Molecular Biology Reports (2024) 51:58
https://doi.org/10.1007/s11033-023-08951-y

ORIGINAL ARTICLE

Epidemiological characterization of clinical isolates of meticillin resistant *Staphylococcus aureus* through multilocus sequence typing and staphylococcal cassette chromosome *mec* typing in Northwest Iran

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Received: 11 October 2023 / Accepted: 7 November 2023

Abstract

Background Methicillin-resistant *Staphylococcus aureus* (MRSA), is considered a potential and aggressive nosocomial pathogen. It accounts for 50% of *S. aureus* isolates in tertiary hospitals in Iran, however, there is no sufficient evolutionary and epidemiological investigation about this medically important bacterium. We aimed to study the lineage and evolution of MRSA in Northwest Iran during 2021–2022 based on the obtained phenotypic and genotypic characteristics.

Materials and methods Seventy-two non-duplicate MRSA isolates were collected from 3 referral hospitals in Tabriz, Ardebil, and Urmia cities. The antimicrobial susceptibility patterns were determined by disk diffusion test and micro broth dilution methods. Thereafter 4 virulence genes (*eta*, *etb*, *pvl*, *tst*) and 5 types of staphylococcal cassette chromosome *mec* (SCC*mec*) were detected by PCR. In the final step, representative isolates were selected to be studied by Multilocus sequence typing (MLST).

Results The highest resistance was observed to erythromycin and clindamycin at a rate of 76.4%, followed by ciprofloxacin (61.1%), gentamicin (54.2%), rifampin (38.9%), and co-trimoxazole (27.8%). All isolates were susceptible to vancomycin. The virulence genes of *etb*, *pvl*, *tst*, and *eta* were detected in 50%, 29.2%, 21.8%, and 13.9% of isolates, respectively. SCC*mec* types III and I were the most prevalent types, followed by types IV, II, and V. MLST analysis revealed 6 sequence types: ST6854, ST5282, ST127, ST7804, ST1607, and ST7784. Two MLST-based clonal complexes (CC8, and CC97) were identified as well.

Conclusion The ST numbers were non-repetitive. CC8 as a pandemic clone and an individual lineage and clinically significant clade was reported as the most prevalent clonal complex. It is essential periodic evaluations of antibiotic susceptibility patterns and study the evolutionary characteristics of medical-challenging microorganisms in particular MRSA to effectively treat and restrict the outbreaks.

Keywords MRSA · SCC*mec* typing · MLST · Antibiotic resistance

Introduction

Staphylococcus aureus is considered an important and accountable pathogen for mild to severe infections such as septicemia, endocarditis, and osteomyelitis in hospitalized patients and a crucial agent in high mortality rates in hemodialysis patients and intensive care unit (ICU) and surgery units in humans and animals, with the potential to cause coincidental nosocomial infections in part with *Pseudomonas aeruginosa*, *Escherichia coli*, and *Acinetobacter baumannii* [1, 2]. The strong pathogenicity and various virulence factors return to the acquisition of resistance agents to antibiotics [3]. In early 1940, penicillin was introduced for the treatment of infections, however, the emerging resistance was immediately reported as a result of penicillinase enzyme production encoded by plasmid genes [4]. In 1959, methicillin the first semisynthetic penicillin was developed and introduced to overcome the existing infections, unfortunately, the rapid resistance was repeated for this antibiotic as well. This event caused to increase in the

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Published online: 02 January 2024

