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Tolerance Increase in *Escherichia coli* O157:H7 and Methicillin-Resistant *Staphylococcus Aureus* USA300 Exposed to Low-Power Continuous Ultraviolet Radiation from Narrow-Wavelength Sources

Saeed Keshani Langroodi[1]; Christopher M. Sales[2]; Chetan Jinadatha[3]; and Gregory Fridman[4]

Abstract: *Escherichia coli* O157:H7 is a major cause of foodborne disease outbreaks throughout the world, while methicillin-resistant *Staphylococcus aureus* (MRSA) is responsible for many difficult-to-treat infections in humans. Ultraviolet (UV) irradiation is commonly used for disinfection in food processing, medical facilities, and water treatment to prevent the transmission of these pathogen. With increased use of UV disinfection technologies over the last few years because of COVID-19 and concerns about other communicable disease, it has become a concern that microbial species could develop tolerance to UV irradiation, especially when it is applied continuously. To elucidate the effect of continuous UV exposure at different wavelengths and power levels on the tolerance development of bacteria, *Escherichia coli* O157:H7 and MRSA)USA300 growths were investigated by continuously exposing inoculated agar plates to six different commercially available UV sources at wavelengths of 222 nm, 254 nm, 275 nm, and 405 nm. The agar plates in these experiments were partially covered by a thin acrylic sheet, which provided either complete protection from the UV to the cells directly under the sheet, no protection if significantly away from the sheet, or partial protection near the edges of the sheet due to shading or small amounts of UV reflection under the sheet at the edges. In these experiments, tolerant cells of *E. coli* and *S. aureus* were found from the 222 nm, the 405 nm, and one of the 254 nm sources. Upon examination of the power of each UV source, it was shown that the 275 nm and 254 nm sources that resulted in no tolerant cells had surface power densities [at 25 cm (10 in.)] that were more than 10–200 times greater than those that had tolerant cells. These results suggests that bacterial cells have a higher chance to develop UV tolerance under lower power UV sources (under the experimental conditions in our laboratory). Genome investigation of the tolerant colonies revealed that there are no significant differences between the cells that developed tolerance and the original organism, hinting at the need to explore the role of epigenetics mechanisms in the development of UV tolerance in these bacteria. **DOI: 10.1061/JOEEDU.EEENG-7182.** *© 2023 American Society of Civil Engineers.*

Introduction

Bacterial resistance against ultraviolet (UV) radiation has been investigated in various studies, including from spacecraft associated spores (Osman et al. 2008), clean room environments (La Duc et al. 2007), and natural environments such as Antarctica and higher altitude environments (Fernández Zenoff et al. 2006; Dib et al. 2008; Yang et al. 2008; Marizcurrena et al. 2017). UV-resistant bacteria that naturally inhabit higher altitudes—the UV exposure is more severe due to the thinner atmospheric layer—were subject of several studies. Yang et al. (2008) studied bacterial isolates obtained from the upper atmosphere, and compared the UV exposure survival rate of those isolates to surface airborne isolates. The results indicated that upper atmospheric isolates have better tolerance to UV radiation than surface isolates. UV tolerance of bacterial isolates from extreme environments in the high altitudes of the Andean wetlands have been investigated by Dib et al. 2008, who exposed two isolates obtained from Andean wetlands to UV-B for 30 and 60 min. They found no decrease in the survival rate after exposure; indeed, the survival rate was enhanced with increased UV doses. In a similar study by Fernández Zenoff et al. (2006) the survival rate of UV resistant bacteria obtained from high altitude Andean lakes was compared to other bacterial isolates from lower altitudes. The results suggest that the UV-B exposure survival rate of bacteria was correlated to their natural environment; in particular, those isolates that inhabited a higher UV irradiation environment had higher survival rates. These studies imply that microorganisms may be able to develop tolerance to prolonged exposure to UV.

