

TITLE: EVALUATION OF THE VIRULENCE OF *Staphylococcus aureus* WITH REDUCED SUSCEPTIBILITY TO VANCOMYCIN IN INFECTION MODEL OF *Galleria mellonella*.

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ABSTRACT: *Staphylococcus aureus* is an important human pathogen, responsible for various infections, among them bacteremia. The emergence of methicillin-resistant *S. aureus* strains (MRSA) has made it difficult to treat patients worldwide. Vancomycin has been used as a last resort for the treatment of infections caused by MRSA. However, the appearance of strains with reduced susceptibility to vancomycin has been reported in several countries, bringing a new problem for the control of these infections. A relationship between staphylococcal pathogenesis and susceptibility to vancomycin has been investigated, but more studies are needed to understand this relationship. This study aims to compare the level of virulence between *S. aureus* subpopulations grown at high concentrations of vancomycin with its respective wild type strain using the *in vivo* infection model of *Galleria mellonella*. Two *S. aureus* strains (23 and 91) isolated from bacteremia in hemodialysis patients were grown at 37°C under selective pressure, at increasing concentrations of vancomycin up to 16 µg/mL. Subpopulations isolated at the last concentration were called derivative (23V₁₆ and 91V₁₆). The virulence assay was performed by inoculating the bacterial strains at different concentrations (10⁴ to 10⁷ CFU/larva) directly into the hemocele of *G. mellonella* (20 larvae per group) and incubating at 37°C. Mortality of the larvae was followed by 24, 48 and 96h. Control groups were composed of larvae inoculated with sterile PBS. The results of the three biological replicates were combined and the survival curve was calculated using the Kaplan-Meier method compared using the Log rank test, considering $P = <0.001$. We observed significant differences ($p=0,000000000175$ and $0,00000000497$, respectively) between the virulence of the parental and his derivative strains at the concentration of 10⁵ CFU/larva for both pair of strains: an increase of about 50% in the survival rate of the larvae that were inoculated with derivatives strains in relation to their respective parental strain. Our results showed that the physiological changes necessary to survive in high concentration of vancomycin have an impact on virulence, probably due to reducing of expression the some virulence factors. In addition, these results indicate that the *in vivo* analysis model with *G. mellonella* is suitable for studies involving alterations of virulence profile related to *S. aureus* strains with reduced susceptibility to vancomycin.

KEYWORDS: bacteremia, hVISA, *Galleria mellonella*, vancomycin, virulence

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