The frequency of qacA/B and smr genes in clinical isolates of methicillin resistance coagulase negative staphylococci

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Abstract

Introduction:
Increasing use of disinfectants biocide cause to appearance of resistant strains of coagulase negative Staphylococcus. Some research confirmed this gene responsible for resistance to methicillin and association with these agents. The aim of this study was to investigate the presence of resistance genes of biocides such as qac A/B and smr in coagulase negative staphylococci.

Materials & Methods:
In this cross-sectional study, 60 samples of Staphylococcus epidermidis and 49 samples of Staphylococcus saprophyticus were collected over 9 months from clinical samples. After the initial biochemical tests and confirmation of genus and species of isolates, specific primers were used to study qacA/B and smr genes through polymerase chain reaction (PCR). Data were analyzed using chi-square test.

Results:
Of 60 isolates of Staphylococcus saprophyticus, 36 isolates (60%) had mecA. Among these, 19 isolates (52.77%) had qacA and 21 isolates (58.33%) had smr. Furthermore, of 49 Staphylococcus epidermidis isolates, 27 isolates (55.1%) had mecA and among those isolates, 11 isolates (22.44%) had qac A/B and 8 isolate (16.32%) had smr.

Conclusions:
The results of our study showed the widespread presence of qac A/B and smr in clinical isolates of methicillin-resistant coagulase-negative staphylococci. Given the low frequency of qacA/B and smr in the isolates sensitive to the antibiotics, it is necessary to evaluate antibiotic resistance for treatment of microbial infections.

Keywords: Drug Resistance, Methicillin-Resistant Staphylococcus, qacA/B

Introduction
Gram-positive cocci cover a very wide range of bacterial genus and species, each of which has in turn a role in primary and secondary infections (1). Coagulase-negative staphylococci (CoNS) are among the most important pathogens that cause catheter-related infections in hospitals (1). They have certain adhesive features helping the organism to bind to and colonize on artificial tools (2). CoNS, a normal flora of the human skin, turn pathogenic in certain conditions and cause...
infection (3). CoNS are responsible for widespread human and livestock infections and can spread numerous infections in hospitals and the community (4). S. saprophyticus and S. epidermidis are the most important CoNS (5). They were considered as saprophytic bacteria for a long time, but they have become aggressive and pathogenic agents due to increased use of medical equipment, such as catheters, prostheses, and cutters in recent decades (4). Now these bacteria, especially the two mentioned species, are classified as nosocomial infection agents (5). In most cases, beta-lactam antibiotics are used for the treatment of infections caused by CoNS. The new-generation cephalosporins have a greater share in this field (6). The treatment of infections caused by these organisms with these medications has failed because of a rapid increase in resistance to them (6). The presence of these resistant bacteria on different surfaces and the use of biocides and disinfectants had led to the emergence of resistant strains to disinfectants among the resistant strains (3). Chlorhexidine and pentavalent antimony compounds are the most widely used hospital bactericides for disinfection of surfaces and in some cases sterilization of the equipment (6). The presence of beta-lactam resistance genes, including mecA can lead to their resistance to treatment (7). These genes along with disinfectant resistance genes can cause the emergence of resistant strains against both antibiotics and biocides (7). The qacA/B and smr are among genes that cause resistance to chlorhexidine and polyvalent antimony (8). Some studies found the emergence of genes responsible for qac resistance along with genes coding for resistance to gentamicin, trimethoprim, penicillin, kanamycin and tobramycin antibiotics on mobile genetic elements (9, 10). An increase has been reported in genetic mutations along with mobile elements linked to plasmids among resistant bacteria carrying mecA gene (11). The smr, qac B, and qac A genes are usually carried on plasmids and thus have high transferability (12). Other genes including qac J, qac H, and qac G in the qac group were also studied in the staphylococci isolated from livestock and dairy samples (13, 14). Given the importance of using bactericides in hospitals for disinfecting medical instruments and surfaces, the emergence of bacteria that are resistant to these chemicals can lead to severe infections. Therefore, the present study was conducted to identify qacA/B and smr genes in methicillin-resistant clinical isolates of CoNS.

