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The Evaluation of Electrolyzed Water, Sodium Dichloroisocyanurate and Peracetic Acid with Hydrogen Peroxide for the Disinfection of Patient Room Surfaces

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Running Title: Hospital Disinfection Assessment Protocol

ABSTRACT

Background: Sporicidal disinfectants are necessary to control *Clostridioides difficile* and *Candida auris*. Novel application methods such as electrostatic sprayers may increase disinfection effectiveness. We employed a standardized protocol to assess three sporicidal disinfectants: electrolyzed water (EW), sodium dichloroisocyanurate (NaDCC) and peracetic acid/hydrogen peroxide (PAA/H_2O_2).

Methods: The study was conducted at two New York City hospitals (1,082 total beds) over an 18-month period. The three chemicals were applied by housekeeping personnel following the hospital protocol; the use of electrostatic sprayers was incorporated into EW and NaDCC. In randomly selected rooms, five surfaces were sampled for microbial colony counts after cleaning. Data analyses were performed using negative binomial logistic regression.

Results: We collected 774 samples. NaDCC-disinfected surfaces had a lower mean colony count (14 CFU) compared to PAA/H_2O_2 (18 CFU, $p=0.36$) and EW (37 CFU, $p<.001$). PAA/H_2O_2 and EW had more samples with any growth (both $p<.05$) compared to NaDCC. NaDCC applied with wipes and an electrostatic sprayer had the lowest number of samples with no growth and <2.5 CFU/cm^2 (difference not significant).

Conclusions: The use of NaDCC for surface disinfection resulted in the lowest bacterial colony counts on patient room high touch surfaces in our study.

KEYWORDS

Environmental surveillance, patient room disinfection, sodium dichloroisocyanurate, electrolyzed water, peracetic acid/hydrogen peroxide

INTRODUCTION

In spite of decades of effort, contaminated environmental surfaces within patient rooms remain a significant cause of healthcare associated infections. In the New York City area, the persistence of *Clostridioides difficile* and emergence of *Candida auris* highlights the importance of routine surface disinfection with sporicidal agents in our region.[1–4]

The Center for Disease Control and Prevention (CDC) urges healthcare systems to implement environmental surveillance to identify vulnerabilities when disinfecting patient care areas, with a particular focus on high touch surfaces.[5] However, the CDC did not release specific protocols for implementing this surveillance and limited research exists on an appropriate standardized assessment protocol of patient room surface disinfection.

Environmental surveillance to assess the adequacy of surface disinfection may be executed by a variety of different methods. Fluorescent marking is useful for tracking disinfection frequency and identifying surfaces missed during cleaning, but cannot indicate the quantified biological load on the surface after disinfection.[6] Adenosine triphosphate (ATP) bioluminescence quantifies the presence of genetic material on surfaces using relative light units (RLU).[7] The ATP bioluminescence method, however, does not distinguish between viable and inactivated organisms, and the evidence surrounding correlation between RLU and colony forming units (CFU) is variable.[7] Microbial culture of surfaces using growth and selective media allows for direct quantification of surface microbial load and identification of pathogens of interest.

The individual elements of a standardized assessment protocol to assess hospital surface disinfection, such as swab type, surface area size, and growth media are reported in the literature.[8,9] Pre-moistened flocked swabs are the favored sampling method compared to contact plates given ease of use and consistent results.[8,9] Limited information exists on the acceptable microbial contamination levels to use when assessing patient room surface disinfection. The US Department of Agriculture (USDA) published standards for acceptable level of surface colonization in food processing areas indicating surface microbial loads above 5 CFU/cm^2 are unacceptably contaminated.[10] Agencies in the United Kingdom (UK) cite a standard of 2.5 CFU/cm^2 as the upper acceptable limit, but there is no accepted standard in the United States.[10–14]

