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Original Research Article

Photoinactivation of extensively drug-resistant gram-negative bacteria from healthcare-associated infections in Venezuela

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ABSTRACT

Background: The overwhelming advance of antimicrobial resistance demands the incorporation of new antibiotics and effective alternative treatments, and among the latter, blue light phototherapy. The objective of this study was to evaluate the photoinactivation effect of irradiation with blue light (405 nm) on extensively drug-resistance (XDR) Gram-negative bacteria from healthcare-associated infections
Methods: A lighting unit was made using a 50W LED fitted to a condenser lens to create an irradiance gradient. After characterization of the lamp and standardization of microbiological procedures, bacterial inocula of 1.5×10^7 CFU of 40 bacterial strains: 27 Enterobacterales and 13 Pseudomonadales were subjected to irradiation for 15 minutes with blue light. The results were analysed with the SPSS program and by applying the Shapiro-Wilk and Mann-Whitney tests. Survival rates were also calculated.
Results: 82.5% of the strains were photoinactivated, with inhibition thresholds between 86 and 126 J/cm^2 for most of the Enterobacterales and below 86 J/cm^2 in large part of the Pseudomonadales, these being the most sensitive group. 17.5% of the strains showed tolerance to irradiation thresholds up to>171 J/cm^2. The survival rate decreased as the halo of inhibition of bacterial growth increased. The photoinactivating effect of blue light on the bacteria studied was a characteristic independent of the complexity of the resistance pattern that these strains presented.
Conclusions: The findings obtained show that blue light has an important photoinactivating effect against XDR Gram-negative bacteria of nosocomial origin and can be considered as a future therapeutic alternative to contain the phenomenon of antimicrobial resistance.

Keywords: Photoinactivation, Blue light, Enterobacterales, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, Extensively drug-resistant strains

INTRODUCTION

Antimicrobial resistance is considered by the World Health Organization (WHO) as one of the most significant public health problems of the 21st century and indeed, one of the biggest challenges in clinical practice.[1] Among the objectives of the Global Action Plan proposed by the WHO to contain antimicrobial resistance, are research and development of new antibiotics, as well as exploration of other options for prophylaxis and treatment that are more effective and less prone to the rapid emergence of antimicrobial resistance.[2] In this regard, in 2017 the WHO released a list of the 12 bacteria that should be considered a priority due to the limited treatment alternatives available and their impact on public health. Among these bacteria, multidrug-resistant (MDR), extensively drug-resistant (XDR) *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and Enterobacterales are considered a critical

priority.[3] These bacteria are especially dangerous in hospitals, nursing homes, or long-stay units and among critically ill patients who need to be cared for with medical devices such as ventilators and intravenous catheters.[2-3]

Unfortunately, these bacteria, known to be resistant to third and fourth-generation cephalosporins, as well as carbapenems, can cause serious infections, such as septicaemia and pneumonia, often with fatal outcomes.[1-3] In this context, considering also the decline in the discovery of new antibiotics, some novel alternatives for the treatment of bacterial infections have emerged, which although still under investigation, are currently showing encouraging results. Among these possible options is the antimicrobial Photodynamic Inactivation/antimicrobial Blue Light (aPDI/aBL) therapy.[4,5] In recent years, several studies have reported the bactericidal effect of visible light, with most of these investigations indicating that the blue part (400-500 nm) is particularly responsible for the inhibitory effect on bacterial pathogens.[5] However, a wide range of microbial cells, including Gram-positive bacteria, Gram-negative bacteria, mycobacteria, yeasts, dermatophytes and moulds in planktonic or biofilm forms, have also proven to be susceptible to blue light.[4-6]

The aPDI/aBL therapy exploits the photoexcitation of intracellular porphyrins by blue light, leading to energy transfer and the production of highly cytotoxic reactive oxygen species (ROS), such as free radicals, singlet oxygen and peroxides.[6] These ROS cause irreversible intracellular damage by simultaneously affecting numerous cellular targets, including DNA, RNA, proteins, and lipids. Wavelengths ranging from 400 to 425 nm can be used for bacterial inactivation.[4] However, the optimal antimicrobial activity occurs at 405 nm, as this is the wavelength in the electromagnetic spectrum where endogenous photosensitizers (PS), like porphyrins and flavins are mostly excited.[5] Due to its multitarget mode of action, the antimicrobial photodynamic inactivation treatment, including aBL inactivation, is considered to have a low impact on the development of resistance. Unlike antibiotics, full resistance to phototreatment with blue light has not yet been reported.[4-6]

