] Department of Drug and Food Control, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[f] Microbiology Department, Islamic Azad University of Tonekabon Branch, Jahrom, Iran
[g] Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 17 August 2014
Received in revised form
26 February 2015
Accepted 13 March 2015
Available online 14 March 2015

Keywords:
Staphylococcus aureus
Superantigen
MRSA
MSSA
Entorotoxin
SCC*mec*

ABSTRACT

Staphylococcus aureus (*S. aureus*) is considered as a notorious nosocomial pathogen among hospitalized patients and community-dwelling subjects. Its increasing morbidity and mortality is believed to be due to antibiotic resistance. However, the data concerning molecular properties of infecting strains are few.

In this study, a total of 192 *S. aureus* strains, including 88 (45.8%) meticillin-sensitive *S. aureus* (MSSA) and 104 (54.2%) meticillin-resistant *S. aureus* (MRSA) were recovered from clinical samples. The prevalence of subtypes containing staphylococcal cassette chromosome *mec* (SSC*mec*), staphylococcal enterotoxins (SEs), toxic shock syndrome toxin (TSST) and exfoliative toxin was assessed by PCR. Antibiotic susceptibility pattern and vancomycin resistance of each isolate were evaluated by disk diffusion method and micro-dilution method, respectively.

9 (2.3%) strains required MIC > 2 mg/l of vancomycin, which significantly increased among multi drug resistant (MDR), MRSA and SCC*mec* type III strains ($p < 0.05$). 171 (89%), 140 (72.91%), 7 (3.6), 78 (48.6%), 5 (2.6%), 151 (78.64%), 129 (67.18%), 178 (92.7%) and 15 (7.8%) of 192 isolates harbored *mec*A, *ent*A, *ent*B, *ent*C, *ent*D, *ent*E, *eta, etb* and *tsst-1* genes, respectively. 31 (16.14%), 5 (2.6%), 95 (49.48%) and 7 (3.64%) of 192 isolates carried SCC*mec* type I, II, III and IV, respectively. We found a significantly higher rate of MRSA and resistance to all tested antibiotics, except to penicillin G, kanamycin and linezolide among the SCC*mec* type III class ($p < 0.05$).

According to our findings, MSSA isolates should be taken as seriously as MRSA strains due to the potential presence of broad spectrum virulence factor genes.

1. Introduction

Staphylococcus aureus (*S. aureus*), as one of the major nosocomial pathogens in various parts of the world, causes a wide range of human infections, including endocarditis, pneumonia, toxic shock syndrome, and bacteremia [1]. The emergence of meticillin-resistant strains along with multi drug resistant (MDR) strains in hospital settings and nursing homes increase the morbidity and mortality rate of staphylococcal infections owing to cumbersome treatment [2]. Resistance to meticillin is acquired via several mechanisms. The expression of an altered penicillin binding protein (PBP2a) is the main mechanism. The highly conserved *mecA* gene is responsible for encoding PBP2a that bears on mobile genetic elements called staphylococcal cassette chromosome *mec* (SSC*mec*) [3,4]. Eleven types of SSC*mec* have been recognized, five of which (I–V) are universally spread. It seems that other types of SSC*mec* appear only in an initial rise region [5,6]. The members of SCC*mec* family possess two indispensable components, conserved terminal

* Corresponding author.
E-mail address: hosseini361@yahoo.com (H.M. Hosseini).

http://dx.doi.org/10.1016/j.micpath.2015.03.013
0882-4010/

inverted repeats and direct repeats [7]. The third region called Junkyard locates between the two described parts and includes several genes and pseudogenes, which do not appear to be necessary for the pathogenesis of the microorganism [8].

A wide range of virulence factors, including exfoliative toxin (ETA and ETB) and potent superantigens such as staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin (TSST) are produced by *S. Areas*, which lead to severe infections and toxemia syndromes. Superantigens are known as a robust T-cell mitogen activator which are responsible for extensive unspecific T-cell proliferation and secretion of pro-inflammatory cytokines, subsequently causing severe sepsis [9,10]. 21 discrepant types of SE have been reported with different sequence homologies [11]. The *tsst-1* gene encodes a 21.9 KDa extracellular toxin causing toxic shock syndrome (TSS) [12]. Although the frequency of the *tsst-1* gene within meticillin sensitive *S. aureas* strains (MSSA) have already been high, recent reports revealed that the number of MRSA harboring this gene has been increased [13–15]. There are three isoforms of ET, ETA, ETB and ETD which are encoded by the *eta*, *etb* and *etd* genes, respectively. The ETA and ETB toxins are associated with the occurrence of staphylococcal scaled skin syndrome (SSSS) [16]. Despite the genetic homology and similar biological activity, ETA and ETB differ in their immunological characteristics and location of encoding genes. The *eta* gene has a chromosomal origin that is integrated by prophages, whereas the *etb* gene is a plasmid gene [17].

To the best of our knowledge, There are few studies surveying the relation between the co-presence of SEs, TSST and exfoliative toxin and their clinical outcomes in patients suffered from *S. aureus* infections. Herein, we attempted to assess the prevalence of *S. aureus* subtypes containing SSC*mec*, SEs, TSST and exfoliative toxin in MRSA and MSSA strains isolated from clinical samples.

2. Materials and methods

2.1. Bacterial isolates

A total of 192 *S. aureus* strains were recovered from clinical samples, including wounds, urine, stool, blood, bail, tracheal aspirates, sputum, bronchoalveolar lavage (BAL), bone, ear infections, chest tube, pleural effusion, synovial fluid during 2009–2012 from one of the educational hospitals in the north of Tehran, Iran. *S. aureus* strains were identified by means of various biochemical tests,Gram-staining, catalase, coagulase and DNAse activity tests, manitol fermentation and the presence of hemolysis on blood agar plates after 24 h incubation at 37 °C. To remove the colonized *S. aureus* isolates, all of the infectious cases were confirmed in accordance with clinical and Para-clinical manifestations along with the results from microbial culture and colony count. The collected strains were freezed and stored at −80 °C for further examinations.

