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ORIGINAL ARTICLE

Evaluation of the BD GeneOhm StaphSR assay for detection of *Staphylococcus aureus* in patients in intensive care units

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Screening

Background: *Staphylococcus aureus* is the major nosocomial pathogen and rapid detection of colonized patients with subsequent precaution is needed to prevent transmission. A new assay, the BD GeneOhm™ SaphSR assay (BD GeneOhm™, San Diego, CA, USA), is a multiplex real-time polymerase chain reaction (PCR) for rapid detection of both methicillin-sensitive *S aureus* (MSSA) as well as methicillin-resistant *S aureus* (MRSA).
Methods: Anterior nasal swab specimens of 273 pediatric and adult patients hospitalized in intensive care units at Chang Gung Memorial Hospital were collected for this assay in parallel with conventional cultures as standard.
Results: Overall, 71 (26.0%) patients were colonized with *S aureus* by conventional culture and MRSA accounted for 67.6% of all isolates. For the detection of MRSA, 79 patients (28.9%) were positive by PCR and 48 (17.6%) were positive by conventional cultures. The sensitivity, specificity, and positive and negative predictive values were 95.9%, 85.3%, 58.5%, 99.0%, respectively. For the detection of MSSA, 48 patients (17.6%) were positive by PCR and 23 (8.4%) were positive by conventional culture. The sensitivity, specificity, and positive and negative predictive values were 91.3%, 89.2%, 43.8%, and 99.1%, respectively.
Conclusion: As a screening method, the BD GeneOhm™ StaphSR assay could rapidly detect and differentiate between MRSA and MSSA colonization. A negative result of the assay could almost exclude *S aureus* colonization.

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Introduction

Staphylococcus aureus, regardless of methicillin-resistance (MRSA) or methicillin-susceptibility (MSSA), is among the major nosocomial pathogens worldwide, including in Taiwan.[1] They can cause wide spectrum of infections from systemic infections, such as sepsis and catheter-related infections, to local infections, such as skin and soft tissue infections or surgical site infections. Patients colonized with *S aureus*, especially MRSA, may spread to other patients and can serve as reservoirs for subsequent infections.[2,3] Compared with MSSA, infections caused by MRSA are associated with higher mortality, hospital stay, and costs.[4,5]

In recent years, hospital infection controls have put emphasis on rapid detection of MRSA-colonized patients to improve patient care. Some reports had showed that active surveillance of MRSA colonization might decrease relevant mortality and morbidity,[6,7] and these measures had became a part of new guidelines by Center for Disease Control and Prevention of the United States.[8] In many European countries, recommendations are made for MRSA screening on cultures from nares, skin, or mucosa.[9] In the Netherlands, the "search-and-destroy" methods even had decreased the MRSA prevalence of all staphylococcal infections to less than 1%.[10]

To detect MRSA/MSSA colonization, conventional culture requires 48—96 hours. Some selective agars, such as CHROMagar MRSA medium (BD Diagnostics, Sparks, MD, USA), MRSA ID (bioMerieux, Portland, USA), MRSASelect (Bio-Rad Laboratories, CA, USA) could shorten turnaround time within 18—24 hours.[11,12] Because early detection of MRSA/MSSA colonization would facilitate identification of carriers and therefore allow early intervention to prevent spread or infections, several nucleic acid amplification-based assays, such as BD GeneOhm MRSA real-time polymerase chain reaction (PCR) assay (BDGeneOhm, San Diego, CA, USA), hyplex StaphyloResist multiplex PCR-ELISA system (BAG, Lich, Germany), GenoType MRSA Direct (Hain Lifescience, Nehren, Germany), and IDI-MRSA assay (Infecto Diagnostics, Inc., Sainte-Foy, Quebec, Canada) have been developed and can yield results within 2 hours.[13—15] However, these assays could only identify MRSA but could not identify MSSA.

A new assay, called BD GeneOhm™ StaphSR assay (BD GeneOhm, San Diego, CA, USA), is a multiplex real-time PCR method that uses primers specific for various staphylococcal cassette chromosome (SCC) *mec* right-extremity sequences and a probes and primers specific for the *S aureus* chromosomal *orfX* gene to the right of the SCC *mec* insertion site.[14,15] This assay is the first product that offers not only rapid detection of *S aureus* but also differentiation between MRSA and MSSA. Preliminary results[16—18] showed fair value in rapidly differentiating bacteremia caused by MRSA and MSSA from bloodstream samples. However, additional specimen claims, including nasal or wound samples, are under study. In this article, we evaluated the performance of this assay in parallel with conventional cultures as standard for detection of MRSA and MSSA from anterior nares specimens of pediatric and adult patients hospitalized in intensive care units (ICUs) to determine the usefulness for rapid screening.

