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Original Article

Molecular Characterization of *Staphylococcal* cassette chromosome mec and Tetracycline Resistance genes in Clinical Isolates

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Abstract

Background & Objectives: *Staphylococcus* species are major causes of hospital and community acquired infections worldwide. This study aimed to investigate the distribution of *Staphylococcal* cassette chromosome mec (SCCmec) types among clinical *Staphylococcus* isolates from Shiraz and evaluated their association with tetracycline resistance determinants. Understanding these resistance mechanisms is important for epidemiological surveillance and infection control.

Materials & Methods: In this cross-sectional study, 93 clinical *Staphylococcus* isolates were collected from different clinical specimens in Namazi hospital in Shiraz, Iran. Antimicrobial susceptibility testing was performed using the agar disk diffusion method and MIC method according to the CLSI guidelines. Polymerase chain reaction (PCR) was employed to detect tetracycline resistance genes (*tetK* and *tetM*) and to determine SCCmec types.

Results: Tetracycline resistance was detected in 51.61% of the examined *Staphylococcus* isolates. Molecular analysis identified the *tetK* and *tetM* genes in 38.70% and 41.30% of isolates, respectively. SCCmec type II was the predominant cassette type, and a significant association was observed between SCCmec type II and tetracycline resistance determinants.

Conclusion: Diverse SCCmec types were identified among clinical *Staphylococcus* isolates, with SCCmec type II being the predominant type. The high prevalence of *tet* genes indicates a substantial burden of tetracycline resistance. Continuous molecular surveillance is essential for monitoring the distribution of resistance determinants and SCCmec elements. Strengthening antimicrobial stewardship programs may help reduce the spread of resistant strains in healthcare settings.

Keywords: *Staphylococcus*, Molecular surveillance, SCCmec, *tetK*, *tetM*, MIC.

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Introduction

Staphylococcus species are among the most important opportunistic bacterial pathogens responsible for a wide range of hospital and community-acquired infections worldwide (1,2). These bacteria can lead to a broad spectrum of clinical conditions, including uncomplicated skin infections and severe invasive diseases such as bacteremia, endocarditis, pneumonia, and septicemia. In particular, *Staphylococcus aureus* and coagulase-negative *Staphylococci* (CoNS) have emerged as clinically significant pathogens, especially among immunocompromised patients and individuals with indwelling medical devices (3,4).

The rapid emergence and dissemination of antimicrobial-resistant *Staphylococci* have become a major global concern on health (5). Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase negative *Staphylococci* (MR-CoNS) exhibit resistance to multiple antimicrobial agents, thereby limiting therapeutic options and increasing morbidity and mortality rates (6,7). Resistance to methicillin is mainly mediated by the *mecA* gene, which is located on the *Staphylococcal* cassette chromosome mec (SCCmec). SCCmec is a mobile genetic element ranging in size from 21 to 67 kbp and is widely distributed among various *Staphylococcus* species. These elements play a crucial role in the evolution, adaptation, and dissemination of antimicrobial resistance among *Staphylococcal* populations (8-10). SCC elements harbor cassette chromosome recombinase (*ccr*) genes, which mediate their integration and excision within the bacterial chromosome. Among the various SCC elements identified up to this time, SCCmec is the most extensively studied because it carries the *mecA* gene, the principal determinant of methicillin resistance, thereby conferring resistance to β-lactam antibiotics. SCCmec elements exhibit remarkable genetic diversity, and numerous SCCmec types and subtypes have been identified based on the combination of the *mec* gene complexes and *ccr* genes (9). According to the International Working Group on the Classification of *Staphylococcal* Cassette Chromosome Elements (IWG-SCC), 13 SCCmec types (I–XIII) have been recognized to date. These types are classified by specific combinations of *mec* gene complexes (A, B, C1, C2, and E) and *ccr* gene complexes (types 1–9), reflecting the extensive structural diversity and evolutionary adaptation of SCCmec elements across *Staphylococcal* populations (9,11).

