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RESEARCH ARTICLE

Prevalence and genetic characteristics of fusidic acid resistant *Staphylococcus aureus* clinical isolates: Emergence of t030 strains carrying *fusB* in Tehran, Iran

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ABSTRACT

The literature on fusidic acid resistant *Staphylococcus aureus* strains is scarce in Iran, although the emergence of these strains in health care settings is increasing. This descriptive cross-sectional study was conducted on 68 fusidic acid resistant *S. aureus* strains to learn about the molecular characteristics and antimicrobial resistance of strains isolated from hospitalized patients. In the present study, the prevalence of resistance to fusidic acid in *S. aureus* isolates was 15.1%. Fusidic acid resistance determinative factors (*fusB*, *fusC* and *fusD*) were identified by multiplex PCR assay. To detect the existence of *fusA* and *fusE* determinants and their mutation status, amplifications and sequencing were performed. Molecular characterization of fusidic acid resistant isolates was investigated by SCC*mec* and *spa* typing methods. All strains were MRSA and multi drug resistant. Two (2.9%) and 31 (45.6%) isolates were resistant to vancomycin and mupirocin respectively. The SCC*mec* type IV was highly prevalent representing 50% followed by types III (51.5%), and SCC*mec* types II (13.2%). *fusB*, was the most predominant acquired gene (66.2%) followed by *fusC* (19.1%), and *fusA* (14.7%). The mutations in *fusA* were present in 10 isolates with 5 (50%) having L461K mutation showing fusidic acid MIC values of ≥256 μg ml^{-1} followed by H457Y (40%), and H457Q (10%) showing fusidic acid MIC values of 128 and 64 μg ml^{-1} respectively. Isolates were allocated to ten particular t030 (22.1%), t037 (14.6%), t408 (11.8%), t064 (11.8%), t008 (10.3%), t002 (8.8%), t005 (5.9%), t790 (5.9%), t318 (4.4%), and t018 (4.4%) *spa* types. *fusA* positive isolates were assigned to particular *spa* types t002 (60%), and t005 (40%). There may be be a spreading of fusidic acid resistance among MRSA, creating worrying public concern. This research notes the importance of adequate data of local prevalence of FA-resistant MRSA in Iran for taking appropriate measures to treat, control and reduce the incidence of these isolates.

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INTRODUCTION

Staphylococcus aureus, methicillin-resistant *S. aureus* (MRSA) in particular, is an opportunistic pathogen responsible for various human infections ranged from mild superficial tissue infection to life-threatening infections such as pneumonia, endocarditis, and sepsis [1]. The genetic architecture of the organism, for example genotype, adhesion profile, phenotype, antimicrobial resistance determinants, virulence and toxin genes, paly key role in

determining the severity of *S. aureus* pathogenicity [1, 2]. Many researches illustrate various mechanisms such as efflux pumps, horizontal gene transfer, and mutations in antibiotics' receptor to develop resistance in this bacterium which can limit therapeutic options and subsequently lead to exacerbation of the infection [3]. In recent decades, rising frequency and simultaneously resistance to multiple drugs among *S. aureus* isolates have become a major issue in healthcare settings worldwide [3–5]. Hence, many researches have recently focused on understanding the resistance pattern and related molecular mechanisms which could be crucial in making the right medical management decisions.

