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Original Article

Molecular Characterization and Antimicrobial Susceptibility of Methicillin-Resistant *Staphylococcus aureus* Isolates from Clinical Samples and Asymptomatic Nasal Carriers in Istanbul (Turkey)

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Received: 11-Nov-2019; **Revision:** 17-May-2020; **Accepted:** 24-Sep-2020; **Published:** 20-Jul-2021

Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) has been a widespread problem in Turkish hospitals. **Aims:** The aim of this study was to investigate the staphylococcal toxin genes of the clinical and nasal MRSA isolates, and their antibiotic resistance profiles. **Materials and Methods:** Isolation of nasal and clinical bacteria was done following standard microbiological methods. The presence of antimicrobial resistance genes (*mec A, pvl, tsst-1,* and *SEs* genes) was determined using the real-time polymerase chain reaction (PCR) assay. **Results:** Among nasal MRSA isolates, 66.7% were toxigenic. The distribution of genes was as follows: *pvl* 26.7%, *tsst-1* 3.3%, and *SEs* 36.7%. Therefore, the nasal MRSA isolates had a rate of 23.3% multidrug resistance (MDR) pattern to the non-beta-lactams antibiotics. All (100%) clinical MRSA isolates were found to be toxigenic. The distribution of genes was as follows; *pvl* 10%, *tsst-1* 6.7%, and *SEs* 100%. The clinical MRSA isolates had a rate of 60% MDR. **Conclusions:** Following detection of *pvl, tsst-1, and SEs* among nasal and clinical MRSA isolates, and the presence of high antimicrobial resistance, the spread of these strains may be an additional factor contributing to the emergence of community-acquired (CA)-MRSA and hospital-acquired (HA)-MRSA. This study is the first to determine the resistance to linezolid and tigecycline in both nasal and clinical MRSA isolates, for the first time in Turkey. All nasal and clinical MRSA isolates were uniformly susceptible to vancomycin and quinupristin-dalfopristin. Our findings show that MRSA infections in Turkey can be empirically treated with vancomycin and quinupristin-dalfopristin based on the lack of demonstrable resistance to these drugs.

Keywords: *Antimicrobial resistance, mecA, MRSA, pvl, SEs, tsst-1*

Introduction

Staphylococcus aureus (*S.aureus*) is an important pathogen whose enzymes and toxins contribute to its pathogenicity. The healthy people who are carriers may be a source of infection with high morbidity and mortality in patients. Asymptomatic nasal *S. aureus* carrier rate changes between 10% and 40% in both community and hospital environments, and furthermore it plays a role in the spreading of multiple-resistant staphylococcus, especially methicillin-resistant *S. aureus* (MRSA).[1,2]

S. aureus has numerous cell-associated and secreted virulence factors that promote cellular adhesion, invasion, bacterial reproduction, and a deficiency of immune responses. Some of the virulence factors include Panton–Valentine leukocidin (*pvl*), toxic shock syndrome toxin 1 (*tsst-1*), hemolysins, exfoliative toxins (*ETs*), and staphylococcal enterotoxins (*SEs*). While *tsst-1*, causes toxic shock syndrome that manifests itself with skin eruptions and multiple organ failure, *pvl* and

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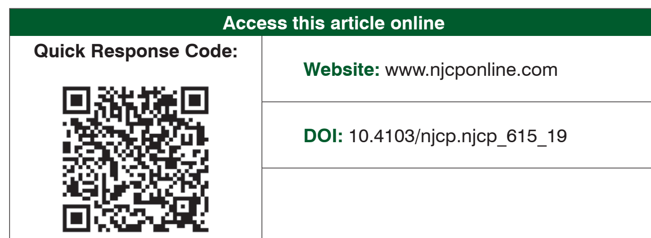

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/njcp.njcp_615_19

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How to cite this article: Dincer SD, Demirci M, Celepler Y, Namal N, Aksaray S, Aktepe OC, *et al.* Molecular characterization and antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* isolates from clinical samples and asymptomatic nasal carriers in Istanbul (Turkey). Niger J Clin Pract 2021;24:997-1004.

