Journal of
Emerging Investigators

The Effects of Ultraviolet Light on *Escherichia coli*

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Summary

Ultraviolet (UV) radiation is known to inhibit cell growth and induce gene damage (1). For these reasons, UV radiation is used as a method to sterilize surgical instruments because it kills the bacteria present and disrupts bacterial reproduction (2). Infections acquired from hospitals, particularly post-surgical infections, have become increasingly common, and require the use of UV disinfection systems. In this study, we investigated the effect of UV light on *Escherichia coli* (*E. coli*). Specifically, this study explored the effects of the small UV lights currently used in school laboratories, in an attempt to extend UV radiation methods to common households. We used the number of colony-forming units (CFUs) to determine whether or not the UV light increases or decreases cell growth. *E. coli* were exposed to UV light with a wavelength of 254 nm. The number of CFUs under control and UV-exposed conditions were measured after 24 and 48 hours. We observed that UV light exposure at 254 nm from a small school laboratory light inhibits bacterial growth.

Received: December 8, 2014; **Accepted:** May 4, 2015;
Published: September 7, 2015

Introduction

Ultraviolet (UV) light is the portion of the electromagnetic spectrum between visible light and x-rays, with a wavelength of 100 to 400 nm. Sources of UV radiation include the sun, lasers, tanning beds, and numerous medical instruments such as dental polymerizing equipment (4). In medical operating rooms, UV radiation is a method of infection control. The UV light is used to sterilize both operating rooms and surgical instruments, which reduces the risk of surgical wound contamination and postsurgical infections. According to a study conducted by Duke University Medical Center and the University of North Carolina Health Care, in which 229 environmental surfaces were sampled from the rooms of 39 patients over a 15-month period, infection rates in medical environments were reduced from 10% to 0.24% using a UV-C lamp (5). In comparison to the lamp used in the experiment, the wavelength of the UV-C lamp implemented by Duke University is capable of stronger penetration of bacteria. Therefore, the effect of UV-C radiation would be greater than that of the UV lamp used in this experiment. UV-C radiation is emitted by the sun and is typically blocked by the atmosphere, particularly the ozone.

Extended exposure to UV radiation can alter the genetic material of a cell, leading to unfavorable mutations and even cell death. Typically, the shorter the UV wavelength, the greater the damage to an organism (6). From a molecular standpoint, cell death could be due to the fusion of thymine bases that are located next to each other (7). This new bond between two of the same nitrogenous bases negatively impacts the structure and overall shape of the DNA molecule, subsequently altering the genetic material of the cell. These cells are unable to reproduce because the dimerization prevents proper DNA replication (8). For this reason, UV radiation is constantly used in medical environments to sterilize instruments and machinery because of its ability to disrupt bacterial division (2).

E. coli, as shown in **Figure 1**, can be used as a simple model system for the effects of UV irradiation. This is due to its considerably smaller genome size of 4,400 genes, compared to the 30,000 genes found in a human cell. Bacteria have a single circular chromosome, with occasional plasmids, rather than multiple chromosomes like a human cell. Replication in bacteria occurs at a faster rate than the replication in human cells, which need to replicate multiple large chromosomes. *E. coli*'s ability to reproduce at a rapid rate makes it an ideal choice for experimentation (9). The K-12 strain of *E. coli*, specifically, is ideal because it is non-pathogenic and can be grown without the use of an incubator (10).

In this study, we investigated the phenotypic changes of living cells in response to a short wavelength of UV light (254 nm). The study mimics the UV sterilization methods of a hospital or other medical environments. *E. coli* (K-12 strain) was used as a model system. We collected data including the number of colony-forming units (CFUs) present in plates exposed to UV light compared to the number of CFUs in the control plate. Although similar

Figure 1: Inoculation of agar plates with *E. coli*.

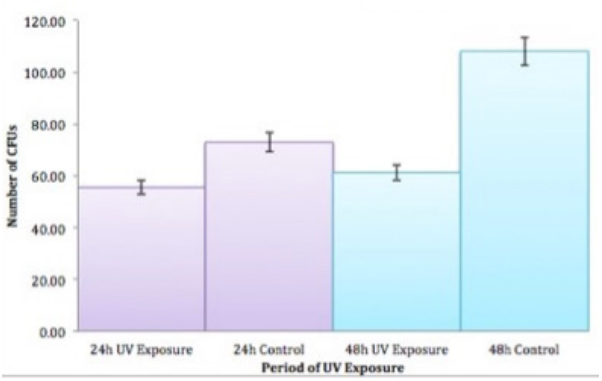

Figure 2: Average number of *E. coli* CFU's for varying UV exposure times. Error bars represent the standard deviation across three trials.

experiments have already been documented, this study specifically investigated the effects of small UV lights currently used in laboratories at schools. The small UV lights were used in an attempt to extend UV radiation methods to common households.

