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Evaluation of aminoglycoside- and methicillin-resistant *Staphylococcus aureus*: phenotypic and genotypic insights from clinical specimens in Ardabil, Iran

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Abstract

Background Combination therapy including an aminoglycoside antibiotic and a cell-wall active agent is considered the most suitable option to treat invasive infections with methicillin-resistant *Staphylococcus aureus* (MRSA). Dual drug therapy enhances the effectiveness of treatment and reduces the risk of resistance development. This study aims to elucidate the phenotypic and molecular resistance to aminoglycosides and methicillin, and the molecular epidemiologic characteristics of *S. aureus* in Ardabil northwest Iran.

Methods Totally, 118 *S. aureus* isolates collected from clinical specimens were investigated. Identification was performed using standard microbiological and molecular approaches. Aminoglycoside and methicillin resistance were evaluated using the disk diffusion assay, and the minimum inhibitory concentrations (MICs) of aminoglycosides were determined via the agar dilution method. The *mecA* gene encoding methicillin resistance and aminoglycoside modifying enzymes (AMEs) genes were detected using PCR. Molecular epidemiologic features of the isolates were determined using staphylococcal cassette chromosome *mec* (SCC*mec*) typing *spa* typing and ERIC-PCR assays.

Results Of the isolates, 42.4% ($n=50$) and 57.6% ($n=68$) were identified as MRSA and MSSA, respectively. All MRSA isolates were *mecA*-positive. Among MRSA isolates, SCC*mec* type IVa (17; 34%) was predominant, followed by types IVc, V, III, II, and I. Resistance rates to gentamicin, kanamycin, tobramycin, and amikacin were 16.1%, 17.8%, 8.5%, and 8.5%, respectively. Overall, the aminoglycoside resistance and most non-aminoglycoside antibiotics were significantly higher in MRSA versus MSSA isolates. The prevalence of AME genes was as follows: *aac(6')-Ie-aph(2")* (30; 76.9%), *aph(2")-Ib* (22; 56.4%), and *ant(4')-Ia* (14; 35.9%). About 60% of aminoglycoside-resistant isolates harbored ≥ 2 AME genes. The t030 type was the most common *spa* type identified. The ERIC-PCR profiles categorized the isolates into 19 unique ERIC types.

Conclusions This study reveals high aminoglycoside and methicillin resistance in *S. aureus* isolates from Ardabil hospitals. Predominant SCC*mec* type IVa and *spa* type t030 indicate specific molecular patterns. These findings highlight the need for continuous surveillance and targeted treatment strategies for MRSA infections.

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Keywords *Staphylococcus aureus*, MRSA, MSSA, Aminoglycoside-resistance, Methicillin-resistance, SCC*mec* typing, *Spa* typing

Introduction

Staphylococcus aureus (*S. aureus*), a Gram-positive coccus, is a significant pathogen responsible for nosocomial- and community-acquired infections. Common manifestations of *S. aureus* infections range from toxin-mediated diseases, such as food poisoning, scalded skin syndrome, and toxic shock syndrome, to invasive conditions like cutaneous infections, bacteremia, pneumonia, osteomyelitis, septic arthritis, and endocarditis [1]. While *S. aureus* infections are traditionally treated with common antibiotics, the bacterium's capacity to develop resistance, particularly to semisynthetic penicillins like methicillin, poses a challenge to effective treatment [2]. Methicillin-resistant *S. aureus* (MRSA) strains resist nearly all β-lactam antibiotics, including penicillins, cephalosporins, carbapenems, and frequently other antibiotics [2]. The emergence of MRSA is attributed to the *mec* genes (*mecA*, *mecB*, and *mecC*), which encode a novel penicillin-binding protein, PBP2a, that exhibits low affinity for β-lactam antibiotics [3]. The *mec*A gene is widespread among staphylococcal species and is carried on a mobile genetic element known as the staphylococcal cassette chromosome *mec* (SCC*mec*). This element comprises two main components: the *ccr* gene complex and the *mec* gene complex. To date, thirteen SCC*mec* (I-XIII) types have been identified in MRSA strains [4]. The prevalence of MRSA varies widely across different regions. In Europe, MRSA rates range from less than 1% to over 50% among European Union (EU) member states [5]. In countries around the Persian Gulf, MRSA regional prevalence is reported as 25–35% [6]. Up to 90% of S. aureus infections in some areas are MRSA [7]. The overall prevalence of MRSA in Iran is notably high (43%), with significant regional variations and a growing trend over time [8].

MRSA isolates are often classified as multidrug-resistant (MDR) organisms, though not all MRSA isolates exhibit multidrug resistance. This characteristic leaves limited therapeutic options for treating these infections [2]. Compared to drug-sensitive forms, MRSA infections significantly increase the risk of death by 64%. They are associated with severe conditions such as septicemia, septic shock, and pneumonia [7]. Vancomycin has historically been the treatment of choice for MRSA-associated infections; however, the prevalence of vancomycin-resistant MRSA strains is rising [9].