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γ-hemolysin target polymorphonuclear cells and immune cells like macrophages, and cause severe skin and soft tissue infections (SSTIs). Also, *SEs* have reported many food-borne diseases with different toxin subtypes.[2,3]

Several toxins such as *tsst-1* and *SEs* belong to the superantigen (SAg) family. Superantigens are proteins that have high mitogenic properties, causing T- and B-cells expansions that result in clonal deletion and massive cytokine production. Consequently, a critical situation is established that involves endothelial leak, hemodynamic shock, multiorgan failure, and possibly death because of intensive secretion of interleukin-1, TNF, and interferon-γ.[3-5]

There are national studies searching virulence and antibiotic resistance genes in MRSA isolated from clinical and nasal samples but there are not any studies comparing the MRSA isolates from the clinical and nasal carriers for *SEs, pvl,* and *tsst-1* genes and antibiotic resistance profile. Therefore, in this study, we aim to investigate the toxin genes and antibiotic resistance profiles in MRSA isolates from both clinical and nasal samples, and determine the distinctness and similarities of two groups in addition to from an empirical and specific treatment guide for MRSA infections

Materials and Method

Bacterial isolates

İn this study, we analyzed 60 MRSA isolates, 30 isolates from various clinic samples of laboratory MRSA-diagnosed hospitalized patients, and 30 isolates from nasal samples of healthy volunteers in four universities and one state health facility, between February 2016 and March 2018. All clinical strains were isolated after 72 h of the admission of the patient to the hospital, thus regarded as hospital-acquired.

Nasal swabs were obtained from both nares of the volunteer participants by using a sterile cotton swab moistened with physiological saline. The nasal and clinical specimens were first inoculated on Columbia agar plates with 5% sheep blood (BioMerieux, France) and Chromagar MRSA (BioMerieux, France) incubated under 5% CO_2 at 35°C for 24 h.

For the purpose of the usual definition, VITEK MS (BioMerieux, France) and conventional methods were used together.[1] Isolates were transferred into a tryptic soy broth medium containing 15% glycerol and stored at - 80°C for phenotypic and genotypic analyses

Phenotypic antibiotic susceptibility patterns of the isolates

Phenotypic antimicrobial susceptibility testing was performed by VITEK 2 Compact (BioMerieux, France), and interpretation was done according to EUCAST-2016 guidelines.[6] MRSA isolates were defined as MRSA using a cefoxitin 30-µg disk screening test and PCR (for *mecA* gene). *S. aureus* ATCC 25923 was used as quality control.[7]

Molecular detection

Bacterial DNA isolation

Template DNA was prepared by a simple and rapid boiling procedure from the suspension of *S. aureus* colonies.[7] DNA was collected and stored at - 20°C until real-time PCR runs.

Molecular detection of the mecA gene of S. aureus

Real-time PCR was used for the detection of *mecA* [Table 1]. As positive controls, *S. aureus* ATCC BAA-41 was used. Light Cycler 480 Probe Master kit (Roche Diagnostics GmBH, Mannheim, Germany) was used with specific primers and probes for *mecA* on Light Cycler 480 II (Roche Diagnostics GmBH, Mannheim, Germany) instrument according to the manufacturer’s instructions. These primers and probes are shown at Table 1. The following real-time PCR ptotocol was used: denaturation step at 95°C for 10 min, followed by 45 cycles, of 10 s at 95°C, 30 s at 55°C, and 1s at 72°C.

