EVALUATION OF GERMICIDAL UV-C LIGHT FOR SURFACE DISINFECTION IN A TERTIARY CARE HOSPITAL

Qanita Fahim, Nasir Uddin, Najam Ul Hassan, Nargis Sabir*, Anam Tariq, Ayesha Bashir, Sarmad Ahmad**

Combined Military Hospital Lahore/National University of Medical Sciences (NUMS) Pakistan, *Combined Military Hospital Nowshera/National University of Medical Sciences (NUMS) Pakistan, **NUST College of Electrical and Mechanical Engineering, Rawalpindi Pakistan

ABSTRACT

Objective: To check the efficacy of 36-Watt Ultraviolet-C tube light, in terms of distance and time against medically important microorganisms (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeroginosa, Candida albicans and Aspergillus species*).
Study Design: Quasi-experimental study.
Place and Duration of Study: Pathology department, Combined Military Hospital, Lahore Pakistan, from Jun to Sep 2020.
Methodology: ATCC control organisms of above mentioned bacteria, yeasts, and fungi were exposed to ultraviolet-C light for different times and distances to ascertain its germicidal effect. Two methods were selected, one in which micro-organisms inoculated plates were exposed to ultraviolet-C light and second in which McFarland suspensions of microorganisms were exposed before inoculation. Both the methods were compared. Observations were noted down after repeated performance of both the procedures.
Results: An exposure time of 15 minutes, mean ± SD (13.8 ± 10.1) at 1-foot distance was proved ideal for all the tested bacteria, but yeasts and fungi required >30 minutes, mean ± SD (17.5 ± 13.5) to be killed. Moreover, distance and time of exposure were found out to be directly proportional irrespective of microbial load. Greater the distance longer the ultraviolet C exposure was required.
Conclusion: Ultrviolet-C light 36-Watt can have efficient inactivation of bacterial, fungal and archaeal species up to 6 feet for >30 minutes exposure time. Ultraviolet-C light disinfection is best for areas like closed rooms, operation theatres, PCR Labs, and bio-safety cabinets keeping bio-safety guidelines in view.

Keywords: Disinfection, Microorganisms, Ultraviolet-C light.

INTRODUCTION

By the end of year 2019, an outbreak of novel human corona virus leading to serious pneumonia emerged in Peoples Republic of China, City of Wuhan, Hubei province, that consequently became a global pandemic[1]. Molecular specialists all over the world endeavored to sequence its genome (29,903 nucleotides). It was identified as a novel corona virus (2019-nCOV)[1]. It is the seventh member of the corona virus family. It infects humans and has been named by the International Committee on Taxonomy of viruses as "SARS-CoV-2". On the 11th of February 2020, the disease caused by this virus was named by the World Health Organization (WHO) as "Corona Virus Disease 19 (COVID 19)"[2,3].

In the wake of ongoing pandemic a continuous search for disinfectants to neutralize surfaces and the environment, infected with corona virus has led to the discovery of using ultraviolet C (UVC) light for the purpose. UVC light has long been known for its antimicrobial effects in water, food items, and ventilator ducts[4]. Many handy devices containing UVC light are available in the markets. Many manufacturers claim the sporicidal/viricidil activity of these products. During COVID-19 pandemic, hospitals are in search of a product that can neutralize or clean the hospital environment with efficiency and consistency against viruses and bacteria. Many studies have evaluated the role of UVC light to reduce the bioburden of Acinetobacter, VRE, MRSA, Ebola and Clostridium difficile in hospital rooms[5-7].