Continuous ultraviolet radiation is widely used for pathogen inactivation in medical facilities, food processing, water treatment, etc. (Gómez-López 2012; Rossitto et al. 2012; Bustos et al. 2014; Gayán et al. 2014; Luo et al. 2014; Sholtes and Linden 2019). The authors previously anecdotally observed UV-tolerant colonies persisting even with longer UV treatments, and decided to investigate this further. In this study we have selected two organisms: *Escherichia coli* O157:H7, and methicillin-resistant *Staphylococcus aureus* (MRSA) USA300. The first is a pathogen responsible for several recent food safety concerns, and has been discussed in food

[1]Bio Lab Director, AAPlasma LLC, 1325 S 33rd St., Philadelphia, PA 19146. Email: saeed@aaplasma.com

[2]Associate Professor, Dept. of Civil, Architectural, and Environmental Engineering, Drexel Univ., 3141 Chestnut St., Philadelphia, PA 19104. Email: cms566@drexel.edu

[3]Chief of Infectious Diseases Section, Central Texas Veterans Health Care System, 1901 Veterans Memorial Dr., Temple, TX 76504. Email: Chetan.Jinadatha@va.gov

[4]CEO, AAPlasma LLC, 1325 S 33rd St., Philadelphia, PA 19146 (corresponding author). ORCID: https://orcid.org/0000-0003-4006-4722. Email: greg@aaplasma.com

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safety literature for decades (Doyle 1991; Lim et al. 2010; Rani et al. 2021). The second is a well-known challenge for medical professionals and is responsible for a number of infections acquired in hospitals (Kallen et al. 2010; Köck et al. 2010; Stefani et al. 2012; Kourtis et al. 2019). For the UV sources, we have chosen those that are employed for continuous use: 222, 254, 275, and 405 nm.

We caution the reader to view these results as preliminary evidence of potential tolerance development in pathogens when UV sources are used continuously in a facility.

Materials and Methods

In the exposure tests, the agar plates were prepared by pouring 50 mL of melted Luria-Bertani (LB) medium agar into 100 mm × 15 mm plastic petri dishes. *E. coli* and MRSA were grown in the LB broth at 37°C for 24 h. Then, the LB agar plates were inoculated with 500 μL*E. coli* or MSRA, and air dried in a biological safety cabinet for 30 min. Black acrylic slides [25.4 mm × 50.8 mm × 1.6 mm (1 in. × 2 in. × 1/16 in.)] were disinfected using 70%/30% ethanol/water. Then, one acrylic slide was placed at the center of each inoculated agar plate (Fig. 1). In this way, the organisms outside of the slide were exposed to UV while those underneath the slide were not. The *Escherichia coli* O157:H7 samples were generously provided to us by Prof. N. Nitin, UC Davis. The MRSA USA 300 (*Staphylococcus aureus* subsp. *aureus* Rosenbach BAA-1717) was purchased from American Type Culture Collection (ATCC #BAA-1717, Manassas, Virginia) in freeze-dried form and was grown in the lab following the ATCC instructions.

Five different UV sources (Table 1) were used to investigate possible UV tolerance development. The sources were selected based on their availability and their claims for continuous use. All sources were purchased by the authors. No funding or incentives were provided by the vendors. Table 1 lists the names we have given these sources throughout this paper. Inoculated agar dishes, without lids, were arranged in the chamber and incubated at 37°C, 25 cm (10 in.) below the UV source (Fig. 1). The moisture of the incubator was maintained at a high level by placing water trays in the chamber. The bacterial growth on the plates was monitored daily. The tolerant colonies—colonies that were visible on the plate after at least four days of UV exposure—were collected, resuspended in 7 mL of LB media in a 15 mL centrifuge tube, and incubated at 37°C overnight.

Next, the tolerance was confirmed by exposing the tolerant colonies to the matching UV sources without the acrylic cover. LB agar plates were inoculated with 500 μL of tolerant *E. coli* or *S. aureus*, air dried in the biological safety cabinet for 30 min, then incubated as previously described, without acrylic cover and lids, under the matching UV source. The bacterial growth on the plates was monitored daily. The tolerant colonies were collected, resuspended in 7 mL LB media in a 15 mL centrifuge tube, and incubated at 37°C overnight. Bacterial cells were separated from the media by centrifuging for five minutes at 5,000 rpm. The bacterial cells were then resuspended in 50% LB media, 25% glycerol, and 25% deionized water, and stored at −20°C.