numbers of Meticillin-Resistanant *Staphylococcus aureus* (MRSA), and therefore, the appropriate treatment option changed to vancomycin [5–7]. In 2005, MRSA infection deaths overtook AIDS mortalities in the USA [8], and in Iran, these infections have dramatically risen and this is considered an important medical challenge in hospitals [9]. Methicillin resistance is confirmed by the presence of *mecA* gene located on the Staphylococcal Cassette Chromosome *mec* (SCC*mec*) which includes the encoding ability of Penicillin Binding Protein 2a (PBP2a) and consequently prevention of β-lactams effects. SCC*mec* typing has been developed to determine the epidemiology, outbreaks, and lineage of MRSA isolates. Until now, 13 SCC*mec* types for MRSA strains have been described based on the combination of *ccr* and *mec* gene complexes, and only I–V types are distributed globally, whereas the others exist in local strains of the origin country [5–7]. Nowadays, the frequency of MRSA strains has risen to 50–60% in some countries, and Iran's different provinces, therefore, microbiological research and special attention to treatment, and control policies seem more necessary [10, 11]. MRSA can be divided into healthcare-associated MRSA (HA-MRSA), and community-acquired MRSA (CA-MRSA), according to the molecular epidemiological features. CA-MRSA is a relatively new clone reported globally since the 1990s with the ability to cause infections in healthy and immunocompromised individuals. The high pathogenicity of CA-MRSA returns to the production of Panton–Valentine leucocidin (*pvl*) [12]. As mentioned earlier, *S. aureus* has extensive storage of virulence factors including adhesins, host-cell damaging agents, and immunomodulatory molecules that are different in specificity and presence between clones, and a major diversity between infections. The superantigen *tst-1* is unique in its ability to cross mucosal surfaces and is the only pyrogenic toxin superantigen known to reactivate bacterial cell wall-induced arthritis and increase the lethal effects of endotoxin on renal tubular cells [13]. Exfoliative toxins (ETs) are defined as extracellular proteins that cause blisters in bullous impetigo and, in the disseminated form of staphylococcal scalded-skin syndrome (SSSS). Previous investigations demonstrate that *eta* gene is harbored on the temperate phage genome integrated into the *S. aureus* chromosome, whereas the *etb* gene is harbored on a large plasmid known as pETB. The *eta*-carrying *S. aureus* strains are frequently isolated from bullous impetigo- patients, whereas *etb*-carrying strains are isolated from SSSS patients [14].

The high incidence of MDR-MRSA isolates suggested a revision of policies for infection control [15]. These strains acquired resistance features to methicillin as a result of SCC*mec* presence and other antibiotics. The existing reports declare that SCC*mec* types I, II, and III are the most prevalent types among HA-MRSA, and SCC*mec* types IV and V among the CA-MRSA [16]. The smallest structural of SCC*mec* types returns to SCC*mec* type IV as the mobile version. The SCC*mec* type IV associated with CA-MRSA can be also pertinent to some nosocomial clones, and studying this type is extremely crucial for molecular and epidemiological investigations [17]. All existing challenges make the treatment of MRSA infections difficult and costlier more than ever [6] and have caused to development of molecular characterization including Pulsed Field Gel Electrophoresis (PFGE), SCC*mec* typing, and Multilocus Sequence Typing (MLST) [18]. These methods have high speed and reproducibility that make them widely used in the classification of infectious agents, particularly in *S. aureus*. MLST is developed in Macro-epidemiological and evolutionary investigations, which is based on analysis of polymorphisms nucleotides in the sequence of 7 housekeeping genes, and obtained sequences from each gene locus in bacterial species known as an allele [19, 20].

The majority of the reports in Iran related to MRSA are data restricted to local regions, and there has not been a comprehensive review in this regard [10, 15, 21]. Therefore, we aim to investigate the evolutionary and epidemiological characterization of MRSA isolates by SCC*mec* typing and MLST methods for the first time in Northwest Iran according to genotyping characteristics and antibiotic resistance profile which will be completely explained in the related sections of the text.

Materials and methods

Study design and setting

This cross-sectional study was conducted at 3 participating referral hospitals in Tabriz (East Azarbaijan province), Ardebil (Ardebil province), and Urmia (West Azarbaijan province) cities (Northwest Iran) from November 2021 to December 2022. All patients who met fulfilled criteria for MRSA infections were included in the study. Patient demographic data including gender, age, and source of infection were obtained from medical unit records in each hospital. (Table 1). The Ethics Committee of Tabriz University of Medical Sciences approved this study (Number: IR.TBZMED.VCR.REC.1400.112).

Bacterial isolation and detection of MRSA

Seventy-two non-duplicate bacterial isolates were identified presumably as *S. aureus* (MRSA) biochemically and morphologically following positive DNase, catalase, mannitol fermentation, and coagulase tests from (Tabriz) (n = 26), (Ardebil) (n = 21), and (Urmia) (n = 25). After reliable confirmation, meticillin resistance was evaluated by detection of *mecA* gene through polymerase chain reaction (PCR), and

Table 1 Patient demographic data including gender, age, and source of infection separately for each city

City sample sources and collection wards		Tabriz n (%)	Ardebil n (%)	Urmia n (%)
Urine	General wards	4 (11.07)	2 (6.85)	5 (14.4)
Wound	ICU and internal wards	7 (19.38)	5 (17.14)	6 (17.28)
Blood	ICU	3 (8.30)	4 (13.71)	5 (14.4)
Trachea	ICU	4 (11.07)	6 (20.57)	5 (14.4)
CSF	ICU	5 (13.84)	4 (13.71)	3 (8.64)
Body fluids	Surgery wards	3 (8.30)	0 (0)	1 (2.88)
Mean age years + SD		44 + 77	45 + 95	49 + 70
Gender (Male)		58.9	59.4	57.4
Gender (Female)		42.1	39.1	44.1
Total isolate number		26 (36.11)	21 (29.16)	25 (34.72)

disk diffusion test (DDT) using cefoxitin (30 µg) disk (Mast, UK), and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) 2020 guidelines. Thereafter, the confirmed MRSA isolates were stored in a Tryptic Soy Broth (TSB) medium in part with 30% glycerol and preserved at − 70 °C for the next steps.

