INTRODUCTION

Control of healthcare-associated infections remains a major clinical concern related to improving mortality and morbidity rates. Since its discovery in 1961, methicillin-resistant *Staphylococcus aureus* (MRSA) continues to be an important healthcare-associated pathogen. MRSA bacteremia, skin and soft tissue infection, and surgical site infections are associated with longer hospitalizations, greater mortality, and higher...
healthcare costs. To date, several different hospital-based strategies have been proposed by infection control personnel and hospital administrators to mitigate the spread and impact of MRSA. Nonetheless, the incidence of MRSA infections remains high in Japan.

Methicillin-resistant *S. aureus* infections typically occur in individuals who are colonized with MRSA. Implementing contact precautions for MRSA carriers is essential for preventing healthcare-associated infections. To implement effective precautions for avoiding MRSA infections, it is important to clarify when, how, and from whom MRSA is transmitted. In other words, early detection of MRSA infections in hospitalized patients is critical. Prior hospital admission is a risk factor for MRSA infection, suggesting that MRSA is acquired during hospitalization.

When MRSA is prevalent in hospitals, it can be transmitted by undetected carriers to uninfected patients in the hospital. However, MRSA transmission rates and routes in the hospital are not completely understood. Multilocus sequence typing (MLST) is useful in clarifying the diversity and epidemiology of MRSA in healthcare settings. The purpose of this study was to evaluate MRSA transmission rates in a hospital, where MRSA infection/colonization density was relatively high and hand hygiene compliance was inadequate. The prevalence of MRSA colonization in patients at the time of hospital admission to and discharge from a medical school hospital in Japan was determined using surveillance cultures.

## 2 | MATERIALS AND METHODS

### 2.1 | Study setting and design

This study was conducted at Kochi Medical School Hospital, a 605-bed tertiary care general hospital with 13 wards. All patients admitted to one of the 50-bed wards from June 2008 to June 2009 were analyzed. Samples were collected from 157 patients at the time of admission and on the day of discharge. Data from clinical medical records were retrospectively reviewed (Table 1). Contact precautions were implemented over standard precautions for patients with MRSA.

### 2.2 | Isolation and identification of *Staphylococcus aureus* and MRSA

After obtaining written informed consent, we used wet (0.85% NaCl) cotton swabs to obtain bilateral anterior cultures of the nares of all participants. These swabs were immediately inoculated in Staphylococcus Medium 110 (Becton Dickinson & Co, Sparks, MD, USA) supplemented with 5% egg yolk (Kyokuto, Tokyo, Japan) and Trypticase soy agar containing 5% sheep blood (Nippon Becton Dickinson & Co, Tokyo, Japan). Selective plates were cultured for MRSA for 48 hours at 36°C. *Staphylococcus aureus* was identified using API Staph (Sysmex-bioMérieux, Tokyo, Japan), and *femA* and *femB* gene detection was performed using polymerase chain reaction (PCR). The presence of *mecA* gene was confirmed using a real-time PCR assay as previously described.

### 2.3 | Laboratory methods

During the study period, MRSA strains collected from participants were stored and evaluated. DNA extraction and MLST were performed according to the methods described by Enright et al. Sequence types were determined by accessing the MLST website (http://www.mlst.net/). MLST was performed using a 3130 Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA).

### 2.4 | Nosocomial transmission events of MRSA

Transmission events in the ward were defined as the transfer of MRSA with the same MLST type from a colonized patient to another patient who was previously negative, hospital stays that overlapped with the stay of a colonized patient, and instances of epidemiological linkage. The linkage was defined as either being a roommate of the index patient in a multibed room or being treated by the same attending doctors.

### 2.5 | Methicillin-resistant *Staphylococcus aureus* prevalence proportion in the ward

We retrospectively analyzed MRSA isolation data from all patients admitted to the ward. MRSA point prevalence proportion refers to the total number of patients with MRSA divided by the total number of patients admitted to the ward in each month. The MRSA prevalence proportion expresses MRSA transmission pressure in the ward; therefore, we used it as a parameter in this study.

