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The Effect of Increased Exposure of UVC Light on Human Skin Microbiota

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The Effect of Increased Exposure of UVC Light on Human Skin Microbiota

by

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Abstract

Many methods have been employed to prevent infections from opportunistic pathogens in immunocompromised individuals. Among these are the use of ultraviolet light (UV). In this study, UVC light, was found to have a deleterious effect on specific skin flora. Organisms tested included *Acinetobacter baumanii, Candida albicans, Candida kefyr, Corynebacterium renale, Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis,* and *Streptococcus pyogenes*. Nutrient agar was used to cultivate most organisms. Blood agar was used for the cultivation of *Streptococcus pyogenes* and *Enterococcus faecalis*. Bacterial suspensions were made and utilized to plate each organism onto a set of 7 nutrient agar or blood agar plates. A portion of each of 6 of the plates was then exposed to UVC light for 15, 30, 45, 60, 75, and 90 seconds respectively. For each organism, increased exposure to UVC light resulted in a decrease of the number of colony forming units observed in the portion of the plate that was exposed to the UVC light. These data suggest that UVC light acts as an efficient bactericidal agent. Results obtained in this study may lead to innovative uses for UVC light in the prevention of disease.

Key terms: microbiota, UVC light, opportunistic pathogens

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List of Abbreviations

ATCC American Type Culture Collection

Spp. Species

UV Ultraviolet

UVC Ultraviolet C

Chapter 1: Problem Statement

The skin is the body's largest organ and its primary role is to serve as a physical barrier to protect the body from foreign organisms and toxins. The skin is also able to form symbiotic relationships with external microorganisms due to different niches produced by folds and invaginations in the skin. Identifying and quantifying these microorganisms have many clinical impacts, considering that some of the microorganisms may become pathogenic when presented with a susceptible host.

Many methods have been employed in an effort to prevent susceptible individuals from contracting an infection from opportunistic pathogens. Some of these methods include endorsing hand-washing campaigns, using commercial disinfectants, and using antimicrobial agents. However, many bacteria are becoming resistant to commercial disinfectants and antimicrobial agents, which results in the proliferation of bacteria on inanimate objects. Due to this, other methods have been explored in an effort to combat the abundance of microorganisms, especially in health care environments. This has become such an important endeavor that an estimated $13.1 billion a year is spent on chemical and physical solutions to infection prevention in the health care setting (Jordan, 2015).

One method employed to decrease numbers of microorganisms includes the use of alcohol-based disinfectants. While some studies have shown that alcohol based disinfectants have proven to be beneficial in reducing both numbers and types of microorganisms, other research has shown that some microbial populations are enhanced through the use of disinfectants and that these alcohol-based disinfectants may damage host defenses.

A study with the purpose of determining the antimicrobial effect of 70% isopropyl alcohol on the normal flora of the hands found that the alcohol reduced the baseline bacterial counts by 84%, 93%, and 98% on days 1, 3, and 5 respectively (Aly and Maibach, 1979). One case in which the use of alcohol-based disinfectants proved to be ineffective is presented with a study focusing on *Acinetobacter baumanii,* a bacteria with a symbiotic relationship on the human skin and a common cause of nosocomial infections (Gandhi et al., 2014). The ethyl alcohol used in this study impaired the antimicrobial activity of neutrophils by decreasing the phagocytosis and killing of bacteria. This damage to the neutrophils caused the disinfectant to be ineffective in preventing pneumonia in human patients (Gandhi et al., 2014). Another study was done using the antiseptic para-chloro-meta-xylenol, an antimicrobial that is safe to use on human skin. The research reported the effects on three important skin microbes - *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Low bactericidal activity was seen among these organisms due to protection provided by the skin. The skin aided in the protection of the organisms by preventing the antiseptic from reaching the organisms (Messager, et al., 2004).

