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Short Communication

Methicillin-resistance *Staphylococcus aureus* detection by an improved rapid polymerase chain reaction (PCR) assay

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In this study, a novel *orfX*-PCR for rapid detection of methicillin-resistant *Staphylococcus aureus* (MRSA) had been developed and applied, with the specific targeting *orfX* on SCC*mec*. PCR amplification generated one specific band with size of 212 bp, and high specificity was obtained. Application of this PCR had been further performed on detection for 262 MRSA and MRCNS strains, and *orfX*-PCR results showed expected products for either MRSA or MRCNS strains, demonstrating the *orfX*-PCR assay to be a useful and powerful MRSA detection method.

Key words: *Staphylococcus*, MRSA, *orfX*-PCR, rapid detection.

INTRODUCTION

Since the first discovery in 1961, methicillin-resistant *Staphylococcus aureus* (MRSA) has become one of the most prevalent pathogens that cause nosocomial infections throughout the world (Chang et al., 1997). Methicillin resistance in staphylococci is caused by penicillin-binding protein 2a (PBP2a) encoded by the *mecA* gene, which is located on a mobile genetic element designated as staphylococcal cassette chromosome *mec* (SCC*mec*) (Katayama et al., 2000). As one example of the leading "Super Bugs", MRSA strains show resistance to practically all β-lactam antibiotics and usually other multiple drugs due to the *mecA* and associated resistance genes carried by SCC*mec*, respectively (Schito et al., 2006). China remains one of the worst areas for antibiotics abuse, with an estimate annual consumption of 140 g per person, which is 10 times higher than that in the United Kingdom and the United States. Increased awareness for the risk and hazard of MRSA strains and demands for test capable of early, cost-effective, timely, and sensitive MRSA detection has made it an urgent necessity. Most recently, a multiplex-polymerase chain reaction (PCR) assay for differentiation of MRSA, methicillin-sensitive *S. aureus* (MSSA), methicillin-resistant coagulase-negative staphylococci (MRCNS), methicillin-sensitive coagulase-negative staphylococci (MSCNS) and non-staphylococci strains, had been developed and applied (Xu et al., 2011c). As clinical MRSA diagnosis is concerned, *Staphylococcus* strains have commonly been identified via routine standard procedures including colony morphology, Gram staining, testing of catalase, hyaluronidase and coagulase,

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as well as the Vitek 2 automated system and the API-Staph commercial kit, which makes the detection of 16S rRNA somehow irrelevant. In addition, despite the advantages of saving time, cost, technical resources, labor and personnel, the further development and broad application of multiplex-PCR has been restricted by its limitations including cross-reaction of different sets of primers, self-inhibition due to formation of dimmers, reduced amplification efficiency caused by the simultaneous and parallel amplification, undetectable influence of different targets, as well as the requirement for standard full use of external and internal quality control (both positive and negative control for each targets) for each assay. As consequence, this study aimed at developing and evaluating a simple and improved MRSA testing method based on *orfX*-PCR assay, and further applying this assay to detection of a large scale of MRSA and MRCNS strains from various clinical samples.

MATERIALS AND METHODS

The protocol was designed for direct detection of MRSA by targeting *orfX* (O1: ACCACAATCMACAGTCATT and O2: CCCGCATCATTTGATGTG, with an expected 212 bp amplicon) located on the site of SCC*mec* and had been considered to be a highly conserved open reading frame in *S. aureus*). For development, evaluation and optimization of the *orfX*-PCR assay, 6 reference strains were used, including MRSA ATCC29212 and 85/2082 (*S. aureus* with *mecA* positive), MSSA ATCC25923 (*S. aureus* with *mecA* negative), MRCNS ATCC700586 (non *S. aureus* with *mecA* positive), MSCNS ATCC12228 (non *S. aureus* with *mecA* negative) and *Escherichia coli* ATCC25922 (non *S. aureus* with *mecA* negative) (Figure 1). Cultural conditions and DNA extraction of Gram-positive and -negative strains were performed as described previously (Xu et al., 2007, 2008a, b, 2009, 2010). The PCR reaction was carried out in a total of 25 µl reaction mixture, and optimization of *orfX*-PCR assay had been performed as described previously, with 6 primer concentrations, 6 dNTP concentrations and 8 annealing temperatures included. PCR amplification and the amplicons determination were carried out as previously. A negative control was performed using sterile water instead of culture or DNA template. The PCR product of *orfX* was cut out from the agarose gel and was ligated with the pGEM-T easy vector, followed by the sequencing and nucleotide sequence analysis as previously. The specific amplification generated a specific single band on agarose gel, with sizes 212 bp for *orfX*.

