Effectiveness of a novel ozone-based system for the rapid high-level disinfection of health care spaces and surfaces

Dick Zoutman, MD, FRCPC,[a] Michael Shannon, MD, MSc,[b,c] and Arkady Mandel, MD, PhD, DSc[c]
Kingston and Ottawa, Ontario, Canada

Background: Vapor-based fumigant systems for disinfection of health care surfaces and spaces is an evolving technology. A new system (AsepticSure) uses an ozone-based process to create a highly reactive oxidative vapor with broad and high-level antimicrobial properties.

Methods: Ozone gas at 50-500 ppm was combined with 3% hydrogen peroxide vapor in a test chamber and upscaled in rooms measuring 82 m^3 and 90 m^3 in area. Test organisms included methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococcus, *Escherichia coli*, *Pseudomonas aeruginosa*, *Clostridium difficile*, and *Bacillus subtilis* spores dried onto steel discs or cotton gauze pads.

Results: The combination of 80-ppm ozone with 1% hydrogen peroxide vapor achieved a very high level of disinfection, with a ≥ 6 $\log_{10}$ reduction in the bacteria and spores tested on steel discs and MRSA tested on cotton gauze during a 30- to 90-minute exposure. The entire system was scalable such that it achieved the same high level of disinfection in both the 81-m^3 and 90-m^3 rooms in 60-90 minutes.

Conclusion: The ozone hydrogen peroxide vapor system provides a very high level of disinfection of steel and gauze surfaces against health care-associated bacterial pathogens. The system is an advanced oxidative process providing a rapid and effective means of disinfecting health care surfaces and spaces.

Key Words: Ozonation; hydrogen peroxide; fumigation.

Recent research has demonstrated that the environment acts as an important reservoir for health care-associated pathogens and contributes significantly to their persistence and spread between patients and health care providers.[1] Common health care-associated pathogens are known to survive for prolonged periods (often months) on inanimate surfaces, and epidemic strains of methicillin-resistant *Staphylococcus aureus* (MRSA) survive longer than nonepidemic strains on environmental surfaces.[2,3] Pathogens can be transmitted from these environmental surfaces to the clothing and hands of health care providers and then to patients.[4,5]

Cleaning of environmental surfaces of a hospital requires a major allocation of resources. For example, recently the UK government announced a £57.5-million program of ''deep cleaning'' for every National Health System hospital.[6] Although important to reduce surface contamination, manual cleaning methods have limited efficacy in reducing the bioburden of pathogens in health care environments.[7-9] The use of hospital disinfectants in the manual cleaning of patient care areas and operating rooms can be problematic. The high level of hospital occupancy and demand for rapid turnaround time of operating rooms and in-patient accommodations can limit the time for cleaning, in turn limiting the time that cleaning products can be left on environmental surfaces to achieve the desired antimicrobial effect claimed by the manufacturer.[10,11] Inadequate mechanical cleaning of surfaces has been shown to actually increase the sporulation of *Clostridium difficile* and to increase its spread during the manual cleaning process.[12]

For the foregoing reasons, there has long been interest in treating hospital operating theaters and patient care rooms with a fumigant that will eliminate resident

From the Department of Pathology and Molecular Medicine, Queen's University, Kingston, ON, Canada[a]; Medizone International Inc, Innovation Park, Queen's University, Kingston, ON, Canada[b]; and Canadian Foundation for Global Health, Ottawa, ON, Canada.[c]

Address correspondence to Dick Zoutman, MD, FRCPC, Department of Pathology and Molecular Medicine, Queen's University, 76 Stuart Street, Kingston, ON K7L 2V7, Canada. E-mail: zoutmand@queensu.ca.

Conflict of interest: Research funded by Medizone International Inc.

