A pilot observational study of hydrogen peroxide and alcohol for disinfection of privacy curtains contaminated by MRSA, VRE and *Clostridium difficile*

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Abstract

Privacy curtains, frequently used in hospitals to separate patient care areas may have an important role in the transmission of healthcare-associated pathogens. In this pilot study, we inoculated curtain swatches with suspensions of clinical specimens of methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococcus (VRE), and *Clostridium difficile* before using a gloved hand to touch the inoculated curtain swatch and transfer to clean agar plates. Three different commonly used disinfectants were then sprayed onto these swatches before using a clean gloved hand to touch the swatch and transfer onto new agar plates. All plates were incubated at 35°C for 24 and 72 h. Bacterial growth before and after disinfection was assessed and compared. 3.1% hydrogen peroxide effectively eliminated transfer of *C. difficile*, MRSA and VRE from inoculated curtains.

Introduction

There is increasing evidence that the physical environment of hospital rooms is contaminated with micro-organisms and that this contamination may play a role in transmission of multidrug resistant micro-organisms and in healthcare associated infections. Environmental surfaces have been found to be colonised with multi-drug-resistant organisms (MDROs) (Otter et al, 2011) and *Clostridium difficile* (Dubberke et al, 2007). Patients in hospital rooms that were previously occupied by patients colonised with methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant enterococcus (VRE), and/or *C. difficile* are more likely to become colonised with these organisms. (Huang et al, 2006; Shaughnessy et al, 2011).

Additionally, it has been shown that privacy curtains are frequently and rapidly colonised with MDROs (Trillis et al, 2008; Ohl et al, 2012; Schweizer et al, 2012) and have been implicated in at least one outbreak due to *Acinetobacter* spp. (Das et al, 2002). As a result, many US hospitals remove and change privacy curtains upon discharge in rooms of patients colonised with MDROs.

There are very little data on the efficacy of disinfecting privacy curtains colonised with MDROs. Two studies demonstrated favourable results by using hydrogen peroxide to disinfect curtains colonised with MRSA and VRE (Neely and Maley, 1999; Burns and Minton, 2011).

The purpose of this study was to evaluate the microbiologic efficacy of three different agents in disinfecting privacy curtains inoculated with MRSA, VRE and *C. difficile*.

Methods

Clinical bacterial isolates of MRSA and VRE spp. previously isolated from patients in our institution were grown on sheep blood agar and incubated for 24h at 35°C (non-CO₂). Similarly, clinical isolates of *C. difficile* (NAP-1 and non-NAP-1), were initially grown for 72h on CDC agar under anaerobic conditions. The exact mechanisms of *C. difficile* sporulation and the rate of sporulation remain unclear at this point; therefore we chose to assess the presence of spores using established laboratory methods (Burns and Minton, 2011). The *C. difficile* suspensions were assessed microscopically prior to use, and the presence of at least 50% spores was confirmed. Since both vegetative cells and spores are present in the healthcare environment, the confirmed presence of spores and vegetative *C. difficile* organisms was considered appropriate and representative of what would be seen in an in vivo clinical setting.

Table 1. Colony counts for *Clostridium difficile* before and after application of two different preparations of hydrogen peroxide as disinfectant

Disinfectant	Mean and range for bacterial colony counts			
	Spray 1 – 3.1% H ₂ O ₂		Spray 2 – activated H ₂ O ₂	
	Before application of spray	After application of spray	Before application of spray	After application of spray
<i>C. difficile</i>; Non-NAP strain (3 swatches each category)	42 (9–73)	0.6 (0–2)	87.6 (54–132)	61 (7–33)

H₂O₂, hydrogen peroxide; NAP, nucleosome assembly protein

Two series of experiments were done. Initially, six swatches of freshly laundered 2x2 inch privacy curtains composed of a cotton/polyester blend that are used at our hospital were inoculated with nine drops of three rows of 0.5 McFarland suspension of non-NAP strain of *C. difficile*. All curtain swatches were allowed to air dry for 20 minutes. A clean gloved hand was used to grasp the curtain swatch and finger imprint cultures were plated on a blood agar plate as described by Bobulsky et al. 2008 (Neely and Maley, 2000). The swatches were then sprayed with an activated hydrogen peroxide from Diversey or a 3.1% hydrogen peroxide solution manufactured by Ecolab using the manufacturer's dispensing containers from 6 inches to 1 foot away as per manufacturer's instructions. After 1 hour, to ensure drying of the saturated cloth, another gloved finger imprint-culture on SBA was obtained. Samples were incubated anaerobically for 48 hours, using the BD GasPak™ EZ Anaerobe Pouch System (Becton, Dickinson & Co., Sparks, MD). After completion of the incubation of agar plates, the presence of spores and Gram positive bacilli (indicative of *C. difficile*) was confirmed by Gram stain and colony counts were obtained. Differences in colony counts before and after use of disinfectants were evaluated and descriptive statistical analyses were performed (StataCorp. College Station, TX).