Molecular detection of specific virulence genes of Staphylococccus spp. (SEs, pvl, and tsst-1 gene)

PCR was used for the detection of species-specific genes to confirm their identities. Targeted genes included the *SEs*, *pvl*, and *tsst 1* gene.[8-12]

Primers and probes of *sea, seb, sec, sed, see, seg, seh, sei* and *sej* enterotoxin, *pvl* and *tsst-1* gene were provided from Integrated DNA Technologies (IDT, Coralville, IA). Table 1 shows the primers and probes that were used. Light Cycler 480 Probe Master kit (Roche Diagnostics GmBH, Mannheim, Germany) was used with these primers and probes on Light Cycler 480 II (Roche Diagnostics GmBH, Mannheim, Germany) instrument according to the manufacturer’s instructions. Primers 0.5 uM and probes 0.1 µM were added in reactions of final concentrations. The following real-time PCR ptotocol was used: denaturation step at 95°C for 10 min, followed by 45 cycles, of 10 s at 95°C, 30 s at 55°C, 1s at 72°C.

All molecular analyses were performed at the Medical Microbiology Laboratory at the Medical School of Bahceşehir University, in Istanbul, Turkey. The strains *S. aureus* ATCC 13565 (*sea, sej*), *S. aureus* ATCC 14458 (*seb*), *S. aureus* ATCC 19095 (*sec, seh*), *S. aureus* ATCC 23235 (*sed, seg, sei*), *S. aureus* ATCC 27664 (*see*), *S. aureus* ATCC 25923 *(pvl*), and *S. aureus* ATCC 51650 (*tsst-1*) were used as a positive control. As a nontoxigenic control, *S. aureus* ATCC 6538 was used.

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Statistical analysis

The prevalence of MRSA and virulence genes was compared using the Fisher exact test. Statistical analysis was performed with SPSS version 16 (SPSS Inc, Chicago, IL). $P < 0.05$ was regarded as statistically significant.

Results

In this study, we evaluated the prevalence in the nasal and clinical isolates of MRSA that contained the *tsst-1*, *pvl*, *SEs* gene, and antimicrobial susceptibility patterns. In total 66.6% (n = 20) of clinical *S. aureus* isolates were recovered from blood infections, and the remaining isolates were from wound infections 20% (n = 6) and 13.4% (n = 4) sputum. The distribution of MRSA isolates toxin genes is presented in Table 2. Of the nasal isolates, 66.7% and of the clinical isolates, 100% were toxigenic. Regarding virulence genes, the most common gene was *SEs* (36.7% rate of nasal MRSA isolates), followed by *pvl* (26.7%) and *tsst-1* (3.3%) genes; and

Table 1: Oligonucleotide primers and hydrolysis probes used in the real-time PCR assay

