Evaluation of Ambulance Decontamination Using Gaseous Chlorine Dioxide

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Abstract

Objective. We evaluated gaseous chlorine dioxide (ClO_2) decontamination of an ambulance using a variety of bacterial biological agents. **Methods.** Spores of attenuated *Bacillus anthracis* and *Bacillus atrophaeus* as well as vegetative cells of *Acinetobacter baumannii, Mycobacterium smegmatis*, and *Staphylococcus aureus* were exposed to ClO_2 gas inside an ambulance. Log reduction in viability was assessed following decontamination using organism plate counts. **Results.** Ambulance decontamination with ClO_2 gas concentrations of 362 to 695 ppm maintained to exposures of 756 ppm-hours with 65% relative humidity (RH) achieved inactivation of all the bacterial agents tested. Decreasing exposure (ppm-hours) and RH (<65%) or restricting air flow reduced inactivation but still achieved greater than 6-log reductions in organism viability. **Conclusion.** Up to 10-log reductions were achieved in an ambulance interior following exposure to ClO_2, indicating that gas concentrations needed to mitigate biological agent contamination can be achieved and maintained safely in an ambulance. Future studies are ongoing to evaluate gaseous ClO_2 in other environments contaminated with biological agents of health care concern. **Key words:** chlorine dioxide; ambulance; decontamination; fumigation; *Bacillus anthracis*

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Background

Standards for environmental cleaning in hospital settings have been established and integrated into infection control efforts aimed at preventing the spread of infections resulting from environmental contamination.[1–3] Chemical solutions containing chlorine are commonly used in surface disinfection during the terminal cleaning of isolation rooms following discharge of patients with infectious diseases. Similar compounds are used for surface cleaning in ambulances following transport and care of infectious patients. Manual application of chemical disinfection is time- and labor-intensive and requires appropriate methods to prevent inadequate organism reduction or spread of contamination throughout an ambulance.[4]

Ambulances represent unique prehospital care environments comprising confined spaces with limited air handling and a multitude of storage compartments, which increase the potential for contamination and complexity of surface decontamination efforts.[4–6]

Importance

Bioterrorism or other biological events resulting in contaminated casualties pose a significant risk for emergency vehicles and may require decontamination methodology beyond standard procedures. The environmental contamination risk of an intentional biological agent deployment was illustrated by the 2001 anthrax attacks, which contaminated multiple buildings with anthrax spores.[7] These attacks underscore the need for timely and effective decontamination methods for prehospital environments prior to the occurrence of intentional contamination events.

Possible methods for decontamination include heating, liquids, gases, vapors, and irradiation. The decontamination capabilities of various gas (ozone, ethylene oxide) and vapor agents (hydrogen peroxide) have been established in small-scale laboratory studies.[8,9] Gas fumigation agents are ideal for ambulance decontamination, since gases are able to penetrate the compartment areas that may harbor contaminants.[8] Only a limited number of studies, however, have evaluated this modality. Aerosolized hydrogen peroxide vapor has also been evaluated for ambulance decontamination and was found to require three decontamination cycles to produce a 6-log reduction in *Bacillus atrophaeus* spores.[10]

Goals of This Study

We evaluated the capacity of chlorine dioxide (ClO_2) gas to decontaminate high concentrations of a variety of bacteria inside an ambulance. Chlorine dioxide was selected as the decontaminating agent since the scientific literature has established this agent as an effective decontaminant in a variety of other environments. Additionally, ClO_2 is one of the few decontaminating agents registered by the Environmental Protection Agency (EPA) as a gaseous decontaminant.[11]

METHODS

Theoretical Model of the Problem

Transportation and care of highly infectious or biologically contaminated patients pose a risk of ambulance contamination. Following contamination, manual ambulance-cleaning techniques require a significant contact time and also present exposure risks to personnel during the process of cleaning. Gaseous fumigation methods have been developed and are used by some ambulance services, but few studies have evaluated the efficacy of ambulance fumigation. The focus of our study was to assess the effectiveness of fumigating an ambulance with gaseous ClO_2.

Study Design

A field study evaluating ClO_2 decontamination using five bacteria placed inside an ambulance was done.

