Investigation of Resistance to Aminoglycosides and Tetracyclines among Methicillin-Resistant and -Sensitive *Staphylococcus* Isolates in Shiraz, Southwestern Iran

Tarbiat Modares University

ARTICLE INFO

Authors
Yeganeh Sadeghi asl, *MSc[1]*
Abolfazl Rafati Zomorodi, *PhD[1,2]*
Abdollah Bazargani, *PhD[1]*
Yalda Malekzadegan, *PhD[3]*
Nafiseh Hosseinzadeh Shakib, *MSc[1]*
Mohammad Motamedifar, *PhD[4*]*

[1] Department of Bacteriology and Virology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
[2] Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran.
[3] Department of Microbiology, Saveh University of Medical Sciences, Saveh, Iran.
[4] HIV/AIDS Research Center, Institute of Health, Shiraz University of Medical Sciences, Shiraz, Iran.

* *Correspondence*
HIV/AIDS Research Center, Institute of Health, Shiraz University of Medical Sciences, Shiraz, Iran.
E-mail: motamedm@sums.ac.ir

ABSTRACT

Background: Methicillin-resistant staphylococci (MRS) are regarded as a global public health threat. Physicians are restricted in their treatment options due to resistance to aminoglycosides and tetracycline derivatives. This study investigated aminoglycoside and tetracycline derivative resistance among *Staphylococcus* isolates in Shiraz, southwestern Iran..

Materials & Methods: Totally, 113 staphylococcal isolates were recovered from different clinical samples in Nemazee Teaching Hospital from October 2019 to January 2020. Kirby-Bauer disc diffusion method was performed to assess the antimicrobial susceptibility of the isolates against aminoglycoside and tetracycline antibiotics. Aminoglycoside-modifying enzymes (AMEs) and *tet* genes were investigated among staphylococci isolates using polymerase chain reactions (PCR).

Findings: MRS prevalence among *Staphylococcus* isolates was 61% (69 of 113). The majority of MRS isolates were obtained from blood (39.1%; 27 of 69) and urine (17.4%; 12 of 69). The highest prevalence of MRS isolates was among emergency room patients (34.8%; 24 of 69). The highest resistance of MRS isolates was against tobramycin (59.4%; 41 of 69) and tetracycline (55.1%; 38 of 69). The prevalence of *tetM* and *aac* (6')-*Ie-aph* (2'') genes was significantly higher among MRS compared with methicillin-sensitive staphylococci (MSS) (87.5% vs 12.5% and 95.6% vs 6.4%, respectively) (*p*= .001).

Conclusion: The prevalence of MRS isolates, including methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci (MRCoNS), was remarkable in Shiraz as the center of medical services in the southwest of Iran. Furthermore, these MRS isolates were highly resistant to aminoglycosides and tetracyclines. Therefore, antimicrobial stewardship is necessary to address health conditions.

How to cite this article
Sadeghi-asl Y., Rafati-Zomorodi A., Bazargani A., Malekzadegan Y., Hosseinzadeh Shakib N., Motamedifar M. Investigation of Resistance to Aminoglycosides and Tetracyclines among Methicillin-Resistant and -Sensitive Staphylococcus isolates in Shiraz, Southwestern Iran. Infection Epidemiology and Microbiology. 2024;10(2): 89-100

CITATION LINKS

[1] Parte AC. LPSN–List of prokaryotic... [2] Marsilio F, et al. Coagulase-positive... [3] Sato'o Y, et al. Optimized... [4] Heilmann C, Ziebuhr W, Becker K. Are coagulase... [5] Adeoye-Isijola M, et al. Vancomycin ... [6] Asante J, et al. Review of ... [7] McClure JA, et al. Novel multiplex... [8] Khan HA, et al. Nosocomial... [9] Tkadlec J, et al. Characterization of... [10] Mlynarczyk-Bonikowska B,et al. Molecular... [11] Watkins RR, et al. Antimicrobial... [12] Seyedi-Marghaki F, Distribution of... [13] Epps QJ, et al. State of... [14] Bourbour S, et al. Identification of major... [15] Beigverdi R, et al. Prevalence of genes... [16] Goudarzi M, et al. Distribution of... [17] Al-Saadi DA, Abd Al-Mayahi FS, editors. Antibiogram... [18] Pal M, Kerorsa GB, Marami LM, Kandi V. Epidemiology... [19] Lollai S, Ziccheddu M, Duprè I, Piras D. Characterization of... [20] Khoramrooz SS, et al. Detection of... [21] Garoy EY, et al. Methicillin... [22] Clinical and Laboratory Standards... [23] Zomorodi AR, et al. Investigation of... [24] Adwan K. Fast DNA isolation... [25] Motamedifar M, et al. Frequency of the... [26] Asokan GV, et al. WHO global... [27] Kranjec C, et al. Staphylococcal... [28] Bahmani N, Kalantar E, Torabi V. Survey of... [29] Abbasi Montazeri E, et al. Prevalence of... [30] Singh S, et al. Coagulase... [31] Gergova RT, et al. Molecular... [32] Asante J, et al. Multidrug-resistant... [33] Shariati A,et al. The global... [34] Perumal N, Murugesan S, Krishnan P. Distribution of... [35] Ogefere HO, et al. Prevalence of... [36] Xu S, et al. Ocular pathogens... [37] Díaz MC, et al. In-vitro activity of... [38] Mahdiyoun SM, et al. Frequency of... [39] Xu Z, et al. Antimicrobial... [40] Li Y, et al. Methicillin-resistant... [41] Ong ML, et al. High prevalence of... [42] Shrief R, et al. Prevalence of... [43] Emaneini M, et al. Distribution... [44] Poulsen AB, et al. Detection of...

Introduction

Staphylococcus genus comprises more than 54 species and 28 subspecies (until July 2018), consisting of several clinically highlighted pathogens [1].

