



In vitro assessment of the bactericidal effect of low-power arsenium-gallium (AsGa) laser treatment *

Avaliação *in vitro* da ação bactericida do laser de baixa potência (AsGa)

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Abstract: The objective of the present study was to perform an *in vitro* evaluation of the bactericidal action of a low-power arsenium-gallium (AsGa) laser at a wavelength of 904nm and energy density of 6 J/cm². Ten petri dishes were seeded with *Pseudomonas aeruginosa* and another ten with *Staphylococcus aureus*. The dishes were then randomly divided into four groups with five plates in each group. Two groups were treated with AsGa laser once a day for 5 days, while the other two groups received no treatment. No halo of growth inhibition was found in any of the groups. It was therefore concluded that laser treatment (AsGa, 904nm, 6J/cm²) had no bactericidal effect.

Keywords: Antibacterial agents; Bacteria; Bacterial growth; Laser treatment; Laser treatment, low-power

Resumo: Verificar, *in vitro*, o efeito bactericida do laser de baixa potência, AsGa, 904nm, na dose 6 J/cm². Foram semeadas 10 placas de Petri com a bactéria *Pseudomonas aeruginosa* e 10 placas de Petri com *Staphylococcus aureus*. Aleatoriamente, foram divididas em quatro grupos (5 placas cada): dois grupos foram tratados com o laser AsGa a cada 24 horas, durante cinco dias, e dois grupos não receberam tratamento. Em todos os grupos, não foi observado qualquer halo de inibição do crescimento. Assim, concluiu-se que a terapia a laser (AsGa, 904nm, 6 J/cm²) não produziu efeito bactericida.

Palavras-chave: Antibacterianos; Bactérias; Crescimento bacteriano; Terapia a laser; Terapia a laser de baixa intensidade

Several studies have been conducted to understand the tissue repair process and the effects of laser treatment on wound healing.¹ However, little has been published in the literature on the effect of low-power laser (LPL) on the phenotypic modulation of different bacterial populations, with ongoing debates on whether LPL kills bacteria or whether radiation can actually increase bacterial growth.^{2,3}

Therefore, the objective of the present study was to perform an *in vitro* evaluation of the bactericidal effect of low-power arsenium-gallium (AsGa) laser at a wavelength of 904nm and energy density of 6 J/cm².

This study was performed at the Laboratory of Microbiology, Santa Maria College, Cajazeiras, Paraíba, Brazil between February and June 2011.

The strains of bacteria used, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, were diluted in sterile saline solution to obtain a turbidity scale of 0.5 on the MacFarland scale (1.5 x 10⁸ UFC / ml). After 15 minutes, seeding was initiated by placing a sterile cotton swab in the suspension and then cultivating the surface of a petri dish of 65 mm in diameter containing blood agar.

The equipment used was a low-power arsenium-gallium (AsGa) laser (Ibramed[®], Laserpulse model), and the protocol parameters followed consisted of a modified version of those used by Coutinho et al²: laser power of 15W, wavelength of 904nm, energy density of 6 J/cm², applying the punctual application technique for 10 minutes on each dish.

A intentional sample of twenty petri dishes was

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prepared with blood agar and randomly divided into four groups, as follows:

Group I: Five petri dishes containing *Staphylococcus aureus* not submitted to laser treatment;

Group II: Five petri dishes containing *Staphylococcus aureus* submitted to laser treatment;

Group III: Five petri dishes containing *Pseudomonas aeruginosa* not submitted to laser treatment;

Group IV: Five petri dishes containing *Pseudomonas aeruginosa* submitted to laser treatment.

All the petri dishes were handled in a laminar flow cabinet previously sterilized with alcohol 70%. The laser was directed in a straight, centrally focused, perpendicular manner onto the surface of the bacteria culture at a standard distance of 1 cm. One application was administered daily for five consecutive days. Following irradiation, the dishes were subjected to aerobic incubation for 24 hours in an oven (37 °C).

Bacterial growth was evaluated as a function of the extent of the growth inhibition zone, verified every 24 hours for a total of 120 hours. The existence of an inhibition zone was assessed and results were classified as resistant, intermediate or sensitive.

No growth inhibition zone was found in the areas submitted to irradiation in any of the groups analyzed with either type of bacteria, (Figures 1 and 2). The bacteria were thus considered resistant, indi-

cating that the low-power AsGa laser had no bactericidal effect.

There is no consensus in the literature with respect to the effects of laser treatment on bacterial growth. While some studies indicate biostimulation or proliferative effects, others have described an inhibitory bactericidal or bacteriostatic effect, indicating that these effects are associated with changes generated by the increase in the energy level provided by the radiation in the respiratory chain of the bacteria.⁴

Carvalho Silva and Silva performed an experiment to evaluate microbiological bacterial growth *in vitro* following application of a helium-neon (HeNe) laser at a wavelength of 632.8 nm and output power of 3 mW. These authors reported a significant reduction in bacterial growth, both in solid and in liquid conditions.⁵ In this study, no inhibitory effect on bacterial metabolism was seen. Growth could be seen in the colonies, suggesting that exposure of the bacteria to a laser beam affects the respiratory chain, promoting precocious cell division.