On the other hand, some factors that favourably influence the microbicidal activity of blue light have been identified, such as the duration of irradiation and the pretreatment of bacterial cells with exogenous photosensitizing substances.[7] These substances are classified according to their origin and chemical structure, where the most commonly used are synthetic dyes, such as methylene blue and toluidine blue.[8] However, in recent years the applicability of nanoparticles and aPDI to improve PS delivery to the bacteria and optimize inactivation kinetics, has been studied. Among these nanostructures are fullerenes and titanium dioxide derivatives.[9] Numerous studies agree on the potential of aBL as a therapy for the treatment of localized infections, particularly those related to skin and soft tissue infections, and also for the decontamination of surfaces, liquids, and food, thus reducing the use and exposure to antibiotics.[10-14] Similarly, other authors confirm that aPDI/aBL is effective in killing MDR and XDR bacteria, indicating that the use of blue light may help in containing the spread of resistance genes.[5,8,10-14] To the best of our knowledge, and after an exhaustive review of the national literature, to date there are no known studies in Venezuela that evaluate the photoinhibitory effect of blue light (405 nm) on hospital-acquired or community-acquired pathogens. For this reason, considering the antimicrobial potential of blue light (405 nm), in this work we investigated its ability to photoinactivate XDR Gram-negative bacteria from healthcare-associated infections in Venezuela.

METHODS

Study setting

An experimental and applied study was conducted from July to December 2022 at the Laboratory of Molecular Microbiology, Faculty of Pharmacy and Bioanalysis, University of The Andes, Mérida, Venezuela.

Bacterial strains

The bacterial collection analysed consisted of 40 extensively drug-resistant Gram-negative strains from adult patients with healthcare-associated infections at the University Hospital of The Andes (UHTA), Mérida, Venezuela, (15 *Klebsiella pneumoniae*; 1 *Klebsiella oxytoca*; 1 *Klebsiella aerogenes*; 10 *Escherichia coli*; 11 *Pseudomonas aeruginosa*; 2 *Acinetobacter baumannii*). These strains, microbiologically and molecularly characterized in previous studies (Table 1), are from the Molecular Microbiology Laboratory collection at the Faculty of Pharmacy and Bioanalysis of the University of The Andes, Mérida, Venezuela.[15-19]

Irradiation unit-technical specifications

A 50W LED light source that emits at a wavelength of 405±10 nm, is attached to a heat sink with a cooling fan that runs constantly during the irradiation process to prevent the unit from overheating (Figure 1). A condenser lens was placed in front of the LED to concentrate light with the purpose of obtaining a high irradiation value in the middle of the Petri dish and, at the same time, to produce a steep irradiation gradient from the center to the edge of the dish, which allows to determine in a single process the inhibition thresholds of the inoculated samples. A constant maximum irradiation of 0.19 J/s/cm^2 at the center of the Petri dish was measured with a LI-250A Radiometer (LI-COR, Lincoln, NE, USA). The light falloff (irradiation gradient) was measured with a spot photometer. Irradiation reaches a negligible level at 36 mm from center. Mechanically, the unit was designed and made to fit precisely over a 90 mm diameter Petri dish, by replacing its cover, to ensure that maximum irradiation occurs always at the center of the dish.

Standardization stage of the irradiation protocol

Bacterial strains: Two international reference strains (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853) and two XDR bacteria representing the collection under study (*K. pneumoniae* LMM-X8 and *P. aeruginosa* LMM-150830) were used at this standardization stage of the study.

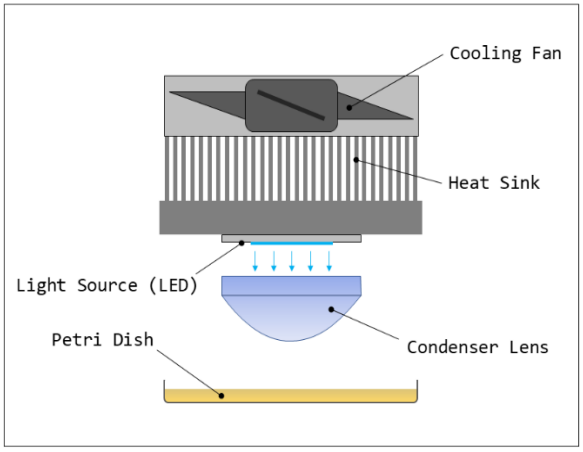

Figure 1: Schematic diagram of the irradiation unit.

