Major article

Evaluation of hydrogen peroxide vapor for the inactivation of nosocomial pathogens on porous and nonporous surfaces

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Sebastian Lemmen MD [a,*], Simone Scheithauer [a], Helga Häfner [a], Saber Yezli PhD [b], Michael Mohr [c], Jonathan A. Otter PhD [b,d]

[a] Department of Infection Control and Infectious Diseases, University Hospital Aachen, Aachen, Germany
[b] Bioquell UK Ltd, Andover, Hampshire, UK
[c] Schülke & Mayr GmbH, Norderstedt, Germany
[d] Centre for Clinical Infection and Diagnostics Research, Department of Infectious Diseases, King's College London and Guy's and St. Thomas' National Health Service (NHS) Foundation Trust, London, UK

Background: Clostridium difficile spores and multidrug-resistant (MDR) organisms, such as methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococcus (VRE), and MDR Acineto-bacter baumannii, are important nosocomial pathogens that are difficult to eliminate from the hospital environment. We evaluated the efficacy of hydrogen peroxide vapor (HPV), a no-touch automated room decontamination system, for the inactivation of a range of pathogens dried onto hard nonporous and porous surfaces in an operating room (OR).
Methods: Stainless steel and cotton carriers containing >4 $\log_{10}$ viable MRSA, VRE, or MDR A baumannii were placed at 4 locations in the OR along with 7 pouched 6 $\log_{10}$ Geobacillus stearothermophilus spore biologic indicators (BIs). HPV was then used to decontaminate the OR. The experiment was repeated 3 times.
Results: HPV inactivated all spore BIs (>6 $\log_{10}$ reduction), and no MRSA, VRE, or MDR A baumannii were recovered from the stainless steel and cotton carriers (>4-5 $\log_{10}$ reduction, depending on the starting inoculum). HPV was equally effective at all carrier locations. We did not identify any difference in efficacy for microbes dried onto stainless steel or cotton surfaces, indicating that HPV may have a role in the decontamination of both porous and nonporous surfaces.
Conclusion: HPV is an effective way to decontaminate clinical areas where contamination with bacterial spores and MDR organisms is suspected.

Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), *Acinetobacter bau-mannii*, and *Clostridium difficile* are serious nosocomial pathogens because of a combination of their environmental resilience, their association with antimicrobial resistance, and their outbreak potential.[1,2] Surface contamination has been implicated in the transmission of these organisms, and previous room occupancy by patients with MRSA, VRE, *A baumannii*, or *C difficile* infection or colonization increase the chances that these pathogens are acquired by patients subsequently admitted to the same room.[1] As a consequence, environmental disinfection is reiterated in various guidelines for the prevention and control of infection with these organisms in health care settings.[3-6] However, these organisms, especially the spores of *C difficile*, can present a particular challenge to effective decontamination in health care settings because of their resistance to desiccation and some disinfectants and the fact that achieving adequate distribution and contact time with liquid cleaning agents is difficult in the complex hospital environment.[7-9] Indeed, even bleach (sodium hypochlorite), which is effective against hospital pathogens in vitro, does not always eliminate pathogens from surfaces. For example, Barbut et al[9] found that 23 of 194 swabs (12%) collected from 5,000 ppm bleach-treated rooms were contaminated with *C difficile*, and Manian et al[7] found that 26.6% of patient rooms

Table 1
In vitro efficacy of HPV against MDR organisms dried on stainless steel disks and cotton

	MRSA		VRE		MDR *A baumannii*	
	Stainless steel	Cotton	Stainless steel	Cotton	Stainless steel	Cotton
Mean bacterial count in the working suspension (CFU/mL)	7.3×10^8	7.3×10^8	4.2×10^8	4.2×10^8	5.8×10^8	5.8×10^8
Mean bacterial count applied on the carriers (CFU per carrier)	7.3×10^6	7.3×10^6	4.2×10^6	4.2×10^6	5.8×10^6	5.8×10^6
Mean bacterial count on the control carriers at the end of HPV exposure (CFU per carrier)	2.6×10^4	6×10^4	3.1×10^4	3.4×10^4	1.5×10^5	6×10^4
Mean bacterial count on the HPV-exposed carriers at the end of HPV exposure (CFU per carrier)	<1	<1	<1	<1	<1	<1
Mean $\log_{10}$ reduction caused by drying and overnight incubation at ambient environment and recovery technique	2.4	2.1	2.3	2.4	1.6	2.0
Mean $\log_{10}$ reduction caused by HPV exposure	4.4[*]	4.7[*]	4.1[*]	4.0[*]	5.1[*]	4.7[*]

