**Frequency of Staphylococci in the Nasal Cavities of Healthy Medical Workers**

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**Abstract**

Infective agents are abundant in hospitals, and so medical staff members are often exposed to them. Although previous studies have highlighted the role played by the nasal flora of medical staff in the development of nosocomial infections, few studies have specifically investigated this issue. Six volunteer medical staff members, who worked at Nihon University Hospital at Matsudo, participated in this study. Nasal samples were obtained from the medical staff, and then the samples were cultured and evaluated using routine bacteriological study methods. Staphylococci were detected in the nasal samples of all of the medical staff. *Staphylococcus epidermidis* was the predominant species in their nasal cavities (71.3%). None of the medical staff had been infected with methicillin-resistant *Staphylococcus aureus* (MRSA), but four of six staff members possessed methicillin-resistant coagulase-negative staphylococci (MR-CNS).

Medical staff members are both at risk of infection and also a potential source of nosocomial pathogens such as methicillin-resistant staphylococci. As a preventive measure against nosocomial infection, it might be necessary to continuously investigate the frequency of methicillin-resistant staphylococci in the nasal cavities of medical staff.

**Introduction**

Staphylococci inhabit human skin, as well as the nasal cavities and oral mucosa(1). *Staphylococcus aureus* (*S. aureus*) is a common cause of invasive and life-threatening infections, but coagulase-negative staphylococci (CNS) mainly cause nosocomial infections, such as catheter-related bloodstream infections, prosthetic valve endocarditis, central nervous system shunt infections, and prosthetic joint infections(2–4). Since the 1980s, methicillin-resistant CNS (MR-CNS) and methicillin-resistant *S. aureus* (MRSA) have been attracting considerable attention(5). Methicillin resistance in staphylococci is caused by the expression of the penicillin-binding protein PBP2', which is encoded by the *mecA* gene. MR-CNS, as well as MRSA, possess *mecA* genes and induce severe infections in compromised hosts. MRSA is uncommon in healthy people and is considered to be an abnormal member of the bacterial flora found in humans, as it is predominantly carried by individuals with underlying diseases(6). MRSA mostly spreads from patient to patient via the colonized hands and noses of medical staff during patient contact or after the handling of contaminated materials(7).

Fatal nosocomial infections have aroused interest in studies of the opportunist microorganisms found in the flora of patients with diseases involving chronic progression, such as immune depression, neutropenia, and diabetes mellitus. Nosocomial infections can result in prolonged hospitalization, which in turn leads to financial loss, or even death(8).

The role of the hands and nasal flora of medical staff in the development of nosocomial infections has been emphasized by many previous studies. Staphylococci are common residents of the nasal cavity, which acts as a natural incubator(9–11). In addition, while people often wash their hands, the nose is rarely subject to meticulous hygiene measures, and thus, the nasal flora plays an important role in contamination during nosocomial infections. It is important to confirm the frequency of staphylococci, especially MRSA.
and MR-CNS strains, in the nasal cavities of hospital workers in order to develop preventive measures against nosocomial infection.

The purpose of this study was to investigate the frequencies of staphylococci, especially MRSA and MRCNS, in the nasal cavities of healthy medical staff working at Nihon University Hospital at Matsudo.

Materials and Methods

Subjects and clinical samples

This study’s protocol was approved by the ethics committee of Nihon University School of Dentistry at Matsudo, Japan (EC-2-014). Six medical workers, who worked at Nihon University Hospital at Matsudo, participated in this study. All of them fulfilled the following inclusion criteria: 1) being systemically healthy, 2) not having received antibiotic treatment in the last six months, and 3) not having been hospitalized. These criteria aimed to ensure that any nasal carriage was associated with the hospital environment. Nasal swabs were taken from both nostrils using sterile cotton swabs and placed in sterile vials containing 0.5 ml of 0.05 M Tris-HCl buffer (pH 7.2). The nasal samples were dispersed by ultrasonication for 30 s in an ice bath (50 W, 20 kHz, Astrason System model XL 2020, NY, USA). One hundred μl of appropriately diluted samples were then plated on mannitol salt agar (MSA, Nissui, Tokyo, Japan) to isolate staphylococci, and brain heart infusion (BHI, Difco Laboratories, Mich., USA) supplemented with 1% yeast extract and 1.5% agar (BHI-Y agar plate) to confirm the total number of bacteria. All plates were incubated at 35 °C for 48 hours in an aerobic environment, and the number of colony-forming units (CFU) was calculated. All experimental samples containing more than 1 × 10³ CFU/ml on BHI-Y agar plates were accepted for clinical analysis.

Identification of staphylococci in the clinical samples

More than 30 colonies on MSA were subcultured from each subject on BHI-Y agar plates. Species identification was performed by Gram-staining and PCR analysis or with the ID 32 Staph system (bioMérieux, Marcy, France). The following Staphylococcus species were identified by PCR analysis using species-specific primers (12, 13): S. aureus, S. capitis, S. caprae, S. epidermidis, S. haemolyticus, S. hominis, S. lugdunensis, S. saprophyticus, S. warneri, and S. xylosus. All other Staphylococcus species were identified using the ID 32 Staph system. All of the Staphylococcus species except S. aureus and S. intermedius were classified as CNS.