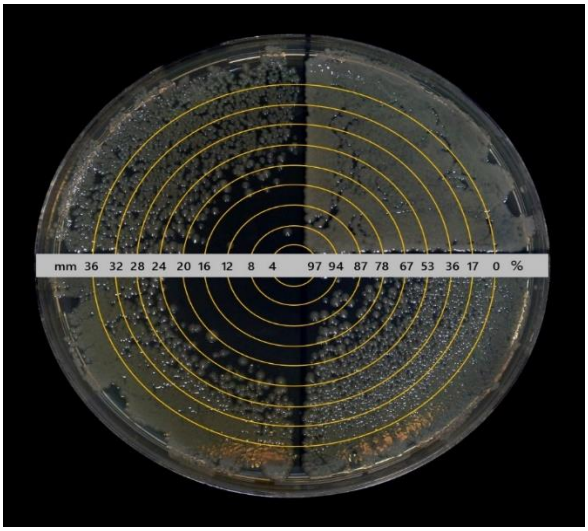

Figure 2: Four strains with different inhibition thresholds. Scale at left indicates distance (mm) from center of the Petri dish. Scale at right shows the corresponding percentage (%) of maximum irradiation. A constant maximum irradiation of 0.19 J/s/cm^2 occurs at the center of the Petri dish. Inhibition thresholds are calculated using radii of inhibition halos to determine the corresponding percentages of maximum irradiation, which are finally multiplied by the exposure time.

Bacterial inoculum: Two basic standards of bacterial inoculum commonly used in antimicrobial susceptibility testing were prepared and tested as described below: The 4 selected strains were sub cultured on trypticase-soy and MacConkey agar (Oxoid) to confirm their viability and purity. Then, from the culture on trypticase-soy agar (Oxoid), 3 or 4 typical colonies were taken and resuspended in 2 ml of sterile 5% saline solution until reaching the turbidity of the 0.5 McFarland standard, equivalent to 1.5×10^8 Colony Forming Units (CFU). This solution constituted the first bacterial inoculum to be tested and is the one commonly used in antimicrobial susceptibility testing by the disk diffusion technique. The second type of inoculum is frequently used in susceptibility testing by dilution techniques (MIC) and consists of diluting 1:10 the suspension initially equivalent to 0.5 McFarland (1.5×10^8) so that the final inoculum will be 1.5×10^7 CFU. Culture medium inoculation: The culture medium used was Mueller Hinton agar (Oxoid); it was dispensed in sterile disposable 90 mm Petri dishes until reaching a thickness of 5 mm. Once the sterility control was verified, these plates were divided into 4 quadrants. The strains were inoculated in triplicate as follows; the 2 upper quadrants were inoculated with 1.5×10^8 CFU and the 2 lower quadrants with 1.5×10^7 CFU. Two plates were selected for irradiation while the other formed the viability control group, which was not irradiated. Irradiation: The plates selected for irradiation were exposed for 15 and 30 minutes, corresponding to maximum irradiation levels of 171 and 342 J/cm^2, respectively, at the center of the Petri dish. Reading of the test: When an evident inhibitory effect was observed, the distance from the center of the Petri dish to the edge of the halo where bacterial growth started to be visible was measured using a ruler. The larger the inhibition halo, the lower the energy required for the photoinactivation of the microorganism (Figure 2). Determination of the inhibition threshold: In the procedure to determine the inhibition threshold (J/cm^2) of the strains studied, a spreadsheet (Microsoft Excel) was used containing all the relevant parameters, such as: name of strains, bacterial inoculum (CFU), maximum irradiance (J/s/cm^2), irradiation time (s), measurement of the inhibition halo radius (mm) and the corresponding relative irradiance (gradient factor), using the equation:

$$\begin{aligned} &\textit{Inhibition threshold} \\ &\quad = \textit{Maximum irradiance at center} \\ &\quad \times \textit{irradiation time} \\ &\quad \times \textit{relative irradiance} \end{aligned}$$

Evaluation stage of inhibition thresholds on XDR bacteria

Bacterial strains and conditions for irradiation assays: In this stage of the study, the total of the 40 XDR bacteria were used. Considering the results obtained in the standardization stage, the following conditions for irradiation were established: Irradiation time of 15 min (900 s). Bacterial inoculum of 1.5×10^7 CFU.