Different SCCmec types are associated with distinct epidemiological features, virulence characteristics, and antimicrobial resistance patterns. Therefore, SCCmec typing has become an essential molecular epidemiological tool for tracking the transmission and evolution of resistant *Staphylococcal* strains in healthcare settings (12,13). Also, SCCmec types serve as key molecular markers for differentiating hospital-acquired methicillin-resistant *S. aureus* (HA-MRSA) from community-acquired methicillin -resistant *S. aureus* (CA-MRSA). In general, HA-MRSA strains are linked to SCCmec types I, II, and III, whereas CA-MRSA strains are predominantly associated with SCCmec types IV and V. The growing prevalence of CA-MRSA in hospitals may be attributed to the relatively smaller size and greater mobility of SCCmec IVa and SCCmec V elements compared with other SCCmec types (14).

Tetracycline is a broad-spectrum antimicrobial agent extensively used in the treatment of *staphylococcal* infections. However, the widespread and sometimes inappropriate use of tetracycline has accelerated the emergence of

resistant bacterial strains (15,16).

In *Staphylococcus* species, resistance to tetracycline is primarily associated with two major mechanisms: (i) active efflux pumps mediated by the plasmid-borne *tet(K)* and *tet (L)* genes, which mainly confer resistance to tetracycline, and (ii) ribosomal protection mediated by the *tet(M)* and *tet(O)* genes, located on transposons or chromosomal elements, which are associated with resistance to both tetracycline and minocycline (17).

The increasing dissemination of these resistance determinants among clinical isolates poses a significant challenge to antimicrobial therapy and infection control programs (5,15). Understanding the relationship between SCCmec types and tetracycline resistance genes can provide insight into the dissemination of resistant clones.

Although several studies have investigated antimicrobial resistance among *Staphylococci*, limited information is available regarding the distribution of SCCmec types and their association with tetracycline resistance genes among clinical isolates in southern Iran. Therefore, this study investigated SCCmec types and their association with tetracycline resistance genes among clinical *Staphylococcus* isolates.

Materials and Methods

A) Staphylococcus isolates: This cross-sectional study was conducted on 93 *Staphylococcus* isolates recovered from a variety of clinical specimens collected from Namazi hospital in Shiraz. Presumptive identification of *Staphylococcus* isolates was performed based on the colony morphology, Gram staining characteristics, and catalase test. Gram-positive cocci arranged in clusters and showing positive catalase reactions were considered as *Staphylococcus* spp. Further differentiation of isolates was carried out using standard biochemical tests, including coagulase, DNase, mannitol fermentation, and novobiocin susceptibility tests. Coagulase-positive isolates were identified as *Staphylococcus aureus*, whereas coagulase-negative isolates were classified as coagulase-negative *Staphylococci* (CoNS). Then, the isolates were frozen and stored at -20ºC for further study. The biochemical identification was more confirmed by a species specific PCR assay (4).

B) Antimicrobial susceptibility testing by disk diffusion method: The antimicrobial susceptibility of 93 *Staphylococcus* isolates against tetracycline was determined using the standard disk diffusion assay based on the recommendations of the Clinical and Laboratory Standards Institute (CLSI). The test was carried out on Mueller-Hinton agar plates (Merck, Germany).

Several well-isolated colonies from fresh 24-hour pure cultures (on blood agar) were transferred into tubes with 5 mL of Mueller Hinton broth, and the bacterial suspensions were adjusted to the turbidity equivalent of a 0.5 McFarland standard. Subsequently, tetracycline disks (30 µg, PadtanTeb, Iran) were placed on the inoculated agar surface. After incubation at 37°C for 24 hours, the diameters of inhibition zones were measured and interpreted according to CLSI criteria (8).

