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Major Article

The effects of a novel, continuous disinfectant technology on methicillin-resistant *Staphylococcus aureus* (MRSA), fungi, and aerobic bacteria in 2 separate intensive care units in 2 different states: An experimental design with observed impact on health care associated infections (HAIs)



Kimberly Trosch RN, BSN^{a,*}, Patricia Lawrence MS, RN, CIC^a, Amy Carezza BBA^a, Katherine Baumgarten MD^b, Beth Ann Lambert MS, CIC^b, Nattie Leger RN, MSN, LSSBB^b, Lori Berthelot RN, BSN, CIC^b, Melissa Woosley RN, CIC^c, Deborah Birx MD^a

^a Clinical Affairs, ActivePure Technologies, Dallas, TX

^b Department of Infection Prevention and Control, Ochsner Health Center-West Bank, Gretna, LA

^c Department of Infection Prevention and Control, Lexington VA Healthcare System, Troy Bowling Campus, Lexington, KY

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Background: Hospitals are exposed to abundant contamination sources with limited remediation strategies. Without new countermeasures or treatments, the risk of health care-associated infections will remain high. This study explored the impact of advanced photohydrolysis continuous disinfection technology on hospital environmental bioburden.

Methods: Two acute care intensive care units in different locations (ie, Kentucky, Louisiana) during different time periods were sampled every 4 weeks for 4 months for colony-forming units (CFUs) of methicillin-resistant *Staphylococcus aureus* (MRSA) and fungi on surfaces and floors and fungi and aerobic bacteria in the air.

Results: At both sites, surface testing showed greater than 98% reduction in mean fungi and MRSA CFUs. Floor results had reductions by more than 96% for fungi and MRSA at both sites. Aerobic bacterial air and fungal CFUs had reductions up to 72% and 89%, respectively. HAIs declined 70% when postactivation data were compared to preactivation data.

Discussion: The continuous nature of advanced photohydrolysis decontamination, its ability to be used in occupied rooms, and its independence of human resources provide an innovative intervention for complex health care environments.

Conclusions: This study is on the pioneering edge of demonstrating that continuous decontamination can reduce surface, floor, and air contamination and thereby reduce the acquisition of HAIs.

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BACKGROUND

Hospital environments are affected by a multitude of factors that can cause pathogenic reservoirs to form, contributing to infection risk.¹ Not only are hospital environments exposed to abundant contamination sources,^{2–13} with limited systems to reduce contamination,^{14–17} multiple studies have shown that prevailing remediation strategies remain suboptimal. In many facilities, only 40% to 50% of required surface cleaning occurs,¹⁸ and widely observed variances persist, including the time spent cleaning, the number of wipes used, and the level of decontamination achieved.^{19–21} Emerging data also suggest the floor surfaces as additional significant reservoirs for pathogens, which can be transferred to hands through

* Address correspondence to Kimberly Trosch RN, BSN, 14841 Dallas Pkwy, Suite 500, Dallas, TX 75254.

E-mail address: ktrosch@activepure.com (K. Trosch).

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high-touch objects often in contact with the floor.²² We must also acknowledge the present limitations, with over 90% of disinfection practices dependent on human labor²³ and hospitals reporting insufficient environmental service (EVS) personnel to maintain cleaning standards.^{24,25}

In addition to the dynamic environmental factors impacting infection risk, the Centers for Disease Control and Prevention has announced antimicrobial resistance as a serious public health threat.²⁶ This continued reduction in pharmacological options demands new treatments or countermeasures to impact the spread of multi-drug resistant organisms.^{27,28} However, greater emphasis on legacy prevention measures only expands reliance on human factors to achieve a consistently clean environment associated with lower infection risk.²⁹

Given the combination of these serious factors, a study to explore the impact of a novel advanced photohydrolysis (AP) technology inside 2 separate high-acuity hospital environments was performed. This technology provides continuous surface and air decontamination independent of human resources. The vital role of photolysis as a moderator of outdoor air chemistry has long been recognized, but far less focus has been placed on the potential benefits photolysis can have indoors.³⁰ The AP technology adapts the science of photolysis for the built environment using a proprietary photocatalyst installed in the ducts of a hospital heating, ventilation, and air conditioning system. The ambient humidity in the conditioned air is transformed through a photochemical reaction as it travels through the matrix of the photocatalyst. Identical to the reactions that occur outdoors, the water (H₂O) is broken down into trace oxidative molecules, which persistently and continuously diffuse into the environment of care and neutralize pathogenic compounds.

