JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Disinfectant/antiseptic resistance genes of Staphylococci and serotyping of clinical *Staphylococcus aureus* isolates

Hamdullah Suphi Bayraktar[1], Fatih Köksal[2], Nizami Duran[3], Tülay Kandemir[2], Başak Baykara[4], Tülin Güven Gökmen[5], Melda Meral Öcal[6]

[1] Hatay Mustafa Kemal University Experimental Researches Application and Research Center, Antakya, Turkey
[2] Çukurova University Faculty of Medicine, Department of Microbiology, Adana, Turkey
[3] Hatay Mustafa Kemal University Faculty of Medicine, Department of Microbiology, Antakya, Turkey
[4] Hakkari University Faculty of Health Science Department of Nutrition and Dietetics, Hakkâri, Turkey
[5] Çukurova University Ceyhan Faculty of Veterinary, Department of Microbiology, Adana, Turkey
[6] Mersin University Faculty of Science and Letter, Department of Biotechnology, Mersin, Turkey

Abstract

Introduction: Staphylococci are commonly isolated from healthcare settings worldwide. Methicillin-resistant *Staphylococcus aureus* (MRSA) is among the most important causes of nosocomial infections.

Objectives: This study aims to evaluate the resistance of Staphylococci to the most commonly used disinfectants and antiseptics in healthcare settings and the detection of the dominant serotype of *Staphylococcus aureus* in Hatay province.

Methodology: The frequency of disinfectant/antiseptic resistance genes was detected by the polymerase chain reaction (PCR) technique in identified staphylococcal strains, and antibiotic susceptibility of strains was evaluated phenotypically. *Staphylococcus aureus* strains were classified in terms of clonal and phylogenetic relationships with Pulse Field Electrophoresis and *spa* sequence typing methods.

Results: The identification rate of coagulase-negative staphylococci was 43.2% and the others (56.8%) were *Staphylococcus aureus*. Among these isolates, 103 methicillin-resistant *S. aureus* were identified. It was determined that 81 (78.64%) of these isolates harbored *qacA/B* and/or *smr genes*. The dominant *spa* serotype was found to be t223.

Conclusions: According to *spa* serotyping results, it was detected the serotype t223 was the dominant clone in our region, unlike the t030 was dominant in Turkey. Since there are many Syrian immigrants living in the Hatay region due to its geographical location, being the dominant clone of a different serotype has an epidemiologically significant importance.

Key words: *Staphylococcus aureus*; *QacA/*B; smr; PFGE; *spa* serotyping.

J Infect Dev Ctries 2026; 20(2):219-228. doi:10.3855/jidc.21412

(Received 03 February 2025 – Accepted 29 July 2025)

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are resistant to all beta-lactam antibiotics except ceftobiprole and ceftaroline [1,2]. MRSA is common in hospitals all over the world, especially in critical units such as intensive care and burn units and oncology services, where patients with natural and acquired immune system defects are followed. However, recently, outbreaks of bacteremia, endocarditis, and community-acquired MRSA (CA-MRSA) infections associated with skin and soft tissue have been reported [3].

HA-MRSA strains also show resistance to most broad-spectrum antibiotics used extensively in hospitals, antiseptics and disinfectants, as well as beta-lactam antibiotics. Indeed, the existence of resistance to antiseptics containing acriflavin and ethidium among HA-MRSA isolates has been discussed for more than 40 years [1,2].

It has been shown that antiseptic resistance genes, which are thought to be carried by mobile genetic elements with genes encoding β-lactam group antibiotic resistance, called Staphylococcal β-lactamase plasmids, are also carried by various *Staphylococcus aureus* aminoglycoside resistance plasmids [1,4]. These plasmids are particularly related to resistance to quaternary ammonium compounds (QACs) and ethidium bromide [5,6]. It has been shown that the pSK1 plasmid, which is frequently seen in *S. aureus* strains and associated with aminoglycoside and trimethoprim resistance genes, also carries genes involved in resistance to QACs and di-amidines [7]. It was reported that *qacA,* encoded in β-lactamase/heavy metal resistance plasmids, with this plasmid, encodes antiseptic resistance [8].

Hatay, located in the southern part of Turkey, is one of the cities most frequently flooded with refugees in Turkey after the Syrian war. In this study, Pulse Field

Gel Electrophoresis (PFGE) and *spa* sequence analysis methods and clonal properties of HA-MRSA strains isolated from patients under treatment in a tertiary university hospital in Hatay, and resistance to disinfectants/antiseptics such as quaternary ammonium compounds and chlorhexidine, which are used extensively in instrument and surface disinfection in hospitals, were investigated. This study aims to evaluate the resistance of staphylococci to the most commonly used disinfectants and antiseptics in healthcare settings, detection of the dominant serotype of *Staphylococcus aureus* in Hatay province, and determine the clonal and phylogenetic relationship between the strains.

Methodology

A total of 429 Staphylococcal strains isolated from clinical samples between 2017-2018 were used. Identification and susceptibility tests of strains were performed by Vitek 2 automated system (bioMerièux, France). The presence of disinfectant resistance genes (*qacA/qacB, smr*) in staphylococci was investigated by polymerase chain reaction (PCR).

Bacterial Isolates

Blood culture bottles from various clinics were incubated for up to seven days after being placed in the routinely used BACT/ALERT 3D automated blood culture device (bioMerièux, France). Gram staining was done from blood culture bottles with positive signals and inoculated on 5% sheep blood agar (COS; bioMerièux), MacConkey agar (MCK; bioMerièux), and chocolate medium (PVX; bioMerièux). The cultured media were incubated for 18-24 hours at 37ºC. Gram staining was made from the media with growth detected, and those with Gram (+) cocci were first subjected to the catalase test. Plasma coagulase test was performed on colonies showing catalase-positive characteristics. Strains with positive plasma coagulase test were accepted as *S. aureus.* The non-clot-forming strains were evaluated as coagulase-negative staphylococci (CNS). Wound, sputum, and urine samples were inoculated onto blood agar and eosin methylene blue (EMB) agar and incubated at 37°C for 24–48 hours. The isolated strains were identified using conventional methods (colony morphology, Gram staining, catalase, and coagulase tests) and the Vitek 2 automated identification system automated system (bioMerièux, France). The *in vitro* antibiotic susceptibilities of the isolates were determined using the Vitek 2 automated system based on the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [9].

