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Epidemiology and molecular characteristics of methicillin-resistant *Staphylococcus aureus* from skin and soft tissue infections in Shiraz, Iran

Samira MEHRSHAD[1], Masoud HAGHKHAH[1,*], Shahin AGHAEI[2]
[1]Department of Pathobiology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
[2]Department of Dermatology, Iran University of Medical Sciences, Tehran, Iran

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Background/aim: Panton–Valentine leukocidin (PVL)-positive methicillin-resistant *Staphylococcus aureus* (MRSA) infections are increasing in some regions of Iran. The aim of the current study was to assess the epidemiology and molecular characteristics of *S. aureus* isolated from patients with skin infections in Shiraz, Iran.

Materials and methods: Swab samples were obtained from patients admitted to the skin and burn units of hospitals. The medical records of each patient were collected via questionnaire. All staphylococcal isolates were collected and examined by conventional methods for detecting *S. aureus* strains. PCR was used to detect *S. aureus* harboring the *mecA* and *pvl* genes.

Results: Out of 243 staphylococcal isolates, 55 (22.6%) *S. aureus* and 91 (37.4%) *S. epidermidis* were detected. Of the 45 patients, 21 (46.7%) were *S. aureus* carriers. The *mecA* gene was identified in 60% of *S. aureus* isolates, and the rest were sensitive to methicillin. Of the *S. aureus* isolates, 54.5% were positive for the *pvl* gene.

Conclusion: This study revealed a high prevalence of PVL-positive MRSA strains in the evaluated hospitals. Thus, early diagnosis of infections caused by this pathogen seems to be necessary by intake screening allowing for proper treatment, especially in high-risk patients and in order to prevent the spread of infection.

Key words: Methicillin-resistant *S. aureus*, methicillin-sensitive *S. aureus*, Panton–Valentine leukocidin, skin infection, Shiraz, Iran

1. Introduction

Among staphylococcal species, *Staphylococcus aureus* has been shown as a clinically relevant human pathogen, and in many individuals it may lead to asymptomatic colonization (1). Various infections from skin and soft tissue infections (SSTIs) to life-threatening infections like endovascular infections, pneumonia, septic arthritis, endocarditis, osteomyelitis, and sepsis are caused by this bacterium (2). Carriage of *S. aureus* is a risk factor for subsequent infection in various settings. Recent investigations suggest that nares-only screening may underestimate the prevalence of *S. aureus* colonization, and accurate determination requires sampling from other body sites (3). The spread of antibiotic resistance among strains of *S. aureus* has been dramatically elevated, such that methicillin-resistant *S. aureus* (MRSA) is recognized as a nosocomial pathogen worldwide. MRSA strains were once exclusively limited to hospital care; however, after outbreaks of infection in hospitals and health care facilities they have increasingly been detected among patients in the community who lack conventional risk factors for MRSA infection (4). MRSA represents a significant cause of morbidity and mortality in both hospital and community settings, which raises serious concerns in the treatment of staphylococcal infections; hence, the accurate and early determination of methicillin resistance is a necessity for the prognosis of *S. aureus* infections (5). Most MRSA and some methicillin-susceptible *S. aureus* (MSSA) isolates produce Panton–Valentine leukocidin (PVL), a pore-forming toxin consisting of two subunits S (LukE) and F (LukD) (6,7). It has become increasingly important as a virulence factor in necrotizing SSTIs such as furuncle and pneumonia (6). Both MSSA and community-associated MRSA can express PVL (8). Contrary to European countries (9), PVL-associated infections are increasing in some regions of Iran (10). This study aimed to assess the microbiological, epidemiological, and clinical features of purulent skin infections caused by *S. aureus* in Shiraz, southern Iran.