METHODS

Study design

The experimental study design investigated the effect of the AP system on environmental surface methicillin-resistant *Staphylococcus aureus* (MRSA) and fungi, as well as aerobic bacteria and fungi in air, using baseline preactivation samples compared to postactivation samples. Sampling occurred at baseline and every 4 weeks for 4 consecutive months in each intensive care unit (ICU) at different time periods (ie, December 2021 to March 2022 at Louisiana and October 2022 to January 2023 at Kentucky) on Tuesday mornings prior to shift change (ie, ~6 AM) and daily EVS cleaning. Staff were not blinded to the installation of the AP system.

The AP technology was installed inside the existing heating, ventilation, and air conditioning system and mechanically delivered continuous diffusion of trace oxidative molecules. The technology was extensively evaluated as part of a Food and Drug Administration (FDA) Class II 510(k) safety clearance evaluation, which was successfully concluded, and clearance was awarded in 2020. The AP device is manufactured by ActivePure Technologies and consists of a patented cell design, containing a 253.8 nm ultraviolet (UV-C) bulb surrounded on both sides by a proprietary, honeycomb-shaped photocatalyst made from a hydrophilic polycarbonate and coated with a blend of metallic semiconductors to function as a UV reactor, augmenting the photonic energy of the UV-C light to sufficiently induce photolysis.

The Lexington, Kentucky VA Healthcare System Research and Development Committees approved the study, and at the Louisiana site, approval by Institutional Review Boards was not deemed necessary. Individual patient data were not used at either study site. All standard infection prevention practices remained in place throughout the study period at both sites.

Prior to the study start, housekeeping staff at each site were observed by ActivePure personnel for compliance with the standard cleaning practices. All EVS protocols remained in place throughout the studies. The primary disinfectant used at both sites was a quaternary-based solution (ie, Virex II 256). Additionally, the Louisiana site used an ultraviolet germicidal irradiation tower after patient discharge, except for the last 4 weeks of the study period when this process was removed to isolate the effects of the AP system.

At the Kentucky site, 2 members of the ICU nursing team, 1 with previous experience as a microbiologist, were trained by ActivePure personnel to collect the environmental samples. At the Louisiana site, environmental samples were collected by staff from Lighthouse Environmental Infection Prevention, an unaffiliated third party.

Areas on the surfaces and floors were selected based on the Centers for Disease Control and Prevention list of high touchpoints³¹ and used throughout the studies. Sampling templates (10 cm × 10 cm), obtained from Environmental Express (C1010) defined the sample areas. Air samples were collected in the same areas as surface and floor samples were collected. All samples from both sites were evaluated by ResInnova Laboratories, an independent laboratory.

Sample collection

Surfaces and floors

A 3M biocide-free, premoistened sponge on a stick (ie, SSL10NB) was used to collect each sample by wiping the sponge across the outlined 10 cm × 10 cm area with a left and right motion, then turning the sponge over and changing direction, 90°, swabbing in an up and down motion. The sponge was then aseptically placed into the sample bag, sealed, and labeled. The process was repeated in all environmental areas using separate sample sponges.

Air

Air sampling was obtained by using the SAS 180 microbial sampler machine (Thomas Scientific). The American Society for Testing and Materials D8068-19 standard was followed. Prior to collecting air samples, the air sampler was calibrated according to the instructions for use and cleaned with 70% isopropyl alcohol. The air sampler with the agar plate was placed at the location of capture, the protective cover was removed, and the pump ran until the flow rate indicated a collection of 1000 L. The protective cover was replaced on the plate and sealed with tape to prevent dislodgement and contamination. The plate was labeled and, along with surface and floor samples, packed on ice and shipped overnight to ResInnova Laboratories.

Quantitative bacterial and fungal enumerations

ResInnova Laboratories recovered each sponge sample from both sites in a 10 mL sterile buffer solution of phosphate buffered saline + Triton X-100 (0.1%) surfactant, which was then homogenized for 1 minute. Each recovery solution was then serially diluted and plated onto selective agar mediums, including, Tryptic Soy to obtain aerobic bacterial counts, Sabouraud dextrose to obtain total fungal counts, and MeReSa to obtain total MRSA counts. Air samples at the Kentucky site were collected directly on inhibiting mold agar to enumerate fungi and tryptic soy agar to enumerate aerobic bacteria. Air samples at the Louisiana site were collected directly on tryptic soy agar plates to enumerate for aerobic bacteria and fungi.

