EFFECTS OF ULTRAVIOLET GERMICIDAL IRRADIATION OF ROOM AIR ON AIRBORNE BACTERIA AND MYCOBACTERIA

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ABSTRACT

As a result of the recent resurgence in tuberculosis (TB), there has been renewed interest in engineering controls to reduce the spread of TB and other airborne infectious diseases in high risk settings. This paper presents the results of experimental studies evaluating the efficacy of ultraviolet germicidal irradiation (UVGI) of room air in reducing the concentration of viable airborne bacteria. Bacterial particles (*Bacillus subtilis* spores and *Mycobacterium parafortuitum*) were continuously generated in a 90 m^3 room. The room was equipped with germicidal lamps, a computer-controlled ventilation system, and temperature and relative humidity controls. Air samples were collected using impingers at 11 locations within the room. Concentrations were quantified using standard culturing and colony plate counts. The effectiveness of the control technique was determined by comparing concentrations of culturable airborne bacteria with and without the control in operation. The germicidal lamps that were evaluated reduced the room-average concentration of culturable airborne bacteria, typically by 40–95% depending on the microorganism used in the experiments, relative to concentrations with no lamps in use.

INTRODUCTION

Many infectious diseases are spread through the air including resurging tuberculosis (TB). Engineering controls are important tools to reduce transmission in many settings [1]. Ultraviolet germicidal irradiation (UVGI) has been proposed and is employed for TB control in healthcare facilities and where persons with unrecognized active disease may be present [2, 3] because of its low cost, ease of application, and potential efficacy to substantially reduce the concentration of airborne microorganisms [4].

UVGI is a form of nonionizing electromagnetic radiation within the UV-C spectrum, <280 nm [5]. Low-pressure mercury-vapor lamps used for germicidal applications predominantly emit radiation at 254 nm. The optimal wavelength for microbicidal effectiveness ranges from 250–270 nm, depending on the microorganism [6].

A room is irradiated by mounting germicidal lamps on walls near the ceiling or suspending them from the ceiling. The emissions from germicidal lamps are directed horizontally and toward the ceiling rather than into the occupied part of a room to inactivate airborne microorganisms in the upper part of the room while minimizing radiation exposure to persons in the lower part of the room. Microorganisms that are exposed to UVGI may be damaged, the effect depending on the dose the cells receive and the lethal dose for each agent.

To expand the limited experimental data on the effect on airborne bacteria of irradiating room air, we conducted experiments quantify the damage UVGI caused to two airborne bacteria. We designed and equipped a chamber with five wall-mounted germicidal lamps, a bacterial aerosol generation system, a computer-controlled ventilation system, temperature and relative

humidity controls, and multiple air sampling locations. Two different surrogate bioaerosols were released—*Bacillus subtilis* (spores) and *Mycobacterium parafortuitum*. With and without the UVGI and ventilation system operating, time-averaged airborne particle concentrations were measured at many points. UVGI efficacy was determined by comparing measurements of the concentration of culturable airborne bacteria with and without UVGI.

METHODS

Test Chamber. A simulated, full-scale health-care room was established at the Joint Center for Energy Management's Larson Building Systems Laboratory, University of Colorado at Boulder (Figure 1). A 90-m^3 chamber housed inside the laboratory was used for testing. The room has a natural infiltration rate of 0.1–0.3 air changes per hour (ACH, h^{-1}). The floor-to-ceiling height is 2.4 m and contains 37 m^2 of clear floor area. The room is capable of maintaining 15–35°C and providing a constant relative humidity from 50–90%. The room has insulated walls, a raised floor, plenum ceiling, one door and no windows. It is furnished with a heated mannequin (108 W) seated in a chair in the middle of the room to simulate a person. The test room is equipped with a computer controlled ventilation system that delivers 2–8 ACH of high-efficiency-particulate-air (HEPA) filtered outside air through two circular diffusers in the ceiling. Air is exhausted through two ports also located in the ceiling. To minimize particles exiting the test room, HEPA filters are installed within the exhaust ducting. The mechanical ventilation system was operated to maintain negative pressure within the room. Two box fans are located on opposite walls to enhance room air mixing.