PCR analyses for species identification of staphylococci

The subcultured isolates were suspended to a 1.0 McFarland standard in 100 μl of distilled water, and 5 μl of the suspension were used as a template for the PCR. The PCR running conditions and species-specific primers for staphylococci used in this study were as described previously (12, 13). Briefly, the PCR mixture contained 0.2 μM of each primer, 10 μl of 2 × Mighty Amp buffer Ver.2, 0.4 μl of Mighty Amp DNA polymerase (Takara Bio Inc., Shiga, Japan), and 5 μl of the template in a final volume of 20 μl. The PCR reactions were carried out in a DNA thermal cycler (Applied Biosystems 2720 Thermal Cycler; Applied Biosystems, CA, USA). The PCR running conditions included an initial denaturation step at 98 °C for 2 min followed by 36 cycles consisting of 98 °C for 30 s, 58 °C for 30 s, and 72 °C for 70 s, and a final extension step of 72 °C for 2 min. The PCR products were analyzed by 2.0% agarose gel electrophoresis and visualized by electrophoresis in 1 × Tris-borate-EDTA on a 2% agarose gel stained with ethidium bromide. A 100 bp DNA ladder (Takara Biomed, Shiga, Japan) was used as a molecular size marker.

Detection of the mecA gene and anti-microbial susceptibility testing

The detection of the mecA gene in the isolates was performed as described previously (14). The PCR procedure was as described above. The PCR running conditions included an initial denaturation step at 98 °C for 2 min followed by 38 cycles consisting of 98 °C for 10 sec, 60 °C for 15 sec, and 68 °C for 1 min. Anti-microbial susceptibility testing of the isolates was performed using the standardized disk diffusion method (Kirby-Bauer) with Mueller-Hinton agar (bioMerieux) (15). The susceptibility of all of the isolates to oxacillin was examined using agar disk diffusion according to the Clinical and Laboratory Standards Institute (CLSI) guidelines in order to detect methicillin-resistant strains (16). In this study, when mecA gene positivity was detected by PCR analysis and resistance to oxacillin was observed during susceptibility testing, the isolates were identified as methicillin-resistant strains. All of the methicillin-resistant strains, except S. aureus and S. intermedius, were classified as MR-CNS.
Results

Frequency of staphylococci in the nasal cavity

The numbers of total bacteria and staphylococci, and the proportion of bacteria in the nasal cavity that were staphylococci are shown in Table 1. The mean number of total cultivable bacteria was $1.69 \times 10^5$ CFU (range: $3.30 \times 10^3 - 5.90 \times 10^5$). The mean number of total cultivable staphylococci was $5.20 \times 10^4$ CFU (range: $1.17 \times 10^3 - 1.34 \times 10^5$). Staphylococci accounted for 30.8% of all bacteria and were detected in the nasal cavities of all six subjects.

Distribution of Staphylococcus species in the nasal cavity

Table 2 shows the distribution of Staphylococcus species in the nasal cavity. A total of five different Staphylococcus species were identified in the nasal swab specimens. S. epidermidis (71.3%) was the species that was most frequently isolated from the nasal cavity, followed by S. aureus (23.1%). Four of the six subjects harbored more than two Staphylococcus species, and S. aureus was isolated from two of these subjects. S. epidermidis was isolated from five subjects. S. capitis (2.3%), S. warneri (1.9%), and S. lugdunensis (1.4%) were rarely isolated. In fact, S. warneri and S. lugdunensis were only isolated from one subject (the same individual). S. capitis was isolated from two subjects.

Frequencies of methicillin-resistant staphylococci in the nasal cavity

Table 3 shows the frequencies of methicillin-resistant staphylococci in the nasal cavity. In this study, MRSA was not detected in the nasal cavity of any subject. In contrast, MR-CNS was isolated from four subjects. All of the MR-CNS strains were classified as methicillin-resistant S. epidermidis (MRSE). MR-CNS; i.e., MRSE accounted for 2.3% of total staphylococci isolates.

Discussion

Our current knowledge of the role of staphylococci in the ecology of the oral flora in health and disease is incomplete. Infective agents are abundant in hospitals; therefore, medical staff, patients’ relatives and friends, and medical students are often exposed to them. Coming into contact with such individuals can result in serious infections and spreads contamination among patients and medical staff. In fact, the main source of staphylococci in humans is human contact, and the most common method of contagion in staphylococcus infections and opportunist enteritis in hospitals is via the hands of hospital staff. Thus, hospital staff members that have been colonized by MRSA and MR-CNS constitute an important source of contamination (8, 15).

