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Original Article

Active Surveillance of Methicillin-Resistant *Staphylococcus aureus* Using a Fully Automated Molecular Test in an Emergency Medical Center

Yoshitomo Morinaga[1], Shuhei Yamano[3], Norihiko Akamatsu[1], Norihito Kaku[1,2], Kentaro Nagaoka[1,2], Yohei Migiyama[1,2], Yosuke Harada[1,2], Naoki Hosogaya[2], Yoshihiro Yamamoto[4], Osamu Tasaki[3], Katsunori Yanagihara[1]*, and Shigeru Kohno[2]

[1]*Department of Laboratory Medicine;*
[2]*Second Department of Internal Medicine, Nagasaki University Graduate School of Biomedical Sciences;*
[3]*Emergency Medical Center, Nagasaki University Hospital, Nagasaki; and*
[4]*Department of Clinical Infectious Diseases, University of Toyama, Toyama, Japan*

SUMMARY: The prevention and control of methicillin-resistant *Staphylococcus aureus* (MRSA) are important, particularly in emergency units. The active surveillance of MRSA was prospectively performed at the emergency medical center of Nagasaki University Hospital. After obtaining nasal swab specimens, a fully automated molecular test (FAMT) and a culture-screening method were utilized for MRSA detection. A total of 150 patients were enrolled in the study, and 366 nasal swab specimens were obtained. MRSA was detected by culture in 11 (7.3%) patients including one new acquisition and by the FAMT in 34 (22.7%) patients including 13 new acquisitions. The sensitivity, specificity, positive predictive value, and negative predictive value of the FAMT at the patient level were 86.7, 85.2, 39.4, and 98.3%, respectively, when compared with the culture-based results. An FAMT can effectively detect MRSA colonization, which may remain undetected with the conventional method, and it may be useful in detecting newly acquired MRSAs.

INTRODUCTION

Hospital-acquired infections represent a clinical concern, and their management and control are important for reducing of hospital-related mortality and morbidity (1). Methicillin-resistant *Staphylococcus aureus* (MRSA) is a common drug-resistant pathogen responsible for severe infections in both the healthcare and community settings (1–3). In the emergency unit, severely infected patients with various conditions are admitted into the hospital, where they can receive aggressive treatments; therefore, monitoring MRSA transmission and infection is an important management strategy for infection control.

Molecular diagnostic techniques have expanded over the years and common technical concerns such as process contamination and inaccurate measurements owing to human error have increased. In this respect, automated systems for microbiological molecular analysis are expected to reduce these concerns. Recently, fully automated molecular tests (FAMTs) for detection of microorganisms have been developed. These tests comprise several protocols including cell lysis, nucleic acid extraction, and the amplification and analysis of target genes using a single platform. Because molecular techniques are usually highly sensitive, FAMTs help to prevent microbiological contamination and minimize technical errors.

We previously reported the utility of molecular diagnostic methods for the active surveillance of MRSA in a respiratory ward (4). A recently developed FAMT for detecting MRSA, BD MAX MRSA, has a high sensitivity and specificity and a low false-positive rate for MRSA detection (5). BD MAX MRSA is a quantitative system based on the real-time polymerase chain reaction (PCR), and it detects MRSA by targeting the SCC*mec*-*orfX* junction.

In this study, we conducted active surveillance of MRSA in an emergency medical center (EMC) in Japan using an FAMT and evaluated its usefulness by comparing the results with those obtained using a standard detection technique.

MATERIALS AND METHODS

Setting: This observational study was conducted between July 9, 2012 and April 16, 2013 in the EMC of Nagasaki University Hospital (NUH). This study was approved by the ethics committee of NUH, and an informed consent form was signed by each patient prior to surveillance.

Microbiological surveillance: Microbiological surveillance of MRSA colonization was performed. Nasal swab specimens were obtained from all enrolled patients within 48 h after admission to the EMC of NUH, All swab specimens were plated directly onto MRSA selective agar with oxacillin (Nippon Becton Dickinson, Tokyo, Japan) and assayed using an FAMT, BD MAX MRSA (Nippon Becton Dickinson). BD MAX MRSA was performed according to the manufacturer's instructions. The same nasal swab specimens were incubated in

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*Corresponding author: Mailing address: Department of Laboratory Medicine, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan. Tel: +81-95-819-7574, Fax: +81-95-819-7422, E-mail: k-yanagi@nagasaki-u.ac.jp

trypticase soy broth (Nippon Becton Dickinson) as a backup culture, and microbial identification was performed when FAMT results were positive but no microorganisms were detected on MRSA-selective agar. The Clinical and Laboratory Standards Institute guidelines were used to confirm the presence of MRSA.

