Disinfection of *Acinetobacter baumannii*-Contaminated Surfaces Relevant to Medical Treatment Facilities with Ultraviolet C Light

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The efficacy of ultraviolet C (UVC) light (100–280 nm) in the decontamination of three hospital-related surfaces, namely, unpainted/painted aluminum (bed railings), stainless steel (operating tables), and scrubs (laboratory coats), was investigated. *Acinetobacter baumannii* cells were inoculated (10^5 or 10^3 cells) on small coupons and dried overnight in a class II biosafety cabinet. Drying resulted in ≤50% loss of viability. The UVC fluence of 90 J/m^2 was observed to be very effective in the decontamination of cells from all metal coupon surfaces (complete killing). However, the same fluence was ineffective in the decontamination of scrubs. The effectiveness of two other common disinfection practices, that is, 15 minutes of boiling or spraying with 70% ethanol, was investigated for the scrubs. Although ethanol treatment was ineffective, the boiling treatment was very effective (complete killing). These results establish that metal surfaces can be decontaminated with UVC irradiation and boiling treatment is effective for scrub decontamination.

Introduction

The emergence and rapid spread of multidrug-resistant isolates causing nosocomial infections have been of alarming concern in recent years.[1] An increasing number of *Acinetobacter baumannii* bloodstream infections in patients at military medical facilities, especially intensive care units, have been reported. Because this bacterium has developed resistance to antimicrobial agents, infection control and disinfection of medical treatment facilities are paramount in ensuring low *Acinetobacter*-related mortality rates. Although infected patients are often the sources of *A. baumannii* infections in health care settings, the ability of this organism to survive for extended periods on environmental surfaces is likely a major contributor to protracted outbreaks in medical treatment facilities.[2]

A. baumannii is a pleomorphic, aerobic, Gram-negative bacterium (similar in appearance to *Haemophilus influenzae*) commonly found in water and soil. It generally is a colonizer of low virulence, but it is capable of causing infections. The bacterium commonly colonizes skin, oropharyngeal secretions, respiratory secretions, and urine; it uncommonly colonizes the gastrointestinal tract and is associated with nosocomial pneumonia, bacteremia, and wound infections. Therefore, *A. baumannii* is commonly referred to as a "hospital opportunist." In one study, the length of survival of a number of Gram-negative bacteria, including *Serratia marcescens*, *Proteus mirabilis*, *Acinetobacter* spp., *Klebsiella pneumoniae*, and *Enterobacter* spp., on seven common fabrics and plastics was investigated.[3] The survival times of bacteria ranged from 1 hour to 8 days at a low inoculation level (~10^2 cells per swatch). At a higher inoculation level (~10^5 cells per swatch), however, the survival times ranged from 2 hours to 60 days. On the basis of these findings, careful disinfection and conscientious contact control procedures in medical treatment facilities are clearly important.

Ultraviolet C (UVC) irradiation (100–280 nm) is most commonly associated with the disinfection of liquids, particularly water. However, there has been an interest in its application for disinfection of surfaces and foods. Ultraviolet germicidal irradiation is a recognized method of inactivating a wide range of biological agents.[4] The efficacy of ultraviolet irradiation is a function of many different locational and operational factors, including intensity, exposure time, lamp placement, and air movement patterns.[5,6] Microorganisms are particularly vulnerable to UVC light of ~254-nm wavelength, because DNA maximally absorbs ultraviolet light in this region, resulting in formation of thymine dimers and other lethal photoproducts.[7]

Methods

Bacterial Strain, Culture, and Viability

A culture of *A. baumannii* (Walter Reed Army Medical Center no. 652) was procured from Walter Reed Army Medical Center. The culture was streaked for single colonies on tryptic soy agar plates and was incubated at 37°C for 24 hours. A single colony was inoculated into 10 mL of tryptic soy broth and cultured at 37°C for 24 hours. Several glycerol stocks were prepared from this culture and frozen at −80°C.

Using a vial of frozen glycerol stock, a tryptic soy agar plate was streaked for single colonies. A flask containing 500 mL of tryptic soy broth was inoculated with a single colony and cultured at 37°C for 24 hours. The cells were concentrated by centrifugation (5,000 rpm for 15 minutes at 4°C) and resuspended in 0.1 volume of dilute (0.5%) buffered peptone water (BPW). The cells were washed twice in BPW, suspended in 20 mL of BPW, and then stored at 4°C in a sterile tube. The viability of the cells was determined for a 2-week period of storage at 4°C. Figure 1 shows a summary of the titers of *A. baumannii* cells in suspension that were stored at 4°C for a >2-week period.

