Molecular Characterizations, Virulence Determinants and Antimicrobial Resistance Profiles of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in the North of Iran

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Research

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Abstract

Background: Emergence and prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) has become a major universal health concern, limiting therapeutic options.

Methods: In the North side of Iran, during the years 2015 to 2017, a total number of 37 MRSA isolates, including 19 clinical isolates from hospitalized patients and 18 colonizing isolates from health care workers were identified from three hospitals, in Gorgan, North of Iran. Antimicrobial susceptibility test was performed using the disk diffusion method and E-test. The presence of virulence and antibiotic resistance determinants were evaluated by PCR. The genotypic characterization was further analyzed using multi-locus sequence, *spa*, *SCCmec*, and *agr* typing.

Results: The frequency of MRSA among *S. aureus* isolates was 38.14% (37/97). The most frequent *S. aureus* resistant isolates were found to be obstinate against penicillin (98%) and gentamicin (82.5%). Additionally, the lowest resistance rates were found against daptomycin (0%), vancomycin (2.7%), and quinupristin-dalfopristin (5.4%). All MRSA isolates were susceptible to daptomycin with MIC₅₀/MIC₉₀ of 0.25/0.5 µg/ml. One isolate belonging to the ST239-SCCmedIII/t037 clone (MIC₅₀≥16µg/ml) was resistant to vancomycin. All but one isolate that shares the ST22-SCCmec IV/t790 strain were positive for both *tsst* and *pv* genes. The most predominant MRSA isolates (27%) were associated with the ST239-SCCmec III/t037 clone; and followed by ST239-SCCmec III/t924 (16.2%).

Conclusions: In our study, circulating MRSA strains were genetically diverse with a high prevalence of the ST239-SCCmedIII/t037 clone. These findings emphasize the need for future and continuous surveillance studies on MRSA to prevent the dissemination of multidrug resistance and existing MRSA clones in an effective manner.

Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) is known to be resistant to various antibiotics and produces many virulence factors, which contribute to high treatment failure [1]. Hence, MRSA is one of the main causes of hospital and community-acquired infections worldwide (HA-MRSA, CA-MRSA) [1–3]. MRSA causes infections ranging from skin and soft tissue to deep-seated and severe life-threatening ones (endocarditis, osteomyelitis, necrotizing pneumonia, meningitis, and toxic shock syndrome) [4–7]. The genetic mobile element of staphylococcal cassette chromosome, *mec* (SCCmec), is a biomarker that is responsible for resistance of *S. aureus* to methicillin and other beta-lactam antibiotics [8, 9]. Additionally, cell surface adhesive components and exotoxins are significant virulence factors of MRSA [9, 10]. MRSA is frequently spread by direct contact with an infected wound or contaminated hands [11]. Previous studies suggested that health care workers' (HCWs) nasal colonization of HA-MRSA strains may also play a significant role in the pathogenesis and epidemiology of infection in both hospital and community settings [11, 12]. There is scarce data regarding MRSA genotypes in Iran.

It is ascertained that the combination of molecular and epidemiological methods in surveillance investigations could be promising for controlling the emergence, colonization, and dissemination of predominant genetic lineages and provide epidemiological data for tracing the source of infection for clinical and treatment purposes

[2, 13]. The geographical differences in the genotypic characteristic of MRSA have been reported [2]. In Asia, there is significant divergence among countries and regions with respect to prevalence of MRSA; in fact, ST22-SCC*mec* IV/t790 and ST239-SCC*mec* III/t037 clones are predominant among patients in Iran [2, 14], and so is ST239-spa t037 and ST5-spa t002 in China [15]. On the other hand, in many regions in Asia [16, 17], sequence type 239 (ST239) is most prevalent, where, in UK, ST36 and ST30 are the most common types [18].

With this background, we are evaluating the molecular characteristics, antibiotic resistance patterns, and virulence genes profiles of MRSA isolates obtained from two kinds of study populations, namely hospitalized patients and health care workers (HCWs) in Gorgan, North of Iran.

Methods

Study Design and Sample Collection of *S. aureus* Isolates

This cross-sectional study was conducted from January 2, 2016 to October 28, 2018 in three hospitals (total of 920-beds) in Gorgan, North of Iran. Written informed consent was obtained from all the patients or HCWs and the study protocol was approved by the Ethics Committee in Golestan University of Medical Sciences (No. 31078693122419), and was conducted in accordance with the Declaration of Helsinki. The demographic profiles of patients and HCWs were recorded. We identified *S. aureus* and MRSA in hospitalized patients and HCWs as well (Table 1). Only the first sample of each patient was included in the study. In case of HCWs, samples were collected from both anterior nares. 302 unduplicated clinical samples (blood, urine, wound, sputum, and others) were obtained from in-patients, out of which *S. aureus* and MRSA were identified in 53 (17.5%), and 19 specimens (6.29%), respectively. Likewise, 351 unduplicated non-clinical samplings from the anterior nares of HCWs were carried out. All the samples were sent for bacterial culturing and identification, using Gram staining, and standard biochemical tests, such as catalase, tube coagulase, DNase test, and mannitol fermentation [19]. The identification process of all *S. aureus* isolates was confirmed by using genotypic methods for the presence of *nucA*, and *femA* genes [2, 20]. Data on department and period of hospitalization, clinical symptoms, antibiotic usages, and underlying conditions were recorded.

Table 1
Demographic characteristics of patients and healthcare workers (HCWs) at the three clinical center of Gorgan, Iran.