When both the conventional culture screening technique and FAMT were negative at the initial screening upon entry into the EMC, subsequent screening for MRSA by culture screening and the FAMT were performed once a week until the day of patient discharge. When MRSA was identified within the first 48 h after EMC admission, culture screening and the FAMT in the following weeks were discontinued. Elevation of inflammatory markers (leukocyte counts, C-reactive protein, and procalcitonin) upon initial screening concomitantly with the presence of symptoms including fever indicated active MRSA infection. Elevation of these inflammatory markers in the absence of the symptoms indicated inactive MRSA infection. Elevation of inflammatory markers 48 h after admission into the EMC concomitantly with the presence of active symptoms indicated hospital-acquired infection. Elevation of the same inflammatory markers in the absence of these symptoms indicated hospital-acquired colonization.

Infection rate analysis: Patient information was acquired at the time of enrollment. Sex, age, and status of admission into the EMC were recorded. The route of admission was recorded on the basis of whether the patient was (i) admitted from another NUH ward, (ii) admitted from another medical facility, (iii) admitted from a nursing home facility, (iv) admitted directly from home with a history of hospitalization in the previous year, (v) admitted directly from home with a history of nursing home admission in the previous year, (vi) admitted directly from home with no hospitalization or admission history in the previous year, or (vii) admitted into the EMC but background admittance data were unknown. The study endpoints were (i) colonization rates of EMC-acquired MRSA, (ii) infection rates of EMC-acquired MRSA, and (iii) evaluation of the effectiveness of the FAMT compared to the conventional culture screening, which is considered the gold standard.

Analysis of discordant results: When the results differed between the conventional culture screening and FAMT, additional tests were performed. For the culture-positive/PCR-negative samples, the isolates were identified using a BD Phoenix100 automated microbiology system (Becton Dickinson). For the culture-negative/PCR-positive samples, the total DNA of the specimen collected from the trypticase soy broth was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and validated by 2 molecular analyses, namely by PCR analysis for *mecA* detection as reported previously (6) and automated multiplex PCR using the BD MAX StaphSR assay (Nippon Becton Dickinson). This is a fully automatic multiplex PCR assay for the detection of MRSA and methicillin-sensitive *S. aureus*. BD MAX StaphSR can detect nucleases (*nuc*), *mecA* genes including *mecC*, and the staphylococcal cassette chromosome *mec* (SCC*mec*) right-extremity junction (MREJ).

Statistical analysis: The categorical variables were analyzed using McNemar's test. A *P*-value (*P*) of <0.05 was considered statistically significant.

RESULTS

Baseline characteristics: A total of 150 patients were enrolled in this study, and their baseline characteristics are shown in Table 1. The median patient age was 61 years (range, 16–92), and 101 patients (67.3%) were male (data not shown). In total, 128 (85.3%) were directly admitted to the EMC from their homes, among whom 24 had a history of hospitalization in the previous year, whereas 104 had no history of hospitalization in the previous year. Sixteen patients were admitted from other medical facilities, and 3 patients were admitted from nursing homes. There were no patients admitted from other wards in the hospital or directly from home with a history of nursing home admission in the previous year. The admittance records of the remaining 3 patients could not be obtained because they did not have a family.

Active surveillance of MRSA: The rate of positive results in the different hospital settings is shown in Table 1. At the initial screening, 10 (6.7%) patients were positive for MRSA using the culture-based method, whereas 21 (14.0%) patients were positive for MRSA using FAMT ($P < 0.01$). Of the 10 patients with a positive culture, 3 were admitted from other medical facilities, 2 had a history of hospitalization, and the remaining 5 had no hospitalization history. Four patients admitted from other medical facilities tested positive using the FAMT (1 was admitted from a nursing home and 3 were admitted from hospitals), whereas 13 patients had no hospitalization or nursing home ad-

Table 1. Results of culture screening and fully automated molecular test at the initial screening and during follow-up

Pre-admittance condition	Initial screening, *n* (%)			Follow-up, *n* (%)		
	No. of patients	Culture	FAMT[1)]	No. of patients	Culture	FAMT[1)]
Other medical facility	16	3 (18.8)	4 (25.0)	12	—	5 (41.7)
Nursing home	3	—	1 (33.3)	3	—	—
Home, with a history of hospitalization in the previous year	24	2 (8.3)	3 (12.5)	12	1 (8.3)	1 (8.3)
Home, with no hospitalization or admission history in the previous year	104	5 (4.8)	13 (12.5)	70	—	7 (10.0)
Unknown	3	—	—	3	—	—
Total	150	10 (6.7)	21 (14.0)	100	1 (1.0)	13 (13.0)

[1)]: FAMT, fully automated molecular test.

