Detection of tetracycline resistance genes, aminoglycoside modifying enzymes, and coagulase gene typing of clinical isolates of *Staphylococcus aureus* in the Southwest of Iran

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**ABSTRACT**

Objective(s): The aim of the present study was to determine the aminoglycoside modifying enzymes (AMEs) encoded genes, tetracycline resistance genes, and the coa based typing of *Staphylococcus aureus* isolates in the Southwest of Iran.

Materials and Methods: Antimicrobial susceptibility of isolates was carried out by agar disk diffusion methods. Two sets of multiplex PCR mixture were used for detection of AME genes and tet genes. All of the isolates were typed with the coagulase gene typing method. Of the 121 isolates, 29.75% and 47.93% were resistant to at least one aminoglycosides and tetracyclines, respectively.

Results: The aac(6')-le-aph(2') was the most frequent gene (97.22%), and aph (3')-IIa and ant (4')-Ia genes were detected in 61.11% and 11.11% of aminoglycoside resistant isolates, respectively. The tetK and tetM genes were detected in 82.75% and 56.9% of tetracycline resistant isolates, respectively. Overall 31.4% of isolates were MRSA. Totally 17 distinct coa gene RFLP patterns, numbered C1 to C17, were observed. The C5 was the most frequent coa type with 31 isolates.

Conclusion: The aac(6')-le-aph(2') and aph (3')-IIa genes were the most important genes contributing to aminoglycosides resistance, while resistance to tetracyclines was mediated by tetK and tetM genes. Interestingly all *S. aureus* with C5 as the most prevalent coa-type were resistant to at least one of the aminoglycoside antibiotics and tetracycline simultaneously. Moreover, 30 out of 31 isolates with this coa type were MRSA, indicating the importance of the C5 coa-type in MRSA strains and also in isolates that were resistant to aminoglycosides and tetracycline.

**Introduction**

*Staphylococcus aureus* is one of the most important causes of nosocomial infections around the world and can cause a variety of diseases such as skin and soft tissue infections, pneumonia, bloodstream infection, osteomyelitis, endocarditis, and also toxin mediated diseases (1). *S. aureus* has shown resistance to different classes of antibiotics, which complicates the treatment of infections (2, 3). Aminoglycosides by inhibiting the bacterial protein synthesis show bactericidal activity. This group of antibiotics especially gentamycin and tobramycin in combination with beta-lactam or glycopeptides antibiotics have synergetical effects on treatment of *S. aureus* infection, particularly endocarditis (4). Resistance to aminoglycosides occurs mainly by drug inactivation via bacterial aminoglycoside modifying enzymes (AMEs) that are encoded by the genes located on plasmids or transposons (4, 5). AMEs are classified into four groups according to the modification imposed on aminoglycoside antibiotics: acetyltransferases (AACs), phosphotransferases (APHs), nucleotidylyltransferases (ANTs), and adenyltransferases (AADs). The most important enzymes which confer resistance to amino-

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glycosides among staphylococci isolates are AAC(6')-APH(2''), APH(3')-III, and ANT(4')-I, which are encoded by aac(6')-le-aph(2''), aph(3')-IIIa, and ant(4')-la genes, respectively (6, 7).

Tetracyclines are broad-spectrum antibiotics used in the treatment and prevention of bacterial infections and can be used for treatment of S. aureus caused infections such as skin and soft tissue infections (SSTIs) (8). Two main mechanisms of resistance against tetracyclines have been identified in S. aureus: active efflux, which is mediated by plasmid encoded tetK and tetL genes and ribosomal protection that is encoded by chromosomal or transposonal tetM or tetO genes (9). It has been shown that S. aureus isolates harboring the tetK gene are resistant to tetracycline but not minocycline while the tetM gene confers resistance to both of them (10).

