Advance Publication by J-STAGE

Japanese Journal of Infectious Diseases

Active surveillance of methicillin-resistant *Staphylococcus aureus* with the fully automated molecular test in an emergency medical center

Yoshitomo Morinaga, Shuhei Yamano, Norihiko Akamatsu, Norihito Kaku, Kentaro Nagaoka, Yohei Migiyama, Yosuke Harada, Naoki Hosogaya, Yoshihiro Yamamoto, Osamu Tasaki, Katsunori Yanagihara, and Shigeru Kohno

Received: August 9, 2014. Accepted: November 17, 2014
Published online: March 13, 2015
DOI: 10.7883/yoken.JJID.2014.352

Advance Publication articles have been accepted by JJID but have not been copyedited or formatted for publication.
Active Surveillance of Methicillin-Resistant *Staphylococcus aureus* with the Fully Automated Molecular Test in an Emergency Medical Center

Yoshitomo Morinaga¹, Shuhei Yamano², Norihiko Akamatsu¹, Norihito Kaku¹,³, Kentaro Nagaoka¹,³, Yohei Migiyama¹,³, Yosuke Harada¹,³, Naoki Hosogaya³, Yoshihiro Yamamoto⁴, Osamu Tasaki², Katsunori Yanagihara¹, Shigeru Kohno³

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

Running title: Automated molecular diagnosis in MRSA surveillance

KEYWORDS: fully automated molecular test, *mecC*, empty-cassette variant

Address correspondence to: Katsunori Yanagihara, MD, PhD
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
ABSTRACT

The prevention and control of methicillin-resistant *Staphylococcus aureus* (MRSA) is an important practice, particularly in emergency units. The active surveillance of MRSA was prospectively performed at the emergency medical center (EMC) in Nagasaki University Hospital. After obtaining nasal swab specimens, a fully automated molecular test (FAMT) and a culture-screening method were performed for MRSA detection. A total of 150 patients enrolled in the study and 366 nasal swab specimens were obtained. MRSA was detected by the 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 FAMT at the patient level were 86.7%, 85.2%, 39.4%, and 98.3%, respectively, when compared with the culture-based results. FAMT can effectively detect MRSA colonization, which may remain undetected with the conventional method, and can be useful in detecting newly acquired MRSAs.
INTRODUCTION

Hospital-acquired infections are a clinical concern and their management and control are important for the reduction 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 and can undergo 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, automation systems for microbiological molecular analysis are expected to reduce these concerns. Recently, fully automated molecular test (FAMT) for detection of microorganisms have been developed and comprises 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 prevent microbiological contamination and minimize technical errors.

We have previously reported the utility of molecular diagnostic methods for the active surveillance of MRSA in a respiratory ward (4). Recently-developed FAMTs for detecting MRSA, BD MAX MRSA, have 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 detects MRSA by targeting the SCCmec-orfX junction.
In this study, we conducted active surveillance of MRSA in an emergency medical center (EMC) in Japan using a 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 the 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

A microbiological surveillance of MRSA colonization was performed. Nasal swab specimens from all enrolled patients were obtained within 48 hours after admission to the EMC of NUH. All swab specimens were plated directly onto MRSA selective agar with oxacillin (Nippon Becton Dickinson) and assayed using a FAMT, BD MAX MRSA (Nippon Becton Dickinson, Tokyo, Japan).

BD MAX MRSA was performed according to the manufacturer's instructions. The same nasal swab specimens were incubated in trypticase soy broth (Nippon Becton Dickinson, Tokyo, Japan) 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 FAMT were performed once a week until the day of patient discharge. When MRSA was identified within the first 48 hours after EMC admission, culture screening and FAMT in the following weeks were discontinued. The 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. The elevation of these inflammatory markers in the absence of the symptoms indicated inactive MRSA infection. The elevation of inflammatory markers 48 hours after admission into the EMC concomitantly with the presence of active symptoms indicated hospital-acquired infection. The 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 following routes of admission were 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 FAMT when 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 with 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 the QIAamp DNA Mini Kit (Qiagen), and validated by 2 molecular analyses, represented by PCR analysis for \textit{mecA} gene detection as reported previously (6) and automated multiplex PCR using the BD MAX StaphSR assay (Nippon Becton Dickinson, Tokyo, Japan). BD MAX StaphSR is a fully automatic multiplex PCR assay for the detection of MRSA and methicillin-sensitive \textit{S. aureus}. BD MAX StaphSR can detect nucleases (\textit{nuc}), \textit{mecA} genes including \textit{mecC}, and the staphylococcal cassette chromosome \textit{mec} (SCCmec) right-extremity junction (MREJ).