Materials and Methods
Sample collection and identification
In this cross-sectional descriptive study, 448 clinical samples were collected through convenience sampling within 9 months (January-December 2015) from health centers in Zahedan, Iran. Samples were collected from urine, blood, exudates, sputum, tracheal tube, chest tube and the catheter tip. Inclusion criteria were being hospitalized for a long time and suffering from bacterial infections. Staphylococcus isolates were identified using the laboratory Gram staining and standard biochemical tests such as catalase, coagulase, mannitol fermentation and dNase. The recognition of S. saprophyticus from S. epidermidis was performed using novobiocin discs and nalidixic acid (MAST, England), PYR tests (Hardy Diagnostics, US), ornithine decarboxylase test, production of acid
from maltose, trehalose, sucrose, mannitol and sucrose sugars, and non-fermentation of glucose in anaerobic conditions (using CO₂ incubator). In the disc diffusion method, first a bacterial suspension was prepared from 12 or 24 hours of culture with a turbidity of 0.5 McFarland, and then it was cultured on Muller Hinton agar media (Merck, Germany) with a thickness of 5 mm. Discs were put on the medium with sterile forceps and then incubated for 24 h at 37°C. Using the novobiocin, bacitracin and polymyxin B discs (MAST, England) S. epidermidis was recognized from S. saprophyticus. Staphylococcus hominis was separated from epidermidis using phosphomycin and desferrioxamine antibiotics. Results were evaluated using the latest version of CLSI. In all tests, S. aureus ATCC 25923 strain was used as a negative control, and S. epidermidis ATCC 12228, S. saprophyticus ATCC 15305 standard strain and S. hominis ATCC 27844 standard strain were used as a positive control. Approved isolates of S. epidermidis and S. saprophyticus were stored using microtubes containing Brain Heart Infusion (BHI) Agar (Merck, Germany) and 10% glycerol at -20°C (13-18).

**Genome extraction**

Clinical isolates stored at -20°C from the microtubes containing BHI Agar (Merck, Germany) were cultured in Muller Hinton agar (Merck, Germany). Then, several colonies cultured from each isolate were inoculated in 5 ml Loria Bertani Broth (Merck, Germany) culture medium in capped glass tubes, that were divided and numbered according to the number of isolates in advance, and incubated for 20 h at 37°C (19). The tubes were removed from the incubator after 20 hours. Then, 1.5 ml of resulted culture media was transferred into capped plastic 5.1 microtubes and genomic DNA extraction was performed using CinnaGen extraction kits. Finally, after qualitative and quantitative measurements by 1% agarose gel, DNA products were stored at -20°C for molecular testing.

**Preparation of primers and PCR testing**

After adding deionized distilled water, the primers were stored at 4°C for 4 hours and then saved dilutions were prepared for further testing and stored at -20°C. The following primers were used in this process to amplify qacA/B, mecA and smr genes in the samples.

In order to perform PCR reactions for each gene, 25 μl of the final solution containing 1 μl of template DNA, 1 μl of each primer at a concentration of 15 pM and 12.5 μl of Master Mix 2x and 1.5 mM MgCl₂ (Ampliqon, Germany) containing Tris-HCl PH8.5, (NH₄) SO₄, 3mM Mgcl₂, 0.2%Tween20, 4/0 MmdNTP 4,2/0 unit Ampliqon polymerase, Insert red dye and stabilizer was used for preparation of the primary mixture. Distilled deionized water was used in order to achieve the final volume (14). The PCR test for genes was performed using a thermocycler (BioRad, USA), according to the following regulation model.
Table 1: The list of specific primers used to identify qacA/B, mecA and smr genes in clinical isolates of CoNS

