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ORIGINAL ARTICLE

Identification of major sequence types among aminoglycoside resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* strains isolated from clinical samples

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ABSTRACT

Background and Objectives: Aminoglycosides have been widely used for treating severe staphylococcal infections. Production aminoglycoside modifying enzymes (AMEs) is the main mechanism of resistance to this antibiotic. The aim of this study was to determine the prevalence of AME genes and molecular characterization of aminoglycoside-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* strains isolated from clinical specimens in Iran.

Materials and Methods: A total of 42 clinical isolates of Gram-positive cocci (20 *S. aureus* and 22 *S. epidermidis*) with resistance to gentamicin were tested for antimicrobial resistance and differentiated by multilocus sequence typing (MLST).

Results: All 42 isolates were resistant to methicillin, kanamycin,and most of them were also resistant to amikacin (98%), tobramycin (98%) and netilmycin (78.5%). Overall, *aac(6')-Ie-aph(2")-Ia* was the dominant AME gene found in 100% of isolates, followed by *aph(3')IIIa* found in 90% of isolates. MLST classified *S. aureus* and *S. epidermidis* into 5 and 9 distinct sequence types (ST), respectively. The majority of the strains belonged to ST239 (50%) for *S. aureus* and ST2 (36%) for *S. epidermidis.*

Conclusion: The resistance to aminoglycosides was mainly due to the presence of the *aac(6')-Ie-aph(2")-Ia* and *aph(3') IIIa* genes as well as the ST239 for *S. aureus* and ST2 for *S. epidermidis* have become the predominant clones in the selected university hospital of Tehran, Iran. Thus, it is critical that clinicians and healthcare workers are aware of the population of *S. aureus* and *S. epidermidis* present in order to make decisions for appropriate treatment and infection control practices.

Keywords: Aminoglycosides; Aminoglycoside modifying enzymes; *Staphylococcus aureus*; *Staphylococcus epidermidis*; Multilocus sequence typing

INTRODUCTION

Aminoglycosides are bactericidal and broad-spectrum antibiotics often used along with either β-lactam or glycopeptides for treatment of serious infections caused by Gram-positive cocci (GPC), particularly *Staphylococcus aureus* and *Staphylococcus epidermidis* (1, 2). These drugs inhibit bacterial protein synthesis by irreversibly binding to the 30S subunit of ribosomes (2). Resistance to aminoglycosides has been reported with increasing frequency in the clinical and microbiological settings (2, 3). There are sev-

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eral mechanisms of aminoglycoside resistance, including the production of aminoglycoside-modifying enzymes (AMEs), target site alterations, decreased permeability, efflux pumps and 16S rRNA methylation (4). AMEs are the most common resistance mechanism against aminoglycosides in GPC, which are classified into four groups according to the modifications they induce: acetyltransferases (AACs), phosphotransferases (APHs), nucleotidyltransferases (ANTs), and adenyltransferases (AADs) (2). AMEs are often located on transposable elements such as transposons and plasmids that can be transferred horizontally from one bacterium to another (4). Recognizing the prevalence of AME genes associated with human infections is highly important to initiate appropriate therapy. Molecular typing methods have become powerful tools for the epidemiological surveillance and control of infectious diseases (5). One of these techniques is multilocus sequence typing (MLST), which relies on polymerase chain reaction (PCR) and sequencing of a number of conserved house-keeping genes (6, 7). MLST shows that a limited number of clone or sequence types (STs) (the Brazilian/Hungarian or ST 239 clone, the Iberian or ST 247 clone and the New York/Japan or ST 5 clone) was responsible for the majority of infections throughout the world (8). The purpose of this study was to determine the predominant clones of aminoglycoside-resistant *S. aureus* and *S. epidermidis* strains in our hospital in Iran by MLST technique.