Fusidic acid (FA), a narrow-spectrum steroid bacteriostatic agent, is widely employed to treat skin and soft tissue infections, septic arthritis, osteomyelitis, staphylococcal nosocomial infections and methicillin-susceptible/resistant *S. aureus* (MRSA and MSSA) induced diseases. Due to its multiple medication administration routes, lack of cross-resistance with other antimicrobial agents, and little side effects, FA has been regarded to be an ideal alternative treatment of severe *S. aureus* infections [6, 7]. Shortly after the introduction of FA in the 1960s, several published data exhibited increased prevalence of resistance to this antibiotic at a low level. However, recently, a significant trend of FA resistance to be on the increase has been reported with increased duration of use which can hamper the effectiveness of this therapeutic option [6–9]. FA via bound to elongation factor G (EF-G) and preventing its release from the ribosome leads to inhibition of protein synthesis. There are two predominant mechanisms to cause decreased susceptibility to FA in *S. aureus*; i) chromosome-mediated resistance which is linked to *fusA* mutations and high-level resistance. ii) plasmid-mediated resistance which is related to *fusB*, *fusc* or *fusD* genes and low-level resistance. iii) *fusE* gene which is related to mutations in *rplF* encoded ribosomal protein L6 and low level resistance [6]. Although, the prevalence, distribution, and characteristics of FA-resistant *S. aureus* strains have been reported in some countries such as USA, Canada, Australia, Europe and Asian countries, epidemiological researches are crucial to plan and assess preventive measures to reduce the incidence of FA-resistant *S. aureus* strains. However, data related to *S. aureus* isolates with FA resistance in Iran are rare. In addition, to establishing a local epidemiological investigation, the present study sought to establish the molecular epidemiology, antibiotic resistance, and genetic characteristics of FA-resistant *S. aureus* isolates derived from clinical samples in Iran.

MATERIAL AND METHODS

Collection of *S. aureus* isolates

This is a cross-sectional, and descriptive study performed between March 2019 and February 2022 in health care settings with affiliated Shahid Beheshti University of Medical Sciences in the city of Tehran, Iran. In this study, 450 non-duplicate *S. aureus* isolates from various clinical samples were selected for determination the frequency of *S. aureus* isolates with FA resistance. Organisms were identified as *S. aureus* by using cultural characteristics and biochemical methods. Further verification of isolates was performed by confirmation of the presence of *nuc*A gene using polymerase chain reaction (PCR) [10]. The research was ethically confirmed by the Ethics Committee of the Shahid Beheshti University of Medical Sciences in Tehran, Iran (IR.SBMU.MSP.REC.1399.333) as well as consent was obtained from participants.

Screening for FA-Resistant *S. aureus*

All the isolates were screened for evaluation of resistance to FA using Kirby Bauer disk diffusion method with 10 μg FA containing disks. The isolates with a zone diameter <24 mm were further analyzed for FA MIC titer by broth microdilution method in concordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST). In line with the EUCAST criteria, *S. aureus* isolates with minimum inhibitory concentration (MIC) cutoff value >1 μg ml^{-1} were considered as FA resistant *S. aureus* strains. For the next step, confirmed FA resistant *S. aureus* strains were saved in 25% glycerol (v/v) in Tryptic Soy Broth (TSB; Merck, Germany) at −70 °C.

Determination of isolates susceptibility

The MIC of tetracycline, clindamycin, erythromycin, penicillin, linezolid, rifampin, ciprofloxacin, nitrofurantoin, vancomycin, mupirocin, and gentamicin (Sigma-Aldrich, St. Louis, Mo) for FA resistant *S. aureus* strains were established using broth microdilution test according to the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI). The interpretive criteria of mupirocin susceptibility is in accordance with the CLSI criteria, in such a way that isolates with mupirocin MIC between 8-256 μg ml^{-1} and ≥512 μg ml^{-1} were confirmed as mupirocin resistant strains at low and high-level (LLMUPR and HLMUPR) respectively. To determine inducible clindamycin resistance phenotype ($iMLS_B$ phenotype) in isolates tested, any growth in well contained 4 μg ml^{-1} erythromycin and 0.5 μg ml^{-1} clindamycin in an incubation time 18–24 h at 35 °C and ambient air was considered as a positive result. Detection of MRSA isolates was done using cefoxitin disks (30 μg) on Mueller Hinton agar plates under incubation time 16–18 h at 33–35 °C and ambient air [10, 11]. Isolates with a zone diameter ≤21 were considered as MRSA, while a zone diameter ≥22 were MSSA. PCR with specific oligonucleotides for the presence of *mec*A gene (583 bp amplicon) was used for definite confirmation [10, 12]. Reference strains of *S. aureus* ATCC 25923, ATCC 43300, and ATCC 29213 were used to control the experiment. Mupirocin and vancomycin resistant isolates were screened for the carriage of *mupA* and *vanA* genes with specific oligonucleotide sequences described by Goudarzi et al.

