In silico PCR verification and simplex real-time PCR detection of methicillin-resistant Staphylococcus aureus (MRSA) from east coast Malaysian clinical isolates

ABSTRACT

The aims of this study were to validate the primers developed for molecular-based detection and identification of Malaysian clinical isolates of methicilin-resistance Staphylococcus aureus (MRSA) using in-silico Polymerase Chain Reaction (PCR) and real-time PCR SYBR with Green I. Rapid molecular diagnostic and risk assessment of the MRSA are possible by real-time PCR SYBR Green I. However, validation of such primers for real-life samples is expensive and time consuming. Hence, development and verification of real-time PCR primers by in-silico PCR can be the first step in the selection of the most appropriate primers. Three species-specific markers were chosen targeting coa (staphylocoagulase), nuc (thermonuclease) and mecA (methicillin-resistance) and were specifically verified against 35 selected S. aureus strains by using in-silico PCR. For the actual laboratory verification, all of the 3 genes were detected with a single specific melting curve peak (Tm at 76.16 ± 0.8 ℃, 78.50 ± 0.4 ℃ and 74.41 ± 0.6 ℃ for a coa, nuc and mecA respectively) in 32 bacterial strains including ATCC reference strains. Thus, there is no disagreement between both in-silico PCR and real-time PCR verification and validation of the primers designed for the detection and identification of MRSA in this study. The potential of using a bioinformatics approach (in-silico PCR) before selecting primer pairs for a given study may enable researchers to accept or reject the potential primer pairs for downstream experimental (in vitro) PCR without wasting any chemicals as well as related cost.

Keyword: In-silico PCR; Methicillin-resistant Staphylococcus aureus (MRSA); Nuclease (nuc) and methicillin-resistant (mecA) genes; Real-time PCR SYBR Green I; Staphylocoagulase (coa)