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Inactivation of vegetative bacterial threat agents on environmental surfaces

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HIGHLIGHTS

- Inactivation of non-sporulating bacterial threat agents on complex surfaces is challenging.
- None of the decontaminants tested were able to completely decontaminate all biological agents on all surfaces.
- Resistance to chemical inactivation differed among the organisms tested only when a weak disinfectant was used.
- These results are intended to help guide decontaminant selection following a biological contamination incident.

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ABSTRACT

Following a wide-area biological terror attack, numerous decontamination technologies, techniques, and strategies will be required for rapid remediation. Establishing an understanding of how disinfectants will perform under field conditions is of critical importance. The purpose of this study was to determine the efficacy of several liquid decontaminants, when used to inactivate vegetative biological agents on environmental surfaces. Aluminum, carpet, concrete, glass, and wood coupons were inoculated with 1×10^8 CFU of *Burkholderia mallei*, *Francisella tularensis*, *Vibrio cholerae*, or *Yersinia pestis*. Using spray-based application methods, decontamination was then attempted with pH-adjusted bleach, 1% citric acid, 70% ethanol, quaternary ammonia, or Pine-Sol®. Results indicated that decontamination efficacy varied significantly by decontaminant and organism. Materials such as wood are difficult to decontaminate, even when using sporicides. The data presented here will help responders develop efficacious remediation strategies following a large-scale contamination incident.

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1. Introduction

Microorganisms have long been used to purposefully inflict morbidity and mortality on enemy populations (Christopher et al., 1997; Poupard and Miller, 1992; Robertson and Robertson, 1995). Relative to other categories of mass destructive weapons (e.g., nuclear weapons) they are inexpensive, able to be quickly manufactured in large quantities, and easily dispersed over large areas (Pearson, 1997). Further, realization of a biological attack is often days to weeks post incident; making criminal investigation, public health protection, and restoration particularly challenging (Broussard, 2001).

The *Bacillus anthracis* (causative agent of anthrax) contamination events of 2001 demonstrated vulnerabilities within the United States to biological terrorism, including the inability to rapidly decontaminate large facilities following such an incident (Canter, 2005b; Canter et al., 2005; Franco and Bouri, 2010). The ultimate result of this incident was five fatalities and a remediation that cost over $650 million and took more than three years to complete (Barakat et al., 2002; Canter, 2005a,b; Jernigan et al., 2002). A biological attack on a larger spatial scale could take decades and billions of dollars to remediate (Franco and Bouri, 2010; US FBI, 2008). Nonetheless, it is believed that another attack is imminent (Graham and Talent, 2008).

While survival of non spore-forming bacterial agents outside of a host is known to be considerably less than that of spore-forming agents such as *B. anthracis* (Gould, 1977; Sinclair et al., 2008; Stuart and Wilkening, 2005), vegetative agents can remain viable for weeks under favorable conditions (Calfee and Wendling, 2012; Williams et al., 2005). In addition, *Brucella suis*, a vegetative select agent, has demonstrated similar resistance to chemical inactivation as *Bacillus* spores when associated with difficult to decontaminate surfaces such as wood (Calfee and Wendling, 2012). Making the assumption that all non spore-forming bacterial agents respond similarly to treatment with decontaminants may lead to unexpected results and incomplete decontamination, as some agents are highly resistant to chemical treatment. For example, *Pseudomonas aeruginosa*, a non spore-former, is notorious for forming persistent biofilms and resisting chemical inactivation (Sagripanti et al., 1997). As such, vegetative bacterial agents remain as a potential threat due to their utility as a bioweapon. Following a biological contamination incident it is imperative that accurate estimates of decontamination efficacy are available, so that recovery activities can commence rapidly. Having data that allow accurate predictions of the effectiveness of

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decontaminants when used against infectious agents on complex surfaces greatly enhances the confidence of federal, state, and local health officials and emergency responders when recommending remediation actions as well as when deciding whether buildings are clean enough for reoccupancy. Further, most disinfection studies have been conducted with the hospital environment in mind. For obvious reasons, hospital environments were designed with surfaces that are easily decontaminated. Remediation activities outside of hospital environments will face numerous materials that are porous, absorbant, and incompatible with liquids. Therefore, it is prudent to determine the decontamination efficacy of available liquid disinfectants when used to inactivate vegetative bacterial threat agents on surfaces likely to be encountered during a bioterror remediation. These data are currently not available, yet would be highly valuable to responders if such an attack were to occur.

The US Environmental Protection Agency's National Homeland Security Research Center is tasked with generating high-quality data that help responders and remediation experts expedite and improve the remediation process. The current study investigated the susceptibility of four vegetative bacterial threat agents (*Burkholderia mallei*, *Francisella tularensis*, *Vibrio cholerae*, and *Yersinia pestis*) to one sporicidal decontaminant (pH-adjusted bleach) and four common disinfectants (70% ethanol, 1% citric acid, quaternary ammonia, and Pine-Sol®). *B. mallei*, *F. tularensis*, *V. cholerae*, and *Y. pestis* are the causative agents of glanders, tularemia, cholera, and plague, respectively. Decontamination efficacy was determined at two temperatures for each agent seeded onto five different material types (aluminum, glass, wood, carpet, and concrete) common to the built environment.