Genomic DNA Isolation

Genomic DNA extraction was performed for staphylococcal strains, which are used in molecular analyses after they are isolated. DNA extraction was performed using the RTA Bacterial Genomic DNA Isolation Kit (RTA LABS) in accordance with the test procedure.

Detection of Disinfectant Resistance Genes

A single-tube in-house PCR method was applied to detect the presence of *qacA/B* and *smr* genes, which are considered indicators for the molecular mechanisms of resistance to chlorhexidine and quaternary ammonium compounds in MRSA strains, and are effective in disinfectants. Primer selections and amplification conditions were adjusted as described by Rouch *et al*., Sasatsu *et al*., and Noguchi *et al*. in Table 1 [10-12].

PCR amplification was performed in a total volume of 25 µL. For PCR amplification, 5 µL of genomic DNA (approximately 50 ng) and 20 µL PCR mix (20 mmol/L TrisHCl, pH 8.4; 50 mmol/L KCl, 10 mmol/L MgCl2, 200 µmol/L each deoxynucleoside triphosphate (dNTPs), 0.6 µmol/L, and 1 U of Taq DNA polymerase from each primer) were mixed. The first denaturation step in the amplification protocol for the *QacA/B* and *smr* genes was performed at 94°C for 5 minutes. Each PCR reaction consisted of 30 cycles. Amplification steps were performed with denaturation at 94°C for 1.5 minutes, binding at 56°C for 30 seconds, and DNA strand elongation at 72°C for 1.5 minutes. The final elongation was performed at 72°C in 7 minutes. PCR products were electrophoresed in 1xTAE buffer solution (40 mmol/L) Trisacetate, 1 mmol/L EDTA) on a 2% (w/v) agarose gel. DNA amplicons stained with ethidium bromide (0.5 200 µg/mL TAE) dye were visualized with a gel imaging system (Wealtec, Dolphin-View, USA).

Spa Typing

Raw data were evaluated with Sequencing Analysis

Table 1. Primer sequences and amplicon lengths of the qacA/B and qacC genes.

Target Gene	Forward (5'-3')	Reverse (3'-5')	Amplicon Lengths (bp)	Reference
*qac*A/B	5′-GCAGAAAGTGCAGAGTTCG-3′	5′-CCAGTCCAATCATGCCTG-3'	361	[10,11,12]
*qac*C	5′-GCCATAAGTACTGAAGTTATTGGA-3′	5′-GACTACGGTTGTTAAGACTAAACCT-3′	195	[10,11,12]

Table 2. Distribution of isolated staphylococci from the clinics and outpatient clinics.

STRAINS	Services and Intensive Care Units			
	Medical	ICUs	Surgery	Pediatrics
S. aureus	95	75	72	2
S. epidermidis	12	25	4	1
S. hominis	5	8	2	2
S. haemolyticus	1	5	6	1
S. saprophyticus	1	-	-	-
S. lugdunensis	1	-	-	-
Other CNS	41	34	30	6
TOTAL	156	147	114	12

ICUs: Intensive care units.

Software version 5.1, and *spa* types were classified with Ridom StphType TM (Ridom GmbH, Würzburg, Germany) software program. Using the BURP algorithm in the software program, the *spa* types were grouped with six or fewer repetition differences.

PFGE

SmaI-PFGE was detailed in a previous study, and band profiles were determined with GelCompar II software (version 4.0; Applied Maths, Sint-Martens-Latem, Belgium).

Statistical Analysis

All data in the study were analyzed with the χ^2 test. A p value < 0.05 was considered significant. Statistical analyses were performed using SPSS (Statistical Package for Social Sciences, SPSS for Windows V. 17.0, Chicago, USA).

Ethical Committee Approval

This study was performed with the permission of the Non-invasive Clinical Research Ethics Committee, with a meeting date of 07.10.2016 and decision number 57.

Results

A total of 429 staphylococcal strains were isolated from various medical services and intensive care units in this study. The majority of the strains were obtained from various medical services and intensive care units. The sources of isolated staphylococcal strains are given in Table 2. The most common isolated staphylococcal strains were *S. aureus,* and 109/244 (44.6%) of them were isolated from wounds and blood cultures [72/244 (29.5%)] in this study. Clinical specimens from which strains have been isolated are given in Table 3.

Among the staphylococcal strains included in the study, 56.77% were *S. aureus* and 43.22% were coagulase-negative staphylococci. Of the strains defined as *S. aureus*, 104/244 (42,62%) were MRSA. While 95 (91.34%) MRSA strains were resistant to clindamycin, the tetracycline resistance rate was found

Table 3. Clinical specimens from which strains have been isolated.

