Significance of Airborne Transmission of Methicillin-Resistant *Staphylococcus aureus* in an Otolaryngology–Head and Neck Surgery Unit

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**Objectives:** To quantitatively investigate the existence of airborne methicillin-resistant *Staphylococcus aureus* (MRSA) in a hospital environment and to perform phenotyping and genotyping of MRSA isolates to study MRSA epidemiology.

**Design:** Prospective surveillance of patients with MRSA infections or colonizations was performed, as was an observational study of environmental air. Airborne samples were taken by an air sampler; samples were obtained from object surfaces by stamping or swabbing. Epidemiological study of MRSA isolates was performed with an antibiotic susceptibility test, coagulase typing, and pulsed-field gel electrophoresis.

**Setting:** Three single-patient rooms in a 37-bed otolaryngology–head and neck surgery unit.

**Patients:** Three patients with squamous cell head and neck cancer were observed to have been colonized or infected with MRSA after surgery.

**Results:** The MRSA samples were collected from the air in single-patient rooms during both a period of rest and when bedsheets were being changed. Isolates of MRSA were detected in all stages (from stage 1 [>7 µm] to stage 6 [0.65-1.1 µm]). About 20% of the MRSA particles were within a respirable range of less than 4 µm. Methicillin-resistant *S aureus* was also isolated from inanimate environments, such as sinks, floors, and bedsheets, in the rooms of the patients with MRSA infections as well as from the patients’ hands. An epidemiological study demonstrated that clinical isolates of MRSA in our ward were of one origin and that the isolates from the air and from inanimate environments were identical to the MRSA strains that caused infection or colonization in the inpatients.

**Conclusions:** Methicillin-resistant *S aureus* was recirculated among the patients, the air, and the inanimate environments, especially when there was movement in the rooms. Airborne MRSA may play a role in MRSA colonization in the nasal cavity or in respiratory tract MRSA infections. Measures should be taken to prevent the spread of airborne MRSA to control nosocomial MRSA infection in hospitals.


*Staphylococcus aureus* is a common pathogen observed in the head and neck region. Antibiotic treatment of most infections in the head and neck region must take into account the prevalence of this organism because *S aureus* is also one of the most common causes of nosocomial infections. During the past 20 years, methicillin-resistant *S aureus* (MRSA) has become an important source of such infections; this pathogen is presently responsible for up to 61% of *Staphylococcus* infections.1 Many patients in the otolaryngology unit have chronic or recurrent infections, such as chronic otitis media or sinusitis. Such patients may be prone to an increased rate of MRSA infections as the result of repeated antibiotic therapy.1 Furthermore, patients who have undergone head and/or neck operations, such as middle ear surgery, usually have long-term packing in their auditory canals or mastoid cavities; the packed gauzes are kept wet by transudates or exudates,2 and these materials are susceptible to contamination by pathogenic bacteria such as MRSA. Therefore, otolaryngology–head and neck surgery units are especially vulnerable to the spread of MRSA infections.3

The principal mode of MRSA transmission within an institution is from patient to patient via transiently colonized hands of hospital personnel who acquire the organism after direct patient contact or after handling contaminated materials.4 Since MRSA isolates have been recovered from many sites, including floors, linens, medical equipment, and hospital furnishings, transmission via inanimate environments may also pose significant risk to the patient.5 In addition, it is thought that MRSA in the form of a bioaerosol can contaminate the air and cause airborne infectious diseases.6 Although airborne transmission of MRSA is generally considered...
PATIENTS AND METHODS

PATIENTS

Characterization of 3 inpatients who participated in this study is given in Table 1. The 3 inpatients who had squamous cell head and neck cancers had been colonized or infected with MRSA during their stay in the hospital. The number of MRSA isolates in the clinical samples was more than 10^5 colony-forming units (CFU) per specimen; no change in this number was observed during the course of the present study.

