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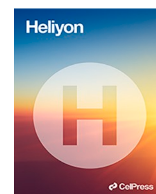

Research article

Kinetics of inactivation of bacteria responsible for infections in hospitals using UV-LED

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ABSTRACT

Controlling the microbial load in the environment is crucial to prevent the spread of organisms. The continuous spread of nosocomial infections in hospital facilities and the emergence of the coronavirus (COVID-19) highlighted the importance of disinfection processes in health safety. This work aimed to evaluate the effectiveness of LED-based disinfection lamps on bacteria from the ESKAPEE group and virus phage in vitro inactivation to be applied in hospital environments and health facilities disinfection.

This study evaluated the effect of different UV wavelengths (275 nm, 280 nm (UVC), 310 nm (UVB) and 340 nm (UVA)) on the disinfection process of various microbial indicators including *E. coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis* and Bacteriophage lambda DSM 4499. Exposure time (5 min–30 min), exposure distance (0.25 m and 0.5 m) and surface materials (glass, steel, and polished wood) were evaluated on the disinfection efficiency. Furthermore, the study determined the recovery capacity of each species after UV damage.

UVC-LED lamps could inactivate 99.99 % of microbial indicators after 20 min exposures at a 0.5 m distance. The exposure time needed to completely inactivate *E. coli*, *S. aureus*, *P. aeruginosa*, *B. subtilis* and Bacteriophage lambda DSM 4499 can be decreased by reducing the exposure distance. UVB-LED and UVA-LED lamps were not able to promote a log reduction of 4 and were not effective on *B. subtilis* or bacteriophage lambda DSM 4499 inactivation. Thus, only UVC-LED lamps were tested on the decontamination of different surface materials, which was successful. *P. aeruginosa* showed the ability to recover from UV damage, but its inactivation rate remains 99.99 %, and spores from *B. subtilis* were not completely inactivated. Nevertheless, the inactivation rate of these indicators remained at 99.99 % with 24 h incubation after UVC irradiation. UVC-LED lamps emitting 280 nm were the most indicated to disinfect surfaces from microorganisms usually found in hospital environments.

1. Introduction

Hospital-acquired infections are considered a critical public health issue worldwide that needs attention due to the continuous appearance of new infectious organisms as the recent SARS-CoV-2 pandemic situation [1,2]. The emergence of the coronavirus (COVID-19) highlighted the importance of disinfection processes in health safety [3]. Therefore, maintaining strategic bacterial

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disinfection in the environment is crucial [4].

T persistence of pathogens on material surfaces often causes severe consequences since in this way, they are available to be transferred from one host to another. Surface contamination has been described before [5] and has also been demonstrated via experimental modelling [6] to be linked to the origin of various infectious disease outbreaks in hospitals and dental offices, or the transfer of pathogenic or spoilage microorganisms from food contact surfaces to food products in food processing facilities [7].

In nosocomial infections, health personnel are crucial since they have to ensure patient care and limit the spread of bacteria and viruses via patient, person, and surface contamination [8]. For this reason, it is relevant to control microorganisms on surfaces since they can be transferred to the hands, and again to various surfaces, from which they can re-infect other people, both patients and medical staff [9].

Manually executed disinfection processes are operator-dependent and tend not to be as efficient as required [4,10]. In addition, many of the contaminated areas are inaccessible by conventional manual disinfection methods in healthcare facilities [4,10]. On the other hand, automated decontamination technologies, such as ultraviolet (UV) light, have long been known to demonstrate antimicrobial effects and are considered “non-touch” methods [1,10].

Unlike traditional chemical sterilization agents, where some can be harmful to human health or produce unsafe by-products, UV light radiation has been proven as a highly efficient and reliable method, without producing unsafe by-products [11–13].

UV inactivation of microorganisms depends on the wavelength range and can occur by several mechanisms [14–16] highlighting the relevance of a simultaneous comparison between UVA, UVB and UVC effectiveness on microbial inactivation [17]. Wavelengths ranging from 310 to 480 nm affect the photolyase enzyme, related to DNA photo-repair in bacteria [18,19].

Damage induced in DNA, that absorbs light with a peak varying between 260 and 270 nm, is more prone to occur due to UVC radiation, as well as bacterial cell repair proteins suppression at 280 nm [20]. In addition, 275 nm UVC light affects RNA and, therefore, inactivates preferably viruses [21,22].

However, some bacteria can repair UV DNA damage induced by replacing the damaged DNA with undamaged nucleotides (DNA repair light-independent) or by photoreactivation [23,24]. The existence of these repairing mechanisms may reduce the effectiveness of UV treatment [23]. Additionally, the physical mode of action of UV disinfection may limit its ability to penetrate different surfaces and materials, such as solid masses or opaque liquids, which could compromise its efficacy. Furthermore, the wavelength of the UV radiation requires testing in case of uncertainty for sensitive materials [25,26]. In addition, in facilities such as hospitals, the irradiation time should be adjusted to account for room dimensions and complexity [25,27].

UVC light (200–280 nm) is more effective at killing microorganisms than UVA (315–380 nm) or UVB (280–315 nm) [28]. Exposing surfaces contaminated with microorganisms to ultraviolet light of wavelengths 260 nm–275 nm (UVC) has already been established as an effective disinfection methodology when used alone in replacement of, in combination with, chemical methods [7,29,30]. Although UVA and UVB are considered less potent bactericides than UVC, there are several studies focused on the development of sterilization systems using UVA or UVB or combinations of these radiation types with UVC, particularly for water disinfection [31].

UV light-emitting diode (UV-LED) is a relatively new and credible UV light source to replace traditional mercury lamps with multiple advantages such as environmental friendliness due to the absence of toxic mercury, high durability and low energy consumption [32,33]. Studies have shown that UVC-LED efficiently inactivated viruses such as HCoV-229E, or *B. subtilis* endospores as demonstrated by Fuchs, [25]. This inactivation efficiency is also reported for *B. subtilis* [34], *S. aureus* [11,34], *E. coli* [11,34], *P. aeruginosa* [35,36], belonging to the ESKAPEE group and involved in nosocomial infections.

Studies have explored the potential of UV-LED in bacterial disinfection as well as the effect of exposure time and exposure distance [37]. In a 2020 study, a 5-log reduction for both tested strains was achieved within a 10-min exposure time to UV-LED at an exposure distance of 4.5 cm [7]. Another study by Ref. [22] efficiently utilized UV-LED to disinfect bacteria at a 2.2 cm exposure distance and 30 s exposure time, while Song et al. [24] utilized UV-LED at an exposure distance of 2 cm for 40 and 180 s to disinfect bacteria. However, comparing the UV-LED effect at different UV ranges is more common in water treatment [24], with a focus on combining it with several chemical agents, as studied by Moreno-Andrés et al., 2023 [47].