CFU, colony forming units; *HPV*, hydrogen peroxide vapor; *MDR*, multidrug resistant; *MRSA*, methicillin-resistant *S aureus*; *VRE*, vancomycin-resistant *Enterococcus*.
[*]Complete inactivation of the organism from the carriers.

remained contaminated with *Acinetobacter* or MRSA after 4 rounds of 5,000 ppm bleach disinfection.

No-touch automated room decontamination technologies, such as hydrogen peroxide vapor (HPV), have therefore been used for the decontamination of health care facilities and to improve the level of surface disinfection.[10] HPV is an Environmental Protection Agency-registered sterilant, does not rely on the operator to achieve adequate distribution and contact time, and has demonstrated in vitro efficacy against various nosocomial pathogens.[8,10] HPV has been shown to eradicate pathogens from environmental surfaces and helps to bring hospital outbreaks under control.[10] The use of HPV mitigates the risk from the prior room occupant with multidrug-resistant organisms (MDROs) and reduces the transmission of *C difficile* in hospitals.[11-13]

Nevertheless, most in vitro studies reported the efficacy of HPV against microorganisms dried onto hard surfaces, and its efficacy against pathogens on porous surfaces (eg, textile, cotton) is not well determined.[8,14,15] Such surfaces can be encountered in the hospital setting and present a harder challenge for effective disinfection than hard nonporous surfaces (eg, stainless steel). Further, most in situ evaluations of HPV have been performed in single rooms or whole wards; few evaluations have been performed in an operating room (OR) setting.[10,16] ORs present a particular challenge for HPV because of complex and often powerful air handling systems combined with sensitive medical equipment. We aimed to determine the efficacy of HPV in an OR against commercially available spore biologic indicators (BIs) as a proxy for *C difficile* spores and against a selection of MDROs dried on both hard nonporous and soft porous surfaces represented by stainless steel disks and cotton, respectively.

METHODS

Microorganisms

Two representative multidrug-resistant (MDR) gram-positive bacteria were used: MRSA strain ATCC 43300 and VRE strain DSM 17050. A MDR *A baumannii* clinical isolate was used to represent environmentally resilient MDR gram-negative bacteria. Tyvek-packaged *Geobacillus stearothermophilus* spore BIs (BAG SporeDisc HPV, Tyvek, BAG Healthcare, Lich, Germany), with a certified population of >6 $\log_{10}$ spores/stainless steel disk, were used as a proxy for *C difficile*.

Carrier preparation and processing

For all bacterial strains, a fresh colony from an overnight growth on blood agar was suspended in 5 mL tryptone soya broth (Biomerieux, Bruchsal, Germany) and was incubated overnight at 37°C.

Then 1 mL of the bacterial solution was centrifuged twice and suspended in 100 μL of sterile distilled water. The suspension was then adjusted to a 1 McFarland standard in a nephelometer to produce a working bacterial suspension. The number of colony forming units (CFU) per milliliter of the working suspension was determined using serial dilutions and incubation on blood agar for 18 hours at 37°C.

Stainless steel carriers (10 mm diameter, circular) were inoculated by applying 10 μL of the working bacterial suspension to the disks, which were then kept in a sterile basin at ambient temperature overnight. For the porous carriers, 1 cm² sterile textile pieces consisting of standard cotton were loaded with 10 μL of the working bacterial suspension. The textile pieces were mounted on extruded polystyrene foam with small needles to assure that they had no contact with the surface beneath and that the textile samples had soaked up the entire amount of the bacterial suspension.

Four of each type of inoculated carrier (stainless steel, cotton) for each of the bacterial strains used were placed in open Petri dishes and distributed in 4 locations (right and left window sill, central [operating table] location, near the door) in an OR. Four of each type of carrier for each of the bacterial strains were kept outside the OR as controls (not exposed to HPV). In addition to the bacterial test disks, 7 Tyvek-packaged 6 $\log_{10}$ *G stearothermophilus* BIs were placed in 4 corner locations plus 3 challenge locations within the OR. BIs were placed in alternating high and low locations in the room corners, approximately 10 cm from the floor or ceiling.