Coupon Preparation and Cell Inoculation

Hospital-relevant surfaces included painted and unpainted aluminum, unpainted steel, and scrub (laboratory coat material). Small pieces (2 × 1 cm), referred to henceforth as coupons, were cut, and metal coupons were precleaned with 70% ethanol and dried. Scrubs and precleaned metal coupons were sterilized through autoclaving in glass Petri plates.

The stock culture of *A. baumannii* was appropriately diluted to achieve two working stocks, with titers of 2×10^6 cells per mL and 2×10^4 cells per mL. Aliquots of 50 μL of the two working stocks

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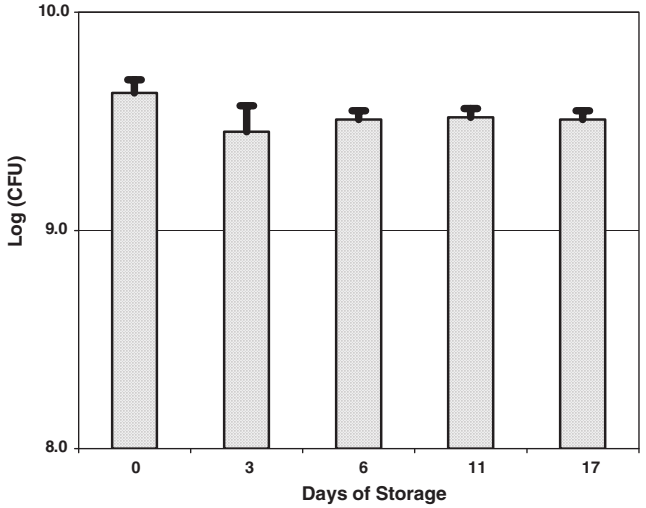

Fig. 1. Stability of *A. baumannii* cells in liquid suspension. A broth culture of *A. baumannii* cells was aseptically stored at 4°C. The number of viable cells was enumerated at the times indicated, through serial tenfold dilution in BPW. It should be noted that the *y*-axis scale has been expanded to highlight the differences over a 2-week period.

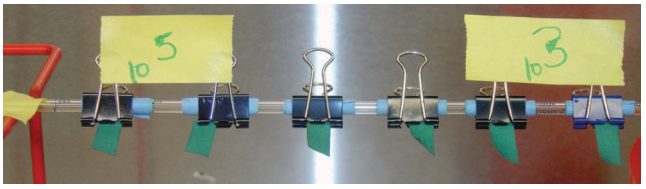

Fig. 2. Air-drying of inoculated scrub coupons after inoculation.

contained 10^5 or 10^3 cells, respectively, and were inoculated on sterile metal coupons by being spread over the entire area. The sterile scrubs were hung from metal clips, autoclaved, and air suspended (Fig. 2). After inoculation, the metal and scrub coupons were dried in a class II biosafety cabinet overnight. Viable cells from the inoculated coupons were recovered in 10 mL of BPW containing 0.01% Tween 80.

UVC Exposure

A special light exposure box, measuring 45 × 19 cm, was designed and fabricated by the Naval Surface Warfare Center (Dahlgren, Virginia). The ultraviolet lamp was hung with a beaded chain, and its vertical height from the floor could be readily adjusted. The floor area that received maximal irradiation with ultraviolet light spanned only 9 × 17 cm (13 cm from both sides of the box, 8 cm from the back side, and 2 cm from the front side). Figure 3 shows a schematic diagram of the floor and demarcation of the area receiving maximal fluence of ultraviolet light. The light bulb was positioned ~4 cm above the floor. The numbers indicated in the inner rectangle are flux values (microwatts per square centimeter) at this height of the bulb. A sheet of corrugated packing material was placed on the floor, and the mapped area was cut out, to ensure placement of the coupons in precise locations every time. In preliminary studies, the flux and energy fluence (ultraviolet dose) were found to be constant for a period of 30 minutes at three different heights of the ultraviolet lamp (results not shown).