Aminoglycoside antibiotics are renowned for their broad-spectrum antibacterial effects and are particularly effective in treating systemic infections caused by Gram-negative bacteria. Recent research has highlighted their potential efficacy against MRSA, positioning aminoglycosides as promising agents against MRSA infections [10]. These antibiotics, often administered alongside other antibiotics such as beta-lactams or glycopeptides, are critical for treating severe life-threatening staphylococcal infections. Such infections include endocarditis, a serious condition, affecting the heart valves [11]. However, aminoglycoside resistance, particularly among MRSA isolates, is a growing concern. A survey conducted in Poland found that 66.7% of MRSA isolates were resistant to at least one aminoglycoside antibiotic [12].

Aminoglycoside resistance emerges through several mechanisms, such as enzymatic drug modification, alterations in ribosomal drug-binding sites, and decreased drug permeability [13]. The primary mechanism of aminoglycoside resistance involves the production of aminoglycoside-modifying enzymes (AMEs), which are categorized into three groups: aminoglycoside-acetyltransferases (AACs) encoded by *aac(6')-Ie/aph(2")-Ia*, aminoglycoside-phosphotransferases (APHs) encoded by *aph(3')-IIIa*, *aph(2′)-Ib*, *aph(2′)-Ic*, *and aph(2′)-Id* and aminoglycoside-nucleotidyltransferases (ANTs) encoded by *ant(4')-Ia*, *ant(6)-Ia* and *ant(9)-Ia* genes [14]. Genes encoding AMEs are often located on mobile genetic elements like transposons (e.g., Tn4001) and plasmids, facilitating horizontal gene transfer among bacteria [15]. The prevalence of specific resistance genes can vary by region. For example, in Iran, a high prevalence of the *aac(6')-Ie/aph(2")-Ia* gene has been reported [16].

Given the high prevalence of MRSA in Iran and the growing concern about aminoglycoside resistance, continuous surveillance and updated evidence-based guidelines are crucial for effective management. In light of the limited data on the molecular characteristics of MRSA isolates and their susceptibility to aminoglycosides in Ardabil hospitals, Iran, this study aims to investigate the prevalence of SCC*mec* types, *spa* genotypes, and aminoglycoside resistance patterns among *S. aureus* isolates from clinical specimens collected at teaching hospitals affiliated with Ardabil University of Medical Sciences. This investigation will provide critical insights for developing targeted interventions, enhancing infection control measures, and improving treatment outcomes. Furthermore, understanding the genetic features of *S. aureus* strains circulating in our region will contribute to global efforts in combating antibiotic resistance.

Materials and methods

Bacterial strains and isolates

In this cross-sectional study, we collected 118 clinical isolates from various specimens obtained from inpatients. The specimens included blood ($n = 38$), urine ($n = 23$), wound ($n = 13$), sputum ($n = 22$), tracheal tube ($n = 19$), and cerebrospinal fluid (CSF) ($n = 3$). The study was conducted at four hospitals in Ardabil, Iran, over 16 months, from February 2017 to June 2018. The hospitals involved in the study included Imam, a 555-bed referral hospital with 23 wards and 11,152 annual admissions; Buali, a children's hospital with 150 beds, 8 wards, and 6,089 annual admissions; Alavi, a women's hospital with 220 beds, 10 wards, and 8,771 annual admissions; and Fatemi, a trauma hospital with 220 beds, 14 wards, and 10,513 annual admissions, all in 2024.

All isolates were cultured on a nutrient agar medium for identification. Confirmation was achieved through Gram staining and biochemical tests, including catalase, tube coagulase, DNase, and mannitol utilization. The isolates underwent further characterization using polymerase chain reaction (PCR). Specifically, we targeted the partial sequence of the *nuc* gene, which encodes the thermostable DNase of *S. aureus* as previously described [17–19]. Genomic DNA was extracted using a commercially available DNA extraction kit (CinaGene, Tehran, Iran), following the manufacturer's instructions—the extracted DNA served as the template for this experiment and subsequent molecular analysis.

Pure cultures were preserved in 10% glycerol at -70 °C in a Tryptic Soy Broth medium (TSB). Furthermore, these isolates were also investigated for macrolide-lincosamide resistance and virulence genes, as documented in our previous study [20].

Susceptibility testing

Antimicrobial susceptibility testing was conducted on 118 *S. aureus* strains using the disc diffusion method. The strains were tested against various antibiotics (Padtan Teb, Tehran, Iran), including penicillin (5 µg), rifampin (5 µg), tetracycline (30 µg), nitrofurantoin, chloramphenicol (30 µg), trimethoprim-sulfamethoxazole (30 µg), cefazolin (30 µg), imipenem (10 µg), ciprofloxacin (5 µg), amikacin (30 µg), gentamicin (10 µg), tobramycin (10 µg), and kanamycin (30 µg). The testing was performed on Muller-Hinton agar (Bio Life Italy) following the Clinical and Laboratory Standards Institute (CLSI) guidelines [21]. Isolates that showed resistance to at least one agent in three or more antibiotic categories were defined as Multidrug-Resistant (MDR) [17].

The agar dilution method determined the minimum inhibitory concentrations (MICs) for gentamicin, tobramycin, and kanamycin (Bio Basics, Inc., Ontario, Canada). According to current CLSI guidelines, only gentamicin is included for susceptibility testing of *S. aureus* for aminoglycosides. Consequently, this study's interpretation of MIC results for amikacin, tobramycin, and kanamycin was based on an earlier version of the CLSI guidelines [22].