Antimicrobial susceptibility

DDT was performed to determine the antibacterial susceptibility patterns on Mueller–Hinton agar using gentamicin (10 µg), ciprofloxacin (15 µg), co-trimoxazole (20 µg), erythromycin (15 µg), clindamycin (2 µg), and rifampin (20 µg) disks purchased from (Mast, UK) according to standard guidelines [22]. The *D*-test technique was conducted by clindamycin (2 µg) and erythromycin (15 µg) disks according to the CLSI recommendations. The minimum inhibitory concentration (MIC) was determined for studying the vancomycin susceptibility pattern using the broth dilution method as previously described [22]. *S.aureus* ATCC 25923 was considered a control strain in described methods. The obtained results were interpreted according to CLSI 2020 guidelines (https://www.clsi.org).

PCR

DNA extraction

DNA extraction was conducted as previously described by Sadeghi et al. [5] with some modifications. Briefly, a primary culture of colonies was prepared. After overnight incubation, 3–5 colonies were dissolved in 450 µL of TE buffer (10 mM Tris, 1 mM EDTA, pH 8). Cell lysis was achieved by treatment with 5 µL of proteinase K (20 mg/mL) for 20 min at 50 °C followed by the addition of 60 µL of 10% SDS for 10 min at 68 °C. Thereafter, 100 µL of 5 M NaCl and 80 µL of cetyltrimethylammonium bromide (CTAB)/NaCl were added and incubated at 65 °C for 10 min. In the next step, 700 µL of chloroform/isoamyl alcohol was added and centrifuged at 12,000×*g* for 8 min. The supernatant was transferred to a fresh tube and the DNA was precipitated with isopropanol, washed with 70% ethanol, dried, and dissolved in 100 µL of deionized water.

The presence of *eta*, *etb*, *tst*, and *pvl* genes was detected by the primers (Metabion-Germany) as previously described [23] (Table 2).

SCC*mec* typing: The optimization of multiplex PCR for SCC*mec* typing (types I, II, III, IV, and V) was carried out by using 5 previously designed primer pairs (Metabion-Germany) [24] (Table 2).

MLST

The protocol of MLST for MRSA was completely implemented as previously described [25] based on seven housekeeping genes (*arc* (carbamate kinase), *aroE* (shikimate dehydrogenase), *glpF* (glycerol kinase), *gmK* (guanylate kinase), *pta* (phosphate acetyltransferase), *tpi* (triosephosphate isomerase), *yqiL* (acetyl coenzyme A acetyltransferase). (Table 2). Allelic profiles and Sequence type designations were assigned by comparison with those previously characterized strains using the MLST database via the internet (http://www.mlst.net). Briefly, amplification of MLST genes, Purification of PCR products, reaction sequencing, electrophoresis, assessment of data quality, assigning allele numbers and STs, comparison of strains by the eBURST, and comparison of strains using concatenated allele sequences had been implemented.

Statistical analysis

Data were calculated by $SPSS_{TM}$ software Version 21.0 (IBM Corp., USA). Chi-square or Fisher's exact tests were employed to determine the significance of the differences. A p-value less than 0.05 was considered statistically significant.

Table 2 Primers used for detecting virulence genes, SCC mec typing, and housekeeping genes for MLST