The present study aimed to answer the question of which UV wavelength between UVA, UVB and UVC, is more effective in bacterial inactivation and virus phage resistance, and to investigate whether this effectiveness was independent of the materials in which the microorganisms are found. To address this question we used microbial indicators belonging to the ESKAPEE group and *Escherichia phage* lambda DSM 4230. It was evaluated the exposure time, exposure distance and surface materials (glass, steel, and polished wood) on the disinfection efficiency. The recovery capacity of each species after UV damage was also determined.

Table 1
Description of the wavelength (nm), number of LEDs composition, total power and flux (W) of the tested lamps.

UV lamps	Number of LEDs	Reference and origin	Wavelength (nm)	Total power (W)	Viewing angle	Flux (mW)
1	24	XMD-FBC-LLCA (seoul)	280 (UVC)	21.6	>135	300
2	12	SU CULEP1.VC (osram)	275 (UVC)	23.4	120	504
3	12	CUD1GF1A (seoul)	310 (UVB)	21.6	115	174
4	12	CUN4GF1B (seoul)	340 (UVA)	17.4	115	648

2. Materials and methods

2.1. UV LED lamps set up and luminaire remote control software (LightControl)

Lightenjin II – Illumination industry, Lda, Águeda, Portugal, developed and optimized four lamps composed of UV LEDs to be evaluated in this study. Each lamp was composed of one or two PCBs, either with 12 or 24 LEDs, with UV-LED integrated radiation with maximum peaks position at 275 nm (UVC), 280 nm (UVC), 310 nm (UVB) and 340 nm (UVA), as shown in Table 1. All lamps have an integrated controller that enables the system to be remotely turned on and off and dynamic support that allows a height variation, with a maximum height of 0.5 m. The fabricant information, viewing angles and other UV lamp specifications are mentioned in Table 1. The UV dose irradiated by the tested lamps didn't vary significantly with the heights tested (data not shown). However, it was exposure time-dependent, showing a cumulative fluence as observed in Fig. 1.

2.2. Microbial indicators cultivation and preservation

Based on the ISO 14937: Sterilization of health care products — General requirements for characterization of a sterilizing agent and the development, validation and routine control of a sterilization process for medical devices (Annex A ISO 14937), *Escherichia coli DSM 30083, Staphylococcus aureus DSM 20231, Bacillus subtillis DSM 10 Pseudomonas aeruginosa DSM 1117* and *Escherichia phage lambda (Enterobacteria phage lambda) DSM 4499* and its host bacterium *Escherichia coli* DSM 4230 were selected to be used in UV-LED inactivation experiments as microbiological indicators of disinfection due their relevance in nosocomial infections [26,38–40]. All the type strains were obtained from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany. Bacteria and phage lambda were grown on a solid culture medium according to the culture conditions described in Supplementary Table 1, and cryopreserved at the University of Coimbra Bacterial Culture Collection (UCCCB), at - 80 °C, in Nutrient Broth (NB) with 15 % (v/v) glycerol.

To prepare a lambda phage stock solution, bacteriophage Lambda DSM 4499 was grown and cryopreserved by promoting the host bacterium *E. coli* DSM 4230 infection on Luria–Bertani (LB) agar medium through the top agar layer method, as described previously [4]. An inoculum of the bacteriophage Lambda DSM 4499 was obtained from the respective stock suspension.

To prepare the inoculum, vegetative cells of *E. coli* DSM 30083, *S. aureus* DSM 20231, *B. subtilis* DSM 10 and *P. aeruginosa* DSM 1117 were prepared in 5 mL sterile 0.85 % (w/v) NaCl. The turbidity of the microbiological indicator suspension was adjusted to five on the McFarland scale.

2.3. Kinetics of UV-LED inactivation in suspension

The effectiveness of UV-LED inactivation was evaluated in three steps. First, the inactivation kinetics were determined using cell suspensions in 96-well microplates. The UV light wavelength effect was tested using four different lamps: UVC-LED 280 nm, UVC-LED 275 nm, UVB-LED 310 nm and UVA-LED 340 nm. Exposure times of 10, 15, 20, 25 and 30 min were tested. Second, the inactivation kinetics were determined using cell suspensions on three common materials in hospitals and health facilities: glass, steel and polished wood. Third, the effect of the exposure distance (energy dose) on the UVC-LED lamp emitting at 280 nm inactivation kinetics was evaluated. The experiments were performed at a distance of 0.5 m–0.25 m between the lamp and the cells. Increasing exposure times were tested from 5, 10, 15, 20, 25 min.

All the inactivation experiments were conducted at room temperature (20 °C - 25 °C), under daylight, with a humidity range from 40 % to 60 %. Before irradiation, the UV-LED lamps were remotely powered on for 20 min to achieve a stable emission stage. In each inactivation experiment, control tests were conducted at room temperature where microbiological indicators were exposed to daylight but not irradiated with UV light. As a control, the microbial indicators suspensions were prepared and settled at room conditions during the same periods of time as in the UV irradiation tests without UV exposure. After the incubation period, both control and irradiated indicators were plated, incubated and enumerated. A flowchart resuming the experimental design is shown in Fig. 2.

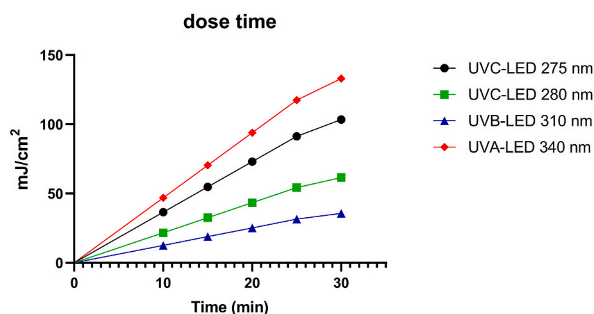

Fig. 1. Cumulative fluence of UVC-LED 275 nm (red), UVC-LED 280 nm (black), UVB-LED 310 nm (green) and UVA-LED 340 nm (blue) lamps depending on exposure time. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.4. Kinetics of UV-LED inactivation on surfaces

To determine the kinetics of inactivation by UV-LED of the indicator microorganisms when laying on the three types of surfaces (1 × 1 cm), these were directly inoculated with 100 μL of a 10^6 CFU/mL concentrated suspension of each indicator. The surfaces were then exposed to UV light with an exposure distance of 0.5 m, for two different periods (20 min and 25 min). In the control condition, the indicators were inoculated in the three types of surfaces, and settled at room conditions for 20 min and 25 min, without exposure to UV light. The control and the irradiated indicators were plated, incubated and the number of microbes present was enumerated.