MRSA detection and molecular analysis

Methicillin resistance was detected using a cefoxitin disk (Fox, 30 µg) following the protocols outlined by CLSI guidelines [21]. Isolates with an inhibition zone diameter of ≤ 21 mm were classified as MRSA [21].

To validate MRSA isolates, we specifically amplified the *mec*A gene using PCR. Additionally, to rule out the oxacillin-susceptible *mecA*-positive *S. aureus* genotype, the *mecA* gene was also screened in MSSA isolates. The DNA extracts a regular form, as *staphylococci* were processed with primers and conditions meticulously outlined in Supplementary Table 1.

SCC*mec* types were identified in MRSA isolates based on previous reports [18, 23]. The primer sequences and PCR conditions are listed in Supplementary Table 1.

DNA from previously identified MRSA isolates with the *mecA* gene and known SCC*mec* types served as the positive controls [17].

Detection of genes encoding aminoglycoside modifying enzymes

The frequency of AMEs encoding genes was investigated in isolates that exhibited resistance to at least one of the aminoglycoside antibiotics. The primer sequences for PCR amplification are detailed in Table S1. Multiplex PCR assays were conducted to detect the presence of common AME-encoding genes, including *aac(6′)-Ie/aph(2′′)*, *aph(2")-Ib*, *ant(4′)Ia*, and *aph(3 '') IIIa*. DNA from AME-confirmed isolate was used as a positive control during PCR testing [24].

spa typing

The X region of the *spa* gene in 20 aminoglycoside-resistant isolates was amplified using PCR, as described [17]. The primer sequences and PCR conditions can be found in Table S1. Both strands of the amplicons were sequenced by Bioneer in South Korea. The *spa* types were identified using the Ridom StaphType software, accessible online at www.spaserver.ridom.de.

Enterobacterial repetitive intergenic consensus (ERIC)-PCR

ERIC genotyping of the isolates was performed using the specified primers outlined in Table S1, adhering to methodologies previously established [18]. To ensure high intra-laboratory reproducibility of our ERIC-PCR assays, we meticulously implemented standardized protocols, utilized consistent batches of reagents, and regularly calibrated our equipment. Our laboratory personnel

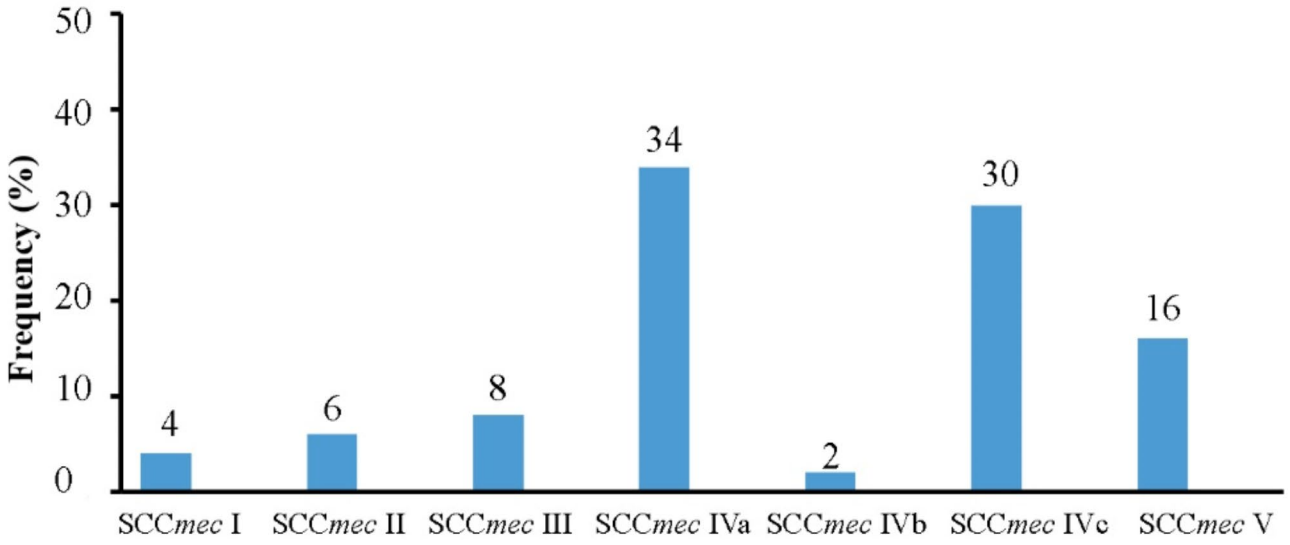

Fig. 1 Frequency of SCC*mec* types in MRSA isolates

Table 1 Resistance phenotypes of different antibiotics determined using the disc diffusion method