Group		Patients		Healthcare workers	
		Total number (N)	<i>S. aureus</i> n (%)	Total number (N)	<i>S. aureus</i> n (%)
Prevalence		302	53 (17.54)	351	44 (12.53)
Characteristics					
Age	< 25 years	51	10 (19.6)	46	5 (10.86)
	25–35 years	59	15 (25.42)	99	11 (11.11)
	36–45 years	70	12 (17.14)	105	14 (13.33)
	46–55 years	63	7 (11.11)	71	9 (12.67)
	≥ 56 years	59	9 (15.25)	30	5 (16.66)
Sex	Female	139	23 (16.54)	202	27 (13.36)
	Male	163	30 (18.4)	149	17 (11.4)
Duration of Hospitalization	≤ 7 days	216	32 (14.8)	-	-
	≥ 7 days	86	21 (24.4)	-	-

Antimicrobial Susceptibility Test

Antibiotic susceptibility test (AST) was performed using the disc diffusion method based on CLSI guidelines for antibiotics [21]; nitrofurantoin (300 µg), gentamicin (10 µg), rifampin (5 µg), chloramphenicol (30 µg), erythromycin (15 µg), clindamycin (2 µg), tetracycline (30 µg), ciprofloxacin (5 µg), penicillin (10units), linezolid (30 µg) ,and quinupristin/dalfopristin(15 µg) (BBL.BD, USA). According to CLSI 2018 [21], the linezolid resistant isolates tested by disk diffusion method were also rechecked using E-test technique.

The MRSA isolates screening was performed using phenotypic (cefoxitin (30 µg)) discs on Mueller-Hinton agar plates and molecular method (*mecA* gene) [20]. Moreover, the minimum inhibitory concentration (MIC) of mentioned antibiotics and also vancomycin, and daptomycin (bioMérieux, France) in MRSA and MSSA isolates were determined by E-test. *S. aureus* ATCC 25923 was used as the control for AST. The clinical MRSA isolates were stored in Tryptic soy broth (TSB; Merck; Germany) containing 20% glycerol at -70 °C for further molecular analysis.

Extraction Of Genomic Dna

Genomic DNA was extracted from fresh overnight cultures of MRSA isolates using the QIAamp DNA mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. DNA purity, quality, and quantity were measured by absorbance spectrophotometry (Nanodrop-1000; NanoDrop Technologies, Wilmington, DE, USA) and agarose gel electrophoresis. Whole extracted DNA from MRSA isolates was immediately stored at -20 °C for further analysis.

Molecular Detection Of Resistance, Adhesion, And Toxin Encoding Genes

All MRSA isolates were screened for drug resistance genes; *ermA*, *ermB*, *ermC*, *lnuA*, *fusB*, *fusC*, *mphA*, *mphC*, *mupA*, *cat*, *aacA-aphD*, *aadC*, *aphA₃*, *tetK*, *tetM*, *tetL*, *vanA*, *vanB*, and *cfr*. [15, 21–24] Detection of 17 virulence genes including *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *sep*, *eta*, *etb*, *hla*, *hld*, *hlg*, *tsst*, *pvl*, *clfA*, and *clfB* in 97 *S. aureus* isolates was performed as previously described [3, 25, 26].

Molecular Characterizations

By *SCCmec* typing analysis, four types (type I, II, III, and IV) were observed among 37 MRSA isolates. The most predominant type was *SCCmec* III, while 23 isolates (62.16%) expressed it, followed by type IV (21.6%, 8/37), type I (8.1%, 3/37), and type II (5.4%, 2/37) respectively. Only one isolate was not typeable. All isolates carrying *pvl* gene were found to be associated with *SCCmec* type IV. Distribution of antibiotic resistance profile, virulence genes profile and *SCCmec* types among 37 MRSA strains isolated from in-patients and HCWs samples have been shown in Table 6.

Results of the *agr* typing method revealed that 64.86%, 24.3%, and 8.1% of 37 MRSA isolates belonged to *agr* type I, *agr* type III and *agr* type II, respectively. Also, one MRSA isolate was non-typeable. The majority (65.2%) of *SCCmec* III isolates harbored *agr* group I. Correspondingly, *agr* group III was present among *SCCmec* III (77.8%), and IV (22.2%) isolates and all of *agr*II group isolates were found in *SCCmec* IV.

The *spa* typing categorized MRSA isolates into 8 *spa* types. Among them, *spa* t37 was the most prevalent type (27%, 10/37), followed by t924 (18.9%, 7/37), t790 (13.5%, 5/37), t383 (13.5%, 5/37), and t030 (10.8%, 4/37). Each of the remaining *spa* types was characterized in ≤ 3 isolates.

According to the MLST method among the 37 MRSA isolates, five different profiles (ST239 in 20 strains, ST22 in 6 strains, ST45 in 5 strains, ST15 in 3 strains, and ST585 in 3 strains) were identified. All 8 isolates harbored *pvl* gene belonged to ST22-*SCCmec*IV/t790 (62.5%), and ST15-*SCCmec*IV/t084 clones (37.5%). Of the total 11 isolates harboring *tsst* encoding gene, 7 (63.63%) belonged to ST239-*SCCmec* III/t037 clone and 4 (36.37%) were member of ST22-*SCCmec* IV/t790 clone. Table 6 lists the characteristics of the 37 MRSA molecular types, studied. ST239-*SCCmec*III/t037, ST22-*SCCmec*III/t016, ST22-*SCCmec*IV/t790, and ST45-*SCCmec*IV/t383 clones were most diverse ones in the three clinical centers in Gorgan. Moreover, Fig. 1 depicts the distribution of MRSA types at three clinical centers in Gorgan, Iran.

