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Molecular detection of the carriers of *Staphylococcus aureus* golden in referred to the Imam Ali Clinic in Shahrekord, Iran

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PEER REVIEW

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Comments

This study is beneficial in which the authors investigated the percentage of patients who were suspicious to the upper respiratory system infections by *S. aureus*. Molecular methods such as PCR can be a fine method to diagnose *S. aureus* the agent of this infection.
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ABSTRACT

Objective: To conduct for the molecular detection of *Staphylococcus aureus* (*S. aureus*) (Golden staph) carriers among patients referred to the Imam Ali Clinic, Shahrekord, Iran.

Methods: This sectional–descriptive study was conducted with 200 persons with suspected upper respiratory tract infections, who were referred to the Imam Ali Clinic in Shahrekord, Iran, in 2012. After culturing the nasal swab samples in mannitol salt agar and blood agar, *S. aureus* colonies were confirmed by biochemical methods. To determine the susceptibility of *S. aureus* strains isolated, molecular methods were used.

Results: Among the 200 investigated samples, 60 cases (30%), comprising 25 men (41.66%) and 35 women (58.33%), were found to be *S. aureus* carriers.

Conclusions: The results of the present study showed that the frequency of the *S. aureus* strain isolated from the nasal swabs of patients with respiratory tract infections admitted to the Imam Ali Clinic in Shahrekord, Iran, was remarkable. Thus, knowing detection of *S. aureus* carriers, who are at a risk of spreading nosocomial infection among the staff, is vital to control and prevent nosocomial infections.

KEYWORDS

Nasal carriers, Respiratory system infections, *Staphylococcus aureus*

1. Introduction

Staphylococcus aureus (*S. aureus*) (Golden staph), a Gram–positive coccus, is the most important pathogens of the Micrococcaceae family causing more than 80% of suppurative diseases, and is the second leading cause of nosocomial infections[1]. Furthermore, this pathogen is known to cause scalded skin syndrome, food poisoning, toxic shock syndrome, bacteremia, endocarditis and pneumonia[2]. Different strains of *S. aureus* and certain coagulase–negative staphylococci can cause nosocomial and community–acquired infections worldwide[3].

S. aureus is a part of the normal flora of the human body, and the anterior nasal region is the most common site of

colonization in humans. Approximately 5%–25% of healthy persons may be temporarily or permanently colonized with *S. aureus*. The location of colonization acts as a reservoir for future infections by *S. aureus*, and people who are colonized with *S. aureus* are at greater risk of infection compared with those who are not colonized[4]. Furthermore, *S. aureus* colonization is more common in patients who come into frequent contact with the bacterium, and whose skin's epithelial integrity is disrupted, such as medical staff, patients on hemodialysis, diabetes, injecting drug users, HIV–positive patients and patients with skin problems[4,5]. Nasal carriers of *S. aureus* are important agents of infection and in 80% of *S. aureus* bacteremia cases, isolated strains have been detected in the anterior nasal region of the

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patients[5].

On an average, 40% of the *S. aureus* strains are methicillin-resistant *S. aureus* (MRSA), and the number is still increasing[6]. Thus, the prevention and control of infections caused by this bacterium are important in reducing nosocomial infections, patient mortality, and health care costs[7]. Some important measures in this regard are to identify the sources of transmission of *Staphylococcus* infections in hospitals, and to detect the nasal carriers of this bacterium, particularly MRSA strains, in hospitals, (medical staff and patients), who are the main sources of nosocomial infections[6,8]. Therefore, to control nosocomial *Staphylococcus* infections, continuous studies on the vectors have been conducted. The purpose of the present study was to perform molecular detection of the carriers of *S. aureus* among patients referred to the Imam Ali Clinic in Shahrekord, Iran.

2. Materials and methods

2.1. Sampling and isolation of *S. aureus*

A total of 200 nasal swabs from patients with suspected upper respiratory tract infections (rhinitis, laryngitis, tracheitis, etc.) were obtained. The samples were taken by using a sterile swab from the anterior nasal region of the patients referred to the Imam Ali Clinic, Shahrekord, Iran, during the latter 6 months of 2012, and cultured for 24 h at 37 °C in peptone water (Merck, Germany). After the initial enrichment, the samples were cultured in solid blood agar (Merck, Germany) and mannitol salt agar (Himedia, India). Subsequently, biochemical tests such as Gram staining, catalase test, coagulase test, and deoxyribonuclease (DNase) test were performed on the suspected *Staphylococcus* colonies, and the bacteria were isolated and maintained in brain heart infusion broth (Merck, Germany) for subsequent studies[9–11].

