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Highlights

- CHRONOlight system showed bactericidal activity with UV-C in 15–45 seconds
- Bactericidal activity depended on bacterial species and UV-C irradiation efflux
- UV-A exposure for 30 minutes did not reduce cell viability
- UV-C resistant isolates had mutations in metabolic, stress and virulence genes

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Title: **Efficacy of the CHRONOlight biodynamic lighting system for control of nosocomial Gram-negative pathogens**

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ABSTRACT

Background:

Healthcare-associated infections are a growing public health concern, with hospitals acting as reservoirs for pathogens. The rise in antimicrobial resistance worsens the issue. This study tested the effectiveness of the biodynamic lighting system (CHRONOlight) at varying UV light times and energy levels to reduce nosocomial Gram-negative pathogens.

Methods:

Eight clinical isolates, representing different Gram negative pathogens, were selected from the Andalusian Reference Laboratory. *E. coli*, *P. aeruginosa* and *A. baumannii* ATCC were used as control strains. Different inoculums were exposed to UV-C light doses 2.0, 1.5 and 1.0 W/m2 (corresponding to an irradiation flux of 100%, 75% and 50%, respectively, with spectral peaks at 270 nm) during 15, 30, 45, 60 and 300 seconds and UV-A (380 nm) during 30 minutes. The bactericidal effect was considered when cellular viability decreased by ≥ 3-log10 CFU/mL. UV-C light-surviving isolates were sequenced (Illumina MiSeq) and analysed to identify mutations associated with UV-C light survival. Development of antibiotic resistance after UV-C exposure were also studied for the same surviving isolates.

Results:

The efficacy of the CBL system varied by exposure time, UV dose, and bacterial species. *E. coli* isolates showed bactericidal effects within 15-30 seconds at 100% UV-C intensity, while *K. pneumoniae* required 30-45 seconds. *P. aeruginosa* and *A. baumannii* showed activity in 15-30 seconds, but clinical *A. baumannii* needed longer exposure, especially at lower UV-C intensities. No reduction in cell viability was seen with UV-A exposure.

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Gene mutations related to metabolism, stress response, motility, and virulence were found in survivors, and, no cross-antibiotic resistance developed in surviving isolates.

Conclusions:

CBL is a novel broad-spectrum lighting prototype designed to effectively reduce the viability of Gram-negative bacteria within short exposure times (30 to 300 seconds) through UV-C emission. This makes it a promising strategy for minimizing nosocomial infections in healthcare settings.

Keywords: UV-C light disinfection, Gram-negative pathogens, Healthcare-associated infections

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Introduction

The rise in healthcare-associated infections (HAIs) is a major public health issue, with significant economic and social impact. Nosocomial infections increase morbidity and mortality rates, reduce life expectancy in hospital populations, and strain healthcare systems [1]. While shortening hospital stays is a strategic goal, the rise in HAIs leads to longer stays and higher costs. Implementing effective infection control programs would reduce financial burdens, improve resource use, and enhance patient quality of life [2]. In addition, the rise of antimicrobial resistance, particularly carbapenemase-producing isolates, exacerbates the problem by reducing therapeutic options and enabling rapid global dissemination, making control measures urgent [3].

Effective control and prevention are critical to reducing HAIs. Intensive surveillance programs have been shown to significantly reduce nosocomial infection rates; however, these programs are still lacking in many hospitals [4]. Strategies to reduce nosocomial infections are extensive and include adherence to hygiene and disinfection measures such as hand washing, surface disinfection and equipment sterilisation [5]. Hospital surfaces, including floors, beds, furniture, monitors, and patient clothing, can harbor pathogens [6]. Cleaning and disinfection primarily rely on manually applied disinfectants, selected based on efficacy, spectrum of activity, environmental compatibility, and the risk of tolerance and resistance development [7]. An ideal disinfectant should have the following characteristics: high bactericidal activity; quick action; resistance to the development of microbial resistance; and safety for people and the environment. However, in practice, disinfectant effectiveness varies depending on the type, application method, and potential uncertainty in manual processes [8]. So, overcoming barriers to effective cleaning remains a challenge [9].