### 2.6 | Hand hygiene practices

Monthly alcohol-based hand rub utilization (volume in mL) was measured during the study period, and the number of times hand hygiene was practiced was calculated from these data.

### 2.7 | Ethical disclosure

The study protocol was approved by the Ethics Committee of Kochi Medical School, and written informed consent was obtained from all participants in this study.
Statistical analysis

Proportions of MRSA colonization were analyzed using the chi-square test.

RESULTS

Methicillin-resistant S. aureus colonization was evaluated in 314 samples obtained from 157 patients both at the time of admission to and at the time of discharge from the ward of our hospital. In total, 11 samples from nine patients were positive for MRSA (Figure 1). MRSA colonization was confirmed in three patients at admission; therefore, the MRSA prevalence proportion on admission was 1.9%. MRSA remained at discharge in two patients (cases #3 and #6) but disappeared in the third patient (case #8). All three MRSA-positive patients were previously admitted to our hospital. The number of times these patients were admitted to the hospital was 1 (case #3), 2 (case #6), and 2 (case #8).

Six patients (3.8%) (cases #1, #2, #4, #5, #7, and #9) were negative for MRSA colonization at the time of admission; however, they were detected positive for MRSA at discharge (Figure 1). Hence, we concluded that they acquired MRSA during hospitalization. Hospital stay lengths for these cases were 4 (case #1), 15 (case #2), 15 (case #4), 35 (case #5), 48 (case #7), and 118 (case #9) days. The mean length of hospital stay in these six patients was 39.2 days; therefore, the MRSA incidence rate was 4.0/1000 patient-days. At discharge, 5.1% of the patients exhibited MRSA colonization; this was significantly higher than the prevalence noted upon admission (P < 0.001).

In total, nine of the 157 (5.7%) patients were positive for MRSA. Clinical characteristics of these nine patients are summarized in Table 2. All patients presented at least one risk factor for MRSA colonization. MLST was performed on 11 MRSA isolates obtained from these patients at both admission and discharge (Figure 1). Identical

| Case | #1 | #2 | #3 | #4 | #5 | #6 | #7 | #8 | #9 |
|------|----|----|----|----|----|----|----|----|----|
| Age, y | 29 | 56 | 77 | 29 | 50 | 79 | 75 | 79 | 69 |
| Male gender | – | + | + | – | + | – | + | + | + |
| Hospitalization history within 1 y | – | + | + | + | + | + | – | – | – |
| Use of antibiotics within 1 mo | – | – | – | – | – | – | – | – | – |
| Presence of infection at the time of admission | – | – | – | + | – | + | – | – | – |
| Diabetes mellitus | + | – | – | + | – | + | – | – | + |
| Primary disease | Skin ulcer | Esophageal cancer | Mediastinal tumor | Skin ulcer | Lung cancer | Thoracic aortic aneurysm | Lung cancer | Lung cancer | Colon cancer |
| MRSA colonization on admission | – | – | + | – | – | + | – | + | – |
| MRSA colonization at discharge | + | + | + | + | + | + | – | + | – |

MRSA, methicillin-resistant S. aureus.

2.8 | Statistical analysis

3 | RESULTS

BASELINE CHARACTERISTICS OF PATIENTS WITH METHICILLIN-RESISTANT STaphylococcus aureus (MRSA) colonization was evaluated in 314 samples obtained from 157 patients both at the time of admission to and at the time of discharge from the ward of our hospital. In total, 11 samples from nine patients were positive for MRSA (Figure 1). MRSA colonization was confirmed in three patients at admission; therefore, the MRSA prevalence proportion on admission was 1.9%. MRSA remained at discharge in two patients (cases #3 and #6) but disappeared in the third patient (case #8). All three MRSA-positive patients were previously admitted to our hospital. The number of times these patients were admitted to the hospital was 1 (case #3), 2 (case #6), and 2 (case #8).