Germicidal Lamps. The germicidal lamp system (Lumalier, Memphis, TN) consists of five luminaries: four mounted in each corner and one hung from the center ceiling (Figure 1). The corner and center luminaries have two and three 9-W lamps respectively. All luminaries have concentric black-painted louvers that generate a band of UVGI with a width of 30 cm in the upper portion of the room. A newly developed and tested technique based on spherical chemical actinometry was used to measure the irradiance in the upper zone of the room in an omnidirectional manner [7].

Bioaerosol Generation. The test aerosol was generated using a six-jet Collison nebulizer (CN 25, BGI, Inc.). Bacterial particles were generated from a suspension of microorganisms in sterile, distilled water containing 10^6–10^8 colony-forming units (CFU's) per ml. Two microorganisms were used: *Bacillus subtilis*, a Gram-positive bacterium and *Mycobacterium parafortuitum*, a rod-shaped fast-growing mycobacterium. To ensure a stable particle size distribution and consistent bacterial concentration over the course of the experiments, the particle suspension in the nebulizer was replaced with fresh solution every 30 min.

Bioaerosol Sampling. To experimentally characterize the spatial distribution, airborne bacteria were sampled with AGI-30 impingers at eleven room locations. Nine of the sampling locations were positioned in the breathing zone, 1.6 m above the floor. One was located near the ceiling and one near the floor. Aerosol was collected for 30 min. at 12 L min^{-1} with a high-flow sampling pump regulated with rotameters. The impingers concentrate the bacteria in 15 ml of sterile, distilled water with minimal damage to the organisms. Before each experiment, the pump and rotameters were calibrated using a bubble meter. Triplicate impingers collected aerosol at every location for two types of analysis: counting of CFU's and epifluorescent microscopy.

The particle size distribution of culturable airborne bacteria was measured using a six-stage, multiple-hole impactor (Grasby Anderson). This impactor samples at 28.3 L min^{-1} and has a cut size of 0.6 μm on the last stage. Plain gelatin with glycerin and water was used for the plate medium, instead of the usual agar medium, allowing for longer, more accurate, sample collection without concerns about overloading the plates.

