

LightCycler® MRSA Advanced Test for Rapid MRSA Detection in Referral Patients Admitted to Intensive Care Units



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OBJECTIVE: We studied the prevalence of Methicillin Resistant Staphylococcus Aureus (MRSA) nasal carrier in patients who were transferred and admitted to the intensive care unit (ICU).

MATERIALS AND METHOD: By using LightCycler® MRSA advanced test for the direct detection of MRSA DNA in nasal colonization by polymerase chain reaction (PCR) and comparing to standard MRSA isolated from plate culture media.

RESULTS: From December 2010 to May 2011, 100 patients were enrolled. They were referred to be admitted in ICUs of Bangkok Hospital Medical Center (BMC) (87), Samitivej Hospital Sukhumvit (12) and Bangkok Hospital Pattaya (1). Seventeen patients were excluded from the study. Of 83 patients, the LightCycler® MRSA test detected MRSA from an anterior nasal swab in 12 (14.5%) patients while concomitant plate culture grew MRSA in 10 (12.1%) (Kappa = 0.7913, 95% confidence interval (CI) = 0.594-0.988).

CONCLUSION: The LightCycler® MRSA advanced test is a diagnostic test for rapid MRSA detection. The test aids in the detection of hospital infection and in the control of MRSA infections by rapid detection, therefore identifying the appropriate and isolated patient whom has MRSA colonization particularly in high risk patients.

Methicillin Resistant Staphylococcus Aureus (MRSA) is now more acknowledged as an important in serious bacterial infection both in healthcare and community acquired settings.

Healthcare-associated MRSA (HA-MRSA) infection is defined as MRSA infection occurring following hospitalization or previously attended healthcare facility (e.g. dialysis, residence in a long term care setting) within 12 months.¹ The prevalence of HA-MRSA was reported as high with up to 60% among Staphylococcus aureus infections in the ICU.²

Community-associated MRSA (CA-MRSA) is defined as MRSA infection occurring in the absence of healthcare exposure. Most case reports of CA-MRSA are associated with skin and soft tissue infection in young adults.³

Transmission of the MRSA strain commonly occurs by transiently contaminated hands of healthcare workers or environmental contamination. Persons with MRSA colonization might have a higher risk of MRSA infection than those without and also serve as a reservoir for transmission.²

The HA-MRSA strain tends to carry the *mecA* gene.^{4,5} This encode of the penicillin binding protein (PBP2A) permits the organism to be resistant to methicillin and other beta-lactam antibiotics.

The *mecA* gene is located on a mobile genetic element called Staphylococcal chromosome cassette (SCCmec). Most HA-MRSA clones are associated with SCCmec type I, II and III which are multidrug resistant⁶ while CA-MRSA strains have SCCmec type IV and V which are formerly susceptible to other antibiotics.⁷ The aim is to prevent and control MRSA infection in hospitals by preventing MRSA cross-infection. Active surveillance culture to detect patients with MRSA colonization would benefit early isolation precautions particularly in high risk settings of MRSA infection, such as patients in ICUs, immunocompromised patients, patients on hemodialysis and patients in long-term care units.^{8,9} Standard plate culture media for bacterial detection and identification usually takes 2-3 days. The LightCycler[®] MRSA advanced test is a qualitative in vitro diagnostic test for the direct detection of nasal colonization with MRSA by polymerase chain reaction (PCR) with amplified MRSA DNA and a fluorogenic target specific hybridization probe for the detection of amplified DNA. The test takes under 2 hours of laboratory time.¹⁰

Our study is to find the prevalence of MRSA nasal carriers of patients whom were referred to our ICUs by using the rapid advanced MRSA test for rapid MRSA detection and comparing this to the bacterial isolation by standard plate culture media.

Materials and Methods

The study was carried out prospectively at three study sites under the Bangkok Hospital Group, i.e., BMC, Samitivej Hospital Sukhumvit and Bangkok Hospital Pattaya. The study included patients who were transferred to be admitted to ICUs at the study sites. They were screened for study eligibility. The eligible patients were patients whose age was > 18 years, who had been hospitalized in transferring hospitals for more than 24 hours prior to the transfer, and provided written informed consent to participate in the study.