MATERIALS AND METHODS

Bacterial isolates. A total of 42 clinical isolates of GPC (20 *S. aureus* and 22 *S. epidermidis*) with resistance to gentamicin from various specimens (blood, wound, urine, respiratory tract, eye) were collected from different inpatients in a teaching hospital of Tehran University of Medical Sciences, during the period of January 31- December 21, 2017. Gentamicin-resistant GPC were identified by disk agar diffusion. The gentamicin discs were used at concentration of 10 and 120 mg. Only one isolate per patient showed resistance to gentamicin was included in the study. Identification of the isolates was performed based on a series of conventional microbiological tests (9). The amplification of *S. aureus nuc* gene (aur-F: TCGCTTGCTATGATTGTGG, aur-R: GCCAATGTTCTACCATAGC) and *S. epidermidis nuc* gene (epi-F: TTGTAAACCATTCTGGACCG, epi-R: ATGCGTGAGATACTTCTTCG) confirmed the identity of isolates (10). The reaction mixture contained 12.5 µL PCR Master Mix 2× (Ampliqon, Denmark), 1 µL of each primer (10 pmol, Metabion, Martinsried, Germany), 3 µL of DNA (10-20 ng/µl) and 8.5 µL of DNase-free water in a total reaction volume of 25 µl per sample. The PCR thermocycling conditions consisted of an initial denaturing step at 94°C × 5 minutes, followed by 35 cycles of denaturation at 94°C × 45 s, annealing at 58°C × 45 seconds, extension at 72°C × 45 seconds and a final extension at 72°C × 5 minutes. The amplified DNA fragments were electrophoresed in a 1.5% agarose gels with 0.5× TBE (Tris/Borate/EDTA) buffer. The DNA bands were visualized by KBC power load dye staining and photographed under UV illumination.

Antimicrobial susceptibility testing. All 42 gentamicin-resistant GPC were susceptibility tested against to other aminoglycosides by a disk diffusion method using, amikacin 30 µg, tobramycin 10 µg, kanamycin 30 µg and netilmycin 30 µg, according to the Clinical and Laboratory Standards Institute guidelines (CLSI) (11). The Minimum Inhibitory Concentrations (MICs) of oxacillin and gentamicin were interpreted according to CLSI guidelines; MIC ≥4 µg/ml and MIC ≥0.5 µg/ml breakpoints for identification of oxacillin resistant *S. aureus* and *S. epidermidis*, respectively (11). Gentamicin resistant strains were detected with MIC ≥16µg/ml breakpoints for both *S. aureus* and *S. epidermidis* (11). The methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant *S. epidermidis* (MRSE) were screened based on susceptibility to cefoxitin (30 µg) and confirmed by molecular detection of *mecA* (12, 13). Results were interpreted according to CLSI (2017) guidelines (11). The interpretive criteria for cefoxitin were: *S. aureus*, sensitive ≥22 mm and resistant ≤21 mm; *S. epidermidis*, sensitive ≥25 mm and resistant ≤24 mm.

All antibiotic disks were purchased from Mast Co, UK and *S. aureus* ATCC 25923 was used as the qualitycontrol organism in antimicrobial susceptibility determination.

DNA extraction and amplification of *mecA* and AME genes. Bacterial genomic DNA was extracted from cultured bacteria with boiling method. For amplifcation of the *mecA* gene, primers mecA1 (5'- TGCTATCCACCCTCAAACAGGATTTA-3') and mecA2

(5'- AACGTTGTAACCACCCCAAGA-3') were used (10). PCR conditions were as follows: initial denaturation at 94°C for 5 min followed by 30 cycles (denaturation at 94°C for 45 s, annealingat 59.5°C for 45s, and extension at 72°C for 45 s) then final extension at 72°C for 5 min. For amplifcation of the AME genes, three sets of primers specific for *aac(6')-Ie-aph(2'')-Ia* (F: CAGAGCCTTGGGAAGATGAAG, R: CCTCGTGTAATTCATGTTCTGGC), *aph(3') IIIa* (F: GGCTAAAATGAGAATATCACCGG, R: CTTTAAAAAATCATACAGCTCGCG) and *ant(4') Ia* (F: CAAACTGCTAAATCGGTAGAAGCC, R: GGAAAGTTGACCAGACATTACGAACT) were used (12). Amplifcation was carried out using following conditions: An initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 95°C for 50 s, annealing at 54°C for 50 s and extension at 72°C for 50 s, followed by a final extension at 72°C for 5 min. All PCR reactions were performed in a T100™ thermal cycler (Bio-Rad).

Molecular typing by MLST. Molecular typing of isolates was performed by MLST, as described on the *S. aureus* MLST Database (https://pubmlst.org/saureus/) for *S. aureus* and *S. epidermidis* MLST Database (https://pubmlst.org/sepidermidis/) for *S. epidermidis.* The Minimum spanning trees (MST) of STs were generated by the geoBURST algorithm using Phyloviz software version 2.0 (13).

Statistical analysis. IBM SPSS Statistics 22 was used for statistical analysis. A *P*-value of ≤ 0.05 was considered significant.

RESULTS

The details of the studied strains concerning their resistance pattern, resistance genes, allelic profile, and their sequence type (ST) are listed in Tables 1 and 2. All isolates were resistant to gentamicin, and kanamycin. Resistance rates were 98% (41/42) for amikacin, 98% (41/42) for tobramycin and 78.5% (33/42) for netilmycin. Twenty (100%) *S. aureus* isolates and 22 (100%) *S. epidermidis* isolates were identified as MRSA and MRSE, respectively, based on the definitions mentioned in Materials and Methods. MLST analysis of the 20 *S. aureus* isolates identified 5 distinct STs of which ST239 was the predominant MLST type (10/20, 50%) and was more likely to be isolated from blood samples. All ST239 isolates were resistant to cefoxitin, amikacin, gentamicin, kanamycin, netilmicin and tobramycin and the results of the antibiotic resistance genes pattern indicated that the pattern *aac(6')-Ie-aph(2''), aph(3')-IIIa* and *ant(4')-Ia in* ST239 was significantly more frequent than other STs ($P< 0.0001$). The MIC against gentamicin in all *S. aureus* isolates ranged from 128 to 512 µg/ml. The MIC against gentamicin in isolates belonging to ST239 were significantly higher than those of isolates belonging to the other STs ($P< 0.0001$). The second most prevalent type was ST30 (6/20, 30%), which was mostly isolated from wound samples. All ST30 isolates were resistant to cefoxitin, amikacin, gentamicin, kanamycin, netilmicin and tobramycin and harbored *mecA, aac(6')-Ie-aph(2'')* and *aph(3')-IIIa.* The MIC of all ST30 isolates against gentamicin was 256 µg/ml. The remaining three types, ST859, ST8, and ST1465 were detected in 2, 1, and 1 isolates, respectively.

The MLST of 22 *S. epidermidis* isolates showed 9 distinct STs of which ST2 (36%; 8/22) and ST5 (23%; 5/22) were the most prevalent types and were more likely to be isolated from blood samples. All ST2 and ST5 isolates were resistant to cefoxitin, amikacin, gentamicin, kanamycin, netilmicin and tobramycin. By comparing antibiotic resistance genes, the pattern *aac(6')-Ie-aph(2''), aph(3')-IIIa* and *ant(4')-Ia* in ST2 was significantly more frequent than other STs ($P< 0.0001$). The MIC of all ST2 and ST5 isolates against gentamicin was 256 µg/ml. The remaining seven types, ST22, ST54, ST581, ST179, ST23, ST588, and ST580 were detected in 3, 1, 1, 1, 1, 1, and 1 isolates, respectively. The minimum spanning trees of the *S. aureus* and *S. epidermidis* isolates are shown in Figs. 1 and 2, respectively.

The most common AME gene was *aac(6')-Ie-aph(2'')-Ia*, found in 100% (42/42) of isolates, followed by *aph(3')IIIa* and *ant(4')Ia),* found in 90% (38/42) and 59.5% (25/42) of these isolates, respectively. The combination of three genes [*aac(6')-Ie-aph(2'')-Ia*, *aph(3')IIIa* and *ant(4')Ia)*] was 52% (22/42). The combination of *aac(6')-Ie-aph(2'')-Ia* with *aph(3')IIIa* and *aac(6')-Ie-aph(2'')-Ia* plus *ant(4')Ia* was observed in 38% (16/42) and 7% (3/42) of these isolates, respectively. Only one isolate of *S. aureus* harbored the *aac(6')-Ie-aph(2'').* No significant differences in the distribution of AMEs genes were found between strains of the various clinical samples.

Table 1. The phenotypic and genotypic characteristics of 20 aminoglycoside-resistant *S. aureus*