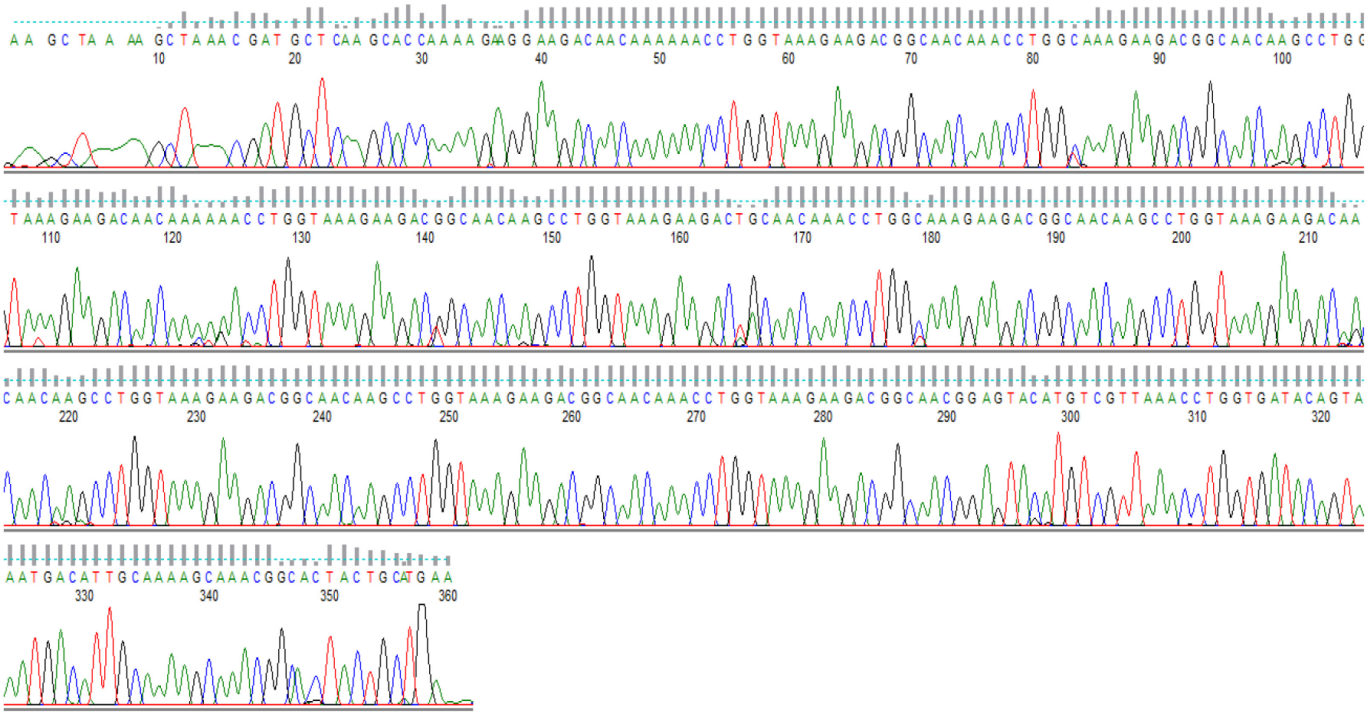
STRAINS	Blood	Wound	Urine	Sputum	TOTAL
S. aureus	72	109	43	20	244
S. epidermidis	11	40	18	3	72
S. hominis	17	-	-	-	17
S. haemolyticus	7	4	-	-	11
S. saprophyticus	1	-	-	-	1
S. lugdunensis	-	1	-	-	1
Stapylococcus spp.	65	10	7	1	83
TOTAL	173	164	68	24	429

Table 4. Antibiotic susceptibility of meticillin resistant *Staphylococcus aureus* strains.

Strain (n)	Antibacterials	≥ 64	32	16	8	4	2	1	0.5	Total
MRSA (104)	DA					75 (R)	20 (R)		9 (I)	104
	DAP						5 (R)			5
	GN		18 (R)	30 (R)			39 (R)			87
	TE		40 (R)	14 (R)		28 (R)	12 (R)			94
	E		15 (R)	28 (R)	23 (R)	10 (R)	16 (I)	9 (I)		101
	CIP			17 (R)	44 (R)	13 (R)				74
	TEC		10 (R)		17 (R)	21 (R)			1 (I)	49
	TMP/SXT	7 (R)		18 (R)	27 (R)	39 (R)				91
	VA		1 (R)							1

MRSA: Meticillin resistant *Staphylococcus aureus;* DA: Clindamycin; DAP: Daptomycin; GN: Gentamycin; TE: Tetracycline; E: Erythromycin; CIP: Ciprofloxacin; TEC: Teicoplanin; TMP/SXT: Trimethoprim Sulfamethoxazole; VA: Vancomycin; R: Resistant; I: Susceptible; Increased exposure; S: Susceptible.

Figure 1. Determination of *spa* types with Ridom Software.



to be 90.38% (94/104). The most effective antibiotics were vancomycin and daptomycin (resistance rates were 0.96% and 4.80%, respectively) against MRSA strains. Aminoglycoside resistance was 83.65% and 76 (73.07%) MRSA strains were found to be resistant to macrolids. On the other hand, fluoroquinolone resistance of MRSA strains was determined as 71.15% (74/104). The antibiotic susceptibility of isolated MRSA strains is given in Table 4.

It was observed that 35 (33.98%) of 103 HA-MRSA strains included in the study harbored only the *qacA/B* gene, and the number of strains harboring only the *smr* gene was 24 (23.30%). The number of strains that co-existed with both genes was 21 (20.38%) (Figure 1,2).

Considering the correlation of the distribution of disinfectant resistance genes with the phenotypically detected antibiotic resistance, all MRSA strains resistant to daptomycin were found to be harboring *qacA/B* (n = 3), *smr* (n = 1), and *qacA/B* and/or *smr* (n = 1). Only one isolate was resistant to vancomycin, harbored both *qac A/B* and *smr* genes. The ratio of

Figure 2. *qacA/B* gene positivity of some strains.

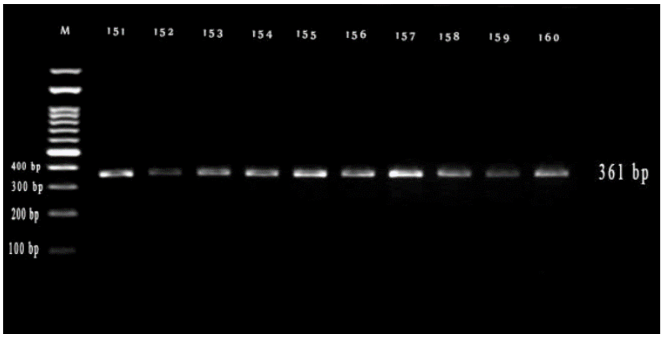


Table 5. Antibiotic susceptibility of MRSA strains and distribution of disinfectant resistance genes.