C) Evaluation of tetracycline resistance by MIC method: The minimum inhibitory concentration (MIC) was determined using the broth microdilution method according to the guidelines of the CLSI. Serial two-fold dilutions of tetracycline ranging from 256 to 0.25 µg/mL were prepared in sterile distilled water. The assay was performed in 96-well microplates containing bacterial suspension and

different antibiotic concentrations. Positive and negative controls were included in each assay. After incubation at 37°C for 18-20 h, the MIC value was defined as the lowest concentration of tetracycline that completely inhibited visible bacterial growth. The obtained results were interpreted according to CLSI standards which proposed susceptible category breakpoints at ≤ 4 µg/mL and resistance higher ≥16 µg/mL for *Staphylococci* (18).

D) Molecular analysis: Molecular identification and characterization of the isolates were performed using polymerase chain reaction (PCR) assays.

Genomic DNA was extracted from bacterial isolates using the DNP^{TM} extraction kit (CinnaGen, Iran) according to the manufacturer's instructions. The extraction procedure required approximately 35-55 min, and the purified DNA obtained was suitable for subsequent molecular biology applications. The quality and concentration of the extracted DNA were evaluated, and the samples were stored at -20° C until further analysis.

Specific primers were used for amplification of antibiotic resistance genes (*tetK* and *tetM*) as well as SCCmec typing. Primers specific for SCCmec types I, II, III, IVa, IVb, IVc, IVd, and V were utilized in this study together with primers targeting the *tetK* and *tetM* genes (Table 1). Amplification of the *tet* genes was performed by individual PCR assays using primer sets previously described by Di Francesco et al. (19), whereas primers for SCCmec typing were selected according to the method described by Jadhav et al. (7).

PCR amplification was performed in a final reaction volume of 50 µL containing 0.2 mM dNTPs, 4 µL of extracted DNA template, 3 mM $MgCl_2$, 10 mM Tris-HCl, 10 mM KCl, 20 pmol of each primer, and 1 U of Taq DNA polymerase. The thermal cycling conditions included an initial denaturation step at 95°C for 5 min, followed by 40 amplification cycles consisting of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72° C for 30 s. A final extension step was carried out at 72°C for 7 min. The amplified PCR products were separated by electrophoresis on 1.5% agarose gel containing ethidium bromide and subsequently visualized under ultraviolet (UV) illumination (7).

Table 1. Primer sequences used for PCR detection of SCCmec and tetracycline resistance (*tet*) genes.

Gene	Primer Sequence (5′–3′)	(bp)	Ref
SCCmec I	F:GCTTTAAAGAGTGTCGTTACAGG R:GTTCTCTCATAGTATGACGTCC	613	7
SCCmec II	F:CGTTGAAGATGATGAAGCG R:CGAAATCAATGGTTAATGGACC	398	7
SCCmec III	F:CCATATTGTACGATGCG R:CCTTATTGTCGTAACAGATCG	280	7
SCCmec IVa	F:CGCCTTATTCGAAGAAACCG R:CTACTCTTCTGAAAAGCGTCG	776	7
SCCmec IVb	F:TCTGGAATTACTTCAGCTGC R:AAACAATATTGCTCTCCCT	493	7
SCCmec IVc	F:ACAATATTTGTATTATCGGAGAG C R:TTGGTATGAGGTATTGCTGG	200	7
SCCmec IVd	F:CTCAAAATACGGACCCCAATAC A R:TGCTCCAGTAATTGCTAAAG	881	7
SCCmec V	F:GAACATTGTTACTTAAATGAGCG R:TGAAAGTTGTACCCTTGACACC	325	7
tetK	F:TCGATAGGAACAGCAGTA R:CAGCAGATCCTACTCCTT	169	19
tetM	F:GTGGACAAAGGTACAACGAG R:CGGTAAAGTTCGTCACACAC	406	19

E) Statistical analysis: The association between SCCmec types and tetracycline resistance was evaluated using the chi-square test. A p-value of less than 0.05 was considered statistically significant. Statistical analyses were performed using *SPSS* version 24 Statistics software.

Results

A) Clinical isolates: In this study, a total of 93

Staphylococcus isolates were collected from Namazi hospital in Shiraz. The species distribution was as follows: *S. epidermidis* 66 (70.97%), *S. aureus* 26 (27.96%), and *S. saprophyticus* 1 (1.07%). The different clinical samples included urine (n = 38; 40.86%), wound (n = 29; 31.18%), blood (n = 20; 21.50%), and sputum (n = 6; 6.45%). This study was approved by the relevant Ethics Committee (Ethics Code: IR.IAU.A.REC.1402.075).