In addition to developing environmental surveillance to assess disinfection of surfaces within patient care locations, new sporicidal chemical disinfectants and application methods with minimal toxicity and surface damage profiles are needed to combat the rise of surface-persistent pathogens. The sporicidal disinfectants electrolyzed water (EW), sodium dichloroisocyanurate (NaDCC) and peracetic acid/hydrogen peroxide (PAA/H_2O_2) are less toxic and corrosive than bleach based products, but efficacy data based on routine use in the healthcare setting is scarce.[15–22] Electrostatic sprayers apply uniformly sized, positively charged disinfectant particle to surfaces. Use of these devices result in a more uniform coating of surfaces and warrant further evaluation in the healthcare setting.

Our study has two main goals: to implement an environmental surveillance protocol to measure microbial contamination of surfaces and apply this protocol to determine the efficacy of three different chemical disinfectants in reducing surface contamination. We further assessed the

efficacy of chemical application by electrostatic sprayer compared to the traditional microfiber cloth method.

METHODS

Setting and Study Design

The study was conducted over an 18-month period at our 700-bed academic medical center in Manhattan and 380 bed major teaching hospital in Brooklyn, both in New York City. In the first phase, both NaDCC and PAA/H_2O_2 were applied in one step by microfiber cloths to high touch surfaces, while EW was applied using a two-step method: first the dilute sodium hydroxide solution was applied by microfiber cloths to high touch surfaces followed by the dilute hypochlorous acid solution by an electrostatic sprayer or pump spray. Each disinfectant was assigned to a unique medical surgical unit of 28 to 34 beds and applied by routine housekeeping personnel following hospital protocols. After analysis of the phase 1 data revealed NaDCC to be the most effective, this chemical was further assessed in phase 2 by comparing application using a two-step application process of microfiber cloths followed by electrostatic sprayer versus microfiber cloth alone.

Standardized Sampling Protocol

Surfaces in rooms randomly selected by study personnel were sampled within one to two hours of discharge cleaning. Housekeeping personnel were unaware of the rooms being selected for sampling. One 10mL bottle of sterile, preservative-free 0.9% sodium chloride (NaCl) solution was used per room/bay. Before sampling a surface, one flocked nylon swab was dipped in the NaCl solution and pressed against the side of the bottle to remove excess. The sample was taken

by moving rolling the swab back and forth over the standardized 36cm^2 surface area to maximize swab contact with the surface. The swab was then turned 90 degrees and rolled as it was moved back and forth over the same area. A template was held over the surface to designate the sample area. The swab was then used to inoculate a TSA 5% blood agar plate (BD Diagnostic Systems, USA) using the same technique. One plate was used per surface and all plates were incubated for 24 hours at 36ºC in aerobic conditions before the number of colonies visible to the naked eye were counted. We did not perform colony species identification. Many bacteria species, as well as *Candida* species, can grow on blood agar plates.[23] Colony counts were performed after incubation, and all results and sampling information were recorded in a survey maintained in OpenREDCap (REDCap 10.0.19, Vanderbilt University).

Classifying Surfaces in Patient Care Areas into Zones

Guided by FDA surface sampling directives and Minnesota Hospital Association recommendations, we divided our sampling sites into zones based on proximity to the patient.[24,25] We classified surfaces in direct contact with the patient as zone 1, for example the over-bed table, call button, mattress and bedframe. Surfaces in indirect contact with patients through provider contact prior to patient interaction were classified as zone 2, such as the oxygen meter on the headwall, the privacy curtain, and the in-room computer. Surfaces in the patient room entrance and those touched predominantly by visitors, such as the room furniture, were classified as zone 3. Surfaces outside the patient room with the potential for transmission to the patient through contaminated hands or supplies were classified as zone 4, for example a phone in the patient unit workroom. We included zone 1 and 2 high touch surfaces due to their use by patients and healthcare providers, but zone 3 and 4 sites are potential pathogen reservoirs requiring regular disinfection.

Chemical Disinfectants and Application Methods

Prior to the study, our daily and post-discharge disinfection protocol incorporated PAA/H_2O_2 (Oxycide, Ecolab, Minnesota) as our standard disinfectant, applied with microfiber wipes for high touch surfaces and cloth mops for floor surfaces. All wipes and mops are for single patient use and a standardized process consistent with best practices for environmental cleaning in healthcare facilities was followed.[26] In phase 1, we compared PAA/H_2O_2 to NaDCC (PURTABS, Earthsafe, Massachusetts) applied using microfiber cloths to high touch surfaces following our standard protocol and EW (Viking Pure Solutions, Florida) using a two-step method: first the dilute sodium hydroxide solution was applied by microfiber cloths to high touch surfaces followed by the dilute hypochlorous acid solution by an electrostatic sprayer or pump spray on high touch surfaces. The use of each disinfectant was assigned to specific floors and not randomized. Staff were trained on appropriate application method but did not remain consistently assigned to the same floor, as per normal hospital workflow. In phase 2, NaDCC was further assessed by comparing application of the chemical using microfiber cloths to high touch surfaces following our standard protocol followed by use of an electrostatic sprayer on all patient room surfaces versus use of microfiber cloths alone. After spraying, the disinfectant was left untouched to allow for protective film formation. All surfaces except the curtain were wiped and then sprayed; the curtain was not wiped in the standard protocol. Throughout the study, disinfectant equipment was inspected to ensure adherence to manufacturer standards.

Data Analysis

We assessed disinfectant efficacy using two colony count thresholds, 0 CFU/cm^2 and 2.5 CFU/cm^2. UK healthcare and US food safety research uses the 2.5 CFU/cm^2 threshold when assessing disinfection, and this threshold has also been suggested for assessing patient surface

disinfection.[12,14,27,28] However, limited research exists on the applicability of this threshold or others as appropriate for assessing patient surface disinfection to meaningfully prevent HAIs.[1] We therefore included 0 CFU/cm^2 as another potential threshold to use in hospital settings, as previous research has suggested the risk of pathogen transmission even when very few colonies are present on the surface.[13,29] We compared the number of samples above each threshold for each disinfectant using negative binomial logistic regression, and adjusted for building, room type, and sample zone. For assessing samples above each threshold by zone and disinfectant, we adjusted for building and room type. Significance was defined as alpha <0.05 for this study. All analyses were performed using SAS, version 9.4 (SAS Institute, Cary NC).

RESULTS

Comparison of disinfectant efficacy after discharge cleaning

We obtained 774 surface samples from patient rooms after discharge cleaning during our study. EW had a significantly higher mean colony count (p<.001) compared to NaDCC and a higher percentage of samples with any growth on culture and above 2.5 CFU/cm^2 compared to NaDCC and PAA/H_2O_2 (Table 1). PAA/H_2O_2 did not have a significantly higher mean colony count (p=0.36) compared to NaDCC, but did have a higher percentage of samples with any growth and above 2.5 CFU/cm^2 compared to NaDCC (Table 1). After an interim analysis on disinfectant effectiveness, EW was discontinued and the first phase was completed with NaDCC and PAA/H_2O_2.

Table 1: Description of Microbial Sampling Results by Disinfection Chemical After Discharge Cleaning (N=774)

Disinfectant Method	Sample Zone	Surface	Median Colony Count (IQR) (CFU per 36cm^2)	Mean Colony Count (CFU per 36cm^2)	Colony Count p-value	Samples Above 0 CFU/cm^2 (%)	Samples Above 2.5 CFU/cm^2 (%)
NaDCC (N=357)			**0 (0-2)**	**14**	**(ref)**	**142 (40)**	**19 (5)**
	1	Over-bed table (N=64)	0 (0-0)	1		12 (19)	0 (0)
	1	Call button (N=68)	0 (0-1)	13		20 (29)	2 (3)
	1	Bedframe (N=64)	0 (0-1)	14		19 (30)	2 (3)
	2	Oxygen meter (N=98)	0 (0-5)	17		46 (47)	12 (12)
	2	Privacy curtain (N=52)	7 (1-23)	27		39 (75)	3 (6)
	2	In-room computer (N=11)	2 (0-26)	11		6 (55)	0 (0)
PAA/H_2O_2 (N=270)			**1 (0-5)**	**18**	**0.36**	**146 (54)**	**16 (6)**
	1	Over-bed table (N=67)	1 (0-3)	15		36 (54)	3 (4)
	1	Call button (N=65)	0 (0-2)	21		22 (34)	2 (3)
	1	Bedframe (N=55)	1 (0-6)	8		32 (58)	1 (2)
	2	Oxygen meter (N=62)	2 (0-8)	21		43 (69)	8 (13)
	2	Privacy curtain (N=16)	5 (0-19)	35		11 (69)	2 (13)
	2	In-room computer (N=5)	0 (0-2)	1		2 (40)	0 (0)
EW (N=147)			**2 (0-25)**	**37**	**<0.001**	**97 (66)**	**21 (14)**
	1	Over-bed table (N=33)	1 (0-6)	16		18 (55)	1 (3)
	1	Call button (N=28)	1 (0-19)	46		16 (57)	4 (14)
	1	Bedframe (N=29)	1 (0-3)	42		15 (52)	3 (10)
	2	Oxygen meter (N=29)	16 (2-100)	68		26 (90)	13 (45)
	2	Privacy curtain (N=28)	8 (2-33)	17		22 (79)	0 (0)

Abbreviations: NaDCC - sodium dichloroisocyanurate solution, PAA/H_2O_2 – peracetic acid/hydrogen peroxide, EW – electrolyzed water

After adjusting for building, room type, and sample zone, both PAA/H_2O_2 and EW were significantly more likely to have any growth on culture compared to NaDCC (Table 2). After adjusting for the same variables, EW was significantly more likely to have cultures above 2.5 CFU/cm^2 compared to NaDCC, but there was no significant difference between PAA/H_2O_2 and NaDCC (Table 2).

Table 2: Assessment of Disinfectant Chemical Effectiveness by Colony Count Threshold After Discharge Cleaning (N=774)

Disinfectant Method	# Samples >0 CFU/cm^2 (%)	>0 CFU/cm^2 OR (CI)	p-value	>0 CFU/cm^2 OR* (CI)	p-value	# Samples >2.5 CFU/cm^2 (%)	>2.5 CFU/cm^2 OR (CI)	p-value	>2.5 CFU/cm^2 OR* (CI)	p-value
NaDCC (N=357)	142 (40)	(ref)	(ref)	(ref)	(ref)	19 (5)	(ref)	(ref)	(ref)	(ref)
PAA/H_2O_2 (N=270)	146 (54)	1.8 (1.3-2.5)	<0.001	2.5 (1.7-3.6)	<0.001	16 (6)	1.1 (0.6-2.2)	0.74	1.4 (0.6-3.3)	0.41
EW (N=147)	97 (66)	2.9 (2.0-4.4)	<0.001	2.8 (1.7-4.6)	<0.001	21 (14)	3.0 (1.5-5.7)	0.001	2.4(1.1-5.4)	0.03

*Adjusted by building, room type, sample zone

Abbreviations: NaDCC - sodium dichloroisocyanurate solution, PAA/H_2O_2 – peracetic acid/hydrogen peroxide, EW – electrolyzed water

Difference in Colony Counts between Zone 1 and Zone 2 Sampling Surfaces

Across all 3 chemical disinfectants, we noted zone 1 sites had lower median colony counts compared to zone 2 sites and had lower percentage of samples with any growth and above 2.5 CFU/cm^2 (Tables 1 and 3). After adjusting for building and room type, zone 2 in general had a greater risk of samples above both thresholds compared to zone 1 across all disinfectants.