Antibacterials	Strains harboring disinfectant/antiseptic genes			Number and rate of antibiotic resistant MRSA strains harboring *qacA/B* and/or *smr* genes
	qacA/B (+)	*smr* (+)	*qac A/B* and/or *smr* (+)	
DA	26	3	22	51/95 (53.68%)
DAP	3	1	1	5/5 (100%)
GN	32	9	18	59/87 (67.81%)
TE	22	1	21	44/86 (51.16%)
E	30	3	18	51/76 (67.10%)
CIP	14	3	22	39/74 (52.70%)
TEC	5	11	11	27/48 (56.25%)
TMP/SXT	28	1	22	51/91 (56.04%)
VA	-	-	1	1/1 (100%)

DA: Clindamycin; DAP: Daptomycin; GN: Gentamicin; TE: Tetracycline; E: Erythromycin; CIP: Ciprofloxacin; TEC: Teicoplanin; TMP/SXT: Trimethoprim sulfamethoxazole; VA: Vancomycin.

gentamicin and erythromycin-resistant and qac gene(s) positive MRSA strains was similar (67.81% and 67.10%, respectively). Teicoplanin and trimethoprim sulfamethoxazole resistance of qac gene(s) positive MRSA strains were also similar, with the percentages of 56.25% and 56.04%, respectively. Table 5 represents the antibiotic susceptibility of MRSA harboring *qacA, qacB,* and/or *smr* genes.

By using the Based Upon Repeat Pattern (BURP) algorithm in Ridom StaphType software, the species were separated into complexes that differ from each other by less than 5 repetitions. Accordingly, 3 *spa* clonal complex (CC) were detected. Cluster 1 (CC005) consisted of two isolates of t005 *spa* type and 19 isolates of t223 *spa* type derived from it. Cluster 2 (CC021) contained 3 isolates of t582 *spa* type and 6 isolates of t030 *spa* type. Cluster 3(CC002) contained a single t002 isolate and four t105 isolates derived from it (Figure 3,4).

As a result of the dendogram analysis of the band polymorphism obtained with PFGE using the GEL-COMPAR-II program, it was determined that they gather in 15 clusters and 9 of our isolates identified with capital letters (A;B;C;D;E;F;G;H and I) with multiple members and 6 isolates were single-membered, and 40 sub-clusters belonging to these clusters. It was observed that 34 strains clustered in 13 sub-clusters, a1,a2,a3,…..a13 in cluster A, which is the largest cluster and which has the most sub-clusters (Figure 5).

Fifteen (44.1%) of the strains in cluster A were isolates from patient samples taken from surgical intensive care units, and this number constituted 36% of HA-MRSA strains. The second largest set is cluster B with 21 members, and the largest has 7 subsets with 4 members. Ten (47.61%) of the strains in this cluster consisted of strains isolated from intensive care unit samples. It was observed that 56.05% of HA-MRSA strains isolated from patients hospitalized in the surgical intensive care unit were collected in clusters within A and B clusters (Figure 5, Table 6). In the clonal distribution obtained by *spa* typing, while t030 was defined as the dominant clone in all studies in our country, t223 was determined as the prominent clone in our study. Similar results were obtained in the surgical intensive care unit, which is considered to be risky,

Figure 3. *smr* gene positivity of some strains.

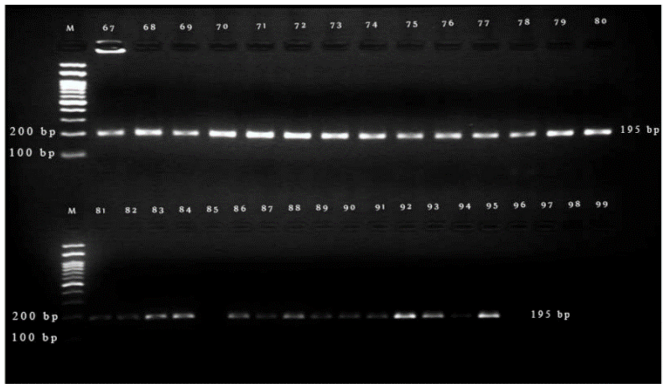


Figure 4. Detection of *spa*CC by BURP algorithm.

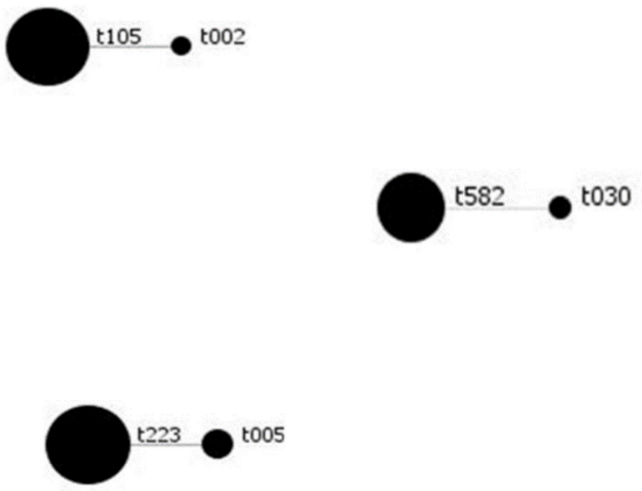
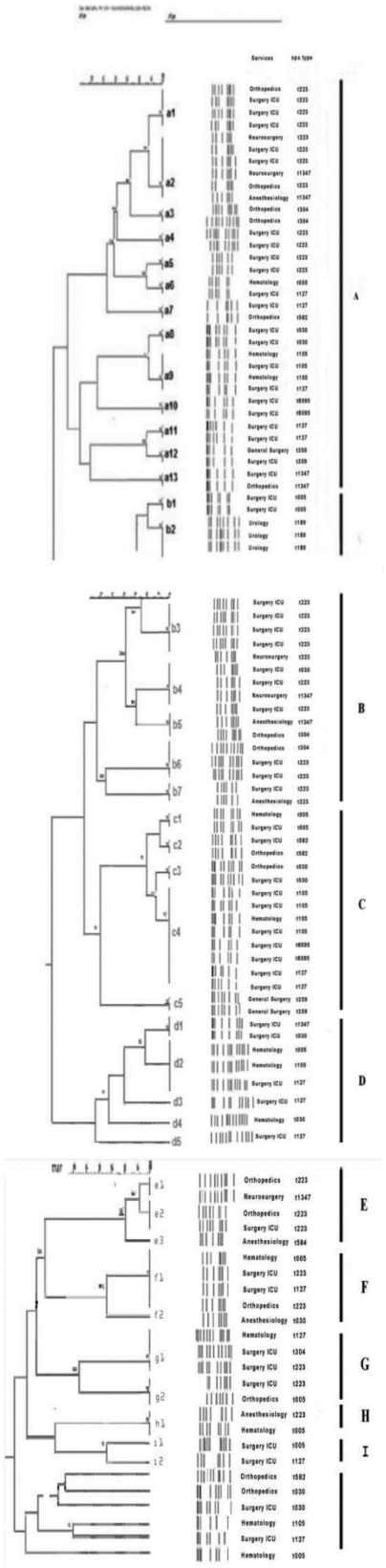