Antibiotic	MSSA *N* = 68 n (%)			MRSA *N* = 50 n (%)			*p*-value	Total *N* = 118 n (%)		
	S	I	*R*	S	I	*R*		S	I	*R*
Penicillin	68(100)	0(0)	0(0)	0 (0)	0(0)	50(100)	0.0001	68 (57.6)	0(0)	50(42.4)
Cefazolin	66(97.1)	0(0)	2(2.9)	35(70)	3(6)	12(24)	0.0001	101 (85.6)	3(2.5)	14(11.9)
Imipenem	68(100)	0(0)	0(0)	0(0)	0(0)	50(100)	0.0001	118 (100)	0(0)	0(0)
Gentamicin	62(91.2)	0(0)	6(8.8)	37(74)	0(0)	13(26)	0.012	99 (83.9)	0(0)	19(16.1)
Amikacin	67(98.5)	0(0)	1(1.5)	41(82)	0(0)	9(18)	0.006	108 (91.5)	0(0)	10(8.5)
Tobramycin	67(98.5)	0(0)	1(1.5)	41(82)	0(0)	9(18)	0.001	108 (91.5)	0(0)	10(8.5)
Kanamycin	64(94.1)	0(0)	4(5.9)	33(66)	0(0)	17(34)	0.0001	97(82.2)	0(0)	21(17.8)
Rifampin	68(100)	0(0)	0(0)	45(90)	0(0)	5(10)	0.008	113 (95.8)	0(0)	5(4.2)
Tetracycline	53(77.9)	0(0)	15(22)	35(70)	0(0)	15(30)	0.328	88 (74.6)	0(0)	30(25.4)
Chloramphenicol	56(82.3)	0(0)	12(17.7)	49(98)	0(0)	1(2)	0.007	105 (89)	0(0)	13(11)
Nitrofurantoin	67(98.5)	1(1.5)	0(0)	48(96)	1(2)	1(2)	0.490	115 (97.5)	2(1.7)	1(0.8)
Trimethoprim-Sulfamethoxazole	64(94.1)	2(2.9)	2(2.9)	45(90)	0(0)	5(10)	0.139	109 (92.4)	2(1.7)	7(5.9)
Ciprofloxacin	59(86.8)	0(0)	9(13.2)	43(86)	0(0)	7(14)	0.905	102 (86.4)	0(0)	16(13.6)

underwent thorough training, and positive and negative controls were included in each run to monitor performance. Multiple replicates of the same sample in each experiment were analyzed, and standardized data analysis methods were employed. Consequently, our ERIC-PCR assays demonstrated high reproducibility within our laboratory, providing reliable and consistent results across different runs. The analysis of ERIC patterns was conducted using GelQuest software version 3.3.5.0. Similarity among ERIC-PCR profiles was assessed using the Dice coefficient and the unweighted pair group method with arithmetic mean (UPGMA). Isolates demonstrating an 80% or greater similarity were grouped into the same clusters and considered clonally related.

Statistical analysis

The chi-square test was used to analyze the data. A *P*-value ≤ 0.05 was considered statistically significant.

Results

According to the cefoxitin disc assay, 42.4% (50 out of 118) of the isolates were identified as MRSA. All MRSA isolates were positive for the *mecA* gene, while no *mecA*-positive results were found among the MSSA isolates. Notably, various SCC*mec* types were observed, including types I, II, III, IVa, IVb, IVc, and V. Specifically, types IVa (34%; $n = 17/50$) and IVc (30%; $n = 15/50$) were the most prevalent. SCC*mec* type IVd was not detected (Fig. 1).

Table 1 illustrates the antibiotic resistance pattern of the isolates. Among non-aminoglycoside antibiotics, the highest resistance rates were observed for penicillin (42.4%), tetracycline (25.4%), cefazolin (11.9%),

Table 2 Resistance phenotypes of aminoglycoside antibiotics determined using the agar dilution method

MIC (µg/ml)	Gentamicin			Kanamycin			Tobramycin		
	Resistant *N* = 19 (%)	Susceptible *N* = 99 (%)	Total *N* = 118 (%)	Resistant *N* = 21 (%)	Susceptible N = 97 (%)	Total *N* = 118 (%)	Resistant *N* = 10 (%)	Susceptible = 108 (%)	Total *N* = 118 (%)
0.125	0 (0)	57 (57.6)	57 (48.4)	0 (0)	58 (59.8)	58 (49.2)	0 (0)	59 (54.6)	59 (50)
0.25	0 (0)	17 (17.1)	17 (14.4)	0 (0)	0 (0)	0 (0)	0 (0)	40 (37)	40 (33.9)
0.5	0 (0)	3 (3)	3 (2.5)	0 (0)	0 (0)	0 (0)	0 (0)	5 (4.6)	5 (4.2)
1	0 (0)	8 (8.1)	8 (6.8)	0 (0)	0 (0)	0 (0)	0 (0)	4 (3.7)	4 (3.4)
2	0 (0)	4 (4)	4 (3.4)	0 (0)	16 (16.5)	16 (13.6)	0 (0)	0 (0)	0 (0)
4	0 (0)	5 (5.1)	5 (4.2)	0 (0)	8 (8.2)	8 (6.8)	0 (0)	0 (0)	0 (0)
8	0 (0)	5 (5.1)	5 (4.2)	0 (0)	5 (5.2)	5 (4.2)	0 (0)	0 (0)	0 (0)
16	4 (21.1)	0 (0)	4 (3.4)	0 (0)	10 (10.3)	10 (8.5)	0 (0)	0 (0)	0 (0)
32	3 (15.8)	0 (0)	3 (2.5)	0 (0)	0 (0)	0 (0)	3(30)	0 (0)	3 (2.5)
64	3 (15.8)	0 (0)	3 (2.5)	5 (23.8)	0 (0)	5 (4.2)	2(20)	0 (0)	2 (1.7)
128	4 (21)	0 (0)	4 (3.4)	3 (14.3)	0 (0)	3 (2.5)	4(40)	0 (0)	4 (3.4)
256	5 (26.3)	0 (0)	5 (4.2)	4 (19)	0 (0)	4 (3.4)	1(10)	0 (0)	1(0.9)
512	0 (0)	0 (0)	-	9 (42.9)	0 (0)	9 (7.6)	0 (0)	0 (0)	-
MIC_{50}	≥ 64	≥ 0.125	≥ 0.25	≥ 256	≥ 0.125	≥ 2	≥ 64	≥ 0.125	≥ 0.125
MIC_{90}	≥ 256	≥ 2	≥ 64	≥ 512	≥ 8	≥ 256	≥ 128	≥ 0.25	≥ 1