TABLE 1. Ambulance Sites Seeded with Bacterial Strains for Chlorine Dioxide Decontamination Testing

		Distance (Meters) from:		
Placement Site	Description	Injection Site	Floor	Ceiling
1	Floor next to injection tubing	0.3	0.0	1.8
2	Countertop	2.2	0.6	1.2
3	Open cabinet	1.7	1.2	0.6
4	Bench	1.6	0.3	1.5
5	Open cabinet	1.3	0.7	1.1
6	Shelf	1.8	1.6	0.2
7	Closed steel door cabinet	2.7	0.0	3.3
8	Seat	1.5	0.3	1.5
9	Floor	3.0	0.0	1.8
10	Floor	0.6	0.0	1.8

The organisms consisted of a commercial preparation of spores from *Bacillus atrophaeus* ATCC 9372 (Raven Labs, Omaha, NE), viable growth containing both vegetative and spores of *Bacillus anthracis* Sterne, viable growth of vegetative cells of *Acinetobacter baumannii* (highly drug-resistant wild-type strain designated BC9782), *Mycobacterium smegmatis* ATCC 14468, and *Staphylococcus aureus* ATCC 43300. Ten organism placement sites within an ambulance were selected to assess complete exposure of the interior by ClO_2 gas (Table 1). The organisms were placed in duplicate pairs at each site (Fig. 1).

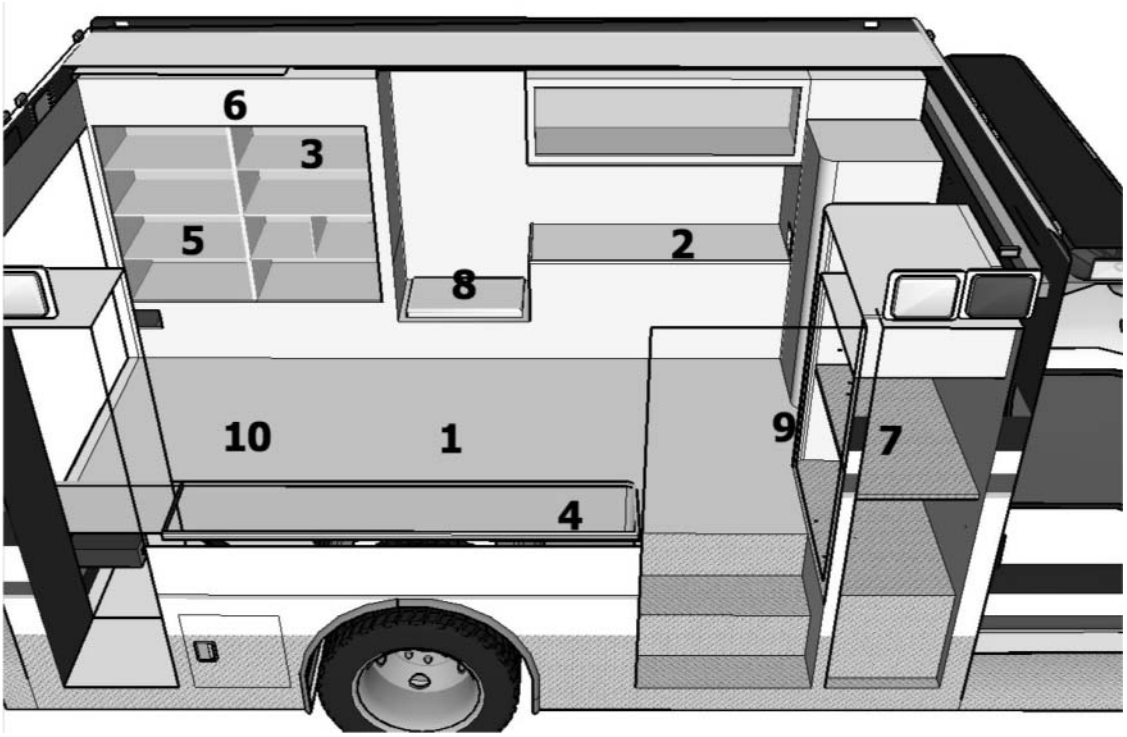

FIGURE 1. Ambulance placement sites where organisms were located during the chlorine dioxide decontamination.

Setting

Chlorine dioxide fumigation was performed using a Ford F 350 type III training ambulance located in an enclosed garage.