* Correspondence: mhaghkha@shirazu.ac.ir

2. Materials and methods

2.1. Sampling and isolation

This was a prospective case-control study involving a convenience sample of inpatients and outpatients presenting with skin infections. The patients were hospitalized in the skin and burn units of academic hospitals or were outpatients referred to the dermatology department of the Shahid-Faghihi teaching clinic in Shiraz, Iran. Patients presenting with active infections at the time of consultation who met the inclusion criteria for infections were included in the study. Infections included in this study were diagnosed as surgical site infections, burn wound infections, pemphigus, ecthyma, psoriasis, and urticaria with the signs of boils, cellulitis, abscess, necrosis, and other infections. Swab samples were obtained from purulent skin lesions requiring incision and drainage or with spontaneously draining purulent fluid from 45 patients admitted from September 2013 to January 2014. In order to detect *S. aureus* carriers, swabs were also taken from four other regions (nose, axilla, umbilicus, and groin). The medical records of all patients were reviewed and information including age, sex, referral reason, underlying disease persistence, anatomical site of infection, clinical symptoms, and previous institutional admission with household history of similar symptoms were collected as well in a questionnaire.

All samples were transferred to the microbiology laboratory in brain heart broth and streaked on blood agar and MacConkey agar plates. Colonies suspected as staphylococci were Gram-stained and subcultured on mannitol salt agar. Collectively, 262 staphylococci isolates were obtained and stored for further experiments. Afterwards, *S. aureus* and *S. epidermidis* were diagnosed by biochemical tests including catalase, oxidase, slide and tube coagulase, mannitol fermentation, DNase, urease, and VP tests (11).

2.2. DNA extraction

All *S. aureus* isolates were cultured on blood agar and incubated overnight at 37 °C. DNA was isolated from the reference strains with a DNA extraction kit (CinnaGen, Iran) according to the manufacturer's instructions. Obtained DNA was used as the template in all polymerase chain reactions (PCRs), which are described as follows.

2.3. Polymerase chain reactions

S. aureus isolates were genetically confirmed by species-specific PCR (12) and all *S. aureus* isolates were screened for the presence of *mecA* and *pvl* genes by PCR, as previously described (6,13). PCR targets, primers used, and sizes of PCR products are summarized in Table 1.

For species confirmation, DNA amplification was carried out on a thermocycler (miniMJ model, Bio-Rad, USA) in a reaction volume of 25 µL, containing 2.5 µL of 10X PCR buffer (500 mM KCl and Tris HCl, pH 8.4) (CinnaGen), 1.25 µL of 50 mM $MgCl_2$ (CinnaGen), 0.2 mM of each deoxynucleotide triphosphate (dNTP) (CinnaGen), 0.25 U of Taq DNA polymerase (CinnaGen), 25 pmol of each primer (CinnaGen), and 5 µL of template DNA (30 ng/µL). After initial denaturation at 94 °C for 3 min, PCR products were amplified by 35 cycles of denaturation at 94 °C for 45 s, annealing at 58.5 °C for 45 s, and extension at 72 °C for 2 min, with a final extension step at 72 °C for 10 min.

For amplification of the *mecA* and *pvl* genes, PCR cycles were run under the following conditions: initial DNA denaturation at 95 °C for 7 min, which was followed by 30 cycles of DNA denaturation at 95 °C for 1 min, primer annealing at 55 °C for 1 min, and DNA extension at 72 °C for 1 min. After the final cycle, the reaction was terminated by keeping it at 72 °C for 7 min for the *mecA* gene and DNA denaturation at 92 °C for 30 s, primer annealing at 58.5 °C for 30 s, and DNA extension at 72 °C for 45 s with final extension at 72 °C for 10 min for the *pvl* gene. *Staphylococcus aureus* strain NCTC 13300 was used as a positive control and negative controls contained all reagents except template DNA.

Table 1. The primer sequences and expected amplicon sizes of each gene investigated by PCR in this study.

Gene name	Primer name	Sequence	Amplicon size (bp)	Reference
23S rRNA	Sau327	5'-GGACGACATTAGACGAATCA-3'	1300	Esmailnezhad et al., 2012 (12)
	Sau1645	5'-CGGGCACCTATTTTCTATCT-3'		
mecA	mecA-F	5'-CTCAGGTACTGCTATCCACC-3'	449	Shitto et al., 2006 (13)
	mecA-R	5'-CACTTGGATTATCTTCACC-3'		
pvl	pvl-F	5'-ATCATTAGGTAAAATGTCTGCACATGATCCA-3'	433	Khosravi et al., 2012 (6)
	pvl-R	5'-GCATCAACTGTATTGGATAGCCAAAAGC-3'		

2.4. Detection of PCR products

The PCR products were resolved by electrophoresis through a 1.5% agarose gel containing ethidium bromide.