2.5. Assessment of recovery capacity after UV exposure

To determine the recovery capacity of the bacterial indicators after UV exposure, the viability of the cells was examined. Bacterial suspensions of the volume and optical density mentioned before were exposed to the UV-LED lamp emitting at 280 nm for surface disinfection for 25 min. The exposed suspensions were collected and incubated in Nutrient Broth (NB) medium for recovering viability during 1 h, 4 h and 24 h, at 37 °C. Cells that survived were enumerated by spread plating according to the culture conditions described in Supplementary Table 1, followed by incubation. These results obtained after the different tested incubation periods were compared with the condition where the indicators were also exposed to UV light and directly plated, without any recovery period.

2.6. Cell survival determination

After the UV-LED irradiation period, serial 10-fold dilutions of the samples were made in sterile 0.85 % (w/v) NaCl, and 100 μL were spread on agar plates for colony-forming unit (CFU) enumeration, or in the case of bacteriophages, plaque-forming units (PFU). The plates were incubated according to the conditions indicated in Supplementary Table 1.

In inactivation experiments with different materials surfaces, those were inoculated exposed to the UV light and subjected to irradiation. Posteriorly the disinfection cycle was completed, the surfaces were placed into 50 mL tubes with 5 mL of sterile 0.85 % (w/v) NaCl, shaken manually, and allowed to incubate for 1 h at room temperature. Serial 10-fold dilutions and 100 μL plated for CFU or PFU quantification were performed. The plates were incubated according to the conditions shown in Supplementary Table 1 as well.

To quantify the PFU, 100 μL of the 10-fold serial dilutions were mixed with the same volume of *E. coli* DSM 4230 host suspension (0.3 OD600nm in 0.85 % (w/v) NaCl). The mixture was then plated using the top agar layer method as described by Henriques and colleagues [4].

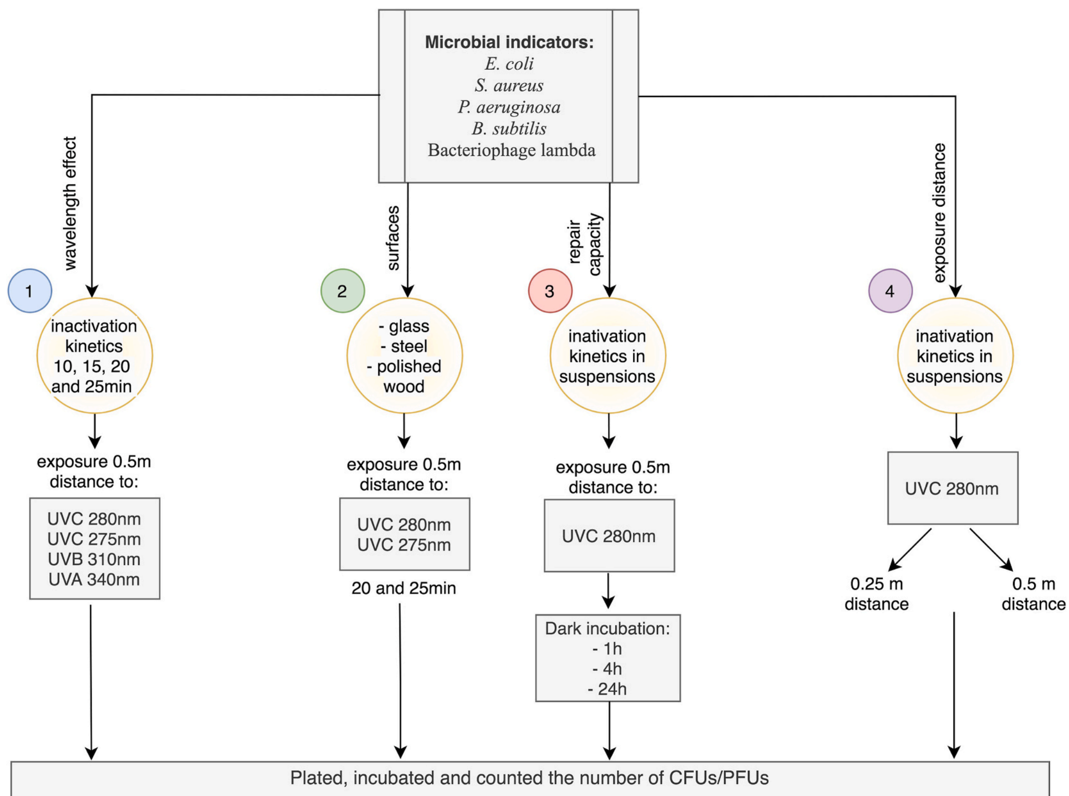

Fig. 2. Diagram of the experimental design performed with the tested UV-LED lamps.

2.7. Statistical analysis

All the experiments and control conditions were performed in triplicates. All the assays with UV exposure were compared to the control tests. Results were presented as mean ± standard deviation and the number of replicates performed in each test is indicated in the respective figure caption.

The results of the comparison were expressed as $\log_{10}$ reduction and elimination rate (%). The $\log_{10}$ reduction after UV-LED exposure was calculated through Equation (1):

$$log_{10}\ reduction = \log_{10}(N_0) - \log_{10}(N_1) \tag{1}$$

where N_0 is the mean of the CFU·mL^{-1} or PFU·mL^{-1} replicate values of the control test in a given experiment, and N_1 is the CFU·mL^{-1} or PFU·mL^{-1} value of one of the replicates of the disinfection test of a given experiment. For its part, the elimination rate after UVC-LED exposure was calculated with formula (2):

$$\text{Elimination rate}(\%) = (N_0\text{-}N_1)\ /\ N_0 \times 100 \tag{2}$$

Statistical differences between groups were evaluated by one-way analysis of variance (ANOVA). After ANOVAs, post hoc comparisons were made by applying Dunnett's test. Differences were considered statistically significant when the associated p-values were lower than 0.05. Statistical analysis was performed by use of GraphPad Prism 9 for Windows 64-bit, version 9.3.1 (GraphPad Software Inc., San Diego, CA, USA).