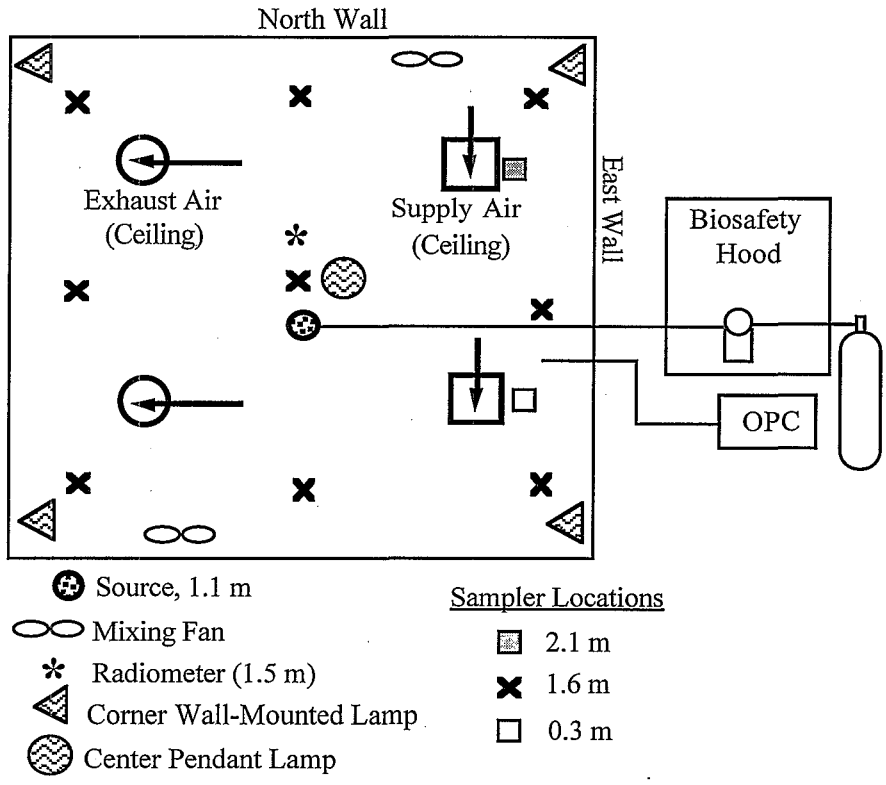

FIGURE 1. Configuration of 90 m^3 test room (plan view). Heights above the floor are given. OPC is an optical particle counter, biosafety hood contains the aerosol generation system.

Bioaerosol Analysis. Two independent methods were used for aerosol quantitation: (i) culturing and colony plate counts and (ii) epifluorescent microscopy. The culturing method identifies the concentration of bacteria that are culturable and able to repair or replicate. Epifluorescent microscopy uses sensitive, biological stains to measure the total numbers of airborne microorganisms.

Experimental Protocol. Eight experiments were conducted (Table 1) using the following protocol. After cleaning the test room by supplying HEPA-filter outside air via the ventilation system, the room was put under negative pressure and the ventilation and germicidal lamp configurations were established. The box fans were turned on to ensure room air mixing. The room door was closed and sealed to reduce infiltration. Aerosol generation was started and continued for 90 min. after which air samples were collected. During the "No Ventilation" experiments, aerosol concentrations continuously changed with time. During the "Mechanical Ventilation, 6 ACH" experiments, aerosol concentrations approached steady state after 30 min. Every pair of "Lamps On/Lamps Off" experimental scenario was replicated at least once.

Typically, for a given ventilation configuration, the experiment in which the lamps were on was conducted in the morning, followed by the experiment with the lamps off in the afternoon. All factors were kept constant between these two experiments, including the bacterial suspension concentration, except the level of UVGI. Each sequence of experiments was then repeated.

Table 1. Experimental Scenarios

Test Organism	Ventilation Configuration	Germicidal Lamp Configuration
B. subtilis	No Ventilation	Lamps On
B. subtilis	No Ventilation	Lamps Off
B. subtilis	Ventilation, 6 ACH	Lamps On
B. subtilis	Ventilation, 6 ACH	Lamps Off
M. parafortuitum	No Ventilation	Lamps On
M. parafortuitum	No Ventilation	Lamps Off
M. parafortuitum	Ventilation, 6 ACH	Lamps On
M. parafortuitum	Ventilation, 6 ACH	Lamps Off

RESULTS

UV Spherical Irradiance with Actinometry. The energy flux in the upper zone of the room (2.1 m) varied from one part of the room to another, peaking near the germicidal lamps at 61-87 μW per cm^2 and decreasing to 23-36 μW per cm^2 between corner and center lamps. The room-averaged fluence rate in the upper zone was 43 (SD = 20) μW per cm^2.

Effectiveness of UVGI. Effectiveness is a relative measure of the impact of UVGI on average airborne bacterial concentrations, C, and is defined as $E = 1-(C_{Lamps\ On}/C_{Lamps\ Off})$. The germicidal lamps that were evaluated were effective, typically reducing the average concentration of culturable airborne bacteria by 40–95% (Figure 2). The effectiveness of UVGI-induced inactivation was dependent upon the microorganism used in the experiment and the ventilation configuration.

Particle Size. Impactor data showed that most of the particles were between 0.65 and 2.1 μm (d_{50}'s for impactor stages 4 through 6). As the particle size decreased from 2.1 to 0.65 μm the effectiveness of UVGI-induced inactivation increased by 30–50%. Many factors could explain why larger particles may have lower inactivation, including larger particles are made up of more cells, larger particles have lower mean room residence times, or larger particles evaporate slower compared to smaller particles.