Ultraviolet (UV) light can be classified based on its wavelength into three types, ordered from lowest to highest energy: type A (315-400 nm), type B (280-315 nm), and type C (100-280 nm), being type C ultraviolet light (UV-C) the most penetrating and therefore the most harmful type [10]. It inactivates a wide range of viruses and bacteria quickly, with minimal environmental impact. The disinfection mechanisms of UV-C disinfects by modifying nucleic acids, preventing replication, and causing cell death [11][12]. The primary mechanisms of action of UV-C in bacterial sterilization include DNA damage and the generation of oxidative stress. DNA damage inhibits replication, leading to cell death, while increased oxidative stress further promotes cell death by damaging key cellular components [13]. Therefore, UV-C light could be a valuable tool in the disinfection and sterilization processes of various hospital environments. The International Commission on Illumination (CIE) has defined its stance on the use of UV-C light for the disinfection of viruses and bacteria, stating that although its effectiveness is well established, UV irradiation should not be conducted in occupied spaces [14]. Consequently, UV lighting systems should only be employed in unoccupied areas, such as hospital rooms during patient turnover. These standards established safety operation but did not define the design criteria for low-spectrum light to neutralize pathogens. The radiant exposure required for the deactivation of an infectious agent by 90% depends on the environmental conditions (such as relative humidity) and the kind of infectious agent and it typically ranges between 2 mJ/cm^2 and 20 mJ/cm^2 for emitting radiation at 254 nm [15].

The Chronolight Biodynamic Lighting System (CBL) is a broad-spectrum lighting system designed to improve the health of hospitalized patients. It aims to regulate chronobiological markers (such as cortisol and melatonin) through a specific spectral distribution. CBL use adjustable white LEDs (2500 K–5500 K), blue LEDs (470 nm),

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and amber LEDs (600 nm) to either suppress or promote melatonin secretion as needed. Additionally, the system includes UV-A LEDs (380 nm) to reduce hospital-related bacteria and UV-C LEDs (270 nm) for disinfection. The design is based on studies evaluating melatonin suppression and bacterial neutralization (Figure 1).

Therefore, the aim of this study is to evaluate the ability of this novel biodynamic lighting system to eliminate or neutralize pathogens in healthcare environments by UV-C and UV-A, respectively emission.

Materials and Methods

Bacteria strains

Multiresistant clones of epidemiological interest of Enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were selected from Reference Laboratory for the Surveillance and Control of Nosocomial Infections and Prudent Use of Antimicrobials Program in Andalucía (PIRASOA program, Hospital Universitario Virgen Macarena Seville Spain). Specific relevant phenotypes studied were: *E. coli* ST131 producing CTX-M-15 and OXA-48, *E. coli* ST131 producing VIM-1, *K. pneumoniae* ST512 producing KPC-3, *K. pneumoniae* ST15 producing VIM-1 and CTX-M-15, *P. aeruginosa* ST175 producing IMP-23, *A. baumannii* ST2 producing OXA-58 and *A. baumannii* ST636 producing OXA-24. *E. coli* ATCC25922, *P. aeruginosa* ATCC27853 and *A. baumannii* ATCC19606 were used as control strains. Specie identification was performed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS) (Bruker).

Whole genome sequencing (WGS)

Genomic characterization was performed by whole genome sequencing (WGS) (Miseq; Illumina). Total DNA was extracted using with automatic system MagCore HF16 Plus

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(RCBBioscience®) and sample library was prepared using Nextera Flex DNA library preparation kit (Illumina®). DNA sequencing was carried out with the MiSeq Reagent Kit V3 (600 cycles) in the Illumina MiSeq sequencer (2x300 paired-end reads). Reads were Reads were following trimmed, quality filtered and assembled de novo using the CLC Genomic Workbench v9 (Qiagen). Clone assignment and resistance determinant detection analysis were performed using the MLSTFinder2.0, ResFinder3.1, respectively.

UV light and pathogens neutralization

To evaluate the effectiveness of the Chronolight Biodynamic Lighting System (CBL) in bacterial elimination and neutralization, a reduced prototype—comprising only the UV-emitting diodes—was designed for use within a laminar flow cabinet. Figure 2 illustrates this reduced prototype, which includes solely the UV-emitting diodes, along with the corresponding irradiance levels relative to the luminous flux. Technically, each bacteria was cultured overnight in MHB at 37ºC. To obtain logarithmic phase of growth, overnight cultures were diluted 1:10 in MHB medium and grown at 37°C with agitation until they reached an OD625 of 0.1, corresponding to about $1x10^8$ CFU/ml (High inoculum). Then, culture was centrifuged at 5,000 g for 10 min at RT and pelleted cells were resuspended in 10 ml of 0.9% NaCl. Three ml of each inocula (high ($1x10^8$), medium ($1x10^5$) and low ($1x10^3$) were placed in each separated wells in Thermo Scientific™ 6 Well Plate. The plate was then exposed to different UV-C light doses, without a lid at a distance of 20 cm, with irradiations of 2.0, 1.5 and 1.0 W/m² for UV-C (corresponding to an irradiation flux of 100%, 75% and 50% respectively in the luminaire, with spectral peaks at 270 nm) for 15, 30,45, 60 and 300 seconds (Table 1). For the UV-A light emission (spectral peak at 380nm) a period of 30 minutes was tested. Appropriate dilutions of cells were plated immediately after UV treatment on MHA medium plates and incubated overnight at 37°C. As control of basal cell counts, each inoculum was seeded without exposure to UV