Resistance breakpoints: Gentamicin ≥ 16 (µg/ml), Kanamycin ≥ 64 (µg/ml), and Tobramycin ≥ 16 (µg/ml)

Table 3 Distribution frequency of aminoglycoside-resistance genes in *Staphylococcus aureus* strains

Organism	Total *N* = 39 (%)	aac(6´)-Ie/aph(2") *n* (%)	aph(2")-Ib *n* (%)	ant(4')-Ia *n* (%)
MSSA	11 (28.2)	7 (63.6)	6 (54.5)	6 (54.5)
MRSA	28 (71.8)	22 (78.6)	18 (64.3)	8 (28.6)
Total	39 (100)	29 (74.4)	24 (61.5)	14 35.9)

-No isolate was positive for *aph (2") Ic*, *aph (2") Id*, and *aph (3") IIIa* genes

ciprofloxacin (13.6%), and chloramphenicol (11%). Conversely, resistance against rifampin (4.2%) nitrofurantoin (0.8%), trimethoprim-sulfamethoxazole (5.9%), and imipenem (0%) was infrequent. Regarding aminoglycoside antibiotics, 17.8% of isolates were resistant to kanamycin, 16.1% to gentamicin, 8.5% to amikacin, and 8.5% tobramycin.

Overall, the resistance rate in MRSA isolates was significantly higher compared to MSSA isolates ($P \leq 0.05$). Furthermore, 24% ($n = 12/50$) of MRSA and 11.86% ($n = 8/68$) of MSSA isolates exhibited the MDR phenotype.

Additionally, the MICs of gentamicin, kanamycin, and tobramycin were determined. The results aligned with the disc diffusion assay, and the resistance frequency rates were similar in both tests (Table 2). MIC_{50} and MIC_{90} values for gentamicin were ≥ 64 µg/ml and ≥ 256 µg/ml, respectively. Similarly, kanamycin had MIC_{50} and MIC_{90} values of ≥ 256 µg/ml and ≥ 512 µg/ml, and tobramycin had values of ≥ 64 µg/ml and ≥ 128 µg/ml.

Molecular analyses revealed out of 39 aminoglycoside-resistant isolates 29 (74.4%) were positive for *aac(6´)-Ie/aph(2´´)*, 24 (61.5%) for *aph(2")-Ib*, and 14 (35.9%) for *ant(4´)Ia* genes (Table 3). Notably, *aph (2 ") Ic*, *aph (2 ") Id*, and *aph (3 ") IIIa* genes were not detected in this study.

Overall, six distinct AME-encoding gene profiles were identified among aminoglycoside-resistant isolates. MRSA isolates exhibited more diverse combination patterns, with 17.9% of isolates coextending all three AME-encoding genes (Table 4).

Furthermore, among the 20 aminoglycoside-resistant strains analyzed in this study and the 19 macrolide-co-resistant isolates from our previous study [20], 11 distinct *spa* types were identified. The most prevalent were *spa* types t030 (28.2%) and t310 and t2018 (each 16%), followed by t078 (12.8%), t026, t02, and t0304 (each 5.1%). Additionally, t14870, t0267, t021, and t790 were each observed in 2.5% of the strains.

Figure 2 displays the genotyping profiles of 39 aminoglycoside-resistant *S. aureus* strains, identified through ERIC-PCR fingerprinting. Each isolate generated between 7 and 17 amplicons, with sizes ranging from 50 to 150 base pairs. Using an 80% similarity threshold, the ERIC-PCR profiles categorized the isolates into 19 unique ERIC types.