Keeping above in view, an in-house UVC disinfection device was designed (fig-1) by a member of our study group from Military Engineers Corp Lahore. It was desired to ascertain its efficiency and potential utility as a disinfectant of surfaces in the settings of health care facilities. Simultaneously, a quasi- experimental study was planned in Microbiology Department, CMH Lahore to ascertain the germicidal effect of UVC light against commonly encountered bacteria, yeasts, and fungi. The concept of this study was based on a hypothesis that if UVC light can kill most of the medically important microorganisms, then it will have a viricidal effect as well. Hence, it can be utilized to disinfect the environment of closed rooms, operation theatres (OT), Laboratory departments especially PCR

Correspondence: Dr Qanita Fahim, Associate Professor, Department of Microbiology, Combined Military Hospital, Lahore Pakistan
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rooms, hospital ITCs and dental procedure rooms against corona virus.

This study was aimed to check the efficacy of 36-Watt UVC tube light, in terms of distance and time against medically important microorganisms, (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeroginosa, Candida albicans, and Aspergillus species*).

METHODOLOGY

This quasi experimental study was conducted at Pathology department, Combined Military Hospital (CMH) Lahore Pakistan, from June to September 2020 after approval from Research Review Board, Combined Military Hospital Lahore (RRB ltr no. 236/2020). All informations in the study were kept confidential. Meanwhile subject Performa had been distributed to health care workers (HCWs) as a pilot project.

A UVC tube, (TUV 36W ISL, Phillips, Holland), UVC radiation 15 watts, 48 inches long, 1 inch in diameter, emitting short wave UV radiation of 253.7 nm was used in the study. In this study, three bacterial (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeroginosa*), one fungal (*Aspergillus sp*) and one archaeal (*Candida albicans*) strains were selected. American Type Culture Collection (ATCC) strains selected, were *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeroginosa* ATCC 27853, *Candida albicans ATCC* 10231, and *Aspergillus sp*[8,9]. All bacteria were grown in nutrient broth and subcultured on blood agar and Mac Conkey agar. Yeast and fungus were subcultured on Sabouraud dextrose agar. The first four were incubated for 18-24 hours at 35 ± 20C in ambient air. Aspergillus sp was incubated at 220C for 6-7 days for spores to mature. Later, spores were suspended in normal saline for UVC light exposure[9].

Method-1

A suspension of each of above-mentioned microbes was made, and the opacity was adjusted to 0.5Mc Farland standards. After inoculation of 100µl of each bacterial suspension on MacConkey agar and fungal and yeast on Sabouraud agar plates (Oxoid, UK) (Pre-exposure control plates), the Same Mc Farland suspensions were then exposed to UV-C light, which was horizontally placed on a table for 15, 20 and 30 minutes at a constant distance i.e., 1 foot. 100µI of these suspensions of bacteria after each exposure to UVC light were then subcultured on appropriate media and incubated as mentioned above. Suspensions of Aspergillussp were incubated at 22°C for 48 hours. This procedure was repeated and again with each increment of distance till the final exposure of suspensions at 6 feet for 15, 20 and 30 minutes were done. This method was less labor intensive and cheap.

Method-2

100µl of each of bacterial suspension was inoculated on 19 plates of MacConkey agar. One (pre exposure control plate) was incubated at standard temp and time as Mc Farland control. Rest were placed vertically facing towards UVC light with lids off at distances of 1-6 feet, exposed for 15, 20 and 30 minutes and incubated at standard time and temperature. Colony count was noted down in the form of colony-forming units per milli litter CFU/ml. *Candida albicans* and *Aspergillussp*. Were inoculated on *Sabouraud agar* and after exposure to UVC light, plates were incubated as mentioned above. This method was labour intensive and required 19 plates of MacConkey agar for each organism.

Mc Farland Control: 0.5Mc Farland means a viable colony count equal to 1.5 x 108 CFU/ml. A suspension of each of a known microbe was made and opacity was adjusted to 0.5 McFarland standards. 0.1µl of this suspension was added to 9.9 ml of distilled water (DW) making 1/100 dilution (Dilution 1) and further 0.1µl of dilution 1 was added to 9.9ml DW to make a dilution of 1/10000. 100 µl of second dilution when inoculated on MacConkey agar (Oxoid, UK) and incubated for 24 hours yielded growth of 10 CFU, confirming its fitness[10]. UVC light of 36 Watt, (Phillips, Holland) of Microbiological safety cabinet (Technico Scientific supply), class II was taken as "Gold Standard". Results were comparable with "Gold Standard" UVC light.