Table 3: Comparison of Disinfection by Zone and Chemical After Discharge Cleaning (N=774)

Disinfectant Method	Zone	Median Colony Count (IQR) (CFU per 36cm^2)	Mean Colony Count (CFU per 36cm^2)	Colony Count p-value	# Samples >0 CFU/cm^2 #1 (%)	>0 CFU/cm^2 OR* (CI)	p-value*	# Samples >2.5 CFU/cm^2 (%)	>2.5 CFU/cm^2 OR* (CI)	p-value*
NaDCC (N=357)	1 (N=196)	0 (0-1)	9	(ref)	51 (26)	(ref)	(ref)	4 (2)	(ref)	(ref)
	2 (N=161)	1 (0-14)	20	0.03	91 (57)	3.8 (1.0-14.1)	0.05	15 (9)	4.3 (1.3-14.7)	0.02
PAA/H_2O_2 (N=270)	1 (N=187)	0 (0-3)	15	(ref)	90 (48)	(ref)	(ref)	6 (3)	(ref)	(ref)
	2 (N=83)	2 (0-8)	22	0.25	56 (67)	0.5 (0.1-3.3)	0.47	10 (12)	3.8 (1.3-11.0)	0.01
EW (N=147)	1 (N=90)	1 (0-7)	34	(ref)	49 (54)	(ref)	(ref)	8 (9)	(ref)	(ref)
	2 (N=57)	13 (2-47)	43	0.54	48 (84)	3.8 (1.1-12.0)	0.03	13 (23)	3.0 (1.2-7.9)	0.02

*Adjusted for building, room type

Abbreviations: NaDCC - sodium dichloroisocyanurate solution, PAA/H_2O_2 – peracetic acid/hydrogen peroxide, EW – electrolyzed water

Comparison of NaDCC disinfectant application method efficacy after discharge cleaning

NaDCC applied with a microfiber cloth and electrostatic sprayer did not produce a significantly different mean colony count for zone 2 samples compared to NaDCC applied with a microfiber cloth alone (p=0.90) (Table 4). NaDCC applied with a microfiber cloth alone did not have a significantly higher risk of sample colony counts with any growth and above 2.5 CFU/cm^2 compared to application with the e-sprayer (Table 4).

Table 4: Comparison of NaDCC Zone 2 Disinfection by Application Method After Discharge Cleaning (N=161)

Disinfectant Method	Median Colony Count (IQR) (CFU per 36cm^2)	Mean Colony Count (CFU per 36cm^2)	Colony Count p-value	# Samples >0 CFU/cm^2 (%)	>0 CFU/cm^2 OR* (CI)	p-value*	# Samples >2.5 CFU/cm^2 (%)	>2.5 CFU/cm^2 OR* (CI)	p-value*
NaDCC E-Sprayer (N=80)	0 (0-6)	20	(ref)	36 (45)	(ref)	(ref)	5 (6)	(ref)	(ref)
NaDCC Mop + Wipe (N=81)	2 (0-20)	19	0.90	55 (68)	2.6 (0.7-9.4)	0.15	10 (12)	3.8 (0.6-26.1)	0.17

*Adjusted for building, room type, swab location

Abbreviations: NaDCC - sodium dichloroisocyanurate solution

DISCUSSION

Surface-persistent pathogens such as *C. difficile* and *C. auris* present an increasing threat to patient safety and prompt the routine use of sporicidal disinfectants in patient care areas.[30] CDC guidance directs infection prevention departments to develop an evidence-based, efficient, and standardized protocol for assessing environmental disinfection. Using previous research and CDC recommendations, we created a simple but effective protocol for sampling surfaces to assess disinfection. In order for our protocol to be useful in routine surveillance, it needed to incorporate readily available materials, and a surface sample size that was attainable over both flat and non-flat surfaces.