B) Phenotypic susceptibility to tetracycline: Results of antibacterial susceptibility tests for the 93 screened *Staphylococcus* isolates (Fig. 1) shows that 15 (57.7%) of *S. aureus* and 41 (61.2%) of coagulase negative *Staphylococcus* isolates were resistance to tetracycline. The only *S. saprophyticus* isolate recovered in the present study exhibited resistance to tetracycline. In contrast, intermediate susceptibility to tetracycline was observed in 6 (6.45%) of *S. epidermidis* isolates and 2 (2.15%) of *S. aureus* isolates.

No statistically significant association was observed in the distribution of resistotype frequencies between coagulase-positive and coagulase-negative *Staphylococcus* isolates ($P > 0.05$).

C) Determination of tetracycline MIC values in Staphylococcus isolates: According to the CLSI guidelines for *Staphylococcus* spp., isolates with MIC values ≤4 µg/mL were considered susceptible, isolates with MIC values of 8 µg/mL were classified as intermediate, and isolates with MIC values ≥16 µg/mL were considered resistant to tetracycline. Following MIC testing, the susceptibility pattern of *Staphylococcus* strains to tetracycline is presented in Table 2. Among the examined isolates, 31 samples (33.3%) exhibited MIC values less than or equal to 4 µg/mL for tetracycline, while 11 isolates (11.8%) showed an MIC value of 8 µg/mL. In addition, 51 isolates (54.9%) demonstrated MIC values greater than or equal to 16 µg/mL, indicating resistance to tetracycline. No statistically significant correlation was identified between the bacterial isolates and the level of tetracycline resistance observed in the present study ($P > 0.05$).

D) Frequency of tetracycline resistance genes: PCR analysis revealed that among the 93 *Staphylococcus* isolates, 38 isolates (41.3%) carried the *tetM* gene, while 55 isolates were negative for this gene. In addition, the *tetK* gene was detected in 36 isolates (38.7%). The amplified PCR products for the *tetM* and *tetK* genes were 406 bp and 169 bp in size, respectively (Fig 2 and 3). No statistically significant differences were detected between the tetracycline resistance genes (P = 0.76).

E) PCR detection of the SCCmec genes in clinical isolates: Assigned SCCmec types by PCR revealed SCCmec type II was predominant with (n = 53; 60%) samples, followed by SCCmec types I (n = 46; 49%), IVa (n = 40; 42.8%), V (n = 40; 42.8%), IVc

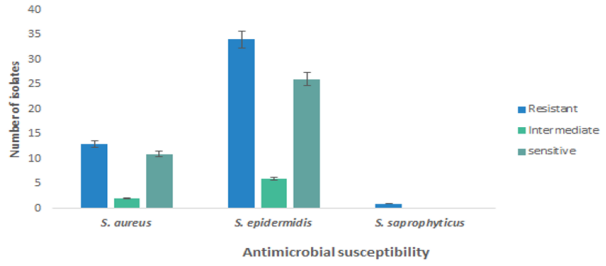

Fig 1. Prevalence and frequency distribution of tetracycline resistant *Staphylococcus* isolates.

Table 2. Tetracycline Susceptibility pattern of *Staphylococcus* isolates by MIC.

Susceptibility Pattern	Number of Isolates (n)	Percentage (%)	MIC value (µg/mL)
Sensitive	31	33.33	≤4
Intermediate	11	11.83	8
Resistant	51	54.84	≥16
Total	93	100	-

(n = 38; 41.3%), IVb (n = 37; 39.7%), IVd (n = 37; 39.7%), and III (n = 36; 38%) (Figure 4). Figure 5 shows the PCR amplification of the SCCmec II gene, which produced a distinct specific band of approximately 398 bp. Also, the significant differences between SCCmec type II with other types was observed ($P < 0.05$). A statistically significant correlation was observed between SCCmec II and the *tetK* ($P = 0.01$) as well as *tetM* ($P = 0.02$) resistance genes. However, no significant association was detected between the *tetK* and *tetM* genes and the other SCCmec types.

