Environmental survey of Methicillin-Resistant Staphylococci in a Hospital in Japan

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We examined the hospital-wide incidence of methicillin-resistant *Staphylococcus* contamination in a hospital environment to predict the risk of the nosocomial spread of infection. Samples were also taken from different surfaces and medical equipment in a general hospital ward and a staff station. The isolates were identified as bacterial strains and analyzed by PCR for detection of the *mecA* gene and staphylococcal cassette chromosome *mec* (SCCmec) types (I–V).

Overall, out of 146 isolates that were screened, 15.7% of the samples in the hospital wards were contaminated with *Staphylococcus aureus* and 74.7% were isolated with coagulase-negative *Staphylococcus* (CNS). The methicillin-resistant *mecA* gene was detected in all oxacillin-resistant *S. aureus*, and 89% of oxacillin-resistant CNS was identified as methicillin-resistant *S. aureus* (MRSA) and MRCNS respectively. All *S. aureus* and CNS from the hospital wards with MRSA patients were detected as MRSA and MRCNS. A widespread distribution of MRSA and MRCNS was detected in the Cuff. The majority of the MRSA and MRCNS isolates in this study were SCCmec type V, which are a community-acquired infection type.

The increased incidence and prevalence of community-acquired MRSA and MRCNS, as well as hospital-acquired MRSA, should be recognized as serious healthcare problems.

Key words : MRSA / MRCNS / SCCmec / infection control.

INTRODUCTION

Human microflora naturally contains *Staphylococcus* species. These bacteria spread and contaminate hospital environments. Methicillin-resistant *Staphylococcus aureus* (MRSA) is resistant to most antibiotics, not only methicillin, causing severe symptoms in immunocompromised patients. MRSA is one of the most common causative agents of opportunistic infections and hospital infections. Recently, methicillin-resistant coagulase-negative *Staphylococcus* (MRCNS) has become one of the most notable microorganisms causing infections in hospitalized patients.

The route of bacterial transmission is thought to be primarily via contact transmission between patients and healthcare professionals and the hospital environment. In particular, the *Staphylococcus* genus is a resident bacterium of the skin and mucous membranes and is often spread through medical equipment and devices in hospital environments (Perdreau-Remington et al., 1995; Uwagawa et al., 1999). Therefore, the medical environment is an important source of bacterial...
transmission. However, it is unclear what places or equipment in the hospital environment are the sources of infection and, thus, which ones require attention. Therefore, the contamination status was examined in a hospital environment to identify the main sources of methicillin-resistant staphylococci, the places at risk of becoming the transmission route, and the medical tools and equipment that can be contaminated.

In this study, we conducted a bacterial survey using methicillin-resistant staphylococci as an indicator in a medical environment. This is important because it is necessary to learn about what medical tools and equipment in MRSA-contaminated hospital wards, general hospital wards, and staff stations should be disinfected. We confirmed carefully when we encountered contaminated surfaces. In addition, meca is located in S. aureus and CNS on a genetic element called the staphylococcal cassette chromosome mec (SCCmec) (Katayama et al., 2000; Wisplinghoff et al., 2003). We analyzed the distribution of SCCmec types in methicillin-resistant staphylococci isolated from the medical environment and medical equipment.

**MATERIALS AND METHODS**

**Sample collection**

Different surfaces and medical equipment in a university hospital in Japan which were frequently touched by patients carrying MRSA, encompassing a ward with one MRSA patients, a general hospital ward (four non-MRSA patients were in the ward), and a staff station, were selected and swabbed (Figure 1, Table 1) for this survey. Samples were collected from each surface with Microbiological Wipe Swab (SmartCheck: AS ONE Co., Ltd, Osaka, Japan) sterilized cotton wipes. Floor and desk surfaces were swabbed as samples (10cm×10cm in area).

**Culture methods**

After swabbing, the cotton wipes used for the samples were collected and placed in the 0.01M phosphate-buffered saline solution (10mL) that is included with SmartCheck and stirred well. A portion (100μL) of the samples was plated on nutrient agar (BD, Franklin Lakes, NJ, USA) and incubated at 37°C for 24 hours to measure the total bacterial counts, which were then converted into colony-forming units (CFU)/mL.

Similarly, 100μL samples were plated on the mannitol salt agar selective for Staphylococci (AS ONE Co., Ltd, Osaka, Japan) and incubated at 37°C for 24 hours. Colonies cultured on mannitol salt agar were plated on the Muller-Hinton agar (BD, Franklin Lakes, NJ, USA) with 5μg/mL oxacillin and cultivated at 37°C for 24 hours to examine the presence or absence of drug resistant Staphylococcus aureus (MRSA) and coagulase-negative-Staphylococcus (MRCONS) (Brown et al., 2005; Loeffer et al., 2005; NCCLS/CLSI, 2007).