Results

Prevalence of Clinical and Non-Clinical *S. aureus* Isolates

In a total number of 97 *S. aureus* isolates, 44/351 (12.53%) and 53/302 (17.54%) cases were from HCWs and hospitalized patients, respectively (Table 1). In samples obtained from HCWs, 10.6% (14/132) and 9.91% (12/121) of all *S. aureus* isolates belonged to the nurses and technicians, respectively (Table 2). The clinical *S. aureus* isolates were collected from different sources, including urine 14 (13.3%); wound 18 (21.95%); blood 9 (17.3%); Sputum 5 (18.51%); Pus 3 (15.8%), and other body fluids 4 (21%) (Table 3). Thirty-seven MRSA and sixty methicillin susceptible *S. aureus* (MSSA) strains were recovered from clinical and nasal carriage of HCWs samples, respectively (Tables 2 and 3). The proportion of HCWs with MRSA nasal carriage was 48.6% (18/37). Most of the MRSA isolates were obtained from wounds [18.9% (7 isolates)], and urine [16.2% (6 isolates)] (Table 3). *S. aureus* along with MRSA was identified in 44 (12.53%) and 18 specimens (5.12%), respectively.

Table 2
Distribution of *S. aureus*, MRSA and MSSA carriage among the different health professions

HCW	No. of samples	<i>S. aureus</i> n (%)	MRSA n (%)	MSSA n (%)
Doctors	34	7 (20.5)	2 (5.88)	5 (14.7)
Nurses	132	14 (10.6)	5 (3.78)	9 (6.81)
Technicians	121	12 (9.91)	6 (4.95)	6 (4.95)
midwives	12	3 (25)	1 (8.33)	2 (16.66)
Radiologist	4	0 (0)	0 (0)	0 (0)
Pharmacist	6	1 (16.66)	0 (0)	1 (16.66)
Others	42	7 (16.66)	4 (9.52)	3 (7.14)
Total	351	44 (12.53)	18 (5.12)	26 (7.4)

Table 3
Distribution of *S. aureus*, MRSA and MSSA carriage in clinical samples

Site of Sample Collection/N.	Ward/N.	<i>S. aureus</i> n (%)	MRSA n (%)	MSSA n (%)
Blood/52	ICU/3	9 (17.3)	3 (5.76)	6 (11.53)
	CCU/5			
	Surgery/10			
	Emergency/8			
	Burn/13			
	Internal/9			
	ENT/4			
Urine/105	ICU/0	14 (13.33)	6 (5.71)	8 (7.61)
	CCU/35			
	Surgery/25			
	Emergency/21			
	Burn/14			
	Internal/10			
	ENT/0			
Wound/82	ICU/8	18 (21.95)	7 (8.53)	11 (13.41)
	CCU/9			
	Surgery/17			
	Emergency/17			
	Burn/8			
	Internal/10			
	ENT/13			
Sputum/27	ICU/3	5 (18.51)	0 (0)	5 (18.51)
	CCU/0			
	Surgery/3			
	Emergency/5			
	Burn/2			
	Internal/6			
	ENT/8			

Site of Sample Collection/N.	Ward/N.	<i>S. aureus</i> n (%)	MRSA n (%)	MSSA n (%)
Body Fluids/19	ICU/0 CCU/0 Surgery/4 Emergency/5 Burn/10 Internal/0 ENT/0	4 (21.05)	2 (10.52)	2 (10.52)
Pus/17	ICU/2 CCU/0 Surgery/5 Emergency/2 Burn/7 Internal/0 ENT/1	3 (15.79)	1 (5.26)	2 (10.52)
Total/302	ICU/16 CCU/49 Surgery/64 Emergency/58 Burn/56 Internal/35 ENT/26	53 (17.54)	19 (6.29)	34 (11.25)

Antimicrobial Susceptibility

S. aureus isolates were found to be predominantly resistant to penicillin (98%, 95/97) and gentamicin (82.5%, 80/97). Similarly, 5.4% (2/37) and 18.9% (7/37) of MRSA isolates were resistant to quinupristin-dalfopristin and linezolid (Table 4). The MIC ranges, MIC₅₀ and MIC₉₀ values for 14 antibiotics in MRSA and MSSA isolates are shown in Table 5. E-test strips results revealed that in entire MRSA isolates only one (2.7%) was resistant to vancomycin (≥ 16 µg/ml) and 6 (16.08%) were obstinate against linezolid (≥ 8 µg/ml). All the MRSA isolates were susceptible to daptomycin with MIC₅₀ of 0.25 µg/ml and MIC₉₀ of 0.5 µg/ml. 5.4% (2/6) of the linezolid resistant MRSA isolates showed high resistance with MIC of 64 µg/ml to the respective antibiotic.

Table 4
Antibiotic resistance of *S. aureus*, MRSA and MSSA isolates among nasal carriage and clinical samples by disk diffusion method

Antimicrobial Agent	<i>S. aureus</i> (n = 97)	MRSA (n = 37)	MSSA (n = 60)
	R, n (%)	R, n (%)	R, n (%)
Penicillin (P)	95(98)	37 (100)	58(96.5)
Cefoxitin (CF)	37 (38.1)	37 (100)	0 (0)
Gentamicin (GM)	80(82.5)	32 (86.5)	48(80)
Erythromycin (E)	46 (47.4)	34 (91.9)	12 (20)
Tetracycline (TE)	35 (36.1)	25 (67.5)	10 (16.6)
Ciprofloxacin (CIP)	33 (34)	23 (62.1)	10 (16.6)
Nitrofurantoin (FD)	39 (40.2)	30 (81.1)	9 (15)
Clindamycin (CC)	35 (36.1)	27 (73)	8 (13.3)
Chloramphenicol (C)	40 (41.2)	30 (81.1)	10 (16.6)
Rifampin (RIF)	26 (26.8)	21 (56.7)	5 (8.3)
Quinupristin-Dalfopristin (SYN)	2 (2.1)	2 (5.4)	0 (0.0)
Linezolid (LNZ)	8 (8.2)	7 (18.9)	1 (1.6)