All agar plates were incubated at 30 °C or 37 °C (as applicable) for 48 to 96 hours before counting the colonies. Serial dilution plate counts were done to calculate viable colony-forming units (CFUs) per sponge for each media type. The minimum detection limit was 10 CFUs.

Statistical methods

Data were analyzed using International Business Machines (IBM) SPSS Statistics version 29.0.1.0. A one-way repeated measures analysis of variance (ANOVA) with post-hoc simple contrasts was used to determine if a significant reduction in mean CFUs occurred in each ICU's surface, floor, and air microbial burdens from baseline to final postactivation #4 (Fig 1). In all data cohorts, there were no extreme outliers deemed impermissible, and the data were either normally distributed, as assessed by boxplots and Shapiro-Wilk tests ($P > .05$) or deemed acceptable as one-way repeated measures ANOVA is robust to violations of normality. The assumption of sphericity was not met by any data cohorts, as assessed by Mauchly's test of sphericity, therefore, Greenhouse-Geisser was used to correct

the one-way repeated measures ANOVA omnibus significance values. Post hoc tests utilize simple contrasts rather than pairwise comparisons with Bonferroni adjustment. Partial eta squared was calculated to measure the effect and strength of the association. A significance of .05 was used throughout.

Methods to evaluate health care-associated infections (HAIs)

One infection preventionist at each facility assessed their respective HAIs according to their standard practice reporting. HAIs were totaled for 21 months prior to activation at Louisiana and 12 months prior at Kentucky and were compared to 21 and 12 months after activation at the Louisiana and Kentucky sites, respectively. Each preactivation observation time period was selected to match