Knowledge about the local epidemiology of S. aureus in a hospital setting is very important for prediction of trends in antibiotic-resistance patterns, detection of outbreaks and tracking the spread of infection, epidemiological surveillance, and hospital infection control (1, 11). Therefore various molecular techniques are available for identification and compression of different S. aureus strains. Of these techniques pulsed-field gel electrophoresis (PFGE), multilocus enzyme electrophoresis [MLEE], randomly amplified polymorphic DNA (RAPD) assay and a repetitive element sequence-based PCR (rep-PCR) and coagulase gene typing as well as sequence-based (multilocus sequence typing [MLST] and spa typing) techniques (12, 13) have been used. The coagulase gene (coa) typing is another technique in which the organism is typed based on the polymorphic region of the coa gene. Although the discriminatory power of the coa typing is lower than PFGE, MLAST, and spa typing methods (14), it is easier, faster, accurate, and enough reproducible for typing of S. aureus isolated from different sources (15). All of the S. aureus isolates produce the coagulase enzyme that is encoded by the coa gene. The coa typing is based on the heterogeneity of 81 bp tandem repeats that are located in the 3' coding region of coagulase gene. Amplification of that region using the PCR method results in DNA bands with diverse size and numbers in each strain. Furthermore, digestion of the amplified fragment with AluI and HaeIII restriction enzymes generates more DNA bands in different isolates which increases the discriminatory power of coa typing method (16, 17).

Considering the high frequency of S. aureus infections and contributed antimicrobial resistance in the region, there are a few studies addressing the AMEs genes and tetracycline resistance genes in accordance with the coa typing of S. aureus in Iranian hospitals. The aim of the present study was to determine the AMEs encoded genes, tetracycline resistance genes, and coa typing of S. aureus strains isolated from two major teaching hospitals in the southwest of Iran.

### Materials and Methods

#### Bacterial isolates

A total of 121 S. aureus isolates were collected from hospitalized patients who were referred to teaching hospitals affiliated to Ahvaz Jundishapur University of Medical Sciences from May 2012 to January 2013. Identification of isolates at species level was done by conventional biochemical tests such as manitol fermentation, catalase, tube coagulase, and the DNase test (18). Confirmation of S. aureus isolates was performed using amplification of the nucA gene (19).

#### Antimicrobial susceptibility testing

Antimicrobial susceptibility of S. aureus isolates against 8 antibiotics was carried out by agar disk diffusion methods according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (20). The antibiotic panel included: aminoglycosides group (amikacin, gentamicin, tobramycin, and kanamycin), tetracyclines group (tetracycline, minocycline, and doxycycline), in addition to cefoxitin and erythromycin. All antibiotics were purchased from MAST, UK. S. aureus ATCC 25923 was used as the control strain.

#### Detection of AME encoded, tet, mecA and nucA genes

Total DNA genomic was extracted by boiling methods according to known procedure with some minor modification (21). The oligonucleotides primers and their expected size of PCR products for AME genes aac(6')-le-aph(2''), aph(3')-IIIa and ant(4')-la, tet genes (tetK, tetL, tetMand tetO), mecA and nucA genes are presented in Table 1. Two sets of Multiplex–PCR mixture for detection of the AME genes and the tet genes were prepared separately. Each set was carried out in a total volume of 25 µl containing 12.5 µl master mix (Amplicon, Denmark), 20 pmol of each primer and 300 ng of template DNA. Also, two series of single PCR were carried out for the mecA and nucA genes in 25 µl reaction consisting of 12.5 µl master mix (Amplicon, Denmark), 20 pmol of each primer, and 200 ng of template DNA. The amplification conditions were as follows: initial denaturation at 95 °C for 5 min followed by 35 cycles of amplification at 94 °C for 45 sec, annealing at (55 °C for tet genes, 56 °C for AME genes, mecA and nucA genes) for 45 sec and extension at 72 °C for 45 sec. A final extension step was performed at 72 °C for 7 min.

The PCR products were separated on 1.5% agarose gel containing ethidium bromide and photographed with a digital camera (Canon, Japan) under UV transilluminator (Major science, Taiwan).