2.2. Antimicrobial susceptibility testing

The antimicrobial susceptibility pattern against 12 antibiotics, including cefoxitin (10 μg), penicillin G (10 U), vancomycin (30 μg), ciprofloxacin (5 μg), erythromycin (15 μg), clindamycin (2 μg), cotrimoxazole (25 μg), gentamicin (10 mg), linezolid (30 mg), cephazolin (30 mg), kanamycin (30 mg) and tetracycline (30 μg) were investigated for each isolate. All the antibiotics were purchased from MAST company, Merseyside, UK. The antimicrobial evaluation was performed using disc diffusion method (Kirby–Bauer) based on clinical and laboratory standard institute (CLSI) guidelines [18].

Moreover, to confirm the findings of disk diffusion susceptibility against vancomycin, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was determined using micro-dilution method according to a guideline recommended by CLSI. Nine different concentrations of vancomycin (0.0625, 0.125, 0.250, 0.5, 1, 2, 4, 8, 16, and 32 mg/l) in Muller Hinton (MH) broth were prepared. One milliliter of each concentration was added to a separate test tube. Then, approximately 1×10^8 CFU/ml (equivalent to 0.5 McFarland) from each isolate were inoculated into an appropriate tube. After 24 h incubation at 37 °C, the turbidity of each sample was evaluated. To assess the MBC, 1 ml of each incubated test tube was cultured on a MH agar plate and incubated at 37 °C for 24 h and the bacterial growth was determined. Tubes without antibiotic and another one without bacteria were used as controls.

2.3. DNA isolation and PCR amplification for gene detection

Polymerase chain reaction (PCR) was used to evaluate the distribution of several virulence factors, including *mec*A, *entA, entB, entC, entD, entE, eta, etb* and *tsst-1* genes among the isolates. Furthermore, SCC*mec* elements were identified based on relevant primers (Table 1). All the primer pairs except for *tsst-1* genes were selected from literature. The *tsst*-1 primers is original that its accuracy was confirmed by sequencing (Macrogen Research, Seoul, Korea). Briefly, Each isolate was cultured in 5 ml of LB broth at 37 °C overnight under aerobic conditions and then bacterial cells were collected by centrifugation at 6000 rpm for 10 min. 100 μl buffer solution (10 mM Tris· HCl, pH 8.0; 1 mM EDTA; 100 mM NaCl) and 15 μl lysozyme (0.25 mg/ml) were added to the bacterial pellet and incubated for an hour at 37 °C. Then, 200 μl GC solution and 15 μl proteinase K solution were added and incubated for 15 min at 60 °C. Finally, The DNA was precipitated using isopropanol. The quality and quantity of purified DNA were determined using gel electrophoresis and NanoDrop spectrophotometer (NanoDrop-1000, Wilmington, DE), respectively. 1 μl of each extracted DNA was amplified in 25 μl of mixture reaction containing 10× reaction buffer, 0.1 mM of the deoxynucleoside triphosphates (dNTPs), 2 mM $MgCl_2$, 10 ρmol of designed primers and 1 U of Taq DNA Polymerase (Cinnagen, Tehran, Iran). PCR was carried out in Eppendorf asterCycker (Hamburg, Germany) followed by an initial denaturation step of 5 min at 94 °C; 35 cycles of 1 min at 94 °C, 2 min for annealing as listed in Table 1 for each gene and 1 min at 72 °C followed by 5 min final extension at 72 °C. To detect the presence of desired amplicon, the 1.5% agarose electrophoresis was performed, stained by ethidium bromide and then products were visualized by UV transilluminator (Kiagene, Tehran, Iran). The genomic DNA isolated from the standard strains named MW2, COL, Mu50, Fri1151m and NRS111was kindly gifted from Dr. Patrice Francois for positive controls of *genes* [19] .

2.4. Statistical analyses

All statistical analyses were done using Chi square and Fisher's exact test in the SPSS version 15 (Chicago, IL). *P* values less than 0.05 were considered as statistically significant.

3. Results

3.1. Clinical isolates

In the current cross-sectional study, we surveyed the prevalence of MRSA and MSSA *S. aureus* among our isolates, their antibiotic resistance pattern and possession of virulence factor genes. Out of 192 strains, 116 (60.4%), 34 (17.7%), 12 (6.2%), 10 (5.2%), 8 (4.2), 4 (2.1%) and 2 (1%) were recovered from the wounds, blood, bail,

Table 1
The sequences of primers.

Gene name	Forward sequence	Reverse sequence	Annealing temperature (C)	Product size (bp)	Ref
mecA	TGAGTTGAACCTGGTGAAGTT	TGGTATGTGGAAGTTAGATTGG	57	857	[39]
SCCmec I	TTTAGGAGGTAATCT CCT TGA TG	TTT TGC GTT TGC ATC TCT ACC	52	154	[40]
SCCmec II	CGT TGA AGA TGA TGA AGC G	CGA AATCAATGG TTAATGGACC	53	398	[41]
SCCmec III	CCA TAT TGT GTA CGA TGC G	CCTTAGTTGTCGTAACAGATC G	49	280	[41]
SCCmec IV	TTTGAATGCCCTCCATGAATAAA T	AGAAAAGATAGAAGTTCGAAGA	55	458	[40]
entA	TTGGAACGGTTAAAACGAA	GAACCTTCCCATCAAAAACA	50	121	[42]
entB	'TCGCATCAAACTGACAAACG	GCAGGTACTCTATAAGTGCC	55	478	[42]
entC	GGAGGAATAACAAAACATGAAGG	AAAGGCAAGCACCGAAGTAC	59	459	[42]
entD	TGGTGGTGAAATAGATAGGAC	TGAAGGTGCTCTGTGGATAAT	51	384	[42]
entE	TGGTAGCGAGAAAAGCGAAG	TGTAAATAATGCCTTGCCTGAA	55.5	495	[42]
tsst-1	CTGGTATAGTAGTGGGTCTG	AGGTAGTTCTATTGGAGTAGG	54	271	Original
eta	TTTGCTTTCTTGATTTGGATTC	GATGTGTTCGGTTTGATTGAC	54	464	[39]
etb	ACGGCTATATACATTCAATT	TCCATCGATAATATACCTAA	50.9	200	[39]