Table 6. The relationship between PFGE patterns and *spa* types of intensive care unit HA-MRSA isolates collected in large clusters.

Clusters	Number of members	Intensive care unit isolates		*spa* type		
		n	%	type	n	%
A	34	15	44.1	t223	9	60
				t127	4	26.7
				t030	2	13.3
B	21	10	47.6	t223	9	90
				t030	1	10
C	16	9	56.25	t105	4	44.4
				t359	2	22.22
				t005	2	22.22
				t030	1	11.11
D	8	5	62.5	t127	3	60
				t030	2	40
E	5	1	20	t223	1	100

Figure 5. Clonal relationships of HA-MRSA isolates included in the study according to PFGE and *spa* typing.



especially for the epidemics of nosocomial infections.

As the data is collected regionally, information about clinical features, treatment strategies, and, more importantly, how to prevent these infections and management of the infection process are of vital importance in local healthcare settings. The epidemiological aspects of the study were thoroughly analyzed, and the PFGE patterns and *spa* types of the MRSA strains were presented according to sample numbers in Table 7. Two groups of ethnic origins (Turkish and Syrian) were detected in the study.

Discussion

MRSA strains are one of the most important causes of high morbidity and mortality, especially in intensive care units. These microorganisms are one of the main factors of nosocomial infections [13]. In Turkey, it has been determined that approximately 27.7-52.1% of nosocomial infections are associated with MRSA [14-16]. In this study, the MRSA isolation rate was found to be 24.06% (103/428). It has been reported that the rate of HA-MRSA, which was determined as 35.1% in 2008 in Turkey, decreased to 18.5% in 2011 [17]. Similarly, there are studies showing that HA-MRSA isolation rates tend to decrease in various studies conducted around the world. For example, it was reported that there was a significant decrease in bacteremias originating from MRSA in the UK as a result of the activity of control programs initiated in 2005, and this decrease was 50% when the 2004 and 2011 data were compared [18-20]. On the other hand, in our study, it was determined that the MRSA isolation frequency from intensive care units was 21.2%. This rate is much higher than 11.2% of all samples.

It was observed that 95% of the types determined as type t223 were collected in A and B PFGE clusters, and 84.21% were isolated from Syrian patient samples by demographic data. These data contradict the literature that t030 spa type is the common *spa* type in Turkey [21]. However, it can be considered important in terms of proving the rightness of the question “The possible contribution of Syrian patients to the MRSA epidemic”, which constitutes the scientific basis of this study. Due to the sustained war since 2011, a large number of Syrian people migrated to different parts of our country, but particularly to our city. The ethnicity, cultural differences, social habits, and habits of communal living may contribute to the spread of infectious diseases as well as clonal and phylogenetic diversities. The Ministry of Health's provision of free medical examination and treatment for Syrian refugees as part of humanitarian assistance has gradually increased the

Table 7. Comparison of Comparison of MRSA strains isolated from various services and intensive care units by PFGE and *spa* types.

PFGE type		*spa* type	Sample Number
A	a1	t223	50, 56, 214, 215
	a2	t223 – t1347	62, 79, 93, 173 - 216, 224
	a3	t304	154, 212
	a4	t223	32, 182
	a5	t223	33, 219
	a6	t005 – t127	36 - 213
	a7	t127 - t582	319 - 3
	a8	t030	304, 411
	a9	t105 - t127	40, 42, 302- 417
	a10	t8095	401, 415
	a11	t127	410, 427
	a12	t359	337, 381
	a13	t1347	97, 282
B	b1	t005,	5, 218
	b2	t189,	102, 121, 125
	b3	t223	60, 83, 234, 235
	b4	t223 - t030 - t1347	82, 397, 420 – 193 - 194
	b5	t1347 – t304	89 - 313
	b6	t304 – t223	137 - 52, 164
	b7	t223	43, 229
C	c1	t005	293, 407
	c2	t582	41, 46
	c3	t030	53, 205
	c4	t105-t8095-t127	90, 202, 203, 208- 309, 383- 406, 414
	c5	t359	81, 207
D	d1	t1347 - t030	1 - 210
	d2	t005 - t105 - t127	6 – 251 - 422
	d3	t127	303
	d4	t030	351
	d5	t127	308
E	e1	t223, t1347	112, 225
	e2	t223	39, 58
	e3	t584	95
F	f1	t005 - t223 – t127	218 – 223, 391 - 69
	f2	t030	99
G	g1	t127 - t304 - t223	51 – 204 - 403
	g2	t223 - t005	301 - 418
H	h1	t223 - t005	12 - 318
I	i1	t005	30
	i2	t127	419

demand for our hospital. The dominance of subtype t223, which differs from the other regions of our country, is thought to be associated with these reasons aforementioned above. The possible relationship between HA-MRSA infections, especially bacteremias, and ethnicity in the United States was investigated in a study. According to a ten-year study conducted in the states of California, Colorado, Connecticut, Georgia, Maryland, Minnesota, New York, Oregon, and Tennessee, incident rates for hospital-associated MRSA infection decreased by over 65% for Black and White people from 2005 through 2013; however, the rate of decrease varied between Black and White patients. Among Black people, incidence rates declined from 18.16 cases per 100,000 Black people to 6.21 cases per 100,000 Black people. In White people, there was a decline from 8.46 cases per 100,000 to 2.94 per 100,000 [22].

In this study, it was determined that there may be *spa* types within a PFGE cluster that are not in the same clonal complex. For example, the presence of t223 and t1347 or t005 and t127 without clonal relationships in cluster A was notable. When David *et al.* examined 149 randomly selected samples, they observed a similar result. In their study, they determined a total of 6 PFGE types and 17 spa types, USA100, USA300, USA500, USA800, USA900, and non-typable (NT), that do not resemble a previously known PFGE pattern. They found that the dominant *spa* type in the USA100 cluster was t002, but there were 3 more *spa* types that were shown to be unrelated by BURP analysis and MLST. Likewise, 3 unrelated *spa* types within the USA500 cluster could not be distinguished from each other by PFGE. In addition, they determined that a total of 8 *spa* types, t008 *spa* type dominant, were included in the USA300 cluster, which is the most frequently detected pulsotype [23]. Clonal and phylogenetic relationships of MRSA t223 serotype, as well as other serotypes, which harbored disinfectant resistance genes, were shown transparently in this study. The major source of

the t223 serotype was various intensive care units, and according to findings, most of the serotype t223 was isolated from Syrian patients by demographical data, when compared with Turkish patients.

In a study performed by Khademi and co-workers, the 24 distinct *spa* types were classified into five *spa*-clonal complex (CC) clusters and 12 singletons, with none assigned to alternative CC groups. These findings emphasize the association between *spa* types and their corresponding CC clusters, underscoring their usefulness in evaluating phylogenetic and clonal links among clinical isolates [24]. In another study, MRSA strains identified as *spa* type t586 were recovered from the blood samples of hospitalized patients in the Czech Republic [25]. In the overall distribution of serotypes, it was determined that nine isolates of the predominant MRSA serotype t223 in the major cluster A were recovered from the surgical intensive care unit. Additionally, five isolates of the t127 serotype were also detected in the same unit. Two t223 serotype isolates were obtained from the orthopedics and traumatology service, and one from the neurosurgery service. In cluster B, which was the second largest, nine MRSA isolates of serotype t223 were identified, along with two isolates of serotype t005 and one isolate of serotype t030, all recovered from the same unit. This indicates that there are clonal and phylogenetic relationships and evolution among the serotypes and that these strains have spread within a single unit.

In infections caused by persistent MRSA strains, it is recommended to immediately start gold standard anti-MRSA antibiotics such as vancomycin and daptomycin, regardless of the antibiogram results [26-30]. In our study, it was observed that 91.26% of HA-MRSA strains were resistant to clindamycin, 72.81% to erythromycin, 87.37% to trimethoprim-sulfamethoxazole, 83.49% to gentamicin, and 82.52% to tetracycline. Among MRSA strains, 4.85% were resistant to daptomycin. It was molecularly investigated the *qacA/B* and *smr* gene distributions for resistance to QACs and chlorhexidine.

Although the distribution of *qac* genes in MRSA and MSSA strains varies around the world, the prevalence is higher in Asia than in Europe and America. It was determined that the frequency of the *qacA/B* gene was 2% and the prevalence of the *smr* gene was 7% in MRSA strains isolated from intensive care patients in North America and Canada (30). In Japan, it was found that 32% of MRSA strains carried the *qacA/B* gene, while this rate was 7.5% in MSSA isolates [31]. In another study conducted in Malaysia, while the *smr* gene carriage was rare in MRSA strains, the prevalence of the *qacA/B* gene was 83% [32]. In a study investigating *qacA/B* and *smr* gene frequencies in MRSA isolates in Europe, *qacA/B* prevalence was found to be 63% and *smr* prevalence to be 6% [33].

In our study, *qacA/B* and/or *smr* genes were found to be positive together in 80 of 103 HA-MRSA strains, and the frequency was found to be 77.66%. This finding is consistent with Malaysian and European data but considerably higher than in Asian countries, North America, and Canada. We think that the increase in disinfectant resistance occurs as a result of frequent use. When we combined the discrimination capabilities of two different epidemiological methods that we used together in our study, we had the opportunity to examine the polymorphism in the total genome and only the repeated region of the *spa* gene in HA-MRSA strains. This gave us a great advantage in revealing the correlation between antiseptic and disinfectant resistance genes and clonal and phylogenetic relationships between isolates.

Conclusions

Molecular typing of *Staphylococcus aureus* plays a crucial role in epidemiological research and the investigation of outbreaks [34]. t223 is the dominant *spa* type in our region, which originated from Syrian patients according to our results. Pulsed-field gel electrophoresis (PFGE) has long been regarded as the reference method for bacterial typing, with a standardized classification system and a well-recognized nomenclature among both researchers and healthcare professionals [35]. The spread of infection and molecular epidemiology are essential to the investigation of hospital outbreaks for the infection committee [36]. Antibiotic resistance was remarkable, especially in strains with both *qacA/B* and *smr* genes. No correlation was found between daptomycin resistance and disinfectant resistance genes. In line with the study findings, the infection control committee was informed, and it was recommended to prevent the excessive routine use of antiseptics and disinfectants, to consider other sterilization and disinfection methods such as UV light and hydrogen peroxide systems as alternatives, to ensure the appropriate selection of antibiotics in MRSA infections, and to provide training for healthcare personnel. These measures aim to prevent clonal spread.

Limitations

The study presents monocentric data with a limited number of staphylococcal strains. So, there is a need for multicenter studies in our region to evaluate data more

comprehensively, which would allow for a clearer determination of the frequencies of the *qacA*, *qacB*, and *smr* genes. Clinical implications, such as the region's demographic structure, the epidemiological status of staphylococcal infections, and the resistance profiles to antiseptics/disinfectants and antibiotics, will shed light on the precautionary measures to be taken by the hospital infection control committee.

Funding
This study is financially supported by Cukurova University.