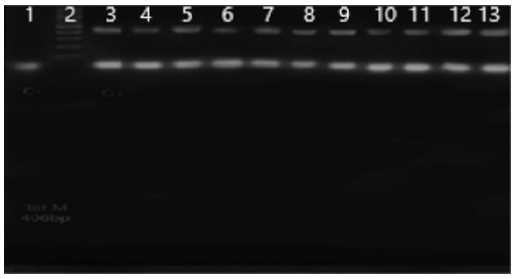

Fig 2. Agarose gel electrophoresis of PCR products for the *tetM* gene. Lane 1: negative control (C $^-$); lane 2: 100 bp Marker (DNA ladder); lane 3: positive control (C $^+$) and Lanes 4-13: positive isolates showing the 169 bp amplified fragment corresponding to the *tetK* gene.

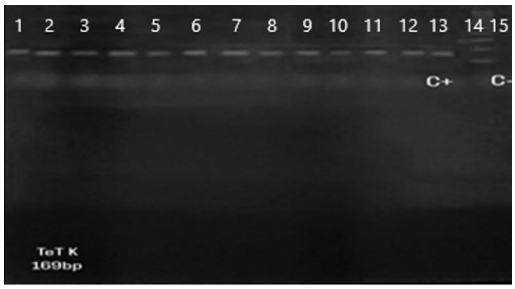

Fig 3. Agarose gel electrophoresis of PCR products amplified for the *tetK* gene. Lanes1-12: positive isolates showing the 169 bp amplified fragment corresponding to the *tetK* gene; lane 13: positive control (C $^+$); lane 14: 100 bp Marker (DNA ladder) and lane 15: negative control (C $^-$).

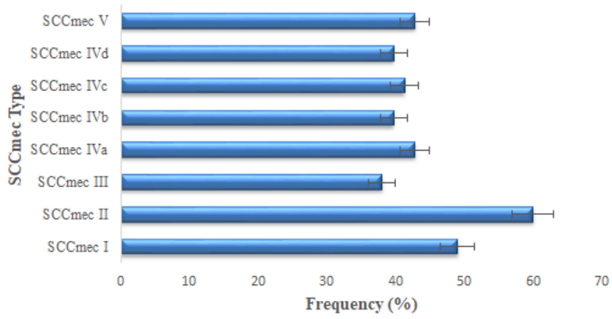

Fig 4. Frequency distribution of SCCmec types among clinical *Staphylococcus* isolates.

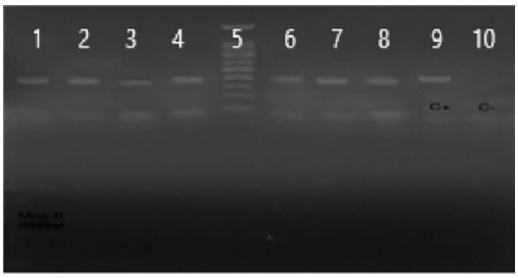

Fig 5. PCR products for the detection of a specific gene for SCCmec type II. Lanes1-4 , 6-8: positive isolates showing the 398 bp amplified fragment corresponding to the SCCmec II gene; lane 5: 100 bp Marker (DNA ladder); lane 9: positive control (C$^+$) and lane 10: negative control (C $^-$).

Discussion

Antibiotic resistance (AR) has emerged as one of the most serious public health challenges worldwide. Recent estimates indicate that nearly 4.95 million deaths are associated with infections caused by resistant microorganisms each year, and this number has potential to increase to 10 million annually by 2050 if effective interventions are not implemented. High levels of resistance have been reported among major bacterial pathogens, including approximately 35% methicillin resistance in *Staphylococcus aureus* (20).