Gene	Amplicon size (bp)	Sequences	References
Virulence genes			
eta	93	Fw: CTAGTGCATTTGTTATTCAA Rv: TGCATTGACACCATAGTACT	[23]
etb	590	Fw: ACGGCTATATACATTCAATT Rv: TCCATCGATAATATACCTAA	[23]
tst	329	Fw: ATGGCAGCATCAGCTTGATA Rv: TTTCCAATAACCACCCGTTT	[23]
pvl	433	Fw:ATCATTAGGTAAAATGTCTGGACATGATCCA Rv: GCATCAASTGTATTGGATAGCAAAAGC	[23]
Staphylococcal sassette chromosome*mec* genes			
SCC*mec*I	613	Fw: GCTTTAAAGAGTGTCGTTACAGG Rv: GTTCTCTCATAGTATGACGTCC	[24]
SCC*mec*II	398	Fw: CGTTGAAGATGATGAAGCG Rv: CGAAATCAATGGTTAATGGACC	[24]
SCC*mec*III	285	Fw: CCATATTGTGTACGATGCG Rv: CCTTAGTTGTCGTAACAGATCG	[24]
SCC*mec*IV	776	Fw: GCCTTATTCGAAGAAACCG Rv: CTACTCTTCTGAAAAGCGTCG	[24]
SCC*mec*V	325	Fw: GAACATTGTTACTTAAATGAGCG Rv: TGAAAGTTGTACCCTTGACACC	[24]
Housekeeping genes			
arcC [carbamate kinase]	456	Fw: TTG ATT CAC CAG CGC GTA TTG TC Rv: AGG TAT CTG CTT CAA TCA GCG	[25]
aroE [shikimate dehydrogenase]	456	Fw: ATC GGA AAT CCT ATT TCA CAT TC Rv: GGT GTT GTA TTA ATA ACG ATA TC	[25]
glpF [glycerol kinase]	465	Fw: CTA GGA ACT GCA ATC TTA ATC Rv: TGG TAA AAT CGC ATG TCC AAT TC	[25]
gmk [guanylate kinase]	429	Fw: ATC GTT TTA TCG GGA CCA TC Rv: TCA TTA ACT ACA ACG TAA TCG TA	[25]
pta [phosphate acetyltransferase]	474	Fw: GTT AAA ATC GTA TTA CCT GAA GG Rv: GAC CCT TTT GTT GAA AAG CTT AA	[25]
tpi [triosephosphate isomerase]	402	Fw: TCG TTC ATT CTG AAC GTC GTG AA Rv: TTT GCA CCT TCT AAC AAT TGT AC	[25]
yqiL [acetyl coenzyme A acetyltransferase]	516	Fw: CAG CAT ACA GGA CAC CTA TTG GC Rv: CGT TGA GGA ATC GAT ACT GGA AC	[25]

Results

In the present study, seventy-two nonduplicate MRSA isolates were collected from 58.4% male and 41.6% female patients from different clinical sources including wounds, blood, CSF, body fluids, and trachea. The mean age of the patients was 48 + 13 years. All isolates were resistant to cefoxitin disk (30 µg/mL) according to the CLSI breakpoints and harbored the *mecA* gene. According to the DDT, the highest resistance was observed to erythromycin and clindamycin at a rate of 76.4%, followed by ciprofloxacin (61.1%), gentamicin (54.2%), rifampin (38.9%), and co-trimoxazole (27.8%) in all isolates. Figure 1 depicts the antibiotic resistance patterns separately in each city, and Table 3 has more information on antibiotic combination resistance in 72 isolates. The multi-drug resistance (MDR) was obtained at a rate of 62.5%. We reported the combination resistance for studied antibiotics, in which this rate was determined at 65.27% for clindamycin-erythromycin. Two isolates had been influenced by the *D*-test phenomenon.

According to the MIC values, all isolates were susceptible to vancomycin. The vancomycin MIC range was recommended 0.25–16 µg/mL by the CLSI, and in the current research, $MIC_{50,}$ and MIC_{90} were 0.5 and 1 µg/mL, respectively in all isolates.

The distribution rate of virulence genes in all isolates was 50% for *etb*, followed by 29.2%, 21.8%, and 13.9% for *pvl*, *tst*, and *eta* genes respectively. The SCC*mec* types prevalence rate was reported as SCC*mec* I 22.2%, SCC*mec* II 9.7%, SCC*mec* III 43.1%, SCC*mec* IV 12.5%, and SCC*mec*