**Identification of Staphylococci**

The colonies detected on the mannitol salt agar plate were subjected to Gram staining, a catalase test, and a coagulase test to confirm the presence of staphylococci. Their bacteria were identified according to biochemical properties using a commercially available staphylococcal identification microplate kit (N-ID TEST SP-18: NISSUI PHARMACEUTICAL Co., Ltd, Tokyo, Japan).

**Detection of methicillin resistant genes and Staphylococcal cassette chromosome mec (SCCmec) types**

A colony isolated on the Mueller Hinton agar with 5μg/mL oxacillin was suspended in 200μL InstaGene matrix (Bio-Rad Laboratories, Hercules, CA, USA), which was used for DNA extraction. Bacterial DNA was extracted according to the manufacturer’s instructions and used for conventional polymerase chain reaction (PCR). The antibiotic-resistant pathogen, MRSA, was identified by PCR to amplify the SCmec types (I–V) using PCR. A type I fragment of 415bp was amplified by PCR using the primers 1272F1 (5′-gccacctcataacatatggaa-3′) and 1272R1 (5′-catcccgagtgaaacccaaa-3′). A type II fragment of 937bp was amplified using the primers 1272F1 (5′-gccacctcataacatatggaa-3′) and 1272R1 (5′-catcccgagtgaaacccaaa-3′). A type III fragment of 937bp was amplified using the primers 1272F1 (5′-gccacctcataacatatggaa-3′) and 1272R1 (5′-catcccgagtgaaacccaaa-3′). A type IV fragment of 518bp was amplified using the primers 1272F1 (5′-gccacctcataacatatggaa-3′) and 1272R1 (5′-catcccgagtgaaacccaaa-3′). A type V fragment of 359bp was amplified using the primers 1272F1 (5′-gccacctcataacatatggaa-3′) and 1272R1 (5′-catcccgagtgaaacccaaa-3′). After staining with ethidium bromide, the amplified bands were observed under UV transillumination.

**RESULTS**

**Bacterial plate counts (BPCs) from the hospital environment**

The results of the BPCs from the hospital environment are shown in Table 2. The places and instruments in the hospital on which the bacterial counts were detected above 0.1×10^5CFU/mL are shown in Table 2.

In the ward where patients had MRSA, most BPC numbers were detected on the call button (2.6×10^5CFU/mL). The same measurements were
TABLE 1. Sample-collected places and medical equipments in a university hospital in Japan

| The hospital ward with patients carrying MRSA                                      | The general hospital ward (including the staff station) |
|-----------------------------------------------------------------------------------|----------------------------------------------------------|
| -Cuff                                                                             | -Cuff                                                    |
| -Pulse oximeter                                                                  | -Pulse oximeter                                         |
| -Sheet                                                                            | -Adhesive tape                                           |
| -Overbed table                                                                    | -Adhesive tape (The nurse carrying)                      |
| -Bed side table                                                                   | -Stethoscope                                             |
| -The arm of bed light                                                             | -Tourniquet                                              |
| -The switch of bed light                                                          | -Glove                                                   |
| -Call button                                                                      | -Workbench                                               |
| -Side-fences for nursing bed                                                       | -Cart                                                    |
| -Bed-side floor                                                                   | -Keyboard                                                |
| -The door knob (Inside hospital ward)                                             | -On the desk of the staff station                        |
| -The door knob (Outside hospital ward)                                            | -The handle of the bed pan washer                        |
|                                                                                  | -The door knob of toilet room                            |
|                                                                                  | -The toilet seat                                         |
|                                                                                  | -The bottle of alcohol for hand disinfection              |
|                                                                                  | (The entrance of hospital ward)                          |
|                                                                                  | -The bottle of alcohol for hand disinfection              |
|                                                                                  | (In the hospital ward)                                   |
|                                                                                  | -Curtain                                                 |
|                                                                                  | -Side-fences for nursing bed                              |
|                                                                                  | -Drip stand                                              |
|                                                                                  | -Bed side table                                           |
found on beside floors \((1.6 \times 10^3 \text{CFU/mL})\), sheets \((800 \text{CFU/mL})\), and over-bed tables \((400 \text{CFU/mL})\). Nursing bed side-fences showed measurements of 400\text{CFU/mL}. Other places and instruments included the doorknob of the outside hospital ward \((200 \text{CFU/mL})\), the Cuff \((100 \text{CFU/mL})\), and the bed light arm \((100 \text{CFU/mL})\).