Extraction of genomic DNA

The genomic DNA was obtained from each strain that had previously grown in blood agar (BA; Merck, Darmstadt, Germany). Briefly, 3–5 pure colonies were suspended in 150 µl deionized water plus 30 µg ml^{-1} lysostaphin (Sigma-Aldrich, St. Louis, Mo). Finally, DNA was extracted with the use of phenol-chloroform method as explained earlier. Qubit fluorometer and Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Carlsbad, USA) were used to confirm the DNA content and purity.

Detection of FA resistance determinants

S. aureus strains were evaluated for the presence of acquired FA resistance genes including *fusB, fusC* and *fusD* by using multiplex PCR assay and specific primers [13]. Strains with negative results for *fusB, fusC,* and *fusD* determinants and/or showing MIC values equal or more than 512 mg L^{-1} for FA, were further analyzed for the presence of *fusA* with specific oligonucleotide primers explained by Chen et al. (primer2). For the *fusB, fusC* and *fusD* genes, the expected amplicon sizes 496bp, 128bp and 525bp were separated after 1.8% agarose gel electrophoresis and perceived by staining with ethidium bromide.

Detection of mutations in *fusA*

fusA gene was amplified by fusA-F 5′-TTTACCCT-GAGTGTGTTCT-3′ and fusA-R 5′-TACATTTAAGCT-CACCTTGT-3′and with protocol published by Chen et al., [14] and sequenced on an ABI Prism 377 automated sequencer (Applied Biosystems, Perkin-Elmer Co., Foster City, CA) to detect the possible mutations as earlier described. The Chromas software (Version 1.45, Australia) was used to edit the resulting sequences. Subsequently, the edited sequences were compared with the already available sequences on the Internet using BLAST tool (http://www.ncbi.nlm.nih.gov/blast/).

Identification of *fusA* L461K mutation

Assessment of the L461K mutation in *fusA* was carried out by the rapid PCR amplification method and four oligonucleotide sequences [13]. In this protocol, isolates that yielded 2 bands (415 bp and 145 bp) were considered as positive results for carriage of L461K and isolates with bands of 415 and 308 bp were negative for L461K.

Genotypic characterization

SCCmec typing. A multiplex PCR was recruited for identifying staphylococcal cassette chromosome mec (SCC*mec*) types based on the oligonucleotide sequences and conditions described by Boye et al., [15]. Obtained banding patterns were analyzed by comparing them with the banding patterns of reference stains as follow: ATCC 10442 (SCC*mec* type I), N315 (SCC*mec* type II), 85/2082 (SCC*mec* type III), MW2 (SCC*mec* type IVa), WIS (SCC*mec* type V), as reference strains.

spa typing. The *spa* gene encoding for surface protein A were amplified using PCR and specific primers according to the same conditions published earlier [10, 12]. The purification of PCR products using the commercial kit (QIAquick PCR Purification) and analysis of nucleotide sequence were performed using an ABI Prism 377 automated sequencer (Applied Biosystems, Perkin-Elmer Co., Foster City, CA). The Chromas software (Version 1.45, Australia) was used to edit the sequences. *spa* types were established by submitting collected data to the Ridom SpaServer database (http://www.spaserver.ridom.de).