2.2. Definitive diagnosis of *S. aureus* using polymerase chain reaction (PCR)

To confirm the presence of *S. aureus* in the cultured samples, PCR was employed. The genomic DNA of the bacteria grown in brain heart infusion broth was obtained by using the DNA extraction kit (Fermentas, Germany), and PCR was performed with the purified extracted DNA.

The presence of *S. aureus* in the samples was confirmed based on the detection of 16s-rDNA gene. The primer pairs (used are as follows: forward: GTA GGT GGC AAG CGT TAC C, and reverse: CGC ACA TCA GCG TCA C, product size:

228 bp). The PCR was carried out in reaction volume of 50 µL, containing 5 µL of PCR buffer (10×), 200 mmol/L dNTP (Cinnagene, Iran), 2 mmol/L MgCl₂, 1 µmol/L each forward and reverse primers, 1 IU DNA *Taq* polymerase enzyme (Cinnagene, Iran), and 5 µL of the DNA sample. The reaction was performed in a thermocycler Mastercycler gradient (Eppendorf, Germany) under the following conditions: initial denaturation at 94 °C for 6 min, 30 cycles of denaturation at 95 °C for 50 seconds, annealing at 61 °C for 70 seconds, extension at 72 °C for 60 seconds, and a final extension at 72 °C for 8 min.

The PCR products were subjected to electrophoresis on 1% agarose gel with ethidium bromide at a constant voltage of 90 V in the presence of 100 bp DNA marker (Fermentas, Germany). The results obtained were analyzed with a gel imaging device (England, Uviteck)[9].

2.3. Statistical analysis

The data were analyzed by using SPSS ver. 16.0 statistical software and a *Chi*-square test. The differences were considered significant if $P < 0.01$.

3. Results

Microbial examination of the 200 samples revealed that 60 cases (30%) had *S. aureus* infection, which was confirmed by PCR. The presence of 16S rDNA primers and observation of a 228 bp band indicated positive result (Figure 1). The 60 cases detected were carriers of *S. aureus*, and comprised 25 men (41.66%) and 35 women (58.33%). The mean age of the study population was 7.5 years.

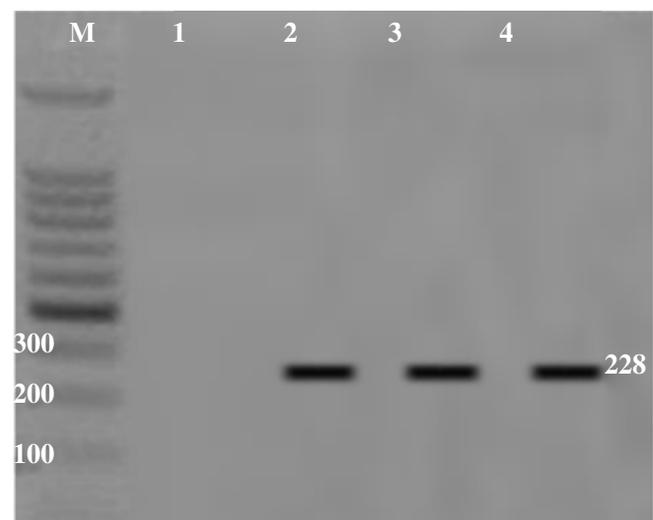


Figure 1. Agarose gel electrophoresis gene 16S rDNA (228 bp) of *S. aureus* isolated from nasal secretions. Column M: 100 bp DNA marker, Column 1: negative control, Columns 2–4: positive samples.

Statistical analysis of the results obtained revealed no significant difference between the sexes and presence of *S. aureus* as well as between infection type and presence of *S. aureus* ($P < 0.01$).

4. Discussion

In this study, among the 200 studied patients, 60 cases (30%) were nasal carriers of *S. aureus*.

In a previous study by Herwaldt, on the prevalence of *S. aureus* nasal carriers among medical staff and hospitalized patients and on the antibiotic resistance patterns of the strains isolated from the nasal and clinical samples in hospitals in Tabriz, 460 medical personnel and patients of different departments of the hospitals were tested for nasal carriage of *S. aureus*[6]. Their results indicated that among the 460 cases detected, 256 were women (55.6%) and 204 were men (44.4%). Furthermore, 160 (34.7%) cases were nasal carriers of *S. aureus*, comprising 92 (35.8%) female carriers and 68 (33.3%) male carriers. In addition, 32.7% of the medical staff and 36.5% of the hospitalized patients were found to be nasal carriers of *S. aureus*[6].

