



Antimicrobial photodynamic therapy against clinical isolates of carbapenem-susceptible and carbapenem-resistant *Acinetobacter baumannii*

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Abstract

Infections caused by *Acinetobacter baumannii* have become a challenge for healthcare professionals because of the rapid increase in Gram-negative bacteria resistant to carbapenem antibiotics. The objective of this study was to evaluate the effect of antimicrobial photodynamic therapy (aPDT) against different strains of *A. baumannii* isolated from patients with infectious process and hospitalized at the intensive care unit of the hospitals of São Jose dos Campos, São Paulo. These isolates were obtained from the Valeclin Clinical Analysis Laboratory (SP, Brazil) and were tested for susceptibility to the carbapenems imipenem and meropenem by determination of the minimal inhibitory concentration (MIC) using the broth microdilution method. The strains susceptible and resistant to these antibiotics were submitted to aPDT using methylene blue and a low-level laser with a wavelength of 660 nm and fluence of 39.5 J/cm² (energy of 15 J and time of 428 s). The number of colony-forming units (CFU/mL) was analyzed by ANOVA and the Tukey test. The laboratory of origin of the clinical isolates identified 1.54% of 13,715 strains tested over a period of 8 months as *A. baumannii*. Among the *A. baumannii* isolates, 58% were resistant to carbapenems by the disk diffusion test. Susceptible isolates exhibited MIC of 0.5 to 1 µg/mL and resistant isolates of 64 to > 128 µg/mL. PDT reduced the number of *A. baumannii* cells for all isolates tested, with this reduction ranging from 63 to 88% for susceptible isolates and from 26 to 97% for resistant isolates. The percentage of viability was dependent on the strain analyzed. In conclusion, these data indicate that PDT could be an alternative strategy for the control of infections caused by carbapenem-resistant *A. baumannii*.

Keywords *Acinetobacter baumannii* · Antimicrobials · Antimicrobial photodynamic therapy · Antimicrobial resistance

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Introduction

Acinetobacter is a nosocomial pathogen represented several species, being *Acinetobacter baumannii* the most important as a healthcare pathogen with a negative impact on patients' outcome of hospitalization [1–3]. The severity of infections caused by these microorganisms is related to virulence factors such as capsular exopolysaccharide, β -lactamase production (carbapenemases), porin alterations, and overexpression of efflux pumps. This diversity of virulence factors permits *A. baumannii* to escape the host immune response and to resist treatment with antibiotics, resulting in the persistence of infection [2–4].

The alert for *A. baumannii* in nosocomial infections is that the resistance profile of this microorganism has changed over the years [1, 5, 6]. Although carbapenems are considered effective antibiotics against *A. baumannii* infections, several studies have reported increasing resistance to these antibiotics. Kuo et al. [7] demonstrated an expressive increase in the resistance of *A. baumannii* strains to imipenem from 3.4% in 2002 to 58.7% in 2010.

These microorganisms encounter in hospitalized patients an ideal environment for multiplication and development of antibiotic resistance [3, 4, 8]. The characteristics of hospitalized patients, particularly those of intensive care units, meet the necessary requisites for the colonization and multiplication of pathogenic microorganisms, as well as immunological vulnerability and imbalance of the resident microbiota [3, 4, 9]. Gales et al. [10] characterized the profile of microorganisms causing nosocomial infections using the database of the SENTRY Antimicrobial Surveillance Program of Latin American medical centers. From 2008 to 2010, more than 5000 clinical isolates were evaluated and *A. baumannii* was a leading species involved in cases of bloodstream infections, pneumonia, and skin infections. In addition, these patients are frequently exposed to antimicrobials, often attributed to episode of recurrent infections and previous hospitalization [9].

The development of new therapies that can destroy microorganisms without inducing the emergence of undesired resistant strains is necessary and urgent since the arsenal of antibiotics against multidrug-resistant microorganisms that are considered safe for the patient has become restricted. In view of this scenario, antimicrobial photodynamic therapy (aPDT) is emerging as a very useful adjunct treatment to conventional antibiotic therapies. PDT is a non-thermal photochemical reaction that involves the simultaneous presence of visible light, oxygen, and a dye or photosensitizer (PS). Several PS have been studied for their ability to bind to bacteria and efficiently generate reactive oxygen species (ROS) upon photostimulation [11]. ROS are formed through type I or II mechanisms and may inactivate several classes of microbial cells including Gram-negative bacteria such as *A. baumannii*, which are typically characterized by an impermeable outer cell membrane that contains endotoxins and blocks antibiotics,

dyes, and detergents, protecting the sensitive inner membrane and cell wall [11–13]. Photosensitizers are light-sensitive molecules and should be biologically stable, photochemically active, and minimally toxic to tissues of the organism. The photosensitizers used include hematoporphyrin derivatives, phenothiazines (toluidine blue and methylene blue), cyanines, phytotherapeutic agents, and phthalocyanines [14].

