Comparison of light cycler PCR and conventional susceptibility testing for detection of MRSA from cultures

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ABSTRACT

Objectives: To compare a duplex light cycler polymerase chain reaction (PCR) assay targeting the mecA gene and a *Staphylococcus aureus* (*S. aureus*) specific marker and the conventional method.

Methods: We evaluated 400 samples sent to the laboratory in Zayed Military Hospital, Abu Dhabi, United Arab Emirates for methicillin-resistant *Staphylococcus aureus (* MRSA) screening and routine bacterial cultures from the period January 2003 to January 2004. All samples were cultured and identified according to the National Committee for Clinical Laboratory Standard guidelines. *Staphylococcus aureus* were tested for methicillin susceptibility according to the guidelines. All *Staphylococcus* positive cultures underwent testing by the new duplex light cycler PCR assay. We used 2 pairs of primers: mecA and nuc. Both targeted the mecA gene and the *S. aureus*-specific marker. Results obtained from the 2 methods (conventional culture method and the real-time PCR method) were compared.

Results: From the 400 samples tested, a total of 9 MRSA were detected by both methods. The real-time PCR method took less than 60 minutes to complete.

Conclusion: This study shows that the duplex light cycler PCR assay method is very sensitive, very specific, and less time consuming in diagnosing MRSA from bacterial cultures.

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Methicillin-resistant *Staphylococcus aureus* (MRSA) has become increasingly prevalent worldwide. In the United States and in some European countries, MRSA accounts for 10-40% of all *Staphylococcus aureus (S. aureus)* isolates.[1,2] Increased surveillance, including screening of high risk patients, has been recognized as an important component of effective infection control programs to limit the spread of MRSA in hospitals. Therefore, rapid and accurate identification of MRSA is essential. The MRSA strains are usually introduced into an institution by an infected or colonized patient or by a colonized health care worker. Traditional antimicrobial susceptibility test methods such as disk diffusion or broth micro-dilution require at least 24 hours to perform. Difficulties in the differentiation of MRSA from borderline oxacillin resistant *S. aureus* strains may also occur.[3,4] Methicillin resistance in *S. aureus* is mediated by the production of an altered penicillin-binding protein (PBP) 2a.[5] The mecA gene complex regulates the production of PBP 2a. Detection of the mecA gene or of PBP 2a appears to most accurately detect MRSA.[6-11] However, the use of these assays is largely restricted to reference centers, and they are not currently utilized by most routine diagnostic laboratories.

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Rapid screening followed by accurate and timely identification of MRSA becomes an elemental procedure in preventive measures. In this study, we compare between 2 screening methods, conventional susceptibility testing and the real-time duplex polymerase chain reaction (PCR) method.

Methods. ***Clinical samples.*** Four hundred consecutive clinical samples sent to our laboratory in Zayed Military Hospital, Abu Dhabi, United Arab Emirates from January 2003 to January 2004 for MRSA screening and routine cultures were investigated. The samples were from wounds or abscesses, the anterior nares, the perineum, urine, catheter insertion sites, skin and soft tissues, sputum, the trachea and other sites. Most specimens were sampled by swabs.

Culture. All swabs were cultured on sheep blood agar and identified by colony morphology, Gram stain characteristics, catalase reaction, coagulase production and the results of the Vitek System using the VITEK GPI 101 card (BioMerieux Vitek, Inc.). Oxacillin susceptibility was determined by the agar screening method with Mueller-Hinton disk diffusion methods (according to the National Committee for Clinical Laboratory Standard) and confirmed by Vitek minimum inhibitory concentrations using the VITEK GPS 101 card.[12,13]

Identification of methicillin-resistant Staphylococcus aureus by duplex real-time polymerase chain reaction . A pure bacterial culture was used in the duplex real-time PCR assay. Only cultures with *Staphylococcus* species characteristics (colony morphology, Gram stain characteristics and catalase reaction) were used.

DNA extraction. A single colony was picked and suspended in 100 µl of MilliQ water. The suspension was then heated at 95°C for 15 minutes. After centrifugation for one minute at 20,800 X g to sediment the debris, the clear supernatant was ready to be used as template DNA in PCR.