2.8. Criteria for disinfection acceptance

In the present work, the disinfection acceptance criteria followed the one referred by the U.S. Pharmacopeia, where the coupon surface disinfection process (no materials specified) is considered effective if it allows a $\log_{10}$ reduction of at least 3 orders for vegetative bacterial cells. In the case of viruses and bacteriophages, the U.S. Pharmacopeia does not clarify the $\log_{10}$ reduction required

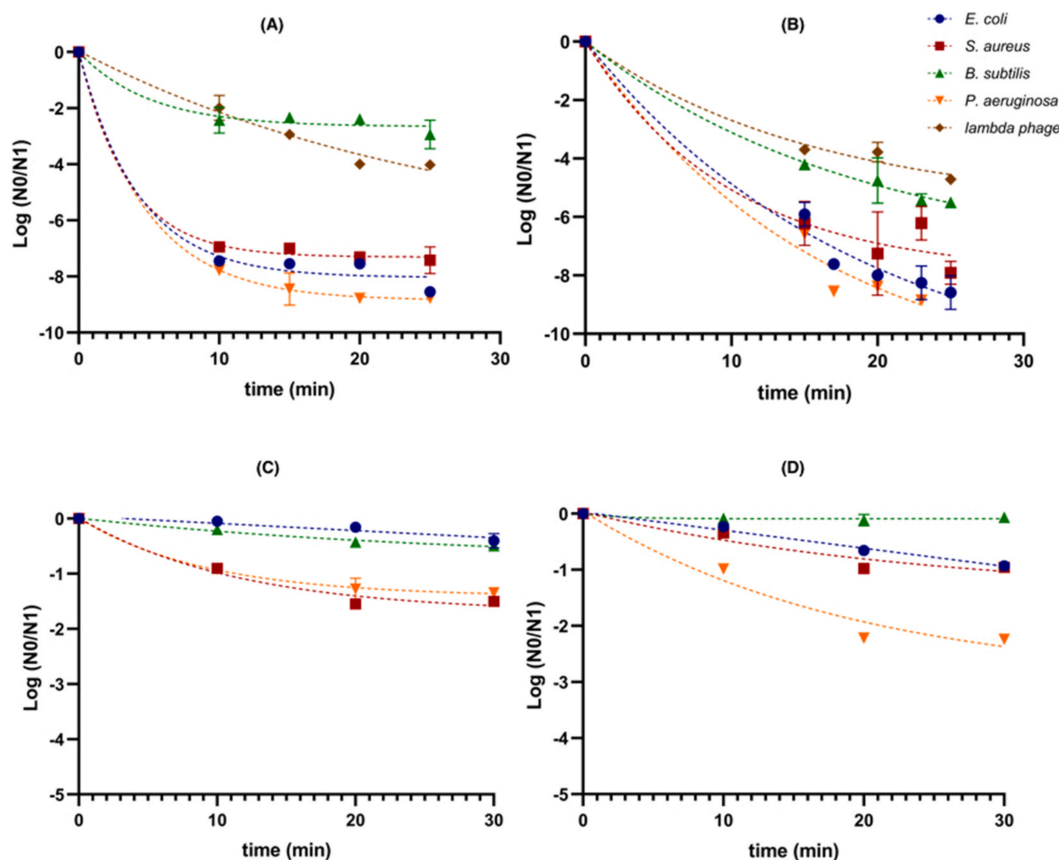

Fig. 3. Inactivation kinetics of the microbial indicators *E. coli* DSM 30083, *S. aureus* DSM 20231, *B. subtilis* DSM 10, *P. aeruginosa* DSM 1117 and Bacteriophage Lambda DSM 4499 by irradiation with (A) UVC-LED 275 nm, (B) UVC-LED 280 nm, (C) UVB-LED 310 nm and (D) UVA-LED 340 nm, with an exposure distance of 0.5 m. The error bars represent the standard deviation of the mean of three replicates (n = 3). The trend line on inactivation patterns was determined with a 95 % confidence interval based on best least squares fit.

to be considered disinfection.

In addition, it also followed the criteria mentioned in the World Health Organization (WHO) [38] manual "Decontamination and Reprocessing of Medical Devices for Healthcare Facilities" and the European Standard EN 4600 11997 which defines that in the case of medical devices and facilities, the sterilization assurance level consists in a log_{10} reduction of 6 orders.

3. Results

3.1. UV-LED inactivation of bacteria in suspension assays

The inactivation curves of microbial indicator suspensions by UV-LED, obtained from 96-well microplate assays, are presented in Fig. 3, as the log_{10} order of magnitude reduction over irradiation time. It was observed that the tested microbial indicators exhibited similar inactivation profiles when UVC-LED lamps emitting at 275 nm and 280 nm were compared.

Irradiation with the UVC-LED emitting at 280 nm during 15 min led to a log_{10} order of magnitude reduction of 4.00 ± 0.00 CFU ml^{-1} for *B. subtilis* and Bacteriophage Lambda DSM 4499 (4.70 ± 0.30 CFU ml^{-1}). The same UVC-LED led to the reduction of *E. coli* by log_{10} 5.90 ± 0.40 CFU ml^{-1}, *S. aureus* by 6.00 ± 0.75 CFU ml^{-1}, and *P. aeruginosa* by log_{10} 6.50 ± 0.00 CFU ml^{-1}.

Moreover, the irradiation with the UVC-LED emitting at 275 nm for 10 min led to a log_{10} reduction between 4 and 6 for *E. coli, S aureus* and *P. aeruginosa* (Fig. 3). However, these same conditions were less effective in inactivating *B. subtilis* and the bacteriophage lambda DSM 4499, because the maximum log reduction achieved was only log_{10} 2.90 ± 0.50 CFU ml^{-1} and log_{10} 4.00 ± 0.03 CFU ml^{-1}, respectively.

When UVC-LED emitting at 275 nm was used for 15 min, a 99.10 ± 1.10 % inactivation rate was measured for the bacteriophage lambda DSM 4499 and 99.50 ± 0.30 % for *B. subtilis*. After increasing the exposure time to 20 min, an inactivation rate of 99.57 ± 0.15 % was achieved for *B. subtilis* and 99.9 % for all other microbial indicators. In addition, UVC-LED emitting at 280 nm achieved a 99.99 % percentage of inactivation of the microbial indicators with 20 min of exposure.