Table 5
Minimal Inhibitory Concentrations (MICs) of MRSA and MSSA isolates

Antimicrobial Agent	MIC Range (µg/mL)	Breakpoints (CLSI) (µg/mL) (S / I / R)	MRSA (n:37)		MSSA (n:60)					
			% S	% R	MIC 50	MIC 90	% S	% R	MIC 50	MIC 90
Penicillin (P)	0.125->256	≤ 0.12 / - / ≥0.25	0	100	64	128	3.5	96.5	16	256
Cefoxitin (CF)	0.25->128	≤ 4 / - / ≥8	0	100	64	128	100	0	0.256	1
Vancomycin (VA)	< 0.063-16	≤ 2 / 4-8 / ≥16	83.8	2.7	1	8	100	0	1	1
Daptomycin (DAP)	0.063-1	≤ 1 / - / -	100	0	0.25	0.5	100	0	0.25	0.5
Gentamicin (GM)	0.5->512	≤ 4 / 8 / ≥16	10.9	89.1	64	512	20	80	32	256
Erythromycin (E)	0.25->128	≤ 0.5 / 1-4 / ≥8	2.1	96.5	128	128	81.6	18.3	1	64
Tetracycline (TE)	0.5-256	≤ 4 / 8 / ≥16	25.87	68.53	32	128	81.6	18.3	8	32
Ciprofloxacin (CIP)	0.25->128	≤ 1 / 2 / ≥4	41.26	52.45	4	32	81.6	18.3	2	32
Nitrofurantoin (FD)	2->512	≤ 32 / 64 / ≥128	21.62	78.37	128	512	86.7	13.3	16	64
Clindamycin (CC)	0.25->128	≤ 0.5 / 1-2 / ≥4	32.43	67.56	4	32	83.3	16.6	0.5	16
Chloramphenicol (C)	8->128	≤ 8 / 16 / ≥32	24.3	75.6	128	128	80	20	8	32
Rifampin (RIF)	< 0.063->128	≤ 1 / 2 / ≥4	35.66	51.05	4	64	80	10	2	16
Quinupristin-Dalfopristin (SYN)	0.25->128	≤ 1 / 2 / ≥4	94.6	5.4	0.5	3	100	0	0.25	1
Linezolid (LNZ)	< 0.063-64	≤ 4 / - / ≥8	83.92	16.08	1	8	100	0	0.5	4

All of the MRSA isolates in the present study were multidrug resistant (MDR). The main MDR profile among the MRSA isolates included a resistance profile to ten antibiotics (40.5%; 15/37), six antibiotics (13.5%; 5/37), nine antibiotics (13.5%; 5/37), eight antibiotics (10.8%; 4/37), seven antibiotics (8.1%; 3/37), five antibiotics (8.1%; 3/37), four antibiotics (5.4%; 2/37), and eleven antibiotics (2.7%; 1/37).

Virulence Gene Profiles

The distribution of 17 putative adhesion and toxin encoding genes diverged in 37 MRSA strains according to MLST, *SCCmec*, *agr*, and *spa* (Table 6). Nine (24.32%, 9/37) MRSA isolates harbored ≥ 10 tested virulence genes, among which were 1 isolate with 12 genes, 4 isolates with 11 genes, and 4 isolates with 10 genes. All adhesion and toxin encoding genes except *etb* gene were identified within several strains and all MRSA isolates exhibited carriage of at least 5 virulence genes. Only one MRSA isolate harbored *etb* gene. Among 37 MRSA strains, the most common toxin encoding genes detected were *hla* (37; 100%), *hlg* (23; 62.16%), *seb* (21; 56.7%), *hld* (20; 54.05%), *sec/sep* (51.3%), and *pvl* gene (21.6%), respectively. All of the *pvl* positive strains expressed resistance profile to at least 5 antibiotics. The majority (5; 62.5%) of *pvl* positive strains belonged to only hospital number 1 and age group of 36 to 56 years. Tested adhesion *clfA* and *clfB* genes were detected in 36 (97.3%), and 19 (51.35%) MRSA strains, respectively.

Table 6

Characteristics of the 37 MRSA strains in hospitalized patients and HCWs samples at the three clinical center in Gorgan, Iran.

MRSA clones	agr type	Adhesion and Toxin profile (No; %)	Antibiotic resistance profile (No; %)	Resistant genes profile (No; %)	Site of sample collection	Strains (No; %)
ST239-SCCmec III/ t037	I	<i>seb</i> (5;50), <i>sec</i> (3;30), <i>sed</i> (5;50), <i>see</i> (1;10), <i>seg</i> (6;60), <i>sep</i> (7;70), <i>hla</i> (10;10), <i>hld</i> (5;50), <i>hlg</i> (5;50), <i>tsst</i> (7;70), <i>clfa</i> (10;10), <i>clfb</i> (6;60)	P, CF, GM, E, TE, CIP, CC, C, RIF, LNZ (2;20) P, CF, GM, E, TE, FD, CC, C, RIF (2;20) P, CF, VA, GM, E, FD, CC, C, RIF (1;10) P, CF, GM, E, CIP, FD, CC, C, RIF, LNZ (1;10) P, CF, E, FD (1;10) P, CF, GM, E, TE, CIP, FD, C (1;10) P, CF, CIP, FD, CC (1;10) P, CF, GM, E, TE, CIP, FD, C, RIF (1;10)	<i>aacA-aphD</i> (7;70), <i>aadC</i> (1;10), <i>aphA</i> ₃ (1;10), <i>ermA</i> (5;50), <i>ermC</i> (4;40), <i>lnuA</i> (4;40), <i>tetM</i> (4;40), <i>tetK</i> (2;20), <i>cat</i> (8;80), <i>cfr</i> (2;33.3), <i>vanA</i> , <i>vanB</i> (1;10)	Nasal Swab, Wound, Blood, and Urine	10;27
ST239-SCCmec III/ t924	III	<i>sea</i> (1;16.6), <i>seb</i> (4;66.6), <i>sec</i> (4;66.6), <i>sed</i> (1;16.6), <i>see</i> (1;16.6), <i>seg</i> (1;16.6), <i>sep</i> (2;33.3), <i>eta</i> (1;16.6), <i>hla</i> (6;100), <i>hld</i> (4;66.6), <i>hlg</i> (5;83.3), <i>clfa</i> (6;100), <i>clfb</i> (3;50)	P, CF, GM, E, TE, CIP, FD, CC, C, LNZ (1;16.6) P, CF, GM, E, FD, C (1;16.6) P, CF, GM, CIP, FD, CC (1;16.6) P, CF, GM, E, TE, CIP, FD, CC, C, RIF (1;16.6)	<i>aacA-aphD</i> (4;66.6), <i>aadC</i> (1;16.6), <i>aphA</i> ₃ (1;16.6), <i>ermA</i> (3;50), <i>ermB</i> (1;16.6), <i>ermC</i> (1;16.6), <i>lnuA</i> (2;33.3), <i>tetK</i> (2;33.3), <i>fusc</i> (1;16.6), <i>cat</i> (5;83.3), <i>cfr</i> (2;33.3)	Nasal Swab, Urine, Body Fluids, and Pus	6;16.2