Gene	Primer and Probe	Sequence	Amplicon Size	Genbank	Ref*
sea	*sea* fw	5-AAAATACAGTACCTTTGGAAACGGTT-3	92	M18970	Klotz *et al*., 2003[8]
	*sea*rv	5-TTTCCTGTAAATAACGTCTTGCTTGA-3			
	sea probe	FAM-AACGAATAAGAAAAATGTAACTGTTCAGGAGTTGGA TC-Tamra			
seb	*seb* fw	5-ACACCCAACGTTTTAGCAGAGAG-3	81	M11118	Klotz *et al*., 2003[8]
	seb rv	5-CCATCAAACCAGTGAATTTACTCG-3			
	seb probe	FAM-CAACCAGATCCTAAACCAGATGAGTTGCACA-Tamra			
sec	*sec* fw	5-AATAAAACGGTTGATTCTAAAAGTGTGAA-3	80	X05815	Klotz *et al*., 2003[8]
	sec rv	5-ATCAAAATCGGATTAACATTATCCATTC-3			
	sec probe	FAM-TAGAAGTCCACCTTACAACAA-Tamra			
sed	*sed* fw	5-TGATTCTTCTGATGGGTCTAAAGTCTC-3	115	M28521	Klotz *et al*., 2003[8]
	sed rv	5-GAAGGTGCTCTGTGGATAATGTTTT-3			
	sed probe	FAM-TATGATTTATTTGATGTTAAGGGTGATTTTCCCGAA-Tamra			
see	*see* fw	5-GCTTTGGCGGTAAGGTGC-3	68	M21319	Chiefar *et al*. 2005[9]
	see rv	5-ATAACTTACCGTGGACCCTTCAGA-3			
	see probe	FAM-AGGCTTGATTGTGTTTCATT-Tamra			
seg	*seg* fw	5-CAATCGACAATAGACAATCACTTGG-3	133	AB060535	Nakayama *et al*., 2006[10]
	seg rv	5-GCAGAACCATCAAACTCGTATAGCT-3			
	seg probe	FAM-TGGCTCACTAAAGAAA-Tamra			
seh	*seh* fw	5-TAAATAGTGAAAAAATTGGCACAGGAA-3	118	AB060536	Nakayama *et al*., 2006[10]
	seh rv	5-AATTCTTGCAATGTCACATTTTTCTT-3			
	seh probe	FAM-CTAATGTTTGGGTAGATGGT-Tamra			
sei	*sei* fw	5-GGGCCACTTTATCAGGACAATACT-3	79	AB060537	Nakayama *et al*., 2006[10]
	sei rv	5-GTTTTATGTTTGCCATTAACCCAAA-3			
	sei probe	FAM-AAATTCTGCTAGAAAAATCCCTAT-Tamra			
sej	*sej* fw	5-GGATTGATAGCATCAGAACTGTTGTT-3	140	AF053140	Letertre *et al*., 2003[11]
	sej rv	5-ATCAAAGGTACTAGGGTTGTATAAATTATATTGT-3			
	sej probe	FAM-CTTCAGGCAAGATATTA-Tamra			
pvl	*pvl* fw	5-ACACACTATGGCAATAGTTATTT-3	176	AB006796	McDonald *et al*., 2005[12]
	pvl rv	5-AAAGCAATGCAATTGATGTA -3			
	pvl probe	FAM-ATTTGTAAACAGAAATTACACAGTTAAATATGA-Tamra			
tsst-1	*tsst* fw	5-TTTTTATCGTAAGCCCTTTGTTG-3	70	AB084255	Chiefari *et al*. 2015[9]
	tsst rv	5-TATTATCGTTTGTAGATGCTTTTGC-3			
	tsst probe	FAM-GATTTTACCCCTGTTCCCTTA-Tamra			
mecA	*Mec*fw	5-GGCAATATTACCGCACCTCA-3			McDonald *et al*., 2005[12]
	*Mec*rv	5-GTCTGCCACTTCTCCTTGT-3			
	*Mec*probe	FAM-AGATCTTATGCAAACTTAATTGGCAAATCC-Tamra			

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Table 2: Status of *mec A* and toxin genes of MRSA isolates

Status of genes	strain number (%)		
	Nasal MRSA	Clinical MRSA	Total MRSA
pvl	8 (26.7)	3 (10)	11 (18.3)
tsst-1	1 (3.3)	2 (6.7)	3 (5)
Ses	11 (36.7)	30 (100)	41 (68.3)
Only one group of toxin genome	19 (63.3)	28 (93.3)	47 (78.3)
More than one group of toxin genome	1 (3.3)	2 (6.7)	3 (5)
Isolates carrying toxin genome	20 (66.7)	30 (100)	50 (83.3)
Isolates not carrying toxin genome	10 (33.3)	0 (0)	10 (16.7)

Table 3: The antimicrobial resistance patterns of nasal and clinical MRSA isolates