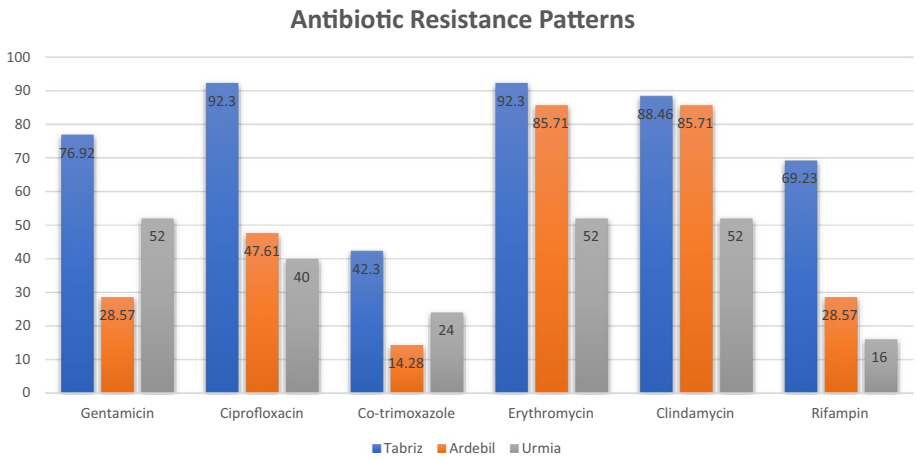

Fig. 1 The antibiotic resistance patterns in Tabriz, Ardebil, and Urmia

Table 3 Antibiotics combination resistance in 72 isolates

Antibiotics combination resistance	Resistance rate n (%)
Gentamicin + Ciprofloxacin	33 (45.83)
Gentamicin + Clindamycin	35 (48.61)
Gentamicin + Erythromycin	33 (45.83)
Gentamicin + Rifampin	21 (29.16)
Gentamicin + Co-trimoxazole	18 (25)
Co-trimoxazole + Clindamycin	18 (25)
Clindamycin + Ciprofloxacin	37 (51.38)
Clindamycin + Erythromycin	47 (65.27)
Clindamycin + Rifampin	26 (36.11)
Erythromycin + Rifampin	25 (34.72)
Co-trimoxazole + Erythromycin	18 (25)
Ciprofloxacin + Erythromycin	37 (51.38)
Ciprofloxacin + Rifampin	25 (34.72)
Co-trimoxazole + Rifampin	11 (15.27)
Clindamycin + Ciprofloxacin, Erythromycin	40 (55.55)
Gentamicin + Ciprofloxacin + Clindamycin + Erythromycin + Co-trimoxazole	15 (20.83)
Gentamicin + Ciprofloxacin + Clindamycin + Erythromycin + Co-trimoxazole + Rifampin	8 (11.11)

V 6.9%. Table 4 describes the gene statistics in each city in detail and Table 5 the genes positive cases in combination.

MLST results revealed diverse ST numbers and 2 CCs (CC8 as a pandemic MRSA clone and CC97) in 18 representative isolates (Table 6; Fig. 2).

Discussion

The mortality rate of MRSA infections surpasses 20,000 cases annually in hospitalized patients in the United States; which is comparable with the death rates resulting from acquired immune deficiency syndrome (AIDS), in part due to viral hepatitis, and tuberculosis. In 2005, MRSA-associated deaths overtook AIDS mortalities in the USA [10]. In Iran, these infections have dramatically risen and this is considered an important medical challenge, particularly in hospitals [9]. The treatment procedures for MRSA infections rely on antibiotics, however, medicine nowadays is faced with a growing problem of antibiotic resistance [26–28]. The main goal of this study was to determine the lineage and evolution of 72 MRSA isolates in three provinces of Northwest Iran based on the obtained critical phenotypic and genotypic characteristics including the most prevalent SCC*mec* types and harboring toxin genes and critical resistance patterns. In this regard, we investigated the resistance rate for 7 antibiotics with DDT and micro broth dilution methods. The results elucidated the equal resistance rate to erythromycin and clindamycin in 72 isolates at a rate of 76.4%, and the lowest resistance rate for co-trimoxazole at a rate of 27.8%. Regardless of intermediate results, rifampin, co-trimoxazole, and gentamicin were reported most effective antibiotics against MRSA isolates. Also, the isolates from Tabriz had a higher resistance to all investigated antibiotics. The resistance in MRSA occurs through drug target alteration, inactivation of drug enzymes, altered drug accessibility, increased efflux of antimicrobial compounds, and a multitude of mobile genetic elements [29]. The MDR rate in the present study was reported at 62.5%, which following the recent research by Ahmadishoar et al. in 2021 in Tabriz that demonstrated a 68.2% MDR and also a whole-susceptible rate for vancomycin [15], and Dibah et al. in 2014 that reported co-trimoxazole as an effective antibiotic against the 41 MRSA isolates with 100% susceptibility for vancomycin in Ardebil [30]. We also reported the combination resistance, in which this rate was determined at 65.27% for clindamycin-erythromycin. This resistance is considered significant because two antibiotics had been influenced by the *D*-test phenomenon. *D*-test has been employed for screening inducible resistance that is affected by *erm* gene. Therapeutic failure for clindamycin