RESULTS

Of the 450 *S. aureus* recovered from different clinical samples, resistance to fusidic acid was detected in 68 isolates (15.1%). Out of which, 51.5% (n = 35) were from male participants and 48.5% (n = 33) were from female participants with a mean age of 41 y (range, 15–73 years). In present study, FA-resistant *S. aureus* strains recovered from blood (26.5%; 18/68), cerebrospinal fluid (CSF) (5.9%; 4/68), and pleural effusion (2.9%; 2/68) were considered as invasive strains and isolates recovered from wound (55.9%; 38/68), urine (5.9%; 4/68), sputum (2.9%; 2/68) were confirmed as non-invasive FA-resistant *S. aureus*. All tested strains including invasive and non-invasive FA-resistant *S. aureus* were susceptible to linezolid. Overall, none of the isolates tested were susceptible to all of the tested antibiotics. All 68 isolates showed resistance to methicillin, possessed the *mecA* gene and were confirmed as MRSA. As presented in Table 1, the highest and lowest resistance rate belonged to penicillin (100; 68/68) and vancomycin (1.5%; 1/68), respectively. In total, all of the isolates were confirmed as multidrug resistance (MDR) strains. In our study, eight resistance patterns were detected, wherein PEN, TET, ERY, CLI, CIP (22.1%; 15/68), PEN, TET, ERY, GEN, CIP (17.6%; 12/68), and PEN, ERY, GEN, CIP, RIF, MUP, CLI (14.7%; 10/68) were the top three frequently detected profile. 40 (58.8%), and 18 (26.8%) isolates were confirmed as $cMLS_B$, and $iMLS_B$, phenotypes. $cMLS_B$ phenotype was higher in non-invasive strains compared to invasive strains (47.1% vs 110.8%), while a higher prevalence of $iMLS_B$ phenotype in invasive strains than those from non-invasive strains were noticed (16.2% vs 10.3%). As described in Table 1, 45.6% of isolates were confirmed as mupirocin resistant, that of which 20 (29.4%) isolates showed HLMUPR and 11 (16.2%) isolates exhibited LLMUPR phenotype. Two vancomycin-resistant *S. aureus* (VRSA) isolates were from wound samples and possessed the *vanA* gene.

The SCC*mec* typing results showed that the IV type was highly prevalent representing 50% (34/68), followed by types III (51.5%; 25/68), and SCC*mec* types II (13.2%; 9/68). Based on the *sap* typing method, isolates were allocated to ten particular spa types, namely t030 (22.1%; 15/68), t037

Table 1. Antimicrobial susceptibility pattern of fusidic acid-resistant MRSA strains

Antibiotic	MIC (μg ml^{-1})			*N* (%)
	Range	50%	90%	
Penicillin	0.125–16	4	8	68 (100)
Vancomycin	0.125–64	32	32	2 (2.9)
Linezolid	0.125–16	0.5	0.5	0 (0)
Tetracycline	0.5–128	16	32	54 (79.4)
Erythromycin	0.5–256	8	16	58 (85.3)
Clindamycin	0.25–64	2	4	40 (58.8)
Gentamicin	1–128	16	32	43 (63.2)
Rifampin	0.5–32	4	4	34 (50)
Ciprofloxacin	0.5–32	4	8	52 (76.5)
Nitrofurantoin	8–1,024	32	32	25 (36.8)
Mupirocin	4–512	128	256	31 (45.6)

(14.6%; 10/68), t408 (11.8%; 8/68), t064 (11.8%; 8/68), t008 (10.3%; 7/68), t002 (8.8%; 6/68), t005 (5.9%; 4/68), t790 (5.9%; 4/68), t318 (4.4%; 3/68), and t018 (4.4%; 3/68). Overall, the most common *spa* types in non-invasive FA-resistant *S. aureus* isolates (44 isolates) were including t030 (22.8%; 10/44), t008 (15.9%; 7/44), t037 (13.6%; 6/44), t002 (13.6%; 6/44), t005 (9.2%; 4/44), t018 (6.8%; 3/44), t318 (6.8%; 3/44), t064 (6.8%; 3/44) and t408 (4.5%; 2/44). t790 was only detected in invasive FA-resistant *S. aureus* isolates (16.7%; 4/24). Other *spa* types identified in invasive strains were including t048 (25%; 6/24), t030 (20.8%; 5/24), t064 (20.8%; 5/24), and t037 (16.7%; 4/24). Isolates with resistance to mupirocin were distributed in invasive isolates with *spa* types t037 (1 isolate), t064 (2 isolates), and t408 (1 isolate) which all were HLMUPR, while non-invasive strains belonged to *spa* type t030 (6 isolates/4 HLMUPR and 2 LLMUPR), t037 (6 isolates/5 HLMUPR and 1 LLMUPR), t008 (7 isolates/4 HLMUPR and 3 LLMUPR), t002 (5 isolates/3 HLMUPR and 2 LLMUPR), and t064 (3 isolates/3 LLMUPR). VRSA strains belonged to non-invasive t030 and t037 isolates (each one isolate) which both harbored *fusB* determinant.

