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Short communication

Antimicrobial blue light inactivation of international clones of multidrug-resistant *Escherichia coli* ST10, ST131 and ST648

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ABSTRACT

Background: International clones of multidrug-resistant *Escherichia coli* have been a leading cause of human and animal infections worldwide. Microbial inactivation by blue light has been proposed as an effective treatment for superficial infections and surface contamination.
Aim: To evaluate the inactivation efficacy of blue light irradiation against high-risk multidrug-resistant strains of *E. coli*.
Methods: Blue LED light (λ = 410 nm) was used to inactivate *in vitro* suspensions of colistin- broad-spectrum cephalosporin-, or carbapenem-resistant *E. coli* strains belonging to sequence types (STs) ST10, ST131 and ST648, carrying *mcr-1*, $bla_{CTX\text{-}M}$ or $bla_{KPC\text{-}2}$ genes.
Results: Our results showed that all *E. coli* strains were susceptible to blue light irradiation, independently of antibiotic resistance and virulence profiles. In addition, blue light irradiation induced a strain-specific and dose-dependent bacterial effect.
Conclusion: Our results support use of blue light as a promising antimicrobial option against MDR pathogens, including high-risk clones of *E. coli* displaying resistance to polymyxins or broad-spectrum β-lactam antibiotics.

1. Background

The rapid dissemination of multidrug-resistant (MDR) pathogens has triggered interest in alternatives to antibiotics therapy for infection control [1]. *Escherichia coli* strains belonging to sequence types (STs) ST10, ST131 and ST648 have been emerging as a versatile prototype of MDR pathogens for human and animal hosts [2]. Worryingly, the convergence of antimicrobial resistance with enhanced virulence also begun to be reported among these *E. coli* strains [2,3].

Microbial inactivation mediated by blue light represents a promising therapeutic procedure to treat superficial infections. Blue light has intrinsic antimicrobial properties as it can be absorbed by natural pigments, such as porphyrins and flavins, leading to photochemical production of reactive oxygen species (ROS). Cellular exposure to ROS allows damage to vital structures and, in sufficient amounts, cause microbial inactivation [4,5].

2. Aims

The aim of the study was to investigate the kinetics of blue light inactivation of high-risk MDR *E. coli* strains.

3. Methods

We used three wild-type *E. coli* strains previously characterized: 1) CTX-M-producing *E. coli* belonging to ST131; 2) KPC-2-producing *E. coli* belonging to ST648 [6]; and 3) MCR-1-producing *E. coli* belonging to ST10 [7]. All strains displayed MDR profile [8]. Additionally, a drug-sensitive strain (ATCC® 25922™) was used as control and an enteropathogenic strain O127:H7 (E2348/69) [9] was used as a typical virulent strain.

Fresh individual colonies were collected from MacConkey agar (Difco, USA) and inoculated into 3 mL of Mueller-Hinton broth (Difco, USA) before being incubated at 37 °C for 24 h under shaking regimen (100 RPM). Then, bacterial cultures were washed twice and suspended

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Table 1
Exposure time and radiant exposure (J/cm^2) of blue light used for inactivation kinetics curves of MDR *Escherichia coli* strains belonging to international clones ST10, ST131 and ST648.

	Irradiation parameters					
Minutes	30	60	90	120	150	180
J/cm^2	68.75	137.50	206.25	275.01	343.75	412.50

in PBS to prepare the experimental inoculum. The inocula were standardized to approximately 10^9 colony forming unit (CFU)/mL by assessing the turbidity of the suspension with a spectrophotometer (λ_{abs} = 625 nm; optical path = 1 cm; optical density = 0.667).

We used a commercial cell-culture plate irradiation system with peak wavelength emission at 410 +/- 10 nm and irradiance level of 38.2 mW/cm^2 (LEDbox, BioLambda, São Paulo, Brazil). One-mL inoculum of each experimental group was individually placed in wells of 12-well plates. All samples were irradiated with more than 95% of beam homogeneity. Each light exposure group of each strain (Table 1) was compared to other strain counterparts at the same light dose.

After treatments, samples were serially diluted (1:10) in sterile PBS, seeded onto Muller Hinton agar plates and incubated at 37 °C for 24 h. CFU quantification was performed according to the methodology described by Jett et al. [10]. All experiments were performed in triplicates and reproduced in three independent experiments.

Survival fraction values were determined as the averages of $\log_{10}$ reduction of CFU normalized in relation to each respective control group. Survival fraction data was tested to confirm normality (Shapiro-Wilk test) and compared in between strains using multiple comparisons of two-way analysis of variance (ANOVA) followed by Tukey test. Statistical tests were performed using Prism software (GraphPad, USA). Significance level was considered at $p < 0.05$.