This genus is further divided into two main categories regarding ability or inability to coagulate rabbit plasma, including coagulase-positive *Staphylococcus* (CoPS) and coagulase-negative *Staphylococcus* (CoNS) [2]. As a CoPS species, *Staphylococcus aureus* (*S. aureus*) is the most clinically important pathogen, causing endocarditis, osteomyelitis, and necrotizing pneumonia [3]. CoNS species have also received worldwide attention because they cause healthcare device-mediated infections (by producing biofilm), abscesses, endocarditis, and urethritis [4].

Since 1961, when they were first detected in the UK, the global expansion of methicillin-resistant *S. aureus* (MRSA) strains has been ongoing [5] and followed by the emergence of methicillin-resistant coagulase-negative staphylococci (MRCoNS) causing serious nosocomial infections [6, 7]. Therefore, the Center for Disease Control (CDC) has acknowledged that MRSA and MRCoNS strains are among the most significant causes of healthcare-associated infections [8]. Furthermore, MRSA and MRCoNS strains display a variety of resistance mechanisms that limit treatment options for related infections. Previous investigations have shown that MRSA and MRCoNS are almost resistant to other antibiotics such as tetracyclines, aminoglycosides, and lincosamides [9-11]. It is more critical to emphasize the emergence of resistance to aminoglycosides and tetracycline derivatives. This is because aminoglycosides are often used synergistically with beta-lactam or glycopeptide antibiotics [12]. Also, tetracycline derivatives are effective for skin and soft tissue infections [13].

The main pathway of aminoglycoside resistance is antibiotic inactivation which occurs through the production of aminoglycoside-modifying enzymes (AMEs) [14]. AMEs are divided into four categories based on the difference in modification type, including acetyltransferases (AACs), phosphotransferases (APHs), nucleotidyltransferases (ANTs), and adenyltransferases (AADs) [15].

Among *Staphylococcus* spp. strains, the three most predominant genes encoding these enzymes are *aac (6')-Ie-aph (2″), aph (3')-IIIa,* and *ant (4')-Ia*; these genes are vitally important because the majority of them are located on plasmids and transposable elements that enable them to evolve by horizontal gene transfer (HGT) [16, 17].

Bacteria with plasmid-encoded *tetK* and *tetL* genes give rise to active efflux pumps, and ribosomal protection mediated by chromosomal or transposon *tetM* or *tetO* genes leads to resistance to tetracyclines [18, 19]. The *tetK* gene causes resistance to tetracycline, while the *tetM* gene develops resistance to tetracycline and minocycline [20]. Nosocomial infections caused by methicillin-resistant staphylococci (MRS) are a major concern globally.

This is due to their high antimicrobial resistance rates. Specifically, resistance to aminoglycosides and tetracycline derivatives limits physicians' treatment options. Therefore, evaluating the prevalence of MRS isolates resistant to aminoglycosides and tetracycline derivatives is recommended to control nosocomial infections.

Objectives: The present study aimed to determine the prevalence of MRSA and MRCoNS isolates and investigate the phenotypic and genotypic resistance to aminoglycoside and tetracycline derivatives among MRS and MSS (methicillin-susceptible staphylococci) isolates in Shiraz, southwestern Iran.

Materials and Methods

Bacterial isolation and identification: In the current cross-sectional study, 113 non-duplicate *Staphylococcus* isolates were collected from patients hospitalized from October 2019 to January 2020 at Nemazee Teaching Hospital, a referral hospital in Shiraz, southwestern Iran. The isolates were obtained from patients who developed nosocomial infection within 48-72 hrs after admission to the hospital. The isolates were transferred to the microbiology laboratory of the School of Medicine, Shiraz University of Medical Sciences. All the isolates were confirmed as *Staphylococcus* spp. using Gram staining, colony morphology on blood agar (Merck, Germany), and catalase/oxidase tests as recommended in a previous study by Garoy et al. (2019) [21]. Also, the isolates were divided into CoPS and CoNS using tube coagulase test [21]. Then CoPS isolates were further tested to identify them as *S. aureus*, including culture onto mannitol salt agar and DNase agar (Merck, Germany).

Antimicrobial susceptibility testing: The isolates susceptibility to six antibiotics was measured using Kirby-Bauer disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2021) [22]. The tested antibiotics were amikacin (AMK, 30 μg), gentamicin (GEN, 10 μg), and tobramycin (TOB, 10 μg) as aminoglycoside groups and tetracycline (TET, 30 μg), minocycline (MIN, 30 μg), and doxycycline (DOX, 30 μg) as tetracycline groups (Conda Pronadisa, Spain). *S. aureus* ATCC 25923 strain was used for quality control. In addition, *S. aureus* and CoNS isolates were phenotypically tested for methicillin resistance using cefoxitin (FOX, 30 μg) and oxacillin (OXA, 1 μg) discs, respectively (CLSI, 2021).

DNA preparation: DNA extraction was performed using a modified boiling method for Gram-positive bacteria as explained in a previous survey [23, 24]. From fresh subcultures, 3-5 colonies were removed and re-suspended in microtubes containing 1 mL of ultrapure sterile water; centrifugation was performed at 6000 ×g for 3 min. After that, the yielded pellets were washed in 1 mL of 1× Tris–EDTA (TE) buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0) (Merck, Germany). Then 500 μL of ultrapure sterile water was added, and the microtubes were transferred immediately into a water bath at 95 °C for 10 min. The microtubes were immediately placed in an ice box for 5 min. Finally, they were centrifuged in a microcentrifuge (Sigma 1-14, Germany) for 5 min at 8000 rpm, and then 100 μL of the supernatant was removed carefully to evaluate purity; for this purpose, the ratio of A260/A280 and A230/A280 was determined by photometric measurements (Nanodrop 2000 UV–Vis spectrophotometer).