#### Coagulase gene typing

PCR amplification of the 3’-end region of the coa gene was performed according to the primers designed previously (22). PCR was performed in a 50 µl reaction mixture, containing 25 µl of master mix
(Amplicon, Denmark), 20 pmol of each primer, and 200 ng of DNA template. PCR reactions were carried out in a thermocycler (Biorad T100, USA) with the minoglycoside antibiotics. There was a significant association between MRSA and resistance to aminoglycoside antibiotics. The isolates that were resistant to 4 aminoglycoside antibiotics were also MRSA, while only one of the four isolates that showed resistance to tobramycin and kanamycin were MRSA. Five (6%) of the MRSA strains were sensitive to aminoglycoside antibiotics. There was a significant association between MRSA and resistance to aminoglycoside antibiotics. P=0.0001. Of 121 S. aureus isolates, 39 were resistant to erythromycin, of which 33 isolates also showed resistance to aminoglycoside antibiotics.

The most prevalent AME gene was aac(6')-le-aph(2') with the frequency of 35 (97.22%) of the 36 resistant isolates. The aph (3')-Illa and ant (4')-Ia genes were detected in 22 and 4 isolates, respectively. Detection profile of AME genes in resistant isolates is shown in Table 2. The most frequent

### Table 1. Oligonucleotide sequences of the primers used for detection of aminoglycosides resistance genes, tetracycline resistance genes, and mecA gene

| Target genes | Oligonucleotide sequences of primers | Size (bp) | Reference |
|--------------|--------------------------------------|-----------|-----------|
| aac(6')-le-aph(2')-I | F-CAGGAAATTGAGAAAATGTTAGAAGG | 369 | (23) |
| aph(3')-Illa | F-GGCTAAATGGAGAATATGACCAGG | 523 | (23) |
| ant(4')-Ia | F-CAAAAGTTCGAAATCGGTAAGGACCC | 294 | (23) |
| tetK | F-GGAGCAATGAGTTGATTAG | 360 | (24) |
| tetM | F-GATGGACGATTACAGAA | 158 | (24) |
| tetL | F-ATACATCCCTGGGTGTCTA | 1077 | (10) |
| tetO | F-AAGTTAGCAATTGCTGCAC | 514 | (25) |
| mecA | F-GTG AGA ATA TAC CAA GTG ATT | 147 | (26) |

### Results

A total of 121 S. aureus isolates from various sources including wound and skin lesions (n=54, 44.62%), blood (n=17, 14.05%), catheter (n=20, 16.53%), tracheal aspirates (n=8, 6.62%), urine (n=3, 2.48%), femur lesions (9, 7.44%), abscess (5, 4.13%) and other clinical specimens (n=5, 4.13%) were analyzed. Considering the positive PCR results for the mecA gene, 38 (31.4%) of isolates were MRSA and 83 (68.6%) were MSSA. Among all of the isolates, 36 (29.75%) strains were resistant to at least one of the tested aminoglycoside antibiotics in this study, while 32 were resistant to all of the tested aminoglycoside antibiotics (tobramycin, gentamicin, glycylide amikacin, and kanamycin) and 4 were resistant against tobramycin and kanamycin. The isolates that were resistant to 4 aminoglycoside antibiotics were also MRSA, while only one of the four isolates that showed resistance to tobramycin and kanamycin were MRSA. Five (6%) of the MRSA strains were sensitive to aminoglycoside antibiotics. There was a significant association between MRSA and resistance to aminoglycoside antibiotics. P=0.0001. Of 121 S. aureus isolates, 39 were resistant to erythromycin, of which 33 isolates also showed resistance to aminoglycoside antibiotics.

The most prevalent AME gene was aac(6')-le-aph(2') with the frequency of 35 (97.22%) of the 36 resistant isolates. The aph (3')-Illa and ant (4')-Ia genes were detected in 22 and 4 isolates, respectively. Detection profile of AME genes in resistant isolates is shown in Table 2. The most frequent