Data was analyzed using the ratio of viable count of respective organisms before and after exposure to UVC light. 100µl of all suspensions adjusted to 0.5 McFarland standard after dilutions (1/100, 1/10,000) were sub cultured on MacConkey agar for quality, growth, and growth condition check and incubated for 18-24 hrs at 35 ± 20C. A colony count of 10 was taken as an indicator of good quality McFarland. Finally, all the observations were noted down after repeated performance of both the procedures and getting the same results.

RESULTS

100µl of initial suspension of *Staphylococcus aureus* when inoculated on MacConkey agar and blood agar yielded growth of approximately 107 CFU /ml of organism and when exposed to UVC light for 15 minutes at a distance of 1 foot yielded no growth and subsequently at 2, 3, 4, 5 and 6 feet yielded growth of nil, 3, 3, 4 and 10 CFU/ml or (six Log10 reduction) i.e., from 107 to 101 CFU/ml (fig-2). Similar results were obtained

against *Pseudomonas aeruginosa* and *Escherichia coli* when 100µl of initial suspension of these organisms were inoculated on blood agar and MacConkey agar yielded approximately 107 CFU/ml of organisms and when exposed to UVC light for 15 minutes at a distance of 1 foot yielded no growth and at 2, 3, 4, 5 and 6 feet yielded growth of nil, 15, 18, 20 and 35 CFU/ml (six Log-10 reduction) i.e from 107 reduced to101 CFU/ml (table-I).

Table-I: Colony counts of micro-organisms on pre-exposure (control plate) and post-exposure MacConkey, and Sabouraud agar plates according to Method-2.

Organisms	Time (min)	CFU/ml Control (Pre-exposure)	Growth of CFU/ ml at Distances in feet I ft	2 ft	3 ft	4 ft	5 ft	6 ft	Log 10 Reduction
Staphylococcus Aureus									
	15	10	Nil	Nil	3	3	4	10	6 Log10
	20		Nil	Nil	01	01	02	08	6 Log10
	30		NIL	Nil	Nil	Nil	Nil	05	6 Log10
E coli and Pseudomonas Aeruginosa									
	15	11	Nil	Nil	15	18	20	35	6 Log10
	20		Nil	Nil	10	12	18	30	6 Log10
	30		Nil	Nil	Nil	Nil	10	25	6 Log10
Candida Albicans									
	15	10	Nil	Nil	7	10	19	22	6 Log10
	20		Nil	Nil	5	8	15	27	6 Log10
	30		Nil	Nil	Nil	6	18	25	6 Log10
***Aspergillus species (approx CFU/ml)**									
	15	13	63	70	77	82	84	90	6 Log10
	20		08	10	17	30	32	35	6 Log10
	30		Nil	Nil	Nil	12	17	28	6 Log10

**Aspergillus sp ; CFU/ml were roughly counted as one can only make subjective assessment in this case.*

100µl of initial suspension of Candida albicans, when inoculated on Sabouraud agar yielded approximately 107 CFU/ml and UVC light post exposure of 15 minutes at a distance of 1 foot yielded no growth and at 2, 3, 4, 5 and 6 feet yielded growth of nil, 7, 10, 19, and 22 CFU /ml (six Log 10 reduction) i.e., from 107 to 101 CFU/ml (table-I). A similar reduction was observed in both. 100 µl of initial suspension of Aspergillus sp, when inoculated on Sabouraud agar yielded approximately 107 CFU/ml and UVC light post-exposure of 15 minutes at a distance of 1 foot yielded no growth and at 2, 3, 4, 5 and 6 feet yielded growth <6 logs (six Log10 reduction). Similar reduction was observed in both the methods (table-I). 100µl of initial suspensions in all the tested microorganisms had yielded 107 CFU/ml but post-exposure of 15 minutes up to a distance of 1 foot from UVC device had given no growth of these microorganisms. However, UVC from the device was effective up to a distance of 6 feet by giving substantial growth reduction in these microorganisms (fig-1). Dose of Ultraviolet-C required to kill these microorganisms has been calculated and shown in (table-II).