Ethical Statement
This study is approved by Non-Interventional Researches Ethics Committee with a decision number of 57/10-Oct7th2016 (Date: 07.10.2016, decision number: 57).

Corresponding Author
H. Suphi BAYRAKTAR
Hatay Mustafa Kemal University Experimental Researches Application and Research Center
31000 Hatay-Türkiye
Tel: +90 539 412 412 2
E-mail: drsuphibayraktar@gmail.com
ORCID: 0000-0003-3400-9292

Conflict of interest
No conflict of interest is declared.

References
1. Mlynarczyk-Bonikowska B, Kowalewski C, Krolak-Ulinska A, Marusza W (2022) Molecular mechanisms of drug resistance in *Staphylococcus aureus.* Int J Mol Sci 23: 8088. doi: 10.3390/ijms23158088
2. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VGJr (2015) *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev 28: 603-61. doi: 10.1128/CMR.00134-14
3. Temiz M, Duran N, Duran GG, Eryilmaz N, Jenedi K (2014) Relationship between the resistance genes to quaternary ammonium compounds and antibiotic resistance in staphylococci isolated from surgical site infections. Med Sci Monit 20: 544-50. doi: 10.12659/MSM.890177
4. Pennone V, Prieto M, Álvarez-Ordóñez A, Cobo-Diaz JF (2022) Antimicrobial resistance genes analysis of publicly available *Staphylococcus aureus* genomes. Antibiotics 16: 1632. doi: 10.3390/antibiotics11111632.
5. Azrad M, Shmuel C, Leshem T, Hamo Z, Baum M, Rokney A, Agay-Shay K, Peretz A (2021) Reduced susceptibility to chlorhexidine among *Staphylococcus aureus* isolates in Israel: phenotypic and genotypic tolerance. Antibiotics 23: 342. doi:10.3390/antibiotics10030342.
6. Rodrigues DF, Borges NHPB, Nogueira CES, Tavares JF, Arcanjo DDR, Barreto HM, Siqueira-Junior JP (2022) Modulation of drug resistance by furanochromones in *NorA* overexpressing *Staphylococcus aureus*. Evid Based Complement Alternat Med 17: 9244500. doi: 10.1155/2022/9244500.
7. Baines SL, Jensen SO, Firth N, Gonçalves da Silva A, Seemann T, Carter GP, Williamson DA, Howden BP, Stinear TP (2019) Remodeling of pSK1 family plasmids and enhanced chlorhexidine tolerance in a dominant hospital lineage of methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 63: e02356-18. doi: 10.1128/AAC.02356-18.
8. Al-Trad EI, Chew CH, Che Hamzah AM, Suhaili Z, Rahman NIA, Ismail S, Puah SM, Chua KH, Kwong SM, Yeo CC (2023) The plasmidomic landscape of clinical methicillin-resistant *Staphylococcus aureus* isolates from Malaysia. Antibiotics 9: 733. doi: 10.3390/antibiotics12040733.
9. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2020) Breakpoint tables for interpretation of MICs and zone diameters, Version 10.0.2020. Available: https://www.eucast.org. Accessed: 30 Dec 2020.
10. Rouch DA, Cram DS, Diberardino D, Littlejohn TG, Skurray RA (1990) Efflux-mediated antiseptic resistance gene *qac*A from *Staphylococcus aureus* – common ancestry with tetracycline-transport and sugar-transport proteins. Mol Microbiol 4: 2051–2062. doi: 10.1111/j.1365-2958.1990.tb00565.x.
11. Sasatsu M, Shima K, Shibata Y, Kono M (1989) Nucleotide sequence of a gene that encodes resistance to ethidium bromide from a transferable plasmid in Staphylococcus aureus. Nucleic Acids Res 17: 10103. doi: 10.1093/nar/17.23.10103.
12. Noguchi N, Hase M, Kitta M, Sasatsu M, Deguchi K, Kona M (1999) Antiseptic susceptibility and distribution of antiseptic resistance genes in methicillin-resistant *Staphylococcus aureus.* FEMS Microbiol Lett 172: 347–353. doi: 10.1111/j.1574-6968.1999.tb13475.x.
13. Pannewick B, Baier C, Schwab F, Vonberg RP (2021) Infection control measures in nosocomial MRSA outbreaks-Results of a systematic analysis. PLoS One 16: e0249837. doi: 10.1371/journal.pone.0249837.
14. Arıdoğan A, Atasever L, Bal Ç (2004) Antibiotic resistance of *Staphylococcus aureus* strains isolated from clinical samples. Türk Mikrobiyol Cem Derg 34: 20-23. [Article in Turkish].
15. Ersoy Y, Fırat M, Kuzucu Ç, Bayındır Y, Kaaarslan Ş, Bilişik G, Dinç But A (2003) Hospital infections at Inonu University Faculty of Medicine Hospital. İnönü Üniversitesi Tıp Fakültesi Derg 10: 133-137. [Article in Turkish].
16. Şanlı K (2020) Susceptibility of hospital-acquired and community-acquired *Staphylococcus aureus* strains to various antimicrobials. İKSSTD 12: 188-93. [Article in Turkish].
17. Cetinkol Y, Çakır Ö F, Enginyurt Ö (2013) Yearly changes in methicillin resistance among Staphylococcus aureus strains isolated from blood cultures. ANKEM Derg 27: 38-42 doi: 10.5222/ankem.2013.038. [Article in Turkish].
18. Johnson AP, Davies J, Guy R, Abernethy J, Sheridan E, Pearson A, Duckworth G (2012) Mandatory surveillance of methicillin-resistant *Staphylococcus aureus* (MRSA) bacteraemia in England: the first 10 years. J Antimicrob Chemother 67: 802–809. doi: 10.1093/jac/dkr561.
19. Stone SP, Fuller C, Savage J, Cookson B, Hayward A, Cooper B, Duckworth G, Michie S, Murray M, Jeanes A, Roberts J, Teare L, Charlett A (2012) Evaluation of the national Cleanyourhands campaign to reduce *Staphylococcus aureus* bacteraemia and *Clostridium difficile* infection in hospitals in England and Wales by improved hand hygiene: four year, prospective, ecological, interrupted time series study. BMJ 344: e3005. doi: 10.1136/bmj.e3005.