<table>
<thead>
<tr>
<th>Reference</th>
<th>Size (bp)</th>
<th>Sequence length</th>
<th>Primer</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raggi et al. (20)</td>
<td>157</td>
<td>CCACTACAGATTCTTCAGCTACATG CTATGGCAATAGGAGATATGGTGT</td>
<td>qacA/B F</td>
<td>qacA/B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AAAATCGATGGTAAAGGTTGTCG AGTTCTGCAGTACCGGATTTCG</td>
<td>mecA-F</td>
<td>mecA</td>
</tr>
<tr>
<td>Nahaei et al. (21)</td>
<td>533</td>
<td>GCCATAAGTACTGAAGTTATGGA GACTACGGTTGTTAAGACTAAACCT</td>
<td>smr F</td>
<td>smr</td>
</tr>
<tr>
<td>Noguchi et al. (22)</td>
<td>195</td>
<td>GCCATAAGTACTGAAGTTATGGA GACTACGGTTGTTAAGACTAAACCT</td>
<td>smr R</td>
<td>smr</td>
</tr>
</tbody>
</table>

Electrophoresis of PCR products on Agarose 1.5% gel

PCR products of qacA/B, mecA and smr genes, each with a length of 157, 533 and 195 bp, respectively, were isolated by electrophoresis using 1.5% agarose gel. For this purpose, 5 μl of PCR final product was electrophoresed in 1.5% agarose gel in the 0.5X buffer. In order to dye the gel, 5 μl Gel Red (Biotium, USA) solution was added to it and well stirred. Then the gels and their bands were observed under the UV at 260 nm by a UV-transilluminator (UVT-20 SML, USA). A fermentas molecular marker (Thermofisher, USA) with 100 bp sequence was used to determine the size of products (17). The obtained gel was photographed by Gel Doc device (model CCD-Tab1, Kiagen, Iran). In all molecular experiments, the standard strain of S. aureus ATCC25923 was used as a negative control, the standard strain of S. aureus ATCC 7644 for the presence of gene qacA/B, and the standard strain of S. aureus ATCC 33591 for the presence of gene mecA (10).

Data Analysis

Data was analyzed using SPSS version 16. Descriptive statistical methods were used to determine the frequency, percentage and mean, and Chi-square test was used to compare the qualitative results, and independent t-test to compare quantitative data. The significance level was considered as P≤0.05.
Results

A total of 60 clinical isolates of S. saprophyticus isolates were obtained from different samples from different wards including 13 isolates from wounds (21.66%), 4 isolates from central vein and other catheters (6.66%), 40 isolates from urine (66.66), and 3 isolates from blood (5%). Most isolates were obtained from female patients (Table 3). There was no significant relationship between obtained species and the gender of patients from whom the clinical samples were collected (P>0.05). Of the 49 obtained S. epidermidis isolates, 5 were from wounds (10.82%), 15 from central vein and other catheters (30.45%), 28 from urine (57.15%) and 1 (2.5%) from blood (Table 4).

Of the 49 isolates of S. epidermidis, 36 isolates had mecA gene (60%), 19 had qacA/B gene (52.77%) and 21 had smr gene (58.33%). Furthermore, of the 60 isolates of S. saprophyticus, 27 isolates had mecA gene (55.1%), 11 had qacA/B gene (22.44%) and 8 had smr gene (16.32%). According to the Chi-square test result, the P values for mecA, qacA/B and smr genes were 0.01, 0.039 and 0.05, respectively, indicating a significant relationship between the presence of antibiotic-resistant genes and genes responsible for resistance to detergents and disinfectants, such that most samples that had mecA gene were positive for qacA/B and smr genes, too. No significant relationship was observed between Staphylococcus species and clinical samples. Furthermore, after gel electrophoresis of the products derived from mecA, qacA/B, and smr genes, the desired bands with a molecular weight of 533, 195 and 157 were identified, respectively (Figures 1 and 2).