### Table 2. Aminoglycoside resistance pattern and profile of AME genes in resistant isolates of Staphylococcus aureus

| Resistance phenotype | aac(6')-le-aph(2') | aph(3')-Illa | ant (4')-Ia | mecA | Isolates number |
|----------------------|-------------------|-------------|-------------|------|----------------|
| AK, GM, K, TN        | +                 | +           | -           | +    | 1              |
| AK, GM, K, TN        | +                 | -           | -           | -    | 19             |
| AK, GM, K, TN        | +                 | -           | -           | +    | 12             |
| K, TN                | +                 | +           | -           | +    | 2              |
| K, TN                | +                 | -           | -           | -    | 1              |
| K, TN                | -                 | -           | -           | -    | 1              |
| Total                | 35                | 22          | 3           | 34   | 36             |

AK: amikacin; GM: gentamicin; K: kanamycin; TN: tobramycin
profile was aac(6')-le-aph(2')+ aph (3')-IIIa, which was detected in 21 (58.33%) isolates. Co-existence of aac(6')-le-aph(2'), aph (3')-IIIa and ant (4')-Ia genes was observed in one isolate. A significant association was observed between aminoglycoside resistance in S. aureus isolates and presence of the aac(6')-le-aph(2') (P=0.0001) and aph (3')-IIIa genes (P=0.001).

Antimicrobial resistance pattern of tetracycline group showed that 58 (47.93%) of S. aureus isolates were resistant to tetracycline, 25 (20.66%) to doxycycline, and 4 (3.3%) to minocycline. Seven resistant patterns were observed against these antibiotics groups, and co-resistance to tetracycline and doxycycline was the most prevalent. Of 58 tetracycline resistant isolates, 36 isolates were MRSA, whilst only two MRSA strain isolates were sensitive to tetracycline. The tetK and tetM genes were detected in 48 (82.75%) and 33 (56.9%) of 58 tetracycline resistant isolates, respectively, in which 23 (39.65%) isolates harbored tetK and tetM simultaneously. None of the isolates were positive for tetL and tetO genes. The pattern of tetracycline resistance genes in accordance with the antibiogram profile is presented in Table 3. The entire of 121 studied isolates were confirmed as S. aureus by detection of the nucA gene using PCR, and all of them gave positive results for amplification of the coa gene with a single band but in five different sizes ranging approximately from 530 to 860 base pair, each size corresponding to one pattern. The coa gene with 700 bp size was seen in 72 isolates and was considered a predominant type. The second common type with 17 isolates had a 530 bp band. Fifteen, eleven, and six isolates had a single 610, 780 and 860 bp band for the coa gene, respectively. Considering the RFLP patterns of the coa gene with HaeIII and AluI enzymes, the HaeIII-RFLP presented higherdiscriminatory power rather than AluI-RFLP and thereby the HaeIII-RFLP pattern was used for the further analysis. All of the PCR products for the coa gene were digested by the HaeIII enzyme. Totally 17 distinct coa gene RFLP patterns, numbered C1 to C17, were observed. The C5 was the most frequent coa type with 31 isolates (Table 4). It is interesting that aminoglycoside resistant isolates were belonging to C5, C6, C13, and C14 coa types. MRSA strains were also found in C3, C5, C7, C13, and C17 coa types. Resistance to the tetracycline group was observed in eight coa types (C1, C3, C5, C7, C8, C13, C14, and C16). All of the 31 S. aureus isolates with C5 coa type were resistant against at least one of the aminoglycoside plus tetracycline antibiotics group simultaneously and except for one, all of them were MRSA. Similar to the C5 coa type, all of the three isolates with C13 coa type were MRSA and also

| Resistance phenotype | Tetracycline gene |
|----------------------|-----------------|
|                      | tetK | tetM | meCA |
| TE                   | +    | -    | +    | 3 |
| TE                   | +    | -    | -    | 20 |
| TE                   | -    | +    | +    | 8 |
| TE                   | +    | +    | +    | 1 |
| TE                   | +    | +    | -    | 1 |
| TE, DXT              | +    | -    | +    | 1 |
| TE, DXT              | +    | -    | -    | 1 |
| TE, DXT              | -    | +    | +    | 2 |
| TE, DXT, MN          | +    | +    | +    | 17 |
| Total                | 48   | 33   | 36   | 58 |