A previous study by Kloos and Bannerman (16) reported that the populations of staphylococci living in moist habitats, such as the anterior nares, axillae, and inguinal and perineal areas, reached densities of $10^3$ to $10^6$ CFU/cm². The mean number of total cultivable staphylococci detected in this study ($5.20 \times 10^4$ CFU) was similar to that found in previous studies.

Becker et al. (17) reported that the nasal cavities of 92.8% of their subjects had been colonized by at least one Staphylococcus species. In this study, staphylococci accounted for 30.77% of all bacteria and were isolated from the nasal cavities of all six subjects. These results demonstrated that staphylococci are common components of the nasal flora (5, 6, 7).

Currently, there are 35 recognized species in the genus Staphylococcus (16). The following 15 Staphylococcus species are found in humans (human-associated staphylococci): S. aureus, S. auricularis, S. capitis, S. caprae, S. cohnii, S. epidermidis, S. haemolyticus, S. hominis, S. lugdunensis, S. saccharolyticus, S. saprophyticus, S. schleiferi, S. simulans, S. warneri, and S. xylosus (18). Some of them are also indigenous to other mammals. In this study, 5 of 15 human-associated staphylococci were isolated from the subjects’ nasal cavities. S. epidermidis, which accounted for 71.3% of all staphylococcus isolates, was the species that was most frequently isolated from the subjects’ nasal cavities. S. epidermidis was isolated from five subjects. S. aureus accounted for 23.1% of all staphylococcus isolates, and was

<table>
<thead>
<tr>
<th>Subject</th>
<th>Total bacteria</th>
<th>Staphylococci</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>$1.49 \times 10^5$</td>
<td>$1.34 \times 10^5$</td>
</tr>
<tr>
<td>B</td>
<td>$3.30 \times 10^4$</td>
<td>$3.10 \times 10^4$</td>
</tr>
<tr>
<td>C</td>
<td>$1.41 \times 10^5$</td>
<td>$1.17 \times 10^3$</td>
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<tr>
<td>D</td>
<td>$1.26 \times 10^5$</td>
<td>$7.20 \times 10^4$</td>
</tr>
<tr>
<td>E</td>
<td>$6.20 \times 10^4$</td>
<td>$3.70 \times 10^3$</td>
</tr>
<tr>
<td>F</td>
<td>$5.90 \times 10^5$</td>
<td>$9.80 \times 10^4$</td>
</tr>
<tr>
<td>Mean</td>
<td>$1.69 \times 10^5$</td>
<td>$5.20 \times 10^4$</td>
</tr>
<tr>
<td>Staphylococci / Total bacteria (%)</td>
<td>30.8%</td>
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</tbody>
</table>
isolated from two subjects. *S. warneri* and *S. lugdunensis* were only isolated from one subject (the same individual), whereas *S. capitis* was isolated from two subjects. These results were similar to those obtained in previous studies (17, 19) and indicated that *S. epidermidis* is the most predominant *Staphylococcus* species in the nasal cavity. Kloos and Bannerman (16) reported that some *staphylococcus* species demonstrate a marked preference for certain habitats. For example, *S. capitis* prefers the human head and produces very large populations on the scalp following puberty (16). *S. warneri* and *S. lugdunensis* are widely distributed over the body, although their population sizes are usually quite small (16, 20, 21). Similarly, our study demonstrated that *S. capitis*, *S. warneri*, and *S. lugdunensis* are not predominant *Staphylococcus* species in the nasal flora. Although more than 30 isolates were identified in this study, the nasal cavities of each subject were colonized by one to three *staphylococcus* species. Therefore, it was suggested that 10 isolates per subject might be sufficient for studies of the distribution of *staphylococcus* in the nasal cavity.

In this study, MRSA colonization was not detected in any subject, but methicillin-sensitive *S. aureus* (MSSA) was detected in two subjects (33.3%). Kirig et al. (22) reported rates of MRSA and MSSA colonization of 21% and 79%, respectively, among medical workers. Likewise, Mert et al. (23) reported rates of MRSA and MSSA colonization of 9% and 91%, respectively, among medical staff. In a previous study by Becker (17), MR-CNS was detected in 41.8% of hospital patients. In this study, the isolation frequency of MR-CNS, which was detected in four subjects (66.7%), was similar to those observed in previous studies, and all of them were classified as MRSE. Pfaller et al. (24) previously stated that *S. epidermidis* has become resistant to many commonly used antibiotics and might be a reservoir for antibiotic resistance genes in hospitals. In order to prevent an outbreak of nosocomial infection, it is essential to continuously monitor the frequency of *staphylococcus*, and especially MRSA and MR-CNS, in the nasal cavities of hospital medical staff.

Nasal *S. aureus* carriage rates are higher in medical staff, and methicillin resistance shows a tendency to increase with increasing clinical exposure (25). Medical staff members are at risk of infection to nosocomial pathogens such as *S. aureus* and methicillin-resistant *staphylococcus*. Therefore, medical staff should take special care to take hygiene measures such as frequent and thorough hand washing and the wearing of face masks where necessary whilst working in hospitals.

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References