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Susceptibility of Bacteria Isolated from Air-Conditioning System Ducts to Different Ultraviolet Radiation Doses

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Abstract

Several bacteria exist in the air that pass through the ducts of air-conditioning systems. This study aimed to determine the effect of ultraviolet germicidal irradiation (UVGI) on six bacterial isolates (*Serratia liquefaciens, Bacillus pumilus, Bacillus cereus, Staphylococcus lentus, Bacillus subtilis,* and *Oligella ureolytica*) from the in-duct of an air-conditioning system. Bacterial species were exposed to UVGI with a wavelength of 264 nm for different durations. The exposed samples were then cultured and evaluated for growth. Growth was evaluated based on the colony-forming units, and the number of bacterial cells was estimated using spectrophotometry. Thereafter, the susceptibility value (Z value) to the UVGI was calculated for each bacterium. Each bacterium showed a different Z value under the same UVGI conditions, depending on the gram characteristics of the bacterium. The findings confirmed that UVGI is a potent technique for reducing the growth rate of multiple species of bacteria isolated from air-conditioning systems and this reduction depends on the structure of the bacteria (gram +ve and gram -ve) as well as exposure time. UV lamps can be placed in air-conditioning ducts to reduce or prevent the transmission of bacteria into the environment.

Keywords: Ultraviolet Radiation, bacteria, susceptibility, Air-Conditioning System.

INTRODUCTION

Living in a healthy environment is an absolute necessity for a wholesome human life. However, this target cannot be achieved without sterilizing the air that passes through the ducts of the now widely used air conditioning (AC) systems, since these ducts connect the outdoor and indoor environments and allow bacteria and other harmful microorganisms to reach human bodies and cause health problems (Thrasher and Crawley 2009; Cowling *et al.* 2013 and Antusheva *et al.* 2016)[1–3].

There is a HEPA filter that has a highly efficient to capture the airborne microbes of different diameters to provide a fresh and clean air[16,21]. The major problem in this technic is once the AC turns off the microbes will find an excellent environment to increase because this filter does not kill the microbes only keep them from passing to the indoor. In contract, using the ultraviolet germicidal irradiation (UVGI) in the AC duct eliminates the airborne microbes (Bintsis et al, 2000; VanOsdell and Foarde 2002; Rudnick *et al.* 2009 And Ryan *et al.* 2010)[4–7], especially in laboratories and medical facilities that require high standards of air quality (Brickner *et al.* 2003)[8]. The wavelength of the light used in this study is in the greatest absorption range for DNA of microbial cells, that is, 260–265 nm (Courcelle and Hanawalt 2001)[9].

Lopez-Malo & Palou 2005 reported that the bactericidal effect of ultraviolet (UV) radiation is due to the shift in electrons caused by photons, which breaks the links in DNA (Lopez-Malo and Palou, 2005)[10]. Furthermore, UV irradiation can reduce the indoor concentration of airborne bacteria (Kujundzic *et al.* 2006)[11]; it has been observed that placing a UV light source in the duct of air conditioning systems can reduce the microbial concentration by 99.9% (Yassin and Almouqatea 2010)[12].

Different doses of UV radiation are required to cause damage to the different types of microbes in the air (Hijnen *et al.* 2006)[13]. Lai *et al.* studied the UV radiation dose versus the survival of *Serratia marcescens* bacteria in 5 different suspending media and recognized that they were most susceptible to UV irradiation in water media (Lai *et al.* 2003)[14]. Another study examined the disinfection efficacy of different UV intensities on five airborne pathogens and experimentally determined the Z-values (Yang *et al.* 2018)[15].

This study aimed to use an effective and safe technology for air disinfection by applying ultraviolet germicidal lamps in air-conditioning ducts. To improve the efficiency of indoor air quality and for whole room disinfection, the effect of UVGI on five bacteria (*Serratia liquefaciens, Bacillus pumilus, Bacillus cereus, Staphylococcus lentus, Bacillus subtilis,* and *Oligella ureolytica*) that have not been studied for their susceptibility value (Z-values) to UVGI, was studied.

MATERIALS AND METHODS
Experimental design

As shown in Fig. 1, a small wooden chamber (70, 50, 50 cm) consisting of a UV lamp (TOSHIPA/ 15WATTS GERMICIDAL GL 15, the output power = 4.9 W) emitting light with wavelength of 264 nm was used for exposing the

Fig. 1. The wooden chamber containing the UV lamp in its ceiling and the bacteria sample placed at the bottom.

bacteria to UV (Fig. 1). The lamp was placed on the ceiling of the chamber, 15 cm from the bacterial samples.

Six species of bacteria (*S. liquefaciens, S. lentus, B. subtilis, B. cereus, O. ureolytica,* and *B. pumilus*), which were the most abundant bacteria in the AC duct, were isolated during the summer season in 2009, from air conditioning systems (homes, schools, hospitals, and shopping centers) in the eastern region of Saudi Arabia (Al-abdalall and Al-abkari 2017: Al-abdalall *et al.* 2019)[16-17], and cultured on nutrient agar media for 24 h at 37°C. Bacterial isolates were preserved purely on NA slants.

