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In Vitro Efficacy of 222 nm UV Light on Antibiotic Resistant Bacteria Isolated from Patients and Standart Cultures Standard Bacterial Strains

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Abstract

Aims: We observed *in vitro* effects of a filtered 222 nm Far-UVC device (Initus-V) on antibiotic resistant bacteria taken from hospitalized patients and standart antibiotic resistant bacterial cultures by exposing them to filtered 222 nm Far-UVC for 5, 15, 30, 45 and 60 minutes. **Method:** Blood culture samples taken from septic patients hospitalized in Istinye University Hospital in 2022 were inoculated into Petri dishes and antibiotic resistance bacteria were grown. Experiment group bacteria were exposed to 222 nm Far-UVC system while control group was not exposed. Initus-V 222 light from emits 42.9 uW/cm^2 energy at a distance of 50 cm from the source. Bacterial growth and Log reduction values at 24 and 48 hours were calculated for both groups. **Conclusion:** In all antibiotic resistant bacterial cultures obtained either from patients or standart commercial sources growth was inhibited to 0 at 30 minutes except *Klebsiella pneumonia* obtained from patients in which there was 8 log reduction at 30 minutes. Filtered Far-UVC can have place in future treatment of antibiotic resistant bacteria and spread of hospital acquired infections because preliminary animal trials show its safety within certain limits.

Keywords

Antimicrobial Activity, Multidrug-Resistant Bacteria, UV Light, Infection Management

1. Introduction

The beneficial effects of the sun, which is the natural source of Ultraviolet Light (UV), have been known since ancient times, and it was called "health-bringing" by ancient European communities and Incas due to its healing effect. Isaac Newton discovered the components of solar radiation in the visible band with the help of a prism in 1666, John Ritter discovered the light bands in the solar radiation spectrum in 1801, and Nielsen-Finsen discovered UV rays as the cause of tanning and sunburn [1].

Electromagnetic waves emitted by the sun are divided into three different environmental effects; Bands of infrared radiation (IR), visible radiation (VL) and ultraviolet (UV) radiation. The UV-radiation spectrum is divided into UVA (315 - 400 nm), UVB (280 - 315 nm) and UVC (100 - 280 nm) bands with different environmental effects. About 10% of sunlight outside the atmosphere is UV radiation. About a third of this reaches the earth. 95% of the radiation reaching the earth is in the UVA and 5% UVB bands. Most of the UVC and UVB are absorbed by ozone, molecular oxygen and water vapor in the upper atmosphere. Living things are not affected by the UVA radiation that reaches the earth by decreasing and being harmless. UVC cannot reach the earth [2].

UV-radiation with a frequency of 253.7 nm, which is closest to 265 nm, the most common wavelength of the UVC band of sunlight, and which can be produced artificially, is used in many different areas from instruments used in medical interventions to disinfection of wastewater and clean water, disinfection of micro-organisms in living spaces is used.

It has been scientifically proven that as the wavelength of UVC radiation decreases, mammalian cells also develop damage their genetic material. Mercury vapor lamps produce ultraviolet radiation between 200 - 280 nm, and it has been shown that it is absorbed by deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) at approximately 254 nm and causes DNA and RNA damage by causing the formation of pyrimidine dimers [3]-[5].

This frequency is also harmful to human cells and tissues containing DNA. It has been shown that UV irradiation to the skin causes lesions such as erythema formation, potential carcinogenic mutations, and photokeratitis [6]-[8].

In a study conducted in 2004, in the environment where Chinese Hamster Ovary Cells (CHO) were used, 100 mJ/cm^2 of UVC short wavelength (206 nm) irradiation caused damage to E. Coli, while CHO for doses up to 400 mJ/cm^2 did not cause detectable damage to cells. The researchers who conducted the study recommended the application of this short wavelength UV light to prevent surgi-

cal site infections and to provide wound decontamination [9]. Similar studies were also conducted by Buonanno *et al.* [9] [10].

Later, the term "far-UVC" was coined for UVC light in the 200 - 230 nm range [11]. It has been shown that the advantage of far-UVC radiation in sterilization is due to its strong protein absorption, and that less than 5% of far-UVC radiation can reach the nucleus of a mammalian cell with a typical diameter of more than 10 μm compared to micro-organisms [12]. Human skin is composed of dead keratinocytes that absorb most of the ultraviolet radiation and is assumed to be further protected against far-UVC radiation by the stratum corneum, the outermost layer of the epidermis. Similarly, the cornea and tear film, which protects the lens by absorbing far-UVC radiation, are assumed to protect the eye [3].