2. Materials and methods

2.1. Test organisms

Four fully-virulent bacterial strains were evaluated in this study, and included: *Y. pestis* (CO92), *F. tularensis* (SchuS4), *B. mallei* (China7), and *V. cholerae* (serogroup 0:1). A gram stain was performed on each organism and the colony morphologies confirmed to be consistent with previous descriptions of the strains. Strains were obtained from documented sources and stored at −80 °C until used to initiate each experiment. Descriptions of each strain are presented in Table 1.

Fresh cultures were prepared in advance of each day that coupons were inoculated by transferring one colony from a streak plate (freshly growing or stored less than two weeks at 2–8 °C) into 10–20 ml of the appropriate liquid growth media for each organism (Table 1). This culture was then incubated overnight at the required temperature on an orbital shaker set to 200 revolutions per minute (rpm). The bacterial culture (late log phase of growth) was diluted with fresh media to an optical density at 600 mm (OD_{600}) of approximately 0.1 to 0.2 (SPECTRAmax Plus^{384} spectrophotometer, Molecular Devices, Sunnyvale, CA). The final titer of the suspension was determined by analyzing 1:10 serial dilutions of the stock suspension prepared using phosphate-buffered saline (PBS) (Sigma Aldrich, St. Louis, MO) and plated onto the appropriate solid growth media for each organism. Plates were incubated at the temperature and duration (Table 1) optimal for colonial growth, and CFU were enumerated.

2.2. Test materials

Materials commonly found within built (indoor and/or outdoor) environments were selected for this study, and are described in Table 2. Test materials included aluminum, glass, wood, concrete, and carpet (Fig. 1). Material coupons were fabricated by cutting identically-sized pieces from larger pieces of stock material. The exception, concrete coupons were made by first following the label instructions to create the mix then pouring the mix into molds to cure. A single lot of each material was utilized to reduce variation among coupons of the same material type. Coupons were sterilized prior to use by either autoclave (121 °C, 103 kPa, 60 min) or gamma irradiation (~40 kGy; Steris Isomedix Services, Libertyville, IL) according to Table 2. Prior to gamma irradiation, coupons were sealed in 6-mil Uline® poly tubing (Uline, Chicago, IL) to preserve sterility until use. Test coupons were inspected prior to testing. Coupons with anomalies on the application surface were discarded. Blank coupons (not inoculated) were used to confirm the sterility of each material type.

2.3. Coupon inoculation

The stock suspension ($\sim 1 \times 10^9$ CFU ml^{-1}) was used to inoculate the surface of test and positive control coupons. During inoculation, coupons were placed lying flat in a Class III biological safety cabinet (BSC) and inoculated with a 100 µl aliquot of stock suspension using a multichannel micropipette as two rows of five droplets (10 µl per droplet) across the surface of the coupons, yielding $\sim 1 \times 10^8$ CFU per coupon. Inoculated coupons were allowed to dry for 1 h in the BSC prior to being extracted (positive controls), or exposed to decontaminants.

2.4. Decontaminants and application procedures

Prior to testing, the volume of each decontaminant applied to each coupon type and the optimal amount of neutralizer needed to quench its biocidal activity were determined according to methods reported previously (Calfee et al., 2011). During the neutralization tests, it was determined that a mean volume of 0.36 ml was applied to aluminum, glass, wood, and carpet coupons during the spray procedure. Concrete coupons were smaller and received on average 0.10 ml of decontaminant. These experimentally determined volumes, along with the optimal neutralizer concentration, were used to calculate the amount of neutralizer required to quench biocidal activities of the decontaminants during the decontamination tests.

Liquid decontaminants, or sterile filtered water (SFW) (Sigma Aldrich) for positive control samples, were applied to coupon surfaces using a hand-held sprayer (part # 7331X, Qorpak, Bridgeville, PA) at a distance of ~30 cm, until the coupon surface was visibly wetted. For each decontamination test, a new batch of decontaminant was prepared from an unopened container. All decontaminants were 20 ± 2 °C when applied.

pH-amended bleach was prepared by mixing 9.4 parts SFW, 1 part Ultra Clorox® (part # 44600, Clorox® Company, Oakland, CA), and 1 part 5% acetic acid (part # 9508, JT Baker, Phillipsburg, NJ). The resulting solution had a mean pH of ~6.8 and a mean total chlorine content, estimated based on dilution, of ~6200 parts per million

Table 1
Media types and incubation parameters for each of the four vegetative threat agents utilized in the decontamination tests.

Organism (strain)	Solid media	Liquid media	Incubation temperature (°C)	Incubation time (hours)
Y. pestis (CO92)	Tryptic soy agar (G60, Hardy Diagnostics, Santa Maria, CA)	Tryptic soy broth	26 ± 2	~72
F. tularensis (SchuS4)	Chocolate II agar (221169, BD Biosciences, Franklin Lakes, NJ)	Mueller Hinton broth	37 ± 2	~72
B. mallei (China 7)	Chocolate II agar	Nutrient broth	37 ± 2	~72
V. cholerae (serogroup 0:1)	Tryptic soy agar	Tryptic soy broth	37 ± 2	18–24