



Simplex, Duplex and Multiplex PCR for detection of up to six *Enterococcus* virulence genes in *Staphylococcus* spp. isolated from burn patients

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Abstract:

The high incidence of Gram-positive cocci, particularly *Staphylococcus aureus*, poses a severe threat in burn patients, causing a wide variety of diseases ranging from minor skin infection to life-threatening sepsis cases, leading to infection treatment difficulties. This study aimed to investigate a genotypic approach to detect up to six virulence genes in *Staphylococcus* spp. that may be passed from *Enterococcus* spp. isolated from the same niches of burn patients. The study analyzed 200 clinical samples (wound, urine, stool, and blood) from 75 burn patients. The six chosen virulence genes, including *asa1*, *gelE*, *cylA*, *esp*, *hyl*, and *efa*, were detected using simplex, duplex, and multiplex PCR alongside phenotypic testing in *Staphylococcus* spp., in addition to antibiotic resistance genes *vanA* and *aph(3')*. Initial identification used selective media and biochemical tests, followed by 16S rRNA sequencing; of these samples, 38 (19%) were confirmed as *Staphylococcus* species. The distribution included *S. epidermidis* (31.5%), *S. aureus* (28.5%), *S. haemolyticus* (23.6%), *S. hominis* (13%), and *S. saprophyticus* (2.6%). Notably, all 38 isolates grew on HiChrome media typically specific for *Enterococcus faecium*. The *asa1* gene was the most prevalent; it was recorded in up to 100% of isolates, with significant differences $p < 0.0001$. Other genes like *gelE* and *hyl* also showed high frequency, while *efa*, *esp*, and *cylA* levels fluctuated across different PCR methods. A key finding the coexistence of these two bacterial species in the same niche raises concerns about the potential horizontal transfer of virulence-associated traits between them, highlighting the importance of rapid and accurate diagnosis to minimize this potential risk.

Key words: Burn infections, *Staphylococcus* spp., Virulence factor genes, VRS, antimicrobial genes.