Isolate	Source	Resistance pattern	MIC µg/ml		Resistance genes	Allelic Profile	ST
			GM	OX			
SA1	Blood	FOX, AK, GM, K, NET, TN	512	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	2-3-1-1-4-4-3	239
SA2	Blood	FOX, AK, GM, K, NET, TN	512	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	2-3-1-1-4-4-3	239
SA3	Wound	FOX, AK, GM, K, NET, TN	512	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	2-3-1-1-4-4-3	239
SA4	RT	FOX, AK, GM, K, NET, TN	512	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	2-3-1-1-4-4-3	239
SA5	Wound	FOX, AK, GM, K, NET, TN	512	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	2-3-1-1-4-4-3	239
SA6	OBR	FOX, AK, GM, K, NET, TN	512	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	2-3-1-1-4-4-3	239
SA7	Blood	FOX, AK, GM, K, NET, TN	512	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	2-3-1-1-4-4-3	239
SA8	Blood	FOX, AK, GM, K, NET, TN	512	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	2-3-1-1-4-4-3	239
SA9	Blood	FOX, AK, GM, K, NET, TN	512	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	2-3-1-1-4-4-3	239
SA10	Wound	FOX, AK, GM, K, NET, TN	512	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	2-3-1-1-4-4-3	239
SA11	Wound	FOX, AK, GM, K, NET, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa*	2-2-2-2-6-3-2	30
SA12	Wound	FOX, AK, GM, K, NET, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa*	2-2-2-2-6-3-2	30
SA13	Wound	FOX, AK, GM, K, NET, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa*	2-2-2-2-6-3-2	30
SA14	RT	FOX, AK, GM, K, NET, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa*	2-2-2-2-6-3-2	30
SA15	Wound	FOX, AK, GM, K, NET, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa*	2-2-2-2-6-3-2	30
SA16	Blood	FOX, AK, GM, K, NET, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa*	2-2-2-2-6-3-2	30
SA17	RT	FOX, AK, GM, K, TN	512	256≤	*mecA, aac(6')-Ie-aph(2"), ant(4')-Ia*	79-1-14-23-12-4-31	859
SA18	Blood	FOX, AK, GM, K, TN	512	256≤	*mecA, aac(6')-Ie-aph(2"), ant(4')-Ia*	79-1-14-23-12-4-31	859
SA19	Wound	FOX, GM, K	128	256≤	*mecA, aac(6')-Ie-aph(2")*	2-220-1-1-1-1-3	1465
SA20	OBR	FOX, AK, GM, K, NET, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	3-3-1-1-4-4-3	8

RT: Respiratory tract, OBR: Other body fluid, FOX: cefoxitin, AK: amikacin, GM: gentamicin, K: kanamycin, NET: netilmicin, TN: tobramycin, ST: sequence type

DISCUSSION

Multiple molecular typing of GPC is a valuable tool in epidemiological studies and is useful for the monitoring and infection control program (14). The overall incidence of aminoglycoside resistance found in the current study was higher than the incidence that has been reported in other reports from Iran. For example, in a recent study in Iran (2019), the percentages of resistance obtained in 102 clinical isolates of *S. aureus* were 83% to kanamycin, 76% to tobramycin, 71% to gentamicin and 59.5% to amikacin (15). In another study in Iran (2009), the percentages of resistance obtained in 100 clinical isolates of *S. aureus* were 68% to kanamycin, 53% to tobramycin, 52% to gentamicin and 48% to amikacin (16). This discrepancy may be due to selecting bacteria with a defined resistance phenotype, rather than unselected isolates. Based on the STs among *S. aureus* isolates, ST239 was the predominant MRSA clone, which accounted for 50% of the isolates. In addition, this clone was the most common in blood samples which proves that ST239 is one of the leading causes of bloodstream infections. Similar finding was observed in Northwestern China (Urumqi), where the ST239 accounted for more than 60.0% of isolates from blood specimens (17). A multicentre study from four hospitals in Hong Kong showed that ST239 predominated among MRSA bloodstream isolates (18). In this study, all ST239 isolates showed high MIC against gentamicin which may be associated with the simultaneous presence of three AMEs genes [*(aac(6')-Ie-aph(2"), aph(3')-IIIa* and *ant(4')-Ia)*]. In current study, all isolates of the ST239 were resistant to amikacin, tobramycin, kanamycin, netilmycin, and methicillin. This is not surprising, because strains of type ST239 clone is typically resistant to various classes of antibiotics such as aminoglycosides, macrolides and tetracyclines (19). In a previous study in a single hospital in Isfahan (Iran), Havaei et al. observed that 47% of clinical MRSA strains belonged to ST239 (20). Another study conducted in 2017 in Iran, Goudarzi et al. reported that 72% of MRSA strains isolated from burn patients belonged to ST239 (21). A multicenter

Table 2. The phenotypic and genotypic characteristics of 22 aminoglycoside-resistant *S. epidermidis*

