

The Influence of UV-C LED on the Colonies of Three Different Species of Microorganisms

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Recent occurrence of the pandemic revived the interest for germicidal ultraviolet (UV) lamps for disinfection. The use of the UV-C Light Emitting Diodes (LED) can deactivate microbes in air and water, but to achieve safe and successful disinfection of surfaces, careful and precisely defined usage is required that needs additional research. Commercially available UV-C LED lamp with the wavelength of 253,7 nm is used on two types of bacteria (one of them drug resistant) and one fungus.

The presented research results show that, in addition to the dependence of the efficacy on the time of exposure and surface distance from the lamp, the relative effectiveness of disinfection for the same conditions varies for the type of microbe. For *E-coli* it was between 72% and 90%, for *Pseudomonas aeruginosa* between 81% and 97%, while for the *Candida albicans* it went from 93% to 98%.

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1. Introduction

Even though UV lamps have been used for disinfection of water and surfaces for the last 40 years, the recent pandemic sparked the interest in preventing and weakening any potential spreading of diseases using the novel UV-C LED lamps. The term germicidal UV (GUV) is also used and refers to the use of ultraviolet radiation that has energy needed to inactivate bacteria, fungi or viruses.

Energy of the UV radiation damages the DNA and RNA, causing the mutations which stop replication and leads to the inactivation and death of microorganisms. Bacteria and viruses vary in susceptibility to the UV radiation, with fungus relatively harder to eliminate compared to the more rapidly replicating bacteria.¹

Studies show that UV-C wave lengths between 200 and 280 nm can disinfect surfaces along the line of radiation and at the right dosage.² However, there are wide disparities between UV-C measurement and actual microbe reductions^{3,4}. These differences may be due to measurement and experimental variables. The lack of correlation between UV-C metrology and actual pathogen reduction can lead to infection control professionals falsely believing a UV-C lamp is achieving the desired effect.

In addition to the question of the relative efficacy, UV-C can also be a hazard for humans² and must be applied carefully with research backing the application with well-defined conditions: Commercially available UV-C devices emit at a variety of UV-C light intensity. The weaker the intensity, the greater time required to reach the desired cumulative dose⁵. Based on the “inverse square law”, increasing the distance between the UV-C source and the target surface reduces the intensity and the time required to achieve the same flow rate.

This study was undertaken to determine how the altering of the distance between the UV-C source and the target, as well as the exposure time affect the actual bacterial and fungus reduction.

2. Experimental set-up

Reference strains of *Escherichia coli* (*E. coli*) ATCC 8739, *P. aeruginosa* ATCC 10231, and *Candida albicans* ATCC 10245 were used to test the microbicidal effect of UV radiation. Bacteria *E. coli* and fungus *Candida albicans* were selected for this study due to their availability and the fact that they grow readily, while bacteria *Pseudomonas aeruginosa*, was chosen for the study due to its increased resistance to the antibiotics.

200 μ l of each referent strains stock solution was inoculated in BHI broth and incubated for 24 hours at 37°C. After incubation, a ten-fold series of decimal dilutions in sterile 0.9 % saline was made from the broth. From the last dilution, 1ml was taken and pipetted into sterile petri dishes, after which it was exposed to UV radiation under different conditions.

One plate was left as a control sample and was not exposed to radiation. After irradiation, a sterile and cooled nutrient medium – Plate Count agar, was poured into each petri dish, whereupon was incubated for 24 hours at 37°C. After incubation, the number of grown colonies on plates that were exposed to radiation was counted and compared with the number of colonies on plates that were not exposed to UV radiation.

A factory-calibrated, commercially available, portable UV-C LED with 253,7 nm wave length is used as a source of UV radiation, as shown in the experimental set-up in Figure 1. Since the lamp manufacturer declared that the exposure time needed to achieve 90 % microbe elimination should be between 10 to 30 s, and distance between the lamp and treated surfaces should be up to 5 cm, in order to test if the declared values match the factual values, the selected times were 10 s, 20 s and 30 s, at the distance of 5 cm from the plates, and 30 s for the distances of 3 cm, 5 cm and 6 cm.