The global emergence of antimicrobial resistant *Staphylococcus* species has become a serious public health challenge, particularly in hospital environments where resistant strains are associated with prolonged hospitalization, therapeutic failure, and increased mortality rates. Numerous studies have focused on uncovering the genetic factors responsible for

antimicrobial resistance (AMR) across different bacterial species. Although genotype-based approaches can successfully predict resistance phenotypes in certain situations, their predictive performance does not always reach complete accuracy (21,22). In most genomic AMR prediction frameworks, resistance is determined relative to predefined thresholds that are established using clinically significant minimum inhibitory concentration (MIC) values obtained from laboratory susceptibility testing. Moreover, even when resistance appears to be associated primarily with a specific gene or allele, the resulting phenotype is influenced by a network of genomic interactions. These interactions can affect both the activation of resistance mechanisms and the magnitude of their expression, highlighting the complexity underlying the relationship between genotype and phenotype (21,23).

In the present study, a substantial proportion of clinical *Staphylococcus* isolates demonstrated resistance to tetracycline, indicating the increasing dissemination of resistant *Staphylococcal* strains in healthcare settings in Shiraz, Iran. Recent epidemiological investigations have similarly reported high levels of tetracycline resistance among both methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase -negative *Staphylococci* (CoNS), suggesting that resistance to tetracycline remains a major concern worldwide (24-26).

Comprehensive analyses of genome wide covariation patterns provide valuable insights into the evolutionary processes shaping pathogenicity in microorganisms. Approaches that focus solely on individual genes without considering their broader genomic context are often insufficient for reliably predicting biological functions, including antimicrobial resistance (21).

Tetracycline antibiotics are generally effective against Gram-positive bacteria. The distribution of disk diffusion inhibition zones and MIC values usually demonstrates a bimodal pattern, distinguishing susceptible wild-type isolates from those with elevated MICs that are associated with either acquired or inherent resistance mechanisms (27).

The results of the minimum inhibitory concentration (MIC) assay demonstrated varying levels of tetracycline resistance among the clinical *Staphylococcus* isolates. Most resistant isolates exhibited MIC values ranging from 8 to 16 µg/mL, while several isolates showed resistance at higher concentrations up to 32 µg/mL. Overall, tetracycline resistance was detected in a considerable proportion of the examined isolates.

Tetracycline resistance in *staphylococci* is mainly mediated through efflux pumps encoded by the *tetK* gene and ribosomal protection proteins encoded by the *tetM* gene. In the current study, the frequency of the *tetM* gene was slightly higher than that of *tetK*. Similar findings have been reported in several recent studies demonstrating that *tetM* is among the predominant tetracycline resistance determinants in clinical *Staphylococcus* isolates. The widespread prevalence of *tetM* may be associated with its localization on conjugative transposons and transferable mobile genetic elements that facilitate horizontal gene transfer among bacterial populations (28). In contrast, the *tetK* gene is commonly plasmid-mediated and has frequently been identified in multidrug-resistant S. aureus and CoNS isolates (17,19). The coexistence of these resistance genes may contribute to increased adaptability and persistence of resistant strains in clinical environments.

Recent studies have demonstrated that SCC and SCCmec elements contribute not only to the dissemination of antimicrobial resistance but also to bacterial adaptation against bacteriophage

predation. These mobile genetic elements frequently harbor a variety of anti-phage defense systems encoded within or adjacent to SCC/SCCmec cassettes. In addition to carrying the *mecA* gene and mediating resistance to β-lactam antibiotics, SCCmec elements may facilitate the acquisition and mobilization of phage-resistance determinants. Consequently, the evolutionary success of SCCmec-positive *Staphylococci* may be attributed not only to enhanced antibiotic resistance but also to improved protection against bacteriophage infection, thereby promoting bacterial survival and persistence in clinical environments (13,29).