20. Johnson AP, Pearson A, Duckworth G (2005) Surveillance and epidemiology of MRSA bacteraemia in the UK. J Antimicrob Chemother 56: 455–462. doi: 10.1093/jac/dki266.
21. Guven Gokmen T, Kalayci Y, Yaman A, Koksal F (2018) Molecular characterization of methicillin-resistant *Staphylococcus aureus* strains by spa typing and pulsed field gel electrophoresis methods. BMC Microbiol 18: 155. doi: 10.1186/s12866-018-1305-6.
22. Gualandi N, Mu Y, Bamberg WM, Dumyati G, Harrison LH, Lesher L, Nadle J, Petit S, Ray SM, Schaffner W, Townes J, McDonald M, See I (2018) Racial disparities in invasive methicillin-resistant *Staphylococcus aureus* infections, 2005-2014. Clin Infect Dis 67: 1175-1181. doi: 10.1093/cid/ciy277.
23. David MZ, Taylor A, Lynfield R, Boxrud DJ, Short G, Zychowski D, Boyle-Vavra S, Daum RS (2013) Comparing pulsed-field gel electrophoresis with multilocus sequence typing, spa typing, staphylococcal cassette chromosome mec (SCCmec) typing, and PCR for panton-valentine leukocidin, arcA, and opp3 in methicillin-resistant *Staphylococcus aureus* isolates at a U.S. Medical Center. J Clin Microbiol 51: 814-819. doi: 10.1128/JCM.02429-12.
24. Khademi F, Ghanbari F, Mellmann A, Najafzadeh MJ, Khaledi A (2016) Phylogenetic relationships among *Staphylococcus aureus* isolated from clinical samples in Mashhad, Iran. J Infect Public Heal 9: 639-644. doi:10.1016/j.jiph.2016.01.003
25. Pomorska K, Jakubu V, Malisova L, Fridrichova M, Musilek M, Zemlickova H (2021) Antibiotic resistance, *spa* typing and clonal analysis of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from blood of patients hospitalized in the Czech Republic. Antibiotics 10: 395. doi: 10.3390/antibiotics10040395.
26. Fowler Jr VG, Sakoulas G, McIntyre LM, Meka VG, Arbeit RD, Cabell CH, Stryjewski ME, Eliopoulos GM, Reller LB, Corey GR, Jones T, Lucindo N, Yeaman MR, Bayer AS (2004) Persistent bacteremia due to methicillin-resistant *Staphylococcus aureus* infection is associated with agr dysfunction and low-level in vitro resistance to thrombin-induced platelet microbicidal protein. J Infect Dis 190: 1140–1149. doi: 10.1086/423145.
27. Xiong YQ, Fowler Jr VG, Yeaman MR, Perdreau-Remington F, Kreiswirth BN, Bayer AS (2009) Phenotypic and genotypic characteristics of persistent methicillin-resistant *Staphylococcus aureus* bacteremia in vitro and in an experimental endocarditis model. J Infect Dis 199: 201–208. doi: 10.1086/595738.
28. Hawkins C, Huang J, Jin N, Noskin GA, Zembower TR, Bolon M (2007) Persistent *Staphylococcus aureus* bacteremia: An analysis of risk factors and outcomes. Arch Intern Med 167: 1861–1867. doi: 10.1001/archinte.167.17.1861.
29. Khatib R, Johnson LB, Fakih MG, Riederer K, Khosrovaneh A, Shamse Tabriz M, Sharma M, Saeed S (2006) Persistence in *Staphylococcus aureus* bacteremia: incidence, characteristics of patients and outcome. Scand J Infect Dis 38: 7–14. doi: 10.1080/00365540500372846.
30. Longtin J, Seah C, Siebert K, McGeer A, Simor A, Longtin Y, Low DE, Melano RG (2011) Distribution of antiseptic resistance genes qacA, qacB, and smr in methicillin-resistant *Staphylococcus aureus* isolated in Toronto, Canada, from 2005 to 2009. J Antimicrob Chemother 55: 2999-3001. doi: 10.1128/AAC.01707-10.
31. Alam MM, Kobayashi N, Uehara N, Watanabe N (2003) Analysis on distribution and genomic diversity of high-level antiseptic resistance genes *qacA* and *qacB* in human clinical isolates of *Staphylococcus aureus*. Microb Drug Resist 9: 109–121. doi: 10.1089/107662903765826697.
32. Shamsudin MN, Alreshidi MA, Hamat RA, Alshrari AS, Atshan SS, Neela V (2012) High prevalence of *qacA/B* carriage among clinical isolates of meticillin-resistant *Staphylococcus aureus* in Malaysia. J Hosp Infect 81: 206–208. doi: 10.1016/j.jhin.2012.04.015.
33. Mayer S, Boos M, Beyer A, Fluit AC, Schmitz FJ (2001) Distribution of the antiseptic resistance genes *qacA*, *qacB* and *smr* in 497 methicillin-resistant and -susceptible European isolates of *Staphylococcus aureus*. J Antimicrob Chemother 47: 896–897. doi: 10.1093/jac/47.6.896.
34. Faria NA, Carrico JA, Oliveira DC, Ramirez M, Lencastre H (2008) Analysis of typing methods for epidemiological surveillance of both methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains. J Clin Microbiol 46: 136–144. doi: 10.1128/JCM.01684-07.
35. McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, Tenover FC (2003) Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. J Clin Microbiol 41: 5113–5120. doi: 10.1128/JCM.41.11.5113-5120.2003.
36. Rodriguez M, Hogan PG, Satola SW, Crispell E, Wylie T, Gao H, Sodergren E, Weinstock GM, Burnham CD, Fritz SA (2015) Discriminatory indices of typing methods for epidemiologic analysis of contemporary *Staphylococcus aureus* strains. Medicine 94: e1534. doi: 10.1097/MD.0000000000001534.