The negative control specimen and the test coupons were placed in the center of the Petri plate (with no contact between

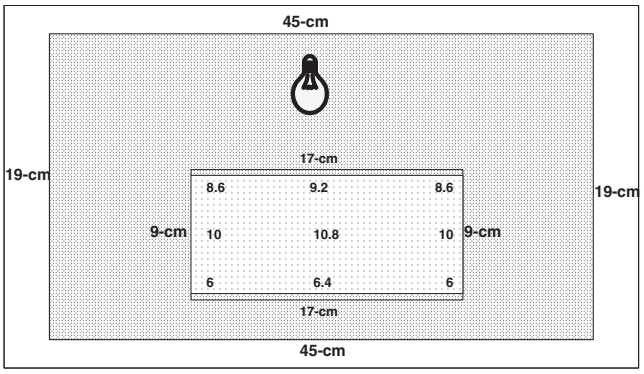

Fig. 3. Diagrammatic sketch of the light box and UVC flux under the lamp (the Petri plate containing the coupons was placed in the center of the lightly shaded area). Numbers indicated in the inner rectangle are flux values (microwatts per square centimeter).

the coupons). The light was turned on for 5 minutes to warm up; the ultraviolet meter was placed in the box during the warm-up period, to record the flux during this period. The sample door was opened after the warm-up period, to introduce the inoculated coupons for ultraviolet light exposure. The lid was removed and the door was closed during the 15-minute exposure period. The coupons were withdrawn from the box for cell extraction.

Recovery of Cells from Control and Test Coupons

The coupons were transferred to sterile 50-mL tubes containing 10 mL of BPW and surfactant (Tween 80). The tubes were vortex-mixed four times, each with a 30-second burst. This approach was found to be optimum for recovery of cells from metal and scrub coupons (results not shown).

Other Disinfecting Treatments for Scrubs

After scrub samples were added to the recovery medium, a set of three tubes were held in boiling water for 15 minutes before extraction. After drying, a set of three scrubs were sprayed with 70% ethanol and kept moistened for 15 minutes. These scrubs were then added to the recovery medium for extraction of cells.

Enumeration of Cells Recovered from Coupons

In the present study, a hierarchical approach to account for viable cells was followed, to achieve a very low detection limit of one to three viable cells. Cells were enumerated through serial 10-fold dilutions to 10^{-2} (for samples inoculated with 10^5 cells) and 10^{-1} (for samples inoculated with 10^3 cells). An aliquot of 0.1 mL was spread-plated on three replicate tryptic soy agar plates. The plates were incubated at 37°C overnight. For samples with low or zero viable cell counts per 0.1 mL, an aliquot of 1 mL was transferred to each of three replicate plates and 20 to 25 mL of molten tryptic soy agar medium (at 48–50°C) was poured into each plate (pour-plating). The samples were swirled to mix and cooled at room temperature for 2 hours before being incubated at 37°C for 24 to 48 hours. The colony-forming units (CFUs) appearing within the set medium were typically smaller than those appearing on the surface of the medium. For samples with no CFUs appearing on the pour-plates, an equal volume of 2× tryptic soy broth was added to the remainder of the recovered medium and incubated at 37°C for 48 to 72 hours, to confirm the absence of a single viable cell. After the incubation period, the presence of at least one viable cell was recorded for all turbid samples.

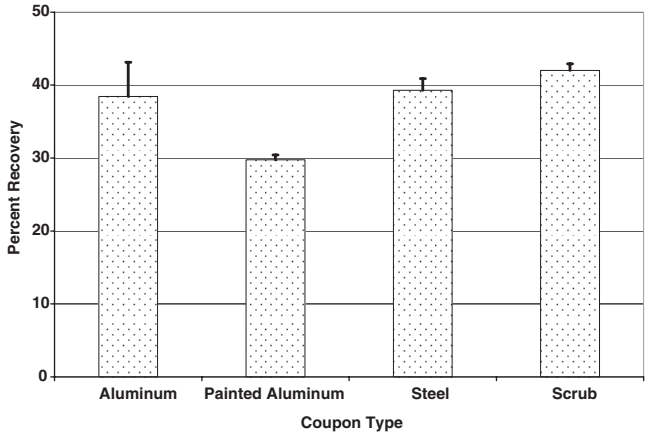

Fig. 4. Recovery of *A. baumannii* cells from metal surfaces and scrub. Aliquots of 50 μL containing 10^5 cells were inoculated on small coupons (three replicates) and dried overnight in a class II biosafety cabinet. The coupons were extracted in 10 mL of BPW containing 0.05% Tween 80 with four vortex-mixing bursts, each for 30 seconds. The cells were enumerated through dilution-plating, as described in Methods. The experiment was repeated twice, and the means ± SDs are indicated.