Material and methods

This study was conducted at Chang Gang Memorial Hospital, a 4000-bed, university-affiliated teaching hospital and was approved by the Institutional Review Board.

Patients

A total of 273 patients, including 167 adults (80 in surgical ICUs and 87 in medical ICUs), 15 children in pediatric ICUs, and 91 newborns in neonatal ICUs, hospitalized in ICUs at Chang Gung Memory Hospital were screened for MRSA/MSSA nasal colonization. Patients who had very unstable vital signs or those with pulmonary tuberculosis were excluded. These patients were not known to previously be colonized or infected with MRSA/MSSA.

Specimen collection

In each patient, nasal swab specimen was collected from the anterior nares using two separate dry Copan Transystem Liquid Stuart swabs (Venturi Transystem; Copan Diagnostics, Corona, CA, USA). Each swab was rubbed inside the anterior nares, first into one side and then into the other, ensuring that each swab contained specimens of both nares of each patient. These swabs were then transported at room temperature and processed within 4 hours.

Conventional culture and broth enrichment

One of two swabs from each patient was inoculated into trypticase soy agar with 5% sheep blood (TSA II 5% SB) plate (Becton, Dickinson and Comapany, Sparks, MD, USA) for conventional culture. Isolates of *S aureus* and MRSA identification by oxacillin susceptibility with the disc diffusion methods were confirmed according to the recommendations of Clinical and Laboratory Standards Institute.

Another swab from the same patient was sent for BD GeneOhm StaphSR assay. If the results showed positive for MRSA or MSSA, this swab would be put into Mueller Hinton Broth (Becton, Dickinson and Company, Sparks, MD, USA) in CO_2 incubator at 37°C overnight and then was subcultured into TSA II 5% SB plate and identified using same methods.

BD GeneOhm StaphSR assay

The BD GeneOhm StaphSR assay uses the same primers and probes for MRSA detection as the BD GeneOhm MRSA real-time PCR assay. These primers and probes have been described in details previously.[19] The MRSA assay uses primers specific for various SCC*mec* right-extremity sequences and a primer and probes specific for the *S aureus* chromosomal *orfX* gene located to the right of the SCC*mec* insertion site.

Specimen preparation

The assay was performed according to the manufacturer's instructions. One of two swabs from one patient was inoculated into 1 mL of buffer in a tube labeled with sample number. The sample buffer tube was subjected to a vortex at high speed for 1 minute, and 600—700 μL of cell suspension

was transferred into a dry lysis tube containing glass beads. The lysis tube was then subjected to a vortex for 5 minutes at high speed, then the supernatant was removed. Fifty microliters of sample buffer was added to the lysis tube and the tube was again subjected to a vortex for 5 minutes at high speed, centrifuged briefly (quick spin), and then heated at 95°C for 2 minutes to inactivate possible PCR inhibitors. The sample was placed on ice for immediate testing or refrigerated at 2°C—8°C for 4 hours or at −20°C until the assay was performed.

Assay procedure

The 25μL Master Mix was reconstituted and aliquoted into labeled SmartCycler tubes (Cepheid, Sunny Vale, CA, USA). Three microliters of the sample from the lysis tube was added to the corresponding labeled SmartCycler tube. Positive and negative PCR controls were also prepared and included with each run. All reagents and samples were kept at 2°C—8°C. The SmartCycler tubes were centrifuged with high speed. To detect inhibition material of the PCR, the assay includes a non-*S aureus* sequence, which serves as an internal control. The PCR results were interpreted using the SmartCycler software with decisional algorithm to interpret the assay result. If the samples contain both MRSA and MSSA, the result would only show MRSA.

Results

Overall results

Overall, 71 (26.0%) patients were colonized with *S aureus* by conventional culture and MRSA accounted for 67.6% of all isolates. For MRSA detection, 79 patients (28.9%) were positive by PCR method and 48 (17.6%) were positive by conventional culture. BD GeneOhm StaphSR assay, compared with conventional agar culture for MRSA detection, had a sensitivity of 95.9%, specificity of 85.3%, positive predictive value of 58.5%, and negative predictive value of 99.0% (Table 1). For MSSA detection, 48 patients (17.6%) were positive by PCR method and 23 (8.4%) were positive by conventional culture. BD GeneOhm StaphSR assay, compared with conventional agar culture for MSSA detection, had a sensitivity of 91.3%, specificity of 89.2%, positive predictive value of 43.8%, and negative predictive value of 99.1% (Table 2). Only two (0.5%) samples produced false negative results for MRSA detection and 2 (0.7%) for MSSA detection.