To better understand if the tolerant colonies had any underlying genetic structural change that could explain the tolerance, *E. coli* isolates exposed to various UV sources and controls were subjected

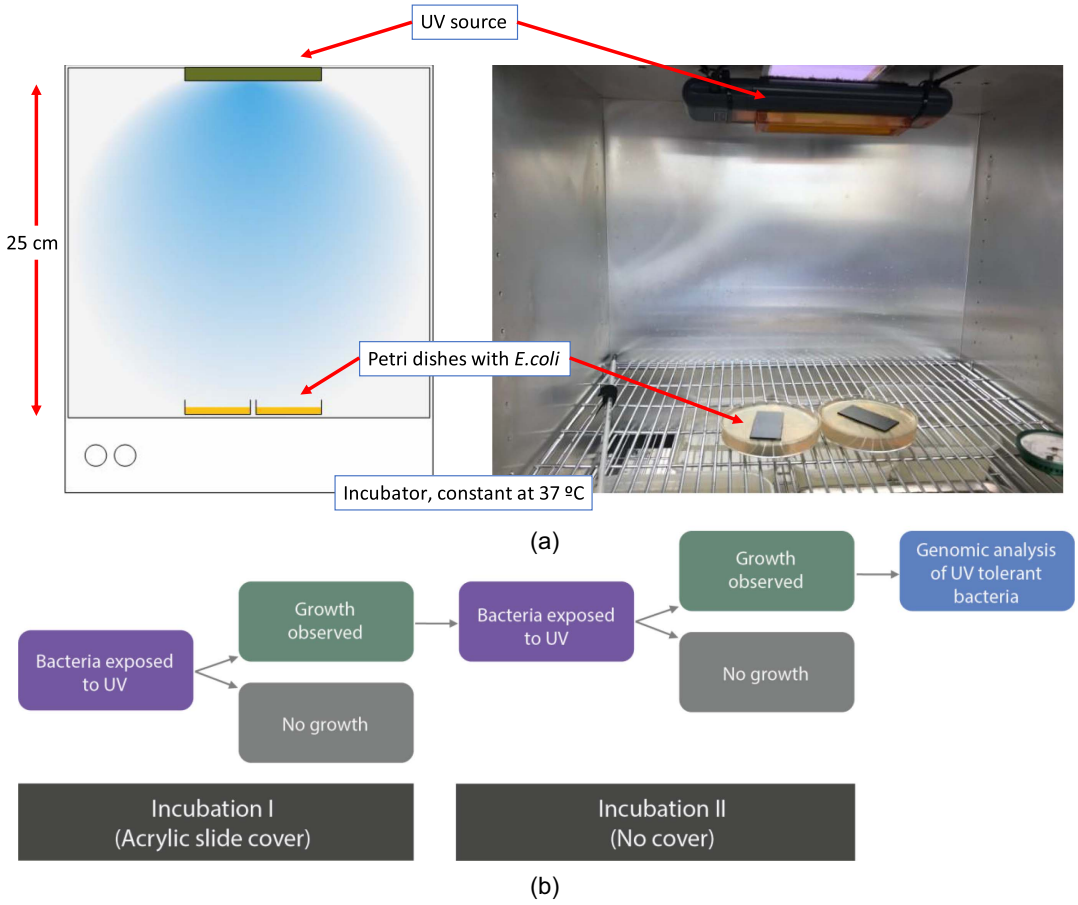


Fig. 1. (a) UV tolerance evaluation experimental setup; and (b) flowchart.

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Table 1. List of different UV sources and their wavelengths

UV source name used here	Wavelength (nm)	Manufacturer
222 Excimer	222	Guangdong Excimer Optoelectronic Technology Co Model: F28-20W DC24V (Heshan City, Jiangmeng, Guangdong Province, China)
254 Biolux	254	Atlantic Ultraviolet Corp Biolux 4 Watt, Model #40-1105 (Hauppauge, New York)
254 MiniMAX	254	Spectroline MiniMAX UV-5GL (Melville, New York)
275 LED	275	Irtronix Model #MD1021-2 2.6W 275nm (9 units of CMD-FSC-COGA) module with PWM dimmable driver (Torrance, California)
405 Black light	405	60 W UV Flood Black Light (Shenzhen, Guangdong Province, China)

to whole genome sequencing, as described in Choi et al. (2022). Whole-genome single nucleotide polymorphism (wgSNP) analysis was conducted using the BioNumerics version 7.6 platform (Applied Maths NV, Sint-Martens-Latem, Belgium). Sequence variation was evaluated by comparing the sequences to the reference genome sequence of *E. coli* and with each other after alignment.