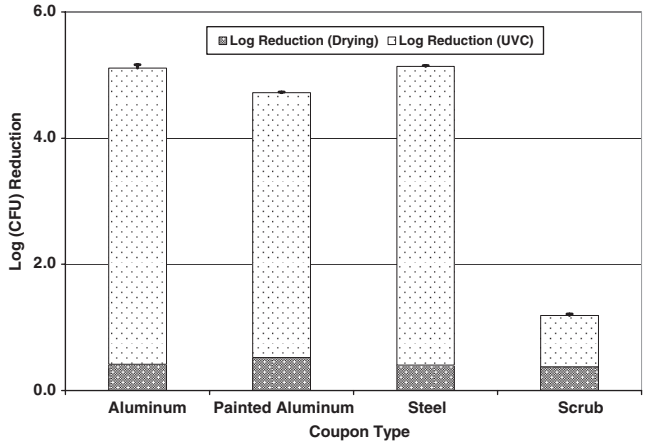

Fig. 5. Reduction of *A. baumannii* cells on metal and scrub surfaces after UVC exposure (90 J/m^2). Aliquots of 50 μL containing 10^5 cells were inoculated on small coupons (three replicates) and dried overnight in a class II biosafety cabinet. The test coupons were irradiated with UVC light (fluence, 90 J/m^2). Viable cells were extracted from control and test coupons in 10 mL of BPW containing 0.05% Tween 80 with four vortex-mixing bursts, each for 30 seconds. The cells were enumerated through dilution-plating, as described in Methods. The experiment was repeated twice, and the means ± SDs are indicated. In each histogram, the reduction resulting from drying is indicated by the shaded area at the bottom.

Cell Counting and Data Reduction

The plates were counted by using a Qcount instrument (Spiral Biotech, Norwood, Massachusetts), and the CFU counts observed in the titer sample and control samples were used to calculate the percentage recovery. The efficacy of ultraviolet killing was computed by subtracting the log(CFU) values of irradiated samples from the log(CFU) values of control samples. The SD was computed from the log(CFU) values of the six replicate coupon samples (from two experimental repeats, each with three replicates).

Results

Recovery of *A. baumannii* Cells from Different Surfaces

Before initiation of the disinfection studies, it was important to quantify the number of viable cells recovered from different surfaces after coupon inoculation and overnight drying. Some losses were expected because of a loss of viability of desiccated cells. However, losses could be attributed to the tight adherence of the cells to different surfaces, contributing to partial recovery. Figure 4 summarizes the percentage recovery of viable cells from aluminum, steel, and scrub coupons when the coupons were inoculated with 10^5 viable cells. The percentage recoveries ranged between 30% and 40%. The desiccation-related losses of *A. baumannii* are difficult to determine, but it appears that such losses could be as much as 40% to 50%. The physical agitation resulting from four 30-second bursts of vortex-mixing appeared to be very effective in releasing the remaining viable cells from all four surfaces.

Efficacy of UVC Irradiation in Decontamination of *A. baumannii* Cells on Different Surfaces

The *A. baumannii*-contaminated coupons were irradiated with 90 J/m^2 fluence of UVC irradiation (258-nm peak emission). Reductions in viable cells resulting from drying and from UVC irradiation were computed from control and test coupons. The data are summarized in Figure 5. As seen in Figure 5, the desiccation-related losses accounted for 0.3 to 0.6 log(CFU). The UVC exposure resulted in ≥4-log(CFU) reductions in viable cells for all three metal surfaces. The killing was complete, because no turbidity was observed when the test coupons were incubated in tryptic soy broth. Complete killing or decontamination of inanimate surfaces may be a desirable goal in intensive care units and patient treatment facilities, because of the prevalence of wounded soldiers who have either open skin surfaces or compromised immune systems. However, <1-log(CFU) reduction in viable cells was observed for scrub material, indicating that UVC irradiation was not effective for scrub decontamination.

Efficacy of Boiling Treatment in Decontamination of Cells on Scrub Surfaces

Because UVC irradiation was ineffective for the disinfection of scrub material, two other common disinfection practices (15-minute incubation in boiling water and 70% ethanol treatment) were evaluated. As shown in Figure 6, although ethanol treatment was ineffective, the boiling treatment was very effective for the disinfection of scrubs.

