Ability of cleaning-disinfecting wipes to remove bacteria from medical device surfaces

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Background: Nosocomial infections are a serious problem in health care facilities. Bacteria can be transferred from patient to patient via contaminated reusable medical devices and equipment.

Methods: An anesthesia machine and objects representative of smooth and ridged machine knobs were contaminated with Staphylococcus aureus, Bacillus atrophaeus spores, and Clostridium sporogenes spores. The ability of 5 commercially available cleaning-disinfecting wipes to remove bacteria was compared with gauze soaked with water or bleach. Gauze soaked with water was used to determine the optimal wetness for bacteria removal, which was then used to evaluate the efficacy of the wipe ingredients.

Results: All of the wipes cleaned the device surfaces significantly better than the no wipe control. Some wipes performed equally well as gauze with water, whereas others performed worse. Overall, the wipe containing sodium hypochlorite was the most effective at removing bacteria. When the wipe ingredients were re-evaluated using the determined optimal wipe wetness on gauze, their effectiveness at cleaning S aureus, but not spores, significantly improved.

Conclusion: Physically removing bacteria from device surfaces with water was often as effective as the cleaning-disinfecting wipes. Of the wipe active ingredients evaluated, sodium hypochlorite was the most effective overall. The wetness of the wipes may also play a role in their effectiveness.

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Hospital-acquired infections remain a serious problem, especially for critically ill or immunocompromised patients. These infections can increase the time and cost of health care and fatality rates.1,3 As new strains of antibiotic-resistant bacteria evolve, treating these hospital-acquired infections becomes more difficult. There are numerous reports of outbreaks in hospitals of multidrug-resistant bacteria, including multidrug-resistant Pseudomonas aeruginosa,4-7 Mycobacterium tuberculosis,8 Acinetobacter baumannii,9,10 and Staphylococcus aureus.10,11

Cleaning is the critical first step in reprocessing reusable medical devices to reduce soil and bioburden. Reducing the soil on a used device is needed to ensure subsequent effective disinfection or sterilization.13,14 Bacteria responsible for nosocomial infections can be introduced directly by a contaminated device or indirectly via the gloved hands of health care personnel who touch a contaminated device and then a patient.15 Therefore, it is important that noncritical reusable devices and equipment, which are not in direct contact with patients (eg, anesthesia machines), are cleaned appropriately between uses. Increasing the level of environmental disinfection has been shown to decrease the spread of both vancomycin-resistant Enterococcus and methicillin-resistant S aureus (MRSA) in health care settings.16,17

Currently, many hospitals use commercially available disinfecting wipes to clean and disinfect their noncritical devices between uses. These wipes make antimicrobial claims on their labels and are
regulated by both the Environmental Protection Agency and the Food and Drug Administration. However, although the ingredients may be effective bactericides when their activity is measured in a test tube, it is unclear how the wipes perform on a device surface. Siani et al demonstrated that there was a discrepancy between the ability of a wipe to kill Clostridium difficile spores in a test tube versus on a surface.16 Previously, our laboratory compared the ability of several cleaning and disinfecting wipes to remove Streptococcus pneumoniae and artificial blood test soil from a medical device surface.19 In this study, S aureus (surrogate for MRSA), C sporogenes (surrogate for C difficile), and Bacillus atrophaeus (surrogate for B anthracis) were used to study the influences of device design and wipe wetness on removing bacteria from device surfaces. C difficile and MRSA are 2 bacteria known to cause nosocomial infections,20 and B anthracis is a Centers for Disease Control and Prevention category A select agent pathogen.22 C difficile and B anthracis are of particular concern because they form spores.

METHODS

Bacteria

S aureus (ATCC 6538) was purchased from American Type Culture Collection (Manassas, VA) and used as a surrogate for MRSA. Liquid cultures were grown in trypticase soy broth in a shaking incubator at 225 rpm at 37°C. Colonies were enumerated after plating on trypticase soy agar (TSA) and incubating for approximately 16 hours at 37°C. B atrophaeus spores (NRRL B4418), surrogate for B anthracis spores, were purchased from Steris (Mentor, OH). Colonies were enumerated after plating on TSA. C sporogenes spores (ATCC 7955), surrogate for C difficile, were purchased from Mesa Laboratories (Lakewood, CO). For propagation, C sporogenes spores were plated on blood agar plates, incubated in an anaerobic growth chamber with GasPak EZ (BD Biosciences, San Jose, CA) at 37°C for 10 days, and then scraped off the plates with a glass rod. The bacteria were then collected as a pellet through centrifugation at 11,000 × g for 15 minutes in a tabletop centrifuge. The pellet was washed with phosphate buffered saline (PBS), centrifuged as previously indicated, and then resuspended in sterile PBS. The bacteria were then incubated in an 80°C water bath for 20 minutes to kill any remaining vegetative cells. Spores were stored at 4°C.