Table 2. Results for PCR confirmation in culture-negative/FAMT-positive samples

Automated multiplex PCR[1)]		*mecA* positive by home-brew PCR (*n*)
Results	No. of samples	
nuc$^+$ *mecA*$^+$MREJ$^-$[2)]	2	1
nuc$^+$ *mecA*$^-$MREJ$^+$	3	—
nuc$^-$ *mecA*$^-$MREJ$^+$	1	—
nuc$^-$ *mecA*$^+$MREJ$^-$	6	5
nuc$^-$ *mecA*$^-$MREJ$^-$	13	1

[1)]: PCR, polymerase chain reaction.
[2)]: MREJ, staphylococcal cassette chromosome *mec* right-extremity junction.

mission history. Of the 11 culture-negative/FAMT-positive patients, 2 were positive for MRSA after broth enrichment culture.

A follow-up was conducted on 100 patients. During follow-up, culture-based screening detected MRSA at the second sampling point in only 1 (1.0%) patient who had a history of hospitalization, whereas the FAMT detected MRSA in 13 (13.0%) patients ($P < 0.01$). Of the FAMT-positive patients, 5 were admitted from other medical facilities, 1 had a hospitalization history, and 7 had no admission history. During follow-up, the FAMT detected MRSA primarily at the second sampling point in 5 (38.5%) patients. Of the culture-positive patients, 2 patients acquired an active MRSA infection on admission and underwent anti-MRSA antibiotic treatment. During the study period, no MRSA infection acquired in the EMC was observed.

Analysis of discordant results: To evaluate the discordant results, additional experiments were performed. Of the 28 culture-negative/PCR-positive samples, 3 were positive for MRSA after broth enrichment culture. Because no bacteria were observed in the enrichment culture, total DNA was extracted from the remaining samples after enrichment culture and analyzed using an automated multiplex PCR, BD MAX StaphSR, and home brew PCR (Table 2). In 12 samples, the following gene patterns were detected using the automated multiplex PCR: *nuc*$^+$ *mecA*$^+$MREJ$^-$ ($n = 2$), *nuc*$^+$ *mecA*$^-$MREJ$^+$ ($n = 3$), *nuc*$^-$ *mecA*$^-$MREJ$^+$ ($n = 1$), and *nuc*$^-$ *mecA*$^+$MREJ$^-$ ($n = 6$). In the other 13 samples, no genes were detected. The *mecA* was detected by home brew PCR in 7 samples; among these samples, 1 was *nuc*$^+$ *mecA*$^+$MREJ$^-$, 5 were *nuc*$^-$ *mecA*$^+$MREJ$^-$, and 1 was *nuc*$^-$ *mecA*$^-$MREJ$^-$.

This study included 3 culture-positive/PCR-negative samples. Of the 3 isolates, 1 was re-identified by a phenotype-based method as a methicillin-resistant coagulase-negative *Staphylococcus* (MRCNS). The other 2 isolates were confirmed to be MRSA, and their counts were 1×10^6 and 1×10^2 CFU/mL. The 2 culture-confirmed MRSA isolates were also confirmed to be positive by the FAMT, but one of the isolates was negative for *mecA* by BD MAX StaphSR and home brew PCR.

Performance testing of the FAMT: In this study, 366 swabs were obtained, and culture screening and FAMT results were compared (Table 3). Compared to culture screening, FAMT results were as follows: sensitivity of 82.3%, specificity of 92.8%, positive predictive value of 35.9%, and negative predictive value of 99.1%. At the patient level, the sensitivity, specificity, positive and negative predictive values during the full observation period were 86.7, 85.2, 39.4, and 98.3%, respectively (Table 4).

Table 3. Performance testing for FAMT at the sample level

		FAMT		Total
		Positive	Negative	
Culture	Positive	14	3	17
	Negative	25	324	349
Total		39	327	366

Table 4. Performance testing for FAMT at the patient level

		FAMT		Total
		Positive	Negative	
Culture	Positive	13	2	15
	Negative	20	115	135
Total		33	117	150

DISCUSSION

Colonization with MRSA is a common risk factor for subsequent MRSA infection. A retrospective cohort study reported that the MRSA infection rate was 14.4% in newly colonized patients in an intensive care unit (7). Another study utilizing a nasal PCR screening test for MRSA demonstrated that patients colonized with MRSA had an increased risk of infection and death, compared to non-colonized patients (8). Therefore, the prevention of MRSA colonization is important for preventing subsequent MRSA infections, and preventing this colonization can lead to decreased morbidity and mortality.