Table 4 Distribution of aminoglycoside resistance genes profile in aminoglycoside-resistant *Staphylococcus aureus* strains

	Genes profile	MRSA *N* = 28 (%)	MSSA *N* = 11 (%)	Total *N* = 39 (%)
R_1	*aac(6´)-Ie/aph(2″)*	8 (28.6)	2 (18.2)	10 (25.6)
R_2	*aph(2″)-Ib*	3 (10.7)	3 (27.3)	6 (15.4)
R_3	*aac(6´)-Ie/aph(2″), aph(2″)-Ib*	6 (21.4)	2 (18.2)	8 (20.5)
R_4	*aac(6´)-Ie/aph(2″), ant(4´)-Ia*	2 (7.1)	4 (36.3)	6 (15.4)
R_5	*ant(4´)-Ia, aph(2″)-Ib*	4 (14.3)	0 (0)	4 (10.2)
R_6	*aac(6´)-Ie/aph(2″), ant(4´)-Ia, aph(2″)-Ib*	5 (17.9)	0 (0)	5 (12.8)

Discussion

Approximately 42.4% of the isolates in our hospitals were identified as MRSA, highlighting the persistent challenge of MRSA infections in clinical settings. A similar prevalence rate was reported for MRSA in clinical specimens in Ardabil [25]. However, our rate is slightly lower than those observed in other Iranian cities, which range from 58 to 64.7% [26–28]. While MRSA prevalence has decreased in some regions, it remains alarmingly high in others, with a staggering 93.9% prevalence reported in southern China [29]. These findings emphasize the need for continued vigilance in infection control practices and highlight the regional and temporal variations in MRSA epidemiology.

This study sheds light on the prevalence of MRSA and the genetic diversity of SCC*mec* types. Understanding these patterns is crucial for infection control strategies, the development of targeted therapies, and surveillance efforts. SCC*mec* types IVa (34%) and IVc (30%) were the most prevalent, suggesting a community origin for most MRSA strains. These findings align with previous reports from Ardabil, where MRSA harboring SCC*mec* types IVa and IVc predominated, even in healthy individuals [17]. Notably, the occurrence of MRSA strains with genetic backgrounds of both HA-MRSA (e.g., SCC*mec* types I, II, or III) and CA-MRSA (e.g., SCC*mec* types IV or V) in either community, hospital, and environmental settings is evident [17, 30]. This cross-occurrence underscores the complexity of MRSA epidemiology from a holistic health perspective.

MRSA is generally more resistant to multiple antibiotics than MSSA, with a higher prevalence of MDR phenotypes. In our study, 24% of MRSA isolates were MDR, showing predominant resistance to beta-lactam antibiotics. MRSA carries the *mecA* gene, which confers resistance to β-lactam antibiotics [31]. In contrast, studies from Iran and Malaysia reported higher MDR rates, with 91.3% and 66% of MRSA isolates classified as MDR, respectively [32, 33]. The lower MDR rate in the current study could be attributed to the SCC*mec* types identified in our MRSA isolates, which mainly belonged to CA-MRSA. HA-MRSA typically shows higher multidrug resistance than CA-MRSA [4]. Furthermore, 11.68% of MSSA isolates in this study were also MDR, with predominant resistance to tetracycline and chloramphenicol. Similar reports from Iran and Malaysia indicated that 8% and 14% of MSSA isolates were MDR, respectively [32, 33]. However, while MDR is generally less common in MSSA isolates, higher rates of up to 84% have been reported in some studies [34].

Although aminoglycoside use declined due to newer antibiotic classes, the emergence of MDR pathogens has reignited interest in this class [35]. Aminoglycosides remain a mainstay for treating severe infections caused by gram-negative bacilli [35]. However, their role extends to life-threatening invasive staphylococcal and enterococcal infections, particularly endocarditis [36]. They often synergize with active antibiotics on the cell wall, improving treatment efficacy [37]. Therefore, aminoglycoside resistance in staphylococci significantly limits therapeutic options. Careful monitoring and consideration of local resistance patterns are crucial for their use. Our study identified low resistance to gentamicin (16.1%) and kanamycin (17.8%), with even lower resistance rates for amikacin and tobramycin (8.5%). Notably, these resistance rates are considerably lower than those reported from other Iranian cities. In various Iranian studies, resistance rates for gentamicin ranged from 84.5 to 98.7% [27, 38, 39], amikacin resistance was reported at 98% [16], kanamycin resistance ranged from 86.3 to 97% [27, 39], and tobramycin resistance ranged from 82 to 98% [16, 27]. Antibiotic resistance exhibits significant geographical variation due to multiple factors, including differences in antibiotic usage, local infection control practices, and environmental conditions [40]. In European countries, the overall resistance to gentamycin was reported at about 3% in MSSA and 23% in MRSA isolates [41]. However, the low incidence of resistance in Ardabil reminds us that aminoglycosides may still be viable for treating many serious *S. aureus* infections in our settings.