The use of aPDT to treat localized infections usually involves the topical application of a PS into the infected tissue, followed by illumination with red or near-infrared light that is able to penetrate the tissue [15]. Because of the delivery of visible light, aPDT has been recommended exclusively to localized infections, as opposed to systemic infections such as bacteremia [15, 16]. *A. baumannii* is an opportunistic pathogen that causes hospital-related infections, especially wound infections. This microorganism has caused 2.1% of intensive care unit-acquired skin/soft tissue infections [17] and was isolated from > 30% of combat victims with open bone fractures [18, 19]. Moreover, the treatment of these infections are very complicated [8, 20]. Thus, aPDT is used to treat localized infections with ability to kill bacteria without interfering with wound healing and it is one of the best advantages of this method [15, 21].

Considering the promising results of aPDT against *A. baumannii* and the need of alternative therapies for the control of nosocomial infections caused by antibiotic-resistant Gram-negative bacteria, the objective of this study was to evaluate the effect of aPDT on carbapenem-susceptible and carbapenem-resistant clinical *A. baumannii* isolates.

Material and methods

Clinical isolates

This study was approved by the Ethics Committee (Process: 24409813.9.0000.0077) of the Institute of Science and Technology of São Paulo State University (UNESP). All isolates were obtained by ValeClin Laboratory from patients with infectious process hospitalized at Hospital Santa Casa of São José dos Campos, São Paulo, during the July 2014 to February 2015 period. In total, 207 *A. baumannii* were isolated from various clinical specimens and identified by biochemical methods (Rugai, Enterokit C, NF Kit) and submitted to antibiotic susceptibility testing by the Kirby & Bauer disk diffusion method at the Valeclin Laboratory according to methodology standardized and validated in this laboratory.

For this study, 21 isolates of *A. baumannii* were collected from clinical specimens such as tracheal aspirates (8), wound infection (6), burn wound (5), and catheter (2) from patients hospitalized at an intensive care unit. Among these isolates, 18 strains were resistant and 3 susceptible to carbapenem antibiotics.

Stocks of the clinical isolates and reference strain were maintained in Brain Heart Infusion broth (BHI, Himedia®, Mumbai, India) with 20% glycerol at $-70\text{ }^{\circ}\text{C}$ in a freezer at the Laboratory of Microbiology and Immunology of ICT/UNESP. For activation, the strains were seeded onto MacConkey agar (Himedia®, Mumbai, India) and cultured in BHI broth. The cultures were incubated in a bacteriological oven for 24 h at $37\text{ }^{\circ}\text{C}$. Reference strain of *A. baumannii* (ATCC 19606) was included in all experiments.

Determination of minimal inhibitory concentration

The minimal inhibitory concentration (MIC) of the carbapenems imipenem and meropenem (Sigma-Aldrich, St. Louis, MO, USA) was determined for the 21 clinical isolates selected for this study. These tests were performed according to the broth microdilution protocol established by the Clinical and Laboratory Standards Institute [22]. The planktonic *A. baumannii* cells were adjusted to an optical density of 0.6 to 0.8 (10^8 bacterial cells/mL), at 625 nm using a spectrophotometer (B582, Micronal, São Paulo, São Paulo, Brazil) and used to prepare the inoculum, with a final concentration of approximately 10^5 cells/mL in each well. Stock solutions of imipenem and meropenem (Sigma-Aldrich, St. Louis, MO, USA) were prepared and diluted serially in Mueller-Hinton broth (cation-adjusted) (Difco, Mumbai, India) in 96-well microtiter plates (Costar Corning, New York, NY, USA), and standard inocula of each strain were added to the wells. After 24 h of incubation in an oven at $37\text{ }^{\circ}\text{C}$, the MIC values were determined by the observation of turbidity of the medium in the microtiter plates. The MIC was defined as the lowest concentration of the antibiotic that inhibits bacterial growth. The breakpoints of susceptibility and resistance to the antibiotics tested were interpreted according to CLSI M100-S23, as followed: imipenem (susceptible ≤ 4 and resistant ≥ 16) and meropenem (susceptible ≤ 4 and resistant ≥ 16). *Escherichia coli* ATCC 25922 was used as a control for susceptibility testing.