Molecular confirmation of *Staphylococcus* isolates: Multiplex polymerase chain reaction (m-PCR) was conducted to confirm all the isolates as *Staphylococcus* spp. and *S. aureus* using previously designed primers for amplification of *16S rRNA* and *nuc* genes, respectively. In addition, MRS isolates were confirmed through determining *mecA* genes. The PCR reaction was conducted using a MJ mini thermal cycler (BioRad Laboratories, Hercules, CA, USA). Table 1 lists the primer sequences.

Molecular detection of AMEs and *tet* genes: The frequency of three AME genes (comprising *aac (6')-Ie-aph (2")-I, aph (3')-IIIa*, and *ant (4')-Ia*) and four *tet* genes (consisting of *tetK*, *tetL*, *tetM*, and *tetO*) was sought among the isolates using monoplex-PCR (Table 1). All PCR reactions were conducted in a final 25 μL volume containing 12.5 μL of PCR 2× Master Mix (Amplicon, Denmark), 1 μL of each primer (2 μM), 2 μL of template DNA, and up to a 25 μL final volume nuclease-free water.

Table 1) Oligonucleotide sequences used for identifying *Staphylococcus spp.* and determining AMEs and *tet* genes

Genes	Primer Sequence (5′→3′)	Size (bp)	Annealing (Temperature)	Reference
aac (6')-Ie-aph (2")-I	F-CAGGAATTTATCGAAAATGGTAGAAAAG R- CACAATCGACTAAAGAGTACCAATC	369	55 ℃ for 1 min	(42)
aph (3')-IIIa	F-GGCTAAAATGAGAATATCACCGG R-CTTTAAAAAAATCATACAGCTCGCG	523	55 ℃ for 1 min	
ant (4 ')-Ia	F-CAAACTGCTAAATCGGTAGAAGCC R-GGAAAGTTGACCAGACATTACGAACT	294	55 ℃ for 1 min	
tetK	F- GTAGCGACAATAGGTAATAGT R- GTAGTGACAATAAACCTCCTA	360	55 ℃ for 45 s	(20)
tetM	F- AGTGGAGCGATTACAGAA R- CATATGTCCTGGCGTGTCTA	158	51 ℃ 1 min	(19)
tetL	F-ATAAATTGTTTCGGGTCGGTAAT R- AACCAGCCAACTAATGACAATGAT	1077	51 ℃ 1 min	
tetO	F-AACTTAGGCATTCTGGCTCAC R-TCCCACTGTTCCATATCGTCA	514	57 ℃ 1 min	
16S rRNA	F- GTGCCAGCAGCCGCGGTAA R- AGACCCGGGAACGTATTCAC	886	55 ℃ for 1 min	(44)
nuc	F- TCAGCAAATGCATCACAAACAG R- CGTAAATGCACTTGCTTCAGG	255		
mecA	R-GGGATCATAGCGTCATTATTC R- AACGATTGTGACACGATAGCC	527		

Abbreviation: AMEs: aminoglycosides modifying enzymes

Statistical analysis: Statistical analysis was carried out using SPSSTM 22.0 software (SPSS Inc., Chicago, Illinois, USA). Additionally, evaluations were performed using Chi-square and Fisher's exact tests; *p*-value at $p < .05$ was considered significant [25].

Findings

Demographic information: Among 113 *Staphylococcus* isolates, the frequency of *S. aureus* and CoNS was 67.2% (76 of 113) and 32.8% (37 of 113), respectively. The age of the study participants ranged from 1 day to 109 years with a mean age of 37.85 ±28.2 years. The proportion of male and female patients was 48.7% (55 of 113) and 51.3% (58 of 113), respectively. Among different hospital wards, most *Staphylococcus* isolates were collected from patients admitted to the emergency room (34.5%; 39 of 113), followed by internal and pediatric wards (23.9%; 27 of 113), and ICU (10.6%; 12 of 113). Also, regarding the various studied specimens, most *Staphylococcus* isolates were isolated from blood (33.6%; 38 of 113), urine (15%; 17 of 113), nasal (11.5%; 13 of 113), and wound (7.1%; 8 of 113) specimens, respectively.

MRS and MSS distribution: In general, 61% (69 of 113) of *Staphylococcus* isolates were MRS, consisting of 56.5% MRSA (39 of 69) and 43.5% MRCoNS (30 of 69). The distribution of MRS isolates in different specimens was as follows: 39.1% (27 of 69) in blood, 17.4% (12 of 69) in urine, 8.7% (6 of 69) in wound, and 7.2% (5 of 69) in nasal specimens. Also, the highest prevalence of MRS isolates was observed among patients admitted to the emergency room (34.8%; 24 of 69), the prevalence among patients admitted to the other hospital wards was as follows: internal 24.6% (17 of 69), pediatric 20.3% (14 of 69), and ICU 10.1% (7 of 69). There was no significant difference in

Table 2) Demographic information about MRS and MSS isolates (N=113)