Purification & identification of isolates
These bacterial strains were identified in the laboratories of Qatif Hospital using BD Phoenix, an automated system for rapid identification of bacteria and for determination of antimicrobial susceptibility to antibiotics. Up to 100 phenotypic tests lead to the ID of microorganisms.

Six bacterial isolates were inoculated in nutrient broth medium separately, and incubated at 37°C for 24 h. Then, bacterial suspensions in 2 mL of Tween 80 per 100 mL distilled water were prepared by transferring 1 ml of the above-mentioned solution to a test tube content 9 ml of the microbial liquid culture and this step was repeated twice to obtain a dilution (10^{-2}). After the exposure, 0.1 mL of the bacterial suspensions was transferred to tubes containing 5 mL of mannitol agar (10 g tryptone + 10 g yeast extract + 5 g mono hydrogen dipotassium phosphate + 3 g mannitol+20 g agar) media1 and incubated at 37°C for 24 h. (Al-abkari 2014)[18].

The UV light dose (Depends on the power of the light and light running time) was calculated using the following equation:

$$D = \text{Intensity of UV} \left[\frac{w}{cm^2}\right] \times \text{Time of Exposure (s)}$$

$$...(1)$$

Where the intensity could be determined from the power of the lamp and the distance between the lamp and the bacterial sample as shown below:

$$I = \frac{P}{4\pi^2} = \frac{4.9[W]}{4 \times 3.14 \times (15\ cm)^2} = 0.0017 \left[\frac{W}{cm^2}\right]$$

$$...(2)$$

Experimental procedure
The bacteria samples were exposed to UV light for different exposure times (5, 10, 15, 20, 60, 180, and 360 min). The experiment was repeated three times and the average of the bacterial concentrations (number of colonies) was calculated using SPSS 16. The concentrations of the bacteria before and after UV exposure were used to calculate the susceptibility (Z) of the bacteria to UV exposure (245 nm)

The number of bacterial cells estimated was measured as described before (Didier *et al.* 2001)[19], using a spectrophotometer to measure the absorbance at a wavelength of 600 nm, and then compared with those of the untreated control
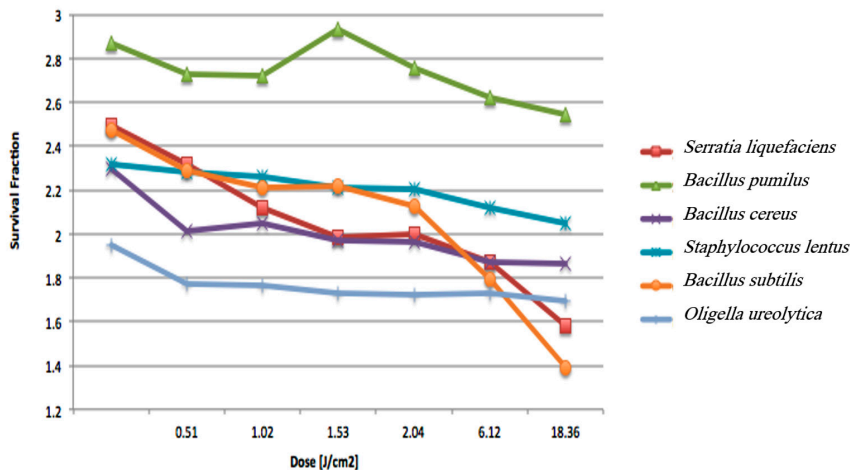
Fig. 2. Survival fraction versus UV dose for the six bacteria isolated from the in-duct of air conditioning systems

group.

The survival fraction F_R was calculated as follows:

$$F_R = \frac{C_{UV}}{C_{NO\ UV}} = e^{-ZD}$$

...(3)

$$Z = \frac{1}{D}\ \log\ \frac{C_{NO\ UV}}{C_{UV}}$$

...(4)

where, C_{UV} and $C_{No\ UV}$ are the bacterial concentrations after and before UV irradiation, respectively, D is the dose of UV that the bacteria received, and Z is the susceptibility expressed in [cm^2/J].

Statistical analysis

Statistical analysis (mean±SD) was carried out using the SPSS 16 software to compare the average Z-values. A *p* value < 0.05 was considered as being a statistically significant difference (George and Mallery 2016) [20].

RESULTS AND DISCUSSION

The results of this study indicated that UV irradiation inhibits the growth of *S. liquefaciens*,

Table 1. Estimated Z-values for *S. liquefaciens*, determined at different times at a 15-cm distance of exposure

Time [s]	Dose [J/cm^2]	$\log\ \frac{C_{NO\ UV}}{C_{UV}}$	Z-value [cm^2/J]
300	0.51	1.09	2.141±0.017
600	1.02	1.189	1.165±0.013
900	1.53	1.332	0.871±0.014
1,200	2.04	1.462	0.717 ± 0.033
3,600	6.12	1.443	0.236±0.006
10,800	18.36	1.592	0.086±0.000
21,600	36.72	2.177	0.059±0.002

B. subtilis, B. cereus, S. lentus, O. ureolytica, and *B. pumilus*, and that this inhibition increases upon longer exposure. The irradiance of the UV lamp is a critical variable and influences the bactericidal activity of UV light (Didier *et al.* 2001)[19].