For each patient, two nasal swabs were performed for MRSA identification using a standard plate culture for MRSA isolation and the LightCycler[®] MRSA advanced test, a real-time PCR based diagnostic testing for MRSA DNA. Both laboratory diagnostic tests were done by “N-Health”, the certified laboratory center of this study.

Clinical information of the enrolled patients was recorded in separate case record forms.

Laboratory Procedure

- Culture and susceptibility testing for MRSA

Standard plate culture and susceptibility tests for MRSA identification were performed according to standard

microbiology laboratory manual references of the National Committee on Clinical Laboratory Standards.

- LightCycler[®] MRSA advanced test

LightCycler[®] MRSA advanced test was performed according to the manufacturer's instructions. The test comprised 3 processes as follows:

- i. Specimen preparation. The nasal swab was processed through mechanical lysis by using the LightCycler[®] Advanced Lysis Kit and the MagNA Lyser Instrument.
- ii. PCR amplification and specific hybridization probes. The lyse specimen was transferred to LightCycler[®] 2.0 Instrument (manufactured by Roche Molecular System, Inc., Branchburg, NS08876 USA) for PCR amplification of targeted DNA and hybridization probes for detection of the targeted DNA.
- iii. Automated result generation. After meeting peak analysis, the PCR result was interpreted and reported by LightCycler[®] Software 4.05 as negative (i.e., no MRSA DNA detected), positive (i.e., MRSA DNA detected), or invalid (i.e., no internal control detected).

Statistical analysis

The estimated sample size of the study population was 71, which was calculated by following formulation.

$$n = \frac{Z_{\alpha/2}^2 P (1 - P)}{e^2}$$

Based on previous surveillance data of MRSA, the proportion of MRSA nasal colonization among patients transferred to BMC (p) was 0.015. The precision of the estimate (e) was 0.03 and the dropout rate was 0.1.

All case record forms were transferred to Bangkok Dusit Medical Service (BDMS) Research Center for data management and data analysis. The Statistical Package for Social Service (SPSS) Software version 19 was used for statistical analysis. The agreement of positive and negative results between the LightCycler[®] MRSA advanced test and the standard plate culture for MRSA isolation were determined by Cohen's kappa coefficient. Risk factors associated with MRSA nasal colonization were determined by the chi-square test and t-test of which *p*-value ≤ 0.05 was considered statistically significant.

Results

From December 2010 to May 2011, a total of 100 patients were transferred to be admitted to ICUs of the 3 clinical study sites. These were in the ICU of BMC (87), Samitivej Hospital Sukhumvit (12) and Bangkok Hospital Pattaya (1). Seventeen (17%) patients excluded from the study were due to were the exclusion criteria of having been admitted less than

24 hours before (6), age less than 18 year old (4), specimen for PCR test was not valid (3), admission outside ICU and no informed consent for specimen collection (2).

The total of 83 patients were eligible for the study, 60 (72.3%) male and 23 (27.7%) female. Their mean age was 62.7±17.7 years. Fifty-eight (69.9%) are of Thai Nationality. While 25 (30.1%) patients are foreigners (Europeans 13, middle eastern patients 4 and Asian patients 8), 11 patients were transferred directly from other countries. Sixty-nine (83.1%) had underlying diseases. Most common diseases are hypertension 65.1%, diabetes mellitus 37.3%, coronary heart disease 21.7%, and chronic renal failure 19.3% (Table 1).

The results of nasal MRSA colonization tested by the LightCycler® MRSA advanced test and the standard plate culture isolation are presented in Table 2.

Table 1: Summary of clinical characteristics of enrolled patients.

Characteristic	n (%)
Total	83 (100)
Male	60 (72.3)
Female	23 (27.7)
Age Mean	62.7±17.7
Nationality	
Thai	58 (69.9)
Foreigner	25 (30.1)
Underlying disease	
Present	69 (83.1)
No	14 (16.9)
Location of referral hospital	
Thailand	72 (86.7)
Other countries	11 (13.3)
Medical treatment before transfer	
Previous infection	
No	43 (51.8)
Yes	40 (48.2)
Previous surgery	
No	66 (79.5)
Yes	17 (20.5)
Clinical active infection on arrival	
No	35 (42.2)
Yes	48 (57.8)
Medical device	
Present	62 (74.7)
None	21 (25.3)

The results showed good correlation between the LightCycler® MRSA advanced test and the standard plate culture. The advance MRSA test detected up to 12 patients (14.5%) while the plate culture isolated MRSA detected 10 patients (12.1%), (Table 3) (Kapp = 0.791, 95% CI = 0.594-0.988).

