

TITLE: ANALYSIS OF PERSISTENCE IN *Staphylococcus aureus* SENSITIVE TO VANCOMYCIN

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ABSTRACT:

Staphylococcus aureus is an important pathogen of nosocomial and community acquired infections. However, sensitive strains have been aroused interest due to reduced susceptibility to vancomycin associated with therapeutic failure and recalcitrant infections. This phenomenon has been attributed to strains with a transitory and heterogeneous metabolic state called antibiotic persistence. Stress conditions, like antibiotic exposure, triggers these reduction of metabolic activity resulting in microbial survival to bactericidal drugs. Therefore, we investigated if subtherapeutic concentrations of vancomycin induce persistence in *S. aureus*. The reference strain ATCC 29213 (wild type) was exposed to 10 mg/L of vancomycin (10xMIC) for six intermittent cycles of six hours. In the end of last cycle, a sample was withdrawn to viable count. The surviving sample (named as derivative sample) and the control one (not exposed to vancomycin) were tested for antibiotic susceptibility and physiological changes by: (i) MIC and minimum bactericidal concentration (MBC); (ii) similarity pattern (PFGE); (iii) time-kill kinetics in 50xMIC; (iv) growth rate, (v) autolysis using Triton X-100, (vi) surface membrane charge by zeta potential and (vii) oxidative stress by TBARs. Antibiotic persistence was not detected by time-kill curve and CBM/MIC ratio but, important physiological changes were observed in derivative strain (E10.6): increased lipid peroxidation, reduced percentage of autolysis and increased surface charge. We also found that exposure to vancomycin by itself increases lipid peroxidation, reduces the percentage of autolysis and reduces surface charge without altering *S. aureus* susceptibility. Drug-induced persistence may present paradoxical results in high concentrations of bactericidal drugs. We hypothesized that persistent fraction is much smaller than the total population, so the phenomenon may be masked in high concentrations. However, additional tests are required to check if the observed physiological changes are isolated or they are related to the development of persistence in sensitive strains. So, we concluded that treatment with vancomycin during six hours does not alter viable count, susceptibility and growth rate. However, vancomycin increases lipidic peroxidation and reduces percentage of autolysis. Though derivative sample does not be persist, it presents surface membrane charge more positive after vancomycin exposure.

Keywords: *Staphylococcus aureus*, antibiotic persistence, vancomycin, subtherapeutic concentrations.

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