0196-6553/$36.00

doi:10.1016/j.ajic.2011.01.012

health care-associated pathogens from all room surfaces, the air, and spaces. The ideal fumigant is cheap, rapid-acting, and provides a high level of microbial kill while leaving no residue or noxious end products or byproducts. Hydrogen peroxide has recently been used in health care settings for this purpose; however, hydrogen peroxide vapor alone can be slow-acting and costly, and has shown only a limited microbial kill effect in field trials.[13-18] Ozone has been used for many years to disinfect municipal water systems, swimming pools, and spas, but has seen only limited use as a fumigant to disinfect health care rooms and surfaces, and has not demonstrated a high level of antimicrobial activity when used for this purpose.[19,20]

The purpose of the present study was to evaluate the conditions for the optimal effectiveness of ozone in combination with hydrogen peroxide vapor as an oxidative process to provide high-level disinfection of surfaces and materials in a test chamber and in full-sized test rooms against common health care-associated pathogens.

MATERIALS AND METHODS

Organisms studied

The bacterial strains used in this study were MRSA (ATCC 33592), *Enterococcus faecium* (high-level vancomycin-resistant [VRE]) clinical strain, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *C difficile* (clinical strain), and *Bacillus subtilis* (ATCC 19659).

Preparation of aerobic non-spore-forming bacteria

Fresh cultures of *S aureus*, *E faecium*, *E coli*, and *P aeruginosa* were grown on Columbia agar with 5% sheep's blood (PML Microbiologicals, St Laurent, QC, Canada) at 35°C in room air for 18-24 hours.

Preparation of spores from *C difficile* and *B subtilis*

C difficile was streaked onto 12 prereduced anaerobic Brucella blood agar plates (PML Microbiologicals) and incubated anaerobically for 48 hours at 35°C. Spores were harvested and cleaned following the methods described by Alfa et al.[21] The spores were stored in 100% ethanol at 4°C. This preparation yielded approximately 1×10^9 to 1×10^{10} spores/mL. Gram stains and malachite green stains of the spore preparation confirmed that the suspension consisted of spores with very few vegetative cells.[21] The method for preparing the spores of *B subtilis* was similar to the above described for *C difficile* except that the bacteria were grown on Columbia sheep's blood agar plates incubated 7 days in room air at 35°C. The spore pellet was heat shocked at 70°C for 15 minutes and treated with 100% ethanol to kill any residual vegetative cells.

Inoculation of stainless steel discs

Bacteria for inoculation to the surface of sterile stainless steel discs were prepared in tryptic soy broth (PML Microbiologicals) to a density of between 5×10^8 and 1×10^{10} cfu/mL as measured with a standardized spectrophotometer. The concentrations of bacteria or spores applied were confirmed by performing serial dilutions in sterile saline and duplicate plating to appropriate media. Brushed stainless steel discs (10 mm diameter and 0.7 mm thick; Muzeen and Blythe, Winnipeg, MB, Canada) were used as the test surface medium. The steel discs were cleaned and then sterilized in a steam autoclave after each use. Then 20 μL of the freshly prepared bacterial inoculum in tryptic soy broth (which acted as an organic soil load) was applied to the steel discs, providing an inoculum of approximately 5×10^6 to 5×10^7 cfu per disc. The inoculated discs were allowed to dry at room temperature while resting in a lidless sterile Petri dish for approximately 45-60 minutes in a biological safety cabinet.[22,23] Three steel discs were placed in each Petri dish, and all exposures of inoculated discs were done in triplicate.

Once the discs were dry, the Petri dish lids were placed over the discs, and the dishes were carefully placed in the test chamber. Then the Petri dish lids were removed, and the discs were exposed to the ozone test conditions. Control discs in triplicate were left covered in the biological safety cabinet and were not exposed to the ozone test conditions.

Inoculation of cotton gauze pads

Using the same bacterial inoculum as above, sterile 5 cm × 5 cm, 12-ply cotton gauze pads (Safe Cross First Aid Ltd, Toronto, ON, Canada) were inoculated and allowed to air-dry in a biological safety cabinet. The sterile gauze pads were aseptically suspended vertically in a small plastic frame in the test chamber, whereas control samples remained covered in the biological safety cabinet. To ensure a flow of the ozone gas mixture through the gauze pads and to provide physical agitation of the fabric, two 40-mm silent mini fans (model SY124020L; Scythe USA, City of Industry, CA) were placed 5 cm in front of the test gauze pads and left to run during the entire test exposure period.

