

**TITLE:** MELITTIN AND ITS SYNERGISTIC ACTIVITY WITH OXACILLIN AGAINST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA)

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**ABSTRACT:**

The spread of resistant bacterias is emerging, with methicillin-resistant *Staphylococcus aureus* (MRSA) being a frequent cause of nosocomial and community-associated infections and with ability to produce biofilms. The search for new antimicrobial agents is required, especially for natural products with an emphasis on antimicrobial peptides (AMPs). Melittin, which represents 50% of the dry weight of the bee venom from *Apis mellifera*, is an AMP with antibacterial activity reported. The synergism of peptides with antibiotics may be able to reduce the dose of antimicrobials, their side effects and have lower cytotoxicity. The aim of this study is to evaluate the antibacterial and antibiofilm activity of melittin and its synergism with oxacillin against MRSA. Resazurin assays was performed to obtain the minimum inhibitory concentration (MIC) of the melittin and oxacillin against an ATCC MRSA 33591 and a clinical isolate of MRSA. The synergism was performed using the time-kill curve. Biofilm inhibition was tested with subinhibitory concentrations (25%, 50% and 75% of MIC) of melittin, oxacillin and their synergism. The studied antimicrobials were also tested on already formed biofilms and their hemolytic percentages were evaluated. Melittin showed MIC of 5.3 µg/mL for ATCC MRSA and 4.0 µg/mL for clinical isolate MRSA and the oxacillin showed MIC of 16.0 µg/mL for ATCC and 8.0 µg/mL for isolate MRSA. The time-kill curve indicated that the combination of 25% MIC melittin + 25% MIC oxacillin was bactericidal synergistic for both MRSA. The combination of melittin with oxacillin showed a higher percentage of biofilm inhibition (up to 60% of reduction for ATCC and up to 52% of biofilm reduction for isolate MRSA), than melittin alone (less than 40% for both strains). Melittin with oxacillin was the only one capable of eradicating the already formed biofilm, requiring 15x its MIC for ATCC MRSA and 20x of MIC to eradicate isolate MRSA. The hemolysis percentages of mellitin and melittin with oxacillin, in their MICs, were 34.9% and 0.8%, respectively, showing a lower percentage when mellitin is in combination with oxacillin. Melittin and its synergism with oxacillin had an antibacterial and antibiofilm activity against MRSA, and melittin with oxacillin had a better antibiofilm action than the melittin alone for both MRSA strains, with a lower percentage of hemolysis. The treatments tested have potential for possible models of new antibacterial agents for MRSA.

**Keywords:** *Apis mellifera*, biofilm, antibacterial activity, time-kill curve

**Development Agency:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) – 2019/24850-0.