

STANDARDIZATION OF AMPLIFICATION AND CLONING OF *vra* AND *gra* GENES IN HETERORESISTANT VANCOMYCIN-INTERMEDIATE *Staphylococcus aureus* (hVISA) ISOLATED FROM CLINICAL SAMPLES OF SOUTHERN BRAZIL

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A brief exposure of *S. aureus* to glycopeptides determines an immediate change in the transcription of several genes involved in cell wall synthesis. Mutations in *graSR* and *vraSR* genes that encode a two-component regulatory system have been described and may be related to heteroresistant vancomycin-intermediate *Staphylococcus aureus* (hVISA). Heteroresistance is characterized by the presence of a rare subpopulations of cells with reduced susceptibility and it is associated with therapy failure in patients treated with vancomycin. In addition, their analytical detection is very difficult. The standardization of molecular techniques to characterize mutations related to glycopeptides resistance is extremely important, since they may lead to the determination of resistance markers which could make laboratory diagnosis earlier, faster and easier. Thus, the aim of this study was to standardize cloning and amplification of *vraSR* and *graSR* genes of 12 hVISA strains obtained from hospitals in Santa Catarina and 3 reference strains (VSSA, hVISA and VISA). For amplification of complete genes (containing both 5' and 3' UTR regions, and the complete CDS of S and R components), specific primers were designed to obtain products of 1,749bp to *vraSR* and 1,993bp to *graSR*. In order to standardize PCR, several parameters were evaluated and the critical steps were: DNA extraction at 100°C in the presence of SDS and NaCl, optimum annealing temperature of primers at 56°C, 100ng of DNA, 1.5 mM MgCl₂ and 0.2 mM dNTPs. The amplicons identity was confirmed by sequencing. For cloning, the *vraSR* and *graSR* genes were ligated into the pGEM®-T Easy vector in the ratio 2:1 vector:insert. The vectors generated were inserted in DH5α calcium-competent cells and transformants were selected by the white color of the colonies. Approximately 6 colonies of each strain were evaluated by toothpick, colony PCR and sequencing. A preliminary analysis of *vra* and *gra* genes sequencing allowed the detection of 8 point mutations. Three isolates analyzed showed amino acids changes at positions 26 and 224 in *graS*. A mutation D148Q in *graR* was more prevalent among hVISA isolates which could be important for resistance development. The cloned genes will be newly cloned in a susceptible *S. aureus* strain to evaluate the relationship between the mutations in the genes and the phenotypic expression of heteroresistance.

Key words: hVISA, two-component systems, *graSR*, *vraSR*.

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