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light in MHA plates. The cell viability was determined by counting the number of colonies and survival was calculated comparing viable cell obtained in absence of UV light and after each different time after UV-C exposure. The bactericidal effect was considered when cellular viability decreased by ≥ 3-log10 CFU/mL, after counting the number of surviving colonies compared to the absence of UV light and the sterilizing capacity was established when the colony count was equal to 0. The detection limit was stabilized at 2-log10. Results were obtained from three independent experiments. Differences of $p<0.05$ were considered statistically significant, using the Student's t-test.

Mutation Analysis of UV-C Exposed Bacterial Strains

For finding mutations, *E. coli* ATCC25922, *P. aeruginosa* ATCC27853 and *A. baumannii* ATCC19606 control strains were subsequently sequenced by Plasmidsaurus (https://plasmidsaurus.com/) using Oxford Nanopore Technology with custom analysis. Up to five surviving isolates of *E. coli* ATCC25922, *P. aeruginosa* ATCC27853 and *A. baumannii* ATCC19606 after exposure to UV-C light at different intensities and times were also sequenced on the Illumina MiSeq sequencer, as described above. Mutations were called using the computational pipeline Breseq variant report v.0.35.7 via Galaxy/Pasteur (https://galaxy.pasteur.fr) using the annotated genome of each control as a reference from Bakta (https://bakta.computational.bio/).

Development of cross antibiotic resistance

To study possible development of cross antibiotic resistance after UV-C exposure, the same surviving isolates selected for mutation studies were analyzed. Disk diffusion was performed for ampicillin, amoxicillin/clavulanic acid, piperacillin, piperacillin/tazobactam, cefazolin, cefuroxime, cefoxitin, ceftazidime, cefotaxime, cefepime, aztreonam, ertapenem, imipenem, meropenem, ciprofloxacin, gentamycin, tobramycin, amikacin and trimethoprim/sulfamethoxazole in accordance with EUCAST

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and CLSI guidelines. *E. coli* ATCC 25922, *P. aeruginosa* ATCC27853 and *A. baumannii* ATCC19606 were used as a control strain (QC, EUCAST).

Results

CBL was highly effective for control of nosocomial Gram-negative pathogens

The efficacy of CBL in eliminate or neutralizing pathogens show a direct dependency on dose and the exposure time of UV light, among the species assessed. In general, CBL was highly effective in eliminating in this class of pathogens. In fact, when *E. coli* isolates (*E. coli* ATCC25922, *E. coli* ST131/CTX-M-15+OXA-48, and *E. coli* ST131/VIM-1) are exposed to UV-C radiation at 100% intensity, bactericidal activity is achieved within 30 seconds from a high inoculum (10^8 CFU/ml) and within 15 seconds from a medium (10^5 CFU/ml) or low (10^3 CFU/ml) inoculum (Figure 3A). When these isolates are exposed to UV-C radiation at 75% and 50% intensities, bactericidal activity was also observed after 30-45 seconds of exposure for all inocula used (Figure 3A). For *K. pneumoniae* ST15/VIM-1 and *K. pneumoniae* ST512/KPC-3, the bactericidal capacity of the CLB, with a UV-C light intensity of 100%, is achieved with exposure times of 30-45 seconds for all tested inocula. For UV-C light intensities of 75% and 50%, the time to achieve bactericidal capacity increase between 60 and 300 seconds (Figure 3B) for all *K. pneumoniae* isolates. When *P. aeruginosa* ATCC27853 and *P. aeruginosa* ST175/IMP-23 were tested, for any inoculum and UV-C light intensity evaluated, the time required to reduce the inoculum to achieve bactericidal capacity was between 15 and 30 seconds (Figure 3C). The same results were obtained for *A. baumannii* ATCC19606, with bactericidal capacities for all three tested inocula in 15-30 seconds. However, for clinical isolates of *A. baumannii* (ST2/OXA-58 and ST656/OXA-24), exposure times of 30-45 seconds were required to achieve bactericidal capacity with 100% UV-C light intensity,