In this experiment, the power of the UV sources was measured 25 cm (10 in.) away from the source using a UV light meter (International Light Technologies Model #ILT770, Peabody, Massachusetts). Optical emission of the UV sources was performed similarly, using an Avantes optical emission spectrometer (OES) (Model #AvaSpec-ULS2048CL-RS-EVO-UA) with a 1 meter optical fiber (Model #FC-UVIR400-1, Apeldoorn, Netherlands).

Results

Tolerance Development

This study aimed to investigate the effect of different UV sources on tolerance development of *Escherichia coli* O157:H7 and MRSA USA300. The results are summarized in Table 2. Fig. 2 shows photographs of the Petri dishes at the time the experiment was stopped, either due to observed growth or due to significant damage and warping of the agar and with no observed growth. After growth was observed around the acrylic plate, the tolerant colonies were collected, inoculated in LB, and grown in the incubator at 37°C. To examine the tolerance of collected colonies, their growth was tested on uncovered LB agar under UV light. Fig. 3 shows the photographs of the growth observed under continuous UV exposure in 222 excimer, 254 MiniMAX, and 405 black light. Scattered *E. coli* O157:H7 and MRSA USA300 colonies were observed on LB agar plates exposed to 222 excimer and 254 MiniMAX, while the agar plates were covered with *E. coli* O157:H7 and MRSA USA300 overnight, for those plates exposed to 405 black light.

Genomic Sequencing

Allele differences between the samples were observed (Fig. 4); however, further analysis showed that there was no genetic (SNP) differences between the unexposed and the tolerant *E. coli*, indicating no genetic changes in the organisms that could explain the observed tolerance. It has been suggested that the resistance could be because of some random events, such as cell shielding by aggregation (Muñoz et al. 2021; Choi et al. 2022). Another explanation could be epigenetic mechanisms. Epigenetic inheritance is the transmission of nongenetic material such as methylation patterns or cytoplasmic components that is inherited alongside genetic traits. This could be more pronounced in prokaryotes, because biomolecular components of the parental cells are passed down to their offspring through their cell binary fission reproduction methods. The role of epigenetic mechanisms in antibiotic resistance has been addressed in several works when the antibiotic resistance could not be explained by gene modifications (Kester and Fortune 2014; Day 2016; Sandoval-Motta and Aldana 2016; Ghosh et al. 2020). Accordingly, because there are no genetic changes observed in the UV tolerant colonies in our study, we can suggest the role of epigenetic regulations in UV tolerance development.

UV Power and Optical Emission Measurement

The UV surface power density meter and the collimating lens with the optical fiber were positioned 25 cm (10 in.) away from the UV source using a 3D-printed holder (Fig. 5). After the UV surface power density meter was zeroed, we turned on the UV source and allowed it to warm up for five minutes. The measurement was then recorded (Table 3).

Fig. 6 shows the optical emission spectra obtained from all the sources. Generally, the title of the UV source corresponds well with its main emission peak; however, other emission peaks can also be observed. For example, the 222 excimer source shows a couple of peaks above 222 nm, which may contribute to inactivation and are sometimes filtered out in other 222 nm sources (Wang et al. 2010; Narita et al. 2018; Kitagawa et al. 2021). We point out this slight discrepancy because we are analyzing tolerance development and should, in the future, consider slight differences between the UV sources claiming a single main wavelength in their marketing materials.

Muñoz et al. (2021) reported that single versus split exposure of the same UV power could affect the germicidal properties of the UV sources. They exposed *E. coli* to the same UV source and UV doses, but with a single versus split exposure pattern. The results showed that UV dose fractionation reduced the number of survived colonies comparing to a single dose application of UV. In our case, the treatment was continuous.

