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Major article

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

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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 $\log_{10}$ 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 baumannii*, 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

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Conflicts of interest: J.A.O. is employed part-time by Bioquell; S.Y. is employed by Bioquell; M.M. is employed by Schülke & Mayr, who distributed Bioquell's products in Germany at the time of this study. All other authors have no potential conflicts of interest to declare.

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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 cm^2 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.