Six patients (3.8%) (cases #1, #2, #4, #5, #7, and #9) were negative for MRSA colonization at the time of admission; however, they were detected positive for MRSA at discharge (Figure 1). Hence, we concluded that they acquired MRSA during hospitalization. Hospital stay lengths for these cases were 4 (case #1), 15 (case #2), 15 (case #4), 35 (case #5), 48 (case #7), and 118 (case #9) days. The mean length of hospital stay in these six patients was 39.2 days; therefore, the MRSA incidence rate was 4.0/1000 patient-days. At discharge, 5.1% of the patients exhibited MRSA colonization; this was significantly higher than the prevalence noted upon admission (P < 0.001).

In total, nine of the 157 (5.7%) patients were positive for MRSA. Clinical characteristics of these nine patients are summarized in Table 2. All patients presented at least one risk factor for MRSA colonization. MLST was performed on 11 MRSA isolates obtained from these patients at both admission and discharge (Figure 1). Identical

| TABLE 3 | Nosocomial methicillin-resistant Staphylococcus aureus transmission events |
|----------|------------------|
| Transmission event | #3 to #2 | #6 to #5 | #8 to #7 |
| Same MLST type | + | + | + |
| Overlap in hospital stay | + | + | + |
| Roommate | – | – | – |
| Same attending doctor | + | + | + |

MLST, multilocus sequence typing.
MRSA strains were obtained from both samples (admission and discharge) in two patients (cases #3 and #6). As confirmed by MLST, one patient had strain ST8, two had ST764, and the remaining six had ST5. Since case #2 was positive for ST764 after case #3 was detected to be positive for the same strain, MRSA transmission could have occurred from case #3 to case #2. Both cases #3 and #2 were treated by the same attending doctors (Table 3). Similarly, two possible transmission events of ST5 strains occurred: from case #6 to case #5 and from case #8 to case #7. In total, three possible transmission routes were documented.

We investigated whether the nine patients who were positive for MRSA on surveillance culture underwent clinical examinations during their hospitalization period. We found that bacterial cultures were not obtained from six of these patients. MRSA infection was detected in case #2 via clinically ordered wound culture on August 11, 2008, and in case #4 via clinically ordered sputum culture on August 14, 2008, both of which were obtained prior to receiving the discharge surveillance cultures. The last patient (case #6) was already known to be a MRSA carrier because MRSA was isolated during previous admission. Overall, of the nine MRSA-positive patients confirmed by surveillance culture, six were detected prior to being identified by conventional clinically oriented examinations.

We calculated the MRSA prevalence proportion in the ward. In total, 42 patients were admitted to the ward on June 5, 2008, and eight of them were MRSA carriers; therefore, the MRSA prevalence proportion was 19.0%. Similarly, the average MRSA prevalence proportion from June 2008 to June 2009 was 13.3% (Table 4).

We calculated the number of times hand hygiene was practiced on the basis of the number of times alcohol-based hand rubs were utilized. On average, there were 4.0 instances of hand hygiene per patient-day (Table 4).

### 4 | DISCUSSION

Methicillin-resistant *S. aureus* transmission events were analyzed by examining surveillance cultures of 157 participants in a tertiary care general hospital in Japan. Results were compared among clinically oriented conventional cultures, MRSA prevalence rates, and hand hygiene compliance data.

Multilocus sequence typing of 11 MRSA isolates was performed to clarify the diversity and epidemiology of MRSA in this healthcare setting. The most frequently detected strain was ST5, accounting for 67% of the isolates; this is consistent with previous findings, with ST5 being the most prevalent clone in Japan. Both ST5 and ST764 strains are closely related and are classified as clonal type CC5, while ST8 is classified as clonal type CC8. Most of the recently isolated healthcare-acquired MRSA (HA-MRSA) isolates in Japan were clonal types CC5 and CC8. These findings suggest that all 11 MRSA isolates were prevalent HA-MRSA strains in Japan and that these patients did not acquire MRSA in community settings.