Measurements and determination of the inhibition threshold, were as the procedure used for the standardization tests. These tests were carried out in three independent events and the average results are described.

Statistical analysis

All the results were analysed with the SPSS statistics 25 program. The values shown are the mean of the tests and their standard deviations. For the calculation of normality, a Shapiro-Wilk test was performed and a p value<0.05 was obtained. The Mann-Whitney U test was used to establish the differences between bacterial groups and inhibition thresholds. The calculation of the survival variable was carried out taking into account that the maximum irradiation coincides with the center of the Petri dish. Bacterial growth in this zone was considered as 100% bacterial survival. All the data corresponding to the measurement of the inhibition halos were analysed using the following equation:

$$x = (45 - n)mm \times 100 \div 45\,mm$$

Where, 45 is a constant (100% bacterial survival); n = inhibition halo (of each strain studied); 100 (% survival of the strain studied).

RESULTS

After establishing the irradiation conditions, the 40 XDR bacteria, hospital-acquired Gram negative were subjected to irradiation assays to determine inhibition thresholds. The results of the effect of irradiation on the total set of bacteria studied are shown in (Figure 3). A total of 82.5% (33/40) of the strains showed inhibition of bacterial growth. This was observed phenotypically by the formation of an inhibitory halo caused by the irradiation gradient. In contrast, 17.5% (7/40) of the strains remained tolerant to irradiation levels exceeding 171 J/cm^2. This group of strains consisted of only Enterobacterales; 4 *E. coli*, 2 *K. pneumoniae*, and 1 *K. aerogenes*. Dose-response analyses of bacterial exposure to aBL showed different sensitivities among bacterial groups and strains of the same species. A distribution of the inhibition threshold values, allowed grouping most of the Enterobacterales in inhibitory ranges between 86 and 126 J/cm^2, while the majority of the non-fermenting Gram-negative bacilli (Pseudomonadales) were below 86 J/cm^2 (Table 2).

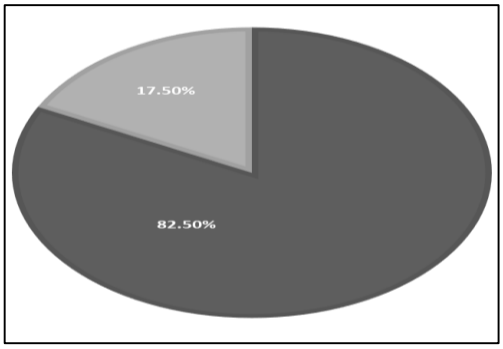

Figure 3: Distribution of 40 XDR Gram-negative bacteria according to the photoinactivation effect of blue light (405 nm). Grey: Tolerant bacteria. Dark Grey: Photo inactivated bacteria.

It was observed in (Table 3) the dependence of the inhibition halo with irradiance values for each group of bacteria. Results allowed us to establish an inversely proportional relationship between the variables studied, i.e., the smaller the inhibition halo, the higher the irradiation threshold, such as in the case of *K. pneumoniae*, *K. oxytoca*, *E. coli* and *K. aerogenes*. In contrast, lower irradiation threshold values were associated with larger inhibition halos, as in the case of *P. aeruginosa* and *A. baumannii*. In this regard, statistical analysis showed that the percentage of survival was also inversely proportional to the size of the inhibition halo. It was also observed that there were variations in the survival percentages among the bacterial groups studied, as well as among strains of the same species. A survival rate of 100% was observed in 7 strains of Enterobacterales that remained tolerant to the effects of blue light. The irradiation threshold values did not show any direct relationship with the antimicrobial resistance profile of each strain or its complexity (Table 4).

Table 1: Bacterial strains used in this study.

Enterobacterales	N	Source	Pseudomonadales	N	Source
***Klebsiella pneumoniae* strains No. LMM: X1, X4, X6, X8, X10, X11, X12, X14, X17, X18, X19, X23, 14524.2, 141060, 14195**	15	15, 18	*Pseudomonas aeruginosa* strains No. LMM: 007, 022, 086, 111, 137, 259, 283, 77923, 150830, 260315, 567.1	11	17, 18
***Klebsiella oxytoca* strain No. LMM: SA26**	1	16	*Acinetobacter baumannii* strain No. LMM: 251, 496	2	17, 18
***Klebsiella aerogenes* strain No. LMM: 200**	1	18	-		
***Escherichia coli* strain No. LMM: A178, 719, 717, 726, 1147, 1194.3, 35218, 15131.1, 15131.2, 15494**	10	15, 18, 19	-		
Control strain			Control strain		
***Escherichia coli* ATCC 25922**			*Pseudomonas aeruginosa* ATCC 27853		

LMM: Laboratory of Molecular Microbiology (University of The Andes, Mérida, Venezuela)

Table 2: Distribution of XDR Enterobacterales and Pseudomonadales strains according to irradiation inhibitory ranges.