Method of Measurement

Gaseous ClO_2 was produced with a commercially available ClO_2-generation system, ClorDiSys Solutions, Inc. (Lebanon, NJ) Minidox-M Decontamination System. The generator was used to measure ClO_2 concentration, relative humidity (RH), temperature, and exposure time during the decontamination process. The ClO_2 gas generator uses a five-phase programmable decontamination protocol to include: 1) *preconditioning phase*, when the RH is elevated to a specified level and the environment is checked for leaks; 2) *conditioning phase*, where the desirable RH is maintained for a specified time; 3) *charge phase*, where the ClO_2 gas is generated to a target concentration; 4) *exposure phase*, where the target concentration of ClO_2 is maintained to the specified exposure time; and 5) *aeration phase*, where the ClO_2 is removed from the decontamination area to 0 parts per million (ppm). The decontamination protocol allowed for a variety of different target RHs, exposure times, and ClO_2 concentrations. Gas concentration was measured in ppm. Exposure was calculated as a function of measured ClO_2 concentrations and time as parts per million hours (ppm-hours).

A portable ClO_2 gas detector (PortSens; Analytical Technology, Inc. Oaks, PA) was used to monitor ClO_2 gas leakage outside of the ambulance during decontamination. Monitoring was conducted on all sides of the ambulance and was used to establish a 5-meter safety perimeter to allow acceptable gas leakage when target ClO_2 concentrations were maintained inside the ambulance while ensuring a safe 0-ppm ClO_2 concentration outside of the safety perimeter. This monitoring was done to ensure the safety of study personnel and others within the facility. The Occupational Safety and Health Administration (OSHA) permissible exposure limit for ClO_2 gas is 0.1 ppm, which is also the ClO_2 odor threshold concentration.[12] The OSHA short-term exposure limit is 0.3 ppm for no more than 15 minutes.[12] Chlorine dioxide gas is a severe respiratory and eye irritant that can produce irritating effects in humans at concentration levels of 5 ppm.

Data Collection and Processing

The *B. anthracis* was inoculated onto tryptic soy agar (TSA) (Remel Labs, Lenexa, KS) and incubated at 37°C for 24 hours. Following incubation, a procedure described by Perdue et al. was adapted to produce *Bacillus* spore suspensions.[13–15] Briefly, single colonies from the agar were inoculated into 100 mL of tryptic soy broth (TSB) and incubated at 37°C for 24 hours. Following incubation, 30 μL of the broth culture was spread onto three modified TSA (M-TSA) plates containing manganese chloride/calcium chloride ($MnCl_2/CaCl_2$) and incubated at 37°C for 48 hours and subsequently incubated at room temperature for an additional 24 hours. Biomass was collected from the M-TSA plates and subsequently suspended into 30 mL of sterile distilled water (dH_2O). This suspension was incubated at room temperature for 72 hours to allow for lysis of the vegetative cells. Following incubation, the spore suspension was centrifuged at 8,000 $\times$ *g* for 10 minutes at 4°C and washed with dH_2O twice. Spore suspensions were microscopically verified via phase-contrast microscopy to distinguish between spores and vegetative cells to verify that at least 90% of the observed cells were spores.

The *A. baumannii* and *S. aureus* strains were grown on TSA plates and incubated at 37°C for 24 hours. The *S. aureus* was incubated with 5% CO_2 and the *A. baumannii* was incubated at ambient air. Isolated colonies were then inoculated into 100 mL of TSB and incubated at 37°C for 24 hours. The broth culture was then washed by centrifugation twice using dH_2O. Following the second centrifugation, the pellet was diluted in phosphate-buffered saline (PBS) and adjusted to concentration.

The *M. smegmatis* strain was grown on TSA plates containing 5% sheep blood (B-TSA) and incubated at 37°C with 5% carbon dioxide (CO_2) for 24 hours. Isolated colonies were inoculated into 100 mL of TSB containing 5% sheep blood and incubated at 37°C with 5% CO_2 for 24 hours. Broth cultures were twice centrifuged to pellet cells and washed with dH_2O. Pellets were diluted in PBS and adjusted to concentration.

The commercially available *B. atrophaeus* spore strip (Raven Labs catalog number 1–6100) was impregnated by the manufacturer with a median value of 10^6 spores and was contained in a sterile Tyvek envelope for aseptic processing. The spore strip indicator was processed according to manufacturer's recommendations by incubation in TSB at 35°C in ambient air for seven days.

For each organism, 300 μL of stock solution was loaded onto a blank X type Petri dish (Fischer Scientific catalog number 08–758–2) in a biosafety cabinet and transported to the ambulance decontamination site in a hard-sided container. Following ClO_2 gas exposure, exposure and nonexposure organisms were retrieved and transported to a Biosafety Level 2 (BSL-2) laboratory for immediate processing .