AIR SAMPLING OF BACTERIA

Sampling sites and methods used in this study are summarized in Figure 1. In 37 beds at an otolaryngology–head and neck surgery unit at a tertiary care medical university hospital, a 6-stage Andersen air sampler (Nihon Kagaku Kogyo Co, Ltd, Osaka, Japan) was used to collect air samples at a rate of 28.3 L/min for 30 min/d (total, 849 L) from the closed, single-patient rooms of the 3 participating inpatients. Each of the samples was collected on both Trypto-soya agar (Nissui Pharmaceutical Co, Ltd, Tokyo, Japan) and MSO agar (salt egg-yolk agar containing 6 mg/L of oxacillin; Nissui Pharmaceutical Co, Ltd). Trypto-soya and MSO agar plates were used to isolate general bacteria and MRSA, respectively. The sampler was placed on a rack 1 m above the floor at a distance of 1 m from the bed. Air sampling was carried out weekly for a month (for a total of 3 sampling times) in the morning, when the patients were at rest and when the bedsheets were changed. After the samples had been collected, the culture media were incubated at 37°C for 48 hours. After incubation, the colonies on the agar plates were counted and the results were expressed as CFU per cubic meter (CFU/m^3) of air. Gram staining of the isolates on Trypto-soya agar was performed. The species of the isolates were identified by the methods described below.

SAMPLING OF INANIMATE ENVIRONMENTS

Samples were collected from 3 sites (bedsheets, floors, and sinks) in areas of 10 cm^2 in the rooms of each of the 3 inpatients (Figure 1). Trypto-soya and MSO agar plates were used and the stamp method was chosen to perform sampling. In this method, agar stamps are rotated several times on the surfaces of the environments. Sampling was carried out 3 times in duplicate at the same time that the air was sampled from the rooms. Agar plates were incubated at 37°C for 48 hours. After the incubation, the number of CFU were counted.

ISOLATION OF BACTERIA FROM THE HANDS AND NASAL CAVITIES OF THE PATIENTS

At the same time, air samples and samples from the nasal cavities of the patients were taken; the latter sampling was performed with sterile cotton swabs moistened with sterile phosphate-buffered saline. The swabs were inoculated on MSO agar and incubated at 37°C for 48 hours. Samples were taken from the hands of the patients via the stamp method with MSO agar plates.

IDENTIFICATION OF MRSA

The MRSA isolates were identified 48 hours after the start of incubation at 37°C on MSO agar plates. The mecA gene was detected by polymerase chain reaction and primers as previously described. Fifty nanograms of bacterial DNA was used as the template DNA. DNA amplification was carried out for 40 cycles in 50 µL of reaction mixture as follows: denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 5 minutes. Ten microliters of the polymerase chain reaction products was analyzed by 2% agarose gel electrophoresis. The presence of a 533–base pair amplier was taken as an indication of the presence of the mecA gene.

PHENOTYPING OF MRSA

Antibiotic susceptibility was determined by the microdilution broth method, in accordance with the National Committee for Clinical Laboratory Standards guidelines. The antibiotics used in the test were ampicillin, piperacillin sodium, oxacillin sodium, cefaclor, imipenem, cefazolin sodium, flomoxef, cefotiam hydrochloride, gentamicin sulfate, arbekacin, minocycline hydrochloride, ofloxacin, erythromycin lactobionate, clindamycin phosphate, vancomycin hydrochloride, and fosfomycin. Coagulase types were also determined by using coagulase antiserum (Denka Seiken, Co, Ltd, Tokyo).