The effectiveness of alcohol-based disinfectants was observed on a solid surface using two different methods: the use of alcohol impregnated wipes and the use of spraying and wiping (Panousi et al., 2008). The use of the alcohol impregnated wipe proved to be significantly more effective than spraying alcohol on the surface and wiping it away. The spray/wipe method did not render any notable changes in the number of viable organisms on the surface (Panousi et al., 2008).

Although there are many methods in which the amount of microorganisms in an area can be reduced, there are limitations that arise. The limitations seen with the previous studies needed to be overcome and other methods of sterilization needed to be considered. More attention has been placed on ultraviolet radiation as a means of combating microbial proliferation (Jordan, 2015). The bactericidal effects of ultraviolet radiation have been recognized under certain conditions such as in aqueous environments (Wang et al., 2012). Waste management systems have utilized ultraviolet radiation in order to sterilize water and additional applications can be accomplished in other settings (Rentschler et al., 1940). The scientific community has realized the beneficial bactericidal qualities of ultraviolet light and much research is being done to document these qualities. Thus, the way is paved for the following research question: the effect of increased exposure to UVC light on human skin microbiota.

Chapter 2: Literature Review

Introduction to the Human Skin Flora

The human skin is host to many types of microorganisms. Some of these microorganisms are resident. These multiply freely on the skin's surface and may be found on the skin at any given time (Evans et al., 1950). These organism have a permanent commensal relationship with the body. Other microorganisms are transient. Transient bacteria may attempt to colonize areas of the body but are unable to do so due to competition from resident microbes, elimination by the body's immune system, and/or physical or chemical changes within the body (Science Prof Online, 2014). Colonization of the skin is dependent upon topographical location, endogenous host factors, and exogenous environmental factors. Additionally, the cutaneous innate and adaptive immune responses play a role in modulating the flora of the skin (Grice & Segre, 2011)

Three major groups of Gram-positive resident bacteria make up most of the microbes inhabiting the skin. These include the coryneform bacteria which encompass the following genera: *Brevibacterium, Corynebacterium, Dermabacter, and Propionibacterium*. *Micrococcus* spp., genetically similar to coryneform bacteria, are also found. Staphylococci species commonly found on the skin include *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, and Staphylococcus hominis.* Although streptococci species are not always found, their presence on the skin has been documented. Gram-negative resident bacteria make up a smaller portion the bacteria inhabiting the skin. *Acinetobacter* spp., *Pseudomonas aeruginosa*, and *Proteus* spp. are examples of these (Noble, 1998).

Several species of fungi also make up a portion of the resident microorganisms that inhabit the skin. According to culture-based analyses, *Malassezia spp*. makes up 53%-80% of the total skin fungal population. *Candida spp.*, although not always found, also contributes to the skin's fungal population. The presence of *Debaryomyces* spp. and *Cryptococcus spp.* has also been documented (Grice & Segre, 2011).

Opportunistic Pathogens

Each of these organisms mentioned have the ability to cause disease and are known as opportunists (Noble, 1998). Opportunists are typically characterized as organisms that usually cause no problems but become pathogenic due to some weakness in the host such as immunosuppression as a result of age or certain disease states. These commensal organisms are easily transmitted to inanimate objects due to their location on the body. Due to these factors, several of these organisms from published lists were used in this study.

Ultraviolet Radiation as a Bactericidal Agent

Over the years, many methods have been employed in an effort to combat the prevalence of harmful microbes. Inactivation of these organisms using exposure to ultraviolet (UV) light is of greater interest in research due to increasing microbial resistance to antibiotics and other bactericidal and fungicidal agents (Maclean et al., 2009). Findings by Jinadatha et al. (2014) reported that exposing a hospital room to UV light was more effective in killing methicillin-resistant *Staphylococcus aureus* and improving the overall cleanliness of the room as compared to traditional cleaning which utilizes chemicals. Additionally, findings by Gayan et al. (2012) reported that UVC light

was effective at killing *Staphylococcus aureus* in the early stages of growth but decreased exponentially as the organisms aged.