The Minidox ClO_2 generator is a registered antimicrobial sterilizer with the EPA (EPA Reg. 80802–1). This ClO_2 gas-generation method utilizes chlorine gas delivery from compressed gas cylinders containing a mixture of 2% chlorine gas in 98% nitrogen balance. Chlorine gas interacts with sodium chlorite contained

in cartridges with other stabilizing compounds to produce pure ClO_2 gas. Target ClO_2 concentration of 360 ppm to an exposure of 720 ppm-hours with 60% RH was established as a target parameter.[16]

The ClO_2 generator was placed outside of the ambulance in a closed garage. Two polyvinylidene fluoride tubing lines were extended from the generator into the ambulance through a small window. One tubing line was used to deliver ClO_2 gas and the other allowed for sampling ClO_2 concentration by a photometer located inside the generator. The access window was sealed with plastic sheeting containing gas-tight connections for the polyvinylidene fluoride tubing. Duct tape was used to seal door seams of the ambulance. The entire process to set up generator tubing lines and seal the ambulance prior to gas generation took approximately one hour. Phase-activated charcoal scrubbers (ClorDiSys Solutions Inc.) were used to remove ClO_2 gas from the ambulance following completion of decontamination.

Outcome Measures

Exposure and control organisms of bacteria were collected from the X type Petri dish using sterile swabbing technique. The sterile swab was used to absorb the organism suspension and was repeatedly drawn across the surface of the X type Petri dish well. Inoculated swabs were deposited into 1.7 mL microcentrifuge tubes containing 700 μL of PBS and vortexed for 30 seconds. PBS solutions were serial diluted and plated in duplicate onto B-TSA, M-TSA, or TSA agar plates and incubated as detailed above for each organism type. Exposed organism serial dilutions were evaluated for reduction in colony-forming units (CFU) against nonexposed control sample serial dilutions to determine log reductions. The *B. atrophaeus* biological indicators were evaluated for growth of the exposure samples and deemed as complete ClO_2 penetration for samples displaying no bacterial growth. Positive and negative controls were performed for all five organisms in each decontamination trial. Percent inactivation is reported to provide a clearer depiction of how much of the stock sample was inactivated. Percent inactivation is reported to add clarity beyond log reductions, which do not indicate the level of total or partial inactivation achieved. Mean log reduction was calculated by measuring the log reduction for each of the 20 organism samples and calculating the geometric mean. Percent inactivation was calculated as the mean percent inactivation of all 20 organism samples.

A site with growth was defined as a site that was less than 100% inactivated, resulting in organism growth in the serial dilution culture.

Data Analysis

The geometric mean log reduction and confidence intervals (CIs) in viable organisms were calculated for each organism using SPSS version 21 (IBM, Armonk, NY). Geometric means were calculated for individual organism placement sites as well as the aggregate of all placement sites within the ambulance for a decontamination trial.

RESULTS

Four decontamination trials were performed with each trial taking less than four hours from start to finish. Gaseous ClO_2 decontamination of an ambulance was evaluated under the parameters outlined in Table 2. The average ClO_2 gas concentrations ranged from 315 ppm to 695 ppm inside the ambulance, with a ClO_2 exposure range of 479 to 850 ppm-hours.

The ClO_2 decontamination trials 1, 3, and 4 resulted in 100% inactivation of *A. baumannii* at all 10 sites, with 98% inactivation at nine placement sites in ClO_2 decontamination trial 2 (Table 3). Although placement site 7 failed to completely inactivate *A. baumannii* in trial 2, the samples at this site displayed 82% and 89% inactivation with 7.4-log and 8.0-log reduction. This site was located in a closed stainless-steel cabinet with a rubber gasket seal around the door.

Complete inactivation of *B. anthracis* spores was not achieved in any decontamination trial and ranged from 88% to 90% inactivation (Table 3). *B. anthracis* spore viability was maintained in the samples at placement site 7 in each of the four ClO_2 trials, likely

TABLE 2. Clorine Dioxide Decontamination Parameters Evaluated during the Decontamination of an Ambulance

ClO_2 Trial	ClO_2 Concentration* (ppm)	Charge Time† (min)	Exposure Time‡ (min)	Aeration Time§ (min)	Exposure¶ (ppm-hours)	% RH
1	362	28	179	33	850	55
2	315	16	92	27	479	65
3	406	9	100	35	763	65
4	695	21	65	22	756	55

*ClO_2 concentration is the average ClO_2 concentration in ppm maintained during the exposure phase.
†Charge time represents the length of time it took to inject ClO_2 gas into the decontamination space to reach the targeted ClO_2 concentration.
‡Exposure time is the length of time the target ClO_2 concentration was maintained in the decontamination space to achieve the target exposure (ppm-hours).
§Aeration time represents the time (min) from ClO_2 gas injection termination until ClO_2 gas concentration was lowered to 0 ppm.
¶Exposure (ppm-hours) is the total exposure achieved throughout the entire decontamination procedure in the charge, exposure, and aeration phases.
ClO_2 = chlorine dioxide; ppm = parts per million; ppm-hr = parts per million hours;% RH = percent relative humidity.