Table 4 The information on detected genes separately in each city

Tabriz					Ardebil					Urmia				
SCC*mec* I	SCC*mec* II	SCC*mec* III	SCC*mec* IV	SCC*mec* V	SCC*mec* I	SCC*mec* II	SCC*mec* III	SCC*mec* IV	SCC*mec* V	SCC*mec* I	SCC*mec* II	SCC*mec* III	SCC*mec* IV	SCC*mec* V
3 (11.53)	5 (19.23)	13 (50)	4 (15.38)	3 (11.53)	7 (33.33)	1 (4.76)	5 (23.80)	5 (23.80)	3 (14.28)	6 (24)	0 (0)	13 (52)	0 (0)	2 (8)
eta	*etb*	*pvl*	***tst***	–	*eta*	***etb***	*pvl*	***tst***	–	*eta*	*etb*	*pvl*	***tst***	–
5 (19.23)	16 (61.53)	11 (42.30)	3 (11.53)	–	1 (4.76)	13 (61.90)	5 (23.80)	5 (23.80)	–	1 (4)	6 (24)	5 (20)	7 (28)	–

Table 5 The positive cases of genes in combination

Genes	Positive cases
eta + *etb*	4 (5.55)
eta + *pvl*	2 (2.77)
eta + *tst*	1 (1.38)
etb + *pvl*	2 (2.77)
pvl + *tst*	7 (9.7)
eta + *etb* + *pvl*	0 (0)
etb + *tst* + *pvl*	5 (6.9)
eta + *etb* + *tst*	1 (1.38)
eta + *etb* + *pvl* + *tst*	0 (0)

is one of the consequences of inducible resistance [31]. A rather new study in the central provinces of Iran is under our research. In this way, Co-trimoxazole had a lower resistance (33.3%) in comparison with erythromycin, ciprofloxacin, gentamicin, and clindamycin [6]. The high resistance to beta-lactams has been reported in other Iranian studies as well [32, 33]. Due to the high prevalence of MRSA, precise and comprehensive policies that describe accurately epidemiological data must be available in each country to control the infections and subsequent medical challenges.

The presence of core genome recombination hotspots in the *S. aureus* genome has been revealed to be pertinent to the existence of mobile genetic elements [34]. Molecular detection of the *mec*A gene using PCR is commonly carried out to confirm the presence of MRSA isolates. This method is still the main recommendation even though it cannot be done routinely. However, the identification of MRSA with disk diffusion is still widely used because it can be done quickly and at a lower cost [35]. In the present study, both techniques were employed for accurate confirmation. 100% of our isolates were resistant to cefoxitin and harbored the *mecA* gene. On the other hand, MRSA isolates harbor various toxin-encoding genes. We screened 29.2% portion for *pvl* gene. It is produced by approximately 5% of *S. aureus* strains, and as the most widely investigated *S. aureus* virulence factor is expressed repeatedly in MRSA more than MSSAs [36]. This gene is commonly studied as a marker for community-acquired MRSA and epidemiologically associated with prevalent CA-MRSA strains carrying SCC*mec* type IV, V, VI, VII, and VIII, accountable for deep dermal and soft-tissue infections. This point should also be taken into account that the increasing prevalence of *pvl*-encoding HA-MRSA is a critical concern that can worsen infections [37]. Goudarzi et al. in Iran reported a 3.9% portion of isolates that harbor *eta*, *etb*, *pvl*, and *tst* genes together [33]. This is despite none of our isolates showing this condition. Another research in Iraq, the neighboring country of Iran, displayed a 3.95% portion for *pvl*, 80.26% *tst*, and 1.31 *etb* genes [38]. We screened *tst* at a rate of 21.8%. More results

Table 6 The complete MLST results. Each row represents 3 isolates with the same characteristics

City	SCC*mec* type	*eta*	*etb*	*tst*	*pvl*	source	ward	Antibiotic resistance profile	*arcC*	*aroE*	*glpF*	*gmk*	*pta*	*tpi*	*yqiL*	ST numbers	Clonal complex
Tabriz	III	–	–	–	–	Trachea	ICU	Gentamicin, Ciprofloxacin, Clindamycin, Erythromycin, Co-trimoxazole, Rifampin	816	601	711	603	4	4	923	6854	CC8
Tabriz	I	–	+	–	–	wound	ICU	Gentamicin, Ciprofloxacin, Clindamycin, Erythromycin, Co-trimoxazole, Rifampin	816	36	149	603	650	4	700	5282	CC8
Ardebil	I	+	+	–	–	Trachea	ICU	Gentamicin, Ciprofloxacin, Clindamycin, Erythromycin, Co-trimoxazole, Rifampin	79	1	4	572	934	415	398	127	CC97
Ardebil	III	–	–	+	–	wound	ICU	Gentamicin, Ciprofloxacin, Clindamycin, Erythromycin, Rifampin	622	490	319	4	934	570	36	7804	–
Urmia	I	+	+	+	–	Trachea	ICU	Gentamicin, Ciprofloxacin, Clindamycin, Erythromycin, Co-trimoxazole	4	716	1	1	5	8	4	1607	CC97
Urmia	III	–	–	–	+	wound	ICU	Gentamicin, Ciprofloxacin, Clindamycin, Erythromycin, Co-trimoxazole	816	696	149	603	802	4	3	7784	CC8