The risk factors for MRSA colonization of these referred patients were significantly related to the history of previous infections from those hospitals (Table 4).

Table 2: Results of the LightCycler® MRSA advanced test and Standard plate culture.

Prevalence of MRSA	Positive n (%)	Negative n (%)	Total n (%)
MRSA plate culture	10(12.1)	73(87.9)	83(100)
MRSA Advanced test	12(14.5)	71(85.5)	83(100)

Table 3: Correlation between results of the LightCycler® MRSA advanced test and Standard plate culture.

LightCycler® MRSA advanced test	Plate culture		Total
	MRSA +ve	MRSA -ve	
MRSA +ve	9	3	12
MRSA -ve	1	70	71
Total	10	73	83

Kapp 0.791, 95% CI = 0.594-0.988

Table 4: Risk factor for MRSA colonization in referral patients.

History	Result of plate culture		p value
	MRSA +ve n (%)	MRSA -ve n (%)	
Previous infection			
No	1(10.0)	42(57.5)	0.006*
Yes	9(90.0)	31(42.5)	
Previous surgery			
No	8(80.0)	58(±9.5)	0.968*
Yes	2(20.2)	15(20.5)	
Length of stay in previous hospital (days)	10.6±58	12.2±29.6	0.866**
Medical device insertion before			
No	1(10.0)	20(27.4)	0.235*
Yes	9(90.0)	53(72.6)	

* Chi-square test ** t-test

Impact of Hand Hygiene improvement with percentage resistance of *Staphylococcus aureus*