The results of the present study are also in agreement with the findings of Ferreira and Souza, who treated skin ulcers with the AsGa laser, 904 nm, 34 mW, at an energy dose of 5 J/cm, with thrice-weekly applications administered over a 90-day period. In microbiological analyses conducted prior to and following laser therapy, these investigators found no change in bacterial colonization, supporting the hypothesis that the AsGa laser has no bactericidal effect.^{2,5,6}

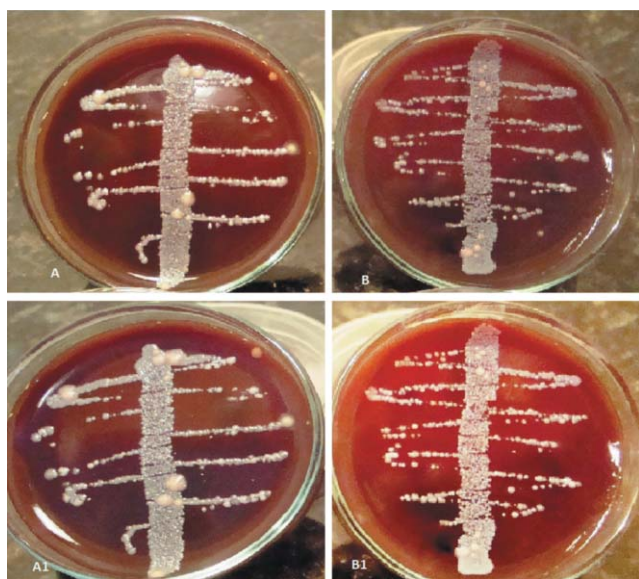


FIGURE 1: Verification of bacterial growth of *Staphylococcus aureus* 24 hours and 120 hours after seeding, with and without laser (AsGa) treatment. **A:** *S. aureus* (Group I) 24 hours after seeding, **A1:** *S. aureus* (Group I) 120 hours after seeding, **B:** *S. aureus* (Group II) 24 hours after seeding, **B1:** *S. aureus* (Group II) 120 hours after seeding

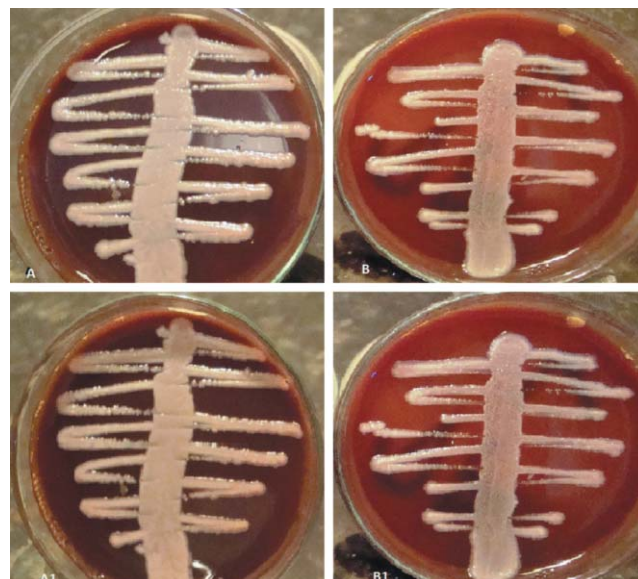


FIGURE 2: Verification of bacterial growth of *Pseudomonas aeruginosa* 24 hours and 120 hours after seeding with and without laser (AsGa) treatment. **A:** *P. aeruginosa* (Group III) 24 hours after seeding, **A1:** *P. aeruginosa* (Group III) 120 hours after seeding, **B:** *P. aeruginosa* (Group IV) 24 hours after seeding, **B1:** *P. aeruginosa* (Group IV) 120 hours after seeding

However, Meral et al. reported a decrease in the number of colony-forming units (CFU) in five strains of bacteria submitted to *in vitro* radiation with low-power yttrium-aluminum-garnet laser (Nd: YAG) 1.060nm and suggested that the bactericidal effect of this exposure would reduce the risk of infection in chronic and postoperative wounds.⁷

Müller reported that without the presence of any photosensitizing agent, laser treatment was unable to promote any change in the number of colony-forming units in bacteria samples (*Staphylococcus aureus*); therefore, low-power laser alone was unable to photo-inactivate those microorganisms, as was also found in the present study.⁸

Regarding the *in vitro* evaluation of the effect of laser therapy on the bacteria, it is clear that the culture conditions are crucial for the growth of the microorganisms.⁹ However, although the participation of hematopoietic cells appears to optimize the defense reaction of the host *in vivo* following the final session of laser treatment, no difference was found in the bacterial growth *in vitro* between the dishes in the groups treated with the ArGa laser and the dishes of the control groups in this study. This fact supports the hypothesis raised by Coutinho, who refutes Meral's theory that laser treatment exerts a strong bactericidal effect when large concentrations of hemoglobin are present.^{2,7,10} □

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