Table 3. The distribution of tetracycline resistance (tet) genes and resistance phenotype in clinical isolates of Staphylococcus aureus

| coa amplification pattern (bp) | RFLP pattern (bp) with HaeII | coa type | S. aureus with coa type (no) | MRSA N=38 | MSSA N=83 | Resistant to aminoglycosides (no) | Resistant to tetracyclines (no) |
|-------------------------------|-------------------------------|----------|-------------------------------|-----------|-----------|-----------------------------|-----------------------------|
| 860                           | 140-170-210-340               | C1       | 3                             | 3         | 3         | 3                           | 3                           |
|                               | 80-210-330                    | C2       | 3                             | 3         | -         | -                           | -                           |
|                               | 140-260-380                   | C3       | 9                             | 2         | 7         | -                           | -                           |
|                               | 320-460                       | C4       | 2                             | -         | 2         | -                           | -                           |
| 700                           | 170-210-320                   | C5       | 31                            | 30        | 1         | 31                          | 31                          |
|                               | 80-170-210-240                | C6       | 5                             | 5         | 1         | -                           | -                           |
|                               | 100-140-460                   | C7       | 6                             | 2         | 4         | -                           | 1                           |
|                               | 130-140-180-250               | C8       | 19                            | -         | 19        | -                           | 4                           |
|                               | 140-180-380                   | C9       | 1                             | -         | 1         | -                           | -                           |
|                               | 80-100-140-380                | C10      | 8                             | -         | 8         | -                           | -                           |
|                               | 140-170-180-210               | C11      | 1                             | -         | 1         | -                           | -                           |
|                               | 80-100-130-140-240            | C12      | 1                             | -         | 1         | -                           | -                           |
| 610                           | 170-210-240                   | C13      | 3                             | 3         | -         | 3                           | 3                           |
|                               | 140-470                       | C14      | 8                             | -         | 8         | 1                           | 2                           |
|                               | 130-140-170-180               | C15      | 2                             | -         | 2         | -                           | -                           |
|                               | 140-180-290                   | C16      | 2                             | -         | 2         | -                           | -                           |
| 530                           | 210-320                       | C17      | 17                            | 1         | 16        | -                           | 9                           |
simultaneously were resistant to aminoglycoside and tetracycline antibiotics groups. In resistant isolates, totally 11 different antibiotics resistant patterns were observed, among which, the pattern including 19 isolates that were resistant to the tobramycin, gentamicin, amikacin, kanamycin, tetracycline, and doxycycline was the most prevalent pattern. It is interesting that all of these isolates belong to the C5 coa type.

**Discussion**

In the present study 26.44% of isolates were resistant to tobramycin, kanamycin, amikacin, and gentamycin simultaneously and in addition, co-resistance to tobramycin and kanamycin was observed in 3.22% of the isolates. Although our results are similar to those by Emaneini et al. from Iran that was conducted on clinical isolates other than burn patients (27), they are lower than the resistance rate of other studies from Iran (28) on different clinical isolates and Emaneini et al. in burn patients (23). These differences may be related to the type of clinical samples and different policies for infection control in different hospitals. In comparison with other countries, the resistance rates to gentamycin and amikacin were similar to Hauschild et al. (29) and Schmitz et al. (4) but lower than the results by Ida et al. (5). Our results showed that 86.1% of aminoglycosides resistant isolates were MRSA, which was 8 times higher than MSSA strains. Like many previous reports, the resistance rate to aminoglycoside antibiotics was higher in MRSA strains than MSSA strains. It has been documented that there is a correlation between resistance against aminoglycoside and methicillin thereby different resistance rates to aminoglycoside in various studies are contributed to the different number of MRSA isolates (30).

