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Detection of Methicillin-Resistant *Staphylococcus aureus* in Healthcare Workers Using Real-Time Polymerase Chain Reaction

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Healthcare-associated infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) have recently become an important issue for healthcare facilities due to high rates of infection, mortality, and high treatment costs. We investigated the frequency of MRSA in healthcare workers (HCWs) via nasal carriage and assessed the performance of the LightCycler® MRSA Advanced test. We tested nasal swabs from the anterior nares of participating HCWs at an intensive care unit. Nasal swabs were identified as *S. aureus*, methicillin-sensitive or methicillin-resistant coagulase-negative *staphylococci* (MSCoNS or MRCoNS), or MRSA by using conventional culture and the LightCycler® MRSA Advanced test. Of the 142 HCWs who participated in this study, only 11 participants (7.8%) were MRSA-positive by conventional culture and MRSA ID, and 24 (16.9%) were positive for *mecA* by real time polymerase chain reaction (PCR). In terms of diagnostic performance, the LightCycler® MRSA Advanced test had a sensitivity of 100%, a specificity of 90.1%, a positive predictive value of 45.8%, and a negative predictive value of 100% compared with conventional culture method. The detection limit of the LightCycler® MRSA Advanced test was 10^3 colony/mL. We concluded that real-time PCR was able to rapidly and sensitively detect MRSA in HCWs. However, MRSA must be confirmed by culture due to false positivity.

Key Words: Methicillin-resistant *Staphylococcus aureus*, *mecA* gene, real time PCR

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major pathogen that causes worldwide healthcare and community-acquired infections.[1] MRSA infections are associated with high morbidity and mortality rates, prolonged hospital stays, increased costs, and increased use of medical and personnel resources.[2,3]

The primary sources of MRSA infections are infected patients, healthcare personnel, and medical devices in healthcare settings.[4] According to meta-analysis, the average rate of MRSA colonization among healthcare workers (HCWs) is approximately 4.6% worldwide, and evidence suggests that HCWs are likely to play a large role in MRSA transmission.[5] Rapid and accurate identification of HCWs and patients carrying MRSA would, therefore, be helpful for preventing transmission and early therapeutic decisions.

Resistance of *S. aureus* to methicillin is primarily mediated by the *mecA* gene,

which codes for the modified penicillin-binding protein 2a (PBP2a) or PBP2'.[6] The LightCycler® MRSA Advanced test (Roche Diagnostics GmbH, Mannheim, Germany) is a qualitative assay used for nasal swab specimens, and involves amplification of the *mecA* gene by polymerase chain reaction (PCR) and detection of amplified DNA using fluorogenic target-specific hybridization probes.

In this study, we investigated the prevalence of *Staphylococcus* nasal carriage among HCW and assessed the performance of the LightCycler® MRSA Advanced test.

Nasal swab samples were collected from the anterior nares of participating HCWs at an intensive care unit (ICU). A double-headed swab (double-headed BBL culture swab, Liquid Stuart, Becton-Dickinson; Sparks, MD, USA) was inserted into the nostril and rotated against the mucosa five times.

One swab head was directly streaked onto a MRSA ID (bioMeriuex, La Balme et Craponne, France) and blood agar medium to identify MRSA. The plates were incubated at 35°C in O_2 and reviewed after 24 hr and 48 hr. Suggestive MRSA colonies were confirmed as *S. aureus* by coagulase and DNase tests. Methicillin resistance was confirmed using the cefoxitin disk diffusion method according to Clinical and Laboratory Standards Institute guidelines.[7]

The LightCycler® MRSA Advanced test was performed on the LightCycler 2.0 instrument using the manufacturer's instructions. The swab extraction and mechanical lysis performed using the MagNA Lyser instrument. The real-time PCR amplification use fluorogenic target-specific hybridization probes for detection of amplified DNA. Each LightCycler MRSA advanced test reaction mixture contained an internal control to detect specimen inhibition and to monitor reagent integrity. The technologist hands-on time was 45 min for the LightCycler® MRSA Advanced test (Roche Diagnostics GmbH, Mannheim, Germany).[1]

The limits of detection were evaluated using *S. aureus* ATCC33591, a methicillin-resistant strain. The isolate was prepared as 10^6 CFU/mL saline suspension and absorbed onto double-headed swabs. The 10^6 suspension was then diluted 10-fold from 10 to 10^6 CFU/mL and also absorbed onto double headed swabs. Each suspension was subjected to duplicate tests.