tracheal aspirates, sputum, ear infections and chest tube samples, respectively. Besides, 1 (0.5%) strain was isolated from each one of synovial fluids, BAL, bone, urine, stool and plural effusion samples. 133 (69.3%) specimens were collected from men and the rest (30.7%) from female. The subjects were in the range of 1–94 years of age, categorized into 10 age groups. Most cases belonged to the age group of 51–60 years. Table 2 lists the frequency of the isolates with respect to different age groups, gender and infection sites. Moreover, as summarized in Table 2, the samples were most frequently collected from special care units (ICU, CCU and VIP) (24%) and less frequently recovered from patients admitted to urology (1.6%) and chemotherapy department (1%).

Table 2
The pattern of MRSA and MSSA isolates distribution according to demographic properties.

	All strains N = 192 (%)	MSSA N = 88 (%)	MRSA N = 104 (%)	P value
Sex				
Female	59 (30.7)	30 (34.1)	22 (27.9)	0.353
Male	133 (69.3)	58 (65.9)	75 (72.1)	
Source				
Wound	116 (60.4)	54 (61.4)	62 (59.6)	0.129
pleural effusion	1 (0.5)	0	1 (1)	
Urine	1 (0.5)	1 (1.1)	0	
Ear	4 (2.1)	4 (4.5)	0	
Blood	34 (17.7)	17 (19.3)	17 (16.3)	
Stool	1 (0.5)	1 (1.1)	0	
Tracheal aspirate	10 (5.2)	1 (1.1)	9 (8.7)	
Sputum	8 (4.2)	3 (3.4)	5 (4.8)	
Bail	12 (6.2)	4 (4.5)	8 (7.7)	
Bone	1 (0.5)	1 (1.1)	0	
Chest tube	2 (1)	1 (1.1)	1 (1)	
BAL	1 (0.5)	0	1 (1)	
Synovial fluid	1 (0.5)	1 (1.1)	0	
Age				
0–10 years	8 (4.2)	4 (4.5)	4 (3.8)	0.326
11–20 years	16 (8.3)	7 (8)	9 (8.7)	
21–30 years	19 (9.9)	5 (5.7)	14 (13.5)	
31-40 years	9 (4.7)	7 (8)	2 (1.9)	
41–50 years	36 (18.8)	21 (23.9)	15 (14.4)	
51–60 years	39 (20.3)	17 (19.3)	22 (21.2)	
61–70 years	33 (17.2)	12 (13.6)	21 (20.2)	
71–80 years	22 (11.5)	10 (11.4)	12 (11.5)	
81–90 years	8 (4.2)	4 (4.5)	4 (3.8)	
91–100 years	2 (1)	1 (1.1)	1 (1)	
Hospital wards				
Internal	37 (19.3)	17 (19.3)	20 (19.2)	0.178
Surgery	30 (15.6)	15 (17)	15 (14.4)	
Orthopedic	33 (17.2)	17 (19.3)	16 (15.4)	
Chemo-therapic dep.	2 (1)	2 (2.3)	0	
Pediatric	7 (3.6)	3 (3.4)	4 (3.8)	
Urology	3 (1.6)	0	3 (2.9)	
Emergency	34 (17.7)	19 (21.6)	15 (14.4)	
ICU/CCU/VIP	46 (24)	15 (17)	31 (29.8)	

3.2. The antimicrobial susceptibility analysis

Antibiotic resistance of each isolate was assessed with antimicrobial susceptibility analysis. The highest resistance was observed against penicillin G (Table 3). Furthermore, resistance to linezolide was observed in 2 (1%) isolates. Results from disc diffusion methods showed prevalent resistance to vancomycin. Therefore, we determined the MIC and MBC for vancomycin using the micro-dilution method for all isolates. According to the micro-dilution, 158 (82.7%), 24 (12.6%) and 9 (4.7%) out of 192 isolates had MIC lower than 2, equivalent to 2 and upper than 2 mg/l for vancomycin, respectively. We showed the significant increase of vancomycin MIC among MDR, MRSA and SCC*mec* type III population ($p < 0.05$) (Table 4).

Multi drug resistance (resistance to more than three antibiotics) (MDR) was found in 102 (53.12%) of the isolates. Statistical analyses

Table 3
The prevalence of virulence factors, SCC*mec* subtypes and antibiotic resistance status among MRSA and MSSA isolates.