The results also showed that UVB-LED emitting at 310 nm and UVA-LED emitting at 340 nm required a minimum irradiation time of 30 min to achieve one order of magnitude reduction of CFUs. UVA LED emitting at 340 nm reduced the CFU log_{10} by almost one order of magnitude for *S. aureus* and *E. coli* (0.95 ± 0.07 CFU ml^{-1} and 0.93 ± 0.05 CFU ml^{-1}, respectively) or around 2.50 ± 0.02 CFU ml^{-1} for *P. aeruginosa.* However, it did not reduce the CFUs of *B. subtilis* regardless of the irradiation time (13.75 ± 1.34 % of inactivation rate). Fig. 3 also presents that a 30 min irradiation with UVB-LED emitting at 310 nm, resulted in CFU ml^{-1} reduction of *S. aureus* and *P. aeruginosa* by a log_{10} order of magnitude of 1.50 ± 0.09 for and 1.34 ± 0.03 CFU ml^{-1}**.** Furthermore, both UVB-LED (310 nm) and UVA-LED (340 nm) were not able to promote bacteriophage lambda DSM 4499 inactivation during the tested exposure times (data not shown).

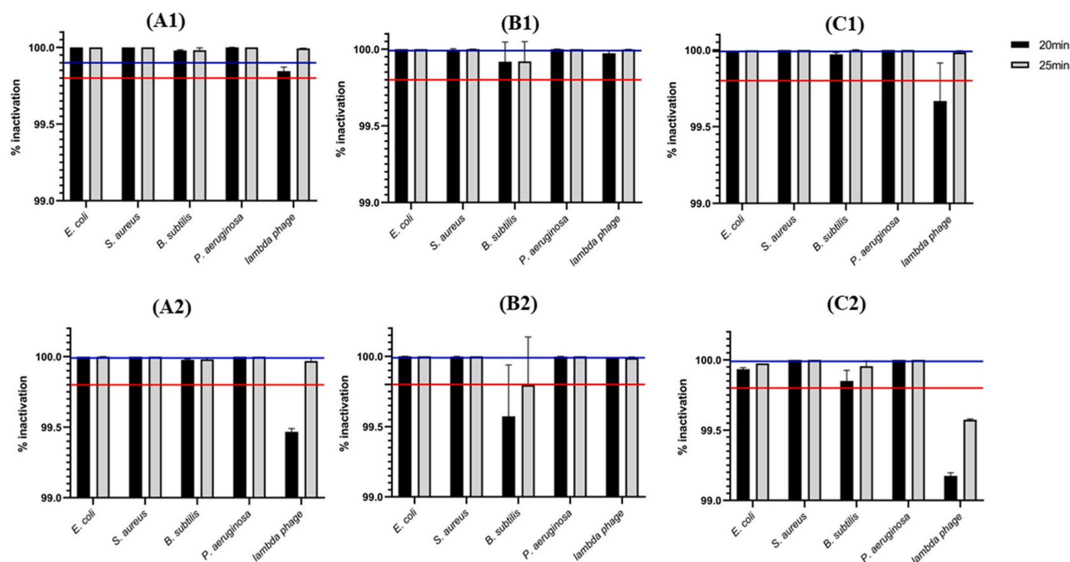

Fig. 4. Inactivation rate of the microbial indicators *E. coli* DSM 30083, *S. aureus* DSM 20231, *B. subtilis* DSM 10, *P. aeruginosa* DSM 1117 and Bacteriophage Lambda DSM 4499, inoculated in glass (A), steel (B) and polished wood (C), after 20 and 25 min irradiation with (A1), (B1), (C1) UVC-LED 275 nm and (A2), (B2), (C2) UVC-LED 280 nm. The error bars in the graph represent the standard deviation of the mean of three replicates (n = 3). The red lines indicate the standard effectiveness established by U.S. Pharmacopeia (corresponding to a reduction of three logs) and the blue lines correspond to the criteria established by WHO and ISO 14937 (corresponding to a six-log reduction). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.2. UVC-LED inactivation of microbial indicators on glass, steel and polished wood surfaces

Both UVC-LED lamps demonstrated a high capacity to inactivate all the tested microbial indicators in multi-well assays within 20 and 25 min of exposure. Considering those results, the UVC-LED lamps were tested to inactivate the same indicators inoculated on surfaces of glass (Fig. 4A1 and 4A2), steel (Fig. 4B1 and 4B2) and polished wood (Fig. 4C1 and 4.C2), using the same exposure times. As shown in Fig. 4, both lamps promoted an inactivation rate of 99.99 % of *E. coli*, *S. aureus* and *P. aeruginosa* with 20 min of exposure (Fig. 4). In addition, the *B. subtilis,* when inoculated on glass and exposed to a UVC-LED emitting at 280 nm, was found to be 99.97 ± 0.01 **%** inactivated after 20 min of irradiation (Fig. 4A2). However, the inactivation of *B. subtilis* was more efficient by increasing exposure time, particularly when inoculated on the steel surface (Fig. 4B2), where the inactivation rate increased from 99.50 ± 0.36 **%** to 99.80 ± 0.34 **%** with 25 min of exposure to UVC-LED emitting at 280 nm.

The results showed that for bacteriophage lambda DSM 4499, it took at least 25 min exposure to UVC-LED emitting at 280 nm to achieve 99.57 ± 0,01 % inactivation when inoculated on polish wood. The inactivation rate for bacteriophage lambda DSM 4499 on steel and glass surfaces, in both wavelengths tested, was higher with a longer incubation period, with 99,99 % achieved (Fig. 4). The inactivation rates are summarized in Table 2.

Based on the results obtained on the inactivation kinetics and the efficiency of surface disinfection of the microbial indicators, the UVC-LED emitting at 280 nm was selected for determining the recovery capacity of the cells after UV exposure and to study the effect of the exposure distance (energy dose) in subsequent experiments.

3.3. Cells ability to recovery after exposure to UVC-LED 280 nm

The ability of bacterial indicators to recover their viability was tested after being exposed to UVC-LED radiation with the wavelength at 280 nm, for 25 min, at a 0.5 m distance. The recovery rate was observed after incubation in a recovery medium for 1 h, 4 h and 24 h. The results were compared with the control condition, where the samples were exposed to the same radiation conditions without incubation in a recovery medium Fig. 5.

Results showed that *E. coli* and *S. aureus* indicators were not able to recover after exposure to UVC-LED emitting at 280 nm radiation even after an incubation period of 24 h in the recovery medium. The viable cell concentration showed no significant differences when compared with the control condition (p-value> 0.05) (Fig. 5A and B).