TABLE 3. Results of Decreased Viability from Clorine Dioxide Ambulance Decontamination Trials

ClO_2 Trial		*Acinetobacter baumannii*	*Bacillus anthracis spores*	*Mycobacterim smegmatis*	*Staphylococcus aureus*
1	% Inactivation	100	90	100	92
	Mean log reduction (95% CI)	9.0 (9.0–9.0)	9.3 (8.6–10.0)	10.0 (10.0–10.0)	8.6 (8.0–9.2)
	Placement sites with growth	*	1, 6, 7	*	7
2	% Inactivation	98	88	98	95
	Mean log reduction (95% CI)	8.9 (8.7–9.1)	8.2 (7.8–8.7)	8.2 (8.1–8.4)	7.7 (7.4–8.1)
	Placement sites with growth	7	2, 5, 6, 7	7	7
3	% Inactivation	100	94	100	99
	Mean log reduction (95% CI)	8.4 (8.4–8.4)	9.0 (8.3–9.7)	10.1 (10.1–10.1)	8.9 (8.8–8.9)
	Placement sites with growth	*	7	*	7
4	% Inactivation	100	96	100	97
	Mean log reduction (95% CI)	9.3 (9.3–9.3)	9.0 (8.4–9.6)	9.6 (9.6–9.6)	8.9 (8.6–9.1)
	Placement sites with growth	*	7	*	7

*Indicates no sites with growth because of complete inactivation.
CI = confidence interval; ClO_2 = chlorine dioxide.

resulting from the sealed cabinet's limiting ClO_2 penetration. *B. anthracis* spores also remained viable at multiple sites after ClO_2 exposure in decontamination trial 1 (sites 1, 6, and 7) and in trial 2 (sites 2, 5, 6, and 7). Trial 1 maintained an average ClO_2 concentration of 362 ppm to the highest exposure evaluated, 850 ppm-hrs, and an RH of 55%. Decontamination trial 1 resulted in complete inactivation (10-log reduction) in one sample from each duplicate pair at placement sites 1 and 6. The single viable *B. anthracis* sample in the placement site 1 duplicate pair displayed an 87% inactivation (8.7 log reduction). Placement site 7 resulted in 5.3- and 5.4-log reductions in decontamination trial 1. The viable sample at placement site 2 displayed 87% inactivation (8.6-log reduction) and the viable sample at site 5 had 73% inactivation (7.2-log reduction) following ClO_2 decontamination trial 2.

Decontamination trials 1, 3, and 4 resulted in complete inactivation of 10.0-, 10.1-, and 9.6-log CFU *M. smegmatis* at all 10 placement sites within the ambulance (Table 3). Decontamination trial 2 achieved complete inactivation at nine of 10 placement sites and incomplete inactivation at placement site 7.

Complete *S. aureus* vegetative cell inactivation (7.9- to 9.0-log reduction) was observed at all placement sites located in the main ambulance compartment in each decontamination trial; however, *S. aureus* vegetative cells at placement site 7 maintained viability in each decontamination trial (Table 3). Decontamination trial 1 achieved 5.0- and 4.8-log reduction of the duplicate-pair *S. aureus* samples at site 7. The duplicate pair of *S. aureus* samples at site 7 in decontamination trials 2, 3, and 4 resulted in 100% inactivation of one sample and CFU log reductions of 4.5, 8.6, and 6.8.

Bacillus atrophaeus spores were evaluated after ClO_2 exposure for complete inactivation or viability. Log reductions representing incomplete inactivation were not assessed.

Chlorine dioxide decontamination trials 1 and 3 had complete inactivation of nine sites, with *B. atrophaeus* spore viability remaining at placement site 7 in both decontamination trials (Table 4). The ClO_2 decontamination trials 2 and 4 had complete inactivation at eight placement sites within the ambulance. *B. atrophaeus* spore samples retained viability at placement sites 1 and 7 in decontamination trial 2 and placement sites 7 and 9 in decontamination trial 4.