			P, CF, GM, E, TE, CIP, CC, C, RIF, LNZ (1;16.6)			
			P, CF, GM, E, FD, CC, C, RIF (1;16.6)			
ST22- SCCmec IV/t790	I	<i>sea</i> (2;66.6), <i>seb</i> (3;100), <i>sec</i> (3;100), <i>sep</i> (3;100), <i>hla</i> (3;100), <i>hld</i> (2;66.6), <i>hlg</i> (2;66.6), <i>tsst</i> (3;100), <i>pvl</i> (3;100), <i>clfa</i> (3;100), <i>clfb</i> (1;33.3)	P, CF, GM, E, TE, FD, C (1;33.3) P, CF, GM, E, TE, CIP, CC, C, RIF (1;33.3) P, CF, GM, E, TE, CIP, FD, CC, C, RIF, LNZ (1;33.3)	<i>aacA-aphD</i> (1;33.3), <i>ermA</i> (1;33.3), <i>ermC</i> (2;66.6), <i>tetK</i> (2;66.6), <i>tetM</i> (1;33.3), <i>cat</i> (3;100), <i>lnuA</i> (2;66.6), <i>aphA</i> ₃ (1;33.3), <i>fusc</i> (1;33.3), <i>cfr</i> (1;16.6)	Nasal Swab	3;8.1
ST239- SCCmec III/t030	I	<i>seb</i> (3;100), <i>eta</i> (3;100), <i>hla</i> (3;100), <i>hld</i> (2;66.6), <i>hlg</i> (3;100), <i>clfa</i> (3;100), <i>clfb</i> (1;33.3)	P, CF, GM, E, TE, CIP, FD, CC, C (1;33.3) P, CF, GM, E, TE, FD, C, RIF (1;33.3) P, CF, E, TE, CIP, FD, CC (1;33.3)	<i>aacA-aphD</i> (1;33.3), <i>ermC</i> (3;100), <i>lnuA</i> (1;33.3), <i>tetM</i> (2;66.6), <i>cat</i> (1;33.3)	Nasal Swab	3;8.1
ST15- SCCmec IV/ t084	II	<i>seb</i> (2;66.6), <i>sec</i> (1;33.3), <i>see</i> (1;33.3), <i>sed</i> (1;33.3), <i>seg</i> (2;66.6), <i>sep</i> (2;66.6), <i>hla</i> (3;100), <i>hld</i> (1;33.3), <i>hlg</i> (1;33.3), <i>pvl</i> (3;100), <i>clfa</i> (3;100), <i>clfb</i> (1;33.3)	P, CF, GM, E, TE, CIP, FD, CC, C, RIF (2;66.6) P, CF, GM, E, TE, FD, C (1;33.3)	<i>aacA-aphD</i> (3;100), <i>ermA</i> (2;66.6), <i>ermC</i> (1;33.3), <i>lnuA</i> (2;66.6), <i>tetK</i> (1;33.3), <i>tetM</i> (2;66.6), <i>cat</i> (3;100)	Nasal Swab, Wound, and Blood	3;8.1
ST45- SCCmec I/ t383	I	<i>sea</i> (1;33.3), <i>seb</i> (2;66.6), <i>sec</i> (2;66.6), <i>sep</i> (2;66.6), <i>eta</i> (1;33.3), <i>hla</i> (3;100), <i>hld</i> (1;33.3), <i>hlg</i> (2;66.6), <i>clfa</i> (3;100), <i>clfb</i> (1;33.3)	P, CF, GM, E, TE, CIP, FD, CC, C, RIF (2;66.6) P, CF, GM, E, TE, FD (1;33.3)	<i>aacA-aphD</i> (3;100), <i>ermC</i> (3;100), <i>tetK</i> (1;33.3), <i>lnuA</i> (2;66.6), <i>tetK</i> (1;33.3), <i>tetM</i> (1;33.3), <i>cat</i> (2;66.6)	Nasal Swab, and Blood	3;8.1

