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

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Key Words: Cleaning Disinfection Decontamination Hydrogen peroxide vapor Vancomycin-resistant Enterococcus Clostridium difficile

Background: Clostridium difficile spores and multidrug-resistant (MDR) organisms, such as methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococcus (VRE), and MDR Acinetobacter 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 log10 viable MRSA, VRE, or MDR A baumannii were placed at 4 locations in the OR along with 7 pouched 6 log10 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 log10 reduction), and no MRSA, VRE, or MDR A baumannii were recovered from the stainless steel and cotton carriers (>4-5 log10 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.

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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. 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. HPV has been shown to eradicate pathogens from environmental surfaces and helps to bring hospital outbreaks under control. 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 known. 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. 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 $\geq 6 \log_{10}$ 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 at ambient environment and recovery technique

Table 1

<table>
<thead>
<tr>
<th></th>
<th>MRSA</th>
<th>VRE</th>
<th>MDR A baumannii</th>
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<tbody>
<tr>
<td></td>
<td>Stainless</td>
<td>Cotton</td>
<td>Stainless</td>
</tr>
<tr>
<td>Mean bacterial</td>
<td>7.3 $\times$ 10^6</td>
<td>7.3 $\times$ 10^6</td>
<td>4.2 $\times$ 10^6</td>
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<tr>
<td>count in the</td>
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<td>working</td>
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<tr>
<td>suspension (CFU/mL)</td>
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<tr>
<td>Mean bacterial</td>
<td>7.3 $\times$ 10^6</td>
<td>7.3 $\times$ 10^6</td>
<td>4.2 $\times$ 10^6</td>
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<tr>
<td>count applied</td>
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<td>on the carriers</td>
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<tr>
<td>(CFU per carrier)</td>
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<tr>
<td>Mean bacterial</td>
<td>2.6 $\times$ 10^4</td>
<td>6 $\times$ 10^4</td>
<td>3.1 $\times$ 10^4</td>
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<td>control carriers</td>
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<td>HPV exposure</td>
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<tr>
<td>(CFU per carrier)</td>
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<tr>
<td>Mean log_{10}</td>
<td>2.4</td>
<td>2.1</td>
<td>2.3</td>
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<tr>
<td>Mean log_{10}</td>
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<td>4.7</td>
<td>4.1</td>
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<td>by HPV exposure</td>
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</table>

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.
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 m² in size) as described previously. 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 periods of time on dry hospital surfaces, and can be transmitted to patients from these surfaces. They are also difficult to eliminate from the hospital environment by standard cleaning and disinfection methods. 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 exposed 5-7 log₁₀ of various strains of MRSA, VRE, A. baumannii, 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 found HPV effective against MRSA, A. baumannii, 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. 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. 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 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 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 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 A. baumannii. For example, in the study by French et al, 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.

We demonstrated that HPV is feasible to apply in the OR setting, and visual inspection of the OR environment and decontamination of loss of viability because of drying and the efficacy of the HPV method for the inactivation of MDROs. Approximately 2 log₁₀ of bacteria was lost because of drying, overnight incubation in an ambient environment, and/or a recovery method because these pathogens are shown to survive for prolonged periods of time on dry surfaces. For instance, Otter and French exposed a range of strains to HPV, 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.
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.

References