However, in order to provide the specific bandwidth (222 nm) of the Initus-V device used in our study, and to reduce the 237 nm and 258 nm UV radiations, which are more harmful to human cells, which occur with the Kr-Cl excimer emission, a special filter has been reported to be used [13]. The current system is reported to emit 42.9 uW/cm^2 of energy at 50 cm from the source. According to the 7th Edition of the ACGIH (American Government Conference of Industrial Hygienists) Biological Exposure Indices Documentation, published in 2020, when the current energy release of the product is calculated from a distance of 50 cm for the eye, the unprotected exposure time is calculated as 160,000/42.9 = 3.729 seconds. The skin's exposure time is calculated using the same method as 11.142 seconds, 185.7 minutes.

2. Methods

Bacterial Strains

Acinetobacter baumannii, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, from multiple antibiotic resistant Gram-negative isolates and Gram-positive isolates *Staphylococcus aureus* and *Enterococcus faecalis* isolates from patients hospitalized in Istinye University Hospital were used.

Bacteria strains observed on selective agar plate after 24 hour incubation. All antibiotic resistant isolates were randomly selected for their identification using Vitek 2 Compact System We applied EUCAST rapid antimicrobial susceptibility testing (RAST) to determine the antimicrobial susceptibilities of the isolates.

Pseudomonas aeruginosa ATCC 27853, *Escherichia coli* ATCC 25922, *Acinetobacter baumannii* ATCC 19606, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 American Type Culture Collection (ATCC) standard bacterial strains were also used in experiments.

Bacteria in the Control and Experimental group were stored at −80˚C and passaged on % 5 Sheep blood Agar (BD) under appropriate conditions of the experiment and incubated 37˚C.

Bacteria in the Control and Experimental groups were prepared from % 5 Sheep blood Agar at 0.5 McFarland turbidity, then spread into a Petri dishe each con-

taining Mueller Hinton Agar was grown for 24 h in a 37˚C incubator. They were harvested by centrifugation and suspended in 0.85% saline. The final bacterial density in each 0.1 mL aliquot used in the experiments was 2×10^8 colony-forming units (CFU)/mL or 1.6×10^9 CFU/mL [14].

While a Mueller Hinton Agar containing Petri dishe was prepared for each strain and the tubes dishes in the control group were removed from the oven without being exposed to any external factors, the dishe of the experimental group containing the same strain were placed in a Nüve brand cabinet (biosafety level 2) with laminar flow, for 5, 15, 30, 45 or 60 minutes under Initus-V 222 light from 50 cm distance. Energy level of the device measured at 50 cm from source is 42.9 uW/cm^2. Afterwards, each Petri dishe was incubated in an oven at 36˚C. The growth of all petri dishes in the control and experimental groups at the 24th and 48th hours after incubation were evaluated and MIC values were calculated [15]. Three independent experiments were performed in triplicate (see **Figure 1**).

The results were provided by calculation of 0.1mL of 2×10^8 CFU/mL and 0.1 mL of 1.6×10^9 CFU/mL at 24 h after incubation at 37˚C. CFU/mL is the number of colonies multiplied by 101 + dilution factor. It takes into account the need to multiply by an extra 10, because of the addition of 0.1 mL to the plate from the tube. The number of CFU/mL was calculated with and without irradiation.

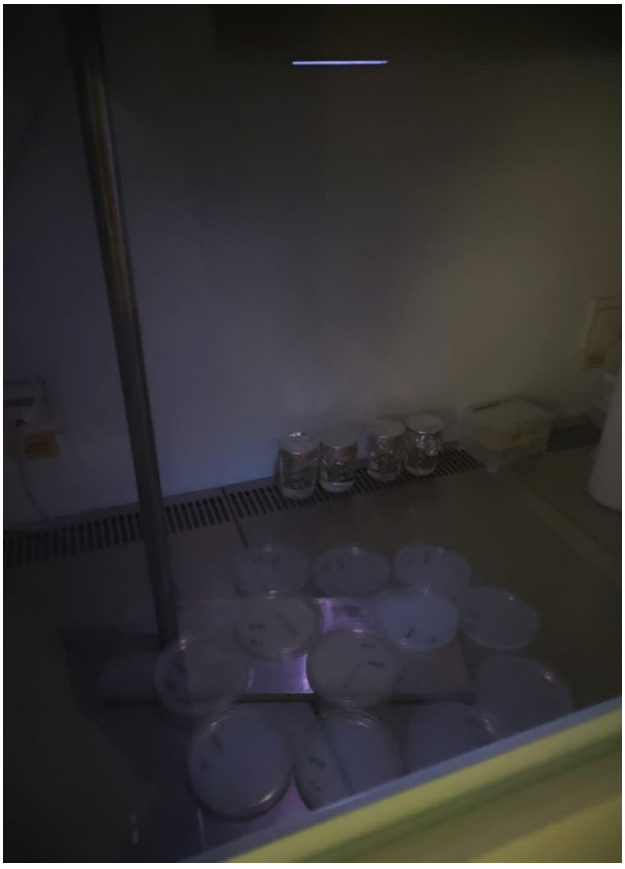

Figure 1. Petri dishes exposed to Far UV from 50 cm distance.