Table 3: The frequency of S. saprophyticus and S. epidermidis isolates based on patients’ gender; smr, qacA/B and mecA in CoNS

<table>
<thead>
<tr>
<th>Gender</th>
<th>CoNS (n=109)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. epidermidis (n=60)</td>
</tr>
<tr>
<td>Female</td>
<td>46 (76.66%)</td>
</tr>
<tr>
<td>Male</td>
<td>9 (23.33%)</td>
</tr>
</tbody>
</table>

Table 4: The frequency of S. saprophyticus and S. epidermidis isolates based on the type of sample and the hospital ward

<table>
<thead>
<tr>
<th>Ward</th>
<th>Urine (%)</th>
<th>Wound (%)</th>
<th>Blood (%)</th>
<th>Central vein and other catheters (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pediatrics</td>
<td>4 (8.1%)</td>
<td>1 (1.6%)</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>ICU</td>
<td>0</td>
<td>0</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Medical-Surgical</td>
<td>1 (2%)</td>
<td>1 (4%)</td>
<td>0</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>P-ICU</td>
<td>0</td>
<td>0</td>
<td>1 (1%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>N-ICU</td>
<td>0</td>
<td>0</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Outpatients</td>
<td>23 (46.93%)</td>
<td>19 (31.66%)</td>
<td>0</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Gynecology</td>
<td>13 (26.53%)</td>
<td>8 (2%)</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Hematology</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

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The frequency of qacA/B and smr

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Figure 1: The results of amplification of mecA, smr and qacA/B genes related to the methicillin-resistance factor, biocides-resistance factor, and tetravalent ammonium compounds in clinical isolates of S. epidermidis. Wells number 1 to 5 contained samples with positive mecA, smr and qacA/B genes. The molecular weight of these pieces was 533, 195 and 157, respectively. M is 100 bp marker. Well 6 is positive control. Well 7 is negative control.

Graph 1: The frequency of smr, qacA/B and mecA in CoNS

Figure 1: The results of amplification of mecA, smr and qacA/B genes related to the methicillin-resistance factor, biocides-resistance factor, and tetravalent ammonium compounds in clinical isolates of S. epidermidis. Wells number 1 to 5 contained samples with positive mecA, smr and qacA/B genes. The molecular weight of these pieces was 533, 195 and 157, respectively. M is 100 bp marker. Well 6 is positive control. Well 7 is negative control.
Figure 2: The results of amplification of mecA, smr and qacA/B genes related to the methicillin-resistance factor, biocides-resistance factor and tetravalent ammonium compounds in clinical isolates of S. saprophyticus. Wells number 1 to 5 contained samples with positive mecA, smr and qacA/B genes. The molecular weight of these pieces was 533, 195 and 157, respectively. M is 100 bp marker. Well 7 is a positive control. Well 5 is a negative control.