Statistical analysis

The categorical variables were analyzed using McNemar's test. A \(P\)-value 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 age was 61 years (range, 16–92) and 101 patients (67.3%) were male. Of the total, 128 (85.3%) were directly admitted to the EMC from their homes, among which 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 2. 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 hospitalization history, and the remaining 5 had no hospitalization history. Four patients admitted from other medical facilities tested positive using FAMT (1 came from a nursing home and 3 came from hospitals), whereas 13 patients had no hospitalization or nursing home admission history. Of the 11 culture-negative/PCR-positive patients, 2 were positive for MRSA after broth enrichment culture.

A follow-up was conducted on 96 patients. During follow-up, the culture-based screening detected MRSA at the second sampling point in only 1 (1.0%) patient who had a history of hospitalization, whereas FAMT detected MRSA in 13 (13.5%) patients.
(p<0.01). Of the PCR-positive patients, 5 were admitted from other medical facilities, 1 had a hospitalization history, and 7 had no admission history. During follow-up, 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 3). In 12 samples, at least one of the following genes was detected using the automated multiplex PCR: \( \text{nuc}^+\text{mecA}^+\text{MREJ}^- \) (n=2), \( \text{nuc}^-\text{mecA}^+\text{MREJ}^+ \) (n=3), \( \text{nuc}^-\text{mecA}^-\text{MREJ}^+ \) (n=1), and \( \text{nuc}^-\text{mecA}^+\text{MREJ}^- \) (n=6). In the other 13 samples, no genes were detected. The \text{mecA} \) gene was detected by home brew PCR in 7 samples: in these samples, 1 was \( \text{nuc}^+\text{mecA}^+\text{MREJ}^- \), 5 were \( \text{nuc}^-\text{mecA}^+\text{MREJ}^- \), and 1 was \( \text{nuc}^-\text{mecA}^-\text{MREJ}^- \).

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 \textit{Staphylococcus} (MRCNS). The other 2 isolates were confirmed to be MRSA and their counts were \( 1 \times 10^6 \) CFU/mL and \( 1 \times 10^2 \) CFU/mL. The 2 culture-confirmed MRSA isolates were also confirmed to be positive for FAMT but one of the two was negative for \text{mecA} \) by BD MAX StaphSR and home brew PCR.
Performance testing of FAMT

In this study, 366 swabs were obtained, and culture screening and FAMT results were compared (Table 3). When 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, sensitivity, specificity, positive and negative predictive values during full observation period were 86.7%, 85.2%, 39.4%, and 98.3%, respectively (Table 4).

DISCUSSIONS

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 involving 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 the reduction of subsequent MRSA infections and can lead to decreased morbidity and mortality.

At NUH, approximately 1,000 S. aureus isolates are detected every year from all clinical specimens and almost 60% are MRSA isolates, and this frequency was similar to that obtained in a 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 above, Japan is known to have a high prevalence of MRSA. In this study, PCR-based screening detected MRSA in 14.0% of patients at the initial screening, and this prevalence was higher than that obtained 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 rate was 27.7% and 19.9% by PCR and culture identification, respectively (9). In an emergency unit, 11% to 31.4% of patients were positive for MRSA by culture screening or molecular testing (10, 11). In 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 carried MRSA most frequently. 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% and 12.5% by culture screening and PCR, respectively, in contrast to 1.9% for those admitted to a respiratory ward. Although we did not evaluate the SCCmec 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 lower number of bacteria (16-18). This advantage could improve 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 other hand, recent studies revealed several problems regarding
molecular-based MRSA detection. At present, targeting the SCCmec-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 SCCmec-orfX junction but a non-functional mecA gene, as well as
empty-cassette variants, can yield culture-negative/PCR-positive results (20). The
identification by targeting the SCCmec-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 with the automated multiplex PCR, 4 strains that were positive for the
SCCmec-orfX junction but negative for mecA could be empty-cassette variants.
Furthermore, a mecA-gene homolog, mecC gene, carried by emerging MRSA strains,
can be considered an additional target in the molecular diagnosis of MRSA (22). The
prevalence of mecC 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 observed a mecA negative MRSA. Further examination will
be required to confirm whether the isolate is a particular one including mecC MRSA.