Initus-V's 222 nm light source was produced by Vestel Inc. (Manisa, Türkiye) and InnowayRG Inc. (Istanbul, Türkiye) consists of a krypton-chloride (Kr-Cl) excimer lamp and an optical filter with emission maximum output at wavelength 222 nm limiting the emission wavelengths to 200 - 230 nm. The lamp unit consists of a lamp, air cooling fan, mirrors and a special band-pass filter. The attached filter was used to remove almost all other wavelengths from the spectrum except the dominant 222 nm emission wavelength. The intensity of 222 nm light was measured using an S-172/UIT250 accumulated UV meter and was found to be maximum 2.23 uW/cm^2 at center and minimum 1.34 uW/cm^2 from 2.5 mt distance [16].

3. Results

In the comparison, it was observed that 222 nm UVC application significantly reduced the growth in both standard cultures (Table 1) and isolated bacterial cultures with antibiotic resistance (Table 2), proportional to the duration of the application.

In addition, it was observed that 222 nm Far-UVC light inhibited all bacterial growth except for *Klebsiella pneumonia* species within 30 minutes, while providing a significant reduction (from Log 12 to 4.2) in *Klebsiella pneumonia* species. According to the information received from the clinic, this strain was obtained from the patient and showed resistance to all known antibiotics and the patient died due to sepsis. The patient was a 61-year-old female living liver transplant recipient. Blood cultures from two bottles were positive for *Klebsiella pneumoniae*. Antibiotic susceptibility testing revealed multidrug resistance (resistant to all antibiotics except amikacin and gentamicin), carbapenemase positive; TEM, SHV, and CTX positive; and ESBL positive masked by AmpC.

Table 1. MIC reduction after Far-UVC exposure from 50 cm at energy level of 42.9 uW/cm^2 in standart antibiotic resistant bacterial cultures with starting number of Log12.

Bacterial type	Far-UVC exposure times					
	0 minute	**5 minute** MIC growth Log Total energy applied 12.87 mj/cm^2	**15 minute** MIC growth Log Total energy applied 38.61 mj/cm^2	**30 minute** MIC growth Log Total energy applied 77.22 mj/cm^2	**45 minute** MIC growth Log Total energy applied 115.83 mj/cm^2	**60 minute** MIC growth Log Total energy applied 154.44 mj/cm^2
A. baumannii ATCC 19606	12	4.4	4	0	0	0
P. aeruginosa ATCC 27853	12	5.19	3.6	0	0	0
E. coli ATCC 25922	12	4.4	3.9	0	0	0
S. aureus ATCC 29213	12	5	4	0	0	0
E. faecalis ATCC 29212	12	4.2	3.7	0	0	0

Table 2. MIC reduction after Far-UVC exposure from 50 cm at energy level of 42.9 uW/cm² in antibiotic resistant bacterial cultures isolated from patients with starting number of Log12.

Bacterial type	Far-UVC exposure times					
	0 minute MIC growth Log	**5 minute** MIC growth Log Total energy applied 12.87 mj/cm²	**15 minute** MIC growth Log Total energy applied 38.61 mj/cm²	**30 minute** MIC growth Log Total energy applied 77.22 mj/cm²	**45 minute** MIC growth Log Total energy applied 115.83 mj/cm²	**60 minute** MIC growth Log Total energy applied 154.44 mj/cm²
A. baumannii	12	5.1	4.2	0	0	0
P. aeruginosa	12	5	4	0	0	0
E. coli	12	5.1	4.2	0	0	0
S. aureus	12	5	4	0	0	0
E. faecalis	12	4.2	3.8	0	0	0
Klebsiella pneumoniae	12	5.2	4.9	4.2	3.6	3.4

4. Discussion

Surgical site hygiene, cleaning of surgical instruments and resistance to antibiotics used after the operation are the most important factors that directly affect the mortality and morbidity of the patient due to the risk of hospital infections. Nosocomial infections constitute one of the most important problems of clinicians due to their resistance to almost all antibacterial drugs, including the most recently developed antibiotics. Even the use of broad-acting antiseptics such as povidone chloride and chlorhexidine could not provide a satisfactory solution to this clinical problem.

In this study, it was observed that UVC light filtered at a wavelength of 222 nm inhibited all bacterial growth within 30 minutes. This time is not expected to adversely affect the human cell within the available data and is not thought to cause a significant increase in the total operation time. As long as internationally established standards are adhered to, intermittent or continuous UVC application during or during the preparation process may result in a reduced risk of wound infection, a reduced risk of antibiotic resistance development, and a significant reduction in mortality, morbidity, or length of stay.

To the best of our knowledge, our current study is the most comprehensive study of the effect of 222 nm UVC irradiation on bacterial growth at specified times. However, there is not enough information about the effect of UVC light on tissues that are not covered with epithelium, factors related to the patient, the quality of the materials used and the effect of the personnel in the environment.

Current exposure limits of humans to 222 nm FarUV radiation in all parts of the world are mostly determined mainly by 5 institutions: International Committee on non-Ionizing Radiation Protection (ICNIRP 14/2007), American Confer-

ence of Governmental Industrial Hygienists (ACGIH 2008), European Commission (2006/25/EC), American National Standards Institute/Illuminating Engineering Society (ANSI/IES RP-27.1-15) and International Electrotechnical Commission (CEI/IEC 62471: 2006). The ACGIH (American Conference of Governmental Industrial Hygienists) is one of the institutions which determines limits for 222 nm Far UV exposure and recently published a new guidelines which are not yet accepted by public regulatory bodies. ACGIH increased in 2022 threshold for 222 nm FarUV exposure from 23 mJ/cm^2 to 161 mJ/cm^2 for the eyes and 479 mJ/cm^2 for skin. Initus-V total irradiance dose at 30 minutes from 50 cm (77.22 mj/cm^2) provides Log 12 to Log 0 reduction in all antibiotic resistance bacteria except Klebsiella Pneumonia (Log12 to 4.2 reduction) [17].

Multiresistant bacteria are frequently transferred from patients to the radiograph machine in the presence of poor infection control practices, and may be a source of cross-infection/ colonization. Improved infection control practices decrease the occurrence of resistant organisms on the radiograph equipment [18]. Bacterial contamination of microphones and computer mice at radiologist workstations is common, with colonization significantly greater than nearby restroom toilet seats and doorknobs [19]. Studies have shown that white coats and ties are a reservoir for colonies of pathogens to grow and spread nosocomial infections; however, there has been limited research on whether lead aprons worn by hospital personnel can be a source of nosocomial infection or a source of infection to themselves. The results showed that there were significant amounts of both *Staphylococcus aureus* and *Tinea sp* residing in lead aprons currently worn by interventional radiology medical staff [20]. Radiology departments may be blind spot for hospital acquired infection spread because almost all patients from in hospital or outpatient clinics visit department. Initus-V in radiology departments may help in reduction of multiresistant bacteria transmissions in hospitals and further clinical studies need to be done.

Far-UVC has same germicidal efficacy but without health hazards of conventional UVC [10] [11] [21] [22]. Far-UVC light (207 or 222 nm) generated by inexpensive excimer lamps which can be deployed in occupied public locations [13] [22]-[25]. Low-dose-rate far-UVC can be a good tool to prevent spread of aeorosolized viruses in public locations [22] or multiresistant bacteria in hospitals.

On the other hand, UVC radiation is only open to external application and may be insufficient to prevent infections that may develop in deep tissues.

This significant effect of UVC application in *in vitro* conditions should be evaluated with clinical studies on the development of antibacterial resistance in patients before, during and after surgery, its effect on hospital infection risk and wound care.

New investigations and trials are frequently encountered in the area of desinfection and sanitation after COVID-19 outbreak. The strong potential of airborne pathogens in contagion of disease implemented the need for a sterilized environment from viruses and bacterias. Many studies are ongoing or being published

recently with Far-UVC.

In a study conducted at Leeds University, five Far-UVC lamps were fixed at the ceiling in a bioaerosol room where an active *Staphylococcus aureus* strain (as a proxy to coronavirus) was sprayed in the air. As the lamps were on, 93.7% of bacterias were suppressed in the room. The investigation was not carried on with SARS-COV-2 due to security reasons but previous surface investigations showed that air suspended *Staphylococcus aureus* strains are less sensitive than Influenza or Coronavirus to Far-UVC inactivation [24]. The study bring the hypothesis that with Far-UVC the rates of decrease for Influenza or Coronavirus will probably be higher and that the inactivation period will be less.

In our study with Far UVC (222 nm), in addition to standard strains we used other strains like strains with high antibiotic resistance or petri plate strains which are more dense than air suspended strains.

Regarding cost savings, numbers are given in a study by R. Raggi *et al.*, emphasizing a cumulative excess length of stay being reduced by 739.3 patient-days during intervention period with an estimated cost savings of $1,219,878 [25].

In conclusion, we presume that Far-UVC strongly supports our hypothesis that it will be more promising.

Conflicts of Interest

All materials, study budget and materials used in execution of this study were obtained from the sources of Istinye University Hospital. No value transfer has been made by manufacturers or third parties. Initus-V Far-UVC light source was supplied for trial purposes by Vestel Inc. (Manisa, Türkiye) - InnowayRG Inc. (Istanbul, Türkiye) consortium.

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