Elution and counting of survivor bacteria

Immediately after exposure to the ozone test conditions, and similarly for the unexposed control discs, the steel discs were vigorously mixed in 10 mL of sterile 0.85% saline solution using a vortex mixer

at high speed for 60 seconds to elute off surviving viable bacteria or spores. The eluted suspension was serially diluted 10-fold in sterile 0.85% saline, and the diluted bacteria were quantitatively plated onto Columbia sheep's blood agar plates for the aerobic bacteria or onto Brucella anaerobic blood agar plates for *C difficile* and incubated under appropriate conditions in duplicate to determine the original inoculum concentration. The same method was used to elute the bacteria from the gauze pads. Calculating the difference in the bacterial counts of the unexposed controls and of the exposed test discs or gauze yielded the logarithmic reduction in bacteria under the test conditions.

Ozone generator and test chamber

The AsepticSure system (Medizone International, Stinson Beach, CA) consists of ozone gas and hydrogen peroxide vapor generation units, related computerized controls, exhaust gas scrubbing units, and a test chamber. Medical-grade oxygen is supplied to the corona discharge ozone generator unit, and the ozone produced passes through polypropylene tubing to the test chamber composed of 5-mm-thick polycarbonate. The chamber is $1.0 \times 0.5 \times 0.5$ m in size (0.25 m^3) fitted with two airtight access doors and a single 40-mm silent mini fan suspended from the ceiling of the chamber to provide gas circulation. Ozone gas exits the chamber through a tube connected to an activated charcoal filter and a small air pump to create a circuit of fresh ozone entering the test chamber, maintaining a stable and constant atmosphere within the chamber. Sterile water and hydrogen peroxide in the desired concentration are added to a sterile reservoir bottle connected to a small humidifier, which delivers aerosols of moisture to maintain a constant predetermined level of humidity and hydrogen peroxide within the chamber. The concentration of the ozone in the test chamber was continuously measured with an ozone meter and probe (EcoSensor 295; Eco Sensors Inc, Newark, CA). The humidity and temperature inside the chamber were measured using humidity and temperature sensor probes (BiOzone Corp, Englewood, CO). Data on ozone levels, humidity, and temperature were continuously uploaded and saved to a customized computer database using a personal laptop computer (model 4233; Lenovo, Morrisville, NC).

In the experiments using hydrogen peroxide, sterile pure pharmaceutical-grade (USP) 3% (vol/vol) hydrogen peroxide was added to the humidification reservoir or diluted in sterile distilled water to the desired final working concentration. Exposure times of the inoculated stainless steel discs or gauze pads varied from 30 to 90 minutes. All experiments were conducted at 21-23°C, ambient pressure, and 80% humidity unless indicated otherwise.

After each experiment, the test chamber interior and humidification system were disinfected with 100% ethanol and allowed to air-dry. The test chamber was always kept closed between uses, to prevent contamination.

Scaled-up testing in rooms

To evaluate the ability to scale up the process to full-sized rooms and to examine the reproducibility in different test room conditions, we sealed off an empty laboratory room (6.1 m × 4.9 m, with a 2.74-m ceiling; total volume, 82 m^3). The entire room except for the cement floor was sealed with 3-mil polyethylene plastic sheeting and polypropylene tape to make it air-tight. During experimental runs, the door was taped shut and the room's air-handling system was turned off.

The generation of ozone for the test rooms by the scaled-up AsepticSure system uses UV light ozone generators instead of corona discharge so as to use the available oxygen in the room to generate the ozone gas. This process does not increase the net amount of gas in the room, and thus the ambient pressure in the test room does not increase. A high-volume humidifier is part of the system, and ozone concentration, humidity and temperature were measured using a 6-channel sensor (model 465L; Teledyne Analytical Instruments, City of Industry, CA). We measured ozone, humidity, and temperature continuously in 5 separate locations inside the room during each experiment and the data for each run were uploaded to the same computer system used for the test chamber. As a further test of the robustness of the system, we later moved the entire apparatus to an unused 90-m^3 laboratory room in a local teaching hospital. This room's walls and ceiling were of solid plaster construction. Penetrations of the walls by pipes, doors, and air ducts were sealed with self-adhering plastic masking film (3M, St Paul, MN).