Name of genes	All strains N = 192 (%)	MSSA N = 88 (%)	MRSA N = 104 (%)	P value
*mec*A	171 (89)	73 (83)	98 (94.2)	0.353
SCC*mec* I	31 (16.14)	11 (12.5)	20 (19.2)	0.207
SCC*mec* II	5 (2.6)	3 (3.4)	2 (1.2)	0.519
SCC*mec* III	95 (49.48)	28 (31.8)	67 (64.4)	**0.001**
SCC*mec* IV	7 (3.64)	2 (2.3)	5 (4.8)	0.35
ent A	140 (72.91)	59 (67)	81 (77.9)	0.058
ent B	7 (3.64)	3 (3.4)	4 (3.8)	0.844
ent C	78 (48.62)	36 (40.9)	42 (40.4)	0.834
ent D	5 (2.6)	3 (3.4)	2 (1.9)	0.527
ent E	151 (78.64)	63 (71.6)	88 (84.6)	**0.019**
tsst-1	129 (67.18)	63 (71.6)	66 (63.3)	0.257
eta	178 (92.7)	81 (92)	97 (93.3)	0.559
etb	15 (7.81)	14 (15.9)	1 (1)	**0.000**
Antibiotic resistance	All strains N = 192 (%)	MSSA N = 88 (%)	MRSA N = 104 (%)	P value
Penicillin G	190 (98.95)	86 (97.7)	104 (100)	0.122
Cephazolin	91 (47.39)	3 (3.4)	88 (84.6)	**0.01**
gentamicin	94 (48.95)	6 (6.8)	88 (84.6)	**0.000**
Kanamycin	122 (63.54)	29 (33)	93 (89.4)	**0.000**
Erythromycin	104 (54.16)	16 (18.2)	88 (84.6)	**0.000**
Tetracycline	123 (64.06)	29 (33)	99 (90.4)	**0.000**
Ciprofloxacin	110 (57.29)	15 (17)	95 (91.3)	**0.000**
Clindamycin	90 (46.87)	8 (9.1)	82 (78.8)	**0.000**
cotrimoxazole	69 (35.93)	2 (2.3)	67 (64.4)	**0.000**
Linezolide	2 (1)	2 (2.3)	0 (0)	**0.000**
MDR	102 (53.12)	7 (8)	95 (91.3)	**0.000**

* $p < 0.05$ is significant in bold.

Table 4
Comparison of vancomycin resistance status of clinical isolates with the presence of virulence factors.

Vancomycin	MIC			P value
	<2 μg N = 158 (%)	2 μg N = 24 (%)	>2 μg N = 9 (%)	
SCC*mec* I	26 (16.5%)	4 (16.7)	1 (11.1)	0.913
SCC*mec* II	5 (3.2)	0 (0)	0 (0)	0.585
SCC*mec* III	71 (44.9)	17 (70.8)	7 (77.8)	**0.014**
SCC*mec* IV	7 (4.4)	0 (0)	0 (0)	0.468
*mec*A	137 (86.7)	24 (100)	9 (100)	0.085
MRSA	73 (46.2)	33 (95.8)	8 (88.9)	**0.001**
MSSA	85 (53.8)	1 (4.2)	1 (11.1)	
entA	113 (71.5)	18 (81.8)	8 (88.9)	0.333
entB	5 (3.2)	1 (4.8)	1 (11.1)	0.473
entC	68 (43.6)	5 (23.8)	4 (44.4)	0.221
entD	3 (1.9)	2 (8.7)	0 (0)	0.144
entE	122 (77.2)	20 (87)	8 (88.9)	0.426
tsst-1	102 (65.8)	19 (79.2)	7 (77.8)	0.347
eta	145 (93.5)	24 (100)	8 (88.9)	0.36
etb	14 (9)	1 (4.2)	0 (0)	0.475
MDR	70 (44.3)	24 (100)	8 (88.9)	**0.001**

* $p < 0.05$ is significant in bold.

showed no significant impact of gender, age, hospital ward and sample types on the incidence of MDR property.

The isolates were phenotypically divided into two groups, MRSA and MSSA, based on resistance or sensitivity to cefoxitin. 88 (45.8%) and 104 (54.2%) out of 192 isolates were MSSA and MRSA, respectively. Apart from linezolide and penicillin G, resistance to other antibiotics significantly increased among MRSA strains compared to MSSA ($p < 0.05$) (Table 3). On the contrary, linezolide resistant isolates significantly belonged to MSSA group ($p < 0.05$).

3.3. Distribution of the virulence factor genes in the clinical isolates

The prevalence of *mec*A, *ent*A, *ent*B, *ent*C, *ent*D, *ent*E, *eta, etb* and *tsst-1* genes was evaluated by PCR. As described in Table 3, 171 (89%), 140 (72.91%), 7 (3.6), 78 (48.6%), 5 (2.6%), 151 (78.64%), 129 (67.18%), 178 (92.7%) and 15 (7.8%) out of 192 isolates harbored *mec*A, *ent*A, *ent*B, *ent*C, *ent*D, *ent*E, *eta, etb* and *tsst-1* genes, respectively.

Prevalence of *mecA* gene and the presence of antibiotic resistance genes are reported in Table 3. Aside from penicillin, vancomycin and linezolid, resistance to other antibiotics significantly prevails in the presence of *mecA* and *entE* genes ($p < 0.05$). Furthermore, we found that the occurrence of *entA* gene is significantly higher among the resistant isolates against cefoxitin, tetracycline, gentamicin, ciprofloxacin, cephazolin and kanamycin ($p < 0.05$). Furthermore, the MDR property was directly correlated to the presence of *mecA*, *entA* and *entE* genes ($p < 0.05$). The frequency of *entC* and *eta* was significantly higher in males ($p < 0.05$). In addition, the frequency of *entE*-carrying strains was significantly high in MRSA samples, while the frequency of *etb* containing isolates was low in the same samples (Table 3). There was no significant correlation between the presence of virulence genes and gender, age, hospital wards or sample types.

3.4. Prevalence of SCCmec genes

31 (16.14%), 5 (2.6%), 95 (49.48%) and 7 (3.64%) out of 192 isolates carried SCC*mec* type I, II, III and IV, respectively. The presence of SCC*mec* type III was significantly higher among MRSA isolates ($p < 0.05$, Table 3). As summarized in Table 5, the ent*A*, ent*C* and ent*E* genes were remarkably more prevalent among SCC*mec* type I ($p < 0.05$). Moreover, the presence of the ent*E* genes was significantly higher within isolates having the SCCmec type III gene ($p < 0.05$). Except penicillin G, kanamycin and linezolide, resistance to all other tested antibiotics was more prevalent among the SCC*mec* type III class ($p < 0.05$). Resistance to cephazolin, kanamycin and ciprofloxacin was observed with high frequency among the SCC*mec* type I ($p < 0.05$) (Table 5). However, we found no significant differences in terms of the pattern of SCC*mec* among different age groups, genders, source of isolates and the hospital wards to which patients were admitted (Table 6).