Isolate	Source	Resistance pattern	MIC µg/ml		Resistance genes	Allelic Profile	ST
			GM	OX			
SE1	Blood	FOX, AK, GM, K, NET, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	7,1,2,2,4,1,1	2
SE2	Blood	FOX, AK, GM, K, NET, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	7,1,2,2,4,1,1	2
SE3	Eye	FOX, AK, GM, K, NET, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	7,1,2,2,4,1,1	2
SE4	Wound	FOX, AK, GM, K, NET, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	7,1,2,2,4,1,1	2
SE5	Wound	FOX, AK, GM, K, NET, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	7,1,2,2,4,1,1	2
SE6	Blood	FOX, AK, GM, K, NET, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	7,1,2,2,4,1,1	2
SE7	Blood	FOX, AK, GM, K, NET, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	7,1,2,2,4,1,1	2
SE8	Blood	FOX, AK, GM, K, NET, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	7,1,2,2,4,1,1	2
SE9	Blood	FOX, AK, GM, K, NET, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa*	1,1,1,2,2,1,1	5
SE10	Blood	FOX, AK, GM, K, NET, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa*	1,1,1,2,2,1,1	5
SE11	RT	FOX, AK, GM, K, NET, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa*	1,1,1,2,2,1,1	5
SE12	OBR	FOX, AK, GM, K, NET, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa*	1,1,1,2,2,1,1	5
SE13	Blood	FOX, AK, GM, K, NET, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa*	1,1,1,2,2,1,1	5
SE14	Urine	FOX, AK, GM, K, TN	128	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa*	7,1,2,2,4,7,1	22
SE15	Blood	FOX, AK, GM, K, TN	128	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa*	7,1,2,2,4,7,1	22
SE16	Blood	FOX, AK, GM, K, TN	128	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa*	7,1,2,2,4,7,1	22
SE17	Blood	FOX, AK, GM, K	512	256≤	*mecA, aac(6')-Ie-aph(2"), ant(4')-Ia*	1,1,2,2,4,1,1	54
SE18	Blood	FOX, AK, GM, K, TN	512	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa*	1,1,1,44,2,1,1	581
SE19	Blood	FOX, AK, GM, K, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa*	1,2,2,2,1,1,1	179
SE20	Blood	FOX, AK, GM, K, NET, TN	128	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	7,1,2,1,3,3,1	23
SE21	Blood	FOX, AK, GM, K, NET, TN	128	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	3,1,16,5,4,7,1	588
SE22	OBR	FOX, AK, GM, K, NET, TN	256	256≤	*mecA, aac(6')-Ie-aph(2"), aph(3')-IIIa, ant(4')-Ia*	3,1,2,2,11,7,1	580

RT: Respiratory tract, OBR: Other body fluid, FOX: cefoxitin, AK: amikacin, GM: gentamicin, K: kanamycin, NET: netilmicin, TN: tobramycin, ST: sequence type

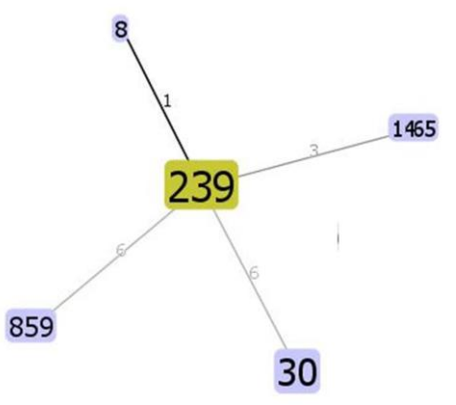

Fig. 1. Minimum Spanning Tree of the 20 aminoglycoside-resistant *S. aureus* showing relationship between different STs assigned by the analysis of MLST data. Each node represents one sequence type and the corresponding ST is given inside the node. The size of each node is directly proportional to the number of strains included under that ST. The number given on the strings connecting the nodes stands for the number of genes by which the strains under those STs differ from each other.