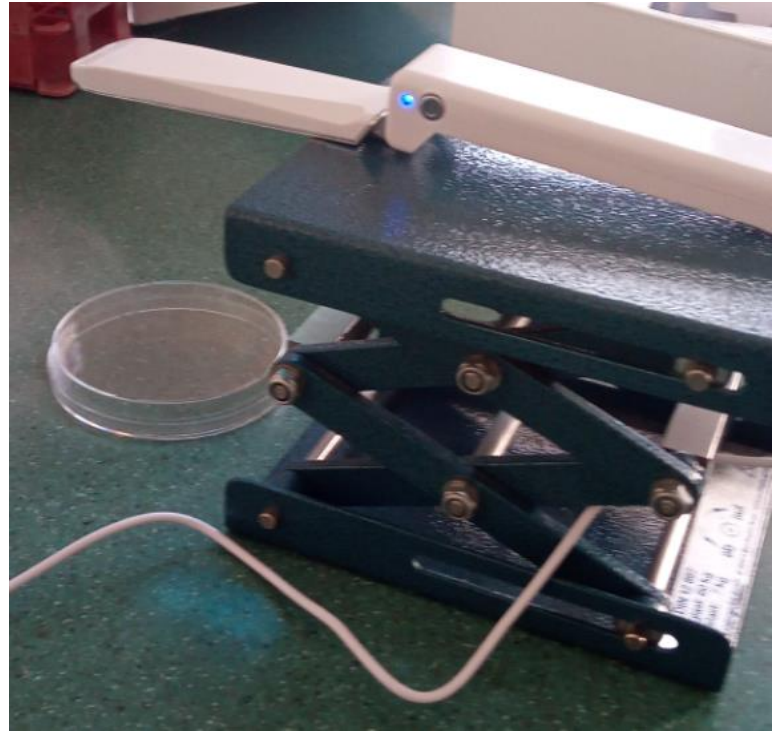
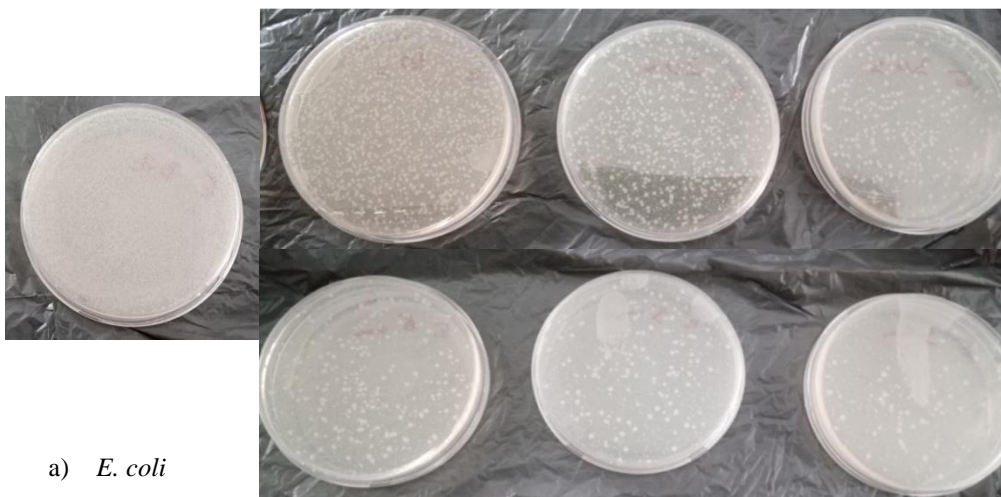


Figure 1. Exposure setup with the horizontally placed lamp

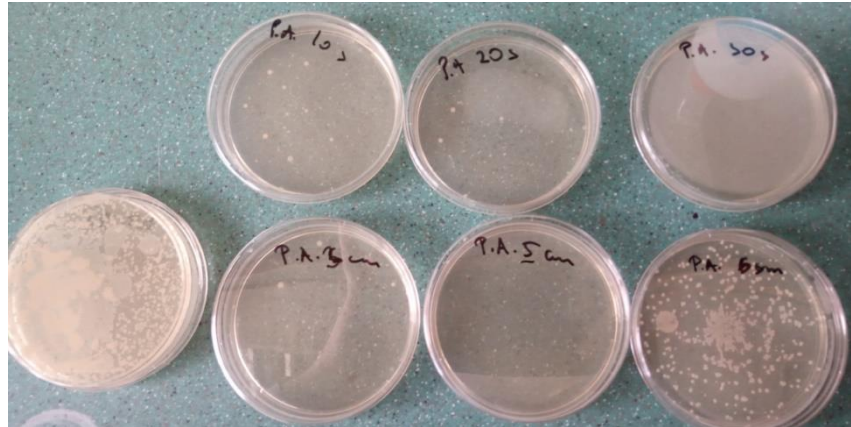
3. Results and discussion

After the plates were removed from the incubator and were left to replicate for 24 hours, photographs were obtained. Two experimenters independently counted colony forming units (CFU) on each plate.