Antimicrobial photodynamic therapy

The methodology for aPDT was performed on 21 isolates of *A. baumannii* according to Souza et al. [23]. Methylene blue (Synth, São Paulo, Brazil), at a concentration of 0.1 mg/mL, was used for the sensitization of *Acinetobacter* strains. The photosensitizer was prepared by dissolution of the dye in physiological solution (0.85% NaCl) and filtration through a sterile 0.22- μm Millipore membrane (São Paulo, Brazil). After filtration, the photosensitizer solution was stored in the dark. The light source used was a gallium–aluminum–arsenide (GaAlAs) Laser (Easy Laser, Clean Line, Taubaté, Brazil) with a wavelength of 660 nm, output power of 0.035 W, spot size area of 0.028 cm^2 , and fluence of 39.5 J/ cm^2 (energy of 15 J and time of 428 s) as shown in Fig. 1. The

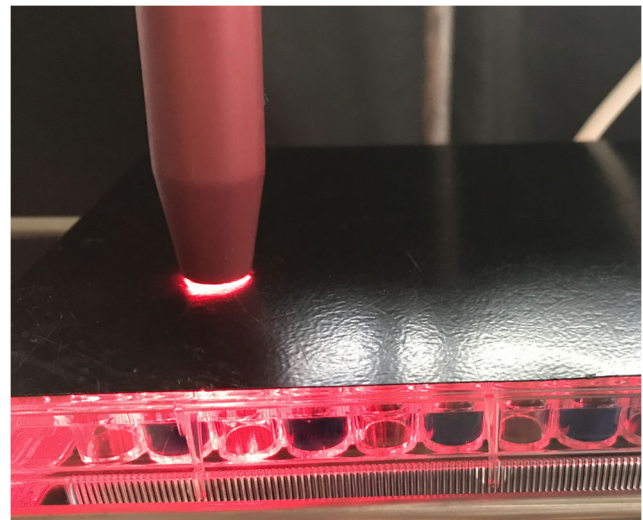


Fig. 1 Photodynamic therapy with methylene blue and a low-level laser performed in the planktonic bacterial cells in the well of a 96-well plate

illuminated area was 0.38 cm^2 . The optical output of the laser unit was measured before, halfway through and after the experiment. The temperature at the bottom of the 96-well microtiter plates (Costar Corning, New York, NY, USA) was monitored using an infrared thermometer (MX4, Raytek, Sorocaba, São Paulo, Brazil), and no increase in temperature was observed after irradiation.

Standard suspensions (10^8 bacterial cells/mL) of the microorganisms were prepared in saline from colonies isolated on BHI agar and adjusted to an optical density of 0.6 to 0.8 at 625 nm using a spectrophotometer (B582, Micronal, São Paulo, São Paulo, Brazil) for all experiments.

Four experimental groups were evaluated: Control group with absence of photosensitizer and laser (P–L–), saline and laser (P–L+), photosensitizer without laser (P+L–) and PDT group with presence of photosensitizer and Laser (P+L+). In the PDT and P+L– groups, 0.1 mg/mL of the methylene blue was added in each well, and the plates were incubated for 5 min on an orbital shaker (Solab Piracicaba, Brazil). Subsequently, the contents of the wells were subjected to irradiation according to the protocol described above. Each assay was performed in aseptic conditions within a laminar flow chamber and with ambient lights turned off. A black mask with a hole matching the diameter of the well opening minimized artifacts related to light scattering during the irradiation procedure. After irradiation, decimal serial dilutions of the suspension (10^{-1} to 10^{-5}) were prepared for each test sample, and 100 μL aliquots of each dilution were spread on plates containing Brain Heart Infusion (BHI) agar. The plates were incubated at $37\text{ }^{\circ}\text{C}$ for 24 h. Afterwards, those plates containing from 30 to 300 colonies were used to calculate the colony-forming units (CFU) and converted into logarithm. Five assays were conducted per group.

Statistical analysis

The CFU/mL data were converted to logarithmic values and submitted to ANOVA, Tukey's test, using the GraphPad Prism 5 program (GraphPad Software, Inc., CA, USA). A p value < 0.05 was considered significant.

The percentage reduction in CFU/mL was calculated for each *A. baumannii* isolate, considering the P+L+ group in relation to the control group (P–L–).

Results

Among the clinical samples registered at the Valeclin Laboratory between July 2014 and February 2015 (8 months), 13,715 were suspicious of an infectious process from hospitalized patients. Of these samples, 13,426 were confirmed to be an infectious process and 207 were positive for *A. baumannii* (1.54%). Fifty-eight percent of the clinical *A. baumannii* isolates were resistant to imipenem and meropenem in the Kirby & Bauer disk diffusion test performed at the Valeclin Laboratory.