Table 2. Summary of the observed E. coli O157:H7 and S. aureus MRSA USA300 growth while continuously exposed to different UV sources during incubation at 37°C

UV source name	Observed growth in *E. coli* O157:H7	Observed growth in MRSA USA300
222 Excimer	Tolerant colonies observed after 2 days	Tolerant colonies observed after 2 days
254 Biolux	No growth after 4 days	No growth after 5 days
254 MiniMAX	Tolerant colonies observed after 5 days	Tolerant colonies observed after 4 days
275 LED	No growth after 7 days	No growth after 4 days
405 Black light	Plates covered with colonies overnight	Plates covered with colonies overnight

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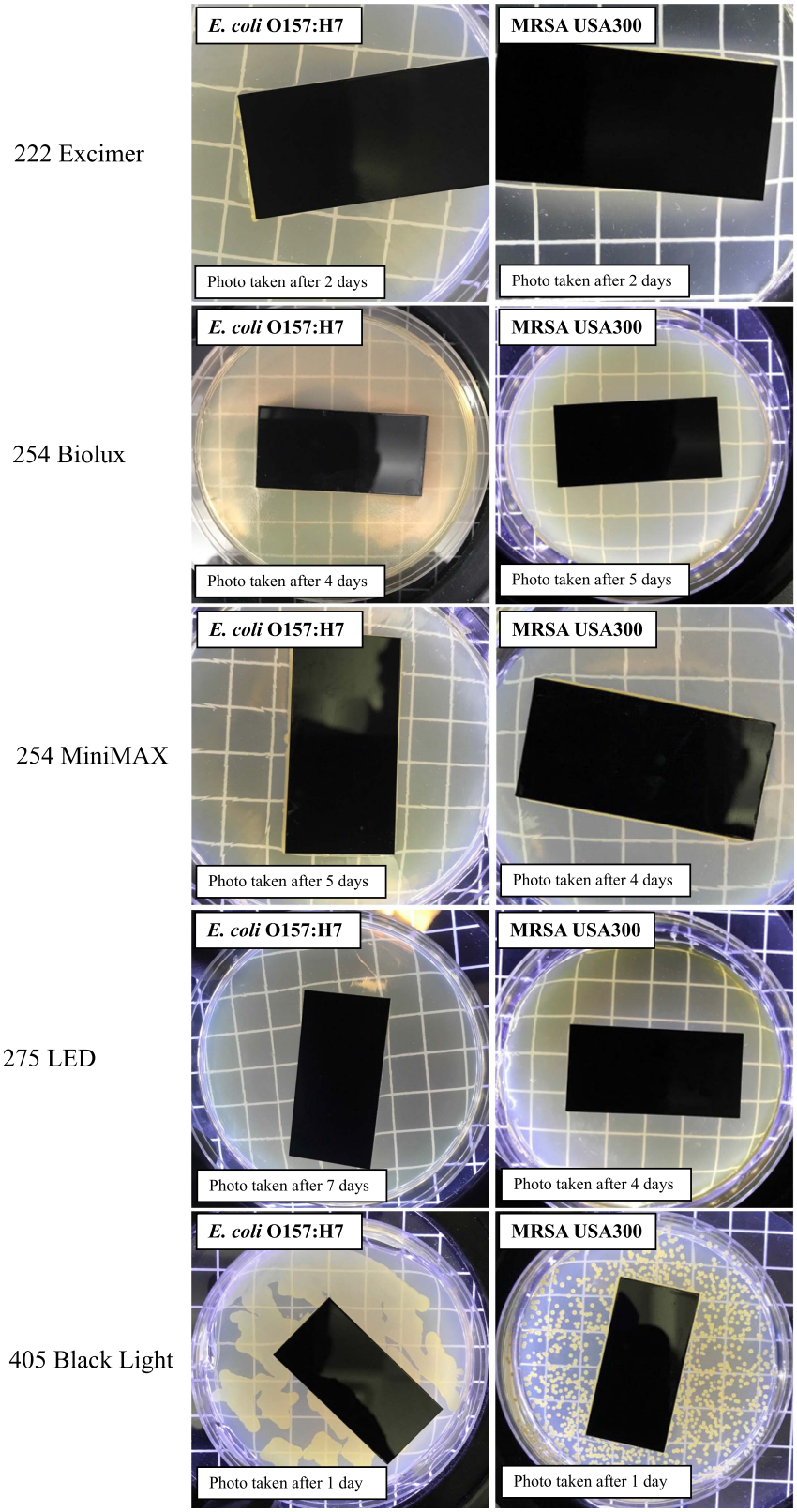