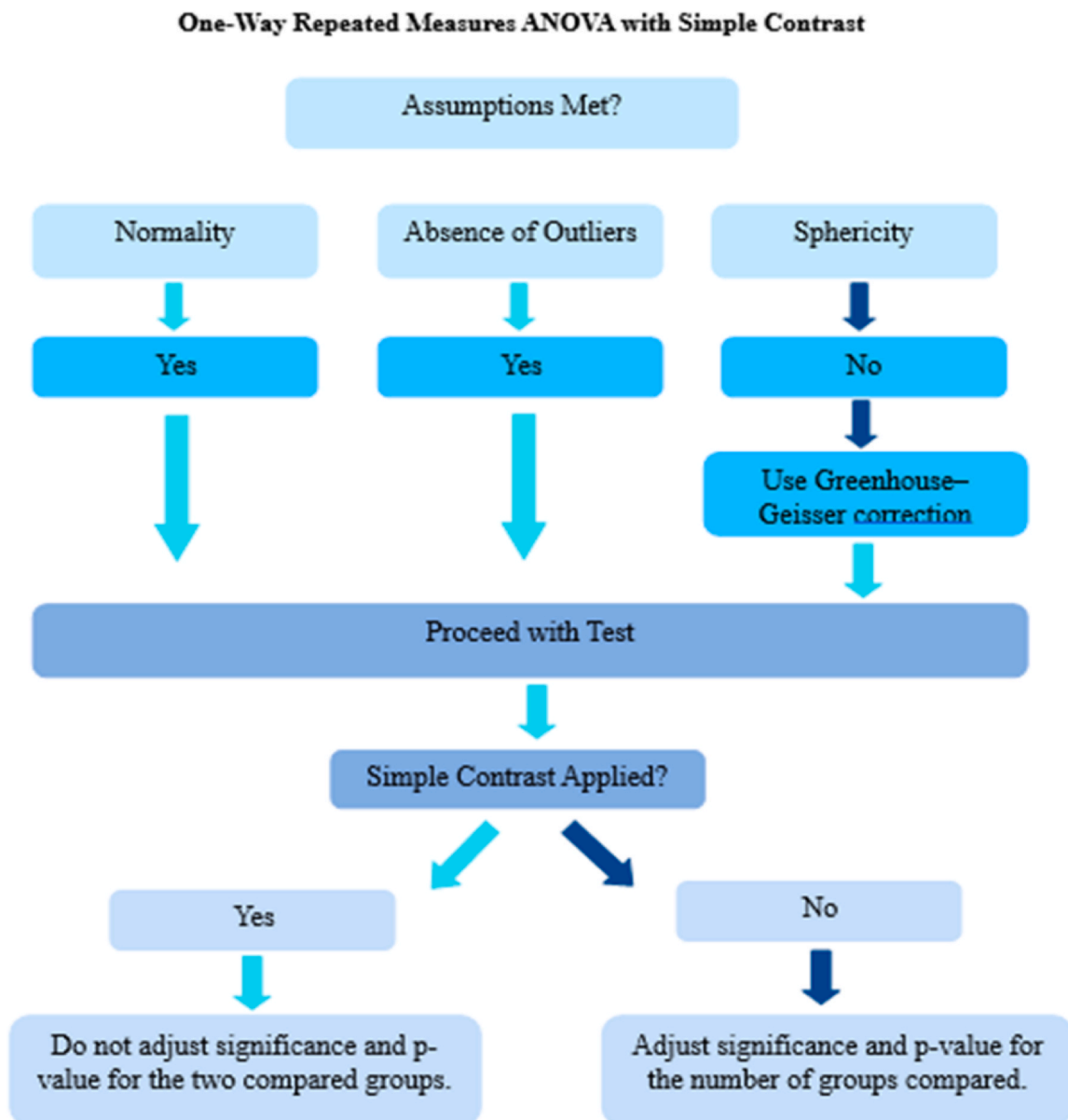


Fig. 1. Statistical analysis flow chart guide for one-way repeated measures ANOVA with post-hoc simple contrasts with the turquoise arrows indicating the methods followed. ANOVA, analysis of variance.

Table 1A
Louisiana surface ANOVA statistics

Variable	Time point	Mean (CFU)	Std. deviation	Std. error	N	95% Confidence interval		Sig. of within-subjects (Greenhouse-Geisser)
						Lower bound	Upper bound	
Louisiana surface fungal burden	Baseline	494.33	925.20	132.17	49	228.58	760.08	P < .001
	Postactivation #1	3.55	11.32	1.62	49	0.30	6.80	
	Postactivation #2	1.18	4.28	0.61	49	0.00	2.41	
	Postactivation #3	5.14	9.17	1.31	49	2.51	7.78	
	Postactivation #4	8.84	17.46	2.49	49	3.82	13.85	
Louisiana surface MRSA burden	Baseline	426.98	1,001.36	143.05	49	139.36	714.60	P = .006
	Postactivation #1	44.02	119.65	17.09	49	9.65	78.39	
	Postactivation #2	0.92	6.43	0.92	49	0.00	2.77	
	Postactivation #3	17.92	91.40	13.06	49	0.00	44.17	
	Postactivation #4	3.00	5.93	0.85	49	1.29	4.70	

ANOVA, analysis of variance; CFU, colony-forming unit; MRSA, methicillin-resistant *Staphylococcus aureus*.

the postactivation observation time period to ensure the matching duration and covering of seasonal respiratory disease months.

The infection site of each HAI reflects the specific vulnerability of individual patients. Each patient does not have the same vulnerable infection site, but rather, the same potential for an infection at a vulnerable site.³² Therefore, aggregate HAI counts were evaluated for each ICU as a reflection of the overall patient risk. The same HAIs (ie, catheter-associated urinary tract infections (CAUTI), central line-associated bloodstream infections (CLABSI), health care onset *Clostridioides difficile* (*C difficile*), MRSA bacteremia, MRSA pneumonia) were defined according to National Healthcare Safety Network protocols³³ and collected for both ICUs.

RESULTS

Environmental surfaces

Mean fungal CFUs were reduced by 98% (494.33 CFUs to 8.84 CFUs) at the Louisiana site and 99% (493.64 CFUs to 6.48 CFUs) at the Kentucky site from baseline to postactivation #4. Mean MRSA CFUs were depleted by over 99% at both sites (Louisiana 426.98 CFUs to 3.00 CFUs; Kentucky 173.61 CFUs to 0.64 CFUs).

A one-way repeated measures ANOVA determined mean surface CFUs were statistically significant at different time points during the

study (Tables 1A and 1B). Post-hoc simple contrasts showed a statistically significant reduction from baseline to final postactivation #4 at both sites for mean fungal (Louisiana P < .001; Kentucky P = .008) and MRSA CFUs (Louisiana P = .005; Kentucky P = .004) (Tables 2A and 2B).