Intriguingly, *fusB*, the most predominant acquired gene, was detected in 45 (66.2%) FA-resistant MRSA strains, while *fusC* was present in 13 (19.1%) isolates. Data displayed that all invasive FA-resistant MRSA isolates were found to carry the acquired *fusB* gene. *fusA* and *fusC* were only detected among non-invasive FA-resistant MRSA isolates. Results also indicated that the isolates *fusA* positive (14.7%; 10/68) were assigned to particular t002 (60%; 6/10), and t005 (40%; 4/10). The distribution of *spa*, SCC*mec*, and resistance profiles among invasive and non-invasive FA-resistant MRSA strains is summarized in Table 2. Nucleotide sequencing of *fusA* indicated three substitutions in EF-G. The amino acid substitution L461K (nucleotide substitution: TTA to AAA; amino acid substitution: leucine to lysine) was the predominant (50%) showing fusidic acid MIC values of ≥256 μg ml^{-1} followed by H457Y (nucleotide substitution: CAC to TAC; amino acid substitution: histidine to tyrosine) (40%), and H457Q (nucleotide substitution: CAC to CAA; amino acid substitution: histidine to glutamine) (10%) showing fusidic acid MIC values of 128 and 64 μg ml^{-1}, respectively. *fusD* and *fusE* were not detected in any of the examined strains. Resistance to FA in high-level was related mostly to point mutations in *fusA*, especially the L461K mutation, whereas *fusC* and *fusB* usually conferred the low-level resistance to FA (Table 3).

DISCUSSION

Although fusidic acid has been licensed for decades in several areas in the clinic, the extensive use of this antibiotic to treat a variety of diseases in humans and the emergence of increased resistance threatens the public health. In the present experiment, the prevalence of resistance to FA in *S. aureus* isolates was reported at 15.1%. In a recently conducted meta-analysis by Hajikhani et al., a low prevalence rates of FA resistance in *S. aureus* isolates was reported (0.5%) [7]. In line with our findings, they also noted a higher prevalence rate of resistance to FA in *S. aureus* isolates obtained from Asia (5.6%) in comparison to other continents which may be due to unrestricted and unscheduled administration of FA, lack of proper alternatives to FA, diverse attitudes towards antimicrobial protocols and the dissemination of FA resistant specific clones. Earlier evidences demonstrated a drastic upraise in the prevalence of FA resistant *S. aureus* isolates during 2000 (4.0%) and 2010–2020 (5.2%) [7]. In a Kuwait based study, increased trends of FA resistance from 22% in 1994 to 92% in 2004 was noted [16]. Compared with the earlier reported rates of resistance to FA by Goudarzi et al. (2.5%) [12], Razeghi et al. (3.6%) [17], Zamani et al. (8.3%) [18], Rahimi et al. (3%) [19], Hasani et al. (3.7%) [20], the rate of FA resistance has had a significant increase in present research which highlights increasing rate in the prevalence of staphylococcal infections and a shift in antibiotic pressures. In our research, the data showed that all FA resistant *S. aureus* isolates were MRSA. This is supported by the observations of Germany (10.3%), Kuwait (9.3%), Ireland (19.9%) and Greece (57%) [7, 13, 21]. In another meta-analysis report, Hajikhani et al also explained a higher prevalence of resistance to FA in MRSA