Discussion

A number of reports in recent years have highlighted the emergence of multidrug-resistant *A. baumannii* isolates in the bloodstreams of soldiers who were injured in Iraq or Afghanistan and were being treated in military treatment facilities.[8,9] Such isolates of *A. baumannii* have been recognized as a leading cause of nosocomial infections. Infection control for environmental surfaces is paramount in keeping the *A. baumannii*-related infection rate low.

There is no Environmental Protection Agency-registered antimicrobial agent for the disinfection of surfaces contaminated with *A. baumannii* cells. In treatment facilities, dilute preparations of hypochlorite (10%) have been used for >100 years as disinfectants with broad antimicrobial activity. Because of possible corrosive actions on metal surfaces, other disinfectants, including quaternary ammonium salts, have been used in recent years.[10,11] However, the efficacy of quaternary salts against *A. baumannii* has not been documented. In this report, the effectiveness of UVC light in the disinfection of metal surfaces intentionally contaminated with

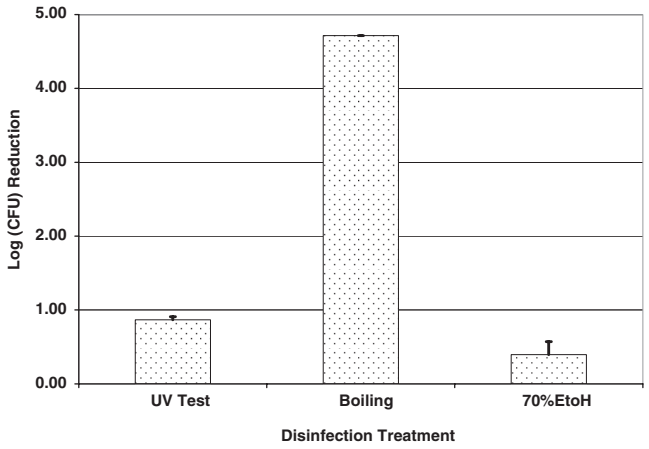

Fig. 6. Reduction of *A. baumannii* cells on scrubs after 15-minute boiling treatment or 70% ethanol treatment. Aliquots of 50 μL containing 10^5 cells were inoculated on small coupons (three replicates) and dried overnight in a class II biosafety cabinet. The test coupons were irradiated with UVC light (fluence, 90 J/m^2), treated for 15 minutes in a boiling water bath, or treated for 15 minutes with 70% ethanol. Viable cells were extracted from control and test coupons in 10 mL of BPW containing 0.05% Tween 80 with four bursts of vortex-mixing, each for 30 seconds. The cells were enumerated through dilution-plating, as described in Methods. The experiment was repeated twice, and the means ± SDs are indicated.

A. baumannii cells was evaluated. UVC irradiation has been used previously in the disinfection of aqueous liquids. A distinct difference exists between the irradiation of microorganisms in liquid suspension and irradiation of those on the surface of a solid object. The difference arises because of complex interactions that may occur between microorganisms on the solid surface and the constituent material of the solid surface. Surface topography may provide "shielding" or physical protection from incident ultraviolet light. For example, UVC inactivation of *Bacillus subtilis* spores on filter papers was less efficient with increasing fiber content per unit area, compared with samples with lower fiber content and more open surface structure.[12] For Gram-negative cells, the energy fluence required for a 1-log(CFU) reduction ranged between 8 and 100 J/m^2; for spores, significantly higher fluence is required to achieve the same level of killing.[13] In this study, the effectiveness of UVC irradiation was clearly shown for disinfection [4–5-log(CFU) reduction] of cells dried on metal surfaces. In addition, the boiling treatment was very effective for the disinfection of scrubs. It should be noted that temperatures of 90°C to 100°C are generally not achieved with current laundry practices. However, use of detergent and the moderately high temperatures (50–60°C) used for routine laundry services may suffice in achieving nearly complete removal and/or killing of *Acinetobacter* cells on scrubs. Future studies should be focused on determining the efficacy of detergent solutions at moderate temperatures for removing and/or killing *A. baumannii* cells.

We suggest that effective disinfection of operating tables and bed railings in military treatment facilities can be achieved with UVC lamps (fluence, 90 J/m^2), which are now commercially available (e.g., Tru-D (Charleston, South Carolina), which is commercially available from UEC Electronics and Automation). UVC irradiation is a cost-effective, easy-to-use, noninvasive, noncorrosive approach, with no adverse environmental effects. All three-dimensional surfaces must be directly exposed to the UVC irradiation, to ensure better infection control in patient treatment facilities.

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