2.5. Statistical analysis

The data were analyzed using SPSS 16 (SPSS Inc., Chicago, IL, USA). The chi-square (χ^2) test or Fisher's exact test was used to compare categorical variables in all cases and $P < 0.05$ was considered statistically significant in the final model.

3. Results

A total of 409 bacteria were isolated from specimens of 45 patients and 243 of these were *Staphylococcus* species. Staphylococci isolates consisted of 55 (22.6%) *S. aureus*, 91 (37.4%) *Staphylococcus epidermidis*, and 97 (39.9%) other staphylococci species (Table 2). *S. aureus* was the cause of skin infection in 53.3% of the patients and 24 (53.3%) of the 45 patients were colonized exclusively by *S. aureus*. The relation between sampling site and staphylococcal species was significant ($P < 0.05$), as given in Table 3.

By use of specialized primers, the standard *S. aureus* NCTC 13300 yielded PCR products of 1300 bp, 449 bp, and 433 bp as expected for the *23S rRNA*, *mecA*, and *pvl* genes, respectively, as shown in Figures 1–3. Among the 55 *S. aureus* isolates, 30 (54.5%) were PVL-positive and 33 (60%) were confirmed as MRSA. Epidemiologically, 16 (66.7%) and 18 (75%) patients out of 24 *S. aureus* carriers had *S. aureus* isolates harboring the *mecA* and *pvl* genes, respectively. *pvl* and *mecA* positivity was higher in *S. aureus* carriers ($P < 0.001$). The *pvl* gene was detected in 20 (66.7%) MRSA and 10 (33.3%) MSSA isolates ($P < 0.001$). The prevalence of *pvl* in *S. aureus* strains isolated from burn-injury cases and patients with skin infection was 53.3% (16/55 *S. aureus*) and 46.7% (14/55 *S. aureus*), respectively. PVL-positive *S. aureus* was detected in 12.5% (1/8) outpatients and 45.9% (17/37) inpatients. Demographic characteristics of the patients included the study and their relationships with *S .aureus* are given in Table 4, while Table 5 compares the percentages of MRSA and PVL-positive *S. aureus* cases from different sampling regions.

Table 2. Distribution of organisms isolated from patients with skin infections.

Organism	No. (%) of isolates (n = 409)
S. aureus	55 (22.6%)
MRSA	33 (8.07%)
MSSA	22 (5.38%)
S. epidermidis	91 (22.25%)
Aerobic gram-negative bacilli[a]	60 (14.67%)
Non-aureus and -epidermidis species of *Staphylococcus*	97 (23.71%)
Other	106 (25.92%)

MRSA: Methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*.
[a]Pure or mixed with organisms other than *Staphylococcus* spp, including *Pseudomonas aeruginosa*, Enterobacter species, Serratia species, Proteus species, and *Escherichia coli*.

Table 3. Distribution of *Staphylococcus* species in different samples obtained to detect carriage.

Sampling region	*Staphylococcus*			Total n (%)
	S. aureus n (%)	*S. epidermidis* n (%)	Other species n (%)	
Lesion	13 (33.3%)	10 (25.6%)	16 (41.0%)	39 (100.0%)
Nose	15 (26.3%)	29 (50.9%)	13 (22.8%)	57 (100.0%)
Axilla	4 (7.1%)	25 (44.6%)	27 (48.2%)	56 (100.0%)
Umbilicus	11 (22.0%)	18 (36.0%)	21 (42.0%)	50 (100.0%)
Groin	12 (29.3%)	9 (22.0%)	20 (48.8%)	41 (100.0%)