At the Laboratory of Microbiology of ICT/UNESP, the 21 isolates from patients hospitalized at an intensive care unit were submitted to the broth microdilution test for determination of imipenem and meropenem MICs and the results are shown in Table 1. The result of the broth microdilution test showed the same antibiotic susceptibility profile as the agar diffusion method. The MIC determined by the broth microdilution method was 0.5 to 1 µg/mL for susceptible isolates and 64 to > 128 µg/mL for resistant isolates.

Firstly, three susceptible (AS1, AS2, and AS5) and three resistant clinical isolates (AR1, AR2, and AR6) were tested to antimicrobial PDT in order to verify the effects of laser or PS alone (P–L+, P+L– groups). For these isolates, four experimental groups proposed in the present study (P–L–, P–L+, P+L–, and P+L+) were performed. Statistically significant differences ($p < 0.0001$) were obtained in the P+L+ group (aPDT) compared with the other groups (P–L–, P–L+, and P+L–), which did not show significant differences among them ($p > 0.05$) as indicated in the Fig. 2. Thus, the 15 carbapenem-resistant *A. baumannii* isolates were investigated regarding their susceptibility to in vitro photosensitization, testing only two experimental groups: control (P–L–) and submitted to aPDT (P+L+).

The percentage of viability in CFU/mL was calculated for all isolates studied: one reference strain (ATCC), 3 antibiotic-susceptible isolates (Fig. 3a), and 18 antibiotic-resistant isolates (Fig. 3b). The percentage of viability was dependent on the strain analyzed. PDT resulted in a reduction of 92% for ATCC strain 19,606. The reduction achieved with aPDT among the susceptible strains was 63% for isolate AS1, 70% for AS5, and 88% for AS2 (Fig. 3a). PDT resulted in a

Table 1 Determination of the minimal inhibitory concentration in clinical *Acinetobacter baumannii* isolates

<i>A. baumannii</i> isolate	Imipenem (µg/mL)		Meropenem (µg/mL)	
	MIC	Reference	MIC	Reference
AS-1	1	S	1	S
AS-2	<0.5	S	<0.5	S
AS-5	1	S	1	S
A1	>128	R	128	R
A2	>128	R	128	R
A3	>128	R	128	R
A4	>128	R	64	R
A5	64	R	64	R
A6	>128	R	128	R
A7	>128	R	64	R
A8	>128	R	128	R
A20	>128	R	128	R
A23	>128	R	64	R
A24	>128	R	64	R
A25	>128	R	64	R
A26	>128	R	128	R
A27	>128	R	>128	R
A28	>128	R	64	R
A31	>128	R	128	R
A32	>128	R	64	R
A33	>128	R	128	R
ATCC	8	I	4	I

Escherichia coli ATCC25922 were used for quality control of antimicrobial susceptibility testing and included in each assay

S susceptible, R resistant, I intermediate susceptibility to the antibiotics

microbial reduction of 24 to 97% in the resistant isolates and this reduction was higher than 50% in 15 out of 18 isolates and higher than 80% in 11 out of 18 isolates tested (Fig. 3b).

Discussion

In the present study, among the 13,426 clinical isolates analyzed at Valeclin Laboratory (Brazil) over an observation period of 8 months, 1.54% was identified as *A. baumannii*. Gaynes and Edwards [17] analyzed 400,000 nosocomial isolates registered at the National Nosocomial Infections Surveillance (NNIS) in Atlanta (USA) from 1986 to 2003 and found 7% of *A. baumannii* isolates, a rate slightly higher than that observed in the present study.

The antibiotic susceptibility profile of these clinical isolates was compared by two different methods: disk diffusion and broth microdilution. The results indicated that both methods were effective in detecting susceptibility of the isolates to the antibiotics since the broth microdilution method confirmed all results obtained in the agar diffusion test. Comparing methods for antibiotic susceptibility testing, Liu et al. [24] obtained reliable results with the disk diffusion and broth microdilution methods. The authors suggested to replace the broth microdilution method, which has been used as a gold standard, with the disk diffusion method because of the low cost of