4. Discussion

Virulence factor profiles, antibiotic resistance pattern and the genetic background of *S. Areas* strains have an impact on the clinical manifestation of infection. It is believed that the expression of genes responsible for virulence factors and toxins alters due to the

Table 5
Frequency of virulence factors and antibiotic resistance among different SCC*mec* subtypes.

Name of genes	SCC*mec* I n = 31 (%)	P value	SCC*mec* II n = 5 (%)	P value	SCC*mec* III N = 91 (%)	P value	SCC*mec* IV n = 7 (%)	P value
ent A	30 (96.8)	**0.001**	3 (60)	0.481	74 (77.9)	0.119	4 (57.1)	0.311
ent B	1 (3.3)	0.888	0	0.653	4 (4.2)	0.668	5 (71.4)	0.653
ent C	21 (67.7)	**0.001**	4 (80)	0.078	43 (45.3)	0.135	3 (42.9)	0.676
ent D	1 (3.2)	0.817	0	0.710	3 (3.2)	0.925	0	0.659
ent E	29 (93.5)	**0.03**	3 (60)	0.289	83 (87.4)	**0.002**	7 (100)	0.165
tsst-1	22 (71)	0.723	4 (80)	0.567	65 (68.4)	0.634	6 (85.7)	0.312
eta	29 (93.5)	0.870	5 (100)	0.573	87 (91.6)	0.715	7 (100)	0.503
etb	3 (9.7)	0.695	2 (40)	0.051	4 (4.2)	0.069	1 (14.3)	0.527
Antibiotic resistance	SCC*mec* **I**	**P value**	SCC*mec* **II**	**P value**	SCC*mec* **III**	**P value**	SCC*mec* **IV**	**P value**
Penicillin G	31 (100)	0.533	5 (100)	0.816	93 (97.9)	0.151	7 (100)	0.782
Cefoxitin	20 (64.5)	0.207	2 (40)	0.519	67 (70.5)	**0.000**	5 (71.4)	0.35
Cephazolin	20 (64.5)	**0.043**	2 (40)	0.720	65 (68.4)	**0.000**	5 (71.4)	0.205
gentamicin	20 (64.5)	0.058	1 (20)	0.189	66 (69.5)	**0.000**	5 (71.4)	0.226
Kanamycin	27 (87.1)	**0.012**	4 (80)	0.735	76 (80)	0.581	6 (85.70	0.460
Erythromycin	18 (58.1)	0.634	3 (60)	0.791	67 (70.5)	**0.000**	5 (71.4)	0.35
Tetracycline	22 (71)	0.64	3 (60)	0.965	77 (81.1)	**0.03**	5 (71.4)	0.907
Ciprofloxacin	23 (74.2)	**0.038**	3 (60)	0.901	74 (77.9)	**0.000**	5 (71.4)	0.441
Clindamycin	16 (51.6)	0.564	2 (40)	0.755	62 (65.3)	**0.000**	5 (71.4)	0.185
cotrimoxazole	10 (32.3)	0.721	1 (20)	0.722	50 (52.6)	**0.032**	4 (57.1)	0.484
Linezolide	0	0.533	0	0.816	0	0.159	0	0.782
MDR	20 (64.5)	0.165	3 (60)	0.755	70 (73.7)	**0.000**	5 (71.4)	0.323

* $p < 0.05$ is significant in bold.

Table 6
Pattern of demographic characteristics among clinical isolates with different SCC*mec* subtypes.

	SCC*mec* I n = 31 (%)	P value	SCC*mec* II n = 5 (%)	P value	SCC*mec* III N = 91 (%)	P value	SCC*mec* IV n = 7 (%)	P value
Sex								
Female	9 (29)	0.823	2 (40)	0.649	32 (33.7)	0.380	3 (42.9)	0.479
Male	22 (74.2)		3 (60)		63 (66.3)		4 (57.1)	
Source								
Wound	23 (74.2)	0.915	4 (80)	0.990	60 (63.2)	0.227	1 (14.3)	0.324
pleural effusion	0		0		1 (1.1)		0	
Urine	0		0		0		0	
Ear	0		0		1 (1.1)		0	
Blood	5 (16.1)		0		14 (14.7)		2 (28.6)	
Stool	0		0		1 (1.1)		0	
Tracheal aspirate	2 (6.5)		0		8 (8.4)		2 (28.6)	
Sputum	1 (3.2)		0		5 (5.3)		1 (14.3)	
Bail	0		1 (20)		3 (3.2)		1 (14.3)	
Bone	0		0		0		0	
Chest Tube	0		0		1 (1.1)		0	
BAL	0		0		1 (1.1)		0	
Synovial fluid	0		0		0		0	
Age								
0–10 years	0	0.475	0	0.017	4 (4.2)	0.930	0	0.601
11–20 years	4 (12.9)		1 (20)		11 (11.6)		1 (14.3)	
21–30 years	1 (3.2)		1 (20)		8 (8.4)		0	
31-40 years	0		0		4 (4.2)		0	
41–50 years	9 (29)		0		19 (20)		3 (42.9)	
51–60 years	6 (19.4)		1 (20)		19 (20)		0	
61–70 years	6 (19.4)		0		16 (16.8)		1 (14.3)	
71–80 years	4 (12.9)		0		9 (9.5)		1 (14.3)	
81–90 years	1 (3.2)		2 (40)		4 (4.2)		1 (14.3)	
91–100 years	0		0		1 (1.1)		0	
Inward								
Internal	4 (12.9)	0.263	0	0.097	21 (22.1)	0.226	3 (42.9)	0.532
Surgery	8 (25.8)		0		14 (14.7)		0	
Orthopedic	3 (9.7)		3 (60)		18 (18.9)		0	
Chemotherapic dep.	0		0		2 (2.1)		0	
Pediatric	0		1 (20)		4 (4.2)		0	
Urology	0		0		2 (2.1)		0	
Emergency	5 (16.1)		0		10 (10.5)		1 (14.3)	
ICU/CCU/VIP	11 (35.5)		1 (20)		24 (25.3)		3 (42.9)	