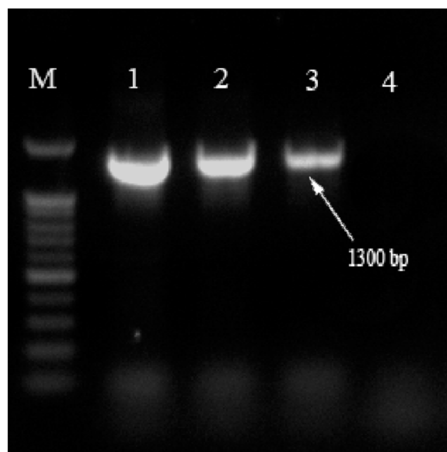

Figure 1. Gel electrophoresis of species-specific *23S rRNA* gene PCR for *S. aureus.* Lanes: M, 100-bp molecular weight marker; Lanes 1 and 2, clinical *S. aureus* isolates; Lane 3, positive control; Lane 4, negative control.

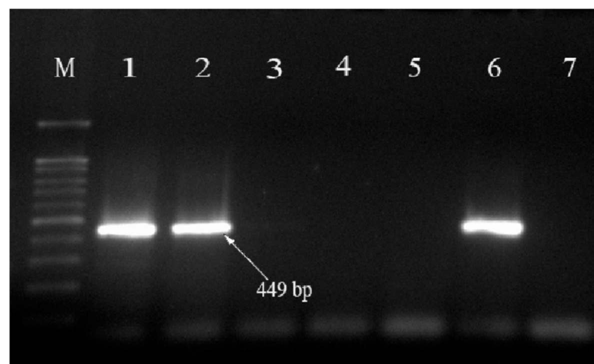

Figure 2. Gel electrophoresis of *mecA* gene PCR. Lanes: M, 100-bp molecular size marker; 1–5, clinical samples, 1 and 2 *mecA*-positive and 3–5 *mecA*-negative; 6, positive control; 7, negative control.

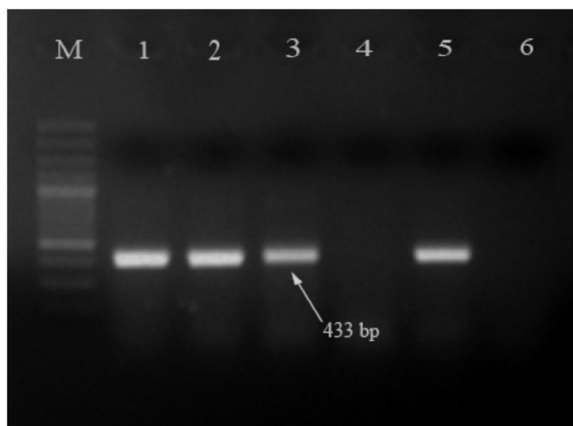

Figure 3. Gel electrophoresis of *pvl* gene PCR. Lanes: M, 100-bp molecular size marker; 1–4, clinical samples, 1–3 *pvl*-positive and 4 *pvl*-negative; 5, positive control; 6, negative control.

The most prevalent lesions among patients were ulcers (n = 20), necrosis (n = 18), scaly plaque/crust (n = 15), and abscess (n = 5). Significant positive association was observed between *S. aureus* infection and necrosis in skin lesions: 54.2% of *S. aureus* infections caused necrotic lesions, and 50% of those were detected as PVL-positive MRSA. *S. aureus* infections were more common in patients who used antibiotics (68.9% versus 31.1%). However, antibiotic exposure did not show significant association with *S. aureus* infections and colonization ($P > 0.05$). *S. aureus* infection and carriage rates were higher in men compared to women (62.5% versus 37.5% for *S. aureus* infections and 66.7% versus 33.3% for *S. aureus* carriage, $P < 0.05$). Among patients hospitalized in burn units, *S. aureus* infection was detected in 73.7%; this rate was significantly higher than in other patients with skin infections ($P < 0.05$). Surprisingly, none of the infections in surgical wounds were caused by *S. aureus.*

4. Discussion

S. aureus is a frequent cause of nosocomial infection. This bacterium is simply acquired and has the potential to become resistant to many commonly used antibiotics; the prevalence of resistant strains is posing serious therapeutic and infection control problems within the hospital environment (6). MRSA remains the predominant cause of acute purulent skin and soft tissue infections. Therefore, accurate and early detection of methicillin resistance has an important role in the prognosis of infections caused by *S. aureus* (14). Identification of the *mecA* gene is the most reliable method to identify MRSA carriers early in their hospitalization. This allows the prompt initiation of

Table 4. Comparison of demographic characteristics in patients with skin infections due to *S. aureus* or other organisms.