In the general hospital ward, including the staff station, most BPC numbers were detected using adhesive tape on a nurse \((4.5 \times 10^3 \text{CFU/mL})\) and applying adhesive tape on a table \((1.85 \times 10^3 \text{CFU/mL})\). The bottle of alcohol for hand disinfection (at the entrance to the ward), a pulse oximeter, a toilet seat, and the Cuff measured \(1.17 \times 10^3 \text{CFU/mL}, 600 \text{CFU/mL}, 530 \text{CFU/mL}, \) and \(450 \text{CFU/mL}\), respectively. BPCs were detected on the computer keyboard \((300 \text{CFU/mL})\) at the staff station and a tourniquet \((150 \text{CFU/mL})\). Nursing bed side-fences showed measurements of less than \(100 \text{CFU/mL}\) in the general hospital ward.

### Identification of isolated bacteria

Figure 2 shows a total of 146 strains isolated on mannitol salt agar plates. The pie chart on the left shows the bacterial species from all the samples isolated on the mannitol salt agar plates. The pie chart on the right shows the isolated CNS bacterial species.

CNS: Coagulase-negative Staphylococcus

| The hospital ward with patients carrying MRSA (CFU/mL) | The general hospital ward (including the staff station) (CFU/mL) |
|------------------------------------------------------|---------------------------------------------------------------|
| Call button                                          | Adhesive tape (The nurse carrying)                           | 4,500 |
| Bed-side floor                                       | Adhesive tape                                               | 1,850 |
| Sheet                                                | The bottle of alcohol for hand disinfection                  | 1,170 |
| Overbed table                                        | (The entrance of hospital ward)                             | 600  |
| Side-fences for nursing bed                          | Pulse oximeter                                              |      |
| Door knob (Outside hospital ward)                   | Toilet seat                                                 | 530  |
| Cuff                                                 | Cuff                                                        | 450  |
| The arm of bed light                                 | Keyboard                                                    | 300  |
|                                                       | Tourniquet                                                   | 150  |

The bacterial counts of other tested places and equipments were 100\text{CFU/mL} or less.
mannitol salt agar plates. Of the identified bacteria, 23 were strains of S. aureus (15.7%), 109 were strains of CNS (74.7%), and 14 were strains of Bacillus spp. (9.6%) (Figure 1). CNS was identified by N-ID TEST SP-18 as S. epidermidis (19.1%), S. haemolyticus (11.8%), S. capitis (11.8%), S. lugdunensis (10.3%), S. hyicus (8.8%), S. hominis (7.4%), S. warneri A (5.9%), S. caprae B (4.4%), and other CNS (20.5%).

Screening of resistant strains on oxacillin-containing plates

Oxacillin-resistant strains were isolated from 56.5% (13 strains) of S. aureus and 50.5% (55 strains) of CNS.

All S. aureus and CNS from the hospital wards with patients carrying MRSA were oxacillin-resistant strains (Figure 3A). In contrast, 16.7% S. aureus and 22.9% CNS from the general hospital ward, including the staff station, were detected as oxacillin-resistant strains (Figure 3B).

Detection of methicillin-resistant mecA gene

The methicillin-resistant mecA gene was detected in all oxacillin-resistant S. aureus and in 89.0% (49 strains) of oxacillin-resistant CNS.

The presence of the mecA gene was confirmed in all oxacillin-resistant S. aureus and oxacillin-resistant CNS from patients with MRSA in the hospital wards. These strains were identified as MRSA and MRCNS (Figure 4A). In contrast, the mecA gene was detected in all oxacillin-resistant S. aureus, but not in all oxacillin-resistant CNS (62.5%) from the general hospital ward, including the staff station (Figure 4B).

Detection of Staphylococcal cassette chromosome mec (SCCmec) types

The SCCmec types of MRSA from patients carrying MRSA in the hospital wards were primarily Type II and Type IV (Figure 5A). Most MRCNS were of the SCCmec Type V, which are of the community-acquired infection type, but Types I and IV were also detected (Figure 5B).
In contrast, the SCCmec type of all MRSA and MRCNS from the general hospital ward, including the staff station, was identified as Type I (Figures 5C, 5D).