Inhibition threshold J/cm^2	Enterobacterales N (%)	Pseudomonadales N (%)
45-85	2 (7,40)	8 (61,54)
86-126	7 (25,93)	5 (38,46)
127-167	11 (40,74)	0
168 and more	7 (25,93)	0
Total	27 (100)	13 (100)

Table 3: Relationship of the size of inhibition halos and inhibition thresholds for blue light (405 nm) determined for each group of bacterial species studied.

Bacterial strains	Inhibition halo (mm)	Inhibition threshold (J/cm^2)
Klebsiella pneumoniae	11.73±8.09	141.73±27.81
Klebsiella oxytoca	7.00±0.00	162.00±0.00
Klebsiella aerogenes	0.00±0.00	171.00±0.00
Escherichia coli	12.20±11.29	133.40±39.70
Pseudomonas aeruginosa	25.63±4.10	76.63±27.45
Acinetobacter baumannii	26.50±3.53	72.00±25.45

DISCUSSION

Blue-light-mediated microbial inactivation represents a promising therapeutic alternative for the treatment of non-systemic infections, such as skin and soft tissue infections, especially those caused by XDR bacteria.[4-6,10,11,20] The growing phenomenon of antimicrobial resistance has forced researchers to look for new therapeutic alternatives.[1-4]

In fact, recent studies on the usefulness of blue light for the control of multidrug-resistant pathogens have raised hopes and it has been considered as a possible effective option for treatment.[4-6] This study showed that blue light (405 nm) was effective in inactivating more than 80% of the XDR Gram-negative strains analysed. Currently, few studies have investigated in detail the relationship between energy dose and bacteria killing.[20] However, thanks to the fact that the irradiation unit was designed in such a way that an irradiation gradient could be generated, we were able to simultaneously determine, in a single run, the inactivation thresholds of different strains. In this respect, most of the strains belonging to the Enterobacterales group were inhibited with irradiation thresholds between 61 to 167 J/cm^2. In relation to *P. aeruginosa* and *A. baumannii*, these strains were more sensitive to photoinactivation (46 to 124 J/cm^2) than the Enterobacterales group. Some studies suggest that the differences in the inactivation of different bacterial species by irradiation at 405 nm are due to the fact that each of these species have a different capacity to generate reactive oxygen species (ROS) and, in turn, to produce different pathways to resolve oxidative stress.[21] Other authors argue that a bacterium's sensitivity to a wavelength of the visible spectrum may be affected by the type and prevalence of endogenous photosensitizing substances such as porphyrins, as well as their peak absorbance for these substances.[4-6,20] In this regard, other reports indicate that doses around to 50 J/cm^2 are sufficient to produce an increase in the amount of reactive oxygen species (ROS) in strains of *P. aeruginosa* and *A. baumannii*, which leads to a considerable reduction of growth in vitro and in vivo, due to the high amount of intracellular porphyrins that these bacteria usually have.[22] In this context, it was possible to observe that blue light irradiation induced strain-specific inactivation kinetics. In fact, statistical analysis revealed significant variation in survival levels within different strains of the same species at the same light doses. In general, it was determined that the survival values per species in this study were directly related to the irradiation thresholds. In other words, lower survival percentages were related to lower irradiance levels. Nevertheless, it is important to emphasise that irradiation doses of up to 171 J/cm^2 were insufficient to inhibit bacterial growth in just 7 strains (17.5%) of the Enterobacterales group, showing 100% survival rate.