Characteristic	*S. aureus* skin infection group (n = 24)	Non-*S. aureus* skin infection group (n = 21)	OR	95% CI	P value
Male sex	15	7	3.57	0.90–14.72	**0.05**
Referral reason [a]			-		**0.016**
Burn infection	14	5	4.48	1.05–20.27	**0.01**
Purulent skin infection	10	12	0.48	0.12–1.87	0.23
Surgical site infection	0	4	0	0–1.27	**0.011**
Comorbidity	14	5	0.82	0.19–3.48	0.76
Lesion type					…
Pustule	6	8	0.54	0.12–2.31	0.34
Abscess	3	2	1.36	0.16–13.29	0.75
Ulcer	11	9	1.13	0.29–4.34	0.84
Plaque	9	6	1.5	0.36–6.35	0.53
Necrosis	13	5	3.78	0.89–16.93	**0.038**
Antibiotic therapy	17	14	1.21	0.29–5.16	0.76
Erythromycin	4	3	1.2	0.19–8.04	0.83
Clindamycin	2	3	0.55	0.06–4.7	0.53
Vancomycin	11	4	3.6	0.79–17.52	**0.055**
Cephalosporins	12	11	0.91	0.24–3.45	0.87

Data are no. of patients. Statistically significant relationships are bolded.
MRSA: Methicillin-resistant *S. aureus*; PVL: Panton–Valentine leukocidin; MSSA: methicillin-sensitive *S. aureus*; OR: odds ratio; CI: confidence interval.
[a]Variable significant in multivariable analysis

Table 5. The percentage of MRSA and PVL-positive *S. aureus* based on sampling regions.

Sampling region	*S. aureus*	
	MRSA	PVL-positive *S. aureus*
Lesion	6 (46.2%)	8 (61.5%)
Nose	8 (53.3%)	6 (40.0%)
Axilla	3 (75.0%)	2 (50.0%)
Umbilicus	9 (81.8%)	5 (45.5%)
Groin	7 (58.3%)	9 (75.0%)

isolation measures to minimize MRSA transmission to other patients and health care providers (15).

In this study, 33 of the 55 (60%) *S. aureus* specimens isolated from skin infections were *mecA*-positive by PCR. The prevalence of MRSA was higher in our study compared to similar studies from Turkey (31.81%) (16), Beijing (3%) (17), Egypt (47.37%) (18), and Sao Paulo (10.6%) (15). It was similar to the rate reported from Torrance, California (66.57%) (3), and lower than the rate found in a general report from the United States (79%) (19).

Although there is high geographic variability in the prevalence of MRSA from different regions of Iran, such

as 36% (20), 53% (21), 60% (22), 87.36% (6), and 90% (23), it should be noted that the MRSA strains of the mentioned reports were isolated from other clinical complications than skin infections. In terms of frequency, our findings indicate a greater difference compared to a previous study in Shiraz (43%) (24). Seasonal changes, which have a direct relation with bacterial colonization and genetic changes of *S. aureus* over time, may also affect the spread of methicillin resistance among *S. aureus* strains. In the present study, the MRSA isolation rate was substantially higher in *S. aureus* carriers compared to the isolation rate from skin lesions (P < 0.001). These varieties may reflect different policies for infection control and other factors involved in these areas (25).