In the second set of experiments three different strains of 0.5 McFarland suspension of *C. difficile* were inoculated onto the freshly laundered curtain swatches along with 0.5 McFarland suspensions of clinically obtained MRSA and VRE strains. The 0.5 McFarland suspensions of MRSA and VRE were diluted to a concentration of 1:100 and also inoculated onto curtain swatches using nine drops in three rows to ensure saturation. The swatches were again allowed to air dry for 20 minutes and a clean gloved hand was used to obtain finger imprint cultures. The swatches were then sprayed with an activated hydrogen peroxide from Diversey; a 3.1% hydrogen peroxide solution manufactured by Ecolab, or a 53% alcohol solution, a common concentration used in many household environmental disinfection sprays, respectively using the manufacturer's dispensing containers from 6 inches to 1 foot away. After 1 hour, to ensure drying of the saturated cloth, another clean gloved finger imprint-culture on SBA was obtained. For test samples of MRSA and VRE, all agar plates were incubated at 35°C for 24 hours. Samples from the test runs with *C. difficile* were incubated anaerobically for 48 hours, using the BD GasPak™ EZ Anaerobe Pouch System (Becton, Dickinson & Co., Sparks, MD). After completion of the incubation of agar plates, the presence of bacterial growth was assessed and colony counts were obtained. The presence of Gram positive cocci in clusters (indicative of *Staphylococcus aureus*), Gram positive cocci in chains (indicative of *Enterococcus*), and spores and Gram positive bacilli (indicative of *C. difficile*) was confirmed by Gram stain. Differences in colony counts before and after use of disinfectants

were evaluated and descriptive statistical analyses were performed (StataCorp. College Station, TX).

Results

The initial hand imprint plates for MRSA and VRE showed confluent growth. After disinfection with alcohol, activated hydrogen peroxide and 3.1% hydrogen peroxide, there was no growth seen on any of the post spray hand imprint plates.

The initial agar plates for the finger imprint cultures for *C. difficile* showed between 9 and 438 colonies. Alcohol spray had a statistically non-significant reduction in the growth of *C. difficile* on the post hand imprint agar plates (average $p = 0.66$). However, activated hydrogen peroxide and 3.1% hydrogen peroxide significantly reduced and eliminated transmission of *C. difficile* respectively ($P < 0.05$) (Tables 1 and 2).

Discussion

Three different disinfectants were tested, and 53% ethanol spray, 3.1% hydrogen peroxide and activated hydrogen peroxide were all equally effective in reducing growth of MRSA and VRE on SBA plates after transfer from curtains to agar plates using a gloved hand imprint culture method. Alcohol did not appreciably reduce the transfer of *C. difficile*. Activated hydrogen peroxide demonstrated very good activity in eliminating transfer of *C. difficile*, but 3.1% hydrogen peroxide performed the best with no significant transfer of *C. difficile* from inoculated curtain swatches.

Hydrogen peroxide has been shown in many studies to effectively decontaminate patient care areas (Rutala and Weber, 2013). In our study we were able to compare the efficacy of three different agents in eliminating transfer of not only MDROs but *C. difficile* from heavily inoculated privacy curtain swatches. Our study results demonstrate that 3.1% hydrogen peroxide spray effectively disinfects privacy curtains inoculated with high concentrations of MRSA, VRE and/or *C. difficile*. Previous studies have shown efficacy of hydrogen peroxide in disinfecting privacy curtains colonised with MRSA and VRE (Neely and Maley, 1999; Price et al, 2012), but none have tested the ability of hydrogen peroxide spray to disinfect curtain swatches inoculated with *C. difficile*.