acquisition of resistance genes [20]. There are scarce data in the literature on the link between genotype/phenotype of *S. aureus* and clinical outcomes. In the context of cumbersome antibiotic therapy, this study presents findings on comparing the presence of some toxin genes, SCC*mec* genes and the pattern of antibiotic resistance between MSSA and MRSA strains for the first time in Iran.

In order to do a thorough screening of MRSA isolates, we tested each isolate both phenotypically and genotypically and determined resistance to cefoxitin using disk diffusion and identified the *mec*A gene by PCR. Our results correspond with the previous Iranian reports published by Sadeghi et al., Moghadami et al., and Habibi et al. which are approximately similar to our findings [21–23]. However, Fatholahzadeh and his colleagues found a lower rate of MRSA incidence (36%) [24]. Inconsistent with previous reports (predominant MRSA strains among blood and pulmonary infections occurred in a hospital settings) [7,25], our MRSA strains were more prevalent among samples recovered from wounds in patients between 50 and 70 years of age and subjects admitted to ICU/CCU/VIP departments.

We revealed that six cefoxitin-resistant isolates did not harbor *mecA*.

The current study underscores the serious concern of spreading MDR strains. 91.3% of MRSA isolates were MDR with the predominant resistance to penicillin G, while only 8% of MSSA strains were identified as MDR with the main resistance to kanamycin, erythromycin and ciprofloxacin. Furthermore, resistance to all antibiotics tested here except to penicillin G and linzolide were more prevalent in MRSA isolates. However, linzolide was significantly more active on MSSA than MRSA. Rising resistance to two new antibiotics, vancomycin and linzolide, is alarming for the treatment of nosocomial infections. Though, they have been known as efficient agents so far, it requires to devise a new effective alternative. In the current study, vancomycin MIC for the minority of isolates was higher than 2 mg/ml which has increased compared to previous Iranian reports and indicates that vancomycin may become inefficient against *S. aureus* in near future. Recent report by Han et al. [26] revealed a strong association between rising vancomycin MIC and MRSA and SCC*mec* type II but studies performed in Korea and China showed no relationship between the type of SSC*mec* genes and *agr* dysfunction [27,28]. Findings obtained here showed a remarkable association between the increase of vancomycin MIC and the occurrence of MDR, MRSA and SCC*mec* type III properties. These findings suggest the emergence of new super resistant strains which can have a negative impact on the public health.

In accordance with previous data from Iran, there is a remarkably higher rate of resistance to clindamycin, erythromycin, gentamicin, kanamycin, tetracycline, ciprofloxacin and cotrimoxazole among our MRSA isolates [21]. This observation suggests that these antibiotics are not efficient enough to combat infections and their prescription should be supported by the knowledge of circulating resistant strains in the community.

Consistent with previous reports in Iran [7,21,24], findings from the current study demonstrated that the most predominant SCC*mec* gene was type III (49.48%) followed by type I (16.4%), which may be due to horizontal gene transfer. Except in Japan and Korea, the SCC*mec* type III is the dominant type in the majority of Asian countries, including Arab countries, Vietnam, Thailand, Indonesia, Singapore, India and China [29]. Resistance to cefoxitin, cephazolin,

gentamicin, erythromycin, tetracycline, clindamycin and cotrimoxazole was significantly higher in SCC*mec* type III. Other significant results were the prevalence of some toxin genes among different SCC*mec* type strains.

Here, the majority of isolates possessed a varied number of toxin genes, ranging from zero to six genes. The main toxin in our study was *eta* (92.7%) followed by *entE* (78.64%) and *entA* (72.91%). This frequency was higher than the one in the reports from Turkey [30]. We showed that the *entA*, *entC* and *entE* genes were significantly frequent in SCC*mec* type I, whereas *entE* was prevalent in SCC*mec* type III. In addition, there were no *entB*- and *entD*-positive strains within the SCC*mec* type II population. None of SCC*mec* type IV carried the *entD* gene. We showed that there are marginal differences in the distribution of toxin genes among four SCC*mec* types which is in contrast to the previous reports [31–33]. Collins et al. and Jimenez et al. reported opposing data showing that antibiotic resistance and virulence factors demonstrated different pattern based on the type of SCC*mec* gene [20,34]. They recognized attenuation of toxin secretion in SCC*mec* type II but more various ranges of toxin expression in SCC*mec* type IV.

This research has verified that *entE* and *etb* were significantly more frequent among MRSA and MSSA strains, respectively. In contrast to the common belief that MRSA strains have higher rate of enterotoxins [30,35–38], we found that both MRSA and MSSA have similar patterns of enterotoxin distribution.

In conclusion, according to our findings, MSSA isolates should be considered as important as MRSA strains due to harboring broad spectrum virulence factor genes which can act as potential sources of infection. . Moreover, discrepancies between our data and other reports in terms of variation in toxin patterns may be due to geographical differences.

The current study includes some limitations. The authors had no access to the information concerning clinical outcomes, mortality and association between clinical manifestations, severity of diseases and *in vivo* detection of toxins.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgment

We Thank Dr. Patrice Francois for kindly gifted the positive control DNAs.

References

[1] M. Hallin, O. Denis, A. Deplano, R. De Mendonça, R. De Ryck, S. Rottiers, et al., Genetic relatedness between methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*: results of a national survey, J. Antimicrob. Chemother. 59 (2007) 465–472.