Overall, the trial 2 ClO_2 concentration of 315 ppm maintained to an exposure of 479 ppm-hours with 65% RH failed to inactivate all five organisms, including both spores and vegetative cells, in the ambulance. Trial 4, maintaining 695 ppm ClO_2 to 756 ppm-hours with 55% RH, was sufficient for complete inactivation of *A. baumannii* and *M. smegmatis* vegetative cells within the ambulance, but failed to achieve total inactivation of *B. anthracis* and *B. atrophaeus* spores as well as *S. aureus* vegetative cells located inside the closed cabinet.

Discussion

Ambulance decontamination represents a critical element in prehospital infection control following contamination with infectious organisms. The potential for contamination in ambulances used for transporting patients is enhanced due to the small patient care compartment, ventilation system, storage compartments, and medical devices. The potential for personnel and environmental contamination of ambulances with methicillin-resistant *Staphylococcus aureus* (MRSA), *Mycobacterium tuberculosis*, and vancomycin-resistant entercocci through transport

TABLE 4. Growth of *Bacillus atrophaeus* Spores from Clorine Dioxide Ambulance Decontamination Trials

ClO_2 Trial	No. Sites with Growth	No. Sites without Growth
1	1	9
2	2	8
3	1	9
4	2	8

of infected patients has been established.[17,18] Additionally, several studies have documented MRSA environmental contamination in ambulances.[4,18–23] Higher rates of MRSA colonization among paramedics and emergency medical technician (EMT) students further highlights the potential for environmental reservoirs in prehospital environments.[17]

Guidelines have been suggested for selecting a protocol for decontaminating buildings following a biological event, but no guidelines have been established for ambulance decontamination based on a lack of data on this subject.[24] Manual-surface-cleaning protocols have been established and practiced for ambulance cleaning, yet some studies suggest these protocols are inadequate to limit routine environmental contamination.[4,18,19]

Andersen et al. 2006 found three successive exposure cycles with hydrogen peroxide aerosol capable of producing 6-log inactivation of *B. atrophaeus* spores in ambulance placement sites located inside devices, in glove boxes, on equipment, under mattresses, and in the driver cabin.[10] Hydrogen peroxide exposure times of 210 minutes achieved complete inactivation of all spore samples in the ambulance. A similar study by French et al. in 2004 conducted in a hospital ward reported 100% inactivation of 6-log *B. atrophaeus* spore indicators and only 98.8% inactivation of MRSA swab samples, indicating that environmental MRSA contamination may be more persistent to hydrogen peroxide aerosol than commercially prepared spore indicators.[25] In the current study, decreased exposure times, <180 minutes, with ClO_2 gas produced similar results to hydrogen peroxide, with the exception of one placement site located in a gasket-sealed stainless-steel cabinet. Additionally, the current study found ClO_2 capable of reducing MRSA 9 log throughout the ambulance and >4 log reductions in the sealed steel cabinet.

Existing protocols for transportation vehicles associated with high-level clinical isolation units in Germany, Sweden, and Italy use a variety of decontamination methods, but specific protocols have not been published.[26] A combination of fumigation and surface cleaning is utilized in Germany based on the biological organism requiring decontamination. Paraformaldehyde fumigation is used to decontaminate Swedish highly infectious patient transport ambulances, and Italy uses manual surface cleaning protocols to decontaminate transport vehicles.[26] Currently, ambulance decontamination protocols from the three high-level patient isolation units in the United States (Maryland, Georgia, Nebraska) have not been published, and no reports of specialized transportation vehicles exist for these units. High-level isolation facilities use a variety of specialized patient transportation methods, including standard ambulances draped with plastic or absorbent sheeting, specialized high-efficiency particulate air (HEPA)-equipped container vehicles with detachable patient care compartments, fixed-wing aircraft transport of HEPA-equipped ambulances, and the use of patient isolation pods.[26] Despite the effort in developing adequate transport isolation, these units still recognize the need for decontamination after patient transport. To date, a limited number of high-level isolation units employ ambulance fumigation protocols utilizing paraformaldehyde gas, and other units carry out manual surface decontamination in full personal protective equipment. Paraformaldehyde gas is a robust decontaminating agent that adequately decontaminates surfaces but may not be appropriate for ambulance decontamination as it is considered a carcinogen, is not registered as a gas-phase decontaminant by the EPA, is highly corrosive, and can deposit residue in decontaminated areas that must be removed by hand.[27]