Broth culture

MRSA PCR showed 30.3% of culture as positive (10/33) and 18.5% showed (5/27) MSSA PCR positive but conventional culture-negative samples showed growth on broth culture. Broth culture increased positive predictive value from 58.5% to 70.9% for MRSA detection and from 43.8% to 54.2% for MSSA detection.

Discrepant results

Three samples showed discrepant results. Sample 1 yielded MRSA in PCR but MSSA in conventional and broth culture. Sample 2 yielded MSSA in PCR but MRSA in conventional culture and no growth in broth culture. Sample 3 yielded MRSA in PCR and conventional culture but MSSA in broth culture.

Discussion

To our knowledge, this is the first report for the performance of the BD GeneOhm StaphSR assay from anterior nares samples to detect staphylococcal colonization and also to differentiate between MRSA and MSSA. Compared with conventional culture (as standard), the BD GeneOhm StaphSR assay showed a high sensitivity (>90%), a high specificity (>85%), a high negative predictive value (>99%) but a relative low positive predictive value (<60%) for both MRSA and MSSA detection. These results were comparable with those reported previously regarding BD GeneOhm StaphSR assay for bloodstream samples, except for positive predictive value.

In the present study, a large portion of samples showed PCR-positive but culture-negative results. A simple reason was that there were no viable bacteria for growth form culture but residual DNA was successfully amplified by PCR. This situation might occur after exposure to antibiotics or decolonization therapy, but it was not evaluated in this study. Secondly, the culture samples were collected from anterior nares harboring polymicrobial colonization environment, which was different from that of bloodstream sample. These microorganisms and their products or cytokines might affect MRSA/MSSA growth in culture. As reported,[16] CoNS (coagulase-negative staphylococci) may obscure or inhibit MSSA growth in culture. In this study, we only identified MRSA or MSSA colonies from culture plate but ignored other colonies. If we analyzed all colonies growing on culture plate, the results might be a little different. The other possibility was non-*mec*A-containing SCC elements[19,20] or unamplifiable *mec*A gene, which were carried by some CoNS or MSSA strains and may result in detectable SCC elements targeted by BD GeneOhm StaphSR assay and false PCR-positive as MRSA or MSSA. As reported by other authors,[14,21] about 5% specimens identified as MRSA by BD GeneOhm MRSA assay were MSSA phenotype. This phenomenon provided the view of heterogenecity of SCC*mec* and needed further investigation.

Because the colonization and disease burden of MRSA/MSSA continue to increase worldwide, a new PCR-based assay with high specificity and positive predicative value must be developed. By BD GeneOhm StaphSR assay, PCR-positive results are only preliminary and require confirmation of final culture reports. In addition, the new BD GeneOhm StaphSR assay costs about $35 per patient, higher than $25 for the old BD GeneOhm MRSA Assay and $4.59 for CHROMagar MRSA.[22] Although MSSA could also cause serious nosocomial infection, nasal carriers of MRSA were much more likely to develop staphylococcal bacteremia than nasal carriers of MSSA.[23] Whether it is worth to spend additional price to simultaneously detect MRSA and MSSA colonization in nonsterile sites is a problem of cost effectiveness.

There were only four false-negative (PCR-negative but culture-positive) samples, including two MRSA and two MSSA. It was possible that because of either low levels of

Table 1 BD GeneOhm StaphSR assay diagnostic performance compared with conventional streaking method for MRSA detection in patients in ICUs

BBL™ TSA II 5% SB plate/ BD GeneOhm StaphSR assay	Positive	Negative[a]	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Adult ICUs ($n = 167$)						
Positive	28	23	100	83.5	54.9	100
Negative[a]	0	116				
Surgical ($n = 80$)						
Positive	7	9	100	87.7	43.8	100
Negative	0	64				
Medical ($n = 87$)						
Positive	21	14	100	78.8	60	100
Negative	0	52				
Pediatric ICUs ($n = 106$)						
Positive	18	10	90	88.3	64.2	97.4
Negative	2	76				
Pediatric ($n = 15$)						
Positive	5	3	100	70.0	62.5	100
Negative	0	7				
Newborn ($n = 91$)						
Positive	13	7	86.6	90.8	65.0	97.2
Negative	2	69				
Total ($n = 273$)						
Positive	46	33	95.9	85.3	58.5	99.0
Negative	2	192				

[a] Results showing negative or growth of MSSA.
ICU = intensive care unit; MRSA = methicillin-resistant *Staphylococcus aureus*; NPV = negative predictive value; PPV = positive predictive value; TSA II 5% SB plate = trypticase soy agar with 5% sheep blood.