Fig. 2. Photographs of the Petri dishes at the final stages of growth.

Discussion and Conclusion

The results indicate that both *E. coli* O157:H7 and MRSA USA300 respond similarly to different UV irradiations. This could be explained by the specifications of the UV sources (Table 4). The UV sources that fall in the range of short-wavelength UV (200–280 nm) are well-known for their germicide ability. However, in our study, UV tolerance developed in bacteria exposed to two of the UV lights. Table 3 indicates that 254 Biolux and 275 LED have the highest power, wherein there was not any growth observed.

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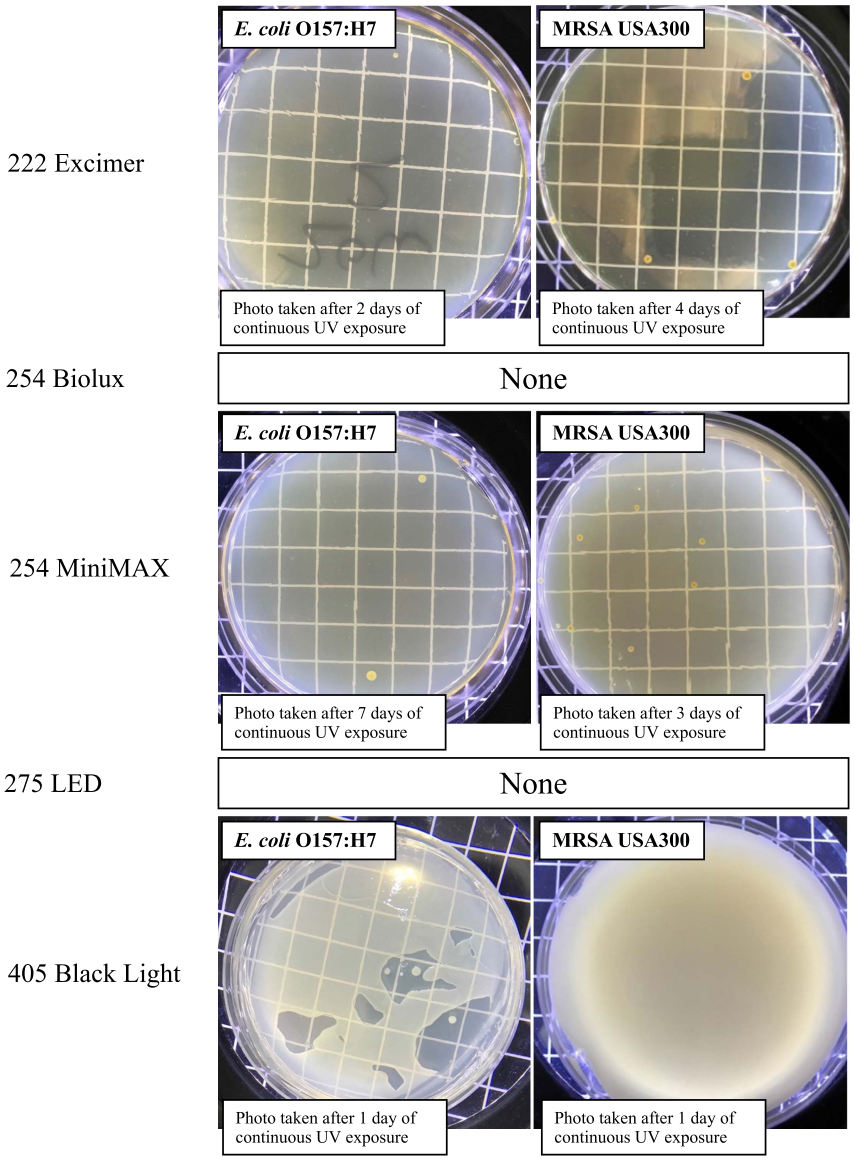