**DISCUSSION**

Depending on which particular medical activity is being conducted, various devices are used. Microorganisms attach to devices that come into contact with humans. The surfaces of medical devices in hospital units are contaminated by both potentially pathogenic and pathogenic bacterial species. In this study, bacterial contamination in MRSA patients' wards and nearby nurse stations was investigated. The highly contaminated equipment and surfaces had been touched by people. In particular, adhesive tapes carried by nurses and tapes that had been left aside had the most bacterial contamination. The tapes carried by nurses are usually attached to various patients; therefore, we have to consider limiting their usage to avoid bacterial contamination. In hospital environments, door handles are contaminated and can be a transmitter of bacteria (Woigani et al., 2012). In a study examining the surrogate markers of nosocomial pathogen transmission, door handles were highlighted as one site that rapidly became contaminated within the context of a neonatal intensive care setting (Oelberg et al., 2000). Our research also indicated that the doorknobs of other hospital wards were contaminated. Not surprisingly, medical staff have to be aware of how doorknobs are a source of infection. In addition, bacterial contamination of a ward depends on the frequency with which it is used by patients or medical staff.

Patients with MRSA are a major concern for hospitals; therefore, such patients are usually confined to single, isolated hospital wards. Patients with MRSA are the number of MRSA-positive body sites of individual patients and the MRSA contamination of the patient's hospital room (Rohr et al., 2009). Our study investigated the environment of wards after patients with MRSA had been discharged. All isolated S. aureus strains from the wards were MRSA ones and, surprisingly, all CNS

**Figure 4.** Detection of the methicillin-resistant *mecA* genes
4A, the hospital ward with an MRSA patient.
4B, the general hospital ward, including the staff station.
MRSA; Methicillin resistant *Staphylococcus aureus*
CNS; Coagulase-negative *Staphylococci*
Figure 5. Detection of the Staphylococcal cassette chromosome mec (SCCmec) types
5A, the SCCmec type of MRSA from the hospital wards with MRSA patients.
5B, the SCCmec type of MRCNS from the hospital wards with MRSA patients.
5C, the SCCmec type of MRSA from the general hospital ward, including the staff station.
5D, the SCCmec type of MRCNS from the general hospital ward, including the staff station.
MRSA; Methicillin resistant Staphylococcus aureus
MRCNS; Methicillin resistant coagulase-negative Staphylococci
strains were also oxacillin resistant and contained the mecA gene. This indicates that patients with MRSA have MRCNS as normal flora and the entire ward becomes contaminated with drug-resistant bacteria. In contrast, the drug-resistant percentages of S. aureus and CNS isolated from the general hospital ward, including the staff station, were 16.7% and 22.9% respectively. The mecA gene was detected in all S. aureus strains but was only 62.5% positive in CNS strains. We hypothesize that drug-resistant Staphylococcus strains can spread by transferring the mecA gene among strains in hospital wards.

The report indicates that the methicillin-resistant mecA gene can transfer from MRSA to methicillin-susceptible S. aureus (MSSA) in vitro (Bitrus et al., 2017). In S. aureus and CNS, mecA is located on a genetic element called the staphylococcal cassette chromosome (SCCmec) (Katayama et al., 2000; Wisplinghoff et al., 2003). A report has implied that the horizontal transfer of SCCmec elements is speculative based on the sharing of SCCmec type V between MRSA and MRSE in the same individual (Ibrahim et al., 2009). However, there are no reports of SCCmec transferring between S. aureus and CNS. Therefore, we analyzed the SCCmec types in the S. aureus and CNS strains. The MRSA strains isolated in the hospital wards with MRSA patients were SCCmec type II and IV, and the MRCNS strains were mainly SCCmec type V. The SCCmec type IV strain was identified in both MRSA and MRCNS. In contrast, all of the strains isolated in the general hospital ward, including the staff station, were identified as SCCmec type I. Analysis of the type of SCCmec revealed that drug-resistant strains were not transferred from the MRSA inpatient wards to other rooms or the nurse stations. On the other hand, the same SCCmec type IV strains were isolated and identified at both hospital wards, including the staff station, from an MRSA patient. Our analysis is limited and is not the only evidence that the mecA gene can be transferred by SCCmec among MRSA and MRCNS strains. All of the strains used in this study were isolated from the hospital environment and not from people. However, further studies are needed on how mecA is transferred by SCCmec between MRSA and MRCNS.

Transmission-based precautions, especially contact precautions and the cleaning of hospital environments, are important for preventing the outbreak of nosocomial infections caused by MRSA and MRCNS. The increased incidence and prevalence of community-acquired MRSA and MRCNS, along with hospital-acquired MRSA, should be recognized as important health care problems. In this study, effective infection control was shown to prevent resistant strains from spreading to other wards and staff from MRSA patient wards.

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