[2] E. Klein, D.L. Smith, R. Laxminarayan, Hospitalizations and deaths caused by methicillin-resistant *Staphylococcus aureus*, United States, 1999–2005, Emerg. Infect. Dis. 13 (2007) 1840–1846.

[3] M.M. Baddour, M.M. AbuElKheir, A.J. Fatani, Comparison of mecA polymerase chain reaction with phenotypic methods for the detection of methicillin-resistant *Staphylococcus aureus*, Curr. Microbiol. 55 (2007) 473–479.

[4] M.G. Pinho, S.R. Filipe, H. de Lencastre, A. Tomasz, Complementation of the essential peptidoglycan transpeptidase function of penicillin-binding protein 2 (PBP2) by the drug resistance protein PBP2A in *Staphylococcus aureus*, J. Bacteriol. 183 (2001) 6525–6531.

[5] E. Ghaznavi-Rad, M. Nor Shamsudin, Z. Sekawi, A. van Belkum, V. Neela, A simplified multiplex PCR assay for fast and easy discrimination of globally distributed staphylococcal cassette chromosome mec types in meticillin-resistant *Staphylococcus aureus*, J. Med. Microbiol. 59 (2010) 1135–1139.

[6] Z. Zong, C. Peng, X. Lü, Diversity of SCCmec elements in methicillin-resistant coagulase-negative staphylococci clinical isolates, PLoS One 6 (2011) e20191.

[7] A. Japoni, M. Jamalidoust, S. Farshad, M. Ziyaeyan, A. Alborzi, S. Japoni, et al., Characterization of SCCmec types and antibacterial susceptibility patterns of methicillin-resistant *Staphylococcus aureus* in Southern Iran, Jpn. J. Infect. Dis. 64 (2011) 28–33.

[8] T. Ito, K. Okuma, X.X. Ma, H. Yuzawa, K. Hiramatsu, Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC, Drug Resist. Updat. 6 (2003) 41–52.

[9] E. Ortega, H. Abriouel, R. Lucas, A. Gálvez, Multiple roles of *Staphylococcus aureus* enterotoxins: pathogenicity, superantigenic activity, and correlation to antibiotic resistance, Toxins Basel 2 (2010) 2117–2131.

[10] S. Holtfreter, B.M. Bröker, Staphylococcal superantigens: do they play a role in sepsis? Arch. Immunol. Ther. Exp. Warsz 53 (2005) 13–27.

[11] D.L. Hu, E.K. Maina, K. Omoe, F. Inoue, M. Yasujima, A. Nakane, Superantigenic toxin genes coexist with specific staphylococcal cassette chromosome mec genes in methicillin-resistant *Staphylococcus aureus*, Tohoku J. Exp. Med. 225 (2011) 161–169.

[12] J. Fraser, V. Arcus, P. Kong, E. Baker, T. Proft, Superantigens – powerful modifiers of the immune system, Mol. Med. Today 6 (2000) 125–132.

[13] G. Durand, M. Bes, H. Meugnier, M.C. Enright, F. Forey, N. Liassine, et al., Detection of new methicillin-resistant *Staphylococcus aureus* clones containing the toxic shock syndrome toxin 1 gene responsible for hospital- and community-acquired infections in France, J. Clin. Microbiol. 44 (2006) 847–853.

[14] A. Tristan, T. Ferry, G. Durand, O. Dauwalder, M. Bes, G. Lina, et al., Virulence determinants in community and hospital meticillin-resistant *Staphylococcus aureus*, J. Hosp. Infect. 65 (Suppl. 2) (2007) 105–109.

[15] J. Parsonnet, R.V. Goering, M.A. Hansmann, M.B. Jones, K. Ohtagaki, C.C. Davis, et al., Prevalence of toxic shock syndrome toxin 1 (TSST-1)-producing strains of *Staphylococcus aureus* and antibody to TSST-1 among healthy Japanese women, J. Clin. Microbiol. 46 (2008) 2731–2738.

[16] O. Yamasaki, T. Yamaguchi, M. Sugai, C. Chapuis-Cellier, F. Arnaud, F. Vandenesch, et al., Clinical manifestations of staphylococcal scalded-skin syndrome depend on serotypes of exfoliative toxins, J. Clin. Microbiol. 43 (2005) 1890–1893.

[17] W.M. Johnson, S.D. Tyler, E.P. Ewan, F.E. Ashton, D.R. Pollard, K.R. Rozee, Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in *Staphylococcus aureus* by the polymerase chain reaction, J. Clin. Microbiol. 29 (1991) 426–430.

[18] M.A. Wikler, Clinical, Institute LS, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard, Clinical and Laboratory Standards Institute, 2009.

[19] A. Fischer, P. Francois, S. Holtfreter, B. Broeker, J. Schrenzel, Development and evaluation of a rapid strategy to determine enterotoxin gene content in *Staphylococcus aureus*, J. Microbiol. Methods 77 (2009) 184–190.

[20] J.N. Jiménez, A.M. Ocampo, J.M. Vanegas, E.A. Rodríguez, C.G. Garcés, L.A. Patiño, et al., Characterisation of virulence genes in methicillin susceptible and resistant *Staphylococcus aureus* isolates from a paediatric population in a university hospital of Medellín, Colombia, Mem. Inst. Oswaldo Cruz 106 (2011) 980–985.

[21] J. Sadeghi, S. Mansouri, Molecular characterization and antibiotic resistance of clinical isolates of methicillin-resistant *Staphylococcus aureus* obtained from Southeast of Iran (Kerman), APMIS 122 (2014) 405–411.

[22] m moghadami, a japoni, a karimi, m mardani, comparison of community and healthcare associated MRSA in Iran, Iran. J. Clin. Infect. Dis. 5 (2010) 206–212.

