Abstract

The genus *Staphylococcus* includes pathogenic and non-pathogenic facultative anaerobes. Due to the
Plethora of virulence factors encoded in its genome, the species *Staphylococcus aureus* is known to be the
most pathogenic. *S. aureus* strains harboring genes encoding virulence and antibiotic resistance are of
public health importance. In clinical samples, however, pathogenic *S. aureus* is often mixed with putatively
less pathogenic coagulase-negative staphylococci (CoNS), both of which can harbor *mecA*, the genetic
driver for staphylococcal methicillin-resistance. In this chapter, the detailed practical procedure for operating
a real-time pentaplex PCR assay in blood cultures is described. The pentaplex real-time PCR assay
simultaneously detects markers for the presence of bacteria (*16S rRNA*), coagulase-negative staphylococcus
(*cns*), *S. aureus* (*spa*), Panton-Valentine leukocidin (*pvl*), and methicillin resistance (*mecA*).