Ventilation. Increasing the air-exchange rate from roughly zero to six ACH decreased the effectiveness of UVGI-induced microbial inactivation by 7–31%. The concentration of culturable airborne bacteria was lower at the higher air-exchange rate, but the average particle room residence time was also lower resulting in less exposure to UVGI.

Total Number Concentration. The number of bacteria aerosolized (10^6–10^8 m^{-3}) was statistically the same (independent *t*-test at the 0.05 level) during the UVGI, based on direct microscopy. The concentration of culturable airborne bacteria was less than the concentration of total airborne bacteria by up to an order of magnitude, suggesting that the bacteria were stressed through the aerosolization process or lost culturability while airborne.

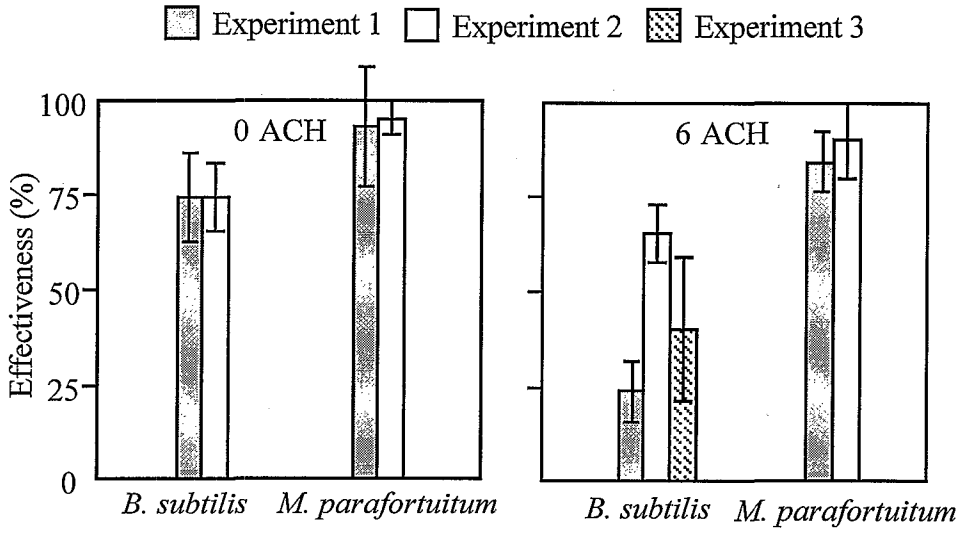

FIGURE 2. Effectiveness of UVGI-induced microbial inactivation for experiments with 0 ACH or 6 ACH ventilation for *B. subtilis* and *M. parafortuitum*. All experiments were replicated two times (no factors changed) except for *B. subtilis* at 6 ACH, which was repeated 3 times.

DISCUSSION

Wells reported the first observation of the efficacy of room air UVGI in 1934 [8]. More recent data suggest that open-air irradiation can achieve large rate constants for inactivation of TB, 10–25 h^{-1}, depending on the total power of the UV lamps [9]. Goldner et al. measured approximately half the concentration of viable, ambient airborne bacteria in two operating rooms when ceiling-mounted lamps were operated [10]. Macher et al. saw a 14–19% reduction in the concentration of viable, ambient bacteria in a well-ventilated hospital waiting room equipped with wall-mounted lamps [11, 12]. Miller et al. saw an effectiveness of 50% for *B. subtilis* spores in a full-sized test chamber equipped with a wall-mounted germicidal lamp and 2 ACH ventilation [13].

Our results show that UVGI plus ventilation can achieve reductions of culturable airborne bacteria, and therefore the potential risk of infection, with effectiveness typically ranging from 40% to 95%. There are, however, many factors that significantly influence the effectiveness of UVGI that our research group will be studying further, including the degree of room air mixing, different ventilation configurations and levels of UVGI, and the type of microorganism. Perhaps more importantly, given that, in theory, infection can occur when a susceptible person inhales a single infectious particle, which is subsequently, deposited deep in the lungs, the degree of protection documented in this study may not suffice for infection control. Complementing UVGI with source-oriented controls may be needed to ensure a very low risk for infection in high-risk settings.

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