The LightCycler® MRSA Advanced test was performed twice for each sample to determine specificity using reference strains ATCC33591 (MRSA), ATCC29213 (MSSA), ATCC14990 (MS *S. epidermidis*), ATCC25922 (*E. coli*), ATCC27853 (*P. aeruginosa*), ATCC700323 (*E. cloacae*), ATCC29212 (*E. faecalis*), ATCC34449 (*C. listiniae*), ATCC700327 (*E. casseliflavus*), and ATCC49619 (*S. pneumoniae*), and five clinical MRSA isolates, five methicilin-susceptible *S. aureus* (MSSA) isolates, five methicilin-resistnat coagulase-negative *Staphylococcus* (MRCoNS) isolates, and five methicilin-susceptible coagulase-negative *Staphylococcus* (MSCoNS) isolates.

Of the 142 HCWs who participated in this study, 51 were resident physicians, 51 were nurses, 19 were nursing auxiliaries, and 21 were physicians. Eleven participants (7.8%) were MRSA-positive according to conventional culture and MRSA ID, while 24 (16.9%) were positive for *mecA* according to real-time PCR. Among the 13 discordant specimens, 6 were MRCoNS, 4 were MSSA, and 3 were MSCoNS. These 13 isolates were retested with coagulase, DNase and cefoxitin disk diffusion test for distinguising from MRSA. The results of these biochemical tests were same as the results of the first tests. Additionally, we tested *mecA* PCR using following primers: primer MR1: ATG AGA TTA GGC ATC GTT CC (645-664); primer MR2: TGG ATG ACA GTA CCT GAG CC (1992-1173).[8] Six MRCoNS among them showed resistance to 4 ug/mL cefoxitin disk and 4 MRCoNS were positive for *mecA* PCR. Therefore, we must consider further workup such as conventional culture, coagulase, and DNase for distinguising them from MRCoNS

Table 1. Performance of the LightCycler® MRSA Advanced Test and ChromID MRSA Compared with Conventional Culture Methods

Test	No. of samples (n=142)*				Results (%)			
	True positive	False positive	False negative	True negative	Sensitivity	Specificity	Positive predictive value	Negative predictive value
LightCycler® MRSA	11	13†	0	118	100	90.1	45.8	100
ChromID MRSA	11	17‡	0	114	100	87.0	39.3	100

MRSA, Methicillin-resistant *Staphylococcus aureus*.

**S. aureus* were 34 (23.9%) cases in conventional culture and 11 (32.4%) of these cases were MRSA.

†4 Methicillin-resistant coagulase negative *staphylococci* (MRCoNS) were positive for *mecA* PCR among 6 MRCoNS.

‡These isolates show pale green colony and identified as coagulase-negative *staphylococci* by futher biochemical tests.

when positive in the LightCycler® MRSA Advanced test. However, we had a limitation about the accuracy of this PCR assay because we performed additional PCR using in-house primer. These in-house primers were not as good as the LightCycler® MRSA Advanced test. Further studies on the prevalence and clinical importance of *mecA*-positive MRCoNS are needed.

In terms of diagnostic performance, the LightCycler® MRSA Advanced test demonstrated 100% sensitivity, 90.1% specificity, 45.8% positive predictive value, and 100% negative predictive value compared with the conventional culture method. The detection limit of the LightCycler® MRSA Advanced test was 10^3 CFU/mL. As for analytical specificity, all tested non-*S. aureus* species were found negative for *mecA* by using the LightCycler® MRSA Advanced test.

Culture-based MRSA detection methods, even those that utilize screening agar, are time consuming and result in a 2-4 day turnaround time for accurate results. Because carriers can spread MRSA during this time period, rapid detection of personnel who has been colonized by this pathogen is crucial in MRSA surveillance programs.[1] A variety of commercial molecular tests for detecting MRSA colonization that have high sensitivity and specificity and have potential for rapid detection were developed.[9-13] In the present study, the LightCycler® MRSA Advanced test was found to be a rapid detection assay of MRSA with high sensitivity. Furthermore, the LightCycler® MRSA Advanced test had high sensitivity (100%) and good specificity (90.1%) compared with conventional culture methods (Table 1). However, the test demonstrated a high rate of false positives. Therefore, the presence of MRSA must be confirmed by culture.

We observed that 34 (23.9%) of the HCWs in our sample were carriers of *S. aureus* strains, and that MRSA represented 11 (32.4%) of these cases (i.e., 7.8% of HCWs surveyed) (Table 1). The carriage rate of *S. aureus* and MRSA in the present study was slightly higher than those reported by previous studies,[4] however, our findings cannot be generalized because our sample consisted of ICU personnel.

In conclusion, the qualitative PCR amplification of *mecA* is a rapid and sensitive method for detecting MRSA in HCW. However, cultures must also be conducted to confirm results because of high rate of false positives when the PCR method is needed.

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