Cleaning bacteria from the surface of an anesthesia machine

As previously reported, the surface of the Dräger Fabius GS anesthesia machine (Draeger Medical Inc., Telford PA) was taped off into 2.5- × 2.5-cm squares.19 Then, 10 μL of bacteria was applied to each square and allowed to dry for 1 hour. S aureus was applied at approximately 1010 colony forming units (CFU)/mL. B atrophaeus and C sporogenes spores were applied at approximately 108 CFU/mL. Squares were cleaned by wiping in a horizontal motion 3 times, with 1 of the 5 commercially available wipes or with sterile gauze soaked in water or in 5% bleach diluted 1:10 in water. The positive control square was not cleaned, and the negative control square was not inoculated with bacteria. After 10 minutes, the caps were placed in 5 mL of sterile PBS and vortexed for 3 seconds. The supernatant was then plated to collect residual bacteria. Plating and calculations were performed as previously described. Experiments were run 3-5 times.

Neutralization

First, it was determined whether Dey/Engley (D/E) Neutralizing Broth (BD Biosciences, San Jose, CA) was capable of neutralizing the disinfectants of interest. To do this, bleach (1:10) and the 5 wipe ingredients were diluted 1:10 in either PBS or D/E Neutralizing Broth, incubated for 10 minutes at room temperature to allow for neutralization. S aureus was then added at approximately 105 CFU/mL. After another 10 minutes of incubation at room temperature, samples were serially diluted, plated, and incubated overnight. Experiments were performed in triplicate.

To determine whether there was significant death from residual disinfectant after serial dilution, bacteria were added to bleach (1:10) or liquid squeezed from 1 of the 5 wipes (Table 1) and then incubated at room temperature for 10 min. S aureus was added at 1010 CFU/mL, whereas B atrophaeus and C sporogenes spores were added at 109 CFU/mL. The bacteria-disinfectant solutions were then diluted 1:10 in either PBS or D/E Neutralizing Broth and incubated at room temperature for 10 minutes. Samples were then diluted serially and plated, and incubated overnight. Experiments were performed in triplicate.

Long-term survival

Bacteria were applied to several squares on the anesthesia machine surface and several smooth and ridged caps as previously described. Bacteria were used at the same concentrations as those used in the cleaning experiments. The initial time 0 sample was taken after 1 hour of drying. Subsequent samples were taken after 1 day, 3 days, 1 week, 2 weeks, and 1 month. All incubations were performed at ambient room temperature. Samples were collected, diluted, and plated as previously described. Experiments were performed in triplicate.

Statistics

All experiments were repeated 3-5 times. Data is reported as the mean ± the standard error of the mean (SEM). Unpaired Student t tests were performed using Microsoft Excel (Microsoft, Redmond, WA).
RESULTS

Wipe effectiveness as packaged

The effectiveness of cleaning-disinfecting wipes to clean an anesthesia machine was measured. This device is reusable, has a variety of surface types, and can be neglected between uses because it is a noncritical device with often no specific person assigned to reprocess it. The 2 device surface types that were compared for cleanability were as follows: (1) a large, flat, horizontal, relatively smooth area and (2) smaller knobs on the device. The anesthesia machine had 3 knobs with varying ridge sizes. The caps of the 15-mL Falcon tubes served as surrogates because the caps are similar to the knobs in size and material. Additionally, we investigated 2 types of caps (flat and ridged) to directly compare the effect added texture, such as ridges, has on cleanability.

D/E Neutralizing Broth neutralized all of the wipe ingredients, and there was no difference in the number of bacteria after exposure to disinfectant whether or not neutralization was performed (data available on request). The 5 commercial wipes and gauze wetted with water and bleach significantly cleaned S aureus, Bacillus atrophaeus, and Clostridium sporogenes spores from both the anesthesia machine surface and the caps compared with the no wipe control. This indicates that the cleaning-disinfecting wipes tested are capable of removing and killing both vegetative and spore-forming bacteria from a variety of device designs.

Even though the commercially available wipes removed all of the S aureus from the surface of the anesthesia machine, their performance was not statistically better than gauze with water, which removed almost all (99.99%) of the bacteria (Fig 1). However, all of the commercial wipes, except wipe 1, signifi cantly outperformed gauze with water when cleaning S aureus from the flat and ridged caps (Figs 2-3). Wipe 4, containing hydrogen peroxide as the active ingredient, was the most effective at removing S aureus from either the flat or ridged caps.