and between 60 and 300 seconds for UV-C light intensities of 75% and 50% (Figure 3D). The sterilising capacity of CBL for *E. coli* strains was achieved within 300 seconds when starting from a high inoculum and within 30-45 seconds for medium or low inocula. For *K. pneumoniae* strains, 300 seconds was required to achieve sterilising capacity for most inoculum and intensities tested, although this condition was not achieved for high inocula and 50% intensity. All *P. aeruginosa* isolates tested required between 15 and 45 seconds to reach sterilisation conditions, except for *P. aeruginosa* ATCC27853 when starting from a high inoculum. Finally, for *A. baumannii* isolates, the control strain *A. baumannii* ATCC19606 required 15-45 seconds to reach sterilisation conditions. However, clinical strains did not achieve sterilisation when starting from high inocula, whereas sterilisation times for medium and low inocula ranged from 60 to 300 seconds. After 30 minutes of UV-A light radiation, no reduction in cell viability was observed in any of the *E. coli*, *P. aeruginosa*, or *A. baumannii* isolates tested.

Mutations found in surviving colonies do not modify antimicrobial susceptibility

Mutations were found in the different surviving colonies of *E. coli* ATCC25922, *P. aeruginosa* ATCC27853 and *A. baumannii* ATCC19606 selected after exposure to UV-C light for different durations and intensities. These control isolates were selected due to their susceptibility profile and absence of resistance mechanisms related to previous exposure. Mutations found in *E. coli* ATCC25922, that survived UV-C exposure, were located in genes related to modulation of metabolism, maintenance of redox balance and cellular homeostasis. Genes related to the Krebs cycle, such as citrate/succinate antiporter (*citT*), trans-aconitate 2-methyltransferase (*tamT*) and acetyl-CoA C-acyltransferase (*fadA*), and genes related to GABA metabolic pathways, such as GABA permease (*gabP*) and glutamate decarboxylase (*gadB*). Genes related to bacterial motility and cell adhesion (flagellar transcriptional regulators (*flhc*, *Ag43/Cah*) and stress response and virulence

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(*YfcC* and *SohB*) were also found to be mutated. In *P. aeruginosa* ATCC27853, several mutations related to the type VI secretion system (*vgr*, *hcpA*) and genes associated with bacterial communication and quorum sensing (*pquiB*) were found in isolates that survived exposure to UV-C light. Mutations were also identified in genes encoding proteins involved in virulence, biofilm formation and bacterial competition (16S ribosomal RNA/DNA repair exonuclease, SbcCD nuclease subunit, methyl-accepting chemotaxis protein (*pctB*), "two-partner secretion system exoprotein" (*fhaB*) and glycine cleavage system aminomethyltransferase (*gcvT*)), which contribute to the survival of isolates in hostile environments. In *A. baumannii* ATCC19606, mutations were found in genes associated with increased survival, stress adaptation, and virulence. Specifically, mutations were identified in genes related to adhesion and biofilm formation (*bapA*), transcriptional regulation of genes involved in stress adaptation and hostile conditions (*gigC*, *YneE*, and *pcKG*), genes associated with fatty acid synthesis (*aac*), and a chaperone involved in lipoprotein transport to the outer membrane (*lolA*).

Interestingly and regarding the possible development of antibiotic resistance following UV-C exposure, no reduction in halo size was observed for any antibiotic in any of the surviving isolates tested. All isolates maintained halo size compared to their control isolate for all antibiotics tested (Supplementary Table S1, S2 and S3).

Discussion

Ultraviolet light, particularly in the UV-C range is a highly effective antimicrobial agent, particularly against Gram-negative bacteria. In this study, we assessed the bactericidal efficacy of a biodynamic lighting system prototype (CBL) capable of emitting both UV-C and UV-A light as different wavelengths offer different advantages in terms of safety, tissue penetration and efficacy. Tests were conducted on control and relevant clinical

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isolates of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii*. Results demonstrated a strong bactericidal effect of UV-C at short to medium exposure times, ranging from 30 to 300 seconds. These durations translate to disinfection cycles of 50 minutes to 8 hours under real conditions, depending on the pathogen. Clinical isolates of *A. baumannii* required longer exposure, while *E. coli*, *P. aeruginosa*, and *K. pneumoniae* were inactivated more rapidly.

The emission spectrum in the UV-A band was also evaluated, as recent studies have explored the potential of UV-A light, particularly at a wavelength close to 365 nm, as a safer and more environmentally friendly alternative for sterilization purposes and, although, UV-A emitting diodes are less effective in bacterial neutralization compared to UV-C light, they can be safely used in occupied spaces [16]. However, as demonstrated in our results, UV-C exhibit significantly higher efficacy compared to UV-A because, in our case, UV-A light did not induce an actual reduction in cell viability in any of the tested strains, even when the exposure time was increased up to 30 minutes. This may be due to its lower efficacy compared to UV-C light, limiting its applicability in hospital environments.