During the experiments in the test rooms, between 3 and 5 Petri dishes with 3 inoculated stainless steel discs identical to those prepared for the experiments with the chamber were placed in immediate proximity to the sample ports of the Teledyne sensor within the test room. The discs were placed 1 m above the ground on small tables located 2-2.5 m from the ozone generator, which was placed in the center of the room. At the end of each experimental run in the test rooms, the charcoal-filled scrubbers were activated; this reduced the ozone level in the rooms to ≤0.02 ppm within 20 minutes.

RESULTS

To evaluate the interactions of ozone concentration, hydrogen peroxide concentration, and exposure time,

Table 1. Effects of ozone, humidity, hydrogen peroxide, and exposure time on bacterial kill of MRSA on stainless steel discs in the test chamber

Variable	Ozone, ppm	Humidity, %	H_2O_2, %	Exposure time, min	Log_{10} reduction	SD
Ozone, 0% H_2O_2	50	80	0	90	0.97	0.03
	80	80	0	90	0.83	0.06
	120	80	0	90	0.81	0.09
	180	80	0	90	0.66	0.13
	500	80	0	90	6.73	0.07
Humidity and time, 0.2% H_2O_2	80	45	0.2	30	0.13	0.03
	80	45	0.2	60	1.17	0.03
	80	45	0.2	90	1.29	0.09
	80	60	0.2	30	0.04	0.06
	80	60	0.2	60	0.99	0.05
	80	60	0.2	90	1.86	0.05
	80	80	0.2	30	1.40	0.03
	80	80	0.2	60	2.40	0.02
	80	80	0.2	90	7.45	0.03
Humidity and time, 1% H_2O_2	80	60	1	30	1.05	0.08
	80	60	1	60	2.50	0.09
	80	60	1	90	7.45	0.03
	80	80	1	30	7.37	0.09
	80	80	1	60	7.37	0.09
	80	80	1	90	7.37	0.09
Time, 3% H_2O_2	80	80	3	30	6.40	0.09
	80	80	3	60	6.40	0.09
	80	80	3	90	6.40	0.09

SD, standard deviation.

we ran serial experiments altering these variables using the MRSA strain as the test organism inoculated to steel discs. After the most optimal conditions for bacterial kill were determined, we applied these conditions to the other test bacterial strains or spores to evaluate the spectrum of antimicrobial activity.

The elution efficiency of recovering the bacteria or spores after drying onto the steel discs was determined by the ratio of the bacterial count of the unexposed control discs to the bacterial count of the original inoculum placed onto the steel discs multiplied by 100. The elution efficiency exceeded 95% in all cases and for all strains and spores.

We examined the effect of increasing ozone concentrations on bacterial kill by exposing the MRSA strain to ozone in increasing concentrations without hydrogen peroxide at 80% humidity for 90 minutes. At 50-180 ppm ozone, bacterial kill was negligible; however, at 500 ppm ozone, there was a >6 $\log_{10}$ reduction in bacteria compared with the unexposed control discs (Table 1).

The addition of hydrogen peroxide at low concentrations (0.2%) combined with 80 ppm of ozone at exposure times of 30-60 minutes produced incremental increases in bacterial kill, but to a maximum reduction of only of 2.4 $\log_{10}$. The bacterial kill was enhanced by increasing the humidity level to 80% compared with 45% or 60% humidity. A >7 $\log_{10}$ reduction was achieved after 90 minutes of exposure to 80 ppm ozone and 0.2% hydrogen peroxide at 80% humidity. Under these same conditions of ozone and humidity but without any hydrogen peroxide, the bacterial kill was minimal, suggesting that hydrogen peroxide was producing a synergistic bacterial kill with the ozone gas compared with the same conditions without hydrogen peroxide. Exposing the MRSA strain to 1% hydrogen peroxide for 90 minutes without any ozone in the chamber produced a negligible bacterial kill (data not shown).