The susceptibility values of the bacteria investigated in this study were smaller than those reported in other studies regarding the difference in the light conditions. In a previous study, the Z value of *B. subtilis* was 200 cm^2/J (Rudnick *et al.* 2009)[6], which is larger than the observation for this bacterium (2.5 cm^2/J) in this study. However, the difference in the Z values could be due to the low UV dose of 0.007509 J/cm^2 used in the previous study compared with the UV dose of 0.51 J/cm^2 used in the current study. The light dose is strongly related to the ability of the bacteria to live longer. Therefore, it makes sense that when the UV dose decreases by two numbers, the Z-value increases by two numbers.

For the Enterobacteriaceae family, Lai *et al.* 2003[14] reported the susceptibility (Z value) of *Serratia marcescens* to be 22000 cm^2/J. In contrast, in this study, *Serratia liquefaciens*, which also belongs to the Enterobacteriaceae family, showed a Z value of 2.137 cm^2/J. The huge difference in the Z values might be attributed to the large difference in the exposure time and the UV dose used. In the previous study, the exposure time was 7.6 s and the dose used was 0.000342 J/cm^2 Lai *et al.* 2003[14], whereas in the current study, the exposure time was 300 s and the dose used was 0.51 J/cm^2.

The Z values indicate the resistance of these bacteria to UV light, with larger Z values suggesting higher resistance. Increasing the time of exposure to the UV light increases the number of photons hitting a bacterial cell, subsequently enhancing the probability of photons reaching the bacterial DNA and damaging it (Courcelle and

Table 2. Z-values for different bacteria for 300 s of UVGI exposure at 15-cm distance of exposure

Microorganism	Gram stain	Z value [cm^2/J]	Family
Serratia liquefaciens	-Gm	2.141±0.017	Enterobacteriaceae
Staphylococcus lentus	+Gm	1.858 ± 0.009	Staphylococcaceae
Bacillus subtilis	+Gm	2.359 ± 0.061	Bacillaceae
Bacillus cereus	+Gm	2.335 ± 0.036	Bacillaceae
Oligella ureolytica	-Gm	2.162 ± 0.000	Alcaligenaceae
Bacillus pumilus	+Gm	2.944 ± 0.011	Bacillaceae

Hanawalt 2001)[9]. When the UV radiation breaks the bonds in bacterial DNA, a new bond between the nucleotide prevents the replication of DNA and that leads to bacterial cell death. At low doses, cells would not die, but accumulate mutations (Courcelle and Hanawalt 2001)[9.] The Z value of *S. liquefaciens* decreased from 2.137 to 0.059 cm^2/J when the dose of light increased from 0.51 to 36.72 J/cm^2, indicating that the bacteria became more susceptible as the UV dose increased.

The structure of bacteria, particularly the thickness of the cell wall, is an essential factor that significantly affects their airborne survival under the effect of UV radiation. Each bacterium needs to be examined separately for its airborne survival under UV irradiation due to differences in the structure of bacteria (Tang 2009)[21]. Table 2 shows the susceptibility of different gram-negative and gram-positive bacteria, which reflect structural differences among the bacteria; the susceptibility ranged from 1.843 to 2.943 cm^2/J. Notably, gram-negative bacteria, such as *S. liquefaciens* and *O. ureolytica* showed low Z values of 2.137 and 2.162 cm^2/J, respectively, which is likely due to the presence of a thinner cell wall in such bacteria (Beveridge 2009)[22]. In contrast, the species of the family Bacillaceae, which comprises gram-positive bacteria, had the highest Z values and were, therefore, more resistant than the other tested bacteria. Thus, the gram characteristics of a bacterium can help to determine the dose of UV radiation required to inhibit its growth.

CONCLUSION

The current study indicates that UV irradiation has the ability to reduce air pollution by reducing the concentration of airborne bacteria. This effect of UV depends on the dose of UV used as well as on the bacterial structure. Since multiple bacteria, possibly pathogenic, exist in ducts of air conditioning systems, it would be a good practice to place UV radiation lamps in the duct to sterilize the air passing through the duct and, thereby, minimize or prevent the transmission of airborne bacteria into indoor spaces.

SIGNIFICANCE STATEMENT

This study discovered that UV radiation can be used inside AC ducts to eliminate the bacteria present inside, which can be beneficial for the indoor environment. This study would help build AC ducts with the exact value of light doses that can prevent bacterial growth and avoid wasting electricity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

AHA: conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft, contributed reagents/materials/analysis tools; SAA: analyzed the data, prepared figures and/or tables, reviewed drafts of the paper, and approved the final draft; HAA: designed and performed experiments, contributed reagents/materials/analysis tools.

DATA AVAILABILITY

Not applicable.

ETHICS STATEMENT

Not applicable.

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