Variable (N=113)	MRS (N= 69)		MSS (N=44)		Total (%)
	MRSA (N=39) N(%)	MRCoNS (N= 30)N (%)	MSSA (N= 37)N (%)	MSCoNS (N=7)N (%)	
Gender					
Male	11 (20)	22 (40)	20 (36.4)	2 (3.6)	55 (48.7)
Female	28 (48.3)	8 (13.8)	17 (29.3)	5 (8.6)	58 (51.3)
Age					
0-10	8 (26.7)	9 (30)	12 (40)	1 (3.3)	30 (26.5)
11-20	3 (50)	2 (33.3)	1 (16.7)	0	6 (5.3)
21-30	6 (37.5)	6 (37.5)	3 (18.7)	1(6.3)	16 (14.2)
31-40	5 (71.4)	1 (14.3)	1 (14.3)	0	7 (6.2)
41-50	5 (38.5)	2 (15.4)	5 (38.5)	1 (7.7)	13 (11.5)
≥51	12 (29.3)	12 (29.3)	15 (36.6)	2 (4.9)	41 (36.3)
Wards					
Emergency Room	10 (25.6)	14 (35.9)	13 (33.3)	2 (5.1)	39 (34.5)
Internal	14 (51.9)	3 (11.1)	10 (37)	0	27 (23.9)
Pediatric	6 (22.2)	8 (29.6)	10 (37)	3 (11.1)	27 (23.9)
ICU	3 (25)	4 (33.3)	4 (33.3)	1 (8.3)	12 (10.6)
Surgery	4 (80)	1 (20)	0	0	5 (4.4)
Transplant	2 (66.7)	1 (33.3)	0	0	3 (2.7)
Sample					
Blood	11 (28.9)	16 (42.1)	9 (23.7)	2 (5.3)	38 (33.6)
Urine	4 (23.5)	8 (47.1)	4 (23.5)	1 (5.9)	17 (15)
Nasal	3 (23.1)	2 (15.4)	7 (53.8)	1 (7.7)	13 (11.5)
Wound	6 (75)	0	2 (25)	0	8 (7.1)
Eye	3 (50)	1 (16.7)	1 (16.7)	1 (16.7)	6 (5.3)
Axillary	1 (20)	1 (20)	1 (20)	2 (40)	5 (4.4)
Sputum	4 (80)	0	1 (20)	0	5 (4.4)
Throat	1 (25)	0	1 (75)	0	4 (3.5)
Pleural	1 (25)	1(25)	2 (50)	0	4 (3.5)
Abdominal	1 (33.3)	1 (33.3)	1 (33.3)	0	3 (2.7)
Tissue	2 (66.7)	0	1 (33.3)	0	3 (2.7)
Pulmonary	0		2 (100)	0	2 (1.8)
ETT	1 (50)	0	1 (50)	0	2 (1.8)
Double Lumen Tube	0	0	1 (100)	0	1 (0.9)
Fluid	0	0	1 (100)	0	1 (0.9)
Joint	1 (100)	0	0	0	1 (0.9)

Abbreviation: MRS: methicillin-resistant staphylococci, MSS: methicillin-sensitive staphylococci, MRSA: methicillin-resistant *Staphylococcus aureus*, MSSA: methicillin-sensitive *Staphylococcus aureus*, MRCoNS: methicillin-resistant coagulase-negative staphylococci, MSCoNS: methicillin-sensitive coagulase-negative staphylococci, ETT: endotracheal tube.

the distribution of MRS isolates between different specimens and hospital wards ($p= .27$ and $p= .52$, respectively). Table 2 provides additional information.

Antimicrobial susceptibility testing: Generally, the highest resistance of MRS isolates was against tobramycin (59.4%; 41 of 69) and tetracycline (55.1%; 38 of 69). Also, MRS and MSS isolates revealed a significant variation in resistance to the tested antibiotics ($p< .05$), except for doxycycline ($p= .21$). The antimicrobial susceptibility patterns of MRSA, MSSA, MRCoNS, and MSCoNS isolates are shown separately in Table 3.

Molecular detection of AME and *tet* genes: The predominant *tet* genes among *Staphylococcus* isolates were *tetM* (42.4%; 48 of 113) and *tetK* (34.5%; 39 of 113). Remarkably, the prevalence of *tetM* and *tetK* genes was significantly higher among MRS isolates (60.9%; 42 of 69, $p= .001$ and 43.5%; 30 of 69, $p= .018$, respectively) compared with MSS isolates. Also, the frequency of AME genes among *Staphylococcus* isolates was as follows: *aac (6')-Ie-aph (2'')* 39.8% (45 of 113), *aph (3')-IIIa* 30.0% (34 of 113), and *ant (4')-Ia* 16.8% (19 of 113). There was a significant difference in harboring *aac (6')-Ie-aph (2'')* and *aph (3')-IIIa* genes between MRS isolates (62.3%; 43 of 69, $p=.001$ and 43.5%; 30 of 69, $p= .001$, respectively). The distribution of detected genes among *Staphylococcus* isolates and their association with antibiotic resistance are presented in Table 4. In addition, 64.6% (73 of 113) of *Staphylococcus* isolates simultaneously contained more than one resistance gene. The prevalence of co-existence of resistance genes was 34.2% (25 of 73) for *tetM* and *tetK* and 20.5% (15 of 73) for *aac (6')-Ie-aph (2'')* and *aph (3')-IIIa* (Figure 1).

Discussion

Staphylococcal isolates, particularly MRSA and MRCoNS, are listed as threats to global health [26]. Although MRSA isolates are more prevalent, MRCoNS has also received worldwide attention [27]. According to previous surveys conducted in Iran, the prevalence of MRSA and MRCoNS was 56-55% in Sanandaj in 2013 [28] and 76.3-65.9% in Ahvaz in 2021 [29]; the prevalence rates of MRSA and MRCoNS in the present study were also in the same range (56.5 and 43.5% respectively). However, an increasing trend in the prevalence of MRCoNS has been reported in other studies conducted worldwide during the period. These studies indicated a prevalence rate of 57.6% in India in 2016 [30], 93.9% in Bulgaria in 2019 [31], and 76.4% in South Africa in 2021 [32]. As the differences could be caused by various factors, such as geographical veracity and sample size, further investigation is necessary to better compare them. Nonetheless, the increasing incidence of MRCoNS seems to be alarming globally.