At the end of the HPV decontamination cycle, each carrier was transferred into a sterile glass tube with 1 mL of distilled water and was left to stand at ambient temperature for 15 minutes before sonication with ultrasound (40 kHz) for 20 minutes to detach the bacteria from the surfaces. Bacterial counts on the resulting solution were performed by serial dilutions of 10 μL of the solution and incubation on blood agar for 18 hours at 37°C. In addition, the remaining 990 μL of the resulting bacterial suspension were plated out on 2 blood agar plates to be able to report a detection limit of <1 CFU. Colonies were counted, and the average cell count was calculated for each material and organism. The efficiency of recovery of bacteria from carriers (measure of loss of viability because of overnight drying combined with the possibility of incomplete recovery of bacteria from the carriers) was determined by calculating the difference between the number of bacteria applied to the disks and the number recovered from the control carriers. The concentration of bacteria recovered from metal and cotton carriers on the 3 replicate runs was compared using a paired *t* test. The efficacy of HPV decontamination was calculated as the difference in bacterial count recovered from the control carriers and the number recovered from the carriers exposed to HPV.

G stearothermophilus BIs were removed following HPV exposure, transferred into test tubes containing 10 mL tryptone soya broth, and incubated for 7 days at 55°C. They were then evaluated visually for opacity. An unexposed BI was treated in the same way and incubated with each batch as a positive control.

HPV decontamination

Three replicate HPV decontamination cycles were performed. Carriers were exposed to HPV in an unoccupied, fully equipped OR (95 m³ in size) as described previously.[11] Briefly, the door to the room was sealed using tape, and all ventilation ducts were closed. An HPV generator (Q-10, Bioquell, Andover, UK) was positioned in the center of the room and was used to vaporize 30% liquid hydrogen peroxide into HPV, which was injected into the room for 50-52 minutes to achieve a dose of 11.2 g/m³ until hydrogen peroxide was deposited on all exposed surfaces. Peak concentrations of hydrogen peroxide measured in the air were approximately 500-600 ppm. After a 20-minute dwell time during which no further HPV was injected, the HPV was converted to oxygen and water vapor by a catalytic converter (R-10, Bioquell, Andover, UK). The OR was re-entered once the concentration of HPV in the room was <0.5 ppm, and carriers were then collected for processing as previously described.

RESULTS

The working solution of all strains contained >8 log₁₀ CFU/mL, and >6 log₁₀ per carrier of each bacterial strain was applied to each carrier with a recovery of >4 log₁₀ per carrier (Table 1). There was no difference in bacterial recovery from stainless steel disks compared with cotton for all bacterial strains ($P > .05$ for all strains).

HPV inactivated bacteria and spores on all carriers, regardless of the organism, location, or surface material (Table 1). It was not possible to compare the efficacy of HPV on stainless steel with cotton because no pathogens were recovered.

HPV cycle times from the start of room preparation to the end of aeration (when the room was ready for reoccupation) ranged from 2-3 hours.

DISCUSSION

C difficile spores and MDROs (eg, MRSA, VRE, MDR *A baumannii*) are important nosocomial pathogens that are able to survive for long period of time on dry hospital surfaces, and can be transmitted to patients from these surfaces.[1,2] They are also difficult to eliminate from the hospital environment by standard cleaning and disinfection methods.[7,9,17] We evaluated the efficacy of HPV for the inactivation of a range of MDROs dried on a hard nonporous surface (stainless steel disks) and a soft porous surface (cotton) in an OR.

HPV eliminated both bacterial endospores and vegetative bacteria dried on stainless steel disks and cotton carriers at various sites in the OR. The efficacy of HPV against these organisms dried on stainless steel disks has been reported previously. Otter and French[8] exposed 5-7 log₁₀ of various strains of MRSA, VRE, *Acinetobacter*, and *C difficile* spores dried on stainless steel disks to HPV. HPV completely inactivated all organisms even when they were dried in 0.3% bovine serum albumin to simulate soiling. Similarly, Fu et al[18] found HPV effective against MRSA, *Acinetobacter*, and *C difficile* spores dried on stainless steel carriers and 6 and 4 log₁₀ *G stearothermophilus* BIs, located at various sites in a test room.

