TITLE: IMPACT OF MUTATIONS IN hVISA ISOLATES ON DECREASED SUSCEPTIBILITY TO VANCOMYCIN, THROUGH POPULATION ANALYSES PROFILE - AREA UNDER CURVE (PAP-AUC)

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ABSTRACT:

More than 20 genes can be related to heteroresistant intermediate-vancomycin *Staphylococcus aureus* (hVISA). Mutations in two-component regulatory systems *graSR*, *vraSR*, *walKR* and in gene encoding RNA polymerase beta unit (*rpoB*) have been described and may be related to hVISA. The genetic mechanisms involved in this phenotype are poorly understood and their analysis may lead to the determination of molecular markers, which could make laboratory diagnosis earlier, faster and accurate. Our study included eight hVISA isolates obtained from four hospitals in Santa Catarina State, Brazil, and aimed to analyze sequences and the transcription levels of *graSR*, *vraSR*, *walKR* and *rpoB* genes. The impact on the phenotype expression was evaluated through the population profile analysis tests - area under the curve (before and after induction), induction of resistance, minimal bactericidal concentration, vancomycin tolerance and slow VISA phenotype. Five isolates showed mutations in at least one of the genes evaluated. The *rpoB* H481N and *graS* T224I were the most frequent mutations, followed by *graR* D148Q and *walK* A468T. The *walK* R222K and *vraR* E59D mutations were observed less frequently, but like other mutations were more involved in the acquisition of resistance in our samples. The PAP-AUC values in these isolates were 1.19 to 1.37 (with two mutations in *graSR* and one in *walKR*), from 0.92 to 1.09 (five mutations in *graSR*, one in *walKR* and two in *rpoB*) and from 0.98 to 1.17 (four mutations in *graSR*, one in *vraSR*, two in *walKR* and one in *rpoB*). Among the genes analyzed, the *graSR* complex (the mutations in L26F and T224I were found in the three isolates) was the one that demonstrated the greatest impact in the decrease of the susceptibility to vancomycin. Our findings demonstrate that no single mutation was found in any isolate, responsible for characterization of the phenotype. Mutations in *walKR* and *vraSR* genes, alone, did not demonstrate relevance. Although mutations in the *graSR* gene have shown the greatest impact on decreased susceptibility, the sum of the performance of other genes may also lead to the development of the hVISA phenotype.

Keywords: hVISA, *rpoB*; Two-component regulatory systems; VISA

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