The B atrophaeus and C sporogenes spores were more difficult to clean from both the anesthesia machine surface and the caps compared with S aureus. On the anesthesia machine surface, cleaning with water and gauze reduced the number of spores by approximately 99%, which is 2 logs less than the reduction of S aureus from the anesthesia machine surface (Fig 1). The only wipe to perform signifi cantly better than gauze with water in removing both spore types from the machine surface was wipe 3 (Fig 1). Wipe 4 signifi cantly removed more B atrophaeus spores, but not C sporogenes spores, from the anesthesia machine surface than gauze with water (Fig 1).

Gauze with water removed approximately 70%-90% of the spores from the flat and ridged caps. When cleaning spores from caps, none of the wipes performed signifi cantly better than gauze with water, and there were 2 wipes that performed signifi cantly worse than the water control (Figs 2-3). Wipe 1 reduced the number of both B atrophaeus and C sporogenes spores on the flat caps by approximately 55%-70% or 0.6 log, which was consistently worse than the water control (Fig 2). Both wipes 1 and 2 were signifi cantly worse than gauze with water at removing C sporogenes spores from ridged caps (Fig 3). The wipes reduced the number of C sporogenes spores by approximately 50%-75% or 0.5 log. Given that gauze with water was meant to represent the physical removal aspect of cleaning, it was unexpected that the cleaning-disinfecting wipes performed worse in some instances. It was noted that one variable that varied between the different wipes used was their wetness. Therefore, we investigated whether the wetness of the wipes in this study may play a role in their effectiveness.
Wipe ingredient effectiveness reapplied at optimal wipe wetness on gauze

The wipes were weighed before and after drying as previously reported and were found to have a wipe wetness of 0.23-0.67 g of liquid/cm³ of wipe (Table 1). To control for the varying amount of wetness in each wipe, the liquid was squeezed from each of the commercial wipes and reapplied on gauze at an optimal wipe wetness. The optimal wipe wetness was first determined for each of the device surfaces using gauze and water. Water was added to sterile gauze at 5 different levels of wipe wetness: 0.2, 0.4, 0.6, 0.8, and 1.0 g of liquid/cm³ of wipe, which encompassed the range of wetness seen in the commercially available wipes. Because B. atrophaeus spores were generally the most difficult organism to remove from the devices, they were used as a worst-case cleaning scenario in this study.

A wipe wetness of 0.6 g of water/cm³ of gauze removed the most B. atrophaeus spores from the anesthesia machine surface and had the smallest SEM (0.33) compared with other degrees of wipe wetness (data available on request). Additionally, the 0.6 g/cm³ wetness performed significantly better than the wettest wipe when removing spores from the anesthesia machine surface. Therefore, 0.6 g/cm³ was chosen as the optimal wipe wetness to clean the anesthesia machine surface. When cleaning the flat caps, 0.8 g/cm³ was used as the optimal wipe wetness because it had both the least bacteria remaining (3.03%) and the smallest SEM (0.89). Additionally, the 0.8 g/cm³ gauze cleaned significantly better than the driest of the wipes. None of the differing degrees of wipe wetness were significantly better than the others at cleaning the ridged caps, indicating that wetness did not play a significant role in the ability to clean the ridged caps (data available on request). To be comprehensive, we decided to include the ridged caps in the optimal wipe wetness experiments and used the flat caps’ optimal wipe wetness value (0.8 g/cm³).

The liquid from the 5 commercial wipes was collected and added to sterile gauze at 0.6 and 0.8 g/cm³ to clean the anesthesia machine surface and caps, respectively. Because the cleaning-disinfecting wipes already removed 100% of the S. aureus from the anesthesia machine surface when they were used as packaged, this experiment was not repeated at optimal wipe wetness. The wipe ingredients used on gauze at optimal wipe wetness behaved similarly to the packaged wipes when cleaning either B. atrophaeus or C. sporogenes spores from the anesthesia machine surface (data available on request). None of the wipe ingredients performed worse than water, and only sodium hypochlorite (active ingredient in bleach and wipe 3) performed significantly better than water (data available on request). The biggest difference in cleaning effectiveness was seen when the wipe ingredients were used at 0.8 g/cm³ to clean S. aureus from the caps. Although most wipes used as packaged removed significantly more S. aureus from the caps compared with water, there were still approximately 10% of the bacteria remaining on the devices (Figs 2–3). However, when the caps were cleaned with the wipe ingredients gauze at optimal wipe wetness, almost all (>99.9%) of the S. aureus was removed (Fig 4).