Antibiotics	strain number (%)		
	Nasal MRSA	Clinical MRSA	Total
Erythromycin	11 (36.7)	28 (93.3)	39 (65)
Clindamycin	7 (23.3)	11 (36.7)	18 (60)
Quinupristin-dalfopristin	0	0	0
Tetracycline	14 (46.7)	19 (63.3)	33 (55)
Gentamicin	5 (16.7)	7 (23.3)	12 (20)
Tigecycline	3 (10)	3 (10)	6 (10)
Ciprofloxacin	5 (16.7)	7 (23.3)	12 (20)
Linezolid	1 (3.3)	2 (6.7)	3 (5)
Vancomycin	0	0	0
Trimethoprim-sulfamethoxazole	8 (26.7)	9 (30)	17 (28.3)
Multidrug resistance	7 (23.3)	18 (60)	25 (41.7)

the most common gene was *SEs* 100% rate of clinical MRSA isolates, followed by *pvl* (10%) and *tsst-1* (6.7%) genes of clinical MRSA isolates. Most of the strains had *sea* (66.7%) as the leading *SEs* genome.

The distribution of SEs genomes in nasal MRSA isolates in our study was 16.7% for *sea* and *sej*; 10% for *seb*; 6.7% for seg; and 3.3% for *sec, sed, see, seh,* and *sei*. *SEs* gene combinations of *seb* + *sei* + *sej, sec* + *seg* + *sej,* and *sea* + *she* was found in 36% of nasal MRSA isolates.

The distribution of *SEs* genomes in MRSA isolates in our study was 66.7% for *sea*, 46.7% for *seg*, 30% for *sec*, 26.7% for *sej,* 23.3% for *seb,* 16.7% for see*,* 13.3% for *sei*, 10% for *sed,* and 6.7% for *seh* in these isolates. In clinical MRSA isolates, *sea* + *seg* + *sej* (10%), *seb* + *se* + *sej* (3.3%), and different *SEs* gene combinations were detected. A statistically significant difference was found between two groups for *SEs* gene carrying frequency. ($P < 0.00001$)

Antimicrobial susceptibility patterns of nasal and clinical MRSA isolates are presented in Table 3. Multidrug resistance (MDR: the presence of resistance against three antibiotics except B lactam group antibiotics) was observed in 7 (23.3%) of nasal isolates and 18 (60%) of clinical isolates. All isolates with MDR had at least one toxin gene**.** Nasal isolates without toxin genes were found to be susceptible to all antibiotics

Discussion

Today MRSA is commonly distributed all around the world, but its prevalence differs among countries.[13] MRSA strains have become a common problem in Turkish hospitals over the years.[14,15] While MRSA has been seen especially as a hospital pathogen until recently, community-acquired MRSA (CA-MRSA) infections have begun to be observed alongside hospital-acquired MRSA (HA-MRSA) infections since the mid-1990s.[16]

This study investigates the presence of *SEs* (A-I), *pvl,* and *tsst-1* in MRSA isolates from nasal carriers and clinical specimens. The development of *S. aureus*-associated infections is a very complex process. A large number of virulence factors and exotoxins contribute to the development of the infection. Currently, it has been accepted that the staphylococcal exotoxins primarily destroy the cells of the host immune system, either with direct cytotoxicity or through superantigen-mediated mechanisms.[17]

In our study, *pvl* gene positivity was found in 26.7% of nasal MRSA isolates and 10% of clinical MRSA isolates. In studies in Turkey, *pvl* -positive MRSA isolates change between 0% and 10.2%.[18-20] PVL prevalence was found to be less than in some studies 5%, *pvl*-positive CA-MRSA clones were found to be 84.4% around the world even 100% in France, Switzerland, the USA, and Oceania.[4,21-24] Increased clonal spread of *pvl*-positive strains in the recent two decades has brought about important public health problems. This has been especially observed in CA-MRSA strains. Studies have been demonstrated that *pvl*-positive strains have been rapidly spreading in the hospital environment.[25] PVL-positive strains have high morbidity and mortality rates, spread in the hospital environment, cause outbreaks. All of these features increase the risk of development of more resistant and viral strains with the acquisition of antibiotic resistance genes.[26,27]

Our results confirm that *pvl* gene positivity is more in CA-MRSA isolates than (hospital-acquired) HA-MRSA

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isolates. *pvl* gene positivity in clinical MRSA isolates makes us think that isolates with this toxin can spread in the hospital environment and increase the ratio of morbidity and mortality. Monitoring is necessary to prevent the spread of *pvl*-positive MRSA isolates in the hospital environment epidemiologically.