On the other hand, *B. subtilis* (Fig. 5C) demonstrated a significant recovery in cell viability (p-value = 0.002) after 1 h of incubation as well as after 4 h, followed by 24 h incubation (p-value <0.0001), resulting in a difference in $\log_{10}$ reduction of - 1.26 CFU ml^{-1} (from - 5.50 ± 0.17 CFU ml^{-1} achieved in the control condition to - 4.24 ± 0.08 CFU ml^{-1} after 24 h incubation). However, the inactivation rate remained at 99.99 ± 0.00 %. The same was verified with *P. aeruginosa* (Fig. 5D) where $\log_{10}$ reduction was significantly decreased

Table 2
Inactivation rates of the microbial indicators *E. coli* DSM 30083, *S. aureus* DSM 20231, *B. subtilis* DSM 10, *P. aeruginosa* DSM 1117 and Bacteriophage Lambda DSM 4499, inoculated in glass, steel and polished wood, after 20 and 25 min irradiation with (A1), (B1), (C1) UVC-LED 275 nm and (A2), (B2), (C2) UVC-LED 280 nm.

UV Wavelength	Materials	Incubation time (min)	Microbial indicators				
			E. coli	*S. aureus*	*B. subtillis*	*P. aeruginosa*	*Escherichia phage lambda*
275 nm	Glass	20	99.99 ± 0.00 %	99.99 ± 0.00 %	99.98± 0.00 %	99.99 ± 0.00 %	99.84 ± 0.03 %
		25	99.99 ± 0.00 %	99.99 ± 0.00 %	99.98± 0.02 %	99.99 ± 0.00 %	99.99 ± 0.00 %
	Steel	20	99.99 ± 0.00 %	99.99 ± 0.00 %	99.91 ± 0.00 %	99.99 ± 0.00 %	99.97± 0.02 %
		25	99.99 ± 0.00 %	99.99 ± 0.00 %	99.92± 0.13 %	99.99 ± 0.00 %	99.99 ± 0.00 %
	polished wood	20	99.99 ± 0.00 %	99.99 ± 0.00 %	99.97± 0.012 %	99.99 ± 0.00 %	99.67± 0.25 %
		25	99.99 ± 0.00 %	99.99 ± 0.00 %	99.99 ± 0.00 %	99.99 ± 0.00 %	99.98± 0.01 %
280 nm	Glass	20	99.99 ± 0.00 %	99.99 ± 0.00 %	99.98± 0.01 %	99.99 ± 0.00 %	99.46 ± 0.023 %
		25	99.99 ± 0.00 %	99.99 ± 0.00 %	99.98± 0.016 %	99.99 ± 0.00 %	99.99 ± 0.00 %
	Steel	20	99.99 ± 0.00 %	99.99 ± 0.00 %	99.57± 0.36 %	99.99 ± 0.00 %	99,98± 0.01 %
		25	99.99 ± 0.00 %	99.99 ± 0.00 %	99.79± 0.34 %	99.99 ± 0.00 %	99.98± 0.01 %
	polished wood	20	99.93± 0.01 %	99.99 ± 0.00 %	99.85± 0.08 %	99.99 ± 0.00 %	99.17± 0.02 %
		25	99.97± 0.00 %	99.99 ± 0.00 %	99.95± 0.04 %	99.99 ± 0.00 %	99.57± 0.01 %

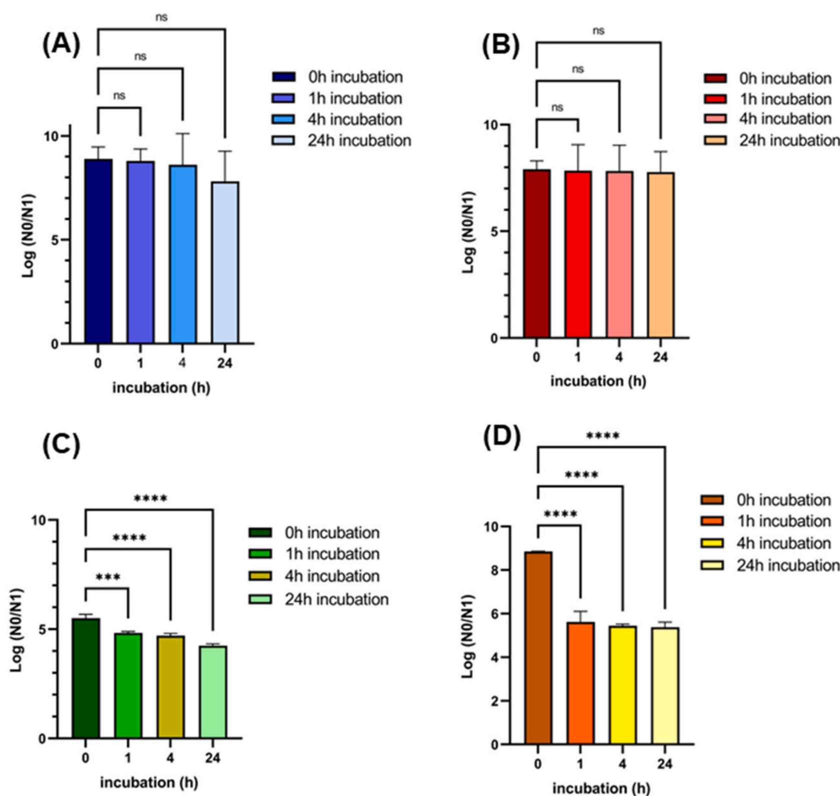

Fig. 5. Inactivation rate of the microbial indicators (A) *E. coli* DSM 30083, (B) *S. aureus* DSM 20231, (C) *B. subtilis* DSM 10 and (D) *P. aeruginosa* DSM 1117 after being exposed to UVC-LED radiation with the wavelength at 280 nm for 25 min at a 0.5 m distance, to determine their ability to recover the viability after incubations of 1 h, 12 h and 24 h. The error bars in the graph represent the standard deviation of the mean of three replicates (n = 3). Statistical difference between groups was evaluated by one-way analysis of variance (ANOVA) with post hoc comparisons made by the Dunnett's test. ns: not significant with p-value >0.05; ****: significant with p-value <0.05.

(p-value <0.0001) from 1 h incubation demonstrating a slight recovery capability after exposure to the UVC radiation. Yet, the inactivation rate also remained 99.99 ± 0.00 %.

