TITLE: **In vitro activity of a nisin formulation with *Melaleuca alternifolia* against MDR *Staphylococcus aureus* isolated from bovine mastitis**

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ABSTRACT:
Staphylococcus aureus is an important pathogen of the dairy cattle industry worldwide that represents significant losses to milk production, and risks to consumer health. This bacteria is highly contagious, with low response to conventional treatments due to the high level of antimicrobial resistance and different virulence factors. In order to develop safer and more effective alternative therapies, new molecules must be investigated. The *Melaleuca alternifolia* essential oil has anti-inflammatory, antifungal, antiviral and antibacterial activities. The nisin, a bacteriocin produced by of *Lactococcus lactis* strains, has shown antimicrobial activity against several of Gram-positive bactéria species. Thus, the aim of this study was to evaluate the in vitro antibacterial activity of bacteriocin nisin, *Melaleuca alternifolia* essential oil and Nisin/Melaleuca complexes against multidrug resistant *S. aureus* (MDR) strains, isolated from bovine mastitis and belonging to an International Collection of Cultures strains (i.e., ATCC). *Melaleuca alternifolia* was solubilized in Muëller Hinton broth supplemented with 0.5% Tween 80, whereas nisin was solubilized in HCl 0,02N, decontaminated by filtration (0.45 µm). The Nisin/Melaleuca complexes were obtained after 1 hour of interaction at 25 °C. The in vitro activity of the nisin, *Melaleuca alternifolia* and Nisin/Melaleuca complexes was evaluated by minimal bactericidal concentration (MBC), checkerboard method and time-kill assays against 27 *S. aureus* strains exhibiting an MDR profile (ampicillin, ciprofloxacin, enrofloxacin, erythromycin, florfenicol, gentamicin, kanamycin, penicillin, streptomycin, trimethoprim-sulfamethoxazole and tetracycline), isolated from bovine mastitis; and *S. aureus* ATCC® 25923™, BAA-976™, BAA-977™, BAA-1026™, MRSA N315 and MU-50. The MBC_{50} value of nisin was 400 µg/mL, whereas the MBC_{50} of *Melaleuca alternifolia* was 10,000 µg/mL. The MBC of the 'Nisin/Melaleuca complexes was 100/625 µg/mL, and this combination was interpreted as synergistic effect ($\Sigma FBC \leq 0.5$). According to time kill assay, for Nisin/Melaleuca complexes, a ≥ 3 $\log_{10}$ reduction (= 99.9% reduction of CFU/mL) of the ATCC® BAA-976™ strain was achieved after 1 hour of interaction. The combination of nisin with *Melaleuca alternifolia* oil (Nisin/Melaleuca) can be an important alternative for the control of bovine mastitis caused by MDR *S. aureus*. The association of distinct molecules with different mechanisms can prevent the activity of bacterial resistance mechanisms, once the interactions of molecules could enhance its actions, decreasing its doses and consequently avoid possible side effects.
Keywords: essential oils, alternative therapy, bacteriocin, dairy cattle.

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