

TITLE: ANTIMICROBIAL AND ANTIBIOFILM ACTIVITY OF TWO NEW SYNTHETIC ANTIMICROBIAL PEPTIDES AGAINST *STAPHYLOCOCCUS AUREUS*

ATHORS: MARTÍNEZ, M.M.B.^{1,2}; FLORES, N.¹; SEMORILE, L.¹; MAFFÍA, P.C.^{1,2}

INSTITUTIONS: 1. LABORATORIO DE MICROBIOLOGÍA MOLECULAR, UNIVERSIDAD NACIONAL DE QUILMES (ROQUE SAÉNZ PEÑA 352, BERNAL 1876BXD, BUENOS AIRES, ARGENTINA). 2. CONSEJO NACIONAL DE INVESTIGACIONES CIENTÍFICAS Y TÉCNICAS –CONICET- (GODOY CRUZ 2290, CABA (C1425FQB), ARGENTINA)

Staphylococcus aureus is a pathogen of clinical importance causing both nosocomial and community infections. Its ability to develop resistance to antibiotics together with its capacity to form biofilms are the main causes of chronic diseases that are difficult to treat. In this context, the research and development of new molecules against diseases associated with biofilms are extremely important. Antimicrobial peptides (AMPs) are good candidates for the development of new therapeutic drugs since they can be *de novo* designed in order to meet the challenge of multiresistant bacteria. Our group has designed and synthesized a series of new cationic, alpha-helical antimicrobial peptides. The objective of this work was to evaluate the antimicrobial and anti-biofilm activity of two of these new synthetic peptides, P5 and P1, against *S. aureus* (ATCC25923). The sequences were designed using a combined rational and computer assisted approach, following an embedded-hybrid peptide design with modifications. In order to determine the MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration), broth microdilution tests were performed according to the CLSI (Clinical and Laboratory Standards Institute). Hemolytic activity was analyzed on mice erythrocytes at different AMPs concentrations. Biofilm inhibition and pre-formed biofilm disruption were evaluated by the violet crystal method. Bacterial viability within the biofilm was also evaluated by metabolic reduction of MTT. A one-way ANOVA was performed with a posteriori Dunnett test. The MIC and MBC values for P1 were 16µg/ml and 64µg/ml respectively and for P5 were 32µg/ml and 128µg/ml. P1 reduced the biofilm formation in 39.35±4.3% and 11.91±5.3% at 0.5xMIC and 0.25xMIC respectively and P5 in 26±4.5% and 22.63±4.0% at 0.5xMIC and 0.25xMIC respectively. Both peptides reduced the amount of pre-formed biofilm at 8xMIC: P1 reduced 43.22±4.2% and P5 44.86±7.5%. It should be noted that in the rupture of pre-formed biofilm, the antibiotic vancomycin was not able to reduce the biofilm. Both peptides showed low hemolytic activity, below 10% in the concentration range tested (0-1054 µg/ml). These results showed that the new synthetic AMPs, P5 and P1, exert good antimicrobial and microbicidal activity in *S. aureus*, with mild hemolytic activity and an interesting anti-biofilm activity, mainly on pre-established biofilms, against which the antibiotic vancomycin showed no activity.

Keywords: Antimicrobial peptides, anti-biofilm, *Staphylococcus aureus*