Table 2. Molecular characteristic of fusidic acid-resistant MRSA isolates

Fusidic acid -resistant MRSA	*spa* type (*n*; %)	SCC*mec* type	FA resistance determinants	Resistance pattern[a] (*n*; %)	Type of Samples (*n*; %)
Invasive (24; 35.3)	t408 (6; 25)	IV	*fusB*	PEN, TET, ERY, CLI, CIP (2; 33.3) PEN, TET, ERY, GEN, CIP (2; 33.3) PEN, GEN, RIF, MUP, ERY (1; 16.7) PEN, TET, RIF, NIT (1; 16.7)	Blood (5; 83.3), Pleural effusion (1; 16.7)
	t030 (5; 20.8)	III	*fusB*	PEN, TET, ERY, GEN, CIP (3; 60) PEN, TET, RIF, NIT (2; 40)	Blood (3; 60), Cerebrospinal fluid (2; 40)
	t064 (5; 20.8)	IV	*fusB*	PEN, TET, ERY, CLI, CIP (3; 60) PEN, GEN, RIF, MUP, ERY (2; 40)	Blood (4; 80), Cerebrospinal fluid (1; 20)
	t037 (4; 16.7)	III	*fusB*	PEN, TET, ERY, GEN, CIP (3; 75) PEN, ERY, GEN, CIP, RIF, MUP, CLI (1; 25)	Blood (4; 100)
	t790 (4; 16.7)	IV	*fusB*	PEN, TET, ERY, CLI, CIP (2; 50) PEN, TET, RIF, NIT (2; 50)	Blood (2; 50), Pleural effusion (1; 25), Cerebrospinal fluid (1; 25)
Noninvasive (44; 64.7)	t030 (10; 22.7)	III	*fusB*	PEN, TET, ERY, CLI, CIP (4; 40) PEN, GEN, ERY, CLI, TET, CIP, RIF, NIT, MUP (3; 30) PEN, ERY, GEN, CIP, RIF, MUP, CLI (2; 20) PEN, ERY, RIF, TET, VAN, GEN, MUP (1; 10)	Wound (7; 70), Sputum (1; 10), Urine (2; 20)
	t008 (7; 15.9)	IV	*fusC*	PEN, GEN, ERY, CLI, TET, CIP, RIF, NIT, MUP (4; 57.1) PEN, GEN, TET, ERY, CLI, CIP, NIT, MUP (3; 42.9)	Wound (7; 100)
	t037 (6; 13.7)	III	*fusB*	PEN, GEN, TET, ERY, CLI, CIP, NIT, MUP (4; 66.6) PEN, GEN, ERY, CLI, TET, CIP, RIF, NIT, MUP (1; 16.7) PEN, ERY, RIF, TET, VAN, GEN, MUP (1; 16.7)	Wound (6; 100)
	t002 (6; 13.7)	II	*fusA*	PEN, TET, ERY, GEN, CIP (1; 16.7) PEN, ERY, GEN, CIP, RIF, MUP, CLI (5; 83.3)	Wound (5; 83.3), Sputum (1; 16.7)
	t005 (4; 9.1)	IV	*fusA*	PEN, TET, RIF, NIT (3; 75) PEN, TET, ERY, GEN, CIP (1; 25)	Wound (2; 50), Urine (2; 50)
	t018 (3; 6.8)	II	*fusC*	PEN, TET, ERY, CLI, CIP (3; 100)	Wound (3; 100)
	t318 (3; 6.8)	IV	*fusC*	PEN, TET, RIF, NIT (2; 66.7) PEN, TET, ERY, GEN, CIP (1; 33.3)	Wound (3; 100)
	t064 (3; 6.8)	IV	*fusB*	PEN, ERY, GEN, CIP, RIF, MUP, CLI (2; 66.7) PEN, GEN, RIF, MUP, ERY (1; 33.3)	Wound (3; 100)
	t408 (2; 4.5)	IV	*fusB*	PEN, TET, ERY, GEN, CIP (1; 50) PEN, TET, ERY, CLI, CIP (1; 50)	Wound (2; 100)

[a]PEN, penicillin; CLI, clindamycin; NIT, nitrofurantoin; ERY, erythromycin; TET, tetracycline; CIP, ciprofloxacin; MUP, mupirocin; GEN, gentamicin; VAN; vancomycin, RIF, rifampicin.