In the present study, the distribution of SCCmec types among MRSA isolates was investigated and compared with previous reports from Iran. The predominance of SCCmec type II among the isolates is notable because this type is commonly associated with hospital-acquired MRSA strains and is frequently linked to multidrug resistance phenotypes. The high prevalence of SCCmec II in this study, may indicate the circulation and persistence of hospital-adapted resistant clones in medical centers of Shiraz. In contrast, the lower prevalence of SCCmec type III observed in the current study may reflect regional variations in clonal dissemination patterns and differences in antimicrobial selective pressure.

The distribution of SCCmec types observed in the present study differed from that reported by Zarkesh Esfahani et al. in Isfahan, Iran. In their study, 50 MRSA isolates collected from various clinical specimens in Isfahan were analyzed using multiplex PCR, and SCCmec type III was identified in 96% of the isolates, and SCCmec type IV accounted for only 4% of cases (30). While their results demonstrated a marked predominance of SCCmec type III, SCCmec type II was the most prevalent type identified in our study. This discrepancy may reflect temporal changes in the molecular epidemiology of MRSA, differences in the study populations, variation in specimen sources, or the emergence and dissemination of distinct hospital-associated clones in different healthcare settings. Nevertheless, both studies indicate the predominance of SCCmec types that are typically associated with healthcare associated MRSA strains, highlighting the continued importance of infection control measures and molecular surveillance programs to monitor the evolution and spread of MRSA within hospital environments.

Ghaznavi-Rad et al. reported a high level of SCCmec genetic diversity among methicillin resistant coagulase-negative *Staphylococci* (MR-CoNS) isolates. Among 70 MR-CoNS isolates, *S. epidermidis* (67%) was the predominant species, followed by *S. saprophyticus* (14.3%), *S. hemolyticus* (13%), and *S. lugdunensis* (5.7%). SCCmec type IVa (27%) was identified as the most prevalent cassette type, whereas SCCmec types III (16%), II (10%), and V (3%) were at lower frequencies (31).

In a study conducted on 200 clinical specimens in Egypt, a total of 124 *S. aureus* isolates were identified by conventional phenotypic methods and subsequently confirmed as methicillin resistant *S. aureus* (MRSA) using PCR analysis. The highest isolation rates were observed among wound, sputum, blood, and urine samples. Antimicrobial susceptibility testing demonstrated that 65.3% of the isolates were resistant to tetracycline. Molecular characterization using multiplex PCR revealed the presence of SCCmec elements in 57% of the isolates. Among the SCCmec-positive isolates, type II was the most prevalent (n = 40), followed by type III (n = 21), type IVa (n = 3), type IVd (n = 2), and type V (n = 1). Additionally, four

isolates were found to harbor both SCCmec type II and SCCmec type IV elements, indicating the genetic diversity of MRSA strains circulating in the studied hospitals (32).

In a study by Goudarzi et al. *tet(M)* was identified as the most prevalent tetracycline resistance determinant (45.3%), followed by *tet (K)* (41.1%), while *tet(L)* and *tet(O)* were detected at substantially lower frequencies (2.1% each). Similarly, the results of the present study demonstrated a higher prevalence of *tetM* compared with *tetK*, confirming the dominant role of these two genes in mediating tetracycline resistance among clinical *Staphylococcus* isolates. Regarding SCCmec typing, five distinct SCCmec patterns were identified in the previous investigation, with SCCmec type III (42.1%) being the predominant type (8). In contrast, SCCmec type II was the most frequently detected cassette chromosome element in the current study. These differences may reflect geographical variation, differences in circulating clones, and local antimicrobial selection pressures. Nevertheless, both studies highlight the extensive genetic diversity of SCCmec elements and the widespread distribution of tetracycline resistance determinants among *Staphylococcal* populations.

The findings of the present study are consistent with those reported by previous investigators (32), who identified a high prevalence of tetracycline-resistant *S. aureus* isolates and a predominance of SCCmec type II among MRSA strains. In the aforementioned study , tetracycline resistance was detected in 65.3% of isolates, and SCCmec type II represented the most common cassette chromosome type (32). Similarly, our results demonstrated a high frequency of tetracycline resistance and revealed SCCmec type II as the predominant SCCmec element among the examined isolates. The comparable distribution of SCCmec types observed in both studies may indicate the circulation of related resistant clones and the persistence of SCCmec type II-associated strains within healthcare settings. These findings further support the role of SCCmec type II as an important genetic determinant contributing to the dissemination of antimicrobial resistance among clinical *Staphylococcus* isolates.