The strains that remained tolerant to the effects of blue light were 4 *E. coli*, 2 *K. pneumoniae* and 1 *K. aerogenes*. It is likely that a longer exposure time or a pretreatment of these strains with a photosensitizer may enhance blue light's inhibitory effect. Dos Anjos et al inactivated with blue light international clones (ST10; ST131 and ST648) of multidrug-resistant *E. coli* with irradiations that ranged between 206.25 J/cm^2 to 412 J/cm^2 in 90 and 180 minutes, respectively.[23] Previous studies have shown that in the pathophysiological processes of enteric bacterial infections, various defence mechanisms of the immune system are activated that promote the appearance of ROS (phagocytosis), and consequently induce cell death of the infectious agent.[22,24,25] As a result of an evolutionary convergence of the mechanisms of elimination of bacterial infections and the survival of infectious microorganisms, these generate adaptive processes that avoid oxidative stress.[24]

Table 4: XDR Gram-negative pathogens from healthcare-associated infections patterns and inhibition threshold values.

Strain Number	Bacteria	Antimicrobial resistance pattern	Inhibition threshold J/cm^2	% Survival
LMM-X1	*K. pneumoniae*	TEM-2, SHV-5, CTX-M-8, CIP, GEN, SXT, FOS	138	67
LMM-X4	*K. pneumoniae*	CTX-M-8, CIP, GEN, AMK, SXT, FOS, NIT	165	89
LMM-X6	*K. pneumoniae*	SHV-5, CTX-M-8, CIP, GEN, SXT, FOS	155	78
LMM-X8	*K. pneumoniae*	CTX-M-2; CIP, GEN, TOB, AMK, SXT, NIT, FOS	114	56
LMM-X10	*K. pneumoniae*	CTX-M-8, CTX-M-9; CIP, GEN, AMK, FOS	138	67
LMM-X11	*K. pneumoniae*	TEM-2, SHV-2, CTX-M-9; CIP, GEN, AMK, SXT	165	89
LMM-X12	*K. pneumoniae*	CTX-M-2; KPC-2, CIP, GEN, AMK, FOS	138	67
LMM-X14	*K. pneumoniae*	CTX-M-15; KPC-2, CIP,AMK,GEN, TOB, SXT	83	44
LMM-X17	*K .pneumoniae*	CTX-M-15; ACN, AMK, GEN, TOB, SXT	167	93
LMM-X18	*K. pneumoniae*	CTX-M-2; KPC-2; CIP, GEN, AMK, NIT, FOS	96	49
LMM-X19	*K. pneumoniae*	CTX-M-8; CIP, AMK, GEN, TOB, SXT, NIT, FOS	146	71
LMM-X23	*K. pneumoniae*	CTX-M-8; KPC-2, CIP, GEN, TOB, STX, FOS	160	82
LMM-X27	*K. oxytoca*	TEM-15, CTX-M-8, KPC-2, GEN, AMK, FOS	162	84
LMM-X29	*K. pneumoniae*	TEM-5, CTX-M-15; CIP, AMK, GEN, TOB, NIT, FOS	119	58
LMM1060	*K. pneumoniae*	KPC-2; CIP, GENT, AMK, TOB, NIT, STX ,FOS	>171	100
LMM416235	*K. pneumoniae*	KPC-2; CIP, AMK, GEN, TOB, STX, FOS	>171	100
LMM200	*K. aerogenes*	AmpC; IMP, CIP; GEN, AMK, STX, FOS	>171	100
LMM038	*E. coli*	CTX-M-15, KPC-2, CIP, GEN, AMK, STX, FOS	90	24
LMM099	*E. coli*	CTX-M-2, VIM-1, CIP, GEN,AMK, NIT, FOS, STX	124	18
LMM316	*E. coli*	TEM-1, CTX-M-15; VIM-1, CIP, GEN,AMK, STX	102	22
LMM726	*E. coli*	CTX-M-15; CIP, GEN, AMK, NIT, STX, FOS	61	28
LMM35218	*E. coli*	CTX-M-15; VIM-1, CIP, GENT, AMK, FOS	149	12
LMM717	*E. coli*	CTX-M-15; VIM-1, CIP, GEN, AMK, STX, NIT	>171	0
LMM719	*E. coli*	CTX-M-15; KPC-2, CIP, GEN, AMK	>171	0
LMM1147	*E. coli*	CTX-M-8; VIM-1, CIP, GENT, AMK, FOS	>171	0
LMM1194	*E. coli*	CTX-M-15; VIM-1, CIP, GEN, AMK	>171	0
LMM15131.1	*E. coli*	CTX-M-2; KPC-2, CIP, GEN, AMK, SXT, NIT, FOS	124	18
LMM007	*P. aeruginosa*	VIM-1, ACN, CIP, AMK, GEN, TOB, FOS	46	30
LMM022	*P. aeruginosa*	VIM-1, ACN, CIP, AMK, GEN, TOB, FOS	96	23
LMM086	*P. aeruginosa*	VIM-1, CIP, GEN, TOB, FOS	46	30
LMM111	*P. aeruginosa*	KPC-2; CIP, AMK, GEN, TOB	124	18
LMM137	*P. aeruginosa*	VIM-1, CIP, GEN, AMK, TOB,	61	28
LMM259	*P. aeruginosa*	VIM-1, CIP, GEN, AMK, TOB, FOS	83	25
LMM283	*P. aeruginosa*	VIM-1; CIP, GEN, TOB, FOS,	46	30
LMM77923	*P. aeruginosa*	IMP; CIP, AMK, GEN, TOB, FOS	76	26
LMM150830	*P. aeruginosa*	KPC-2, CIP, GEN, AMK, TOB	90	24
LMM260315	*P. aeruginosa*	KPC-2, CIP, GEN, AMK, TOB	61	28
LMMGP567	*P. aeruginosa*	VIM-1, CIP, GEN, AMK, TOB	114	20
LMM251	*A. baumannii*	VIM-1, CIP, GEN, AMK, TOB, DOX, SXT	54	29
LMM496	*A. baumannii*	VIM-1, CIP, GEN, AMK, TOB, DOX, SXT	90	24