ST45- SCCmec II/ t383	I	<i>seb</i> (2;100), <i>sec</i> (1;50), <i>sep</i> (2;100), <i>hla</i> (2;100), <i>hld</i> (2;100), <i>hlg</i> (2;100), <i>clfa</i> (2;100), <i>clfb</i> (1;50)	P, CF, GM, E, TE, CIP, FD, CC, C, RIF (1;100) P, CF, E, FD, CC, C (1;100)	<i>aacA-aphD</i> (1;50), <i>ermA</i> (1;50), <i>ermC</i> (1;50), <i>lnuA</i> (2;100), <i>tetK</i> (1;50), <i>cat</i> (2;100)	Wound, and Body Fluids	2;5.4
ST22- SCCmec IV/t790	III	<i>sea</i> (1;50), <i>seb</i> (1;50), <i>sec</i> (2;100), <i>seg</i> (1;50), <i>sep</i> (2;100), <i>eta</i> (1;50), <i>hla</i> (2;100), <i>tsst</i> (1;50), <i>pvl</i> (2;100), <i>clfa</i> (2;100)	P, CF, GM, E, TE, CIP, CC, C, RIF (1;50) P, CF, GM, E, FD, C (1;50)	<i>aacA-aphD</i> (1;50), <i>aadC</i> (1;50), <i>ermA</i> (1;50), <i>ermC</i> (1;50), <i>tetM</i> (1;50), <i>cat</i> (2;100)	Wound, and Urine	2;5.4
ST22- SCCmec III/t016	I	<i>seb</i> (1;100), <i>sec</i> (1;100), <i>sed</i> (1;100), <i>sep</i> (1;100), <i>hla</i> (1;100), <i>hld</i> (1;100), <i>hlg</i> (1;100), <i>clfa</i> (1;100), <i>clfb</i> (1;100)	P, CF, GM, E, TE, CIP, CC, C, RIF (1;100)	<i>aacA-aphD</i> (1;100), <i>aadC</i> (1;100), <i>ermA</i> (1;100), <i>lnuA</i> (1;100), <i>tetM</i> (1;100), <i>cat</i> (1;100)	Nasal Swab	1;2.7
ST239- SCCmec III/t030	NT	<i>seb</i> (1;100), <i>sed</i> (1;100), <i>seg</i> (1;100), <i>sep</i> (1;100), <i>eta</i> (1;100), <i>etb</i> (1;100), <i>hla</i> (1;100), <i>hld</i> (1;100), <i>hlg</i> (1;100), <i>clfa</i> (1;100), <i>clfb</i> (1;100)	P, CF, GM, E, TE, CIP, CC, C, RIF, LNZ (1;100)	<i>aacA-aphD</i> (1;100), <i>ermA</i> (1;100), <i>lnuA</i> (1;100), <i>tetK</i> (1;100), <i>cat</i> (1;100), <i>cfr</i> (1;16.6)	Nasal Swab	1;2.7
ST585- SCCmec NT/ t713	I	<i>seb</i> (1;100), <i>sec</i> (1;100), <i>sep</i> (1;100), <i>hla</i> (1;100), <i>clfa</i> (1;100)	P, CF, GM, E, FD, C (1;100)	<i>aacA-aphD</i> (1;100), <i>ermC</i> (1;100), <i>cat</i> (1;100)	Nasal Swab	1;2.7
ST585- SCCmec III/ t924	III	<i>see</i> (1;100), <i>hla</i> (1;100), <i>clfa</i> (1;100), <i>clfb</i> (1;100)	P, CF, GM, E, FD (1;100)	<i>aacA-aphD</i> (1;100), <i>ermC</i> (1;100)	Nasal Swab	1;2.7
ST585- SCCmec III/ t713	I	<i>sec</i> (1;100), <i>hla</i> (1;100), <i>clfa</i> (1;100), <i>clfb</i> (1;100)	P, CF, CIP, FD (1;100)	-	Urine	1;2.7

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; agr, accessory gene regulator; HCW, Health Care Worker; P, Penicillin; CF, Cefoxitin; VA, Vancomycin; GM, Gentamicin; E, Erythromycin; TE, Tetracycline; CIP, Ciprofloxacin; FD, Nitrofurantoin; CC, Clindamycin; C, Chloramphenicol; RIF, Rifampin; LNZ, Linezolid.

Note: *Sea~sep*, gene encoding staphylococcal enterotoxins; *eta* and *etb*, gene encoding exfoliatin; *hla~hlg*, gene encoding α -hemolysin- γ -hemolysin; *tsst*, gene encoding toxic shock syndrome toxin 1; *pvl*, gene encoding Panton-Valentine leukocidin; *clfA~clfB*, gene adhesion factor.

Molecular Characterizations

By *SCCmec* typing analysis, four types (type I, II, III, and IV) were observed among 37 MRSA isolates. The most predominant type was *SCCmec* III, while 23 isolates (62.16%) expressed it, followed by type IV (21.6%, 8/37), type I (8.1%, 3/37), and type II (5.4%, 2/37) respectively. Only one isolate was not typeable. All isolates carrying *pvl* gene were found to be associated with *SCCmec* type IV. Distribution of antibiotic resistance profile, virulence genes profile and *SCCmec* types among 37 MRSA strains isolated from in-patients and HCWs samples have been shown in Table 6.

Results of the *agr* typing method revealed that 64.86%, 24.3%, and 8.1% of 37 MRSA isolates belonged to *agr* type I, *agr* type III and *agr* type II, respectively. Also, one MRSA isolate was non-typeable. The majority (65.2%) of *SCCmec* III isolates harbored *agr* group I. Correspondingly, *agr* group III was present among *SCCmec* III (77.8%), and IV (22.2%) isolates and all of *agr*II group isolates were found in *SCCmec* IV.

The *spa* typing categorized MRSA isolates into 8 *spa* types. Among them, *spa* t37 was the most prevalent type (27%, 10/37), followed by t924 (18.9%, 7/37), t790 (13.5%, 5/37), t383 (13.5%, 5/37), and t030 (10.8%, 4/37). Each of the remaining *spa* types was characterized in ≤ 3 isolates.