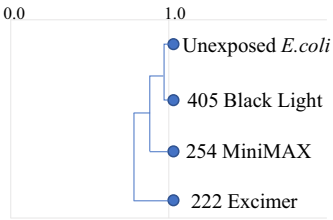
Fig. 3. Photographs of the Petri dishes at the final stages of growth of the tolerant colonies.

Minimum spanning tree

Sample ID	Sample ID	Allele Differences
405 Black Light	Unexposed *E.coli*	0
254 MiniMAX	Unexposed *E.coli*	1
222 Excimer	Unexposed *E.coli*	3

(a)

Dendrogram



(b)

Fig. 4. (a) Minimum spanning tree table; and (b) dendrogram comparing the allele difference of the sequences of genomes exposed to UV sources versus unexposed *E.coli*.

In 254 MiniMAX and 222 excimer, which are almost at the same power range (12.3 $\mu W/cm^2$ and 7.3 $\mu W/cm^2$, respectively), scattered colonies were observed after a few days of incubation. Colonies covered the agar plates overnight for the 405 black light. The 405 black light is the only light in this study that is in the range of UV-A (315–400 nm), and it has the lowest power density (0.532 $\mu W/cm^2$) among our sources. Here, again, the reader should be cautioned: There are commercial devices that utilize this

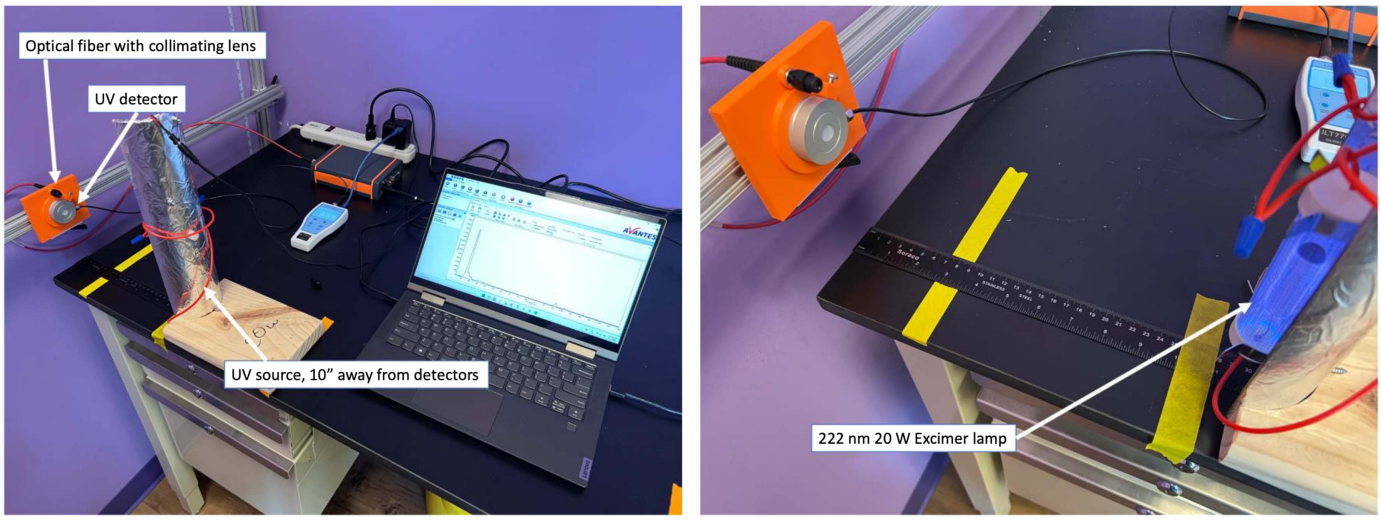


Fig. 5. Photograph of the optical emission and surface power density measurement setup [(a) entire setup; and (b) close-up].