However, practical implementation in real environments presents challenges. The CBL system was tested *in vitro*, and its effectiveness *in situ* may be affected by architectural and operational constraints. Under biosafety cabinet boundary conditions, 30 seconds at 20 cm is sufficient in most cases to achieve bactericidal effect, so, at 210 cm the required exposure time scales approximately 110, yielding approximately 55 minutes. Even so, the applicability of this type of UV luminaires could be useful for hospital environments. Specifically, a ceiling-mounted configuration in hospital rooms places the UV source approximately 160 cm from target surfaces, resulting in a roughly 100-fold decrease in irradiance. Consequently, exposure durations must be proportionally increased to

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maintain bactericidal efficacy. Despite this, adjusted exposure times may still be compatible with hospital operational protocols, particularly during periods when rooms are unoccupied between patients. In addition, line-of-sight limitations in a hospital room create shadowed areas that the luminaire cannot directly irradiate (e.g., under the bed). While such areas are typically less accessible to occupants, a complementary cleaning and disinfection protocol remains necessary to ensure an adequate and safe environment.

The dual functionality of the CBL system represents a major innovation. It not only regulates circadian rhythms through biodynamic lighting based on biomarkers such as cortisol and melatonin [17-18] but also integrates UV-C emission to contribute to pathogen elimination. This integration enhances patient well-being while simultaneously supporting infection control strategies.

Inanimate surfaces, particularly high-contact surfaces, have often been described as the source of nosocomial infection outbreaks, making their effective disinfection crucial to ensuring the safety of healthcare professionals and patients [19]. The use of UV-C light, both in portable devices and robots, has been tested as a complement to standard cleaning protocols, showing very positive results in reducing the presence of organisms on high-contact surfaces in hospitals [20-22] but meaningful restrictions due to relative positioning [23].

The validation of short sterilization times for common nosocomial pathogens like *E. coli, K. pneumoniae, P. aeruginosa,* and *A. baumannii* offers a significant advantage to the prototype, enabling it to sterilize surfaces and fomites during patient absence without compromising safety. However, translating the system to real hospital environments presents challenges, including ensuring proper dosing, coverage, and safety of UV-C radiation through suitable control and usage procedures.

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Several studies have tested UV-C light sterilization in hospitals using different wavelengths, intensities, and exposure times. Some found a 100% reduction in bacterial growth of *E. coli* ATCC25922, *P. aeruginosa* ATCC27853, and *A. baumannii* ATCC19606 with UV-C (254-265 nm) at 912 mJ/cm² and 5.5 W/cm² for 10-20 seconds [22; 24]. These results align with those obtained in our study, showing similar exposure times for the same bacterial strains to achieve reductions in cell viability. However, previous studies have focused on testing reference strains, not including clinical isolates commonly found in hospital environments. According to our results, clinical isolates require a longer exposure time to achieve a comparable reduction in cell viability compared to the reference strain, showing our system high efficacy against these clinically relevant strains. Further studies evaluating such devices using actual clinical isolates from hospital settings are needed to optimise UV-C exposure time and intensity.

Surviving UV-C light exposure involves complex genetic adaptations related to metabolic regulation, cellular homeostasis, redox balance, stress adaptation, and virulence. In *E. coli*, resistance to UV-C is partly due to mutations in genes involved in DNA repair and cell membrane structure maintenance, linked to the growth phase of the isolate [25]. Mutations in our *E. coli* UV-C-surviving isolates were found in genes related to the Krebs cycle and redox balance, affecting membrane permeability and metabolic activity [26]. The SOS response, which involves the upregulation of genes controlled by the LexA repressor, plays a key role in the repair of UV-C induced DNA damage [27]. However, no mutations in genes related to the SOS response or DNA repair were found in surviving *E. coli* isolates, possibly due to the SOS response's transient hypermutation [28]. *P. aeruginosa* adapts to UV-C light through mechanisms like biofilm formation, quorum sensing, DNA repair, and reactive oxygen species management [29]. Mutations in our UV-C-surviving *P. aeruginosa* isolates were found in genes associated with type VI

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secretion systems, quorum sensing, bacterial competition, and survival in hostile environments. However, like the UV-C-surviving *E. coli* isolates, no mutations were detected in DNA repair or SOS response genes. The *sbcD* gene, involved in DNA repair and genomic stability, was the only mutated gene. Regarding *A. baumannii* adaptation to UV-C, resistance is mainly attributed to biofilm formation, oxidative stress regulation, and DNA repair mechanisms, similar to *P. aeruginosa* [30]. In our UV-C-surviving *A. baumannii* isolates, we identified mutations in genes related to adhesion, biofilm formation, and stress adaptation. Additionally, mutations associated with lipid and fatty acid metabolism were also detected. Nevertheless, no mutations were found in the *BlsA* gene, a key regulator of a wide range of *A. baumannii* functions, including biofilm formation and cell motility, which has been linked to oxidative stress response upon light exposure [31]. In any case, and as a limitation of this work, the role of these mutations in survival under UV light remains to be confirmed.