The MRSA prevalence proportion on admission was 1.9%, and all three MRSA-positive patients were previously admitted to our...
hospital, suggesting that previous hospital admission was a risk factor for MRSA infection: this is in agreement with the findings of previous studies.\textsuperscript{2,5} Our results suggest that patients who were colonized with MRSA acquired it during a previous visit to our hospital. The MRSA prevalence proportion in our hospital was 13.3\%, which is similar to that reported (19.9\%) at other facilities in Japan.\textsuperscript{2} The prevalence of MRSA colonization in Japanese hospitals is much higher than that observed in American or European hospitals.\textsuperscript{10,11}

In our study, MRSA incidence rate was 4.0/1000 patient-days. MLST revealed three possible nosocomial transmission routes of MRSA—one case with ST764 strain and two cases with ST5 strains—suggesting that patients who were negative for MRSA at the time of admission acquired nosocomial infections during their respective hospitalizations. The mean number of times hand hygiene was practiced was 4.0 times per patient-day. The hand hygiene compliance rate, which was evaluated using direct observation, was not high during this period.\textsuperscript{12} MRSA infections could have occurred due to insufficient infection control practices. Hence, to prevent the transmission of MRSA, quality control practices should be improved by implementing multifaceted interventions, including hand hygiene, contact precautions, and universal decolonization of inpatients.\textsuperscript{13–15}

Methicillin-resistant \textit{S. aureus} colonization was detected on active surveillance cultures prior to being detected on clinically oriented conventional examinations in six of the nine patients, indicating that some MRSA infections were missed during clinically oriented bacterial cultures.\textsuperscript{16} Some MRSA infections were transmitted by yet-unknown MRSA colonization.\textsuperscript{6} Therefore, active surveillance cultures are an important strategy for elucidating MRSA transmission.

The MRSA prevalence in all patients in the ward was 13.3\%, indicating that MRSA transmission pressure was high. Additionally, this proportion (13.3\%) was higher than the proportion of MRSA carried within the nares of 157 patients (5.1\%), suggesting that MRSA carriage did not often involve the nares. On an average, ward personal exhibited only four instances of hand hygiene per patient-day. Such a poor compliance increases the risk of nosocomial MRSA transmission. On an average, ward personal exhibited only four instances of hand hygiene per patient-day. Such a poor compliance increases the risk of nosocomial MRSA transmission. This reinforces the central role of infection control practices for preventing nosocomial MRSA transmission in hospitalized individuals. The pronounced risk of transmission emanating from undetected MRSA carriers suggests that increasing the frequency of microbiological diagnosis could help reduce MRSA transmission.

**ACKNOWLEDGEMENT**

This study was supported in part by a grant from JSPS KAKENHI (Grant No. 15K08846).