DISCUSSION

Primarily SARS-CoV-2 was transmitted to humans through bat according to one of the study while other suggested transmission through snakes, turtles and pangolins[1,11]. However, a well known mode of transmission of SARS-CoV-2 infection among community is through respiratory droplets formation by infected persons. Other known potential sources of transmission are contaminated surfaces made up of metal, glass and plastic. The virus can survive for 2-3 days on

Table-II: Dose calculation of ultraviolet C light.

Watts x Intensity Factor =µWs/cm² or Ws=J (Joules) or µJ/cm²
UV Dose = UV density x time in sec
UV Dose / UV Density = Time in sec
UV Density is multiplied by area which is πr²
π= 3.14 x r2 (r is radius in meter)
UV Dose required for *Staphylococcus Aureus* is 5786 µWs/cm² or µJ/cm² as per American air and water company 11 5786 x 4 x 3.14 x 1 / 15 = 5526.4/ 60 = 81 min for 1 meter distance Or 5786 x 4 3.14 x 0.5 / 15 = 2763.2 / 60 = 40 min for ½meter distance 5786 x 4 x3.14 x 0.25 /15 = 1381.6 / 60 = 20 min for ¼ of meter

these surfaces[1,11]. As most of the surfaces frequently touched by health care workers in the hospitals are made up of these materials. There is a potential hazard of contracting coronavirus via touching these surfaces. It has been shown that the virus survives better at 30-50% humidity for 2 hours to 9 days at room temperature in a closed room environment and surfaces[12].

Figure-1: Ultraviolet-C emitting device, perceived and designed by engineer Najam Ul Hassan.

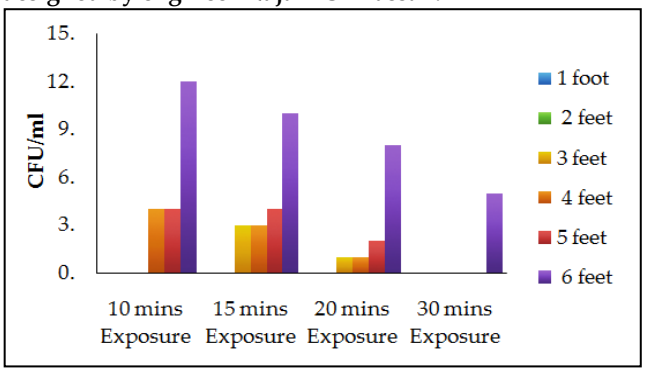

Figure-2: Number of CFU/ml of staphylococcus aureus against exposure time.

In corona virus pandemic, humanity is in search of suitable treatment and prevention. The additional thing that can limit its spread among people is a dry, clean and disinfected environment[4,12,13]. Many methods of disinfection have been suggested by CDC and other professional bodies for environmental disinfection. These include natural ventilation having minimum of six air changes per hour (ACH), use of chlorine and alcohol based disinfectants through spray or defogger for 35-45 minutes, HEPA filters 13/14 with minimum 12 ACH and Ultraviolet germicidal irradiation (UVGI) [4,12,13]. Although preventive strategies like vaccines prove promising yet practically an effective vaccine and vaccination of masses is still in clinical trials and may require 8-9 months. Finally, disinfection is the last resort which is of paramount importance.