Results

A total of three trials were completed. Each trial consisted of four petri dishes inoculated with *E. coli* using an isolated streak from a starter plate of healthy bacteria: a plate exposed to UV for 24 hours, a plate exposed to UV for 48 hours, a 24 hour control plate, and a 48 hour control plate. The control ensured that the bacteria used throughout the experiment were alive and that no other factors affected the reproduction of the *E. coli*. For each trial of UV exposure, we used a new control to compare the number of CFUs present. The two independent variables were UV radiation and the time of exposure. The results are shown in **Figure 2**.

After three trials, it was evident that the number of CFUs in the UV-exposed petri dish was less than the number of CFUs in the control, as shown in (**Figure 3**). The results revealed that the UV exposure did decrease the number of bacterial colonies formed. In fact, there were fewer CFUs in the petri dishes exposed to UV for 48 hours than in those exposed for 24 hours. The longer the duration of exposure resulted in a lower amount of CFU's.

Discussion

In this experiment, the effects of UV radiation on *E. coli* strain K-12 were investigated. We compared the number of CFUs on plates exposed to UV for 24 and 48 hours to that of the control plates. We predicted that there would be fewer CFUs of *E. coli* on the plates exposed to UV light for varying amounts of time compared to control plate.s The following conclusions were drawn from this experiment:

1. UV radiation inhibited the growth of the *E. coli* K-12 strain and possibly killed the bacteria.
2. The growth patterns reveal that the longer the period of UV exposure, the greater the decrease in the number of CFUs.
3) UV laboratory lights of short wavelengths are capable of inhibiting bacterial growth.

The results of the experiment support the initial hypothesis: a small UV light commonly found in high school laboratories is capable of reducing bacterial growth.

The number of CFUs on the plates exposed to UV for 24 and 48 hours was substantially lower than the number of CFUs on the control plates. As shown in **Figure 2**, there were fewer CFUs on the plates exposed to UV light, indicating that the UV radiation suppressed the development and reproduction of *E. coli* cells. The inactivation of the bacterium is likely a result of mutated genetic information induced by UV exposure. In a medical environment, UV light at a wavelength between 200 and 320 nm is used for sterilization (13). Laboratories utilize UV-C lamps for germicidal irradiation (13). As a result, the wavelength is stronger and more penetrable than the high school laboratory lamp used in this experiment. The 254 nm wavelength UV lamp used in this experiment was small in terms of radiation coverage, but still reduced bacterial cell growth. Thus, it is evident that high school laboratory lamps have the ability and potential to disinfect.

Several research studies have investigated the effectiveness of UV sterilization. A study conducted by the Pacific Northwest National Laboratory found that UV radiation has the potential to sterilize tools, which can then be surgically implanted into fish to observe their bodily functions and behavior (12). Multiple experiments have been conducted in which surgical tools were contaminated with and exposed to varying concentrations of bacteria and then exposed to UV radiation (15). This was done to determine the effectiveness of UV sterilization. Based on the data gathered, the researchers deduced that UV sterilization is effective at killing many strains of bacteria in a short period of time. This makes UV sterilization ideal for implementing into surgical procedures. Researchers have also conducted experiments testing the effects of specific doses of UV radiation on the survival of *E. coli* (12). Researchers showed that UV exposure reduced the number of CFUs and that the dose of UV necessary to reduce the amount of CFUs increases exponentially as the wavelengths increases. These findings, namely that the UV reduces the number of CFUs of *E. coli*, support the results of this experiment.

It should be noted that there were several limitations to this experiment. First, only three trials were conducted for each of the UV exposure times, thereby limiting statistical analysis to standard deviation. The standard deviation as shown in **Figure 2**, is small, indicating there was little variation between each trial. The number of CFU's that formed during each trial was fairly consistent. There were no outliers observed during this experiment. The extent of variation with regards to the mean of the CFUs is small which proves that the reliable data was collected. To improve this experiment, the number of trials should be increased. More data points should be collected, over larger periods of time, such as 12, 36, 60,