4. Results

In this study, all tested *E. coli* strains were susceptible to blue light inactivation. All irradiated groups presented significant differences in relation to their respective non-irradiated control groups. In Fig. 1, it is possible to observe that blue light irradiation induced strain-specific

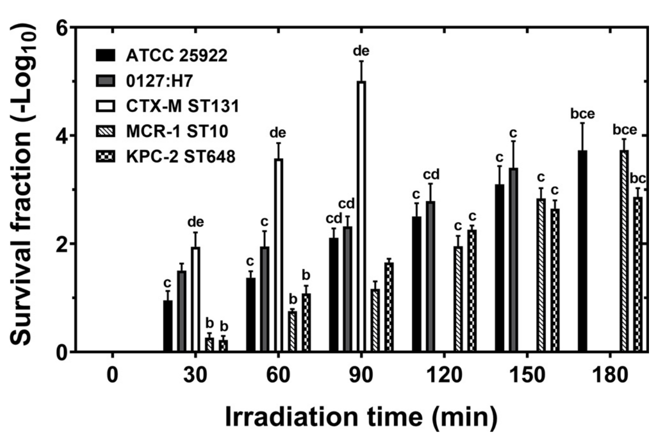

Fig. 1. Survival fraction of MDR *Escherichia coli* strains inactivation induced by blue light irradiation. The results are presented as normalized survival fractions of $\log_{10}$ reduction in function of irradiation time. The error bars are standard deviation of each mean. Statistical differences are indicated by lower-case letters: a) represent differences in relation to the control strain, b) to the 0127:H7 strain, c) to the CTX-M-producing *E. coli* ST131 strain, d) to the MCR-1-positive *E. coli* ST10 strain and e) to the KPC-2-producing *E. coli* ST648 strain. Data is omitted in points where inactivation rate is greater than $7\log_{10}$ such as for CTX-M ST131 at irradiation times greater than 120 min and for KPC-2 ST648 at 180 min.

inactivation kinetics. In fact, statistical analysis revealed significant variation in survival fraction levels within different strains at the same light doses. The CTX-M-producing *E. coli* ST131 (ICBECMO) presented more than 5 log inactivation after exposure to 90 min (206.25 J/cm^2). This strain was the most susceptible when compared to all other tested strains, including the *E. coli* ATCC 25922 strain. The virulent *E. coli* 0127:H7 strain (E2348/69) presented more than 3.4 log of inactivation at 150 min (343.75 J/cm^2). *E. coli* ATCC 25922 and MCR-1-producing *E. coli* ST10 (ICBEC7P) strains exhibited more than 3.7 log of inactivation at 180 min (412.50 J/cm^2). The KPC-2-producing *E. coli* ST648 (ECSIC19) was the most tolerant strain, however, 2.8 log of inactivation was achieved after 180 min of irradiation (412.50 J/cm^2). At the same dose, we observed complete inactivation of CTX-M-producing ST131 (ICBECMO) and 0127:H7 (E2348/69) *E. coli* strains.

5. Discussion

Regardless of the vast literature about the use of blue light to inactivate pathogenic bacteria [11], no studies have yet reported its use against international clones of high-risk *E. coli*. Likewise, there is no comparison of blue light inactivation of MDR *E. coli* carrying the clinically important resistance genes $bla_{CTX\text{-}M}$, $bla_{KPC\text{-}2}$ and *mcr-1*, which emphasizes the novelty of this investigation.

Infections caused by such high-risk strains are often resistant to most commercially available antibiotics, including to the last resort drugs (*e.g.* carbapenems and polymyxins) [2,3,12,13]. Interestingly, even though the CTX-M-producing *E. coli* ST131 is a serious concern worldwide, it was the most sensitive strain to blue light exposure. These findings suggest that susceptibility to blue light irradiation is probably associated with intrinsic metabolic characteristics that are not related to drug-resistance profile. In fact, each tested *E. coli* strain may have intrinsic biochemical characteristics that determine their sensitivity to blue light unique. This uniqueness could be related to concentration of endogenous photosensitive pigments (*eg.,* porphyrin and flavin derivatives), which could range among bacterial strains from the same species.

After 180 min of irradiation, we observed that all tested *E. coli* strains presented more than $3\log_{10}$ inactivation by blue light. In this regard, hospitalized patients infected by MDR strains frequently stay isolated in intensive care units and can be continuously irradiated throughout the entire day. Additionally, there may not be any other therapeutic options available.

Other studies corroborate to our results demonstrating that blue light irradiation is able to inactivate drug-resistant pathogens. Studies performed with bacterial suspensions, biofilms and infections confirmed the successful use of blue light in the treatment of MDR *Staphylococcus aureus*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* [14–16].

In summary, our results showed that blue light irradiation induced a dose-dependent bactericidal effect against all tested *E. coli* strains regardless of antibiotic resistance and virulence profiles. Therefore, blue light represents an effective approach against high-risk MDR *E. coli* isolates opening a new avenue for innovative therapeutic platforms.

Conflict of interest

C. P. Sabino is an associate at BioLambda company but declares to only have scientific interest on this study. There are no further conflicts of interest to be declared.

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