UV irradiation is divided into three different spectral areas UV, UVA, UVB and UVC having a wavelength of 100-200, 315-400, 280-315 and 200-280nm[14]. The best wave length absorbed by nucleic acid of microorganisms is UVC15, it ranges from 250-270 nm and damages the nucleic acid (DNA, RNA) of the microorganism completely[15,16]. The process is called dimerization of pyrimidine molecules which makes replication of nuclear material of microorganisms impossible[17].

Germicidal effect of UV radiation has been known for decades. The researchers, however, have been reluctant to use it for disinfection of hospital environment. Studies have explicitly shown its high germicidal capacity against hospital contained resistant bacteria/viruses[12,13,15,16]. It kills rapidly multiplying microorganisms in the indoor environment. Moreover, it reduces the bioburden of airborne pathogens especially, those resting on inanimate surfaces.

We tried a simple model to find out whether UVC light could be used for inactivation of microbes lying on the surfaces frequently encountering health care workers or not and whether it had a good germicidal activity or not. Our simple quantitative assessment proved the use of UVC light having 253.7 nm wavelength of 36 Watts effective against common hospital microbes.

The UVC light emitting device was mounted on a circular frame with tyres to roll on and was remote control operated. It moved around in the room on its own. If command were given to stand by each surface for almost 20 minutes, it would definitely decontaminate the surface by killing all vegetative bacteria present on it (fig-1). Its germicidal effect was best achieved (100%) at a distance of 1 foot for 20 minutes. it can still kill all bacterial forms to maximum (i.e., 95-98%) with increase in exposure time (i.e., 30 minutes) up to a distance of 6 feet. Fungal, and yeast forms were also reduced. However, it requires an exposure of >30 minutes as it is evident from our results (table-I). Similar findings have been described by Adebiyi *et al* that yeast and fungi require greater exposure time and have 0% survival rate at longer exposures to UVC light[18]. G katara and coworkers also proved an exposure time of 40 minutes up to 8 feet distance[19].

It has been observed that distance and exposure time both are directly proportional to each other i.e., with smaller distance lesser exposure time was required and with greater distance longer exposure time is required; Distance (D) is directly proportional to time

of exposure (T) provided that area is thoroughly exposed without any object in-between that shadows (fig-2)[20]. Moreover, microbial bio-load also proved to have no direct effect on UVC exposure time i.e., greater load requires same exposure times provided required dose of UVC light has been given. According to Mahida and Linblad *et al*, UVC energy of 12000m Ws/cm^2 is required to kill vegetative bacteria but UVC energy dose of 22000 µWs/cm^2 is required against all pathogens[12,20].

In our device, (fig-1) UVC lights were mounted/ fixed in vertical position on the device, in that case light waves would have fallen from sideways, which would have hidden half of tubes behind the working bench in the laboratory, had it been possible to change the position from vertical to horizontal larger area of working benches would have been exposed and disinfected the lab working benches fully.

RECOMMENDATIONS

- UVC fixed devices can be easily used to disinfect environment and surfaces of hospital wards, operation theatres, laboratory rooms, ICUs, ITCs, Corona PCR rooms. It should be remote control operated and rooms and areas to be decontaminated should be vacated first before it starts functioning.
- Device should be operated at appropriate distance. Adequate exposure time should be allowed for contact on the surface to be disinfected.
- Standard safety guidelines should be kept in mind before its use as UVC is known for its carcinogenic effect on human eyes and skin.

Note: UVC light disinfection is best for areas like closed rooms, operation theatres, PCR Labs and biosafety cabinets.

Warning: National Institute of Occupational safety and Health (NIOSH) recommends that the time of exposure for humans to an intensity of UVC 100 µwatts/cm^2 at wave length 254 nanometers should not exceed 1 minute.

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CONCLUSION

UVC light 36 Watt can have efficient inactivation of vegetative microorganisms up to 6 feet if exposure time is >30 minutes. This Ultraviolet-C light disinfection experience can be reciprocated against corona virus but needs further evaluation.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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