Ultraviolet Light

There are three different types of UV light and each has a different biological effect. Ultraviolet A light, which has a wavelength of 320-400 nm, is what is usually referred to as sunlight. These are the rays that we "feel." Ultraviolet A light's bactericidal effects are attributed to oxidative damage to lipids, proteins, and DNA. Ultraviolet B light, which has a wavelength of 280-320 nm, makes up 1% of sunlight. The percentage that does not make up sunlight is absorbed by the ozone layer. Ultraviolet B light causes direct damage to biological systems by creating DNA lesions that block the replication and transcription of DNA. Ultraviolet C light, which has a wavelength of 10-280 nm and does not reach the Earth's surface. It is completely absorbed by the ozone layer. UVC light is potent and is well known for its bactericidal potential (Santos, et al., 2012).

UVC Light

Ultraviolet C light is highly bactericidal, and this quality allows it to be commonly used for sterilization. Ultraviolet C light is commonly associated with the sterilization of liquids such as water but is also used in the sterilization of surfaces and air (Valero et al., 2007). Many factors play a part in UVC light's efficiency as a bactericidal agent. These factors include intensity, exposure time, lamp placement, and air movement patterns. Additionally, the light owes its bactericidal capabilities to the fact that DNA

maximally absorbs UV light within the wavelength of 10-280 nm, the range specified for UVC light (Rastogi et al., 2007).

These factors all play a part in my research project. I believe that it is valuable to know the effect that UVC light will have on organisms known to inhabit the skin because these organisms are easily transmitted and may become pathogenic. My specific research question is the effect of increased exposure to UVC light on specific microorganisms.

Chapter 3: Methodology

Sample

The organisms used in this study includes selected bacteria and yeasts known to inhabit the human skin that have been obtained from the American Type Culture Collection (ATCC), a nonprofit global bioresource center that provides biological products, technical services, and educational programs (ATCC, 2014). The organisms and their ATCC strain numbers are listed in Table 1.

Table 1. Organisms Used for Study

Organism	**ATCC Strain Number**
Acinetobacter baumanii	19606
Candida albicans	10231
Candida kefyr	66028
Corynebacterium renale	19412
Enterococcus faecalis	29212
Pseudomonas aeruginosa	27853
Staphylococcus aureus	43300
Staphylococcus epidermidis	49134
Streptococcus pyogenes	12384

Variables

The dependent variable in this study is identified as colony forming units. A colony forming unit is defined as the number of viable bacterial or fungal cells in a sample (Biology-Online, 2010). The independent variable in this study is identified as the UVC light. This light emits a wavelength of 10-280 nm. The light source was stationed at 44 inches from the cultures in a fume hood throughout various lengths of exposure.

Procedures

Media. In order to cultivate or grow organisms, nutrient agar was made using Difco Nutrient Agar (0001-01). The composition of the agar includes 3 g of beef extract, 5 g of peptone, and 15 g of agar per liter. Twenty-three grams of the nutrient agar powder was mixed with 1L of distilled water in an Erlenmeyer flask. The mixture was heated to 100°C. The mixture was removed from the heat and autoclaved to achieve sterile media. The media was then cooled to about 60°C and poured into Petri plates. The plates were incubated at 36.7°C and observed for contamination. Contaminated plates were discarded in biohazard waste containers while uncontaminated plates were refrigerated at 4-10°C and kept for future use. Commercially prepared blood agar plates (Remel blood agar, 111007) were refrigerated previous to the cultivation of *Streptococcus pyogenes* and *Enterococcus faecalis*.

Reconstitution of Organisms. Using the strains listed in Figure 1, bacteria were reconstituted using Difco nutrient broth (234000). Composition of the broth includes 3 g of beef extract, and 5 g of peptone per liter. The nutrient broth was made by mixing 2.2 g of nutrient broth powder with 300 ml of distilled water in an Erlenmeyer flask and

heating it until boiling temperature was reached. Five milliliters of the broth was pipetted into 10 ml glass tubes and autoclaved. The tubes were incubated at 36.7°C for 24 hours and observed for contamination after that time period. Contaminated tubes were discarded in biohazard containers while uncontaminated tubes were refrigerated at 4-10°C for future use.