Table 3. UV surface power density at 25 cm (10 in.) from the source

UV source	Power
222 Excimer	7.3 μW/cm^2
254 Biolux	137 μW/cm^2
254 MiniMAX	12.3 μW/cm^2
275 LED	110 μW/cm^2
405 Black light	0.532 μW/cm^2

wavelength for water disinfection, for example, and there are studies claiming its effectiveness (McDonald et al. 2013; Maclean et al. 2014; Boyce 2016).

UV irradiation can lead to mutations that in turn can induce tolerance or resistance (Shibai et al. 2017). In our experiments, there were no genetic differences observed at the base pair level to explain the tolerance or predict the emergence of future resistance. Similarly, Aichi et al. (2001) aimed to determine the UV resistance

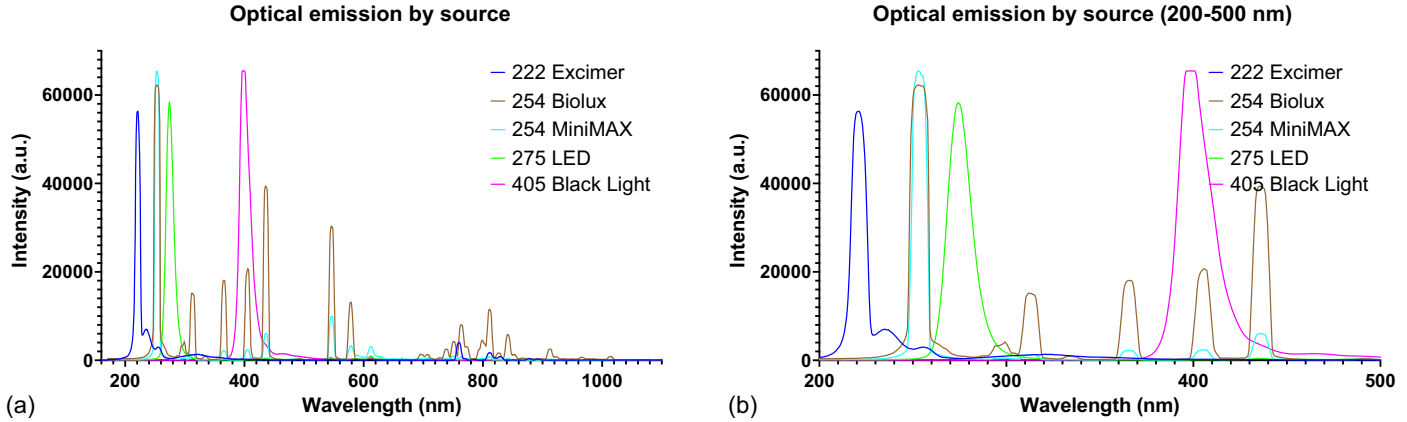


Fig. 6. Optical emission from all UV sources [(a) 160–1,100 nm; and (b) 200–500 nm].

Table 4. Summary of the results of the selected UV sources on E. coli O157:H7 and MRSA USA300 growth

UV source	Wavelength (nm)	UV type	Power	Summary
222 Excimer	222	UV-C	7.3 μW/cm^2	Tolerance demonstrated
254 Biolux	254	UV-C	137 μW/cm^2	No tolerance observed
254 MiniMAX	254	UV-C	12.3 μW/cm^2	Tolerance demonstrated
275 LED	275	UV-C	110 μW/cm^2	No tolerance observed
405 Black light	405	UV-A	0.532 μW/cm^2	No germicidal effect

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development of multidrug-resistant bacteria after serial UV exposure. However, whole genome sequencing (WGS) analysis of the exposed bacteria indicated that there was no UV resistance after 25 UV exposure cycles. They suggested that the survival of some colonies could be due to cell shielding by aggregation or epigenetic inheritance of some phenotypes.

Clearly, a more detailed and in-depth analysis of tolerance development to UV irradiation in pathogens should be performed. The results presented in this paper are meant as an initial indication of this possibility and we caution readers to be careful in selecting the appropriate disinfection source for their particular application, and to monitor the performance of this source over time.

Data Availability Statement

All data, models, or code that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgments

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