Duplex real-time polymerase chain reaction. The duplex real-time PCR was run using the light cycler system(Roche). Primers MECA1 (5-GCA ATC GCT AAA GAA CTA AG-3) and MECA2 (5-GGG ACC AAC ATA ACC TAA TA-3) and primers NUC1 (5-GCG ATT GAT GGT GAT ACG GTT-3) and NUC2 (5-AGC CAA GCC TTG ACG AAC TAA AGC-3), were used targeting the mecA gene and the nuc gene. The nuc gene is a *S. aureus*-specific marker. All Primers were published in previous study.[14] Amplification mixtures contained 2 µl of DNA template, 3mM MgCl2, 1 µM (each) MECA1 and MECA2, 0.25 µM (each) NUC1 and NUC2, and 2 µl of 10X light cycler fast start DNA Master SYBR Green I mixture (Roche) in a final volume of 20 µl. Following an initial denaturation at 95°C for 10 minute to activate the FastStart Taq DNA polymerase, the 32-cycle amplification program consisted of heating at 20 - 95°C with a 0-s hold, cooling at 20 - 55°C with a 5-s hold, and heating at 20 - 72°C with an 8-s hold. Then the one-cycle melting curve program consisted of heating at 20 - 95°C with a 0-s hold, cooling at 20 - 58°C with a 60-s hold, and heating at 0.1 - 95°C with a 0-s hold.

Finally, the experiment protocol ended with one cycle of cooling at 20 - 35°C with a 30-s hold. The fluorescence channel was set at F1 (530 nm).

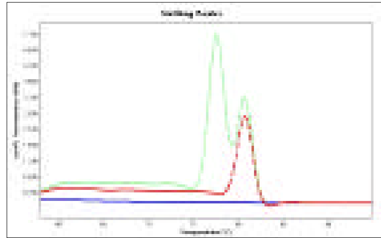

Figure 1 - Temperature curves for: MRSA (dot line) with 2 peaks specific for mecA and nuc genes, MSSA (dash line) with one peak specific for nuc gene, and negative control (solid line) with no peaks. MRSA - methicillin-resistant *Staphylococcus aureus,* MSSA - methicillin-susceptible *Staphylococcus aureus.*

Data analysis. Melting curve analysis was performed to determine which specific gene(s) had been detected from the samples. All MRSA strains tested in the study presented 2 peaks in the melting curve analysis; one peak was specific for the mecA gene with a temperature (Tm) of 77.5 - 79°C, and one was specific for the nuc gene with a Tm of 79.9 - 81.6°C.

Results. From the 400 samples tested, total of 9 MRSA were detected by both methods. Among those 400, 9 (100%) MRSA were detected by the conventional susceptibility testing, and the same numbers were detected by the real-time duplex PCR method. One hundred and twenty samples were methicillin-susceptible *S. aureus (*MSSA) positive and 271 were methicillin-resistant *Staphylococcus epidermidis* (MRSE) positive by both methods. There was no PCR inhibition.

All MRSA strains tested in the study presented 2 peaks in the melting curve analysis; one peak was specific for the mecA gene with a Tm of 77.50 - 79°C, and one was specific for the nuc gene with a Tm of 79.90 - 80.60°C. MSSA strains had only a nuc peak, MRSE strains had only a mecA peak, and MSSE strains had no peak (**Figure 1**).

Discussion. The incidence of nosocomial infections caused by MRSA continues to increase worldwide. Rapid assessment of clinical specimens for the presence of MRSA is an important part of the infection control measures taken to control the spread of MRSA and, thus, to decrease hospitalization costs. In hospitals with low rates of MRSA, it is probably important to detect each patient colonized or infected with MRSA. Thus, the negative predictive value of the screening test should be high.[15,16] The conventional culture methods are time and labor consuming, and the diagnostic values are not as good as those of the new MRSA screening method. In this study we compared the performance of a published real time method and the standard conventional method to detect MRSA in general microbiology laboratory. We found that the real-time PCR method is specific and sensitive as the gold standard method. In addition, the real time PCR method is less time consuming. Finally, we can conclude that, the real time PCR method can be used as a routine method to detect MRSA in a routine microbiological laboratory.