Aminoglycoside-modifying enzymes (AMEs) are crucial in resistance to aminoglycosides among various bacterial species. The distribution and host range of genes encoding these enzymes show significant geographical variations. In our aminoglycoside-resistant isolates, the most common AME-encoding genes were *aac(6')-Ie/aph(2")-Ia* (MRSA: 78.6%, MSSA: 63.6%), *aph(2")-Ib* (MRSA: 64.3%, MSSA: 54.5%), and *ant(4')-Ia* (MRSA:

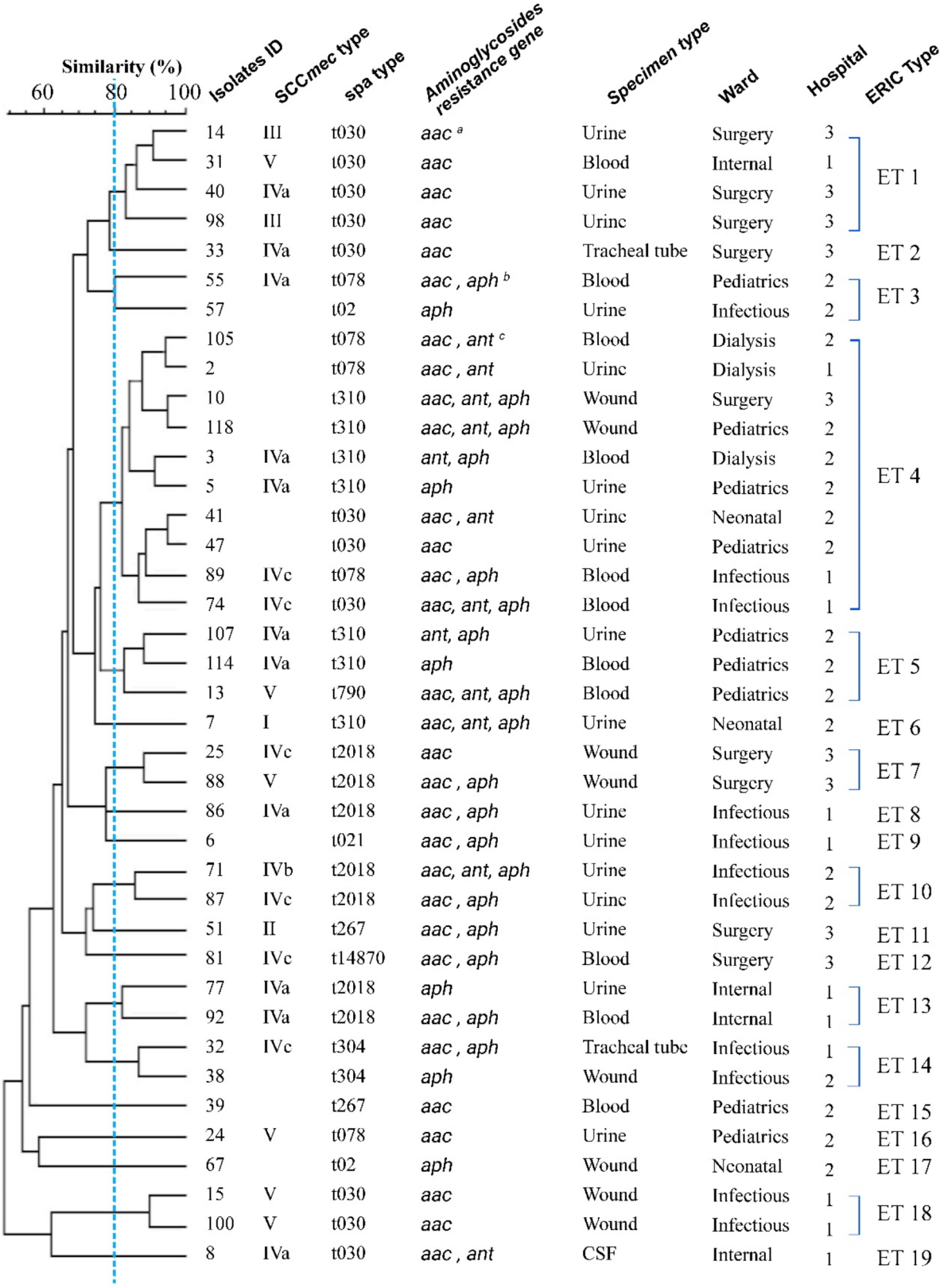

Fig. 2 Dendrogram of the ERIC-based DNA fingerprint analysis using GelcomparII software. [a].aac: *aac(6´)-Ie/aph(2´´)*, [b]. *ant*: *ant(4´)Ia*[c]*aphB*: *aph(2")-Ib*
Hospitals: 1: Imam, 2: Boali, 3: Fatemi, 4: Alavi

28.6%, MSSA: 54.5%). This partially aligns with findings from similar domestic and international studies.

A meta-analysis indicated that *aac(6')-Ie/aph(2")-Ia* is the most prevalent resistance gene, present in 67.7% of MRSA and 55.2% of MSSA isolates, with *ant(4')-Ia* showing a prevalence of 45.3% in MRSA and 35.3% in MSSA across Iran [42]. The *aph(3')-IIIa* gene is also commonly found, with prevalence rates of up to 84.3% in a study in Tehran [43]. Similar results have been reported worldwide.

In Poland, the most prevalent AME genes identified in staphylococci isolates include *aac(6')-Ie + aph(2')* (28.9%), *ant(4')-Ia* (26.7%), and *aph(3')-IIIa* (15.6%) [44]. In another study in Turkey, the *aac(6')/aph(2")* gene was determined in 66% of the isolates, *ant(4')-Ia* gene in 24%, and *aph(3')-IIIa* gene in 8% [45]. However, in our study, *aph(2")-Ib* emerged as the second most prevalent gene among MRSA and MSSA isolates. This discrepancy underscores the potential variations in aminoglycoside resistance mechanisms among different bacterial populations and geographical regions.

