BIO_09 - *In vitro* characterization of aptamers which bind PBP2a from Methicillin-resistant *Staphylococcus aureus* (MRSA) – preliminary results

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Introduction: Infections caused by MRSA are a serious public health problem, being difficult to treat and having high morbidity. Resistance to beta-lactam antibiotics in MRSA is due to the presence of PBP2a, an enzyme with a very low affinity for these antibiotics. The absence of new antibiotics and limited treatment options mean that new therapeutic strategies are considered highly relevant. Aptamers are *in vitro* selected oligonucleotides able of binding with high affinity and specificity to targets, thus being a viable alternative for the development of strategies for the diagnosis and treatment of MRSA infections. Our group previously selected aptamers that bind to PBP2a, and here we present results of in vitro neutralizing activity and binding affinity of these molecules with PBP2a.

Objectives: Characterize the binding of aptamers against MRSA PBP2a proteins and demonstrate *in vitro* activity of these molecules.

Methodology: An inoculum of about 10^4 CFU grown in the exponential phase of a clinical MRSA strain was added to 1 mL of Luria broth (LB) containing 10 micrograms of oxacillin. Different amounts of aptamers were added in a volume of 100 microliters, incubated at 37ºC, with agitation at 60 RPM for 4 hours. A sample without aptamers was used as a positive control. After incubation, serial dilutions were performed and plated in duplicate on Luria agar. Plates were incubated ON and colonies were counted the next day. The dissociation constant (Kd) for the interaction between PBP2a and MRSA aptamer was measured by the Isothermal Titration Calorimetry (Nano ITC) assay. The amount loaded into the syringe and cell was 113,63 µM and 10 µM, respectively. The assay was carried at 250 rpm and 25ºC.

Results: The results obtained showed that the aptamers were able to promote a bacterial reduction that varied from 50 to 80%. It was observed that smaller amounts of aptamers (10^8 molecules) provided better protection results than a higher number of molecules (10^{18} molecules). Aptamers showed KD values of approximately 31 nM, indicating a strong binding to the target protein.

Conclusion: The results demonstrate that the aptamers are able of consistently binding to PBP2a and they are is capable of generating a reduction in the number of bacteria *in vitro* assays.

Keywords: Aptamers, PBP2a, MRSA