Table 3. Distribution of mutations of *fusA* and acquired genes of *fusC* and *fusB* in FA MIC levels

Mutation/acquired genes	No. of strains with various FA MIC (μg ml^{-1})				
	2–16	32	64	128	≥256
L461K	0	0	0	0	5
H457Y	0	0	0	4	0
H457Q	0		1	0	0
fusB	33	12	0	0	0
fusC	13	0	0	0	0
Total (68)	46	12	1	4	5

strains (5.8%) compared to MSSA strains (3.0%). The high prevalence of FA resistant MRSA in our study is in line with increasing trends of FA resistant MRSA isolates, hence, a worldwide emphasize urgent need for management of FA in the clinic to avoid treatment failure and dissemination of resistance.

The emergence of VRSA makes it a threat to control of staphylococcal infections. It is worth noting that in present study, two isolates had resistance to vancomycin (2.9%) which carried *vanA* gene. Results of the meta-analysis performed in 2020 depicted an upward trend in VRSA around the world. Shariati et al. displayed an increasing trend of 2-fold of VRSA after 2010 compared to before. Meanwhile, Asian countries, especially Iran and India, included the

highest rates of incidence of VRSA (67%) which can be due to easy availability of antibiotics, scarcity of appropriate alternatives to vancomycin, paucity of continuous monitoring of drug resistance patterns and diverse attitudes towards antimicrobial protocols [22]. Increasing resistance of *S. aureus* to vancomycin alarms the potential emergence of VRSA strains in our clinical settings.

According to the literature, two predominant pathways to cause decreased susceptibility to FA in *S. aureus* have been defined i) target site alteration mediated by *fusE* (encoding ribosome protein L6, *rplF*), or *fusA* mutations (encoding elongation factor G, EF-G) which is at the chromosomal level and related to high-level FA resistance. ii) target site protection caused by *FusB* family proteins, incorporating *fusB*, *fusC*, and *fusD* which is at the plasmid level related to low-level FA resistance. In this study, we have reported *fusB* in 45 (66.2%) FA-resistant strains examined (MIC 2–64 μg ml^{-1}). Similarly, in China, a recent research displayed that *fusB* was the main mediator in causing FA resistance in all FA resistant *S. aureus* isolates [23]. In another study conducted by Rijnders et al. in Netherlands, it was found that approximately 90% of FA-resistant *S. aureus* isolates harbored *fusB* [24]. A previous study conducted by McLaws and colleagues in Denmark on 291 FA resistant MRSA, showed that near 87% of FA resistant isolates harbored *fusB* or *fusC*, and remaining 13% carried mutations in the *fusA* gene [25]. Conversely, in Castanheira et al's study in 28 medical centers of European countries, *fusC* was also more prevalent than *fusB* (16.9% vs. 10.1%) [13]. In Chen et al's study in Taiwan on 71 FA resistant *S. aureus* strains, *fusA* was reported as the most predominant gene (84%) among FA resistant isolates followed by fusc (16%). None of the isolates harbored *fusB* gene [14]. In another study conducted in Taiwan, *fusC* was reported as the most common FA resistance determinant among tested isolates [26].

Although various alterations have been identified in *fusA* related mutations, the L461K mutation is more frequently found in *S. aureus*. In the current research, amino acid substitutions L461K was the most common carried mutation in *fusA*, representing 50% (5/10) of isolates showing fusidic acid MIC values of ≥256 μg ml^{-1}, followed by H457Y (40%; 4/10), and H457Q (10%; 1/10) with MIC value 128 μg ml^{-1} and 64 μg ml^{-1}, respectively. In a 2021 study in China, mutations in *fusA* on 74 FA-resistant MRSA isolates were investigated. Zhao et al. indicated that amino acid substitutions L461K was found to exist in 54 out of 74 strains (73%) showing MIC value more than 128 μg ml^{-1}. Other detected mutations in *fusA* included H457Q mutation (4.1%), H457Y mutation (4.1%), and L461S mutation (4.1%) [27]. The results presented in current research were in agreement with earlier reports from European countries [13], Kuwait [8], and Taiwan [14]. Other *fusA* mutations identified in present report (H457Y), amino acid change at position 457 from the histidine residue to tyrosine, were described earlier [8, 13, 27]. In contrast to the observations of Castanheira et al., [13], Zhao et al., [24], and McLaws et al., [25], we reported high prevalence of H457Y mutation which was related to elevated fusidic acid MIC values 128 μg ml^{-1}. Although this data should only be extrapolated with further investigation, it seems substitutions at this position could have more subtle effects in the rise of final fusidic acid MIC value compared with other detected mutation (H457Q). Iranian FA-resistant MRSA isolates also exhibited H457Q mutation in one isolate with MIC value 64 μg ml^{-1} which is in agreement with Castanheira et al's work, reporting this mutation in one isolate obtained from Canada [28]. This mutation was also reported earlier from China, Turkey, Denmark, and Taiwan [13, 14, 25, 27].

