

**TITLE:** DETECTION OF CONJUGATIVE PLASMIDS CARRYING GENTAMICIN RESISTANCE GENES IN *STAPHYLOCOCCUS HAEMOLYTICUS*

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**ABSTRACT:**

*Staphylococcus haemolyticus* has emerged in recent years as an important human pathogen related to nosocomial infections associated with bloodstream, invasive medical devices and immune-compromised patients. An important feature related to this microorganism is its ability to develop resistance to multiple antimicrobial drugs. Furthermore, it has been proposed that coagulase-negative *Staphylococcus* group to which *S. haemolyticus* belongs, constitutes a reservoir of resistance and virulence genes, that could favor the horizontal transfer to *Staphylococcus aureus* strains. In *S. aureus*, the most pathogenic species of the *Staphylococcus* genus, it is found the gentamicin resistance gene *aac(6')-Ie-aph(2'')-Ia/aacA-aphD*, encoding an aminoglycosides modifying bifunctional enzyme. This gene may be located on the chromosome or in plasmids that also carry other resistance genes to various antimicrobials. In this context, the aim of this study is to evaluate the presence of gentamicin resistance (Gm<sup>R</sup>) genes and correlate them with conjugative Gm<sup>R</sup> plasmids in nosocomial *S. haemolyticus* strains. For this purpose, the detection of *aacA-aphD* gene was carried out by PCR. This method was applied to 45 strains of *S. haemolyticus* Gm<sup>R</sup> provided by the Hospital Naval Marcilio Dias. Of these, 41 contained the *aacA-aphD* gene. These strains were investigated, by PCR, for the presence of genes related to the conjugation process (*traK*, *traL*, *traM* e *nesF*). Of these, two strains contained the *traM*; one *traK*; three *traL* and 23 *nesF* genes. To infer the correlation of Gm<sup>R</sup> with plasmids, Gm<sup>R</sup> strains with different genes involved in the conjugation process were selected. Then, the temperature cure technique was performed at 43°C. Of these strains, six were cured of Gm<sup>R</sup> and the loss of Gm<sup>R</sup> gene was confirmed by PCR. To analyze the impact of the cure process, susceptibility antimicrobial test was performed, and it was observed that the cured Gm<sup>S</sup> strains became sensitive concomitantly to other antibiotics tested. This suggests that Gm<sup>R</sup> gene in *S. haemolyticus* could be located in plasmids harboring other resistance genes as previously described in other species of *Staphylococcus*. Thus, these plasmids can constitute a powerful armamentarium that could spread rapidly among the *Staphylococcus* genus. To corroborate this hypothesis, DNA hybridization and bacterial conjugation experiments will be performed with the purpose of identifying such plasmids and characterize them as conjugative.

**Keywords:** *Staphylococcus haemolyticus*, plasmids, gentamicin, resistance, conjugation.

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