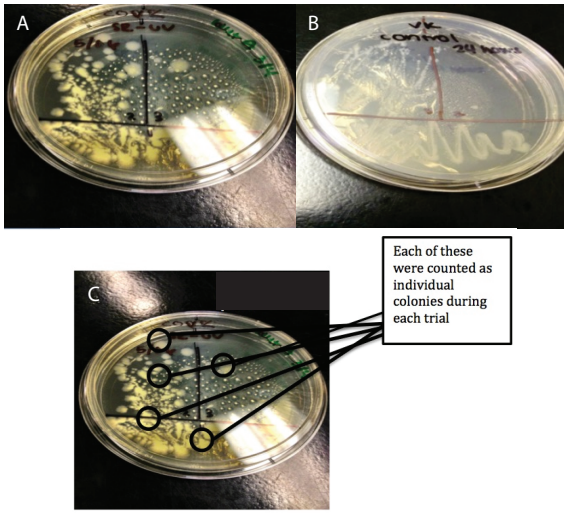

Figure 3: A) Bacterial growth for trial 2 in which the plate was exposed to 254 nm for 24 hours. 55 CFUs were present. B) Bacterial growth for trial 2, in which the plate remained untreated for 24 hours. 67 CFUs were present. C) The method by which the CFUs were identified and counted for data collection.

and 72 hours to help determine what effect, if any, UV light might have on *E. coli* growth over time. Furthermore, the high school laboratory ultraviolet lamp utilized in this experiment can be useful in further experimentation. To confirm the hypothesis that the decreased *E. coli* growth in this experiment was due to UV-induced genetic mutations, genetic analysis via the Sanger sequencing method could be performed. Future work also includes exposing human cells to the UV lamp, instead of bacteria, and observing the effects. Additionally, we plan to experiment with varying wavelengths of UV radiation. The information gathered from this experiment will help to determine which wavelength is most detrimental to a cell. A spectrophotometer could be used to develop a growth curve that can display the relationship between the number of colonies and the absorbance over time. Since it is evident that high school laboratory lamps have the ability to disinfect, by extending experimentation using the high school laboratory UV lamp, extensive data can be collected on the lamp's effectiveness. Specifically, we can investigate small-scale laboratory lamps can be for their precision in reducing the number of bacteria present over time and disrupting bacterial reproduction, and by varying periods of exposure; the amount of time needed to disinfect an area completely can be determined. This concept of small-scale, UV-radiation-based sanitation systems can be applied to households as well. Harmless to humans with the proper protection, UV radiation could rid homes of bacteria, viruses, and other contaminants (14).

Methods

In order to streak a bacterial culture, it is essential to have agar containing all the nutrients required for the bacterium to thrive. The liquid broth agar was heated for 1 minute before it began to boil. It was heated for an additional 30 seconds to ensure that no solid pieces of agar remained in the bottle. We poured the agar quickly into each of the 7 petri dishes, enough to cover the bottom of the plate. To prevent contamination from the air, the petri dishes were closed with a lid and left to cool into a firm substance for approximately 1 hour (11). Initially, we created a "starter plate" to ensure that the *E. coli* K-12 strain was viable. First, an inoculation loop was sterilized by passing it through a Bunsen burner flame multiple times until the entire instrument was red-hot. Once the inoculation loop was cool, we dipped it into the test tube containing the *E. coli* liquid broth. Using the inoculation loop as a spreader, the liquid was spread across the agar plate evenly and gently so that the agar did not tear. Lastly, we placed the lid on the petri dish and stored upside down in an incubator at 37 degrees Celsius for 1 day. The petri dish was stored upside down so that condensation on the lid of the plate would not drip down and contaminate the specimen (11). Each experimental trial required 4 petri dishes. One was a control (for either 24 or 48 hours), labeled with "control", the date, the period of exposure (24 or 48 hours), and the experimenter's initials. The other was labeled with "UV-SR", the date, the period of exposure (24 or 48 hours), and the experimenter's initials. Additionally, for the isolation streak, the bottom of the petri dish was divided into 3 sections, and each section was labeled 1, 2, or 3, as shown in **Figure 1**. To create the isolation streak, we sterilized the inoculation loop and scraped a colony of *E. coli* off of the starter plate. The loop was streaked from one end of sector 1 to the other end of sector 1. We then sterilized the loop, streaked it back and forth between sectors 1 and 2, and re-sterilized it. Lastly, the loop was streaked back and forth between sectors 2 and 3 (11).

To test the effects of the UV light, the variable petri dish was placed exactly 6 inches beneath the UV lamp within a fume hood. The UV lamp was set to 254 nm. The control petri dish was placed within the fume hood, unexposed to the UV lamp.

Acknowledgments

Acknowledgement and gratitude are due to the following contributors: Laboratory supervisor, Dr. Meenakshi Bhattacharya for overseeing all experiments and. Dr. Sophia Gershman for providing the necessary materials to facilitate the research.

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