The potential development of antibiotic resistance due to UV-C light exposure is a concern. While UV-C is an effective microbial inactivation tool, further research is needed to assess UV-C induced resistance risks. Genetic changes linked to increased resistance in *P. aeruginosa*, such as mutations in *gyrA* and *rpsL*, have been reported [32]. However, in our study, none of the UV-C-surviving isolates developed resistance to any tested antimicrobials, maintaining the same susceptibility profile as control isolates. This phenotype data correlates with the mutation profile of survivors, which were not associated with known antimicrobial resistance mechanisms.

This study has several limitations. Different Gram-negative bacterial isolates were tested with varying UV light intensities and exposure times. Further studies are needed to evaluate the CBL's effectiveness against other clinical isolates, including Gram-positive bacteria, and its impact on more resistant structures like biofilms. While the system shows

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promise, its implementation must be carefully assessed for benefits, limitations, and potential ethical impacts. One challenge is its application in isolation rooms or open hospital spaces. UV-C technology should complement, not replace, existing hygiene protocols and strict safety measures are required due to potential harm to humans. Future research should focus on long-term effects, durability, and integrating the system with other technologies for better outcomes. Additionally, this prototype was tested under *in vitro* conditions, and future studies will adapt these conditions to more realistic settings, testing the system in actual hospital rooms to optimize exposure times and UV intensity.

Conclusions

The CBL is an innovative broad-spectrum lighting system prototype that utilizes UV light within biodynamic luminaires. It shows strong potential for controlling nosocomial disease transmission among patients, carers, and hospital staff, especially when rooms are unoccupied. The system aids in pathogen elimination or neutralization in hospital areas prone to infections. Evidence supports its potential to effectively control healthcare-associated infections (HAIs) by adding an extra layer of pathogen control through UV light. The concept proposes installing the equipment in critical hospital areas, such as patient rooms and ICUs, and incorporating mobile systems for ease of access and use.

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Authors contributions

PPP contributed to performed research experiments, data curation, writing and editing the manuscript. PB, SMA and IA contributed to resources finding, design formal analysis, writing and editing the manuscript. MJCA performed research experiments and JMRM

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contributed to data curation, writing and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

Ethical approval

Not required

Declaration of Competing Interest

The authors declare that they are not aware of any conflicts of interest associated with this publication, nor have they received any significant financial support that could have influenced its outcome.

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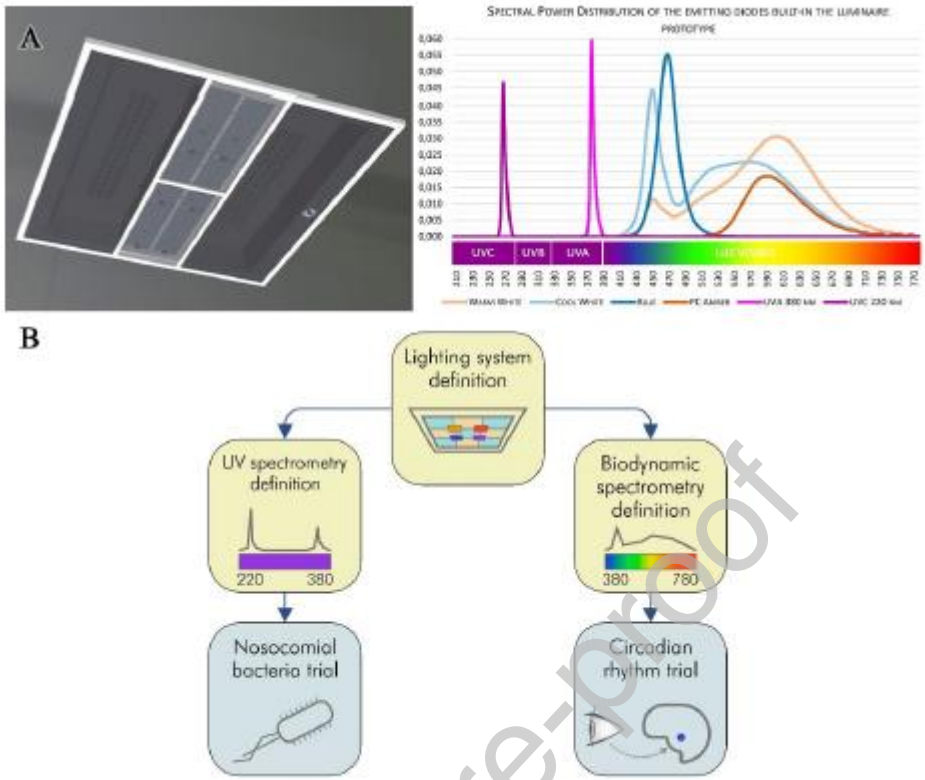