Inoculum: 1.5×10^7; Irradiation time: 15 minutes (900 seconds). Extended spectrum beta-lactamases (CTX-M, SHV, TEM), Carbapenemases (VIM-1, IMP, KPC-2); CIP: ciprofloxacin; GEN: gentamicin; AMK: amikacin; TOB: tobramycin, FOS: fosfomycin; SXT: triethoprim/sulfamethoxazole; NIT: nitrofurantoin.

It is known that KatG and AhpF proteins enable the removal of intracellular hydrogen peroxide in bacteria, thus reducing the oxidative stress, which explains why a higher concentration of these proteins is found in *Enterobacterales* after being exposed to blue light-associated photodynamic therapies.[25]

The inhibitory effect of blue light on multidrug-resistant bacteria, without inducing damage to human tissue, is becoming a promising therapeutic alternative.[5,6,20]

The results of this study indicate that the susceptibility of XDR Gram-negative bacteria to the effects of blue light

does not depend on the species or the resistance mechanisms, which suggests that the photoinactivation is probably associated with intrinsic metabolic characteristics of each strain studied. This could be particularity related to the concentration of endogenous photosensitive substances (e.g., porphyrin and flavin derivatives), which may vary between bacterial strains of the same species.[6-9] Our results agree with other studies where aPDI/aBL therapies lead to the effective eradication of XDR Gram-negative bacteria in hospital-acquired infections, in addition to the synergistic activity that can be achieved by associating photoinactivation and clinical use of antimicrobials.[6,8,10,13]

In fact, Wozniak et al[11,26] demonstrated the usefulness of the synergistic effect of different antibiotics (colistin, tetracyclines or imipenem), with sublethal doses of irradiation (63.6 J/cm^2) against XDR *E. cloacae*, *K. pneumoniae* and *A. baumannii* strains.

Limitations

Some limitations observed in this work were: not being able to include a greater variety of both Enterobacterales and Pseudomonadales bacterial species, as well as testing pan-resistant strains and Gram-positive bacteria of clinical interest. This is due to the unavailability of these biological samples at the time the study was carried out. However, the group of bacteria selected and tested in this work were adequate enough to evaluate the usefulness of the photoinactivating effect of blue light in XDR Gram-negative bacteria from healthcare-associated infections.

CONCLUSION

Our findings showed that blue light (405 nm) has an inhibitory effect against Gram negative XDR from healthcare-associated infections when irradiated from 61 to 165 J/cm^2 for the Enterobacterales and from 46 to 124 J/cm2 for the Pseudomonadales. Tolerant strains survived to irradiation levels upto≤171 J/cm^2. The use of the condenser lens placed in front of the light source of the irradiation unit represented an important technical advantage, since it allows to determine, in a single process, the sensitivity of several strains by quantifying the photoinactivation threshold of each one. It is worth mentioning that this is the first study conducted in Venezuela showing that blue light can inactivate XDR Gram-negative pathogens from healthcare-associated infections. We believe that these results open a new line of research to explore the practical applications of blue light as an alternative therapeutic asset to contain the phenomenon of antimicrobial resistance.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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