Increasing the hydrogen peroxide vapor concentration to 1% in the test chamber in combination with 80 ppm ozone produced a high level of bacterial kill, with a >7 $\log_{10}$ reduction in as little as 30 minutes of exposure at 80% humidity. Lowering the humidity to 60% significantly reduced the bacterial kill, and 90 minutes of exposure was required to achieve a high level of bacterial reduction. The combination of 3% hydrogen peroxide vapor and 80 ppm ozone did not produce a higher level of bacterial kill of MRSA in the test chamber compared with 1% hydrogen peroxide.

Exposure to ozone without hydrogen peroxide did not efficiently kill VRE at ozone concentrations of 80 or 400 ppm, even with 90 minutes of exposure (Table 2). However, exposure to 80 ppm ozone with 1% hydrogen peroxide vapor at 80% humidity produced a 5.79 $\log_{10}$ reduction in VRE. This high level

Table 2. Effects of ozone, hydrogen peroxide, and exposure time on bacterial kill of VRE, *E coli*, *P aeruginosa*, *C difficile*, and *B subtilis* on stainless steel discs in the test chamber (80% humidity throughout)

Organism	Ozone, ppm	H_2O_2,%	Exposure time, min	Log_{10} reduction	SD
VRE	80	1	30	5.79	0.05
	80	1	45	5.79	0.05
	80	1	60	5.79	0.05
	80	1	90	5.79	0.03
E coli	80	1	30	6.77	0.09
	80	1	45	6.77	0.09
	80	1	60	6.77	0.09
	80	1	90	6.77	0.09
P aeruginosa	80	1	30	7.36	0.13
	80	1	45	7.36	0.13
	80	1	60	7.36	0.13
	80	1	90	7.36	0.13
C difficile	80	1	45	7.90	0.07
	80	1	60	7.90	0.07
	80	1	90	7.90	0.07
B subtilis	80	1	90	7.23	0.06
	80	3	90	7.23	0.06

SD, standard deviation.

of bacterial kill for VRE was achieved at 30, 45, 60, and 90 minutes of exposure. Similarly high reductions in *E coli* (>6 log_{10}) and *P aeruginosa* (>7 log_{10}) also were achieved with exposure to 80 ppm ozone and 1% hydrogen peroxide at 80% humidity for 30-90 minutes (Table 2).

Exposing *C difficile* spores to 80 ppm ozone and 1% hydrogen peroxide vapor and 80% humidity resulted in a 7.90 log_{10} reduction in as little as 45 minutes of exposure. Similarly, *B subtilis* spores achieved a 7.3 log_{10} reduction with 90 minutes of exposure to 80 ppm ozone and 1% as well as 3% hydrogen peroxide at 80% humidity (Table 2).

The results for experiments conducted in the test rooms are presented in Table 3. The rooms were fully charged with 80 ppm ozone within 20 minutes of activating the UV ozone generators. The results for MRSA, VRE, and *E coli*, were very close to those achieved with the test chamber and were similar in the 2 test rooms. At 80 ppm ozone and 1% hydrogen peroxide, a ≥6 log_{10} reduction was achieved for each of these pathogens after 60 minutes of exposure. Under these same condition, a 5.75-6.37 log_{10} reduction of spores of *C difficile* and *B subtilis* was achieved after 90 minutes of exposure.

To examine whether the combination of 80 ppm ozone and 1% hydrogen peroxide vapor at 80% humidity can effectively disinfect fabric, sterile cotton gauze pads inoculated with MRSA were placed in the test chamber for 30 minutes. Placing an additional mini fan directly in front of each of the inoculated gauze pads as they were vertically suspended in the test chamber was found to be necessary for effective bacterial kill. Without a mini fan, the bacterial kill was low at 1.37 ± 0.11 log_{10}, whereas adding the mini fans increased the kill to 7.1 ± 0.55 log_{10}.