Currently, many US hospitals remove and exchange privacy curtains in isolation rooms at the time of discharge for terminal room cleaning. This can be a labour-intensive and time-consuming process that impacts on rapid availability of patient beds and patient rooms. Also, many US hospitals do not routinely perform active routine surveillance for MRSA, VRE and *C. difficile* and therefore many patients who may be colonised with these organisms remain undetected throughout the duration of their hospitalisation and may contaminate privacy

Table 2. Bacterial colony counts for *Clostridium difficile*, meticillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *enterococcus* (VRE) before and after application of hydrogen peroxide and alcohol disinfectants

Disinfectant	Mean and range for bacterial colony counts					
	3.1% H ₂ O ₂		Activated H ₂ O ₂		ETOH	
	Before application of spray	After application of spray	Before application of spray	After application of spray	Before application of spray	After application of spray
C. difficile Non- NAP strain (4 swatches each category)	266 (182–328)	0 <i>p</i> = <0.001	396 (238–614)	4.5 (1–14) <i>p</i> = <0.001	258 (210–469)	49.2 (0–150) <i>p</i> = 0.930
C. difficile NAP strain 1 (4 swatches each category)	299 (145–428)	0 <i>p</i> = <.001	346 (150–478)	22.7 (1–69) <i>p</i> = 0.029	354 (271–484)	279 (104–492) <i>p</i> = 0.306
C. difficile NAP strain 2 (2 swatches each category)	227 (116–339)	0 <i>p</i> = <0.001	277 (115–439)	0 <i>p</i> = <0.001	191 (116–267)	267.5 (160–431) <i>p</i> = 0.762
MRSA (3 swatches each category)	Confluent growth	0	Confluent growth	0	379 – confluent growth	0 (0–0)
MRSA, 1:100 dilution (3 swatches each category)	283 – confluent growth	0	380 – confluent growth	0	303 – confluent growth	0
VRE (3 swatches each category)	48 – confluent growth	0	165 – confluent growth	0	63 – confluent growth	0
VRE, 1:100 dilution (3 swatches each category)	26 – confluent growth	0	221 – confluent growth	0	18 – confluent growth	0.33 (0–1)

H₂O₂, hydrogen peroxide; ETOH, alcohol (%); NAP-1, nucleosome assembly protein 1

curtains. Disinfecting privacy curtains by using hydrogen peroxide spray may be an adjunctive or alternative method to terminally clean isolation rooms. This could have a significant impact on room turnover time and bed flow as well as potentially reducing the transmission of MDROs and healthcare associated infections due to privacy curtains in hospitals.

Our in vitro study has several limitations. The first limitation is that we inoculated the curtain swatches with a mix of vegetative and spore forms of *C. difficile*. This more closely mimics real-world contamination with *C. difficile*, but is a less standardised approach. By using a high inoculum of organisms on well-defined small sized curtain swatches we have likely overestimated the organism burden per square inch. Furthermore, the use of small, well-defined curtain swatches allows for maximal application of disinfectant, which may not represent the amount of disinfectant applied to curtains in actual practice. In practice, it is less likely that the entire curtain will be evenly saturated with the tested disinfectants. In addition, the length of time of organisms present on the curtain prior to application of the disinfectant may influence the total organism burden (Neely and Maley, 2000); our study was limited to a total of 1–2 h duration between inoculation and hand-imprint culture. The presence of organic material and its interaction with the disinfectants used was not evaluated in this study. Another limitation is the inherent difficulties in standardising anaerobic growth in the laboratory. This can be seen by the low

yield of recovery for *C. difficile*. However, the same swatch was used for the before and after experiment to help control for variation. Lastly, *C. difficile*, MRSA and VRE were identified by colony morphology and Gram stain. While this is not definitive identification, given the high concentration of pure organisms inoculated and the controlled lab settings in which these cultures were incubated, we felt that this form of identification was reasonable for our pilot study.

The findings of this study suggest that privacy curtains may be adequately disinfected in controlled lab settings. This may be a useful method to help prevent transmission of MRSA, VRE and *C. difficile* in clinical settings. In vivo studies looking at the efficacy of spraying the entire curtain in real life hospital settings are needed to evaluate if privacy curtains can be disinfected by spraying rather than being removed and changed for terminal cleaning of isolation rooms. Additionally, risks to healthcare workers using sprayed disinfectants and costs of training and products need to be evaluated and compared to laundering.

Declaration of conflicting interest

The author declares that there is no conflict of interest.

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