Fig. 2 Dendrogram using average linkage between groups (All isolates)

of these toxin-encoding genes have been implied in other studies from different countries [39, 40].

Direct analysis of the bacterial genome recently has been performed in epidemiological research [41]. The importance of molecular strain typing as an integral part of epidemiological studies is in the investigation of common sources and spread of infection from one patient to another, sporadic and nosocomial infections, tracking pathogenic strains, distinguishing endemic strains from epidemic strains, determining the antibiotics resistance and susceptibility patterns and proper strategy in the treatment and effectively managing and controlling of the related infections [24]. When comparing different molecular methods, it is important to know what region of the genome each method evaluates, for example, PFGE is considered a gold standard for epidemiological classifications in challenging nosocomial pathogens and assesses the entire chromosome based on the total size of the restriction fragments. Hence, minor genetic changes may go undetected. This is also useful to determine the genetic relatedness of MRSA strains isolated during a relatively short period (1–3 months), where presumably, the genetic variability is limited. On the other hand, PCR-based methods detect a specific region of the chromosome at a specific location [42, 43]. The genetic background based on the MLST and SCC*mec* typing is a better understanding of the epidemiology of long-term population relatedness of MRSA and defines the epidemic clones appropriately according to nucleotide variations [44].

The existing reports declare that SCC*mec* types (I, II, III) and (IV, V) are the most prevalent types among HA-MRSA and CA-MRSA respectively [16]. In the present study, we screened SCC*mec* III at 43.1%, followed by SCC*mec* I at 22.2%. Sedaghat et al. conducted a similar study and found the SCC*mec* III as the most prevalent type [45], which is under our results. Another Iranian research reported SCC*mec* type III as the most prominent type [46]. The high prevalence of SCC*mec* III probably highlights the nosocomial origin of MRSA isolates. The smallest structural of SCC*mec* types returns to SCC*mec* type IV as the mobile version. Besides being frequently related to CA-MRSA, SCC*mec* IV can be associated with some nosocomial clones, and studying type IV is extremely crucial for molecular epidemiological investigations of CA-MRSA strains [17]. However, this type is considered the third most common type in the present study. Indian research reported that all of the major SCC*mec* types, particularly SCC*mec* type III are distributed among (HA) MRSA isolates [47]. More results about SCC*mec* types are also available in other studies [48, 49].

In the current study, 18 representative isolates (details of isolates and selection criteria mentioned earlier) had been selected for the MLST analysis, and 6 ST numbers (6854, 5282, 127, 7804, 1607, 7784), and 2 colon complexes as CC8 (an individual lineage and clinically significant clade) and CC97 were identified. The CC8 consisted of SCC*mec* types I and III harboring isolates, however, CC97 included SCC*mec* type I carrying isolates. CC97 is one of the important *S. aureus* CCs in bovines, and recently, a livestock origin of the human pandemic CC97 MRSA strains has been displayed, resulting in two emergent human epidemics CC97 (CA-MRSA) clones [50]. Other studies approve the validity of this matter [50, 51].

An Iranian investigation screened the ST239 as the prevalent ST number in the majority of isolates in two central provinces [52]. Phylogenetic evidence displayed a hospital transmission and intercontinental spread of CC8 (ST239) isolates through South America, North America, Europe, and Asia [53]. The spread of CC8 (ST239) was from South America to Europe and from Thailand to China in the 1990s [54]. The most frequently reported MRSA CCs worldwide between 1961 and 2008 were CC5, CC8, CC30 CC22, and CC45. In brief, CC5 and CC8 are the most prevalent global clonal complexes [55]. Recent shreds of evidence from Africa are limited, however, suggested a predominance of CC8, and infrequently CC5 and CC8 [56].