In another study, Askarian *et al.* investigated the prevalence of nasal carriage of MRSA and its antibiotic susceptibility pattern in healthcare workers at Namazi Hospital, Shiraz, Iran, carriers among 186 clinical staff. Of the 186 nasal carriers of *S. aureus*, 154 (82.8%) carried methicillin-sensitive *S. aureus* and 32 (17.2%) carried MRSA[11].

Rahbar *et al.* reported that *S. aureus* carriers of 40% clinical staff was calculated[12]. In another study conducted by Alghaity *et al.*[13], the rate of *S. aureus* carriers was 24.5% in the patients, while Kenner *et al.* reported a *S. aureus* carrier rate of 38%[14].

In addition, in a study carried out by Paul *et al.* in Nigeria, the rate of *S. aureus* carriers was reported to be 53.8%[15].

The obvious difference in the rate of *S. aureus* carriers in hospitals in different locations may be related to the disinfection of the work surfaces and hospital equipment, as well as the staff's attention to personal hygiene, including hand washing and use of masks and gloves in hospitals, particularly, in high-risk areas. Similarly, the observation of increased percentage of contamination in the division of infectious diseases in hospitals may also be associated with the above mentioned reasons. Thus, it can be concluded that isolation of such high-risk departments of hospitals increases could be a good choice.

Prevention of *S. aureus* infections is achieved by the

implementation of strict monitoring. In some countries such as New Zealand and Denmark, serious policy of isolation is common and has been effective in preventing the spread of MRSA. However, in the United States of America and England, the infection control procedures have been less successful. The existing infection control programs to prevent the transmission of MRSA have revealed that a more serious approach of infection control is needed, because nasal carriers of *S. aureus* increase the risk of subsequent infections[16]. Thus, molecular methods such as PCR appear to be necessary for the definitive diagnosis of bacteria, including *Staphylococcus* spp. The results obtained in the present study could contribute to the careful selection of appropriate antibiotics for the treatment and prevention of further spread of antibiotics resistance in bacteria.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

S. aureus is a part of the normal flora of the human body and anterior nasal is the most common site of colonization in humans. Approximately 5% to 25% of healthy persons may be temporarily or permanently colonized with *S. aureus*. Location of colonization acts as a reservoir for future infections by *S. aureus* and persons who are colonized with *S. aureus* than those who are not colonized are at greater risk of infection. Colonization in patients who have frequent contact with *Staphylococcus* and those who epithelial integrity of skin is disrupted, is more common. Thus, colonization rate among medical staff, patients on hemodialysis, diabetes, injecting drug users, HIV-positive patients and patients with skin problems is more common. Nasal carriers of *Staphylococcus* are important sources of infection and in 80% of cases of *S. aureus* bacteremia look for isolated strains of anterior nasal of patients.

Research frontiers

This study is performed in order to investigate patients who are suspicious to the upper respiratory system infections by *S. aureus*. Among 200 patients who are suspicious to this infection, 60 cases (30%) are positive for such infection by *S. aureus* (male 41.66% and female 58.33%).

Related reports

The data of this study is in agreement with Nikbakht *et al.*, Karamstaji *et al.* and Sadari *et al.* But there is some little differences which can be due to the disinfection of work surfaces and hospital equipment, attention to the principles of personal hygiene among staff, including hand washing and the use of masks and gloves in hospitals, particularly in high risk areas.

Innovations & breakthroughs

Molecular methods such as PCR seems to have been necessary for definitive diagnosis of bacteria, including *Staphylococcus* and results obtained in this study can be contributed to careful selection of appropriate antibiotics to treat and prevent the further spread of antibiotic resistance in bacteria.

Applications

The results of this study have showed frequency of *S. aureus* strain isolated from nasal of patients with respiratory system infections, so knowing carriers with respect to risk of resulted epidemy in nosocomial infection among staff, is vital to program, control and prevent from nosocomial infections.

Peer review

This study is beneficial in which the authors investigated the percentage of patients who were suspicious to the upper respiratory system infections by *S. aureus*. Molecular methods such as PCR can be a fine method to diagnose *S. aureus* the agent of this infection.

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