We applied our protocol to evaluate three chemical disinfectants and two application methods in routine use by hospital personnel. NaDCC produced significantly lower colony counts compared to PAA/H_2O_2 and EW, and its application with a microfiber cloth plus an electrostatic sprayer reduced colony counts compared to microfiber cloth alone, however the

difference did not achieve statistical significance. In addition, zone 1 sites closer to the patient had a lower colony count average compared to zone 2 sites across all disinfectants.

We also sought to address the lack of a quantitative standard of disinfection in hospital rooms through our protocol. We used two different colony count thresholds as the maximum microbial load allowed on a surface, to observe which threshold seemed attainable in the hospital setting. We found that NaDCC was significantly more likely to have samples at the 0 CFU/cm^2 threshold compared to other disinfectants, and that NaDCC application with the electrostatic sprayer also produced more results at this threshold compared to the mop/wipe method. The 2.5 CFU/cm^2 threshold did not produce significantly different results between PAA/H_2O_2 and NaDCC, but EW was significantly more likely to produce results above this threshold, precluding its use in our setting. This threshold proved useful for assessing between zones for the same disinfectant, and between disinfectant application methods.

The microbiological and clinical implications of these thresholds is less clear, as multi-drug resistant organisms (MDROs) surface presence does not appear correlated with microbial load.[27,29] However, decreasing colony counts on a surface after disinfection could decrease the likelihood of contamination of future patients, healthcare providers, and equipment,[31] and is therefore an important goal in hospital disinfection. More research will need to be conducted to determine what colony count threshold is most applicable in preventing HAIs that is also attainable in the context of regular hospital room disinfection. Pairing surface microbial load assessment with specific testing for MDROs has been suggested as a more comprehensive method of hospital disinfection surveillance, but further research is necessary.[27,29]

In the context of the regular hospital disinfection workflow, we captured a holistic view of patient room disinfection, allowing for application of our results to hospital disinfection policy

and protocol. We determined that NaDCC applied with the standard microfiber cloth and mop method plus an electrostatic sprayer is the most effective chemical disinfectant method, and that zone 2 sites frequently touched by healthcare providers have a higher microbial load after disinfection compared to zone 1 sites. Potential causes of this difference in colony counts between zones could include ease of access to these sites for building service staff, focus on zone 1 sites by disinfection procedures, or difference in microbial load pre-disinfection affecting post-disinfection outcomes. Electrostatic sprayers allow for touchless disinfection and could be more effective at targeting sites that are difficult to reach or to disinfect with the standard mop + wipe method as the device produces positively charged particles of equal size which distribute uniformly over surfaces. Further research is needed to establish the true explanation for this difference in colony count post-disinfection.

LIMITATIONS

There were several limitations to our study. It was critical that our results be obtained in the context of the regular hospital disinfection workflow; however, this approach introduced potential confounders in the comparison of chemicals and application methods. Confounders include staff awareness of our project and potential impact on intensity of cleaning, differences in cleaning staff across floors and hospitals, and variable timing of sample collection without disrupting workflow or patient care. We also did not randomize the assignment of disinfectants to different floors and cleaning staff. However, there was a consistent rotation in staff assigned to these floors which should minimize possible difference in work practices. Additionally, our research was conducted surrounding the initial wave of the COVID-19 pandemic, as we paused

our sample collection during the initial 6 months of the pandemic. Most of our samples were collected prior to the COVID-19 pandemic. With all these considerations in mind, our results still have important implications for guiding hospital disinfection policy, as well as proposing a useful standardized disinfection assessment protocol.

CONCLUSIONS

A standardized environmental surveillance protocol is a key component when evaluating room disinfection processes and may identify locations and surfaces presenting greater risk for cross contamination. NaDCC proved to be the best sporicidal disinfectant in our study. Although the results did not reach statistical significance, the incorporation of an electrostatic sprayer in the disinfection process resulted in the lowest number of samples above the 2.5 CFU/cm^2 threshold.

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