According to the MLST method among the 37 MRSA isolates, five different profiles (ST239 in 20 strains, ST22 in 6 strains, ST45 in 5 strains, ST15 in 3 strains, and ST585 in 3 strains) were identified. All 8 isolates harbored *pvl* gene belonged to ST22-*SCCmed*IV/t790 (62.5%), and ST15-*SCCmed*IV/t084 clones (37.5%). Of the total 11 isolates harboring *tsst* encoding gene, 7 (63.63%) belonged to ST239-*SCCmec* III/t037 clone and 4 (36.37 %) were member of ST22-*SCCmec* IV/t790 clone. Table 6 lists the characteristics of the 37 MRSA molecular types, studied. ST239-*SCCmed*II/t037, ST22-*SCCmed*III/t016, ST22-*SCCmed*V/t790, and ST45-*SCCmed*/t383 clones were most diverse ones in the three clinical centers in Gorgan. Moreover, Figure 1 depicts the distribution of MRSA types at three clinical centers in Gorgan, Iran.

Discussion

S. aureus is one of the most frequent bacterial pathogens in Iran, causing a variety of infections. Genotypic background and antibiotic susceptibility of MRSA strains vary in terms of geographical locations dynamically [2].

In this study, the genotypic characterization, virulence determinants patterns and antimicrobial resistance profile of 37 MRSA strains isolated from three hospitals in Gorgan, Northern Iran were analyzed. HA-MRSA associated with high morbidity and mortality has developed worldwide, specifically in Iran [31, 32]. A dramatic emergence and expansion of MRSA in different regions of Iran (20.4%-93.3%) leads to an increase in the costs of antibiotic therapy and reduction of treatment choices [2, 33, 34].

The frequency of MRSA strains diverges in various geographic areas. The relative prevalence (38.14%) of HA-MRSA strains in our study was comparable to the result obtained by Darban et al. (35%) [35]. However, this prevalence was lower than that reported in America, Europe, Africa, and Asia [26, 36–41]. The reasons for this discrepancy in the prevalence of MRSA may be related to the dissimilar antibiotic usage patterns, contrary infection control policies, sources of the isolates, and the characteristics of the subjects (HCWs and patients). Results from the present study showed that the frequency of MRSA was similar in hospitalized patients (51.4%) and HCWs (48.6%). In Iran, the indiscriminate consumption of beta-lactam antibiotics contributes to the spread of resistance of MRSA to these antibiotics [2].

MRSA can arise from MSSA upon specific site integration of *SCCmec* into the *orfX* locus in the chromosome of a susceptible isolate [42].

In the present study, prevalence of MSSA was 61.8% (92.6% among the various health professions and 88.7% in clinical samples), which is in line to the study in Gorgan [43], but was lower compared to Khosravi et al. [44], Heidari et al. [45], and Sepehriseresht et al. [46] surveys.

The gentamycin resistance rate in the 37 MRSA strains was found to be 86.5%, which is higher than rates (60%, 77%, 78.2%, and 40.5%) reported in other similar studies [2, 8, 38].

However, low gentamycin resistance rates in Chinese and Iranian MRSA isolates have been reported [3, 15, 47]. All of the MRSA isolates in the present study were MDR which is in relative line with previous reports from Iran [2, 14] and Taiwan [38]. Our findings also suggested that vancomycin and linezolid are potent and effective treatment options for MRSA [35, 40, 41].

In our study, based on MIC results, 8.3% (5/60) of MSSA strains were found to express intermediate resistance to vancomycin (VISA). However, of these MSSA strains, 3 (60%) had MIC = 3 mg/L and the rest (40%) had MIC = 4 mg/L. Although, Hasani et al. study [48] in the Northwest of Iran reported 19 (23.4%) of MSSA isolates were VISA. Besides, of 60 MSSA isolates, 20 (33.3%) were MDR, and our findings also proposed that vancomycin, quinupristin-dalfopristin, daptomycin, and linezolid are potent and effective against MSSA isolates.

The diverse genotypic characteristics of HA-MRSA in distinct geographic regions have been established [2]. Consistent to previously published data [49], MRSA *SCCmec* type III has been found the most prevalent isolate in our study.

Inconsistent with the previous reports, our research in Iran [50, 51] revealed that *SCCmec* type III was the major *SCCmec* type among MRSA strains in the present study. Similar to Parhizgari et al. [51] and Zetola's et al. [52] The high frequency of MDR-MRSA in our study belonged to *SCCmec* type III.

Historically, the expression level of the most virulence factors of *S. aureus* is regulated by *agr* locus [3]. In agreement with our previous reports [14, 53], the most common MRSA isolates belonged to *agr* I (64.86%), followed by *agr* type III (24.3%). According to the previous data, there is a substantial association between *agr* type and certain bacterial virulence determinants [54]. In addition to that, various *agr* types disseminate from one geographic area to another. Similar to our results, the frequency of bacterial virulence determinants including toxin and adhesive genes in MRSA isolates with *agr* type I was higher than type III [2]. The *agr* type I could have a crucial role in the control of staphylococcal virulence determinants. Nevertheless, in contrast with our findings,

Nowrouzian et al. [54] showed high frequency of toxin coding genes in MRSA isolates belonged to harboring *agr* type III.

The prevalence of *spa* types differs with geographic regions, type of samples, and the time of sampling [55]. The most predominant *spa* types were t032, t008, and t002 in Europe plus t037 and t002 in Asia. In Iran, most MRSA isolates were associated with *spa* types t701, t12311, t021, t037, and t790. Our study showed that t037 was the most prevalent *spa* type. However, t030 has been reported as the major *spa* type in Iran, other than t037 [56], suggesting that t037 has been replaced by t030 *spa* type in the hospitals in Iran. This finding has been similarly reported in China in the year 1994 to 2008 [57, 58].