Our observations about SCC*mec* types are in line with other studies that confirmed the relationship of SCC*mec* types I, II, and III with HA- *S. aureus* infections, whereas IV and V types are prominent in community-associated (CA)- *S. aureus* infections [29]. SCC*mec* type IV was the most prevalent among our isolates (50%). This emergence has been reported regionally to higher levels in reports of the nearby countries, showing a dominance in SCC*mec* type IV ranging from 19 to 90% [5]. Furthermore, we found that the *spa* types of FA resistance in present work were various. Ten *spa* types were identified among the 68 FA-resistant MRSA strains, among which t030-SCC*mec* type III (22.1%, 15/68) carrying *fusB* determinant was the most prevalent *spa* type despite the emergence of diverse *spa* types. FA-resistant t030-SCC*mec* type III strains was reported in a previous study carried out by Goudarzi et al., in Iran [10]. *spa* type t030-SCC*mec* type III which harbored *fusB* was detected among 7.1% of MRSA isolates from clinical samples at a China hospital in 2010–2013 [23]. The current research highlighted the emergence of *spa* type t037-SCC*mec* type III (7.6%), as the second predominant genotype in FA-resistant MRSA strains with a notable prevalence of mupirocin resistance (70%) and carriage of *fusB* (100%) confirming the earlier finding of Chen et al. in Taiwan who indicated t037-SCC*mec* type III and t002-SCC*mec* type II as the most prominent detected genotypes in FA-resistant MRSA strains isolated from hospitalized individuals, accounting for 62% and 29% respectively [14]. Our findings highlighted a relatively high frequency of t408-SCC*mec* type IV and t064-SCC*mec* type IV carrying *fusB* (each 11.8%), as the third predominant FA-resistant MRSA type identified. Heijer et al. investigated resistance to FA on 6,814 clinical *S. aureus* strains from nine European countries. They revealed that *fusC* was the predominant determinant of resistance to FA. Consistent with our study, Heijer et al indicated the emergence of *spa* types t002 ($n = 2$) and t005 ($n = 2$) carrying *fusA*, t408 ($n = 2$) carrying *fusB*, and t008 ($n = 17$) carrying *fusC* in tested strains [9]. We also detected *spa* types t790-SCC*mec* type IV, t018-SCC*mec* type II, and t318-SCC*mec* type IV in tested isolates. To the best of our knowledge, this study is the first to report these unusual types in Iran. This displayed that the common genotypes of FA-resistant MRSA may be diverse from country to country. Therefore, fusidic acid should be supervised worldwide, and their inadvertent or rampant use should be limited.

In conclusion, the current research has a notable strength as it provides data about the local molecular epidemiology of

FA-resistant MRSA in Iran. Our findings propose dissemination *fusB* determinants among FA-resistant MRSA with a relationship with the t030 in Iran that can trigger its epidemic in our area in the future. This research also demonstrated the emergence of FA-resistant *spa* types t018 and t318 for the first time in Iran, raising great concern in spread and dissemination and transfer from and to hospitals. The heterogeneity found in the *spa* typing of FA-resistant MRSA strains studied in the current research does not suggest an outbreak. Given to global expansion and dissemination of FA-resistant determinants particularly *FusB* in MRSA, surveillance and infection control measures need to be specifically performed and updated.

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