colonization or poor swabbing technique, the quantity of MRSA/MSSA on the swabs from anterior nares were below the detection limit of the BD GeneOhm StaphSR assay but were enough to grow in culture. The limit of BD GeneOhm StaphSR assay had been reported to be 15 DNA copy numbers per reaction mixture or 10 colony forming units per reaction mixture for both MRSA and MSSA[16] or to be 10^3 colony forming units/specimen for MRSA.[24] Besides, these samples were collected from anterior nares where other microorganisms, such as CoNS or *Streptococcus* species may also colonize; some inhibitors may interfere with PCR. Because BD GeneOhm StaphSR assay was designed to target a highly conserved sequence that bridges the *orfX* and the major variant SCC*mec* Types I to IV, a novel SCC*mec*-harboring MRSA strain may also produce false-negative results. Contamination of the culture during procedure may produce false-positive culture report but it was least likely. The low false-negative rate produced almost 100% negative predictive value (99.0% in MRSA and 99.1% in MSSA), which were compatible with other studies in literature.[25–27] The high negative predictive value suggests that in a population with a low prevalence of MRSA, BD GeneOhm StaphSR assay can serve as a rapid method for screening people who are not colonized; and for people who are tested PCR-negative, culture might be spared to save unnecessary costs and decrease complexity of assay algorithms.

Besides conventional culture using TSA II 5% SB plate, we added broth enrichment culture for PCR-positive samples. PCR-negative samples were not sent for broth enrichment culture in our study. By adding broth culture, we detected additional 10 MRSA and 5 MSSA cultures from PCR-positive but conventional culture-negative samples and therefore increased positive predictive value from 58.5% to 70.9% in MRSA and 43.8% to 54.2% in MSSA. However, broth culture required additional overnight incubation, technique, and costs. Because of high negative predictive value of BD GeneOhm MRSA assay, we suggested broth culture only in PCR-positive samples to decrease false-positive rate.

Three samples showed discrepant results. As aforementioned, non-*mec*A-containing SCC elements[19,20] or unamplifiable *mec*A gene may explain the two samples showing MRSA in PCR but MSSA in culture. Another explanation is the MRSA/MSSA co-colonization at anterior nares and therefore PCR and culture detected different strains respectively. Although study showed that MRSA and MSSA competed for colonization space, MRSA/MSSA co-colonization at anterior nares did occur in few patients.[28,29]

There are some limitations in this study. We did not collect patients' background data, including age, diseases, previous MRSA/MSSA colonization status, and antibiotics exposure. For samples of false-positive, false-negative, and discrepant results, we neither performed double check nor further molecular test, such as *mec*A PCR or PBP2' assay to confirm the PCR or culture results. Besides, if the swabs contained both MRSA and MSSA, the BD GeneOhm StaphSR Assay used in our study only showed MRSA.

Table 2 BD GeneOhm StaphSR Assay diagnostic performance compared with conventional streaking method for MSSA detection in patients in ICUs

BBL™ TSA II 5% SB plate/ BD GeneOhm StaphSR assay	Positive	Negative[a]	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Adult ICUs ($n = 167$)						
Positive	9	18	100	83.5	54.9	99.3
Negative[b]	1	139				
Surgical ($n = 80$)						
Positive	7	9	100	87.7	43.8	100
Negative	0	64				
Medical ($n = 87$)						
Positive	2	9	66.7	89.3	18.2	98.7
Negative	1	75				
Pediatric ICUs ($n = 106$)						
Positive	12	9	92.3	90.3	57.1	98.8
Negative	1	84				
Pediatric ($n = 15$)						
Positive	2	0	66.7	100	100	92.3
Negative	1	12				
Newborn ($n = 91$)						
Positive	10	9	100	88.9	52.6	100
Negative	0	72				
Total ($n = 273$)						
Positive	21	27	91.3	89.2	43.8	99.1
Negative	2	223				

[a] Results showing negative or growth of MRSA.
[b] Results showing negative or growth of MRSA.
ICU = intensive care unit; MRSA = methicillin-resistant *Staphylococcus aureus*; NPV = negative predictive value; PPV = positive predictive value; TSA II 5% SB plate = trypticase soy agar with 5% sheep blood.

In summary, as a screening method, the BD GeneOhm StaphSR assay could rapidly detect and differentiate between MRSA and MSSA colonization in humans. A negative result of the assay could almost exclude *S aureus* colonization, but a positive result should require culture to confirm it. Although this assay was relatively expensive, there may be an overall cost savings, especially for high-risk populations. Adequate prevention strategy, such as mucipurin decolonization should be implemented to avoid subsequent infection or spread once detected.

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