Aminoglycoside modifying enzymes in S. aureus isolates are encoded by aac(6\(^\prime\))-Ie-aph(2\(^\prime\)), aph (3\(^\prime\))-Ila, and ant (4\(^\prime\))-la genes and confer resistance to aminoglycosides. In this study 97.22% of aminoglycoside resistance isolates, harbored the aac(6\(^\prime\))-Ie-aph(2\(^\prime\)) gene as the most prevalent gene, which is similar to findings of the majority of studies from Iran as well as different European countries, South Korea, and Poland in which the aac(6\(^\prime\))-Ie-aph(2\(^\prime\)) has been reported as a most prevalent gene ranging from 28.9 to 93.7% of their isolates (2, 4, 23, 29, 30). However, in some of these studies the prevalence of the aac(6\(^\prime\))-Ie-aph(2\(^\prime\)) positive strains was lower than what we found. The aph(3\(^\prime\))-Ila was the second common AMEs gene (61.11%) among our studied strain, which is higher than other reports from Iran as well as Japan, South Korea, Poland, and 19 European countries in which this gene has been identified in 8.9%-46% of their isolates (4, 5, 28-30). In contrast to our results, the aph(3\(^\prime\))-Ila was the most prevalent AMEs gene in two different studies conducted by Emaneini et al. (82.78% and 93.7%) and Mahdiyoun et al. (77%) from Iran (23, 27, 31) and Liakopoulos from Greece (73.7%) (7). 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. The lowest frequency among AMEs genes belonged to ant (4\(^\prime\))-la gene which was detected in 11.11% of S. aureus isolates. Although our results are approximately similar to those of Liakopoulos et al. (13.6%) and Perumal et al. (9%) (7, 32), they are in contrast with many studies in which the ant (4\(^\prime\))-la gene has been identified in 26.7% to 89.24% of isolates (4, 5, 23, 27-30). This gene was not detected in two other studies (33, 34). It is quite interesting that 2 of the 3 isolates that harbored ant (4\(^\prime\))-la were MSSA. It is probable that the ant (4\(^\prime\))-la gene has a role in resistance to aminoglycosides antibiotics in MSSA strains although further studies are needed to analyze this matter. Regarding the comparison between various studies, different aminoglycoside resistance rates and also different prevalence of AME encoded genes were observed between countries and even in hospitals and cities of one country. We inferred that these differences may be related to the source of clinical isolates, geographical location, bacterial genotype, prescription of aminoglycosides, and the rate of MRSA isolates. Since aac(6\(^\prime\))-le-aph(2\(^\prime\)) causes resistance to most of the aminoglycosides (5), in our study this gene was found in all of the aminoglycoside resistance isolates except for one. In the present study two out of 3 isolates with the ant (4\(^\prime\))-la gene, showed resistance to tobramycin and kanamycin and the other isolate in addition to these antibiotics was also resistant to gentamicin and tobramycin. It is interesting that all of the isolates with the ant (4\(^\prime\))-la gene were resistant to tobramycin. These results are in accordance with other studies that reported the ant (4\(^\prime\))-la gene inactivates tobramycin (5, 7). The aph (3\(^\prime\))-Ila gene modifies kanamycin and amikacin; in our study all 22 isolates that harbored aph (3\(^\prime\))-Ila were resistant to kanamycin and 90.9% were also resistant to amikacin.

