Multiplex PCR assays for the detection of clinically relevant antibiotic resistance genes in Staphylococcus aureus isolated from Malaysian hospitals.

ABSTRACT

Multiple drug resistant Staphylococcus aureus is one the most common nosocomial pathogen worldwide. The timely identification of this hospital acquired pathogen and detection of the various antibiotic resistant genes harbored is one of the most important function of the microbiology laboratory. In this study, we report the development of a multiplex PCR system for the diagnosis of S. aureus and the detection of clinically relevant antibiotic resistance genes harbored by some isolates. This system was designed to identify S. aureus at species level and to detect methicillin, gentamycin, erythromycin, vancomycin and mupirocin resistant genes, respectively from a single colony in a single tube reaction. All isolates amplified a 108 bp fragment (conserved in S. aureus) confirming the identity of S. aureus, 23 isolates produced a band at the position of 533 bp, 28 isolates at 139 bp and 30 isolates at 174 bp evidencing the presence of mecA (methicillin or oxacillin resistance), ermA (erythromycin resistance), aac (6')-aph (2'') (gentamycin resistance) genes. None of the isolates amplified van A (vancomycin resistance) and ileS-2 (mupirocin resistance) genes showing the absence of their resistance in the isolates studied. These genotypic results when compared with classical antibiotic susceptibility tests showed less correlation. Overall, we found a correlation between phenotypic and genotypic methods of 60% for methicillin, 36.7% for gentamycin, 43.3% for erythromycin, 100% for vancomycin and mupirocin. This suggests that classical antibiotic sensitivity test is not accurate, but need to be supplemented with other methods to be applied in a clinical laboratory. The system developed in this study offers a rapid, simple specific and accurate detection of multiple antibiotic resistant genes in clinical S. aureus isolates and thus could be systematically applied as a diagnostic test in clinical microbiology laboratories, facilitating the design and use of antibiotic therapy.

Keyword: Staphylococcus aureus; Antibiotic resistance; Multiplex PCR; Primer; Nosocomial; Malaysia.