AMEs exhibit a preferred affinity for specific aminoglycoside antibiotics. Notably, the bifunctional enzyme *aac(6')-Ie/aph(2")-Ia* provides resistance to nearly all aminoglycosides, except for streptomycin [46]. This enzyme is crucial in conferring high-level gentamicin resistance to *Enterococcus* and *Staphylococcus* isolates [16, 24, 47, 48]. In our study, 17.6% of MRSA isolates harbored three AME-encoding genes, which aligns with previous findings by Fatholahzadeh et al. who reported a 21% prevalence of such isolates [39]. The coexistence of multiple AME genes in single isolates complicates treatment options, as bacteria can exhibit resistance to a broad spectrum of aminoglycosides [43].

This investigation identified 10 distinct *spa* types among the aminoglycoside-resistant *S. aureus* isolates. Among these, the *spa* types t030 and t310 were the most prevalent, including MRSA isolates. It is suggested that certain *spa* types may confer an advantage to the bacteria, such as increased virulence or resistance to the host immune system [49]. Although a direct correlation between specific spa types and distinct antibiotic resistance profiles has not been conclusively established, our findings align with those reported in Shiraz and Ardabil, Iran, where spa type t030 was predominantly observed among clinical and foodstuff-derived MRSA strains [50, 51]. Conversely, a parallel study conducted within the same demographic—healthy teenage students—yielded different results, with spa types t11332 (14.3%) and t012 (11.4%) emerging as the most common [18]. The technique of *spa* typing represents a formidable technique for the high-resolution and quick identification of the genetic diversity in *S. aureus* strains [52]. It plays a key role in tracking and recording outbreaks, contributing to public health efforts. It is used to manage and prevent possible outbreaks and gain insights into the route of staphylococcal infection [52]. Additionally, ERIC-PCR analyses, alongside *spa* typing results, revealed a dispersed clonal diversity among the aminoglycoside-resistant *S. aureus* isolates in this study. The method is simple, quick, cost-effective, and versatile, with high discriminatory power, making it an accessible and precise tool for differentiating closely related bacterial strains in various epidemiological and genetic studies [53]. ERIC-PCR results often correlate well with other molecular typing methods. In a study, ERIC-PCR has been compared with Pulsed-Field Gel Electrophoresis (PFGE) for typing *Shigella* isolates. ERIC-PCR identified more types (42) than PFGE (37) and showed even higher discrimination power. Both methods showed a 90.4% correlation in designating isolates as clonal or non-clonal, indicating that ERIC-PCR can provide similar and supplementary data to PFGE, with the added benefits of being more rapid and cost-effective [54].

Conclusions

Our study underscores the significant prevalence of MRSA in our hospitals and the ongoing challenge of MRSA infections in clinical settings. The high genetic diversity of SCC*mec* types highlights the complexity of MRSA epidemiology, with strains exhibiting genetic backgrounds of both HA-MRSA and CA-MRSA. Additionally, we identified significant resistance to aminoglycosides, with a notable prevalence of AME-encoding genes, particularly *aac(6′)-Ie/aph(2′′)-Ia*. This underscores the importance of monitoring local resistance patterns to guide effective treatment strategies.

Abbreviations

MRSA Methicillin	Resistant staphylococcus aureus
MSSA	Methicillin susceptible staphylococcus aureus
CA MRSA	Community-associated methicillin-resistant staphylococcus aureus
HA MRSA	Healthcare-associated methicillin-resistant staphylococcus aureus
ATCC	American type culture collection
CLSI	Clinical and laboratory standards institute
DNA	Deoxyribonucleic acid
MIC	Minimum inhibitory concentration
PCR	Polymerase chain reaction
AMEs	Aminoglycoside-modifying enzymes
AACs	Aminoglycoside-acetyltransferases
ANTs	Aminoglycoside-nucleotidyltransferases
MDR	Multidrug-resistant
PBP	Penicillin-binding protein
SCC*mec*	Staphylococcal cassette chromosome *mec*
CSF	Cerebrospinal fluid
TSB	Tryptic soy broth
ERIC	Enterobacterial repetitive intergenic consensus
SPA	Staphylococcal protein A

Supplementary Information

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Supplementary Material 1

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Author contributions
SH: Methodology, Investigation, Formal Analysis, and Original Draft Preparation. HPD: Review, and Editing. MA: Conceptualization, Supervision, Project administration, and Manuscript Revision.

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Data availability
The datasets generated and analyzed during the current study are included in this published article and its supplementary information file (Table S1).

Declarations

Ethics approval and consent to participate
This research was approved by the Ardabil University of Medical Sciences Ethics Committee (Reference number: IR.ARUMS.REC.1396.53). All methods followed relevant guidelines and regulations under the Helsinki Declaration (https://www.wma.net/policies-post/wma-declaration-of-helsinki/). Clinical isolates were collected from the hospital's bacterial repository solely for research purposes, and no patient samples or patient data were used in this study. Consequently, the requirement for informed consent from participants was waived by the Regional Research Ethics Committee of Ardabil University of Medical Sciences.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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