3.4. The effect of exposure distance on the inactivation kinetics

The effect of the exposure distance on the inactivation kinetics was evaluated by comparing the logarithmic reduction in CFUs of the bacterial indicators caused by UVC-LED 280 nm lamp irradiation at two different heights: 0.25 m and 0.5 m high. The results are shown in Fig. 6. After 10 min of exposure at 0.25 m, the *E. coli* (Fig. 6.**A**) log reduction was - 8.35 CFU ml^{-1}. An identical logarithmic reduction was observed after 20 min of exposure when the UV lamp was at a distance of 0.5 m. The results also show that *S. aureus* (Fig. 6B) and *B. subtilis* (Fig. 6C) were efficiently inactivated with 15 min of irradiation at 0.25 m distance, a log reduction of – 9.00 CFU ml^{-1} was reached, and it was superior to the values obtained with 25 min at 0.5 m distance. Yet, in both cases, the inactivation rate was around 99.99 %. *P. aeruginosa* (Fig. 6D), after 25 min of exposure at both of the tested distances, reached an equal log_{10} reduction (-9 CFU ml^{-1}). However, this reduction is obtained a maintained after 10 min, 0.25 m of exposure. Considering these results is possible to observe that with both tested distances, the UVC-LED 280 nm lamp is efficient on bacterial indicators inactivation (99.99 %). Nevertheless, by half-reducing the exposure distance, is possible to decrease the necessary exposure time to achieve a total inactivation of the bacterial indicators.

4. Discussion

Surfaces are often contaminated with microbial organisms, posing a risk to public health, especially in hospitals and health facilities [8]. The recent pandemic of COVID-19 put in evidence that is critical to develop new disinfecting surface methodologies that are non-operator dependent, to minimize the contamination and the dissemination of illnesses [3,4].

Our study goes beyond the state of the art, by giving new insights into the use of more sustainable lamps (LED) to produce UV for promoting disinfection. Instead of the traditional mercury lamps, this work evaluated the use of UV-LED lamps which is an environmentally friendly and more economically feasible technology for disinfection. Furthermore, it answered different points by comparing the disinfection efficiency of different wavelengths when disinfecting different materials such as glass, steel and polished

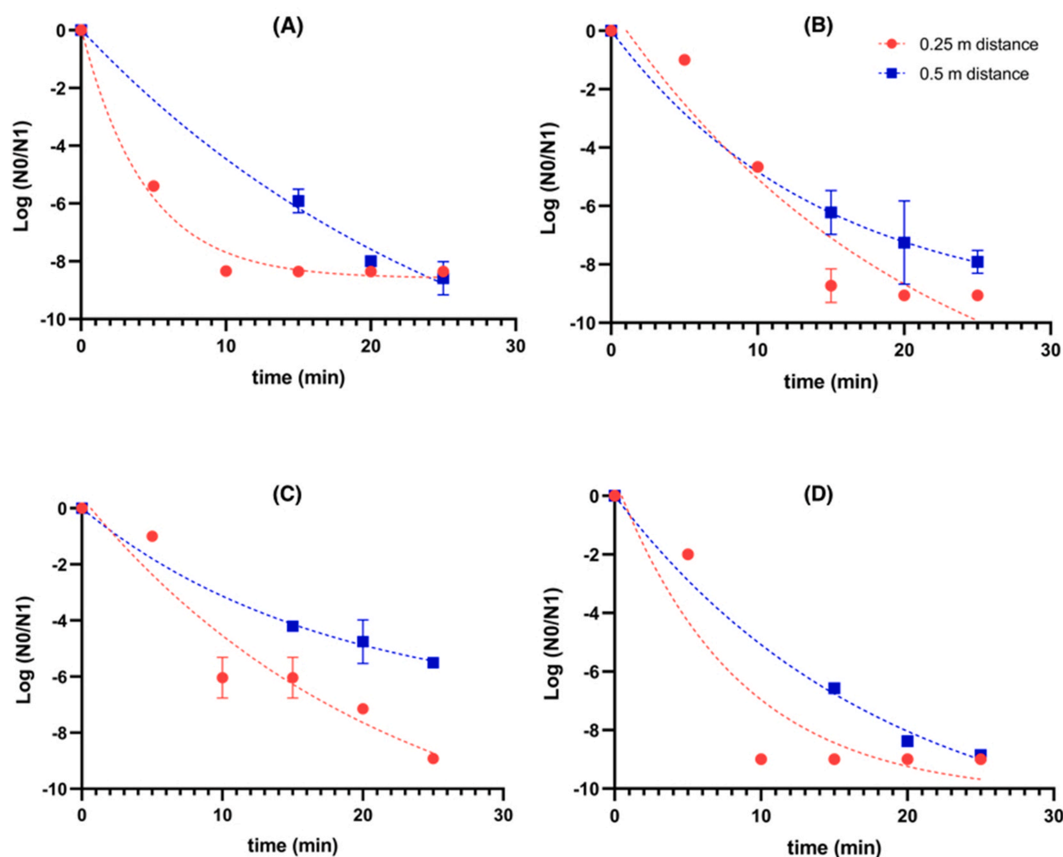

Fig. 6. Inactivation kinetics of the microbial indicators (A) *E. coli* DSM 30083, (B) *S. aureus* DSM 20231, (C) *B. subtilis* DSM 10 and (D) *P. aeruginosa* DSM 1117 with exposure to the UVC-LED 280 nm lamp light at a 0.25 m and 0.5 m distances. The error bars in the graph represent the standard deviation of the mean of three replicates (n = 3). The trend line on inactivation patterns was determined with a 95 % confidence interval based on best least squares fit.

wood. Our study also took into consideration the distance to which the UV should be applied to achieve disinfection efficiency.

The study design may not allow for some generalizations since the virucidal model that was used in the study was not based on human viruses. Therefore, caution should be taken on the generalization of the results to other viruses regarding UV-LED inactivation [4]. Nevertheless, it should be taken into consideration that bacteriophages are widely used as model organisms to evaluate the virucidal activity of UV-C light, particularly for surface disinfection [27]. In addition, according to ISO 14937, we fulfil the criteria of microorganism selection by testing not only strains belonging to the ESKAPEE group but also representative Gram-positive and Gram-negative bacteria, spores and biofilm bacteria producers [26]. According to the literature, UVC-LED lamps emitting at both 280 nm and 275 nm wavelengths can effectively inactivate a variety of microbial indicators. In fact, our study has demonstrated that both lamps achieved nearly 99.99 % inactivation rate in most cases, after 15–25 min utilization. This result was expected because both lamps are in the same UV range affecting DNA and RNA in the cells. Inagaki, Hiroko and colleagues inactivated ≥90 % of *E. coli*, *S. aureus*, *B. subtilis* and P. *aeruginosa* strains with a UVC-LED emitting at 280 nm [34].