In this study, 30 of the 55 (54.5%) *S. aureus* isolates were PVL-positive. Miller et al.(3) in Torrance, California, also reported high PVL positivity (76%) in *S. aureus* causing SSTIs. However, other reports employing the same criteria showed lower prevalences of 15.1%, 15.79%, and 41.46% in Brazil, Egypt, and China, respectively (15,17,18). Although our results of *pvl* gene positivity among *S. aureus* strains is in concordance with the findings of Momtaz and Hafezi (46.34%) (10), it should be considered that a lower prevalence of *pvl* was observed in other reports from Iran (6,26–28). This difference might be due to the fact that PVL-positive *S. aureus* isolates are more prevalent in cutaneous and pulmonary isolates (7), while the sampling population in the mentioned studies was not limited to patients with SSTIs. Another reason might be the type of assay used for detecting the gene. The majority of MRSA isolates (66.7%) in our study harbored the *pvl* gene (P < 0.001). This finding is in agreement with the results of Talan et al. from the United States (19).

Given our finding of a strong association between S. *aureus* skin infections and necrotic lesions when compared to other skin infections caused by other organisms, it is distinctly possible that lesions infected with *S. aureus* are prone to become necrotic. Therefore, these kinds of lesions need more care and appropriate medical treatment. The rate of *S. aureus* infection was significantly higher in patients hospitalized in burn units than patients diagnosed with skin infections (P < 0.05). Hence, targeted intervention measures including hand hygiene, isolation procedures, cleaning of the hospital environment, screening, and decolonization of patients are required to reduce the MRSA acquisition rate in burn-injury hospitals. It is interesting that no surgical wound infection caused by *S. aureus* was observed. This might be due to the appropriate control of *S. aureus* elimination or the use of broad-spectrum postoperative antibiotics. The incidence of *S. aureus* infection in patients receiving vancomycin showed a considerable decrease with a P-value of 0.055 (P > 0.05).

We assessed four body sites other than skin lesions for *S. aureus* colonization and undertook an epidemiologic survey in comparison with other studies performed in Iran (6,29). Our investigation showed a significant association between sampling sites and isolated staphylococci (P = 0.004). Among the 243 specimens of *Staphylococcus* species isolated in this study, the most common were nasal and axillary samples with a rate of 46.5% (113 isolates). *S. aureus* and *S. epidermidis* were most commonly isolated from nasal specimens. Colonization of *S. aureus* in patients was very common (46.8%) and higher than in those studies that cited prevalences of 20%–35% in only nasal colonization (29,30,31). In our study, and similar to other reports (3), additional anatomic sites were assessed for *S. aureus* colonization. Results might have been more reliable if oropharyngeal samples had also been collected. However, the yields of these additional sites might be low (3).

We failed to determine a significant association between antibiotic exposure and *S. aureus* colonization (P > 0.05), although the *S. aureus* colonization rates were higher in patients with a history of antibiotic use. Our finding is in accordance with a report of Miller et al. in the United States (3) that assessed a significant number of criteria as hypothesized MRSA risk factors and did not find any relation between MRSA colonization and antibiotic exposure (P = 0.18).

There are limitations to our study that are going to be considered in future studies. First, we lacked reliable data on previous history of skin infection or treatment for burn patients. Although this was an important risk factor for *S. aureus* colonization, such data could not be obtained reliably and were excluded from our survey. Second, we failed to include some other risk factors in our questionnaire such as the history of any contact with animals or the history of any skin disease in other family members and last referral time to hospitals for any reason. Third, our population may not represent other populations in Iran. It is probably a matter of the geographical variation that affects the *S. aureus* genetic profile. Strain typing could be included with other aims of this study in order to express more aspects of skin infections caused by *S. aureus*.

In conclusion, this study provides baseline information on the epidemiology and molecular characteristics of *S. aureus* isolated from patients with skin infections in Shiraz, southern Iran. Apart from the high prevalence of MRSA isolates in our study, the considerable rate of *pvl* gene positivity is a matter of concern. Since there is strong evidence of involvement of the *pvl* gene in the pathogenesis of *S. aureus* infections, early determination of PVL-positive MRSA during hospitalization by intake screening in high-risk patients will prevent transmission of infection and also necessitates some control strategies for decolonization of carriers in hospitals.

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