Since the emergence of MRSA and MRCoNS, aminoglycosides and tetracycline-derived antibiotics have been highly prescribed for patient management in hospitals [33]. In the current study, MRSA and MRCoNS isolates were resistant to aminoglycoside antibiotics: 69.2 and 46.7% to tobramycin, 66.7 and 33.3% to gentamicin, and 61.5 and 23.3% to amikacin, respectively. Also, the phenotype of resistance to tobramycin, gentamicin, and amikacin was significantly higher among MRSA ($p= .001$) isolates compared to MSSA, MRCoNS, and MSCoNS isolates. This finding is in line with prior observations, reporting a significant correlation between MRSA and aminoglycoside resistance [34]. However, previous investigations have suggested that gentamicin is a more effective antimicrobial agent among aminoglycoside antibiotics against MRSA and MRCoNS isolates with sensitivity frequency in the range of 70-89%, respectively [35, 36]. The high frequency of the

Table 3) Antimicrobial susceptibility patterns and the frequency of *AMEs* and *tet* genes among MRS and MSS isolates (N=113)

Antibiotics	MRS (N=69)						MSS (N=44)						P Value
	MRSA (N=39) N (%)			MRCoNS (N=30) N (%)			MSSA (N=37) N (%)			MSCoNS (N=7) N (%)			
	R	I	S	R	I	S	R	I	S	R	I	S	
TET	25 (64.1)	0	14 (35.9)	13 (43.3)	0	17 (56.7)	14 (37.8)	0	23 (62.2)	1 (14.4)	0	6 (86.6)	.029
MIN	9 (23.1)	6 (15.4)	24 (61.5)	0	1 (3.3)	29 (96.7)	0	0	37 (96.7)	0	0	7 (100)	.001
DXT	9 (23.1)	7 (17.9)	23 (59)	3 (10)	6 (20)	21 (70)	3 (8.1)	4 (10.8)	30 (81)	0	0	7 (100)	.212
TOB	27 (69.2)	0	12 (30.8)	14 (46.7)	2 (6.7)	14 (46.7)	26 (70.3)	7 (18.9)	4 (10.8)	3 (42.8)	0	4 (57.2)	<.001
GEN	26 (66.7)	0	13 (33.3)	10 (33.3)	5 (16.7)	15 (50)	34 (91.9)	0	3 (8.1)	7 (100)	0	0	<.001
AMK	24 (61.5)	2 (5.2)	13 (33.3)	7 (23.3)	7 (23.3)	16 (53.3)	17 (45.9)	15 (40.5)	5 (13.5)	0	3 (42.9)	4 (57.1)	<.001

Genes	MRSA (N=39) N (%)	MRCoNS (N=30) N (%)	MSSA (N=37) N (%)	MSCoNS (N=7) N (%)	P Value
tetM	27 (69.2)	15 (50)	6 (16.2)	0	<.001
tetK	20 (51.3)	9 (30)	8 (21.6)	1 (14.4)	.018
tetL	0	3 (10)	0	0	.28
tetO	1 (2.5)	0	0	0	-*
aac (6')-le-aph (2")	24 (61.5)	19 (63.3)	2 (5.4)	0	<.001
aph (3')-IIIa	11 (28.2)	19 (63.3)	2 (5.4)	2 (28.6)	<.001
ant (4')-Ia	4 (10.3)	11 (36.7)	3 (8.1)	1 (14.3)	.08

Abbreviation: MRS: methicillin-resistant staphylococci, MSS: methicillin-sensitive staphylococci, TET: tetracycline, MIN: minocycline, DOX: doxycycline, TOB: tobramycin, GEN: gentamicin, AMK: amikacin, MRSA: methicillin-resistant *Staphylococcus aureus*, MSSA: methicillin-sensitive *Staphylococcus aureus*, MRCoNS: methicillin-resistant coagulase-negative staphylococci, MSCoNS: methicillin-sensitive coagulase-negative staphylococci, AMEs: aminoglycoside modifying enzymes
*: It is not calculable.

Table 4) Distribution of AMEs and tet genes regarding antimicrobial susceptibility patterns of Staphylococcus spp. isolates (N=113)

Positive *tet* Genes	Tetracycline				Minocycline				Doxycycline			
	R (N=77) N (%)	I (N=0) N (%)	S (N=56) N (%)	P Value	R (N=9) N (%)	I (N=9) N (%)	S (N=95) N (%)	P Value	R (N=14) N (%)	I (N=18) N (%)	S (N=91) N (%)	P Value
tetK (N=39)	22 (56.4)	0	17 (43.6)	.039	4 (10.2)	4 (10.2)	31 (79.6)	.752	9 (23)	6 (15.5)	24 (61.5)	.037
tetM (N=48)	31 (64.6)	0	17 (35.4)	.001	9 (18.7)	9 (18.7)	30 (62.6)	.001	9 (18.7)	7 (14.6)	32 (66.7)	.1
tetL (N=3)	2 (66.6)	0	1 (33.4)	-*	0	0	3 (100)	-*	0	1 (33.4)	2 (66.6)	-*
tetO (N=1)	1 (100)	0	0	-*	0	1 (100)	0	-*	0	0	0	-*

Positive AME genes	Tobramycin				Gentamicin				Amikacin			
	R (N=49) N (%)	I (N=10) N (%)	S (N=54) N (%)	P Value	R (N=36) N (%)	I (N=0) N (%)	S (N=72) N (%)	P Value	R (N=37) N (%)	I (N=30) N (%)	S (N=50) N (%)	P Value
aac (6')-Ie-aph (2'') (N=45)	35 (77.8)	2 (4.4)	8 (17.8)	.001	28 (62.2)	5 (11.1)	12 (26.7)	.001	26 (57.8)	6 (13.3)	13 (28.9)	.001
aph (3')-IIIa (N=34)	18 (53)	2 (5.9)	14 (41.1)	.176	13 (38.2)	2 (5.9)	19 (55.9)	.725	11 (32.4)	7 (20.6)	16 (47)	.811
ant (4')-Ia (N=19)	16 (84.2)	0	3 (15.8)	.001	7 (36.8)	2 (10.5)	10 (52.7)	.168	10 (52.7)	3 (15.8)	6 (31.5)	.251