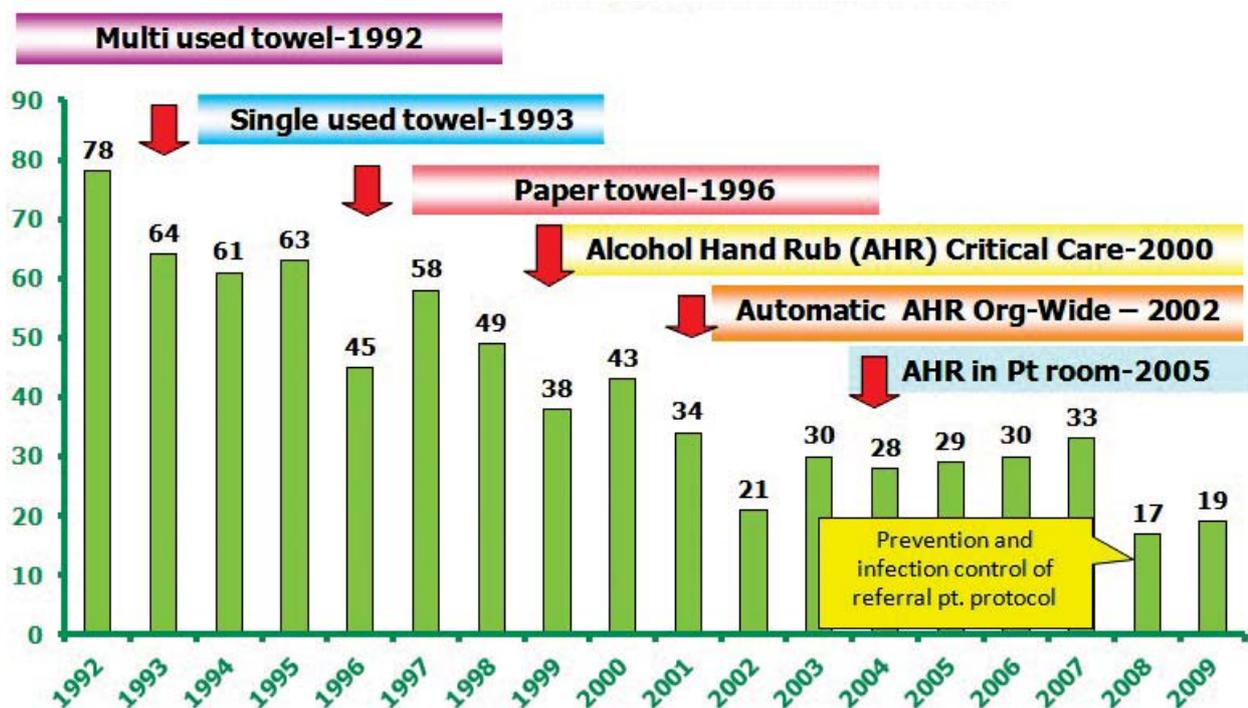


Figure 1: Impact of Hand Hygiene.

Discussion

Our experience of controlling MRSA in hospitals¹¹ requires a multi-strategy approach to good infection control practices including hand hygiene, patient isolation and preventive transmission activities particularly by improving hand washing compliance methods. The improvement of hand hygiene by supplying more equipment for hand washing and the introduction of alcohol hand rub alternatives for hand hygiene that is easily accessible is important. We found that the prevalence of MRSA gradually declined (Figure 1).

Active surveillance culture of MRSA is a method to identify the patient who has MRSA colonization or infection before entering the hospital. The benefit of active surveillance culture appears to be useful in the setting of outbreaks in hospitals and in certain patients at high risk for MRSA infection such as patients in intensive care units, immunocompromised patients, long term care facility patients and patients on chronic hemodialysis.^{8,9} Thompson RL¹² reports MRSA could be controlled over a 12-month period after the implementation of an active surveillance culture to detect and control a strain of MRSA outbreak.

The limitation of an active surveillance culture is the required timing of at least 24–48 hours wait for the culture result. During this period the patient should be placed in isolation as a precaution.

However, the effectiveness to perform universal active surveillance culture is limited, to certain countries where MRSA has a low prevalence e.g. many European countries, for example the Netherlands, Finland and France.^{12–16} They found that universal active surveillance culture is successful in controlling MRSA but studies involved multi strategies beside universal active surveillance culture, such as contact isolation, screening for healthcare workers with decolonization, closing units and comprehensive cleaning. Therefore, which intervention or which combination of interventions is most beneficial?

The LightCycler[®] MRSA advance test is a rapid test tool for direct detection of nasal colonization with MRSA. The test has a short turnaround time of testing of about 2 hours in which the patient will be given appropriate isolation care to reduce transmission and infection in health care settings. It also reduces unnecessary costs for isolation.

Peterson et al.¹⁶ evaluated the use of the LightCycler[®] MRSA advanced test as a rapid tool for detection of MRSA by nasal surveillance swabs. The test showed relative sensitivity and specificity of 95.2% (95%CI = 91.1% - 97.8%) and 96.4% (95%CI = 91.7% - 98.1%) respectively. In comparing with BD gene Ohm assay, for discrepancy analysis, the LightCycler[®] MRSA Advanced test demonstrated significantly more specificity.

Our study found the high prevalence of MRSA colonization in critically ill patients up to 12.1 percent who were transferred to our ICUs.

Most of the patients (90%) have had a history of previous infection before admission.

The LightCycler® MRSA advanced test detects the MRSA gene in up to 14.5% of cases. The result showed good correlation with MRSA isolation by plate culture (Kappa = 0.791, 95%CI = 0.594-0.988).

Our study demonstrated that the LightCycler® MRSA advanced test is a useful rapid screening test to detect MRSA colonization. Future study should evaluate the cost benefit and cost effectiveness of the test for MRSA detection and infection control of MRSA infection in hospitals.

Conclusion

We studied the prevalence of MRSA colonization in critically ill patients who were transferred to the ICU in the Bangkok hospital group. The prevalence of MRSA colonization was found up to 12.1%. The LightCycler® MRSA advanced test showed good results and correlation with standard plate culture. The test might be useful for rapid detection MRSA colonization especially in high risk patients. This will be extremely useful in controlling MRSA infection.

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