The statistically significant association observed between SCCmec II and tetracycline resistance determinants (*tetK* and *tetM*) suggests that SCCmec II-positive isolates may possess a greater capacity for accumulation and dissemination of multiple antimicrobial resistance genes. Previous studies have reported that SCCmec II frequently coexists with resistance determinants against non-β-lactam antibiotics, including tetracycline, aminoglycosides, and macrolides, thereby contributing to multidrug resistance phenotypes (11,33). The coexistence of SCCmec elements and tetracycline resistance genes may complicate treatment strategies and reduce the effectiveness of commonly used antimicrobial agents.

Another notable finding of the present study was the predominance of coagulase-negative *Staphylococci* among the collected isolates. Although CoNS were previously regarded mainly as opportunistic contaminants, they are currently recognized as important nosocomial pathogens, especially among immunocompromised patients and individuals carrying indwelling medical devices. In addition, CoNS are considered significant reservoirs of SCCmec elements and antimicrobial resistance genes that may be horizontally transferred to more pathogenic staphylococcal species such as *S. aureus*. Therefore, continuous surveillance of resistance determinants among both CoNS and *S. aureus*

is essential for understanding the molecular epidemiology and transmission dynamics of *Staphylococcal* infections.

Molecular epidemiological surveillance plays a crucial role in monitoring the dissemination of resistant clones and improving infection control policies. Identification of SCCmec types together with tetracycline resistance genes provides valuable information regarding the genetic diversity, transmission pathways, and resistance mechanisms of clinical *Staphylococcus* isolates. The findings of the present study emphasize the importance of rational antimicrobial prescription, implementation of antimicrobial stewardship programs, and strict infection prevention measures to reduce the spread of multidrug-resistant *Staphylococci* in healthcare settings.

The impact of antimicrobial resistance is especially pronounced in low- and middle-income countries, where inappropriate antibiotic use, inadequate surveillance systems, and limited healthcare resources contribute to the spread of resistant organisms. Notably, antibiotic resistance is not solely a consequence of modern antibiotic consumption. Evidence from environmental samples and ancient microbiomes has revealed the presence of resistance genes long before the introduction of antibiotics into clinical practice, suggesting that resistance represents a naturally occurring evolutionary strategy within microbial populations (20).

Despite the valuable findings obtained in this investigation, several limitations should be considered. The relatively limited sample size and the absence of advanced molecular typing methods such as multilocus sequence typing (MLST) or whole-genome sequencing restricted the detailed characterization of clonal relationships among isolates. Future studies involving larger sample populations and high-resolution genomic analyses are recommended to provide a more comprehensive understanding of the molecular epidemiology and evolutionary dynamics of resistant *Staphylococcus* strains in Iran and neighboring regions.

As the availability of large-scale genomic datasets continues to expand and knowledge of gene function becomes more refined, it becomes increasingly feasible to develop more accurate genotype–phenotype relationships. Such models can incorporate the influence of interconnected genetic networks, thereby offering a more complete representation of the mechanisms underlying phenotypic traits (21).

Conclusion

This study revealed a high prevalence of tetracycline resistance among clinical *Staphylococcus* isolates in Namazi hospital in Shiraz. The *tetM* and *tetK* genes were the predominant tetracycline resistance determinants. SCCmec typing identified substantial genetic diversity, with SCCmec type II representing the most prevalent cassette chromosome element. A significant association between SCCmec type II and tetracycline resistance genes suggests its potential role in the dissemination of antimicrobial resistance. These findings highlight the importance of continuous molecular surveillance, antimicrobial stewardship, and effective infection control measures to limit the spread of resistant staphylococcal strains.

Conflicts of interest

The authors declare that there are no conflicts of interest associated with this manuscript.

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