RESULTS AND DISCUSSION

The PCR product showed highest levels of resolution of DNA when 0.5 µM of each primer, 250 µM of dNTP and amplification at an annealing temperature of 50°C when compared with other conditions (data not shown). MRSA ATCC29212 and 85/2082 yielded specific amplification for *orfX*; MSSA ATCC25923, MRCNS ATCC700586, MSCNS ATCC12228, and *E. coli* ATCC25922 had been detected to be negative for *orfX*. Application of the established *orfX*-PCR assay was further performed on a total of 262 *Staphylococcus* isolates (209 MRSA and 53 MRCNS strains), which were isolated from various clinical samples and had been preliminarily described or identified (Xu et al., 2007, 2008a,b 2009, 2010, 2011a,b). PCR amplicons were evaluated by electrophoresis as the aforementioned and experiments were replicated to ensure reproducibility. According to the results, all 209 MRSA strains yielded the specific single band of 212 bp corresponding to *orfX*; for 53 MRCNS strains, all isolates were detected to be negative for *orfX*. No false positive amplification was observed, indicating the high specificity of the established *orfX*-PCR assay.

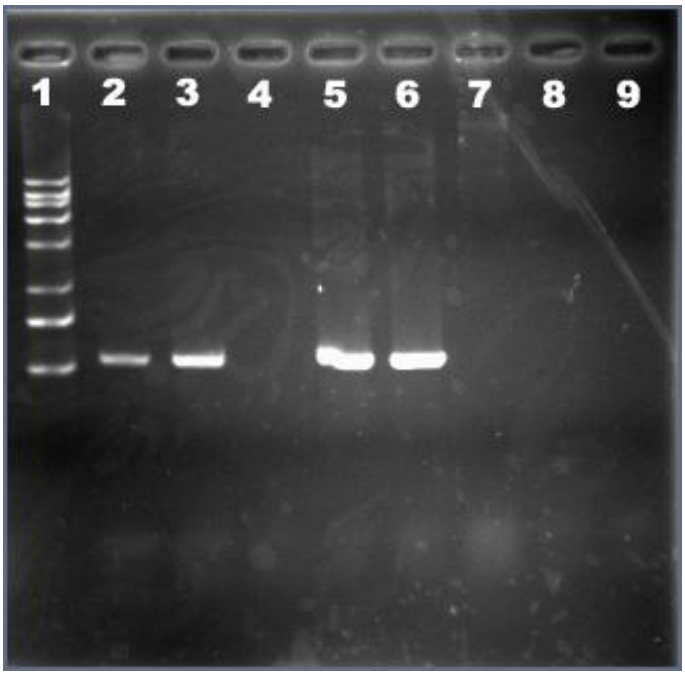

Figure 1. The *orfX*-PCR amplification of reference strains. Lane 1: DNA Marker; lane 2 and 3: MRSA ATCC29212, with DNA amount of 10 and 100 ng, respectively; lane 4: MSSA ATCC25923, with DNA amount of 100 ng; lane 5 and 6: MRSA 85/2082, with DNA amount of 10 and 100 ng, respectively; lane 7, 8 and 9: MRCNS ATCC700586, MSCNS ATCC12228 and *E. coli* ATCC25922, respectively, with DNA amount of 100 ng.

MRSA are currently widespread pathogens throughout the world and have prompted a heightened interest and concern for the rapid detection of these pathogens as well as its related antibiotic resistance determinant. Thus, rapid and accurate detection approaches are needed to reduce risk of MRSA nosocomial infection. In this study, the *orfX*-PCR assay has been demonstrated to be a simple, rapid, specific, and sensitive MRSA detection method, which is also important in the further development through the simplification of result determination.

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