Sequence type 239 (ST239) in *SCCmec* III is found to be the most predominant mobile genetic element in Iran. ST239-*SCCmec* III is characterized into three clades: South American, European, and Asian [2]. The ST239-*SCCmec*III/t037 clone, which is the oldest pandemic MRSA strain is a major HA-MRSA clone predominated in Iranian hospitals [59]. It seems that this clone could have been transferred from neighboring countries. This finding suggests that the frequency of ST239 clone may be closely related to MRSA infections. In our study, the MRSA strains which are PVL positive belonged to ST22 and ST15, moreover, MDR-MRSA was detected among the STs and this result is partially consistent with a study conducted in UK [32] disclosing the same matter that MDR-MRSA was found in STs, however, PVL positive MRSA strains belonged to ST772, ST5, ST8, ST22, ST59, and ST8t0 in that paper. Nonetheless, in contrast to the current results, Havaei et al. [60] did not identify any MRSA in ST22 strains. In our research, 62.5% and 37.5% of MRSA strains carrying the *pvl* gene belonged to ST22-*SCCmec* IV/t790 and ST15-MRSA IV/t084 clones, respectively. Additionally, all but one isolate that shares the ST22-*SCCmec* IV/t790 strains were positive for both *tsst* and *pvl* genes. This finding is relatively in agreement with recent study conducted in Iran [2] declaring all but one of the ST22-*SCCmec* IV/t790 strain, harbored the *tsst* and *pvl* genes. The antimicrobial resistance profiles frequently differ in ST clones of MRSA [57]. In accordance with results of a study in UK by Ellington et al. [32], in the current study, isolates with ST22-*SCCmec* IV/t790 were drastically resistant to multiple antibiotic groups.

There was a powerful correlation observed between ST and *spa* types: ST239 type was principally related to t037 (50%, 10/20), ST22 type to t790 (83.3%, 5/6), ST45 type to t383 (100%, 5/5), ST15 type to t084 (100%, 3/3) and ST585 type to t713 (66.6%, 2/3), respectively. ST239-t037 (10/37, 27%) was observed to be the most common type in current study, followed by ST239-t924 (6/37, 16.2%). There is a diversity of HA-MRSA clones worldwide, for example, the most predominant clones have developed in many different regions of Asia [2, 59, 61–63] are ST6-*SCCmec* IV, ST239-*SCCmec* III, ST239-*SCCmec* IV, ST239- *SCCmec* II, ST5- *SCCmec* II, and ST30- *SCCmec* IV. Especially, six genetic types (ST22, ST859, ST291, ST239, ST6, and ST30) are the main clones dominating in Tehran. In this study, 27.01% of the MRSA isolates belonging to the ST239-*SCCmec* III/t037 clone were detected as the main clone followed by ST239-*SCCmec* III/t924 (16.2%) and ST22-*SCCmec* IV/t790 (8.1%) and that have been reported in other previous studies in Iran [2, 14]. The most VRSA strains separated from clinical samples in USA belonged to ST5 [2].

In line with Shekarabi et al.'s study in 2017 [64], in the current study, one vancomycin resistant (MIC $\geq$ 16 μ g/ml) isolate was obtained from a 45-year-old woman that showed *SCCmec* type III, *spa* type t037, and ST239 as its genetic characteristics. In the recently published data from Iran Azimian et al. [20] and Goudarzi et al. [2] reported the ST1283-*SCCmec* III/t037 and ST22-*SCCmec* IV/t790 clones as VRSA isolates, respectively. On other hand, VRSA strains have certain discrepancies in genetic characteristics [65]. The emergence of reduced or full -

resistance to vancomycin in bacterial strains in Iran may be attributed to the selective pressure caused by unsuitable consumption of drugs as the main treatment option available for MRSA-infections [31, 38].

Conclusions

There is a simultaneous carriage of virulence determinants, multidrug-resistance genes and high genetic diversity among the MRSA strains isolated from patients and HCWs in North of Iran. Sequence typing analysis showed that ST239-SCC*mec* III/t037 and ST22-SCC*mec* IV/t790 clones have a high expression level of *tsst* and *pvl* genes with multidrug resistance genes. Hospital infection control policies and nationwide surveillance efforts are highly demanded to monitor the clonal expansion of MRSA species in the North of Iran.

Abbreviations

S. aureus

Staphylococcus aureus

MRSA

methicillin-resistant *Staphylococcus aureus*

MSSA

methicillin-susceptible *Staphylococcus aureus*

HA-MRSA

hospital-acquired methicillin-resistant *Staphylococcus aureus*

CA-MRSA

community-acquired methicillin-resistant *Staphylococcus aureus*

SCC*mec*

staphylococcal cassette chromosome *mec*

VISA

vancomycin intermediate *Staphylococcus aureus*

VRSA

vancomycin resistance *Staphylococcus aureus*

MDR

multi-drug resistant

HCW

health care worker

ICU

intensive care unit

CCU

cardiac care unit

ENT

ear, nose, and throat

MIC

minimal inhibitory concentration

CLSI

clinical and laboratory standards institute

AST

Antibiotic susceptibility test

R

resistant

I

intermediate

S

susceptible.

agr

accessory gene regulator

P

Penicillin

CF

Cefoxitin

VA

Vancomycin

GM

Gentamicin

E

Erythromycin

TE

Tetracycline

CIP

Ciprofloxacin

FD

Nitrofurantoin

CC

Clindamycin

C

Chloramphenicol

RIF

Rifampin

LNZ

Linezolid

SYN

Quinupristin-Dalfopristin

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of Golestan university of medical sciences (No. 31078693122419). The study protocol was in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. For hospital personnel, written informed consent was obtained before sampling.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no conflict of interest.

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Figures

Figure 1

Distribution of MRSA types at the three clinical centers in Gorgan, Iran. Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*.