Table 3. Effects of ozone, hydrogen peroxide, and exposure time on bacterial kill of MRSA, VRE, *E coli*, *C difficile*, and *B subtilis* on steel discs in the 113-m^3 test room (80% humidity throughout)

Organism	Ozone, ppm	H_2O_2, %	Exposure time, min	Log_{10} reduction	SD
MRSA	80	1	60	6.43	0.06
	80	1	90	6.43	0.06
	80	3	90	6.43	0.06
VRE	80	1	60	6.08	0.07
	80	1	90	6.08	0.07
E coli	80	1	60	6.02	0.08
C difficile	80	1	90	5.75	0.10
B subtilis	80	1	90	6.37	0.09

SD, standard deviation.

DISCUSSION

Ozone's antiseptic qualities have been known since the 19th century. The first ozone disinfection experiment was conducted in France in 1886, when de Meritens demonstrated that diluted ozonized air could sterilize polluted water.[24] Ozone gas (O_3) with a molecular weight of 48 is highly reactive with a large excess of energy (~143 KJ/mol) and a high level of oxidizing power, derived from its marked tropism for extracting electrons from other molecules and simultaneously releasing one of its own oxygen atoms in the process.[25]

Ozone is a broad-spectrum disinfectant that can be used to inactivate a wide range of microorganisms, including viruses and bacteria that may be resistant to other disinfectants.[20,26] Ozone's mode of action against microorganisms is not completely defined. Some studies on bacteria suggest that ozone alters proteins and unsaturated bonds of fatty acids in the cell membrane, leading to cell lysis.[27] Ozone may disrupt cellular enzyme activity by reacting with thiol groups, and it modifies purine and pyrimidine bases in nucleic acids.[28] Both lipid-enveloped and non-lipid-enveloped viruses are susceptible to disruption by ozone through lipid peroxidation with subsequent lipid envelope and protein capsid damage.[29]

Ozone is widely used to disinfect municipal water distribution systems of water-borne pathogens. When applied as a gas to disinfect surfaces and room spaces in laboratory and field settings, ozone has demonstrated modest antibacterial and antiviral properties. During field tests with a prototype of the Viroforce ozone dispersal system (Viroforce Systems, Kelowna, BC, Canada), the authors demonstrated a 3-4 log_{10}

reduction in bacterial pathogens including MRSA, *Acinetobacter baumannii*, and *C difficile* spores applied to various surfaces, both hard and soft, and as dry or wet films. High relative humidity was associated with superior antimicrobial effects of the ozone.[19,20,25]

An important finding in our research reported here is the synergistic effect of the addition of low concentrations of hydrogen peroxide vapor to the ozone gas in both the test chamber and the test rooms. We found a significant increase in bacterial kill with the addition of 1% hydrogen peroxide, with bacterial reduction rates of 6-7 $\log_{10}$. This synergistic effect was noted for all 4 vegetative bacteria that we tested (MRSA, VRE, *E coli*, and *P aeruginosa*) such that only 30 minutes of exposure was required. Such extremely high bacterial kill rates have not been reported previously with the relatively low concentration of ozone and short exposure durations that we used in this study. Likewise, spores of *C difficile* and *B subtilis* were reduced by 6-7 $\log_{10}$ under the same test conditions with slightly longer exposure periods of 45-90 minutes. The AsepticSure system's ability to achieve such high-level disinfection of both surfaces and different room spaces is an important and unique feature in its applications to health care and other settings.

The basis for the chemical interaction between ozone and hydrogen peroxide is known as the peroxone process.[30] The combination of ozone and hydrogen peroxide results in the formation of highly reactive radicals, including trioxidane (H_2O_3). These radicals are much more reactive than either ozone or hydrogen peroxide alone in treating soil, groundwater, and contaminated waste water.[31]

Hydrogen peroxide vapor, which is used in various systems to decontaminate clinical spaces, is an aerosolized liquid and as such is not expected to penetrate crevices and distribute throughout a room as well as a gas, such as ozone. Ozone gas has excellent penetrating characteristics and comes into contact with all contaminated spaces and surfaces, especially when gas circulation is enhanced (by, eg, adding mini fans to the test chamber during the gauze pad treatment as in these experiments). Furthermore, ozone is capable of inactivating a wide range of bacteria, bacterial spores, mycobacteria, fungi, parasites, and viruses. Ozone is safe for use in sealed rooms and can be rapidly deployed in busy health care settings. With its UV ozone-generating technology, the AsepticSure system does not positively pressurize the room in which it is deployed, minimizing the risk of ozone gas escaping from the desired space undergoing the disinfection treatment. The end product after the catalytic conversion (ie, scrubbing) of the ozone and hydrogen peroxide is oxygen.