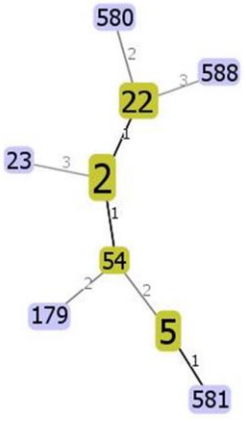

Fig. 2. Minimum Spanning Tree of the 20 aminoglycoside-resistant *S. epidermidis* showing relationship between different STs assigned by the analysis of MLST data. Each node represents one sequence type and the corresponding ST is given inside the node. The size of each node is directly proportional to the number of strains included under that ST. The number given on the strings connecting the nodes stands for the number of genes by which the strains under those STs differ from each other.

study from eight hospitals in Tehran revealed that ST22 was the dominant clone among MRSA strains isolated from ICU patients (22). Evidence shows that ST239 was predominant hospital-acquired MRSA clone in many Asian countries (Indonesia, Singapore, Sri Lanka, Thailand, Vietnam, and Hong Kong), while ST5 was predominant clone in Japan and Korea (19, 20). The second most prevalent type was ST30, which accounted for 30% of the isolates. ST30 was the predominant community-associated MRSA clones in Oceania and Europe (23). The other MRSA clones detected during this study included ST859, ST1465 and ST8, which previously reported by others (22, 24). Based on the STs among *S. epidermidis*, the most predominant type was ST2, which accounted for 36% of the isolates. ST2 is a well-recognized clone that causes nosocomial infections worldwide. A previous study conducted in Tabriz (north-western Iran) during 2012 to 2013 revealed that ST2 and ST5 were the most common clone circulating in hospitals (25). In China, Li et al. studied 80 *S. epidermidis* isolates from clinical samples and observed that ST2 (31%) was the major prevailing clone (26). In USA, Wong et al. evaluated 40 linezolid-resistant *S. epidermidis* (LRSE) isolates and reported that ST2 (45%) and ST5 (10%) were the major hospital-associated MRSE (27). A study done at Hospital of Besancon (France) that showed all LRSE isolates responsible for nosocomial infections belonged to ST2 (28). In this study, ST2 and ST5 were the most common in blood samples. In Sweden, Ahlstrand et al. observed that the predominant STs of *S. epidermidis* blood culture isolates obtained from patients with hematological malignancies were ST2 and ST215 (29). In this study, the most common AME gene was *aac(6')-Ie-aph(2'')-Ia*, which observed in 42 gentamicin-resistant GPC isolates. These results confirmed those of Schmitz et al. who documented that *aac(6')-Ie-aph(2'')-Ia* has been the gene most frequently found in clinical isolates of staphylococci from 19 European hospitals (1). The high prevalence of this gene among our isolates is a major concern, because it confers resistance to the majority of aminoglycosides which commonly used in clinical practice (2, 15). The prevalence of these genes in GPC varied from 62% to 87.5% at different countries (30-32). The second AMEs gene observed in the current study was the *aph(3')IIIa* (90%) followed by *ant(4')Ia* (59.5%). In a previous study in Iran, Marghaki et al. observed that 19% and 14% of the *S. aureus* isolates harbored *aph(3')IIIa* and *ant(4')Ia* genes, respectively (15). In Korean study, 27% of CoNS isolates carried ant *(4')Ia(33).* Reports from Turkey (8%) and Japan (9%) have shown a low prevalence of the *aph(3') IIIa* gene (34). Different policies for prescription of antibiotics, infection control program, and monitoring among hospitals in different regions and countries result in different rates of antibiotic resistant strains reflecting the diversity in the distribution of resistant genes (34-36). Another problem in our country is that many clinicians are unfamiliar with AME genes and this issue facilitates the emergence of resistance organisms (37). In the current study, all 42 isolates were resistant to methicillin which was higher than similar reports from Iran (MRSA=41%; 42/102), Lebanon (MRSA=72%; 93/130), Norway (MRSA=42.6%; 2255/5289), China (MRSA=54.2%; 228/421), USA (MRSE= 73.2%; 52/71) and China (MRSE= 34.4%; 54/157) (15, 38-42). Possible explanation for these high frequency of MRSA and MRSE can be explained with the inappropriate use of antibiotics and poor infection prevention and control measures in Iranian hospitals. The major limitation of our study is the small sample size, lack of clinical data and location (all isolates examined were from a single hospital in Tehran), which may lead to possible bias.

In conclusion, although the results of current study are based on specimens collected from one hospital, our study indicates that the high prevalence of aminoglycoside resistance is mainly due to the presence of the *aac(6')-Ie-aph(2'')-Ia* and *aph(3')IIIa* genes and ST239 for *S. aureus* and ST2 for *S. epidermidis* were the major clones in the selected university hospital of Tehran. Therefore, it is critical that clinicians and healthcare workers to be aware of the population of *S. aureus* and *S. epidermidis* present in order to make decisions for appropriate treatment and infection control practices.

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