At NUH, approximately 1,000 *S. aureus* isolates are detected every year from all clinical specimens, with MSRA isolates comprising almost 60% of this total, and this frequency was similar to that obtained in Japanese surveillance data (2). All patients admitted to the emergency unit are routinely screened for MRSA independently of the occurrence of nasal staphylococcal colonization, and infected patients are managed by an infection control team to avoid cross-transmission. As described previously, Japan is known to have a high prevalence of MRSA. In this study, FAMT-based screening detected MRSA in 14.0% of patients at the initial screening, and this prevalence was higher than that reported in a similar study conducted in a respiratory ward (4). The active surveillance of MRSA performed in 4 university hospitals in Japan revealed that the MRSA-positive rates were 27.7 and 19.9% by PCR and culture identification, respectively (9). In an emergency unit, 11.2–31.4% of patients were positive for MRSA by culture screening or molecular testing (10,11). By contrast, similar reports, primarily from European countries and the United States, indicated a lower prevalence of MRSA, with rates of approximately 10% or less (12–14).

The risk factors for colonization with MRSA can vary

according to the hospital setting and local MRSA prevalence. Nursing home admittance and hospitalization are known risk factors for MRSA colonization, and similar findings were observed in a Japanese hospital (9). Similarly, the present study indicated that the patients admitted from other medical facilities were most frequently colonized with MRSA. Accordingly, community-acquired MRSA is uncommon in Japan, and the prevalence of Panton-Valentine leukocidin (PVL)-positive strains is approximately 0.5% (15). Among the patients with no hospitalization history, the prevalence of MRSA at the initial screening was 4.8% by culture screening and 12.5% by FAMT, in contrast to 1.9% for those admitted to a respiratory ward. Although we did not evaluate the SCC*mec* types or PVL gene, the high prevalence of MRSA in the EMC may be a result of the large number of medical services received by patients, including emergency care and medical examinations.

In general, molecular diagnosis is more sensitive than culture-based detection or antigen testing. Moreover, molecular testing for MRSA and other pathogens can detect a smaller number of bacteria (16–18). This advantage could have improved MRSA detection during the study follow-up. In addition, the data obtained from molecular diagnosis can increase staff awareness about standard precautions against nosocomial infections.

On the contrary, recent studies revealed several problems regarding molecular-based MRSA detection. At present, targeting the SCC*mec-orfX* junction for MRSA detection has been recommended to avoid MRCNS detection because *mecA* is present in both MRSA and MRCNS (19). However, variant *S. aureus* strains that carry the SCC*mec-orfX* junction but a non-functional *mecA* gene, as well as empty-cassette variants, can yield culture-negative/PCR-positive results (20). Identification performed via by targeting the SCC*mec-orfX* junction can include these variants as false-positive results. Accordingly, the prevalence of empty-cassette variants was 7.7% in a junction-targeted molecular identification (21). In this study, among the strains tested using the automated multiplex PCR, 4 strains that were positive for the SCC*mec-orfX* junction but negative for *mecA* could be empty-cassette variants. Furthermore, a *mecA* homolog, *mecC* carried by emerging MRSA strains, can be considered an additional target in the molecular diagnosis of MRSA (22). The prevalence of *mecC*-positive MRSA strains varied from 0.06% in all MRSA strains identified in the UK, to 2.8% in all MRSA strains identified in Denmark (22,23). In the re-evaluation process in our study, we identified a *mecA*-negative MRSA isolate. Further examination will be required to confirm whether the isolate is *mecC*-positive MRSA or another type.

A limitation of this study was that variant prevalence was not assessed. Considering that this study did not aim to compare BD MAX MRSA and BD MAX StaphSR assays, the results obtained from each test were based on different samples. The former was performed using clinical specimens, whereas the latter was performed using extracted DNA. Therefore, the results of BD MAX StaphSR may have been affected by the extraction efficiency. Concerning the samples that were positive by the FAMT but negative by additional PCR testing, we could not determine whether they included *S. aureus* strains carrying unique sequences that were undetectable by the methods used in the present study or whether they included *S. aureus* strains in which *mecA* was not present. Further studies are warranted to solve these discordant results.

In the re-evaluation process, a discrepancy between the molecular methods was observed in 12 samples in the present study. We believe that this finding might be mainly associated with the use of different samples; namely clinical specimens or extracted DNA. Therefore, the discrepancy can contain false-negatives of BD MAX Staph SR or home brew PCR as well as false-positives of FAMT. In the present study, 2 false-positives were observed for the FAMT. In contrast to assays using cultured strains, direct molecular analysis using clinical specimens can contain certain substances that inhibit the process of DNA extraction or PCR. In addition, one of the 2 false-negatives contained an extremely small number of bacteria. Therefore, this sample might be under the detection limit.

In summary, the FAMT is a useful tool for screening nasal colonization by MRSA. Compared to culture-based methods, the FAMT was highly sensitive for the detection of MRSA. As EMC patients are at high risk for MRSA carriage, the active surveillance of MRSA using molecular diagnosis can be an effective tool for infection control.

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