**TITLE:** EXPOSURE TO OXACILLIN SUBINHIBITORY CONCENTRATIONS AND *IN VITRO* INDUCTION OF RESISTANCE EXPRESSION IN HETERORESISTANT AND NON-HETERORESISTANT OXACILLIN-SUSCEPTIBLE MECA-POSITIVE *STAPHYLOCOCCUS AUREUS* (OS-MRSA)

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## ABSTRACT

*Staphylococcus aureus* is naturally susceptible to antibiotics, but is known for its ability to easily acquire resistance. Resistance expression can occur as a consequence of irrational use of antibiotics, since this implies dissemination of subinhibitory concentration (sub-MICs) in different environments and generates a selective pressure on bacteria, favoring its genetic evolution in response to the environmental stress. For this reason, the study aimed to evaluate the *in vitro* influence of selective pressure on antibiotic resistance expression in oxacillin-susceptible *S. aureus* strains, through exposure to oxacillin sub-MICs. Five strains with different genotypic profiles, isolated from nasal colonization were exposed to two-fold serial dilutions of oxacillin (0,125 a 256 μg/mL) during five to ten consecutive days. At every 24 hours strains were reexposed to antibiotic, using visible growth at the highest oxacillin concentration. Before and after induction, disc diffusion test was used to stablish the susceptible profile to several antibiotics; and population analysis profile (PAP) was determined to evaluate the expression of oxacillin resistance. Susceptibility to non-β-lactam antibiotics was not changed. On the other hand, changes in oxacillin and cefoxitin resistance expression were observed. Two strains (SA607 and SA786) began to express homoresistance (minimum inhibitory concentration, oxacillin MIC equal to 256 μg/mL), among them, SA607, the mecA-positive oxacillin-susceptible strain (OS-MRSA). The others (mecA and mecC negative) reached MICs of 8 μg/mL (SA177) and 32 μg/mL (SA799) and with the exception of one (SA292), were classified as heteroresistant after induction. Changes for homoresistant profile in relation to the OS-MRSA strain (SA607) were attributed to mecA activation and the stimulation of bla locus. In relation to SA786, resistance expression was attributed to mecA activation, because this gene was detected by PCR after oxacillin exposure. β-lactamase hyperproduction and modifications in native PBPs of S. aureus were correlated to changes observed for the other strains. *In vitro* simulation of antimicrobial selective pressure changed the phenotypic expression of oxacillin resistance. This reinforces the impact that irrational use of antibiotics has on individuals colonized by *S. aureus* and in population, emphasizing that emergence and spread of resistance represent an evolution process in response to antimicrobial selective pressure.

**Keywords**: Staphylococcus aureus. OS-MRSA. Oxacillin. Subinhibitory concentration. Antibiotic resistance.

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