References

1. Panlilio AL, Culver DH, Gaynes RP, Banerjee S, Henderson TS, JS Tolson, et al. Methicillin-resistant *Staphylococcus aureus* in US hospitals, 1975–1991. ***Infect Control Hosp Epidemiol*** 1992; 13: 582–586.
2. Voss A, Milatovic D, Wallrauch-Schwarz C, Rosdahl VT, Braveny I. Methicillin-resistant *Staphylococcus aureus* in Europe. ***Eur J Clin Microbiol Infect Dis*** 1994; 13: 50–55.
3. Hiramatsu K, Kihara H, Yokota T. Analysis of borderline resistant strains of methicillin-resistant *Staphylococcus aureus* using polymerase chain reaction. ***Microbiol Immunol*** 1992; 36: 445–453.
4. Liu H, Buescher G, Lewis N, Snyder S, Jungkind D. Detection of borderline oxacillin-resistant *Staphylococcus aureus* and differentiation from methicillin-resistant strains. ***Eur J Clin Microbiol Infect Dis*** 1990; 9: 717–724.
5. Archer GL, Pennell E. Detection of methicillin resistance in staphylococci by using a DNA probe. ***Antimicrob Agents Chemother*** 1990; 34: 1720–1724.
6. Chambers HF. Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. ***Clin Microbiol Rev*** 1997; 10: 781–791.
7. Gerberding JL, Miick C, Liu HH, Chambers HF. Comparison of conventional susceptibility tests with direct detection of penicillin-binding protein 2a in borderline oxacillin-resistant strains of *Staphylococcus aureus*. ***Antimicrob Agents Chemother*** 1991; 35: 2574–2579.
8. O'Hara DM, Harrington CR, Reynolds PE. Immunological detection of penicillin-binding protein 29 in clinical isolates of methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. ***FEMS Microbiol Lett*** 1989; 48: 97–103.
9. Tokue Y, Shoji S, Satoh K, Watanabe K, Motomiya M. Comparison of a polymerase chain reaction assay and a conventional microbiologic method for detection of methicillin-resistant *Staphylococcus aureus*. ***Antimicrob Agents Chemother*** 1992; 36: 6–9.
10. Towner KJ, Talbot DC, Curran R, Webster CA, Humphreys H. Development and evaluation of a PCR-based immunoassay for the methicillin-resistant rapid detection of methicillin-resistant *Staphylococcus aureus*. ***J Med Microbiol*** 1998; 47: 607–613.
11. Brakstad OG, Aasbakk K, Maeland JA. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. ***J Clin Microbiol*** 1992; 30: 1654–1660.
12. Smyth RW, Kahlmeter G, Olsson-Liljequist B, Hoffman B. Methods for identifying methicillin resistance in *Staphylococcus aureus*. ***J Hosp Infect*** 2001; 48:103–107.
13. Kloos WE, Bannerman TL. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, editors. Manual of clinical microbiology. 7th ed. Washington (DC): American Society for Microbiology, *Staphylococcus* and Micrococcus; 1999. p. 264–282.
14. Fang H, Hedin G. Rapid Screening and Identification of Methicillin-Resistant *Staphylococcus aureus* from clinical samples by selectibe-broth and real-time PCR assay. ***J Clin Microbiol*** 2003; 41: 2894-289.
15. Francois P, Pittet D, Bento M, Pepey B, Vaudaux P, Lew D, et al. Rapid detection of methicillin-resistant *Staphylococcus aureus* directly from sterile or nonsterile clinical samples by a new molecular assay. ***J Clin Microbiol*** 2003; 41: 254–260.
16. Huletsky A, Gagnon F, Bach VR, Truchon K, Picard FJ, Bergeron MG. Less than one-hour detection of methicillin-resistant *Staphylococcus aureus* directly from nasal swabs by real-time PCR using the smart cycler. ***Clin Microbiol Infect*** 2002; 8: 85.