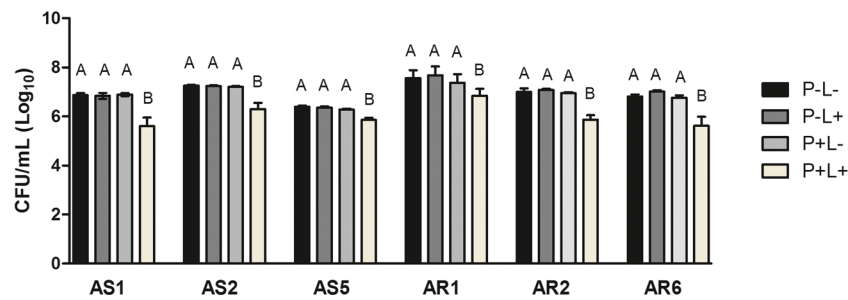


Fig. 2 Mean and standard deviation CFU/mL (\log_{10}) obtained for photosensitization of clinical isolates of *A. baumannii* with methylene blue and a low-level laser. One-way analysis of variance (ANOVA) and Tukey's test for each strain were performed. Different letters (A and B) indicate a significant difference between groups P-L- (control group), P

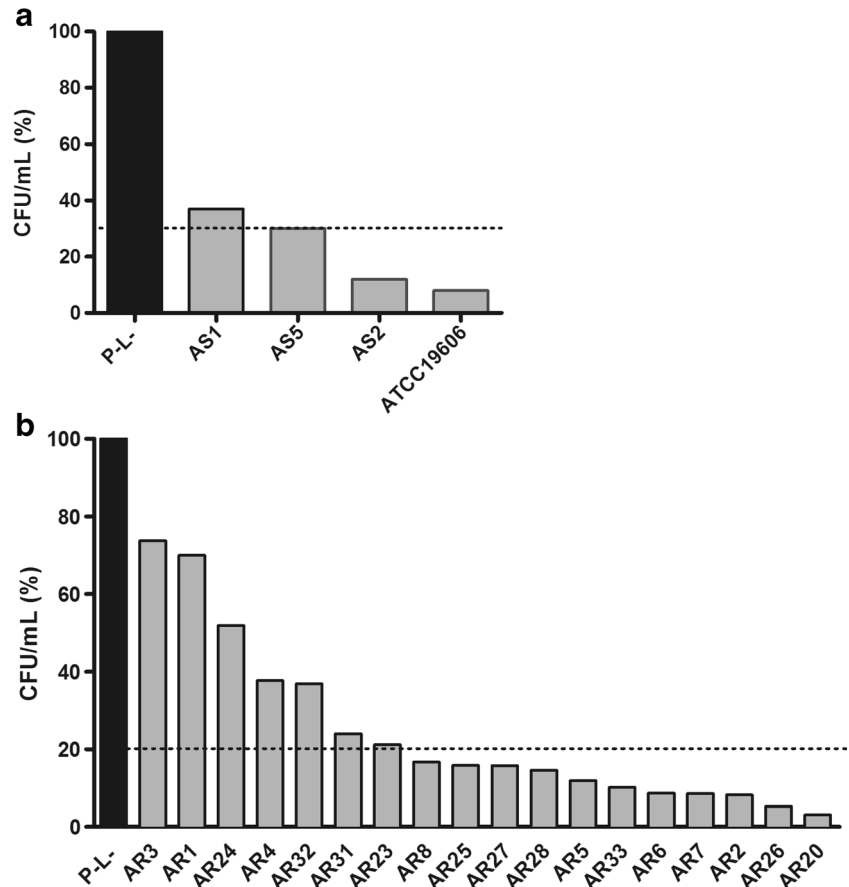
-L+ (only laser), P+L- (only photosensitizer), and P+L+ (laser and photosensitizer) ($p < 0.0001$). Equal letters denote statistical similarity between the groups ($p > 0.05$). Three carbapenem-susceptible clinical isolates: AS1, AS2, and AS5 and three carbapenem-resistant clinical isolates: AR1, AR2, and AR6 were evaluated in this assay

the latter. However, although the disk diffusion method can be used for identifying antibiotic susceptibility of clinical isolates, the determination of MIC has become increasingly more necessary, which is done by the broth microdilution method. According to Jiang et al. [25], to control the increase in nosocomial infections caused by multidrug-resistant *A. baumannii* strains, the determination of MIC of antibiotics and screening for metallo- β -lactamase producers among *Acinetobacter* spp. isolates are extremely important.