GENOTYPING OF MRSA

Genomic DNA analysis was done with pulsed-field gel electrophoresis (PFGE). Pulsed-field gel electrophoresis was performed by the procedure described by Struelens et al with some modifications. Cells were treated using Gene Path Group 1 Reagent Kits (Nippon BIO-RAD Laboratories, Tokyo) and digested with Smal (Takara Shuzo Ltd, Shiga, Japan). Electrophoresis was performed on a 1% agarose gel (Nippon BIO-RAD Laboratories) in a CHEF MAPPER system (Nippon BIO-RAD Laboratories) at 4°C for 22 hours in 0.5 X Tris-borate-EDTA buffer at 170 V; initial and final pulse times were 5 and 80 seconds, respectively. The gels were stained with ethidium bromide, visualized in a transilluminator, and photographed with Polaroid film (type 665) in a Polaroid Land camera (Nippon Polaroid, Tokyo).
RESULTS

SAMPLING OF AIRBORNE BACTERIA DURING THE RESTING PERIOD

Bioaerosol particles were separately collected in a 6-stage Andersen air sampler according to their aerodynamic diameters (from ≥7 to 0.65 μm) (Table 2). The mean ± SD total CFU/m³ of air on Trypto-soya agar were 130.4 ± 20.2, 117.0 ± 10.3, and 95.8 ± 10.6, respectively, in each of the single rooms of each of the 3 MRSA-infected patients during the resting period (Table 2). The main particles were collected between stage 4 and stage 5 of the air sampler. From 48.1% to 76.1% of the bioaerosol particles were within a respirable range of less than 4 μm (stages 4, 5, and 6 in Table 3). Methicillin-resistant Staphylococcus aureus was detected in a few CFU/m³ of the air samples during the resting period (Table 2).

SAMPLING OF AIRBORNE BACTERIA WHEN BEDSHEETS WERE CHANGED

Next, we collected bioaerosols from the single rooms of each of the 3 MRSA-infected patients when the bedsheets were changed; samples were collected with an Andersen air sampler. As shown in Table 2, the mean ± SD total CFU/m³ of air on MSO agar was 62.4 ± 8.4, 73.4 ± 14.1, and 58.5 ± 6.2. During this test period, there were approximately 50 times the number of CFU as during the resting period. Methicillin-resistant S aureus was isolated in each stage on MSO agar, and the aerodynamic diameters of the MRSA isolates were mainly more than 5 μm (Table 3). About 20% of the MRSA particles were within a respirable range of less than 4 μm (Table 3).

SAMPLING OF BACTERIA ON INANIMATE OBJECTS

As shown in Table 2, the mean ± SD total CFU/10 cm² on the bedsheets from each of the 3 patients' rooms, as observed on MSO agar, were 3.0 ± 0.6, 3.2 ± 1.1, and 3.3 ± 3.1. Methicillin-resistant S aureus was also isolated from the floors and the sinks. The CFU of all the environmental samples incubated on Trypto-soya agars exceeded the upper detection limit of the 1 × 10⁵ CFU/plate (data not shown).

SAMPLING OF MRSA ON HANDS AND IN THE NASAL CAVITIES OF INPATIENTS

Methicillin-resistant S aureus was detected on the hands and in the nasal cavities of all of the patients. The mean ± SD total CFU/10 cm² on the hands and nasal cavities of all of the patients, as observed on MSO agar, were 4.0 ± 3.1, 3.7 ± 2.4, and 8.3 ± 4.1 (Table 4).

ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF MRSA

One clinical isolate from patient 1, 5 of all isolates from the air, 5 of all isolates from the inanimate environments (floor, bedsheets, and sink) in the room of patient 1, and 1 of all of the isolates from the hands and nasal cavity of patient 1 were tested for antibiotic susceptibility. As shown in Table 5, the environmental isolates had the same susceptibility patterns as the isolates taken from the room of patient 1. Furthermore, the antibiotic susceptibilities of the 3 clinical isolates from each

Table 1. Characteristics of Patients With Methicillin-Resistant Staphylococcus aureus (MRSA) Infection

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Tumor Location</th>
<th>Treatment</th>
<th>Source of MRSA</th>
<th>Nasal Carriage of MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/82</td>
<td>SCC, larynx</td>
<td>S, CH, RT</td>
<td>Wound, sputum</td>
<td>Positive</td>
</tr>
<tr>
<td>2/M/71</td>
<td>SCC, hypopharynx</td>
<td>S, CH, RT</td>
<td>Wound, sputum</td>
<td>Positive</td>
</tr>
<tr>
<td>3/M/77</td>
<td>SCC, hypopharynx</td>
<td>S, CH, RT</td>
<td>Sputum</td>
<td>Positive</td>
</tr>
</tbody>
</table>

*SCC indicates squamous cell carcinoma; S, surgery; CH, chemotherapy; and RT, radiotherapy.

