

**TITLE:** TRANSMISSION ELECTRON MICROSCOPY ON CELLS OF *Staphylococcus aureus* METHICILLIN RESISTENT (MRSA) TREATED WITH NISIN, OXACILLIN AND THEIR COMBINATION

**AUTHORS:** ALVES, F.C.B. <sup>1\*</sup>; ANDRADE, B.F.M.T.<sup>1</sup>; ALBANO, M.<sup>1</sup>; PEREIRA, A.F.M <sup>1</sup>; FURLANETTO, A.<sup>1</sup>; LYRA, L.P.S<sup>1</sup>, RALL, V.L.M<sup>1</sup>, BARBOSA, L.N. <sup>1</sup>, FERNANDES JUNIOR, A.<sup>1/2</sup>

**INSTITUTION:** <sup>1</sup>DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY. <sup>2</sup>ELECTRONIC MICROSCOPY CENTER, INSTITUTE OF BIOSCIENCES OF BOTUCATU- UNESP, SÃO PAULO STATE UNIVERSITY (RUBIÃO JUNIOR S/N- CEP: 18.610-307- BOTUCATU-SP);

**ABSTRACT:**

The limited development of antimicrobial agents in recent times has compounded the situation and increased the necessity to search for alternative drugs complement or replace antibiotics. Bacteriocins such nisin have many properties which suggest that they are viable alternatives to antibiotics, that include their action broad spectrum and their low toxicity. To reduce bacterial resistance, studies are needed with new antimicrobial drugs and especially studies to understand how these drugs act on the pathogen physiology, their targets and mechanisms of action and the response of this to different treatments. In previously study in our group the combination of nisin and oxacillin showed bactericidal effect and reduced bacterial count of MRSA. The aim of this study is to analyze the damage on morphology of MRSA cells treated with nisin, oxacillin and the combination of these antimicrobials. The value of the Maximum Sublethal Concentration (MSC) of nisin and oxacillin against MRSA was previously determined using the REMA (Resazurin microtiter assay) methodology. After overnight growth in 4 mL of BHI at 37 ° C, the bacterial culture was contacted with the tested antimicrobials nisin and oxacillin and their combination ( $\frac{1}{4}$  MSC nisin +  $\frac{1}{4}$  MSC oxacillin) for 4 hours using 1x and 4x the total value of their concentration (MSC). Finally, the samples after being prepared according to protocols already defined, were analyzed and photographed (digital image) with Electron Transmission Microscope (Tecnai Spirit- FEI Company). In cells treated with the combination of antimicrobials it was possible to detect cell wall damage, loss of plasma membrane integrity and consequent loss of cytoplasmic content, distortion in cell structure and total lysis of the cell. Nisin acts on the plasma membrane of the cell, while oxacillin target on the cell wall and this can facilitating the entrance of nisin, that induces pores formation in the plasma membrane and consequently causing cell death.

**Key words:** MRSA, Nisin, Oxacillin, Synergism, Bacterial morfology

**Development agency:** Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP)