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Review Article

A Study on the Reduction of Airborne Microbial Bioaerosols at Indoor air of Hospital's Intensive Care Unit by Using Novel Air Filtration and UV Irradiation Technology

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Abstract

The monitoring of microbial quality and quantity of indoor air in Intensive Care Units is important to make progress at controlling nosocomial infections. This study's aim was to monitor the presence of pathogenic bacteria and fungi inside an operating Intensive Care Unit and to observe the real-time microbial reduction caused by the application of a prototype air filtration-UV irradiation sterilization device.

Air samples of the indoor air of a hospital's ICU operating room were taken using air sampler technology at opposing sampling spots. The colony formed units were counted, then isolated for the purposes of PCR and molecular sequencing procedures.

The microbial burden recovered from the initial sampling was counted at 141 cfu/m3 regarding the bacterial colonies, with species and sub-species of Pantoea, Priestia, and Bacillus genre having a dominant presence. As for the fungi, 112 cfu/m3 were counted, mainly from the Mucor and Aspergillus family. The testing of prototype UVC-ULTRAPURE displayed particularly promising results on the reduction of active airborne microbial particles, with the sterilization success rate being up to 70%, within 1 hour of the device's performance. The need of microbial monitoring in regular basis, inside hospital rooms should be considered, as patients are prone to secondary infections. Future research and surveillance assessing the importance of UVC radiation technology in terminating pathogens.

Keywords: real time reduction of airborne bioaerosols; air filtration; uvc-ultrapure technology

1. Introduction

At a worldwide pandemic such as COVID-19, bacteria and fungi are reported to cause co-infections to critically ill patients, which increases morbidity and mortality of the virus [1,2]. Airborne viruses, bacteria, and fungi spores usually exist in the form of Bioaerosols. A bio-aerosol is an airborne collection of biological materials. Ubiquitous indoors and out, bio-aerosols in suspended, aerosolized liquid droplets typically contain microbes, like viruses, bacteria and fungi combined with byproducts of cellular metabolism. Inhalation of microbial aerosols can elicit adverse human health effects including infection, allergic reaction, inflammation, and respiratory disease [3]. Hospitalization in ICU is highly associated with microbial infections, leading particularly, to ventilator-associated pneumonia (VAP) and bloodstream infections (BSI) [4]. Furthermore, it is observed that within the first few days after virus infection, critically ill patients often develop respiratory tract distortion or pulmonary dysbiosis, which can further progress into a secondary bacterial or fungal infection just a few weeks later [5].

The rapid and accurate identification of bacteria or fungi, that present as pathogenic or resident microorganisms inside hospital wards, during the period of COVID-19 should be an important step towards the management of patients, especially when there are several published papers [6, 7, 8] which

claim, that bacteria and fungi can act as vessels for viruses and by doing so, these microorganisms can demonstrate a coinfection ability (aiming their own proliferation) or can act supportively to virus's transmission. Another crucial factor for the reduction of the virus's spread is the de-contamination of the hospital's Covid-ward indoor air, by means which do not harm the patient and also do not impede the daily routine of the personnel.

In this work we performed a two-scale experimental procedure, which aim was to initially identify the genre of the microbial load inside a hospital's Intensive Care Unit and furthermore, to investigate the decontamination potential of a prototype air sterilization device, UVC ULTRAPURE, invented at the Department of Mechanical Engineering at the University of West Attica Greece. The device's operating principle is based on the fact that pathogens are being captured on the surface of a wide angle (WA) HEPA FILTER, where this new type of HEPA filter has the capacity to restrain 99.7% of pathogens (bacteria, fungi, and virus etc.) due to the 0.1 to 0.3 micrometer (μm) size of WA HEPA filter holes. It is worth to mention that the size of virus particles is at the scale of 0.1μm, but they are present almost always within larger droplets (>0.1μm), generated by infected hosts and therefore almost always contained by (WA) Hepa Filter.

Contrary to a regular HEPA filter which has dense folds, WA HEPA filter has larger angle between folds to avoid the creation of shading effects during its irradiation and therefore its entire surface can be continuously UV irradiated. Furthermore, the irradiation is also being reinforced by a quartz grid –optical grating which creates a multi – focus effect of UV light on both the surface and holes of HEPA through an optical multi diffraction effect. The capture of microorganisms and the subsequent UV continuous irradiation results in the total distraction of pathogen's organic material without leaving behind any organic residues on the filter. Consequently, any saturation of the filter will be due only to inorganic material (i.e., dust), thus eliminating the health risk related with the handling of filter replacement and disposal.

The need for search for a better UV technology air sterilizer aroused after realizing that most of the devices that are available in the market using UV irradiation for pathogen neutralization and destruction are using the technique of irradiating an air stream which passes through the device and contains these pathogens. According though to published results, the dwelt time of pathogens in the irradiated zone for these devices is extremely small, thus the UV radiation doesn't have the necessary time to kill these pathogens. To the contrary, the proposed new technology is not based on the irradiation of an air stream, but instead on the capturing first of the pathogens on a flat WA HEPA filter surface and subsequently through intense and continuous multi focused irradiation, eliminates them.

2. Methods

2.1 Monitoring of Microbial Load

The microbial load's identification process consists of several steps, starting with air sampling from the hospital's ward space, which had a size of approximately 90 m3 and was occupied by 2 patients at their recovery phase. Through air sampling, it is possible to evaluate microbial contamination in environments with considerable risk of infection. Microbial load measurements with air samplers are performed in accordance with the EU GMP for proper manufacturing principles for microbial monitoring of ambient air in controlled environments up to clean rooms [9]. The site's microbial load was measured by using MAS-100 air sampler (Figure 1), which consists of a radial fan, controlled by a flow sensor, that accurately regulates the real time air flow at 100 liters/min for a 10 min time interval. A specified amount of air is aspirated through a perforated lid and impacted onto the surface of growth media inside a 90-100 mm Petri dish. This procedure is called 'active air monitoring', in contrast with 'passive air monitoring', where the sampling is performed through gravity force only and is a more time-consuming technique [10].

Figure 1: ***The MAS-100 air sampler and Petri dishes positioning on the room and sampling.***

Two types of Petri dishes are used (Figure.1), one that contains TSA (Tryptone soya agar) growth medium for bacteria promotion and another with SAB (Sabouraud agar) medium for fungi promotion. After the standard Petri dish's incubation time (according to ISO 14698-1 instructions), the results found regarding the number of bacteria or fungi, are expressed in cfu (colony forming units) / m3. For a better estimation of the microbial load inside the ICU, air samples were taken in two distinct spots, the first one locating between the patient's head high and the second one at the room's center (Figure 2).

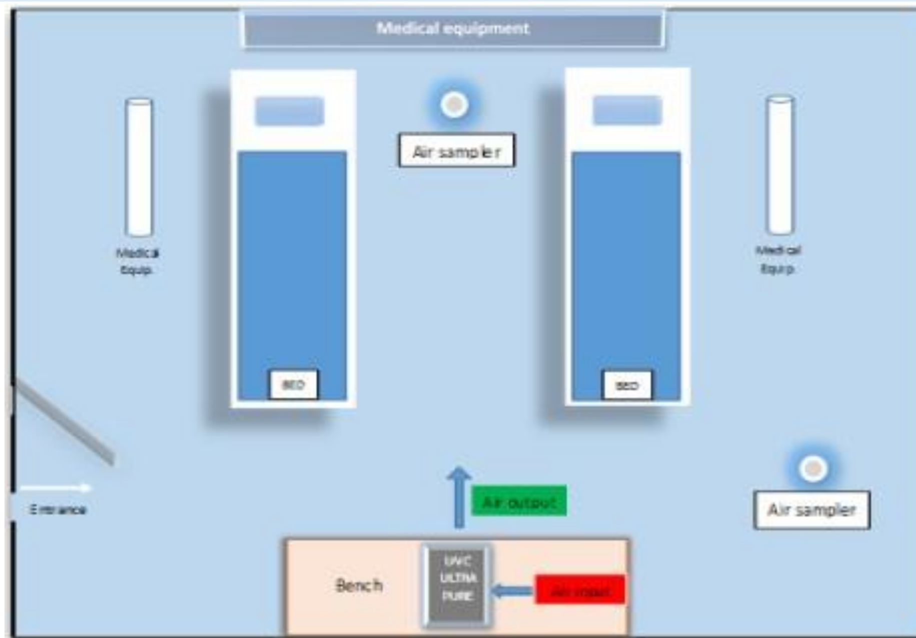

Figure 2: Graphical representation of the Intensive Care Unit (ICU). The air sterilizing device (silver) filtrates air input (red) through WA HEPA, radiates it with UVC and air output (green) is released back in the room.

All doors and windows were kept locked during the sampling, in order to keep the experiment conditions unaltered from exterior factors.

2.2 Decontamination Potential of a Prototype Air Sterilization Device

UVC ULTRAPURE, as the device is called, can filtrate air and apply UVC radiation using a novel patterned technique. The air passes through HEPA 13 filters, which have the ability of retaining microbial particles [13]. These particles are subsequently UVC irradiated, a procedure that can kill most of the retained microbes [14] and moreover, the emitted radiation does not encounter individuals around it, because of the device's steel plated protection. Herein we evaluated the performance of UVC ULTRAPURE prototype, which apply cutting edge technologies, mentioned in the above references.

The second scale of this work was to examine the sterilization potential of UVC ULTRAPURE, inside a rather contaminated environment, as an ICU theoretically is and at real life conditions (patients inside experimental area) between two different time points, 30 and 60 min after the device's application. These 30-minute interval between measurements was decided upon the fact that each measurement takes approximately 10 minutes to complete. As in the first sampling, two distinct sampling spots were used, specimens for TSA and Sabouraud plates were taken and also all doors and windows were kept shut. Following the sampling procedure, the plates were incubated at the appropriate conditions (32.5 °C for TSA plates and 22.5 °C for SAB).

2.3 Molecular Identification

The last step of the experiment's procedure was to identify the genre of these microbial colonies and by doing so to have an estimation of the microorganism's pathogenicity, which if existing, has a crucial effect on the patient's health and recovery. To achieve that, single colonies from agar plates were isolated and diluted in 2ml PBS, so that to be processed for DNA extraction using the Mag Core automated nucleic acid extractor, according to the manufacturer's protocol. DNA samples extracted from the TSA and Sabouraud agar plates were subjected to Polymerase Chain Reaction using primers targeting the 16S ribosomal RNA and 18S ribosomal RNA respectively. PCR products were analyzed using the QIAxcel Advanced System. All three DNA samples extracted from TSA agar plates colonies were found positive for 16S ribosomal RNA and 2 out of 5 DNA samples extracted from Sabouraud agar plates colonies were found positive for 18S ribosomal RNA. Once validated, the PCR products were purified using the QIAquick PCR purification kit according to the manufacturer's protocol. DNA concentration was measured using the Nanodrop spectrophotometer. Sanger sequencing was performed in 120-150 ngr/ reaction of DNA using the internal primers 536F, 536R, 800F, 800R, 1050F and 1050R for 16S ribosomal RNA as previously described and the NS5F, NS6R for the 18S ribosomal RNA. Sequencing data were processed using the BioEdit Software and consensus sequences were analyzed by BLAST, with over 90% successful identification rate for each of the isolated microbial colonies that were recovered.

3. Results

At the end of incubation period the number of microorganisms before and after the application of the prototype device was recorded and also the percentage for the reduction of microorganisms due to the use of the sterilization system, was measured. From the mean values of sampling, came the following results of air samples measurements (Figure.3).

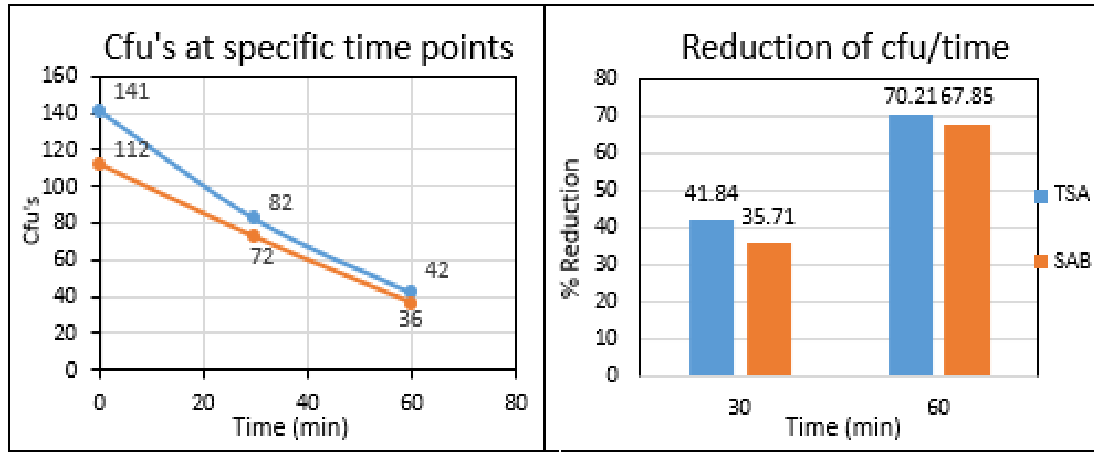

Figure 3: Representation of the reduction of counted colonies and of % cfu's reduction as sterilization time proceeds (cfu/time) by air samples Measurements of bacterial and fungal colonies counted at standard time points before and after device's application.

Specifically, it was found that the device contributed to a 70.21% reduction in bacterial load, as well as a 67.85% reduction in fungal load. At this point, it is worth mentioning that our initial measurements (before the application of the sterilization system) present to us a space with a fairly heavy microbial load, as for both bacteria and fungi more than 100 cfu / plate were retrieved for 1 m3 of air sample. After 1 hour of the device's application and with 4 people always present inside the room (2 patients and 2 research analysts) one can observe that the sterilization system proceeds to a steadily reduction of the air's microbial load.

According to the number of cfu/m3 that were recovered from the sampling procedure, it is safe to claim that the ICU's microbial load was relatively high, in relation to clean room standards for GMP (Good Manufacturing Practice), which is justified when patient's contamination biomaterial is constantly spreading inside the room. The results from BLAST sequencing which came out, showed that the recovered colonies from sampling belonged to the following genera-species of bacteria and fungi (Table 1 and Table 2).

Bacterial colonies	
• *Pantoea agglomerans*	• *Priestia flexa*
• *Pantoea vagans*	• *Neobacillus ginsengisoli*
• *Pantoea deleyi*	• *Bacillus halotolerans*
• *Priestia megaterium*	• *Bacillus mojavensis*
• *Priestia aryabhattai*	• *Bacillus subtilis*
• *Priestia qingshengii*	

Table 1: Genera-Species of Bacteria from the recovered Colonies of Sampling.

The habitat of the recovered bacterial species is placed either in the microfauna of the human digestive system, or in the external environment (coming from the soil) or found in certain foods (such as vegetables). Due to the nature of the experimental field (ICU unit), it was considered necessary to examine the pathogenicity of the identified microorganisms in the human immunological system. As previously mentioned, among the bacterial genera recovered there were several species-representatives of the genus Pantoea, which [11] are potentially pathogenic in immunocompromised patients. Examining the rest of the bacterial genera (Priestia, Neobacillus and Bacillus) no specific reference to the existence of pathogenicity was found, but this does not exclude the occurrence of pathogenicity in case of an overpopulation of the said bacterial genera.

Fungal colonies	
• *Mucor lanceolatus*	• *Mucor janssenii*
• *Mucor circinelloides*	• *Mucor griseocyanus*
• *Mucor racemosus*	• *Circinella simplex*
• *Mucor spinosus*	• *Aspergillus niger* (or *brasilliensis*)

Table 2: ***genera-species of fungi from the recovered colonies of sampling.***

The fungal genera-species that were recovered are all microorganisms of wide and even global distribution, coming mainly from food, from the microbiome of the human skin, or from the external environment. Regarding their potential pathogenicity, the genus Aspergillus [12], in specific conditions of aspiration of large amounts of spores of the fungus, can lead to the appearance of aspergillosis, a serious disease of the respiratory system. The species of the genus Mucor do not show pathogenicity in humans, due to their lack of ability to reproduce at 36-37 ℃, apart from some thermo-resistant strains, which were not found in the fungi we examined.

4. Discussion

Admittedly, this study faced limitations regarding the repeatability of results, which can be justified due to bureaucratic issues that made it difficult to repeat the experiment. The aim of this study, as mentioned above, was to initially perform a monitoring of microbiological material inside an ICU and furthermore to assess the sterilization potential of UVC ULTRAPURE device. As for the first step, the results from the air sampling indicate that inside the ICU the microbial load was relatively high, something that can be justified from the existence of 2 patients inside the room. Although the

hospital's personnel made efforts to keep the environment clean with daily cleaning procedures, the continuous emission of biological material from the patients and the importation of microbes from external environment, kept the bioburden at high values. According to the molecular sequencing of colonies recovered from TSA and SAB plates, a range of potential pathogenic bacteria and fungi was found, which can (under specific circumstances) have a severe impact on the immune system of already immunosuppressed patients. Those findings strengthen the opinion that the conventional means of cleaning are insufficient and extra sterilizing of the ICU is indeed needed.

5. Conclusion

The prototype UVC ULTRAPURE sterilization device had undergone before similar testing inside areas of public interest (classroom, bus, metro) with worth mentioning results, achieving microbial reduction at a range of 45 up to 93% within 30 minutes of system's application [15]. In this work the device was evaluated inside a specific nosocomial area (ICU) with continuous feedback of biological matter coming from the patients and the results were once more encouraging with an estimated reduction up to 70% within 60 minutes of usage. These results imply that, despite the presence of patients and the personnel conducting the experiment, the device has a sterilization potency which is not hindered by the emission of an amount of biological matter inside the experimental zone.

Author Contributions: All authors have contributed to this work equally, both in the execution of the experiment and in the writing process.

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Conflicts of Interest: "The authors declare no conflict of interest.

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