Journal of Microbiology, Immunology and Infection (2014) 47, 484–490

Available online at www.sciencedirect.com

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journal homepage: www.e-jmii.com

ORIGINAL ARTICLE

Rapid detection of methicillin-resistant *Staphylococcus aureus* by a newly developed dry reagent-based polymerase chain reaction assay

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Received 22 February 2013; received in revised form 28 May 2013; accepted 20 June 2013
Available online 6 August 2013

KEYWORDS
Methicillin-resistant *Staphylococcus aureus*;
Nasal carriers;
Nosocomial infection;
Thermostabilized polymerase chain reaction

Background/Purpose: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major pathogen responsible for significant numbers of nosocomial and community-acquired infections worldwide. Molecular diagnosis for MRSA nasal carriers is increasingly important for rapid detection and screening of MRSA colonization because the conventional methods are time consuming and labor intensive. However, conventional polymerase chain reaction (PCR) tests still require cold-chain storage as well as trained personnel, which makes them unsuitable for rapid high-throughput analysis. The aim of this study was to develop a thermostabilized PCR assay for MRSA in a ready-to-use form that requires no cold chain.
Methods: The thermostabilized PCR assay detects the following targets simultaneously: (1) 16S rRNA of the *Staphylococcus* genus; (2) *fem*A gene specific for *S. aureus*; (3) *mec*A gene conferring methicillin resistance; and (4) *luk*S gene, which encodes the virulent toxin. The thermostabilized PCR incorporates an internal amplification control that helps to verify the presence of PCR inhibitors in samples. PCR reagents and specific primers were lyophilized into a pellet form with an enzyme stabilizer.

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http://dx.doi.org/10.1016/j.jmii.2013.06.004

Results: The PCR was validated with 235 nasal swabs specimens and was found to be 100% sensitive and specific. The stability of the thermostabilized PCR was evaluated using the Q_{10} method and it was found to be stable for approximately 6 months at 24°C. The limit of detection of thermostabilized PCR assay was determined by probit regression (95% confidence interval) was 10^6 colony forming units at the bacterial cell level and 10 ng of DNA at the genomic DNA level, which is comparable with conventional PCR methods.
Conclusion: A rapid thermostabilized PCR assay that requires minimal pipetting steps and is cold chain-free was developed for detecting MRSA nasal carriers.

Introduction

Staphylococcus aureus is a major pathogen responsible for nosocomial and community-acquired infections. Methicillin resistance in *S. aureus* (MRSA) is mediated primarily by the *mec*A gene, which encodes the modified penicillin-binding protein 2a (PBP 2a).[1] MRSA incidence varies between countries; in Malaysia, the MRSA infection rate was 10.0/1000 hospital admissions and the incidence density of MRSA infection was 1.8/1000 patient days.[2] The major reservoirs of MRSA are colonized or infected patients and, occasionally, hospital personnel.[3] These data support the view that early detection and prevention of colonization of intensive care unit (ICU) patients with MRSA could reduce the frequency of MRSA infection and assist in the design of effective prevention strategies against MRSA infection.[4] Rapid detection and isolation of patients colonized with MRSA and rapid implementation of contact precautions should result in a reduction of nosocomial MRSA transmission.[5] Guidelines for the identification of MRSA recommend culture.[6] Chromogenic media yield reliable negative results at 20 hours,[7] but isolation, sensitivity testing and the confirmation of MRSA takes 48—72 hours. Diagnosis of MRSA within a few hours by the use of molecular approaches could lead to the early implementation of appropriate antibiotics and reduced hospital bed stays. As molecular methods become commonplace for MRSA, screening of all patients admitted to ICU wards becomes a viable option.

Molecular methods, particularly polymerase chain reaction (PCR), have become more important in the diagnosis of MRSA and have greatly improved the speed, sensitivity, and specificity of diagnostic tests, which in turn facilitates early detection of MRSA patients and helps in infection control and prevention.[8] PCR methods for detection of the *mec*A gene, the Panton—Valentine leukocidin (PVL)-encoding gene *luk*S and the species-specific gene *fem*A have required the use of separate assays.[8,9] Multiplexed PCR techniques to identify the methicillin-resistance gene *mec*A, the *S. aureus*-specific gene *fem*A, and the PVL virulence gene simultaneously have been developed by our research group and others.[10—13] A PCR test was designed on the basis of the distribution of virulence determinants *seh*, *etd*, and *arc*A within the *S. aureus* population.[14] but these genetic determinants are most likely to be specific for community-acquired MRSA clones. However, another PCR test was developed to discriminate *S. aureus* from coagulase-negative staphylococci and to determine methicillin resistance from blood cultures[15]; this assay is used for blood culture only and requires further incubation of clinical samples on blood culture broths. Conventional PCR assays require multiple pipetting steps, skilled personnel for master mix preparation, and cold-chain transportation and storage. Handling of PCR reagents requires dealing with microvolumes, where processing variation can detrimentally affect a test result. Furthermore, PCR error is compounded by the multiple pipetting steps of small volumes of reagents, which may lead to errors. These factors adversely influence the use of PCR as a screening and diagnostic tool of choice for MRSA, even though PCR provides higher levels of sensitivity and specificity. In this study, a thermostabilized PCR assay that is cold chain free and requires only the addition of DNA samples and PCR-grade (DNase-free) water was developed for the detection of MRSA. A PCR assay with specific primers was designed for detection of the MRSA gene *mec*A[10]; the 16S rRNA gene of the *Staphylococcus* genus, the *S. aureus*-specific gene *fem*A, and the virulence gene *luk*S uses an internal amplification control (IC) for monitoring PCR inhibitors. The whole PCR mix containing the specific primers was thermostabilized into a dried pellet, using a technique similar to that described earlier.[16,17] The ready-to-use dried reagent mix was analyzed to determine the limit of detection (LoD) and evaluated with clinical specimens.

Materials and methods

Bacterial strains

The reference strains used in this study (Table 1) were obtained from: the Belgian Coordinated Collections of Microorganisms, Ghent, Belgium; the Institute for Medical Research, Malaysia; and the Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia.

Clinical samples

The study was approved by the Research and Ethics Committee of the School of Medical Sciences, Hospital Universiti Sains Malaysia (HUSM), in accordance with the Helsinki Declaration. Nasal swabs (235) were collected from inpatients in all of the wards in the HUSM from March to August 2008. Informed consent was obtained from all participants before specimen collection. Samples were