IVD_03 - Assessment of the activity of the anti-PBP2a monoclonal antibody from methicillin-resistant *Staphylococcus aureus* (MRSA) against *Enterococcus spp*

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Introduction: Infections caused by *Enterococcus spp.* are a serious public health problem. They are difficult to treat and have high morbidity. Therapeutic monoclonal antibodies (mAbs) have been used to treat diseases in general. In the absence of new antibiotics for the treatment of multidrug-resistant bacteria, immunotherapy using monoclonal antibodies is a promising alternative.

Objectives: Characterize the binding of a mAb against PBP2a of MRSA to PBP5 of *Enterococcus spp*.

Methodology: An *E. coli* BL21 strain, previously transformed with a vector containing the PBP5 gene sequence from Enterococcus faecium, was cultivated at 30°C and had its expression induced with 1 mM IPTG for 4 hours. The expression and solubility analysis of the recombinant protein was evaluated by SDS-PAGE and then purification was carried out by affinity liquid chromatography using His Trap HP column in an Akta Pure system. To evaluate the binding affinity of the anti-PBP2a mAb to the recombinant target protein, the Isothermal Titration Calorimetry (Nano ITC) technique was performed, considering that the protein only interacts with one antibody binding site, the calorimetric titration modeling was carried out using the independent model. The Western Blot technique was performed to evaluate whether the anti-PBP2a mAb would be able to bind to the native proteins of Enterococcal clone strains containing polymorphisms in PBP5.

Results: The recombinant PBP5 protein (rPBP5) was expressed in inclusion bodies and, after solubilization with urea, was successfully purified obtaining a yield of 0.7 mg/mL. The Nano ITC assay demonstrated that the anti- PBP2a mAb is also capable of recognizing rPBP5 and the molecular affinity was measured as KD = 441.2 nM. Regarding the results of western blotting, it was demonstrated that the antibody was capable of recognizing PBP5 in E. faecium strains with and without polymorphisms.

Conclusion: The results obtained demonstrate that the molecular affinity between the PBP5 protein and the anti- PBP2a monoclonal antibody occurs with intense affinity and specificity. Therefore, expanding the spectrum of application of this promising therapeutic alternative.

Keywords: Monoclonal Antibody - PBP2a MRSA – Enterococci; PBP5