Data for cleaning the ridged caps with wipe ingredients at optimal wipe wetness are available on request. When the wipe ingredients used to clean spores from the caps at optimal wipe wetness, only ingredients from wipe 3 cleaned significantly better than water. Interestingly, when the ingredients from wipes 1 and 2 were used at 0.8 g/cm³ on gauze, they were no longer significantly less effective than water at removing the spores from the caps (Fig 4). Because cleaning endpoints are typically normalized to the area of the device, we also calculated the bacteria remaining on the device surfaces after cleaning at optimal wipe wetness (CFU/cm²) (data available on request). After cleaning the bacteria from the device surfaces, there was 2-3 log CFU/cm² remaining on the anesthesia machine surface and 2-5 log CFU/cm² on the caps. In all but one of the experiments, ingredients from wipe 3 left the fewest number of bacteria on the surfaces.

Long-term survival on device surfaces

Both B. atrophaeus and C. sporogenes are spore formers and are resistant to environmental hazards, such as temperature and drying. S. aureus has also been shown to survive on surfaces for several months. We determined if these bacteria were capable of remaining viable after approximately 10⁶-10⁷ CFU were dried on the anesthesia machine surface and caps. Both spore types remained relatively constant in number over a period of 1 month on all 3 surface types. S. aureus were reduced by approximately 1 log after 3 days at room temperature on the surfaces and 4 log after a month (data available on request). These data indicate that if bacteria were not removed or killed immediately after contamination, they could remain on the device for a long time and be a source for cross-contamination.

CONCLUSION

In general, when the wipes were used as packaged, they performed either as well or significantly better than gauze with water to clean the bacteria from the devices. When cleaning vegetative bacteria from a smooth flat surface, the wipes were successful at removing bacteria to the limit of detection. Interestingly, there were a few instances when the wipes performed significantly worse than the gauze-water control while cleaning the caps. We hypothesized that this could be caused by a difference in the wipes’ wetness. Using gauze soaked with water we determined that a moderate wipe wetness appeared to be optimal for cleaning both the anesthesia machine surface and the flat caps. There did not appear to be a clear effect of wipe wetness in cleaning the ridged caps. Of the 5 commercial wipes that were used, wipes 1–4 had very similar degrees of wipe wetness, whereas wipe 5 was noticeably drier.

When the wipe ingredients were used at optimal wetness to clean the anesthesia machine surface, there were between 2 and 3 log of bacteria remaining per cm² of the device surface (data available on request). Currently, there is no accepted cleaning benchmark for bacteria on medical devices. However, Alfa et al found that soiled
endoscopes had approximately 2.5 log CFU/cm² after performing manual cleaning,24 a number that is consistent with what we found on the anesthesia machine surface. When cleaning *Staphylococcus aureus* off of the caps, all of the wipe ingredients left well below 2.5 log CFU/cm², with most leaving no detectable bacteria. However, when cleaning spores from the caps, most of the wipe ingredients were not able to meet 2.5 log CFU/cm², leaving behind between 4 and 5 log of spores/cm². However, it is difficult to draw any conclusions based on the raw CFU values alone because the number of bacteria in the positive controls vary greatly between the anesthesia machine surface and the caps (data available on request). We have taken these differences into account by calculating the percent CFU remaining compared with the positive control to compare the data proportionally across the bacteria and surface types.

While analyzing the data as percent CFU remaining, wipes 1 and 2 cleaned the caps significantly worse than water. However, the ingredients in wipes 1 and 2 performed equally well as water when they were applied on gauze at the optimal wipe wetness. Therefore, we can conclude that the wipe ingredients themselves are not inferior to water. However, it does not appear that wipe wetness is the only factor driving the cleaning effectiveness of the wipes because the 2 wipes that cleaned significantly worse than water when used as packaged (wipes 1 and 2) were not particularly wetter or drier than wipe 3, which performed the best in those experiments. It is possible that another secondary factor may also be playing a role in a wipe’s effectiveness: texture. Although we did not directly measure the effect of texture on the wipes’ cleaning ability, it may be that the texture and composition of gauze are more effective at removing bacteria than wipes 1 and 2.

This is not to say that wipe wetness is an unimportant variable. We demonstrated that gauze with a moderate amount of liquid was superior in cleaning than wetter or drier gauze. Interestingly, 4 out of the 5 wipes have a wipe wetness ratio around 0.6 g/cm³, which is superior in cleaning than wetter or drier gauze. Although we have not included them in this study for technical reasons, users should realize that, based on our studies, a wetter wipe is not necessarily a more effective wipe. Additionally, wet wipes may seep into the electronic components in reusable devices, which could lead to device failures.

Finally, we have reaffirmed the importance of actively cleaning surfaces between uses by demonstrating that both spores and *S. aureus* can remain viable after being dried on the surface of a reusable medical device for a month. The hardiness of *Staphylococcus* bacteria and *Clostridium* spores may contribute to them being a continual source for cross-contamination in the health care environment. If these bacteria are not successfully removed from device surfaces, they can remain viable and ready for transfer to the next patient.

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**References**