Catalase-positive bacteria have been shown to be less susceptible to HPV in other studies; catalase is able to degrade hydrogen peroxide explaining this reduced susceptibility.[8,19] We did not identify any difference in the efficacy of HPV against the catalase-positive *A baumannii* and MRSA compared with the catalase-negative VRE and metabolically inert *G stearothermophilus* spores. The studies that have identified a reduced susceptibility of catalase-producing bacteria to HPV have taken samples from an enclosure during an HPV cycle.[8,19] In contrast, we only collected endpoint carriers; therefore, we were less likely to detect a reduced HPV susceptibility in catalase-producing bacteria.

We did not identify any difference in efficacy on porous cotton compared with nonporous stainless steel. Barbut et al[14] did not identify a difference in the activity of HPV against several strains of *C difficile* spores dried on polyvinyl chloride (representative of a patient room's floor) or laminate (representative of a patient room's furniture) carriers at mean concentrations of 4.7-6.9 log₁₀ spores per carrier. Few studies have evaluated the efficacy of HPV against pathogens dried on porous surfaces. The AOAC (Association of Official Analytical Chemists) carrier test[20] for the Environmental Protection Agency-sterilant test includes spores dried onto porous materials (suture loops); HPV has demonstrated a >6 log₁₀ reduction on spores dried onto these materials. A study by Rogers et al[15] found that the efficacy of HPV for the inactivation of various spores was substantially lower on industrial carpet (range, 1-3 log₁₀ reduction) compared with hard surfaces, such as glass (4-6 log₁₀ reduction). This study was performed using a different HPV generator in a small chamber; therefore, results are not directly comparable with our results, but these findings suggest that further work evaluating the efficacy of HPV on porous surfaces is warranted. In addition, in situ sampling of various hospital surfaces, including porous surfaces, indicates that HPV eliminates various pathogens, including *C difficile* spores, MRSA, VRE, and *Acinetobacter*.[10] For example, in the study by French et al,[21] 50% of 16 fabric chair arms were contaminated with MRSA after terminal cleaning, whereas none were contaminated after HPV. This corroborates older findings showing that liquid hydrogen peroxide sprayed onto fabric materials eliminated pathogens.[22]

We demonstrated that HPV is feasible to apply in the OR setting, and visual inspection of the OR equipment and furniture (including the operating table, storage cabinets, and equipment, such as the operating light) following the 3 HPV exposures did not reveal any noticeable damage or alteration. A recent study[23] showed that regular use of HPV over 8 years at a U.S. hospital did not adversely affect electronic medical equipment. However, the cycle time for HPV (2-3 hours) means that it is not suitable for decontaminating ORs between cases except in the case of high-risk pathogens, which are often held until the end of the day.

Approximately 2 log₁₀ of bacteria was lost because of drying, overnight incubation in an ambient environment, and/or a recovery technique. It was not possible to determine the relative contribution of each of these factors to the results. Nevertheless, it is likely that most of the log₁₀ reductions noted were caused by a combination of loss of viability because of drying and the efficiency of the recovery method because these pathogens are shown to survive for prolonged periods of time on dry surfaces. For instance, Otter and French[8] dried various strains of MRSA, VRE, and *A baumannii* on stainless steel disks and followed their survival in the ambient environment. Minimal loss of viability (<1 log₁₀) was noted after 24 hours for all strains, and all survived for over a month with VRE and MRSA surviving better than *A baumannii*.

Our study is one of the first to evaluate the efficacy of HPV for a range of pathogens on porous surfaces and provides evidence that HPV is feasible in an OR setting. However, limitations include testing 1 strain for each organism and only testing 2 surface types. Further studies should explore efficacy against a wider range of strains on various surfaces types and finishes.

HPV achieved a >6 log₁₀ reduction on *G stearothermophilus* spore BIs and a >4-5 log₁₀ reduction on MRSA, VRE, and MDR

A baumannii dried on stainless steel and cotton carriers located at various sites in an OR. We did not identify any difference in efficacy for microbes dried onto stainless steel or cotton surfaces, indicating that HPV may have a role for the decontamination of both porous and nonporous surfaces. HPV is an effective way to decontaminate clinical areas where contamination with bacterial spores and MDR organisms is suspected.

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