In comparison to the Japanese research that reported the prevalence of enterotoxin genes and drug-resistance profiles differ according to STs/CCs. Most CC5 clones represented enterotoxin gene cluster and *tst-1*, while *pvl*/ACME-positive ST8/SCC*mec* IVa-*spa t*008-*agr*I (USA300 clone) harbored *sek*, *seq*, *sak*, and *speG* genes [57], our findings reveal a separate ST number (ST7804 and ST7784 respectively) for *tst*-positive and *pvl*-positive isolates, and inclusion in the CC8 for the *pvl*- positive isolates and a SCC*mec* type III profile.

Harris et al. sequenced 63 isolates of ST239 as a globally disseminated HA clone defined by MLST. Their reports showed that this ST number is considered a global geographic sequence number and demonstrated the possibility of employing CGS analysis to track transmission within a single hospital [58]. Evaluation of genetic variation at single MLST loci displayed that *yqiL* as one of the housekeeping genes of *S. aureus* had various more polymorphic sites when compared with the other 6 gene fragments and there was suggestive evidence of a signature of recombination within the *yqiL* locus [59].

Overall, epidemiological studies are a broad discussion, and detected ST numbers in the current study were non-repetitive and had not been reported in any other Iranian investigations. We collected the isolates from 3 vast provinces in Northwest Iran, and their phenotypic and genotypic characteristics were studied. The profile of antibiotic resistance was still worrying and increasing. Fortunately, vancomycin has not shown any resistance. Considering the importance of the issue, it is suggested to evaluate the typing patterns of the isolates in each region alternately with applying different methods together, which

can provide more useful and comprehensive information. With the availability of epidemiological and lineage data, the management and prevention of nosocomial infections that pose a serious burden on the health system and immunocompromised patients, have become easier. If these points are achieved, the mortality rate and the economic and psychological burden will have a significant reduction.

Conclusion

Our study presents an overview of antibiotic resistance profiles, and epidemiological information on MRSA isolates in Northwest Iran. MRSA rates continue to rise rapidly in many countries and have a globally dynamic spread. Its clones are defined by lineage and SCC*mec* variant types. They usually display successful and widely spread variants. Similar to the global evidence, CA-MRSA is reported as more invasive, transmissible, and increasingly difficult to differentiate from HA-MRSA. Many MRSA isolates originate from a restricted number of historically dominant clonal lineages, and some other clones are found worldwide, or restricted to certain geographic areas. They imply differences in transmission routes. Therefore, the Harmonization of surveillance will improve epidemiological studies in the future.

ST numbers were exclusive and CC8 as a pandemic, individual lineage, and clinically significant clade clone was reported as the most prevalent clonal complex. Generally, CC5 and CC8 are the most prevalent CCs across the world.

The flexibility of the *S. aureus* genome allows for the adaptive radiation of successful lineages. Mobile genetic elements that carry many of the virulence factors in *S. aureus* are often lineage-associated.

Continuous efforts to understand the variable epidemiology of *S. aureus* infection in humans and animals as a clinical pathogen are crucial steps for effective infection control, appropriate antimicrobial treatment, and monitoring the species' evolution and organism's phylogeny to the prevention of the spread of virulence lineages and for characterizing outbreaks, however, there have been few analyses employing whole genome sequencing, looking at the overall relationships among different clonal groups of this organisms. According to a remarkable raising in MRSA prevalence, a precise and updated report that describes accurately epidemiological data must be available in each country to control the infections and improve national policies.

Acknowledgements We would like to all hospitals for their assistance and all institutes for providing expertise that greatly assisted.

Author contributions ES: Conceptualization, Data curation, Writing—original draft, Writing—review & editing. AA: Formal analysis, Writing—original draft, Writing—review & editing. MYM: Writing—review & editing, Writing—original draft, Data curation. HBB: Writing—original draft, Data curation. RG: Writing—review & editing, Writing—original draft, Data curation, Conceptualization. DL: Writing—review & editing, Writing—original draft. JS: Project administration, Conceptualization, Data curation, Writing —original draft, Writing—review & editing. FYS: Data analysis, review. YS: Review, Data Curation.

Funding This research was financially supported by the Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, and was written based on a dataset of a Ph.D. thesis registered at Tabriz University of Medical Sciences.

Data availability The data supporting this study's findings are available and included in the article.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval The Ethics Committee of Tabriz University of Medical Sciences approved this study (Number: IR.TBZMED.VCR.REC.1400.112). All ethical considerations have been observed during this research. The collection of isolates was conducted with the full consent of the patients and parents were legally authorized representatives of the minor subjects.

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