Our study represents preliminary research evaluating the efficacy of gaseous ClO_2 as a potential method for ambulance decontamination. Our results indicate that although gaseous ClO_2 provides effective decontamination of the majority of the sites evaluated, sealed ambulance storage compartments may pose a challenge to gaseous decontamination methods. In spite of this challenge, we found that ClO_2 was capable of producing >4-log reductions of bacterial organisms inside a gasket-sealed compartment. In locations not protected by sealed doors, reductions >6 log were observed. These results suggested that protocols for gaseous ambulance decontamination should include provisions for opening all compartments with door seals to ensure that sufficient reduction in viable organisms is achieved. Andersen et al. 2006 utilized a fumigation protocol where all ambulance doors were open and hydrogen peroxide was generated into the entire garage space requiring the garage to be sealed.[10] The ClO_2 protocol evaluated in this study consisted of sealing and generating gas into the ambulance in a heated garage, with a 5-m safety perimeter around the ambulance for gas monitoring. Alternative approaches could utilize outdoor decontamination or a cloaking canvas placed around the ambulance in an outdoor setting similar to residential fumigation tents. Our approach allowed decontamination personnel to closely monitor the decontamination process and monitor gas concentration around the exterior of the ambulance in subfreezing outdoor temperatures.

LIMITATIONS AND FUTURE RESEARCH

Since this was a pilot study, only a single ambulance model was assessed, which limits the study. In addition, the ambulance was located in a closed garage to prevent potential drift of ClO_2 to adjacent areas, a factor that helped limit the spread of ClO_2 gas, which may not be possible in all situations. Although an

effort was made to assess damage to materials after the decontamination process, formal structural testing of synthetic materials and electronic equipment located within the ambulance was not conducted. However, no damage or alterations were observed in materials and electronics inside the ambulance, and the vehicle remained completely operational following fumigation. In spite of these limitations, our study adds to the limited published data on gaseous sterilization of ambulances contaminated with hazardous infectious agents. Our study focused on inactivation of bacterial organism, and further investigation of inactivation of fungal or viral organisms is needed. Additionally, a single ambulance was evaluated and the application to other ambulance types may require additional considerations. The results of our study are encouraging, and demonstrate that ClO_2 may be useful for decontaminating environmental surfaces in ambulances exposed to infectious organisms. Further study is necessary to determine the effects of gaseous ClO_2 on high-level contaminations in multiple ambulance models. Future studies should be conducted to assess the cost-effectiveness of and methods for sealing different ambulance models to prevent gas leakage as well as investigations of the effectiveness of ambulance fumigation for dry, wet, and moist contaminants in blood, urine, and other solutions.

Conclusion

Results of the current ClO_2 ambulance decontamination assessment suggest that key considerations be included in ambulance-fumigation protocol development. A safety perimeter should be determined prior to decontamination to ensure personnel safety, and fit-tested personal protective equipment should be carried by personnel at all time. In the current study, unacceptable leakage was determined to be 0.1 ppm beyond the established 5-m perimeter. The safety perimeter requires that fumigation be conducted at a location that can be securely maintained to prevent gas exposures. Interior ambulance compartments should be assessed for seals, and left open for fumigation exposure to allow maximum exposure. The ClO_2 generator used in this evaluation contained PVC connections and tubing that shrink in temperatures below freezing, which may result in gas leakage from the generator or any connection points between tubes. Fumigation conducted when outdoor temperatures are below freezing may require a heated space.