Abbreviation: R: resistance, I: intermediate, S: susceptible, AME: aminoglycoside modifying enzyme
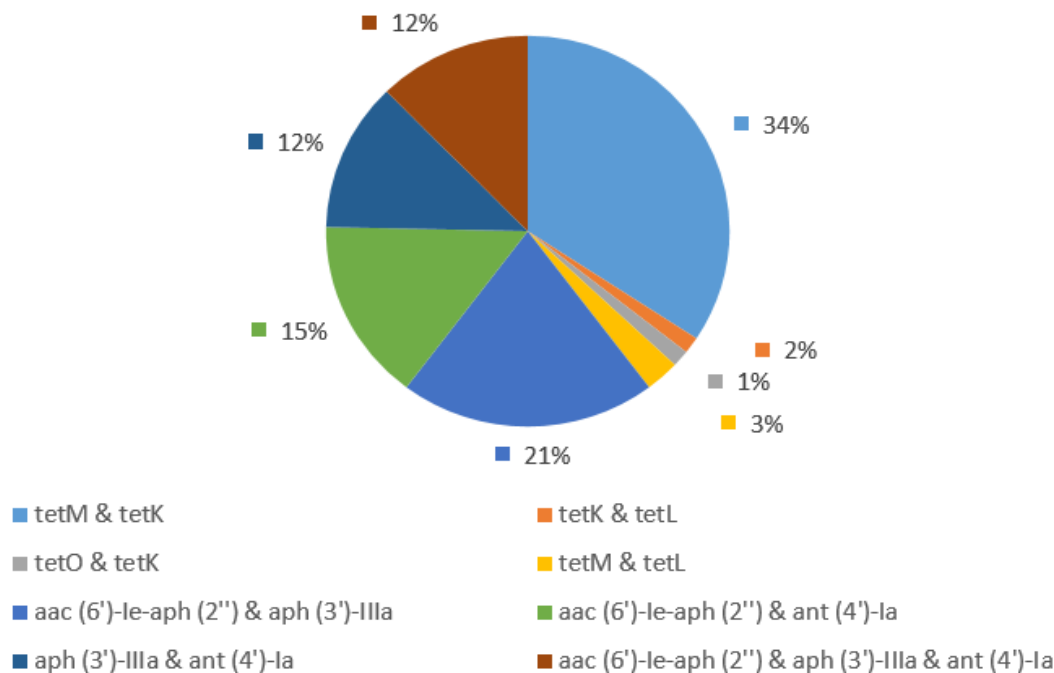*: It is not calculable.

Figure 1) Prevalence of co-existence of AMEs and *tet* genes among *Staphylococcus spp.* isolates (N=73).
Abbreviation: AME: aminoglycoside modifying enzyme

aac (6')-Ie-aph (2") gene among MRCoNS and MRSA isolates (63.3 and 61.5%, respectively) is consistent with previous observations [37, 38]. Therefore, it may be considered as one of the most relevant AME genes for gentamicin resistance.

On the other hand, tetracycline resistance among MRSA and MRCoNS isolates seems to demand careful attention. This is based on the results of other studies, reporting tetracycline resistance in the range of 35-58% among MRS isolates and 34-37% among MRCoNS [39, 40]; these findings are similar to the present study results (MRSA 64.1% and MRCoNS 43.3%). The high prevalence of antibiotic resistance among MRSA and MRCoNS isolates could lead to an increase in the proportion of MRS strains. This is done through transmission of resistance genes to MSS strains.

The relationship between *tet* genes and resistance to tetracycline-derived antibiotics was also assessed. In this regard, the presence of *tetM* and *tetK* genes as the most

prevalent genes among MRSA (69.2 and 51.3%) and MRCoNS (50 and 30%) isolates was significantly associated with resistance to tetracycline, minocycline, and doxycycline. In contrast, *tetL* and *tetO* genes are rarely found in MRSA and MRCoNS strains. These results are in line with previous experiments, establishing the *tetM* gene as the most prevalent gene among tetracycline-resistant MRSA isolates [16, 41-43].

To the best of our knowledge, several pieces of evidence suggest that AMEs and *tet* genes are widespread among *Staphylococcus* species. For instance, the role of integron classes as well-known genetic elements responsible for resistance gene spread among Gram-negative bacteria, especially *Staphylococcus* strains, is well documented [10]. Therefore, it would be beneficial to examine how to target the dissemination of genes encoding antimicrobial resistance. The present investigation suffers from some limitations such as not determining the genotype of *Staphylococcus* isolates and

the species of CoNS isolates. Thus, further investigations are suggested to assess how the prevalence of MRS and MSS isolates has changed over time.

Conclusion

In conclusion, the prevalence of MRS isolates, including MRSA and MRCoNS, was remarkable in Shiraz as the center of medical services in the southwest of Iran. Furthermore, these MRS isolates were highly resistant to aminoglycosides and tetracyclines. For this reason, antimicrobial stewardship is necessary to address at-risk health conditions. Eventually, the increasing prevalence rate of MRCoNS should be considered seriously globally.

References

1. Parte AC. LPSN–List of prokaryotic names with standing in nomenclature (bacterio. net), 20 years on. Int J Syst Evol Microbiol. 2018;68(6):1825-9.

2. Marsilio F, Di Francesco CE, Di Martino B. Coagulase-positive and coagulase-negative staphylococci animal diseases. In: Savini V, editor. Pet-to-man travelling staphylococci: A world in progress. US: Elsevier Science Publishing; 2018, pp. 43-50.

3. Sato'o Y, Aiba Y, Kiga K, Watanabe S, Sasahara T, Hayakawa Y, et al. Optimized universal protocol for electroporation of both coagulase-positive and-negative staphylococci. J Microbiol Methods. 2018;146:25-32.

4. Heilmann C, Ziebuhr W, Becker K. Are coagulase-negative staphylococci virulent? Clin Microbiol Infect. 2019;25(9):1071-80.