Stock cultures. Stock cultures were made from the reconstituted organisms. Stock cultures are defined as cultures of the microorganisms maintained solely for the purpose of keeping the microorganisms in a viable condition by subculture, as necessary, into fresh medium (Merriam-Webster, 2014). The organisms were plated onto either nutrient agar plates or blood agar plates (*Streptococcus pyogenes* and *Enterococcus faecalis* were inoculated on blood agar plates) using the isolation method. This method allows one to obtain isolated colonies which are discrete areas of growth that are expected to originate from a single organism. All bacteria in the colony are clones of the original bacterium, called the colony forming unit. The plates were then incubated at 36.7°C for 24 hours. The plates were taken out of the incubator and preserved for subculture.

Bacterial Suspensions. Bacterial suspensions were made in order to prepare the organisms for UV exposure. A bacterial suspension contains organisms that are dispersed but not dissolved in a fluid (Biology-Online, 2010). Using the stock cultures, several colonies of each organism were placed into respective tubes of sterile water. The 0.5 McFarland turbidity standard, a standard that measures microbial concentration, served as a reference to adjust the turbidity of the bacterial suspensions. This standard ensures that the number of bacteria will be with a given range as to reproduce results.

Experimental Design

Table 2. Experimental Design

Organism	*Control*	*Intervention*
1	O_0	O_{15} O_{30} O_{45} $O_{60}$$O_{75}$$O_{90}$
2	O_0	O_{15} O_{30} O_{45} O_{60} $O_{75}$$O_{90}$
3	O_0	O_{15} O_{30} O_{45} O_{60} $O_{75}$$O_{90}$
4	O_0	O_{15} O_{30} O_{45} O_{60} $O_{75}$$O_{90}$
5	O_0	O_{15} O_{30} O_{45} O_{60} $O_{75}$$O_{90}$
6	O_0	O_{15} O_{30} O_{45} O_{60} $O_{75}$$O_{90}$
7	O_0	O_{15} O_{30} O_{45} O_{60} $O_{75}$$O_{90}$
8	O_0	O_{15} O_{30} O_{45} O_{60} $O_{75}$$O_{90}$
9	O_0	O_{15} O_{30} O_{45} O_{60} $O_{75}$$O_{90}$

UVC Exposure. In order to further prepare the organisms for exposure to the UV light, the organisms from the bacterial suspensions were plated onto seven plates using the lawn of bacteria plating method. This method provides for contiguous growth of organisms. One of the seven plates served as an unexposed control. Each other plate was exposed to the UVC light for either15, 30, 45, 60, 75, or 90 seconds. A template that exposed one-fourth of the inoculated plate was used to block the other three-fourths of the plate from being exposed to the light, making the light's effects more visible.