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 with clinical specimens and the latter with
extracted DNA. Therefore, the results of BD MAX StaphSR may have been affected by the extraction efficiency. With regards to the samples that were positive by 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, the discrepancy between molecular methods was observed in 12 samples in the present study. We believe that this finding might be mainly due to different samples; 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 of FAMT were observed. In contrast to assays using cultured strains, the direct molecular analysis using clinical specimens can contain certain substances that inhibit the process of DNA extraction or PCR. In addition, 1 of the 2 false-negatives contained extremely small number of bacteria. Therefore, this sample might be under the detection limit.

In summary, FAMT of MRSA, is a useful tool for screening nasal colonization by MRSA. Compared to culture-based methods, FAMT was highly sensitive for the detection of MRSA. Since the 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.

ACKNOWLEDGEMENTS
We would like to thank A. Nagatani, K. Yamashita, T. Hirao, T. Inokuma, and H. Izumino, G. Tajima for sample collection and data acquisition. We would like to thank the EMC nursing staff for sample collection.

CONFLICTS OF INTEREST AND FINANTIAL DISCLOSURE

This study was supported by a grant from Nippon Becton Dickinson Co., Ltd. (Tokyo, Japan).

REFERENCES

9 Kanemitsu K, Yamamoto N, Imafuku Y, et al. The capability of mrsa active surveillance to reduce...

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

<table>
<thead>
<tr>
<th>Pre-admittance conditions</th>
<th>Initial screening, n (%)</th>
<th>Follow-up, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All patients</td>
<td>Culture</td>
</tr>
<tr>
<td>Other medical facilities</td>
<td>16</td>
<td>3 (18.8)</td>
</tr>
<tr>
<td>Nursing home</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Home, with a history of hospitalization in the previous year</td>
<td>24</td>
<td>2 (8.3)</td>
</tr>
<tr>
<td>Home, with no hospitalization or admission history in the previous year</td>
<td>104</td>
<td>5 (4.8)</td>
</tr>
<tr>
<td>Unknown</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>10 (6.7)</td>
</tr>
</tbody>
</table>

FAMT: fully automated molecular test
<table>
<thead>
<tr>
<th>Automated multiplex PCR</th>
<th></th>
<th>meca positive by home-brew PCR,</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results</td>
<td>Number of samples</td>
<td>n</td>
</tr>
<tr>
<td>nuc⁺meca⁺MREJ⁻</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>nuc⁺meca⁻MREJ⁺</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>nuc⁻meca⁻MREJ⁺</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>nuc⁻meca⁺MREJ⁻</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>nuc⁻meca⁻MREJ⁻</td>
<td>13</td>
<td>1</td>
</tr>
</tbody>
</table>

MREJ: staphylococcal cassette chromosome meca right-extremity junction

PCR: polymerase chain reaction

MRSA: methicillin-resistant *Staphylococcus aureus*
Table 3. Performance testing for FAMT at the sample level

<table>
<thead>
<tr>
<th>Culture</th>
<th>FAMT</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>14</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>Negative</td>
<td>25</td>
<td>324</td>
<td>349</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>327</td>
<td>366</td>
</tr>
</tbody>
</table>
Table 4. Performance testing for FAMT at the patient level

<table>
<thead>
<tr>
<th>Culture</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>13</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Negative</td>
<td>20</td>
<td>115</td>
<td>135</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>117</td>
<td>150</td>
</tr>
</tbody>
</table>