References

1. Boyce JM, Havill NL, Lipka A, Havill H, Rizvani R. Variations in hospital daily cleaning practices. Infect Control Hosp Epidemiol. 2010;31:99–101.
2. Dancer SJ. The role of environmental cleaning in the control of hospital-acquired infection. J Hosp Infect. 2009;73:378–85.
3. Dumigan DG, Boyce JM, Havill NL, Golebiewski M, Balogun O, Rizvani R. Who is really caring for your environment of care? Developing standardized cleaning procedures and effective monitoring techniques. Am J Infect Control. 2010;38:387–92.
4. Nigam Y, Cutter J. A preliminary investigation into bacterial contamination of Welsh emergency ambulances. Emerg Med J. 2003;20:479–82.
5. Roline CE, Crumpecker C, Dunn TM. Can methicillin-resistant *Staphylococcus aureus* be found in an ambulance fleet? Prehosp Emerg Care. 2007;11:241–4.
6. Stacey R, Morfey D, Payne S. Secondary contamination in organophosphate poisoning: analysis of an incident. QJM. 2004;97:75–80.
7. Centers for Disease Control and Prevention. Update: investigation of bioterrorism-related anthrax—Connecticut, 2001. Morb Mortal Wkly Rep. 2001;50:1077–9.
8. Rogers JV, Choi YW, Richter WR, Stone HJ, Taylor ML. *Bacillus anthracis* spore inactivation by fumigant decontamination. Appl Biosaf. 2008;13:89.
9. Spotts Whitney EA, Beatty ME, Taylor TH Jr, et al. Inactivation of *Bacillus anthracis* spores. Emerg Infect Dis. 2003;9:623–7.
10. Andersen B, Rasch M, Hochlin K, Jensen FH, Wismar P, Fredriksen JE. Decontamination of rooms, medical equipment and ambulances using an aerosol of hydrogen peroxide disinfectant. J Hosp Infect. 2006;62:149–55.
11. Environmental Protection Agency. List A: Antimicrobial Products Registered with the EPA as Sterilizers. Arlington, VA: U.S. Environmental Protection Agency Office of Pesticide Programs, 2011.
12. Occupational Safety and Health Administration: Occupational Safety and Health Guideline for Chlorine Dioxide. Available at: http://www.osha.gov/SLTC/healthguidelines/chlorinedioxide/recognition.html. Accessed April 23, 2013.
13. Baweja RB, Zaman MS, Mattoo et al. Properties of *Bacillus anthracis* spores prepared under various environmental conditions. Arch Microbiol. 2008;189:71–9.
14. Perdue ML, Karns J, Higgins J, Van Kessel JA. Detection and fate of *Bacillus anthracis* (Sterne) vegetative cells and spores added to bulk tank milk. J Food Prot. 2003;66:2349–54.
15. Xu S, Labuza TP, Diez-Gonzalez F. Thermal inactivation of *Bacillus anthracis* spores in cow's milk. Appl Environ Microbiol. 2006;72:4479–83.
16. Lorcheim P, Lorcheim K, Czarneski MA. Case study: exhaust duct decontamination using chlorine dioxide gas. Control Environ Magazine. 2009;12:15–8.
17. Miramonti C, Rinkle JA, Iden S, et al. The prevalence of methicillin-resistant *Staphylococcus aureus* among out-of-hospital care providers and emergency medical technician students. Prehosp Emerg Care. 2012;17:73–7.
18. Mahomed O, Jinabhai CC, Taylor M, Yancey A. The preparedness of emergency medical services against occupationally acquired communicable diseases in the prehospital environment in South Africa. Emerg Med J. 2007;24:497–500.
19. Alves DW, Bissell RA. Bacterial pathogens in ambulances: results of unannounced sample collection. Prehosp Emerg Care. 2008;12:218–24.
20. Rago JV, Buhs LK, Makarovaite V, Patel E, Pomeroy M, Yasmine C. Detection and analysis of *Staphylococcus aureus* isolates found in ambulances in the Chicago metropolitan area. Am J Infect Cont. 2012;40:201–5.
21. Roline CE, Crumpecker C, Dunn TM. Can methicillin-resistant *Staphylococcus aureus* be found in an ambulance fleet? Prehosp Emerg Care. 2007;11:241–4.
22. Noh H, Do Shin S, Kim NJ, et al. Risk Stratification-based surveillance of bacterial contamination in metropolitan ambulances. J Korean Med Sci. 2011;26(1):124.

23. Brown R, Minnon J, Schneider S, Vaughn J. Prevalence of methicillin-resistant *Staphylococcus aureus* in ambulances in Southern Maine. Prehosp Emerg Care. 2010;14: 176–81.
24. Franco C, Bouri N. Environmental decontamination following a large-scale bioterrorism attack: federal progress and remaining gaps. Biosecur Bioterror. 2010;8:107–17.
25. French GL, Otter JA, Shannon KP, Adams NM, Watling D, Parks MJ. Tackling contamination of the hospital environment by methicillin-resistant *Staphylococcus aureus* (MRSA): a comparison between conventional terminal cleaning and hydrogen peroxide vapour decontamination. J Hosp Infect. 2004;57(1):31–7.
26. Schilling S, Follin P, Jarhall B, et al. European concepts for the domestic transport of highly infectious patients. Clinl Microbiol Infect. 2009;15:727–33.
27. Luftman HS, Regits MA, Lorcheim P, Lorcheim K, Paznek D. Validation study for the use of chlorine dioxide gas as a decontaminant for biological safety cabinets. Appl Biosaf. 2008;13:199–212.