Figure1.

A)Theluminaireprototypesincorporatevariouslight-emittingdiodesoperatingwithinthevisiblespectrum,specificallyatuneablewhiteLEDwithacorrelatedcolourtemperature(CCT)rangingfrom2500Kto5500K,ablueLEDwithapeakemissionat470nm,andaphosphor-converted(PC)amberLEDwithapeakat600nm.Thevisiblelightchannelswillbesupplementedbytwoadditionalemittingdiodes:thefirstemitsUV-Aradiationwithaspectralpeakat380nm;andthesecondemitsUV-Cradiationwithadominantspectralpeakat270nm,bothintendedtoreducetheactivityofbacteriaassociatedwithnosocomialinfections.B)Protocolsbasedontrialsevaluatingtheeffectivenessofmelatoninsuppressionandbacterialneutralization.

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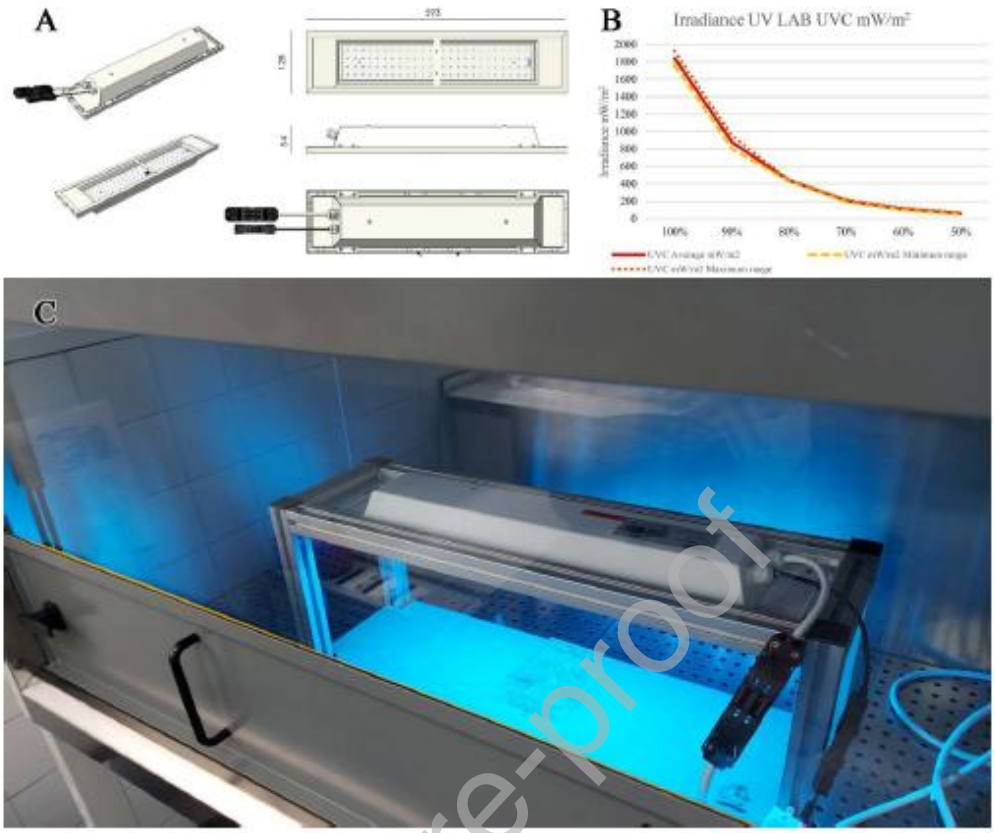

Figure 2.
A) Planimetry of the reduced prototype of the UV luminaire. B) Ratio between the power radiated per UV-C radiation surface and the luminous flux, at the height of the prototype above the plate (about 20 cm). C) Image of the prototype inserted in the laminar flow cabinet.