Fifty-eight percent of all clinical *A. baumannii* isolates identified in this study were resistant to imipenem and

meropenem. Similar results have been reported by Tien et al. [26], who analyzed 1381 clinical *Acinetobacter* spp. isolates from the Teaching Hospital of the China Medical University (Taiwan) and observed resistance rates of 65 and 68% to imipenem and meropenem, respectively. Within this context, antimicrobial PDT has emerged as an alternative and adjunct treatment for the control of pathogenic microorganisms, including Gram-positive and Gram-negative bacteria and fungi, especially those resistant to antibiotic therapy [11, 27–29]. Control measures of *A. baumannii* have become increasingly more necessary and urgent since its potential of nosocomial

Fig. 3 Percentage reduction, expressed as mean values (CFU/mL), in the viability of clinical isolates of *A. baumannii* exposed to laser and photosensitizer (P+L+) compared to the control group (P-L-). **a** Three carbapenem-susceptible clinical isolates and reference strain ATCC 19606. **b** Eighteen carbapenem-resistant clinical isolates



dissemination has reached elevated numbers in recent years [6, 30–34]. Jiang et al. [25] identified *A. baumannii* as the main contaminating agent of catheters in hospitalized patients, leading to the development of bacteremias and subsequent infections. In this respect, PDT may be an important tool to reduce skin bacterial cells and the contamination of catheters.

In addition to the antimicrobial effects, the light source selected for this study (laser at 660 nm) has been widely used to stimulate cellular functions with physiological and clinical benefits [35, 36]. Low-level laser/light therapy (LLLT), also known as photobiomodulation, is based on the assumption that red (600–700 nm) and near-infrared (770–1200 nm) light at low irradiance excites specific chromophores, such as the mitochondrial cytochrome C oxidase, that represents the main source for intracellular ROS generation [35, 36, 37]. In turn, LLLT alters the cellular redox state which induces the activation of numerous intracellular signaling pathways, affecting the transcription factors related to cell proliferation, survival, tissue repair, and regeneration [38–40]. The main medical applications of LLLT include the reduction of pain and inflammation, promotion of repair and regeneration of different tissues and nerves, and prevention of tissue damage in situations where it is likely to occur [36, 38, 39–42]. Based on these facts, Santos et al. [43] evaluated the immunomodulatory effects of PDT on the treatment of *Porphyromonas gingivalis* infections in *Galleria mellonella* animal model. Using red light (laser 660 nm) associated to methylene blue, the authors found that PDT was able to kill the cells of *P. gingivalis*, and also activate the *G. mellonella* immune system by increasing the number of circulating immune cells.

In the present study, the clinical *A. baumannii* isolates that were susceptible and resistant to imipenem and meropenem exhibited significant susceptibility to in vitro photosensitization, confirming that PDT is a promising adjunct technique to resistant infections and has the further advantage of not leading to the selection of resistant strains. In a recent study, Zhang et al. [21] evaluated the bactericidal action of PDT on a multidrug-resistant clinical isolate of *A. baumannii* in a mouse burn model. The results showed significant inactivation of bacterial cells in the treated group after a single exposure to PDT, while the degree of infection remained stable in the control group, with no reduction in the infectious process [21].

In order to test the efficiency of aPDT on different nosocomial *A. baumannii* isolates, 18 carbapenem-resistant clinical isolates were analyzed in this study. Our data showed that PDT resulted in bacterial reduction higher than 50% in 15 isolates. In addition, microbial reduction higher than 80% was achieved in 11 of the 18 isolates submitted to PDT. These data demonstrate the potential of aPDT for the treatment of infections caused by antibiotic-resistant *A. baumannii* strains.

In addition to assisting in the treatment of these infections, PDT can help tackle the transition of susceptible to resistant strains in the hospital setting. In the present study, 42% of the

clinical *A. baumannii* isolates were susceptible to imipenem and meropenem, indicating that despite the increase of resistant strains in recent years there is still a high percentage of antibiotic-susceptible strains that should be viewed with caution. In these cases, aPDT could be important to reduce antibiotic doses and to avoid the use of different classes of antimicrobials. In conclusion, aPDT exerted significant antimicrobial activity against carbapenem-susceptible and resistant clinical *A. baumannii* isolates from patients hospitalized at an intensive care unit, indicating that aPDT could be an alternative strategy to treat superficial infections of *A. baumannii* and to control nosocomial infections.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by the Ethics Committee (Process: 24409813.9.0000.0077) of the Institute of Science and Technology of Univ. Estadual Paulista (ICT/UNESP).

This article does not contain any studies with animals performed by any of the authors.

Informed consent Not applicable.