Discussion

The use of disinfectants and biocides has found a special importance since 1940, in that biocides are the first line attempt for eliminating surface and obvious pollution in most cases in health centers and hospitals (18). Today, the excessive use of antimicrobial biocides led to the emergence of strains with genetic resistance to such material (18). CoNS, as one of the causative agents of nosocomial infections, can play an important role in creating infections in hospitalized patients through contamination of surfaces and medical equipment (19). In this study, the largest number of clinical samples with S. saprophyticus and S. epidermidis were obtained from clinical samples of women. This would explain the presence of some bacterial species of Staphylococcus in female reproductive system infections. Furthermore, most of such bacteria were isolated from urine samples. This reflects the role and presence of a wide range of CoNS in creating urinary tract infections, especially in women, as 13 isolates of S. epidermidis and 8 isolates of S. saprophyticus were isolated from urine samples from the gynecology ward. What makes the present study different from other Iranian studies is the examination of the presence of resistance genes, as well as evaluation of the sensitivity of resistant strains obtained from different clinical samples of CoNS in relation to QACs-containing biocides. The results of this study showed that of 448 analyzed clinical samples, 60 samples were S. epidermidis and 49 samples were S. saprophyticus. This shows the prevalence of S. epidermidis among CoNS which is consistent with Koksal et al. (2009) conducted in Turkey on different species of Staphylococcus and showed that among the coagulase-negative species of Staphylococcus genus, S. epidermidis and S. saprophyticus had the highest frequency and S. epidermidis had the highest frequency (20). Of the 60 isolates of S. epidermidis, 19 isolates had qacA/B gene, 21 had smr gene, and 36 had mecA gene. The results were similar to the results of a
survey by Skovgaard et al. in 2013 in Denmark. In this study, the presence or absence of a relationship between the genes responsible for resistance to biocides, and resistance to methicillin was assessed.Analyzing the data and P-values obtained from smr (0.0056), qacA/B (0.0019), and mecA (0.00136) genes, a significant relationship was found between the presence of genes responsible for resistance to detergents and the genes responsible for methicillin resistance. Most samples that were positive for the presence of mecA gene, were positive for the presence of qacA/B and smr genes, too. Moreover, Skovgaard et al. found that isolates positive for the presence of qacA/B and smr genes had the highest frequency in terms of resistance to penicillin, cefoxitin, and erythromycin (21). The highest frequency of mecA, qacA/B, and smr genes was in clinical samples obtained from urine and wounds, which was similar to a study by Mokhtarian et al. in Gonabad in 2014 in urine samples. They reported S. saprophyticus as the most important factor responsible for urinary tract infections in women. In addition, the highest and lowest resistance in this group pertained to penicillin and vancomycin, which is similar to the present study (22). Arabestani et al. (2014) in Hamadan showed that S. saprophyticus and S. epidermidis had the largest role in coagulase-negative infections in women, and saprophyticus had the largest role. Furthermore, the greatest resistance to antibiotics such as penicillin was observed in this group. That study did not find resistance to vancomycin in S. saprophyticus, which is similar to the present study (23). Most of the 49 S. saprophyticus isolates obtained from clinical samples were isolated from urine and catheter samples. In terms of distribution of genes, 27 isolates had mecA gene, 8 isolates had smr gene, and 11 isolates had qacA/B gene. These results were similar to Zmantar et al. (2011) in Tunisia, which reflected the high proportion of mecA gene and then qacA/B gene (24). The simultaneous presence of qac A/B genes indicates the relationship between these genes with the gene responsible for resistance to methicillin (mecA). Horner (2012) reported a relationship between genes responsible for resistance to some antibiotics and genes responsible for resistance to some biocides in a review study (25). Longtin et al. (Canada 2005), McGann et al. (US, 2003), Vali et al., Noguchi et al. (Japan 1992) reported no relationship between genes responsible for methicillin antibiotic resistance and genes responsible for resistance to biocides, which were not consistent with the present study. In the above studies, the frequency of smr gene was more than qacA/B, which is not consistent with the present study, where the S. saprophyticus bacteria had a higher frequency of qacA/B gene than smr gene (26-28). Zmantar et al., Leelaporn et al., and Sidhu et al. reported a higher frequency of qacA/B gene than smr gene in separate studies on genes responsible for resistance to methicillin and biocide. The results of this study on the S. epidermidis bacteria showed a lower frequency of qacA/B gene than smr gene (29, 30). Differences in the results of this study and other local and foreign studies could be due to different bacterial strains in different places. The high presence of this gene in clinical samples, particularly blood and urine samples indicates the emergence of infections that
are based on CoNS with both genes responsible for resistance to antibiotics and genes resistant to disinfectants.

**Conclusion**
The results of this study suggest the frequency of genes that have the potential for resistance to disinfectants. The present study, as one of the few studies in Iran examining the presence of qac A/B, and smr genes in CoNS, indicates the need for more attention and more extensive research on the type and amount of biocides used in biocide products. Furthermore, this study showed a significant relationship between resistance to QACs and resistance to methicillin. As a result, the presence of each of these resistance indicators can lead to the shared selection of these two antibiotics or the use of disinfectants containing quaternary ammonium compounds.

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**Conflict of interest**
The authors have no conflict of interest with regard to the compilation or publication of this study.

**References:**


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