Several previous studies have also successfully reduced microbial counts in hospitals using hydrogen peroxide vapor.[15-17] However, the use of hydrogen peroxide vapor systems remains controversial in terms of its efficacy against bacterial spores. Prolonged treatment periods and multiple treatment cycles may be necessary to adequately decontaminate rooms and equipment.[13,14] Otter et al[18] demonstrated the operational feasibility of a hydrogen peroxide vapor system in a modern hospital in-patient environment.

In this study, we have demonstrated that a system using a synergistic combination of ozone and hydrogen peroxide that yields reactive oxygen intermediates such as H_2O_3 achieves a very high level of disinfection, with a $\geq$6 $\log_{10}$ reduction in bacterial and spore counts in a short period of time. The system is scalable and reproducible to different full- sized rooms with no reduction in efficacy or efficiency. With due diligence to ensure air-tightness of the test rooms, no leakage of the ozone gas was detected by sensitive external ozone meters outside the rooms. Our findings indicate that just like hydrogen peroxide vapor fumigation systems, ozone-hydrogen peroxide vapor disinfection systems can be successfully deployed in functioning health care facilities, but will produce higher levels of bacterial and spore kill.[18] More studies are needed to examine the applicability and compatibility of this system in actual hospital facilities, such as operating rooms, patient rooms, laboratories, and morgues, and with the materials and equipment that might be exposed to this ozone-based disinfection process.

The authors wish to thank the administration and staff of the Hotel Dieu Hospital, Kingston, Ontario, for allowing us to use space in their hospital for this study, and Kelly Brown for her excellent technical assistance.

References

1. Hamel M, Zoutman D, O'Callaghan C. Exposure to hospital roommates as a risk factor for nosocomial infection. Am J Infect Control 2010;38:173-81.
2. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect Dis 2006;6:130.
3. Wagenvoort JH, Sluijsmans W, Penders RJ. Better environmental survival of outbreak vs sporadic MRSA isolates. J Hosp Infect 2000;45:231-4.
4. Duckro AN, Blom DW, Lyle EA, Weinstein RA, Hayden MK. Transfer of vancomycin-resistant enterococci via health care worker hands. Arch Intern Med 2005;165:302-7.
5. Bhalla A, Pultz NJ, Gries DM, Ray AJ, Eckstein EC, Aron DC, et al. Acquisition of nosocomial pathogens on hands after contact with environmental surfaces near hospitalized patients. Infect Control Hosp Epidemiol 2004;25:164-7.
6. Brown CA, Lilford RJ. Should the UK government's deep cleaning of hospitals programme have been evaluated? J Infect Prevent 2009;10:143-7.
7. McMullen KM, Zack J, Coopersmith CM, Kollef M, Dubberke E, Warren DK. Use of hypochlorite solution to decrease rates of