İn our study, *tsst-1* gene was found in 3.3% of nasal MRSA isolates and 6.7% of clinical isolates. İn Turkey, a study in which the ratio of MSSA in *S. aureus* nasal carriers was 97% found the ratio of TSST-1 toxin to be 27.9%,[18] and a study using clinical samples found this ratio to be 0.7% in MRSA isolates,[28] whereas another study found no toxin.[19] John E. Warner *et al*.[29] investigated *S. aureus* colonization and TSST-1 toxin positivity in nasal, anal, and vaginal regions, they detected the highest colonization in the nasal samples and found TSST-1 toxin positivity in 7% of all samples. Another study searching the presence of TSST-1 in MRSA and MSSA samples found it to be 69% in MRSA isolates and 56% in MSSA isolates and it was not statistically significant.[30] While the prevalence of TSST-1 toxin in nasal and clinical MRSA isolates is detected as lower in foreign countries, it is higher than other studies in our country.

We detected *SE*s gene in 11 (36%) and 30 (100%) of nasal and clinical MRSA isolates, respectively. Though *sea* gene is the most frequent in both sample types, different gene combinations have been detected as well.

In Turkey, the studies using nasal and clinical *S. aureus* samples have demonstrated that the ratio of *SEs* gene is about 40%–95% and *sea* gene is more common. İn our study and the other studies in Turkey have shown that a high ratio of *SEs* gene is found especially in clinical MRSA isolates.[18,19,20,28,31] Zarakolu *et al*.[19] detected SEs toxin in 88% of the MRSA strains isolated from bacteremia patients in Turkey, and in 74% of the strains there were different gene combinations like *sea* + *see* + *sei, sea* + *sej,* and *sea* + *see* + *sei*. In our study groups, the presence of SEs toxins in clinical isolates was higher than nasal isolates ($P < 0.00001$), and it was statistically significant. SAgs are unique because they are resistant to heat, proteolysis, and desiccation due to their extreme stability and high toxicity in humans, some of them are classified as select agents for bioterrorism.[32] Enterotoxigenic *S. aureus* strains with a combination of different SE genes can also promote the incidence and severity of *S. aureus* infections. The presence of at least one SE gene in all clinical isolates and different SEs gene combinations is an ominous status for Turkey.

The comparison of antibiotic resistance between nasal MRSA isolates and clinical MRSA isolates revealed that the highest resistance among clinical MRSA isolates was against erythromycin with 93.3% while erythromycin ranked second among nasal MRSA isolates with 36.7%. Tetracycline resistance was revealed in nasal isolates with 36.7% in the first line.

In global studies, while erythromycin resistance in nasal MRSA isolates changes between 19.7% and 100%, this ratio is between 49% and 60% for clinical MRSA samples.[1,2,33,34] In the studies in Turkey, erythromycin resistance in nasal MRSA isolates was about 20%, this ratio changes between 63.9% and 84.3% in clinical samples.[14,35,36] Macrolide group of antibiotics is an alternative treatment option that is commonly used in patients with MRSA infections and penicillin allergies. As our study has also found, however, the resistance against macrolide is gradually increasing due to its widespread use.

Tetracycline resistance was 10%–16.5%[15] in Turkey and it ranked second with 55% in our study group.

