



Effects of antimicrobial photodynamic therapy with photodithazine® on methicillin-resistant *Staphylococcus aureus* (MRSA): Studies in biofilms and experimental model with *Galleria mellonella*

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ABSTRACT

Staphylococcus aureus infections are a severe health problem due to the high mortality rate. Conventional treatment of these infections is via the administration of antibiotics. However, its indiscriminate use can select resistant microorganisms. Thus, it is necessary to develop alternatives for antibiotic therapy. Antimicrobial Photodynamic Therapy (aPDT), a therapeutic method that associates a photosensitizer (PS), a light source with adequate wavelength to the PS, interacts with molecular oxygen generating reactive oxygen species responsible for cell inactivation, is a viable alternative. This work aimed to analyze, *in vitro* and *in vivo*, the action of aPDT with PS Photodithazine® (PDZ) on the methicillin-resistant *S. aureus* (MRSA) strain. In the *in vitro* method, the *S. aureus* biofilm was incubated with PDZ at 50 and 75 $\mu\text{g}\cdot\text{mL}^{-1}$ for 15 min, adopting the light dose of 25, 50, and 100 J/cm^2 . In addition, PS interaction, formation of reactive oxygen species (ROS), bacterial metabolism, adhesion, bacterial viability, and biofilm structure were evaluated by scanning electron microscopy. Subsequently, the strain was inoculated into models of *Galleria mellonella*, and the survival curve, health scale, blood cell analysis, and CFU/mL of *S. aureus* in the hemolymph were analyzed after aPDT. In the *in vitro* results, bacterial reduction was observed in the different PDZ concentrations, highlighting the parameters of 75 $\mu\text{g}\cdot\text{mL}^{-1}$ of PDZ and 100 J/cm^2 . As for *in vivo* results, aPDT increased survival and stimulated the immune system of *G. mellonella* infected by *S. aureus*. aPDT proved effective in both models, demonstrating its potential as an alternative therapy in treating MRSA bacterial infections.

1. Introduction

Staphylococcus aureus is responsible for several infections, from boils to sepsis, which can cause death [1]. The conventional treatment is carried out by administering antibiotics. However, indiscriminate use is responsible for the emergence of resistant bacteria. Strains of *S. aureus* resistant to several antibiotics have already been described, the most clinically important being methicillin-resistant *Staphylococcus aureus* (MRSA) [2].

The Center for Disease Control and Prevention (CDC) identified in

2019 that the rate of MRSA strains exceeded 41% among adults and 28% among pediatric patients, with approximately 323,700 cases and reaching about 10,600 deaths, resulting in 1.7 billion dollars in expenses [3]. These high mortality rates in hospitalized patients have caused worldwide concern.

In 2017, a list of the 12 bacteria most at risk to human health due to their high resistance was divided into three categories of urgency, in which MRSA was reported as a high priority [4]. In 2019, WHO reinforced the alert about the shortage of developing new antibiotics for MRSA. Currently, only seven antibiotics are in the preclinical phase for

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