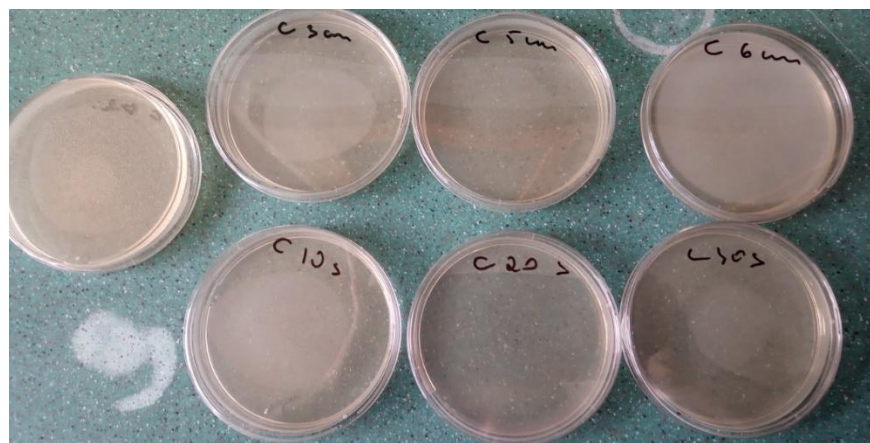
The three-part Figure 2. shows the appearance of the control and the exposed plates at selected exposure times and distances for *E-coli* a), *Pseudomonas aeruginosa* b), as well as *Candida albicans* c). In all three cases, the effect of the UV-C exposure is visible with the naked eye.



a) *E. coli*



b) *P. aeruginosa*



c) *Candida albicans*

Figure 2. The appearance of control and exposed plates for the selected exposure times and distances

The table 1. gives the count for each of the treated microbe plates under above defined conditions. For the untreated plates, since the count was greater than 300 CFUs per plate, the plates were visually divided into 8 parts, counted per each section and the counts was added up. The count for the untreated *E. coli* was over 2000, for *Pseudomonas aeruginosa* – over 1120, and for *Candida albicans* – over 3200 per plate.

Table 1. The count of the CFU per plate for the UV-C treated

<i>E. coli</i> – control over 2000 CFU		
3 cm (30 s)	200	90%
5 cm	360	82%
6 cm	400	80 %
10 s (5 cm)	560	72 %
20 s	520	74 %
30 s	480	76 %
<i>Pseudomonas aeruginosa</i> –control over 1120 CFU		
3 cm (30 s)	30	97%
5 cm	40	96%
6 cm	240	81 %
10 s (5 cm)	150	86 %
20 s	80	92 %
30 s	40	96 %

Candida albicans – control over 3200 CFU

3 cm (30 s)	50	98%
5 cm	80	97%
6 cm	100	96 %
10 s (5 cm)	200	94 %
20 s	50	96 %
30 s	40	97 %

The presented research results show that, the relative effectiveness of disinfection for the same conditions varies for the type of microbe. For *E-coli* it was between 72% and 90%, compared to the reported previous studies of 85%¹. For *Pseudomonas aeruginosa* it was between 81% and 97%, while for the *Candida albicans* it went from 93% to 98%. Furthermore, the dependence of the relative effectiveness of disinfection on the time of exposure, was the highest for 30 s (between 72% and 94 %), and for the lamp distance of 3 cm (between 90% and 98 %), which was expected considering the inverse square law.

It is necessary to point out that the bacteria *Pseudomonas aeruginosa* showed good susceptibility to the UV radiation, which is very important since this pneumonia causing bacteria is showing increasing signs of resistance to the antibiotics⁶ and the use of UV-C radiation can be an alternative way of its elimination in unoccupied spaces and on surfaces.

The relative effectiveness of disinfection was the highest for the treated fungus, which is in the discrepancy with known studies¹, for which the researchers do not have a technical explanation.

Even though there is a public skepticism about how effective disinfection can be realized with hand-held, compact GUV lamps¹, the study performed showed that, if the manufactures instructions are followed, the declared disinfection can be achieved.

4. Conclusion

Due to the coronavirus pandemic, GUV is likely to continue to develop in technology and application, UV-C emerging technologies should be studied to better understand these relationships. Since the time required for disinfection can be critical, it is important to understand the germicidal efficacy of high-dose, high-intensity, short-duration UV-C application.

The presented research results show that, in addition to the dependence of the efficacy on the time of exposure and surface distance from the lamp, the relative effectiveness of disinfection for the same conditions varies for the type of microbe. Also, a limitation of the study is that the researchers tested only at a small range of intensities depended on the distance between the lamps and the target surface and exposure times, and future research studies need to confirm these conclusions over a wider range of intensities tested.

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