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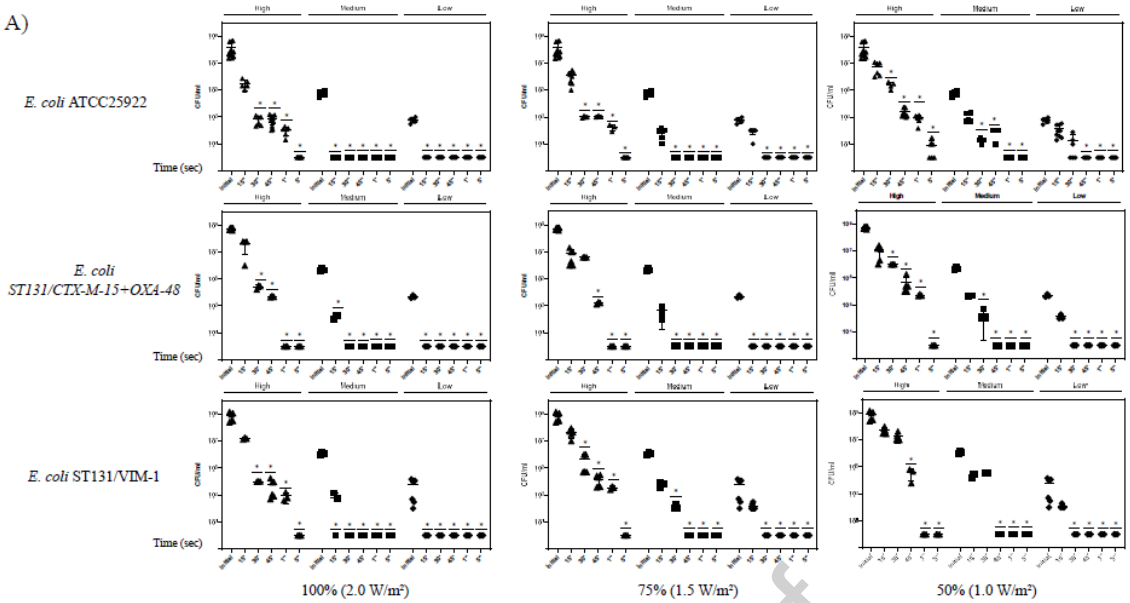

Figure 3. A) Survival graph. CFU reduction before and after application of Chronolight biodynamic luminaires with UV-C intensity doses of 100% (2,0 W/m2), 75% (1,5 W/m2) and 50% (1,0 W/m2) for 15, 30, 45, 60 and 300 seconds to Escherichia coli (ATCC25922, ST131/OXA-48 and ST131/VIM-1). Initial inoculum: High (10_8), Medium (10_5) and Low (10_3). Data are shown as mean. CFU (colony forming units). *p < 0,05.

Table 1: Trial protocol for UV-C light. The table shows each exposure trial time tested with its corresponding equivalence in intensity, irradiance and energy per surface area.

Trial	Irradiation flux	Irradiance	Exposure time	Energy per surface
UVC1	**100%**	**2.0 W/m²**	**15 s**	3 mJ/cm²
UVC2	**100%**	**2.0 W/m²**	**30 s**	6 mJ/cm²
UVC3	**100%**	**2.0 W/m²**	**45 s**	9 mJ/cm²
UVC4	**100%**	**2.0 W/m²**	**60 s**	12 mJ/cm²
UVC5	**100%**	**2.0 W/m²**	**300 s**	60 mJ/cm²
UVC6	**75%**	**1.5 W/m²**	**15 s**	2 mJ/cm²

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UVC7	**75%**	**1.5 W/m²**	**30 s**	5 mJ/cm²
UVC8	**75%**	**1.5 W/m²**	**45 s**	7 mJ/cm²
UVC9	**75%**	**1.5 W/m²**	**60 s**	9 mJ/cm²
UVC10	**75%**	**1.5 W/m²**	**300 s**	45 mJ/cm²
UVC11	**50%**	**1.0 W/m²**	**15 s**	2 mJ/cm²
UVC12	**50%**	**1.0 W/m²**	**30 s**	3 mJ/cm²
UVC13	**50%**	**1.0 W/m²**	**45 s**	5 mJ/cm²
UVC14	**50%**	**1.0 W/m²**	**60 s**	6 mJ/cm²
UVC15	**50%**	**1.0 W/m²**	**300 s**	30 mJ/cm²

Declaration of Competing Interest

The authors declare that they are not aware of any conflicts of interest associated with this publication, nor have they received any significant financial support that could have influenced its outcome.

Likewise, the authors declare that the manuscript does not has not been published previously and it is not under consideration for publication elsewhere

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