Clostridium difficile-associated diarrhea. Infect Control Hosp Epidemiol 2007;28:205-7.
8. Hayden MK, Bonten MJ, Blom DW, Lyle EA, van de Vijver DA, Weinstein RA. Reduction in acquisition of vancomycin-resistant enterococcus after enforcement of routine environmental cleaning measures. Clin Infect Dis 2006;42:1552-60.
9. Denton M, Wilcox MH, Parnell P, Green D, Keer V, Hawkey PM, et al. Role of environmental cleaning in controlling an outbreak of *Acinetobacter baumannii* on a neurosurgical intensive care unit. J Hosp Infect 2004;56:106-10.
10. Worsley MA. Infection control and prevention of *Clostridium difficile* infection. J Antimicrob Chemother 1998;41(Suppl C):59-66.
11. Eckstein BC, Adams DA, Eckstein EC, Rao A, Sethi AK, Yadavalli GK, et al. Reduction of *Clostridium difficile* and vancomycin-resistant enterococcus contamination of environmental surfaces after an intervention to improve cleaning methods. BMC Infect Dis 2007;7:61.
12. Wilcox MH, Fawley WN. Hospital disinfectants and spore formation by *Clostridium difficile* [research letter]. Lancet 2000;356:1324.
13. Shapey S, Machin K, Levi K, Boswell TC. Activity of a dry mist hydrogen peroxide system against environmental *Clostridium difficile* contamination of elderly care words. J Hosp Infect 2008;70:136-41.
14. Andersen BM, 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.
15. 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;57:31-7.
16. Hall L, Otter JA, Chewins J, Wengenack NL. Use of hydrogen peroxide vapor for deactivation of *Mycobacterium tuberculosis* in a biological safety cabinet and a room. J Clin Microbiol 2007;45:810-5.
17. Otter JA, French GL. Survival of nosocomial bacteria and spores on surfaces and inactivation by hydrogen peroxide vapor. J Clin Microbiol 2009;47:205-7.
18. Otter JA, Puchowicz M, Ryan D, Salkeld JA, Cooper TA, Havill NL, et al. Feasibility of routinely using hydrogen peroxide vapor to decontaminate rooms in a busy United States hospital. Infect Control Hosp Epidemiol 2009;30:574-7.
19. Hudson JB, Sharma M, Petric M. Inactivation of Norovirus by ozone gas in conditions relevant to healthcare. J Hosp Infect 2007;66:40-5.
20. Sharma M, Hudson JB. Ozone gas is an effective and practical antibacterial agent. Am J Infect Control 2008;36:559-63.
21. Alfa MJ, Dueck C, Olson N, Degagne P, Papetti S, Wald A, et al. UV-visible marker confirms that environmental persistence of *Clostridium difficile* spores in toilets of patients with *C difficile*-associated diarrhea is associated with lack of compliance with cleaning protocol. BMC Infect Dis 2008;8:64.
22. Best M, Kennedy ME, Coates F. Efficacy of a variety of disinfectants against *Listeria* spp. App Env Microbiol 1990;56:377-80.
23. De Cesare A, Sheldon B, Smith K, Jaykus L. Survival and persistence of *Campylobacter* and *Salmonella* species under various organic loads on food contact surfaces. J Food Prot 2003;66:1587-94.
24. Paulouë J, Le Langlais B. State-of-the-art of ozonation in France. Ozone Sci Eng 1999;21:153-62.
25. Guanche D, Zamora Z, Hernández F, Mena K, Alonso Y, Roda M, et al. Effect of ozone/oxygen mixture on systemic oxidative stress and organic damage. Toxicol Mech Methods 2010;20:25-30.
26. Victorin K. Review of the genotoxicity of ozone. Mutat Res 1992;277: 221-38.
27. Thanomsub B, Anupunpisit V, Chanphetch S, Watcharachaipong T, Poonkhum R, Srisukonth C. Effects of ozone treatment on cell growth and ultrastructural changes in bacteria. J Gen Appl Microbiol 2002;48: 193-9.
28. Russell AD. Similarities and differences in the responses of microorganisms to biocides. J Antimicrob Chemother 2003;52:750-63.
29. Murray BK, Ohmine S, Tomer DP, Jensen KJ, Johnson FB, Kirsi JJ, et al. Virion disruption by ozone-mediated reactive oxygen species. J Virol Methods 2008;153:74-7.
30. Elovitz MS, von Gunten U, Kaiser H. Hydroxyl radical/ozone ratios during ozonation processes, II: The effect of temperature, pH, alkalinity, and DOM properties. Ozone Sci Eng 2000;22:123-50.
31. Xu X, Goddard WA 3rd. Peroxone chemistry: formation of H_2O_3 and ring-$(HO_2)(HO_3)$ from O_3/H_2O_2. Proc Natl Acad Sci USA 2002;99: 15308-12.