Image 1. Template Placement

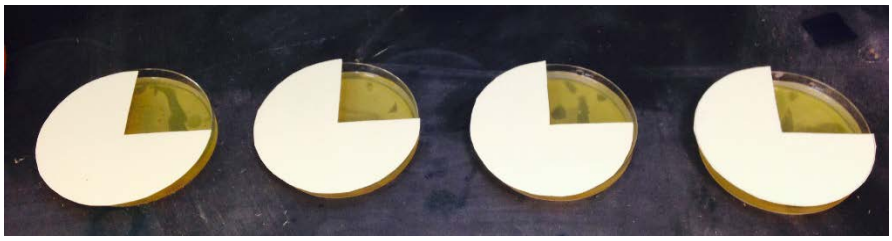
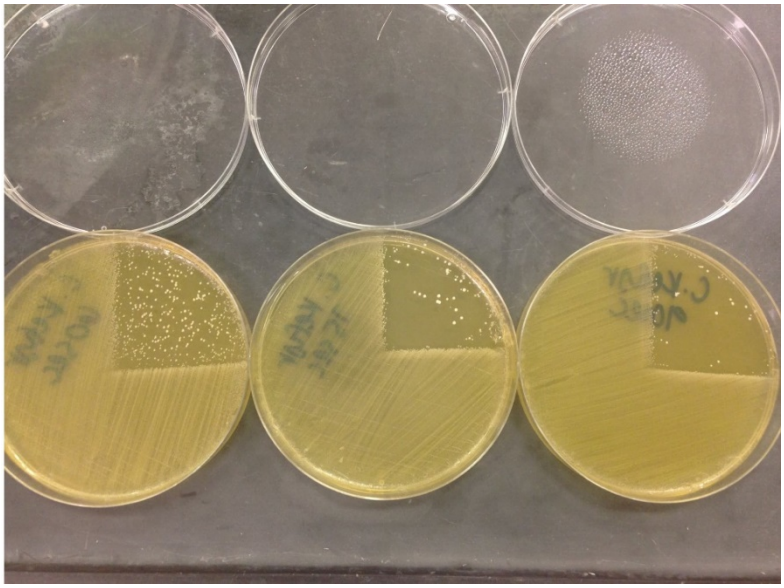


Image 2. Comparison of Growth



Data Collection. In order to determine the effectiveness of the UV light, the exposed plates were incubated for 24 hours. The plates were observed for growth after that time period. Colony forming units in the exposed section of the plate were counted up to 100 colony forming units to document the effect that increased exposure has on microbial growth.

Chapter 4: Results

Data showed that exposure to ultraviolet light had a detrimental effect on the growth each of the organisms in the study. As the length of exposure increased, the number of colony forming units observed decreased. The findings of this study are reported in the Table 2.

Table 3. Results of bacterial growth at specific times for exposure to UVC light

Organism	15 sec.	30 sec.	45 sec	60 sec.	75 sec.	90 sec.
	Results are reported as colony forming units (CFU)					
Acinetobacter baumannii ATCC Strain 19606	>100	17	10	3	1	1
Candida albicans ATCC Strain 10231	>100	>100	>100	56	15	6
Candida kefyr ATCC Strain 66028	>100	>100	>100	>100	46	31
Corynebacterium renale ATTC Strain 19412	>100	>100	94	54	34	4
Enterococcus faecalis ATCC Strain 29212	>100	>100	>100	72	14	12
Pseudomonas aeruginosa ATCC 27853	>100	62	3	2	2	0
Staphylococcus aureus ATCC Strain 43300	>100	>100	84	19	13	7
Staphylococcus epidermidis ATCC Stain 49134	>100	>100	84	19	5	5
Streptococcus pyogenes ATCC Strain 12384	>100	>100	>100	94	4	1

Chapter 5: Discussion and Conclusion

Ultraviolet radiation acted as an efficient bactericidal agent. The UVC light that was utilized in this study was able to decrease the growth of all the microorganisms that were used in this study. Had the length of exposure been carried out for another 15 second increment, it is likely that growth would have been further decreased. Perhaps, no growth may have been observed with additional organisms, as was demonstrated with *Pseudomonas aeruginosa*. As expected, *P. aeruginosa*, and the other Gram negative rod, *Acinetobacter baumanii*, were the organisms most affected by the radiation due to having a thin layer of peptidoglycan. This difference in cell wall structure is an essential difference between the Gram negative organisms and the Gram positive organisms which have a significantly thicker cell wall of peptidoglycan. Future research involving bacteria and ultraviolet radiation may include observing the effects of different wavelengths on the viability of organism used in this study. Additionally, research may be done to determine novel uses for ultraviolet light in the prevention of disease such as providing commercial ultraviolet devices to people which would allow them to efficiently sterilize potential vectors.

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