Table 2. Air Contamination With General Bacteria and Methicillin-Resistant Staphylococcus aureus (MRSA) in the Single Rooms of Inpatients With MRSA Infections During the Period of Rest

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>General Bacteria Level of Contamination, CFU/m³</th>
<th>MRSA Level of Contamination, CFU/m³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>1</td>
<td>130.4 ± 20.2</td>
<td>62.4 ± 8.4</td>
</tr>
<tr>
<td>2</td>
<td>117.0 ± 10.3</td>
<td>73.4 ± 14.1</td>
</tr>
<tr>
<td>3</td>
<td>95.8 ± 10.6</td>
<td>58.5 ± 6.2</td>
</tr>
</tbody>
</table>

*Data are given as mean ± SD. CFU indicates colony-forming units.

Figure 1. Sampling sites and methods for collection of bacteria.
patient had the same pattern (data not shown). Most of the trial antibiotics were not effective against MRSA. Infection or colonization with MRSA was often resistant to minocycline treatment; however, all MRSA infections were sensitive to arbekacin and vancomycin therapy. All isolates had type II coagulases.

**GENOMIC DNA ANALYSIS OF THE MRSA BY PFGE**

As shown in Figure 2, the environmental isolates, the isolates from the inpatient, and the 3 clinical isolates exhibited identical restriction fragment patterns after PFGE of Smal-digested genomic DNA. This result was consistent with findings from the antibiotic susceptibility assay and coagulate typing.

**COMMENT**

In this study, we demonstrated that MRSA was recirculated among the patients, the air, and the inanimate environments, especially when there was movement in the rooms. This suggests that airborne MRSA may play a role in MRSA colonization in the nasal cavity or in respiratory MRSA infections.

A standard 6-stage Andersen cascade sampler was used for collecting MRSA isolates, which were separated according to aerodynamic dimensions from the air in the rooms of MRSA-infected or colonized inpatients. The sampler is widely used in aerobiological studies. As a rule, 6-stage cascade Andersen samplers allow for precise microbiological evaluation as well as for the separation of the organisms according to particle size. Larger microorganism-carrying particles, in the range of 4 to 8 \( \mu m \), are separated in the first 3 stages, whereas smaller particles (<4 \( \mu m \)) are separated in the 3 lower stages. This allows for the detection and differentiation of respirable (stages 4-6) and non-respirable (stages 1-3) particle-adsorbed microorganisms present in the ambient air. The particles of stages 5 and 6 reach the alveoli. Particles smaller than 5 \( \mu m \) may exist in the air for a long time and can reach the lungs and cause infection in susceptible tissues if inhaled. As shown in Figure 1 and Table 3, MRSA was detected both during the rest period and when bedsheets were being changed; the latter period was particularly of interest, as MRSA of all stages was observed in that period. This finding suggests that MRSA is able to colonize in the nasal cavity or even reach the lungs. Thus, it is crucial to design an efficient control system to limit the accumulation of bacterial cells in environments in which recirculation of air is performed.

The number of CFU of MRSA from air sampling during the changing of bedsheets in MRSA-infected patient rooms was higher than that observed during the resting period. When medical staff were present in the rooms of patients, the number of CFU of MRSA increased in and around the rooms, indicating that MRSA on surface environments spreads during periods of movement, such as when bedsheets are changed in hospitals. In such cases, there is also the potential danger of medical staff acquiring the epidemic strain from a patient by direct contact and then further risk of transmitting it to other patients. Moreover, MRSA may be transferred from one patient to another by airborne transmission and by direct hand-to-hand contact. Therefore, to prevent the spread of MRSA, it is recommended that gloves be worn routinely by all personnel entering the rooms of patients with MRSA. More careful disinfection of inanimate hospital environments is also required for the prevention of airborne transmission of MRSA. Such disinfection procedures might promote a decline in the nosocomial MRSA infection rate.

