Development and evaluation of a Quadruplex Taq M an real-time PCR assay for simultaneous detection of clinical isolates of Enterococcus faecalis, Enterococcus faecium and their vanA and vanB genotypes

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ABSTRACT

Background and Objectives: We developed and evaluated the utility of a quadruplex Taqman real-time PCR assay that allows simultaneous identification of vancomycin-resistant genotypes and clinically relevant enterococci.

Materials and Methods: The specificity of the assay was tested using reference strains of vancomycin-resistant and susceptible enterococci. In total, 193 clinical isolates were identified and subsequently genotyped using a Quadruplex Taqman real-time PCR assay and melting curve analysis. Representative Quadruplex Taqman real-time PCR amplification curve were obtained for Enterococcus faecium, Enterococcus faecalis, vanA-containing E. faecium, vanB-containing E. faecalis.

Results: Phenotypic and genotypic analysis of the isolates gave same results for 82 enterococcal isolates, while in 5 isolates, they were inconsistent. We had three mixed strains, which were detected by the TaqM an real-time PCR assay and could not be identified correctly using phenotypic methods.

Conclusion: Vancomycin resistant enterococci (VRE) genotyping and identification of clinically relevant enterococci were rapidly and correctly performed using TaqM an real-time multiplex real-time PCR assay.

Key words: Enterococci, Vancomycin, Multiplex TaqM an real-time PCR

INTRODUCTION

Enterococci are one of the major causes of hospital-acquired infections although they can also cause human infections in the community (1). Hospital acquired infection is defined as an infection which develops 48 h after hospital admission not being the reason of the admission. Enterococci are individual, paired, or short-chain Gram-positive, catalase-negative cocci. This organism is mainly commensals in gastrointestinal tract of healthy individuals but may become opportunistic pathogens in immune-compromised hosts and in patients who have received broad-spectrum antimicrobial therapy or had a prolonged hospital stay (2).

Enterococci display both intrinsic and acquired resistance patterns to many antimicrobials, such as glycopeptides, β-lactams, fluoroquinolones and aminoglycosides which dramatically reduce the remaining therapeutic options among patients infected with these organisms (3). Along with E. faecalis, the genus Enterococcus includes E. faecium which found less frequently than Enterococcus faecalis in clinical isolates and are significantly more resistant to vancomycin than E. faecalis.

E. faecalis and E. faecium are the main causative agents for serious relevant nosocomial infection in humans, thereby it is necessary to discriminate between the low-level vancomycin resistant E. faecalis and E. faecium isolates with the other low-virulence motile enterococcal species (4, 5). Moreover, since vancomycin offers as last line of