In previous studies in Turkey, resistance against linezolid or tigecycline was not reported.[37,38] However, in the present study, we determined the resistance level to tigecycline to be 10% and linezolid to be 6.7%. This important finding is to determine the resistance to linezolid and tigecycline in both nasal and clinical MRSA isolates, for the first time in Turkey. In a review by Shariati *et al*.[39] on reports from various regions of the world, linezolid, daptomycin, and tigecycline effectively (99.9%) inhibit MRSA. MRSA and coagulase-negative staphylococci had greater resistance to quinupristin-dalfopristin with 0.7% and 0.6%, respectively. There was no resistance against vancomycin or kinupristine-dalfopristine in both sample types. Vancomycin is still an important treatment option for especially heavy and complicated MRSA infections in Turkey. On the other hand, monitoring of this antimicrobial activity needs to be continued because there is a slow but continued increase in vancomycin resistance in many global studies.[15,34,40]

Resistance genes in staphylococci, which can usually be transmitted to other *Staphylococcus* sp. through plasmid-mediated exchange mechanisms, result in strains with resistance against many antibiotics, and methicillin resistance in these bacteria is considered to be an indicator of multiple resistance.[40] As the chromosomal region encoding methicillin resistance also encodes resistance against many other antibiotics except methicillin and ß-lactams such as macrolide, clindamycin, fluoroquinolone, tetracycline, mupirocin, and SXT, these strains are observed more commonly in hospital environments and the importance of these

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MRSA strains is increasing.[41] Studies in different geographic regions around the globe have demonstrated MDR in MRSA is changing between 25% and 90.2% for antibiotics other than ß-lactams.[42,43]

The growing increase in multidrug resistance among MRSA strains aggravates this problem and represents a great challenge for the management of severe MRSA infections. In our study, while the ratio of MDR was 23.3% in nasal MRSA isolates, it was 60% in clinical MRSA isolates. The highest MDR resistance was revealed in nasal MRSA isolates with 20% to tetracyline + genatmycine + siprofloksasin combination while it was to tetracycline + clindamycin + erythromycin combination in clinical MRSA isolates with 33%. While being a nasal MRSA carrier is a risk factor for HA-MRSA infections, for HA-MRSA infections in different parts of the body is gaining importance.[44,45] It was found 87.5% clonal relatedness of MRSA isolates in the blood and nasal samples of patients with bacteremia.[46] Although healthy volunteers in our study were asymptomatic nasal carrier, 23.3% of isolates were MDR, this fact may cause a problem in HA- MRSA infections in the future

The enterotoxin producing strains may survive immune system attacks better than those isolates not producing it. The presence of SEs toxin in 100% of clinical isolates with an MDR ratio of 60% suggests a correlation between MDR development and *SEs* toxin presence. However, more advanced studies are needed to confirm this correlation.

The studies conducted show that PVL, the important virulence factor of CA-MRSA strains, has started to spread rapidly in hospital environments.[21,45] Antimicrobial susceptibility algorithms have been used for presumptive identification of *pvl*-positive MRSA isolates.[47,48] In our study, all the *pvl* gene-positive nasal and clinical MRSA isolates were ciprofloxacin, gentamicin, and linezolid sensitive. These results suggest that the sensitivity to ciprofloxacin of MRSA strains may be a presumptive marker for *pvl* gene detection in Istanbul (Turkey). İn addition, it should be investigated with further studies whether it can be a putative marker for detecting *pvl* gene in gentamicin and linezolid susceptibility

This study concluded that MRSA isolates in different geographic regions carry toxin genes with different frequencies and show different antibiotic sensitivity patterns. Infection control mechanisms should be done to prevent, *S. aureus* strains from spreading in hospital environments rapidly and efficiently. Future surveillance studies will be guiding in investigating the toxin and antibiotic sensitivity patterns of *S. aureus* strains, in the understanding of the epidemiology of CA and HA- *S. aureus* strains and the effects of their toxins on host response, and in taking preventive measures against the development of virulent strains.

Financial support and sponsorship

Nil

Conflicts of interest

There are no conflicts of interest.

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