[23] m habibi, h Saderi, p owlia, m asadi karam, detection of methicillin resistance in *staphylococcus aureus* by disk diffusion and PCR methods, Iran. J. Pathol. 3 (2008) 11–14.

[24] B. Fatholahzadeh, M. Emaneini, G. Gilbert, E. Udo, M. Aligholi, M.H. Modarressi, et al., Staphylococcal cassette chromosome mec (SCCmec) analysis and antimicrobial susceptibility patterns of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in Tehran, Iran, Microb. Drug Resist. 14 (2008) 217–220.

[25] M. Falcone, P. Carfagna, M. Cassone, E. Pistella, G. Pavoni, I. Nofroni, et al., *Staphylococcus aureus* sepsis in hospitalized non neutropenic patients: retrospective clinical and microbiological analysis, Ann. Ital. Med. Int. 17 (2002) 166–172.

[26] J.H. Han, P.H. Edelstein, E. Lautenbach, Reduced vancomycin susceptibility and staphylococcal cassette chromosome mec (SCCmec) type distribution in methicillin-resistant *Staphylococcus aureus* bacteraemia, J. Antimicrob. Chemother. 67 (2012) 2346–2349.

[27] S.Y. Park, I.H. Oh, H.J. Lee, C.G. Ihm, J.S. Son, M.S. Lee, et al., Impact of reduced vancomycin MIC on clinical outcomes of methicillin-resistant *Staphylococcus aureus* bacteremia, Antimicrob. Agents Chemother. 57 (2013) 5536–5542.

[28] W. He, H. Chen, C. Zhao, F. Zhang, H. Li, Q. Wang, et al., Population structure and characterisation of *Staphylococcus aureus* from bacteraemia at multiple hospitals in China: association between antimicrobial resistance, toxin genes and genotypes, Int. J. Antimicrob. Agents 42 (2013) 211–219.

[29] P. Chongtrakool, T. Ito, X.X. Ma, Y. Kondo, S. Trakulsomboon, C. Tiensasitorn, et al., Staphylococcal cassette chromosome mec (SCCmec) typing of methicillin-resistant *Staphylococcus aureus* strains isolated in 11 Asian countries: a proposal for a new nomenclature for SCCmec elements, Antimicrob. Agents Chemother. 50 (2006) 1001–1012.

[30] C. Demir, Ö. Aslantaş, N. Duran1, S. Ocak3, B. Özer, Investigation of toxin genes in *Staphylococcus aureus* strains isolated in Mustafa Kemal University Hospital, Turk J. Med. Sci. 41 (2011) 343–352.

[31] M. Liu, J. Liu, Y. Guo, Z. Zhang, Characterization of virulence factors and genetic background of *Staphylococcus aureus* isolated from Peking University People's Hospital between 2005 and 2009, Curr. Microbiol. 61 (2010) 435–443.

[32] U. Nübel, P. Roumagnac, M. Feldkamp, J.H. Song, K.S. Ko, Y.C. Huang, et al., Frequent emergence and limited geographic dispersal of methicillin-resistant *Staphylococcus aureus*, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 14130–14135.
[33] A. Mellmann, T. Weniger, C. Berssenbrügge, U. Keckevoet, A.W. Friedrich, D. Harmsen, et al., Characterization of clonal relatedness among the natural population of *Staphylococcus aureus* strains by using spa sequence typing and the BURP (based upon repeat patterns) algorithm, J. Clin. Microbiol. 46 (2008) 2805–2808.
[34] J. Collins, J. Rudkin, M. Recker, C. Pozzi, J.P. O'Gara, R.C. Massey, Offsetting virulence and antibiotic resistance costs by MRSA, ISME J. 4 (2010) 577–584.
[35] M. Mehrotra, G. Wang, W.M. Johnson, Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance, J. Clin. Microbiol. 38 (2000) 1032–1035.
[36] K. Becker, A.W. Friedrich, G. Lubritz, M. Weilert, G. Peters, C. Von Eiff, Prevalence of genes encoding pyrogenic toxin superantigens and exfoliative toxins among strains of *Staphylococcus aureus* isolated from blood and nasal specimens, J. Clin. Microbiol. 41 (2003) 1434–1439.
[37] A. Tekeli, E. Koyuncu, I. Dolapçi, O.A. Akan, Z.C. Karahan, Molecular characteristics of methicillin-resistant *Staphylococcus aureus* strains isolated from blood cultures between 2002–2005 in Ankara University Hospital, Mikrobiyol. Bul. 43 (2009) 1–10.
[38] J.M. Fueyo, M.C. Martín, M.A. González-Hevia, M.C. Mendoza, Enterotoxin production and DNA fingerprinting in *Staphylococcus aureus* isolated from human and food samples. Relations between genetic types and enterotoxins, Int. J. Food Microbiol. 67 (2001) 139–145.
[39] R.Z. Koosha, A.A. Fooladi, H.M. Hosseini, E.M. Aghdam, Prevalence of exfoliative toxin A and B genes in *Staphylococcus aureus* isolated from clinical specimens, J. Infect. Public Health 7 (2014) 177–185.
[40] Y. Kondo, T. Ito, X.X. Ma, S. Watanabe, B.N. Kreiswirth, J. Etienne, et al., Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: rapid identification system for mec, ccr, and major differences in junkyard regions, Antimicrob. Agents Chemother. 51 (2007) 264–274.
[41] K. Zhang, J.A. McClure, S. Elsayed, T. Louie, J.M. Conly, Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*, J. Clin. Microbiol. 43 (2005) 5026–5033.
[42] T. Dadgar, E. Ghaemi, N. Bahador, A.A. Imani Fooladi, F. Kamareie, Detection of *Staphylococcus aureus* enterotoxin genes A-E in healthy carriers and the patients, Med. Lab. J. 7 (2014) 1–8 (in Persian).