5. Adeoye-Isijola M, Olajuyigbe O, Adebola K, Coopoosamy R, Afolayan A. Vancomycin intermediate resistant Staphylococcus aureus in the nasal cavity of asymptomatic individuals: A potential public health challenge. Afr Health Sci. 2020;20(3):1109-17.

6. Asante J, Amoako DG, Abia AL, Somboro AM, Govinden U, Bester LA, et al. Review of clinically and epidemiologically relevant coagulase-negative staphylococci in Africa. Microb Drug Resist. 2020;26(8):951-70.

7. McClure JA, Zaal DeLongchamp J, Conly JM, Zhang K. Novel multiplex PCR assay for detection of chlorhexidine-quaternary ammonium, mupirocin, and methicillin resistance genes, with simultaneous discrimination of Staphylococcus aureus from coagulase-negative staphylococci. J Clin Microbiol. 2017;55(6):1857-64.

8. Khan HA, Ahmad A, Mehboob R. Nosocomial infections and their control strategies. Asian Pac J Trop Biomed. 2015;5(7):509-14.

9. Tkadlec J, Vařeková E, Pantůček R, Doškař J, Růžičková V, Botka T, et al. Characterization of Staphylococcus aureus strains isolated from Czech cystic fibrosis patients: High rate of ribosomal mutation conferring resistance to MLSB antibiotics as a result of long-term and low-dose azithromycin treatment. Microb Drug Resist. 2015;21(4):416-23.

10. Mlynarczyk-Bonikowska B, Kowalewski C, Krolak-Ulinska A, Marusza W. Molecular mechanisms of drug resistance in Staphylococcus aureus. Int J Mol Sci. 2022;23(15):8088.

11. Watkins RR, Holubar M, David MZ. Antimicrobial resistance in methicillin-resistant Staphylococcus aureus to newer antimicrobial agents. Antimicrob Agents Chemother. 2019;63(12):10-128.

12. Seyedi-Marghaki F, Kalantar-Neyestanaki D,

Saffari F, Hosseini-Nave H, Moradi M. Distribution of aminoglycoside-modifying enzymes and molecular analysis of the coagulase gene in clinical isolates of methicillin-resistant and methicillin-susceptible Staphylococcus aureus. Microb Drug Resist. 2019;25(1):47-53.

13. Epps QJ, Epps KL, Young DC, Zobell JT. State of the art in cystic fibrosis pharmacology—Optimization of antimicrobials in the treatment of cystic fibrosis pulmonary exacerbations: I. Anti-methicillin-resistant Staphylococcus aureus (MRSA) antibiotics. Pediatr Pulmonol. 2020;55(1):33-57.

14. Bourbour S, Beigverdi R, Beheshti M, Jabalameli F, Emaneini M. Identification of major sequence types among aminoglycoside resistant Staphylococcus aureus and Staphylococcus epidermidis strains isolated from clinical samples. Iran J Microbiol. 2022;14(3):305-12.

15. Beigverdi R, Sattari-Maraji A, Jabalameli F, Emaneini M. Prevalence of genes encoding aminoglycoside-modifying enzymes in clinical isolates of Gram-positive cocci in Iran: A systematic review and meta-analysis. Microb Drug Resist. 2020;26(2):126-35.

16. Goudarzi M, Razeghi M, Dadashi M, Miri M, Hashemi A, Amirpour A, et al. Distribution of SCCmec types, tetracycline and aminoglycoside resistance genes in hospital-associated methicillin-resistant Staphylococcus aureus strains. Gene Rep. 2019;16:100454.

17. Al-Saadi DA, Abd Al-Mayahi FS, editors. Antibiogram susceptibility patterns of Staphylococcus aureus harboring of mecA gene and prevalence aminoglycoside modifying enzymes (AMEs) genes in Iraq. IOP Conf Ser Earth Environ Sci. 2021;923(1):1-8.

18. Pal M, Kerorsa GB, Marami LM, Kandi V. Epidemiology, pathogenicity, animal infections, antibiotic resistance, public health significance, and economic impact of Staphylococcus aureus: A comprehensive review. Am J Public Health Res. 2020;8(1):14-21.

19. Lollai S, Ziccheddu M, Duprè I, Piras D. Characterization of resistance to tetracyclines and aminoglycosides of sheep mastitis pathogens: Study of the effect of gene content on resistance. J Appl Microbiol. 2016;121(4):941-51.

20. Khoramrooz SS, Dolatabad SA, Dolatabad FM, Marashifard M, Mirzaii M, Dabiri H, et al. Detection of tetracycline resistance genes, aminoglycoside modifying enzymes, and coagulase gene typing of clinical isolates of Staphylococcus aureus in the southwest of Iran. Iran J Basic Med Sci. 2017;20(8):912-9.

21. Garoy EY, Gebreab YB, Achila OO, Tekeste DG, Kesete R, Ghirmay R, et al. Methicillin-resistant Staphylococcus aureus (MRSA): Prevalence and antimicrobial sensitivity pattern among patients—A multicenter study in Asmara, Eritrea. Can J Infect Dis Med Microbiol. 2019;2019.

22. Clinical and Laboratory Standards Institute. CLSI supplement M100: Performance standards for antimicrobial susceptibility testing. 31st ed. Clinical and Laboratory Standards Intitute; 2021.

23. Zomorodi AR, Mohseni N, Hafiz M, Nikoueian H, Hashemitabar G, Salimizand H, et al. Investigation of mobile colistin resistance (mcr) genes among carbapenem resistance Pseudomonas aeruginosa isolates from bovine mastitis in Mashhad, Iran. Gene Rep. 2022;29:101695.

24. Adwan K. Fast DNA isolation and PCR protocols for detection of methicillin-resistant staphylococci. Folia Microbiol. 2014;59:5-8.