**CONFLICT OF INTEREST**

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

**REFERENCES**

1. Simor AE, Pelude L, Golding G, et al. Determinants of outcome in hospitalized patients with methicillin-resistant \textit{Staphylococcus aureus} bloodstream infection: results from National Surveillance in Canada, 2008-2012. Infect Control Hosp Epidemiol. 2016;37(4):390–7.
2. Kanemitsu K, Yamamoto N, Imafuku Y, et al. The capability of MRSA active surveillance to reduce MRSA infection in Japan. Am J Infect Control. 2013;41(5):470–1.
3. Kunishima H, Yamamoto N, Kobayashi T, et al. Methicillin resistant \textit{Staphylococcus aureus} in a Japanese community hospital: 5-year experience. J Infect Chemother. 2010;16(6):414–7.
4. Mullen A, Wieland HJ, Wieser ES, Spannhake EW, Marinos RS. Perioperative participation of orthopedic patients and surgical staff in a nasal decolonization intervention to reduce \textit{Staphylococcus} spp surgical site infections. Am J Infect Control. 2017;45(5):554–6.
5. Jernigan JA, Pullen AL, Flowers L, Bell M, Jarvis WR. Prevalence of and risk factors for colonization with methicillin-resistant \textit{Staphylococcus aureus} at the time of hospital admission. Infect Control Hosp Epidemiol. 2003;24(6):409–14.
6. Nübel U, Nachtnebel M, Falkenhorst G, et al. MRSA transmission on \textit{Staphylococcus aureus} strain, another such strain carrying a multiple-drug resistance plasmid, and other more-typical \textit{Staphylococcus aureus} strains isolated in 11 Asian countries: a proposal for a new nomenclature for SCCmec elements. Antimicrob Agents Chemother. 2006;50(3):1001–12.
7. Chongtrakool P, Ito T, Ma XX, et al. \textit{Staphylococcus} coccus genome (SCCmec) typing of methicillin-resistant \textit{Staphylococcus aureus} strains isolated in 11 Asian countries: a proposal for a new nomenclature for SCCmec elements. Antimicrob Agents Chemother. 2006;50(3):1001–12.
8. Takizawa Y, Taneike I, Nakagawa S, et al. A Panton-Valentine leucocidin (PVL)-positive community-acquired methicillin-resistant \textit{Staphylococcus aureus} (MRSA) strain, another such strain carrying a multiple-drug resistance plasmid, and other more-typical PVL-negative MRSA strains found in Japan. J Clin Microbiol. 2005;43(7):3356–63.
9. Kuehnert MJ, Krous-Moran D, Hill HA, et al. Prevalence of \textit{Staphylococcus aureus} nasal colonization in the United States, 2001-2002. J Infect Dis. 2006;193(2):172–9.
10. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Emergence and resurgence of methicillin-resistant \textit{Staphylococcus aureus} as a public-health threat. Lancet. 2006;368(9538):874–85.
12. Arise K, Nishizaki S, Morita T, Yagi Y, Takeuchi S. Continued direct observation and feedback of hand hygiene adherence can result in long-term improvement. Am J Infect Control. 2016;44(11):e211–4.

13. Kawamura I, Ohmagari N, Noda S, Sugiyama T, Kurai H. Preventing the transmission of methicillin-resistant Staphylococcus aureus at a tertiary care cancer center in Japan: quality improvement report. Am J Infect Control. 2013;41(11):1105–6.

14. Huang SS, Septimus E, Kleinman K, et al. CDC Prevention Epicenters Program; AHRQ DECIDE Network and Healthcare-Associated Infections Program. Targeted versus universal decolonization to prevent ICU infection. N Engl J Med. 2013;368(24):2255–65.

15. Chun JY, Seo HK, Kim MK, et al. Impact of a hand hygiene campaign in a tertiary hospital in South Korea on the rate of hospital-onset methicillin-resistant Staphylococcus aureus bacteremia and economic evaluation of the campaign. Am J Infect Control. 2016;44(12):1486–91.

16. Leonhardt KK, Yakusheva O, Phelan D, et al. Clinical effectiveness and cost benefit of universal versus targeted methicillin-resistant Staphylococcus aureus screening upon admission in hospitals. Infect Control Hosp Epidemiol. 2011;32(8):797–803.

17. Chipolombwe J, Török ME, Mbelle N, Nyasulu P. Methicillin-resistant Staphylococcus aureus multiple sites surveillance: a systematic review of the literature. Infect Drug Res. 2016;9:35–42.

How to cite this article: Matsumoto K, Takeuchi S, Uehara Y, et al. Transmission of methicillin-resistant Staphylococcus aureus in an acute care hospital in Japan. J Gen Fam Med. 2019;20:13–18. https://doi.org/10.1002/jgf2.216