Antibiotic effectiveness against MRSA infection was low. However, a few antibiotics, (minocycline, arbekacin, and vancomycin) were still effective. All MRSA isolates identified in the ward had one origin, as determined by the antibiotic pattern. Occurrence of MRSA in the ward was effected by spreading of a clone. These results indicate that MRSA isolates from the patients, the air, and the inanimate objects might share a common origin. The classification of genomic DNA fingerprints by PFGE is proposed as a useful and effective means for the purpose of epidemiological studies of nosocomial infection of MRSA. Therefore, we confirmed by PFGE that isolates from the patients, the air, and the inanimate environments had a common origin (Figure 2). The present findings suggest that MRSA was recirculated among the patients, the air, and the inanimate objects in the rooms; transmission was especially likely when there was movement in the rooms.

In this study, we confirmed that MRSA could be acquired by medical staff and patients through airborne

### Table 3. Percentage of General Bacteria-Carrying Particles During the Rest Period and Bedsheet Changing, by Aerodynamic Size*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Stage 1, 7 µm</th>
<th>Stage 2, 4.7-7 µm</th>
<th>Stage 3, 3.3-4.7 µm</th>
<th>Stage 4, 2.1-3.3 µm</th>
<th>Stage 5, 1.1-2.1 µm</th>
<th>Stage 6, 0.65-1.1 µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.3 (40.9)</td>
<td>7.1 (31.3)</td>
<td>9.5 (13.1)</td>
<td>29.2 (5.2)</td>
<td>38.5 (5.0)</td>
<td>8.4 (4.5)</td>
</tr>
<tr>
<td>2</td>
<td>11.7 (38.1)</td>
<td>14.3 (30.1)</td>
<td>14.0 (10.2)</td>
<td>25.2 (12.9)</td>
<td>29.0 (6.1)</td>
<td>5.8 (2.6)</td>
</tr>
<tr>
<td>3</td>
<td>15.7 (40.1)</td>
<td>28.6 (24.9)</td>
<td>7.4 (12.0)</td>
<td>20.9 (14.2)</td>
<td>11.0 (3.9)</td>
<td>16.2 (3.9)</td>
</tr>
</tbody>
</table>

* Data in parentheses are percentages during the changing of bedsheets.

### Table 4. Methicillin-Resistant *Staphylococcus aureus* (MRSA) Environmental Contamination in the Single Rooms of Inpatients With MRSA Infection*

<table>
<thead>
<tr>
<th>Level of MRSA Contamination, CFU/10 cm²</th>
<th>Patient No.</th>
<th>Floor</th>
<th>Bedsheet</th>
<th>Sink</th>
<th>Patient’s Hands</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>5.7 ± 2.4</td>
<td>3.0 ± 0.6</td>
<td>1.3 ± 1.1</td>
<td>4.0 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12.0 ± 2.2</td>
<td>3.2 ± 1.1</td>
<td>0.8 ± 0.7</td>
<td>3.7 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.8 ± 0.7</td>
<td>3.3 ± 3.1</td>
<td>0.5 ± 0.5</td>
<td>8.3 ± 4.1</td>
</tr>
</tbody>
</table>

* Data are given as mean ± SD. CFU indicates colony-forming units.
transmission. The findings suggest the importance of protecting patients against cross-infectious agents existing in aerosols. Although measures for prevention and control of nosocomial infection with MRSA include handwashing with an antimicrobial agent; wearing a gown, gloves, and a mask; and removing MRSA from the nasal vestibule, few measures have been established to control airborne bacteria. Laminar unidirectional airflow, air ventilation, and air filtration could also be beneficial in hospital environments and should be considered. Further studies will be needed to assess the levels of MRSA contamination of air and to develop more effective means of controlling and removing airborne MRSA.

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