25. Motamedifar M, Ebrahim-Saraie HS, Alfatemi SM, Zalipour M, Kaveh M, Khoshkharam-Roodmajani H. Frequency of the toxic shock syndrome toxin-1 gene in methicillin-susceptible and-resistant Staphylococcus aureus isolates from teaching hospitals in Shiraz, Iran. Rev Soc Bras Med Trop. 2015;48(1):90-3.

26. Asokan GV, Ramadhan T, Ahmed E, Sanad H. WHO global priority pathogens list: A bibliometric analysis of Medline-PubMed for knowledge mobilization to infection prevention and control practices in Bahrain. Oman Med J. 2019;34(3):184-93.

27. Kranjec C, Morales Angeles D, Torrissen Mårli M, Fernández L, García P, Kjos M, et al. Staphylococcal biofilms: Challenges and novel therapeutic perspectives. Antibiotics. 2021;10(2):131.

28. Bahmani N, Kalantar E, Torabi V. Survey of methicillin-resistant strains of staphylococci from neonatal septicemia for mecA gene. Life Sci J. 2013;10(10s):303-6.

29. Abbasi Montazeri E, Khosravi AD, Khazaei S, Sabbagh A. Prevalence of methicillin resistance and superantigenic toxins in Staphylococcus aureus strains isolated from patients with cancer. BMC Microbiol. 2021;21(1):1-9.

30. Singh S, Dhawan B, Kapil A, Kabra S, Suri A, Sreenivas V, et al. Coagulase-negative staphylococci causing blood stream infection at an Indian tertiary care hospital: Prevalence, antimicrobial resistance, and molecular characterisation. Indian J Med Microbiol. 2016;34(4):500-5.

31. Gergova RT, Tsitou VM, Mitov IG. Molecular-genetic method for fast direct detection of Staphylococcus aureus and methicillin resistance in blood cultures and punctures. Folia Microbiol. 2019;61(4):559-65.

32. Asante J, Hetsa BA, Amoako DG, Abia AL, Bester LA, Essack SY. Multidrug-resistant coagulase-

negative staphylococci isolated from bloodstream in the uMgungundlovu district of KwaZulu-Natal province in south Africa: Emerging pathogens. Antibiotics. 2021;10(2):198.

33. Shariati A, Dadashi M, Chegini Z, van Belkum A, Mirzaii M, Khoramrooz SS, et al. The global prevalence of daptomycin, tigecycline, quinupristin/dalfopristin, and linezolid-resistant Staphylococcus aureus and coagulase–negative staphylococci strains: A systematic review and meta-analysis. Antimicrob Resist Infect Control. 2020;9(1):1-20.

34. Perumal N, Murugesan S, Krishnan P. Distribution of genes encoding aminoglycoside-modifying enzymes among clinical isolates of methicillin-resistant staphylococci. Indian J Med Microbiol. 2016;34(3):350-2.

35. Ogefere HO, Umaru G, Ibadin EE, Omoregie R. Prevalence of methicillin-resistant staphylococci among apparently healthy students attending a tertiary institution in Benin city, Nigeria. Nig J Basic Appl Sci. 2019;27(1):114-21.

36. Xu S, Guo D, Liu X, Jin X, Shi Y, Wang Y, et al. Ocular pathogens and antibiotic resistance in microbial keratitis over three years in Harbin, northeast China. Acta Ophthalmol. 2021;99(8):909-15.

37. Díaz MC, Ríos E, Rodríguez-Avial I, Simaluiza RJ, Picazo JJ, Culebras E. In-vitro activity of several antimicrobial agents against methicillin-resistant Staphylococcus aureus (MRSA) isolates expressing aminoglycoside-modifying enzymes: Potency of plazomicin alone and in combination with other agents. Int J Antimicrob Agents. 2017;50(2):191-6.

38. Mahdiyoun SM, Kazemian H, Ahanjan M, Houri H, Goudarzi M. Frequency of aminoglycoside-resistance genes in methicillin-resistant Staphylococcus aureus (MRSA) isolates from hospitalized patients. Jundishapur J Microbiol. 2016;9(8):e35052.

39. Xu Z, Liu S, Chen L, Liu Y, Tan L, Shen J, et al. Antimicrobial resistance and molecular characterization of methicillin-resistant coagulase-negative staphylococci from public shared bicycles in Tianjin, China. J Glob Antimicrob Resist. 2019;19:231-5.

40. Li Y, Lin J, Li L, Cai W, Ye J, He S, et al. Methicillin-resistant coagulase-negative staphylococci carriage is a protective factor of methicillin-resistant Staphylococcus aureus nasal colonization in HIV-infected patients: A cross-sectional study. Can J Infect Dis Med Microbiol. 2021;2021.

41. Ong ML, Ho WY, Ng WW, Chew CH. High prevalence of tetM as compared to tetK amongst methicillin-resistant Staphylococcus aureus (MRSA) isolates from hospitals in Perak, Malaysia. Jundishapur J Microbiol. 2017;10(6):e13935.

42. Shrief R, El-Kholy RM, Rizk MA, Zaki ME. Prevalence of tetracycline resistant genes in Staphylococcus aureus isolates from surgical site infections Egypt. Biosci Biotechnol Res Asia. 2019;16(2):221-8.

43. Emaneini M, Bigverdi R, Kalantar D, Soroush S, Jabalameli F, Khoshgnab BN, et al. Distribution of genes encoding tetracycline resistance and aminoglycoside modifying enzymes in Staphylococcus aureus strains isolated from a burn center. Ann Burn Fire Disasters. 2013;26(2):76-80.

44. Poulsen AB, Skov R, Pallesen LV. Detection of methicillin resistance in coagulase-negative staphylococci and in staphylococci directly from simulated blood cultures using the EVIGENE MRSA detection kit. J Antimicrob Chemother. 2003;51(2):419-21.