Similar $\log_{10}$ reduction factors to those obtained by us have been described [27]. Those used a bacteriophage model and an exposure distance of 150 mm, achieving a 4.20 and 5.90 order of magnitude reduction after 50 and 75 s of UVC exposure. Nowadays, UVA and UVB are commonly used in medicine and food industry, agriculture and water purification [12]. Nevertheless, here the capacity to inactivate the microbiologic indicators was not demonstrated for UVA and UVB-tested lamps, since 30 min was necessary to achieve a CFU ml^{-1} $\log_{10}$ reduction from −1 to −2.5 of *E. coli, S. aureus* and *P. aeruginosa*. These lamps were not able to promote inactivation of *B. subtilis* and bacteriophage lambda DSM 4499. Higher UV doses could be achieved by decreasing the irradiation distance or by extending the irradiation time in order to reach inactivation levels with implications for the real world application.

This work did not evaluate the UV damage to the surfaces caused by UV exposure. Nevertheless, the irradiation of the cells was performed at an exposure distance of 0.5 m, considered a “safe” distance, larger than the exposure distances used in other studies where the exposure distance is in the order of centimeters [35,41].

An inactivation rate of 99.99 % was achieved for *E. coli*, *S. aureus* and *P. aeruginosa* after 20 min of exposure to both UVC-LED lights. However, it is worth noting that bacteriophage lambda DSM 4499 and *B. subtilis* required a minimum of 25 min of irradiation exposure to achieve the same level of inactivation. The results from *B. subtilis* were not unexpected. Although we used vegetative cells of *Bacillus*

subtilis to perform the tests, it cannot be overruled the presence of some structures of resistance in the suspension known to be highly resistant to inactivation when subjected to stress conditions like UV exposure [13].

During the assessment of the kinetics of inactivation of the indicator organisms in suspension, it was concluded that UVC-LED 280 nm was the most suitable option to evaluate the effectiveness on surfaces. This decision was made because both UVC lamps were found to be very similar considering the inactivation performance but also because a slightly less energetic UV light might induce less long-term exposure damage on the different tested surfaces [35,42].

The mechanisms of cellular repair are characteristics of cells and were never reported in viruses and bacteriophages. Therefore, for the determination of cell recovery ability after the UV-induced damage after 25 min UVC-LED exposure, only the bacterial indicators were evaluated [26]. *E. coli* did not show significative recovery after incubation during 1 h, 4 h or 24 h in the present study, as demonstrated by Romero-Martínez and colleagues [23]. *S. aureus* showed similar behavior, in line with the published results of Zhao and colleagues [11] when they exposed this strain to UVA-LED light.

On the other hand, as mentioned in several studies, *P. aeruginosa* can restore UV-induced DNA damage [36,43]. This metabolic ability justifies the significant differences in the inactivation (log reduction) observed for this strain when compared to the other indicators when cells were allowed to recover for 1 h after UV exposure. However, the capability to repair the genetic material demonstrated by the strain did not change the final inactivation rate of this indicator, which was maintained at 99.99 %. Significant differences in cell inactivation (log reduction) after UV exposure followed by recovery in the medium were observed when comparing *B. subtilis* with the other indicators. In this case, the recovery of the number of cells after incubation, may not necessarily be due to cell repair mechanisms but rather to the ability of *B. subtilis* to produce endospores, as mentioned before. The endospores are resistant to UV inactivation and will be able to develop into vegetative cells during the period of incubation during recovery [13]. Furthermore, it is stated in different studies with *E. coli* [44] and *S. aureus* [45] that the irradiation with UVC-LED emitting at a wavelength closer to 285 nm reduces the percentage of reactivation capacity [23], which could be attributed to protein damage caused at this wavelength [44].

In regards to the evaluation of exposure distance, in the present work, by reducing the exposure distance to half, the exposure time necessary to achieve the complete inactivation of the bacterial indicators was also decreased as previously observed [46].

In practical applications, the assemblage of the UV lamps into a mobile system (robot) may address the limitations of the UV disinfection process which are the shadow angles, UV dose and the existence of different materials in the hospital space. Whereas contaminated surfaces are a critical factor for the spread of infections, this study, by presenting a long-distance disinfection methodology able to inactivate bacteria belonging to the ESKAPEE group and a virucidal model present in different types of materials, should be taken into consideration in the decision-making process on the use UV-LED for disinfection scenarios in hospitals.

5. Conclusions

Finding and developing an efficient method for surface disinfection using automatic non-operator dependent systems can be decisive for controlling the infections spread in health facilities. UV-LED disinfection has been emerging as an alternative. The results of the present study show that UVC-LED lamps emitting at 280 nm and 275 nm could inactivate 99.99 % of the microbial indicators on glass, steel and polished wood when applied for 20 min, at a 0.5 m distance. The exposure time needed to inactivate those microbial indicators can be decreased by reducing the exposure distance. The ability of the cells to recover from UV-induced damage was apparently observed in *P. aeruginosa*. In *B. subtilis* recovery of vegetative cells after UV exposure was most probably related to the presence of endospore. In both situations, the inactivation rate remained at 99.99 % after 24 h of incubation.

As final remarks, the results obtained in the present work demonstrate that the tested UVC-LED 280 nm lamp is indicated to be applied on surface disinfection at hospital facilities or on medical equipment. These findings had a positive impact in a real-world context by contributing to the development of a disinfection device, non-operator dependent, environmentally friendly and able to ensure high elimination rates, being relevant in the reduction of nosocomial infections spread.

Data availability statement

The type strains used in this study were obtained from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany, and cryopreserved at the University of Coimbra Bacterial Culture Collection (UCCCB https://ucccb.uc.pt/) under the accession numbers *Escherichia coli* UCCCB 113, *Staphylococcus aureus* UCCCB 115, *Bacillus subtillis* UCCCB 117, *Pseudomonas aeruginosa* UCCCB 116, *Escherichia* phage lambda (Enterobacteria phage lambda) UCCCB 120 and *Escherichia coli* UCCCB 114.

CRediT authorship contribution statement

Beatriz Rito: Writing – original draft, Methodology, Investigation. **Leonor Matos:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Diogo N. Proença:** Writing – review & editing, Supervision, Conceptualization. **Paula V. Morais:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing

interests:Paula V. Morais reports financial support was provided by Foundation for Science and Technology. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30738.

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