In the present study 47.93%, 20.65%, and 3.3% of S. aureus isolates were resistant to tetracycline, doxycycline, and minocycline, respectively. Approximately 62% of tetracycline resistant isolates were MRSA, which is similar to the results of Schmitz et al. (4) and Emaneini et al. (27) studies in which they found 57.1% and 61% of MRSA strains were tetracycline resistant. Based on studies from the United States and Canada the rates of occurrence of tetracycline resistance among MRSA isolates has been reported as 15.6% and 14.8%, respectively,
whereas in Latin America and the Western Pacific regions the rates were considerably higher, exceeding 60% (35). Two main resistance mechanisms against tetracycline have been explained in S. aureus; the first active efflux which is mediated by plasmid encoded tetK and tetM genes and the second ribosomal protection which is mediated by transposon or chromosomal tetM and tetO determinants (36). In 58 tetracycline resistant isolates, tetK and tetM were detected in 82.75% and 56.9% isolates, respectively, while none of the isolates were positive for tetL and tetO genes. Although except for one, all of the tetM positive isolates were MRSA, the tetK gene was detected in 45% of MSSA strains. In accordance with our results, Schmitz et al. have shown that tetM was more frequent in MRSA isolates (9). In a multicenter study (37) the tetK gene was detected in higher numbers in MRSA isolates. They showed that all of the tetracycline resistant MRSA isolates from North America harbored solely the tetK gene and in Eastern and Western European countries, 86% of isolates encoded only the tetK gene (37). Although in the present study the tetK gene was the predominant gene, 54.16% of them were MRSA, while this rate for the tetM gene was 97%. In another study tetK and tetM genes were detected in 14.3% and 50% of MRSA isolates, respectively (33). Thereby the tetK gene may have an important role in resistance to tetracycline antibiotics in MRSA in comparison with MSSA strains. One study showed that MRSA isolates harbored tetK and tetM in 31.8% and 36.4%, respectively (10). Co-existence of tetK and tetM genes was observed in 23 isolates, and except for one, all of them were MRSA. On the other hand the combination of the tetM and tetK was approximately 22 times more prevalent in tetracycline-resistant MRSA isolates rather than in tetracycline-resistant MSSA isolates; this is in agreement with the previous report of Schmitz et al. as their MRSA strains also harbored both tetK and tetM simultaneously 10 times more than MSSA isolates (9). Similar to many reports, tetL and tetO were not detected in any of the isolates in the present study, indicating that these genes have no role in tetracycline resistance in our isolates (9, 38-40).

The coagulase gene amplification has been considered as a simple and accurate method for typing of S. aureus (17). In the current study 17 distinct coa gene RFLP patterns (coa-type) were identified, among which, the C5 as a prevalent coa-type was identified in 31 (25.61%) isolates. All of the isolates with C5 coa-type were resistant to at least one of the aminoglycoside antibiotics and also all of them showed resistance to tetracycline antibiotics. In addition 30 out of 31 isolates were MRSA. This finding all together showed the importance of S. aureus with C5 coa type in isolates that were resistant to aminoglycosides and tetracycline and also acquisition of resistance to methicillin (MRSA). On the other hand, the majority of MRSA (78.94%) belonged to the C5 coa type indicating the spread of a specific type of MRSA in these hospitals. Similar to our results, another study found that 77% of MRSA strains belonged to the specific coa type, the L21 coa type (14). From the 17 different coa types, only 4 types were found in aminoglycoside resistant isolates, of which most of the isolates belonged to the C5 and C13 types representing the relationship among specific coa types and resistance to aminoglycoside antibiotics. Hence, it may be specific coa types (genotype) of S. aureus that acquire resistance to aminoglycosides and cause spreading antibiotic resistance in a hospital setting. Among aminoglycoside resistance isolates the aph(3')-IIIa gene was identified only in the C5 coa type, while aac(6')-Ile-aph(2") and ant (4')-Ia were identified in other coa types. It is possible that there is a relationship between aph(3')-IIIa and certain coa types, nonetheless, further research is needed. Tetracycline resistance isolates were found in 8 different coa types, where the tetM gene was found in only three coa- types, while tetK was found in 7 coa types. Probably in spite of the tetK gene which can be present in tetracycline resistant isolates with any coa type, the tetM genes prefer certain coa types.

Conclusion

According to our study, there were significant associations between MRSA strains and resistance to aminoglycoside and tetracycline antibiotics. The aac(6')-le-aph(2") and aph (3')-IIIa genes have an important role in resistance to aminoglycoside antibiotics and tetK and tetM confer tetracycline resistance in S. aureus. The coa gene RFLP patterns showed 17 distinct coa types, among which limited coa types were found to be aminoglycoside resistant or MRSA. It is interesting that all of the S. aurei with C5 as the most prevalent coa-type were resistant to at least one of the aminoglycoside antibiotics and also all of them showed resistance to tetracycline antibiotics. Moreover, nearly all of the isolates with this coa type were MRSA indicating the importance of the C5 coa-type in MRSA strains and also in isolates that were resistant to aminoglycosides and tetracycline.

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Conflict of interest

Authors declare no conflict of interest in this works.
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