References

1. Cisneros JM, Rodríguez-Baño J (2002) Nosocomial bacteremia due to *Acinetobacter baumannii*: epidemiology, clinical features and treatment. *Clin Microbiol Infect* 8:687–693
2. Karamatakis T, Antachopoulos C, Tsakris A et al (2017) Molecular epidemiology of carbapenem-resistant *Acinetobacter baumannii* in Greece: an extended review (2000–2015). *Future Microbiol* 12:801–815
3. Howard A, O' Donoghue M, Feeney A, Sleator RD (2012). *Acinetobacter baumannii*: an emerging opportunistic pathogen. *Virulence* 3:243–250
4. Lee CR, Lee JH, Park M et al (2017) Biology of *Acinetobacter baumannii*: pathogenesis, antibiotic resistance mechanisms, and prospective treatment options. *Front Cell Infect Microbiol* 13:55
5. Lemos EV, Ia Hoz FP, Einarson TR et al (2014) Carbapenem resistance and mortality in patients with *Acinetobacter baumannii* infection: systematic review and meta-analysis. *Clin Microbiol Infect* 20(5):416–423
6. Perez F, Hujer AM, Hujer KM et al (2007) Global challenge of multidrug-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 51:3471–3484

7. Kuo SC, Chang SC, Wang HY et al (2012) Emergence of extensively drug-resistant *Acinetobacter baumannii* complex over 10 years: nationwide data from the Taiwan Surveillance of Antimicrobial Resistance (TSAR) program. *BMC Infect Dis* 12:200
8. Izadpanah M, Khalili H (2015) Antibiotic regimens for treatment of infections due to multidrug-resistant Gram-negative pathogens: an evidence-based literature review. *J Res Pharm Pract* 4:105–114
9. Winter JS, Santos RP, Azambuja AZ et al (2013) Microbiologic isolates and risk factors associated with antimicrobial resistance in patients admitted to the intensive care unit in a tertiary care hospital. *Am J Infect Control* 41:846–848
10. Gales AC, Castanheira M, Jones RN et al (2012) Antimicrobial resistance among Gram-negative bacilli isolated from Latin America: results from SENTRY Antimicrobial Surveillance Program (Latin America, 2008–2010). *Diagn Microbiol Infect Dis* 73:354–360
11. Sperandio FF, Huang YY, Hamblin MR (2013) Antimicrobial photodynamic therapy to kill Gram-negative bacteria. *Recent Pat Antiinfect Drug Discov* 8(2):108–120
12. Ragàs X, Dai T, Tegos GP et al (2010) Photodynamic inactivation of *Acinetobacter baumannii* using phenothiazinium dyes: *in vitro* and *in vivo* studies. *Lasers Surg Med* 42(5):384–390
13. Maisch T, Hackbarth S, Regensburger J et al (2011) Photodynamic inactivation of multi-resistant bacteria (PIB)—a new approach to treat superficial infections in the 21st century. *J Dtsch Dermatol Ges* 9(5):360–366
14. Wainwright M (1998) Photodynamic antimicrobial chemotherapy (PACT). *J Antimicrob Chem* 42:13–28
15. Demidova TN, Hamblin MR (2004) Photodynamic therapy targeted to pathogens. *Int J Immunopathol Pharmacol* 17:245–254
16. Dai T, Tegos GP, Lu Z et al (2009) Photodynamic therapy for *Acinetobacter baumannii* burn infections in mice. *Antimicrob Agents Chemother* 53(9):3929–3934
17. Gaynes R, Edwards JR (2005) Overview of nosocomial infections caused by gram-negative bacilli. *Clin Infect Dis* 41(6):848–854
18. Johnson EN, Burns TC, Hayda RA et al (2007) Infectious complications of open type III tibial fractures among combat casualties. *Clin Infect Dis* 45:409–415
19. Sebeny PJ, Riddle MS, Petersen K (2008) *Acinetobacter baumannii* skin and soft-tissue infection associated with war trauma. *Clin Infect Dis* 47:444–449
20. Moradi J, Hashemi FB, Bahador A (2015) Antibiotic resistance of *Acinetobacter baumannii* in Iran: a systemic review of the published literature. *Osong Public Health Res Perspect* 6(2):79–86
21. Zhang Y, Zhu Y, Gupta A et al (2014) Antimicrobial blue light therapy for multidrug-resistant *Acinetobacter baumannii* infection in a mouse burn model: implications for prophylaxis and treatment of combat-related wound infections. *J Infect Dis* 209:1963–1971
22. (2013) Clinical and Laboratory Standards Institute Performance standards for antimicrobial susceptibility testing [M100-S23]. Clinical and Laboratory Standards Institute, Wayne, PA, USA
23. Souza LC, Brito PR, de Oliveira JC et al (2010) Photodynamic therapy with two different photosensitizers as a supplement to instrumentation/irrigation procedures in promoting intracanal reduction of *Enterococcus faecalis*. *J Endod* 36:292–296
24. Liu JW, Ko WC, Huang CH et al (2012) Agreement assessment of tigecycline susceptibilities determined by the disk diffusion and broth microdilution methods among commonly encountered resistant bacterial isolates: results from the tigecycline *in vitro* surveillance in Taiwan (TIST) Study, 2008 to 2010. *Antimicrob Agents Chemother* 56(3):1414–1417
25. Jiang H, Hu H, Ren H et al (2016) Retrospective data about the catheter-related complications and management in massive bus burn casualties. *J Vasc Access* 17(4):353–359
26. Tien N, You BJ, Chang HL et al (2012) Comparison of genospecies and antimicrobial resistance profiles of isolates in the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex from various clinical specimens. *Antimicrob Agents Chemother* 56:6267–6271
27. Garcez AS, Núñez SC, Azambuja N Jr et al (2013) Effects of photodynamic therapy on Gram-positive and Gram-negative bacterial biofilms by bioluminescence imaging and scanning electron microscopic analysis. *Photomed Laser Surg* 31:519–525
28. Misba L, Zaidi S, Khan AU (2017) A comparison of antibacterial and antibiofilm efficacy of phenothiazinium dyes between Gram positive and Gram negative bacterial biofilms. *Photodiagn Photodyn Ther* 18:24–33
29. de Figueiredo Freitas LS, Rossoni RD, Jorge AO et al (2017) Repeated applications of photodynamic therapy on *Candida glabrata* biofilms formed in acrylic resin polymerized. *Lasers Med Sci* 32(3):549–555
30. Altun HU, Yagci S, Bulut C et al (2014) Antimicrobial susceptibilities of clinical *Acinetobacter baumannii* isolates with different genotypes. *Jundishapur J Microbiol* 7:e13347
31. Izadpou F, Ranjbari N, Aramesh MR et al (2016) An investigation of antibacterial resistance patterns among *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates collected from intensive care units of a university-affiliated hospital in Ahvaz, Iran. *Jundishapur J Microbiol* 9:e35624
32. Clark NM, Zhanel GG, Lynch JP (2016) Emergence of antimicrobial resistance among *Acinetobacter* species: a global threat. *Curr Opin Crit Care* 22:491–499
33. Maraki S, Mantadakis E, Mavromanolaki VE et al (2016) A 5-year surveillance study on antimicrobial resistance of *Acinetobacter baumannii* clinical isolates from a tertiary greek hospital. *Infect Chemother* 48:190–198
34. Wong D, Nielsen TB, Bonomo RA et al (2017) Clinical and pathophysiological overview of *Acinetobacter* infections: a century of challenges. *Clin Microbiol Res* 30:409–447
35. Wang X, Tian F, Soni SS et al (2016) Interplay between up-regulation of cytochrome-c-oxidase and hemoglobin oxygenation induced by near-infrared laser. *Sci Rep* 6:30540
36. Rupel K, Zupin L, Colliva A et al (2018) Photobiomodulation at multiple wavelengths differentially modulates oxidative stress *in vitro* and *in vivo*. *Oxidative Med Cell Longev* 2018:6510159
37. Chung H, Dai T, Sharma SK et al (2012) The nuts and bolts of low-level laser (light) therapy. *Ann Biomed Eng* 40(2):516–533
38. Gupta A, Avci P, Sadasivam M et al (2012) Shining light on nanotechnology to help repair and regeneration. *Biotechnol Adv* 31(5):607–631
39. Avci P, Gupta A, Sadasivam M et al (2013) Low-level laser (light) therapy (LLLT) in skin: stimulating, healing, restoring. *Semin Cutan Med Surg* 32(1):41–52
40. Peplow PV, Chung TY, Ryan B, Baxter GD (2011) Laser photobiomodulation of gene expression and release of growth factors and cytokines from cells in culture: a review of human and animal studies. *Photomed Laser Surg* 29(5):285–304
41. Kuffler DP (2016) Photobiomodulation in promoting wound healing: a review. *Regen Med* 11(1):107–122
42. Wang X, Reddy DD, Nalawade SS et al (2018) Impact of heat on metabolic and hemodynamic changes in transcranial infrared laser stimulation measured by broadband near-infrared spectroscopy. *Neurophotonics* 5(1):011004
43. Dos Santos JD, de Alvarenga JA, Rossoni RD et al (2017) Immunomodulatory effect of photodynamic therapy in *Galleria mellonella* infected with *Porphyromonas gingivalis*. *Microb Pathog* 110:507–511

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