To isolate the effects of the AP system, UV tower disinfection use at the Louisiana site was discontinued for the last 4 weeks of the study. Mean surface fungal and MRSA burden reductions between postactivation tests 3 and 4 were maintained compared to baseline testing (Table 1A) and statistically significant between postactivation test 4 and baseline (Table 2A).

A separate evaluation of MRSA surface reduction was performed using heat maps. Each map shows the swab locations and is color-coded to denote CFUs of MRSA. At the Louisiana site, surface locations with more than 500 CFU/100 cm² were reduced by 90%. The only location with more than 500 CFU/100 cm² was the bedrail of a patient with an active community-acquired MRSA infection (Fig 2A and B). At the Kentucky site, there was a 100% reduction in sample locations with more than 500 CFU/100 cm² of MRSA (Fig 2C and D).

Environmental floors

Mean fungal and MRSA CFUs were reduced by 99% (fungi 11,537.30 CFUs to 99.90 CFUs; MRSA 2,520.70 CFUs to 8.70 CFUs) at

Table 1B
Kentucky surface ANOVA statistics

Variable	Time point	Mean (CFU)	Std. deviation	Std. error	N	95% Confidence interval		Sig. of within-subjects (Greenhouse-Geisser)
						Lower bound	Upper bound	
Kentucky surface fungal burden	Baseline	493.64	988.73	172.12	33	143.05	844.23	P = .008
	Postactivation #1	4.36	25.07	4.36	33	0.00	13.25	
	Postactivation #2	1.36	4.72	0.82	33	0.00	3.04	
	Postactivation #3	6.58	30.08	5.24	33	0.00	17.24	
	Postactivation #4	6.48	21.68	3.77	33	0.00	14.17	
Kentucky surface MRSA burden	Baseline	173.61	320.87	55.86	33	59.83	287.39	P = .038
	Postactivation #1	73.18	222.92	38.81	33	0.00	152.22	
	Postactivation #2	59.48	330.49	57.53	33	0.00	176.67	
	Postactivation #3	19.97	86.28	15.02	33	0.00	50.56	
	Postactivation #4	0.64	3.66	0.64	33	0.00	1.94	

ANOVA, analysis of variance; CFU, colony-forming unit; MRSA, methicillin-resistant *Staphylococcus aureus*.

Table 1C
Louisiana floor ANOVA statistics

Variable	Time point	Mean (CFU)	Std. deviation	Std. error	N	95% Confidence interval		Sig. of within-subjects (Greenhouse-Geisser)
						Lower bound	Upper bound	
Louisiana floor fungal burden	Baseline	11,537.30	13,522.02	4,276.04	10	1,864.23	21,210.37	<i>P</i> = .026
	Postactivation #1	215.90	214.70	67.90	10	62.31	369.49	
	Postactivation #2	166.50	372.15	117.68	10	0.00	432.72	
	Postactivation #3	33.70	56.53	17.88	10	0.00	74.14	
	Postactivation #4	99.90	233.11	73.72	10	0.00	266.66	
Louisiana floor MRSA burden	Baseline	2,520.70	2,715.55	858.73	10	578.11	4,463.29	<i>P</i> = .040
	Postactivation #1	2,238.60	2,859.76	904.34	10	192.85	4,284.35	
	Postactivation #2	81.00	177.79	56.22	10	0.00	208.18	
	Postactivation #3	240.10	260.84	82.48	10	53.51	426.69	
	Postactivation #4	8.70	10.92	3.45	10	0.89	16.51	

ANOVA, analysis of variance; CFU, colony-forming unit; MRSA, methicillin-resistant *Staphylococcus aureus*.

Table 1D
Kentucky floor ANOVA statistics

Variable	Time point	Mean (CFU)	Std. deviation	Std. error	N	95% Confidence interval		Sig. of within-subjects (Greenhouse-Geisser)
						Lower bound	Upper bound	
Kentucky floor fungal burden	Baseline	1,299.50	578.16	289.08	4	379.52	2,219.48	<i>P</i> = .041
	Postactivation #1	936.50	665.53	332.76	4	0.00	1,995.50	
	Postactivation #2	459.00	411.13	205.57	4	0.00	1,113.20	
	Postactivation #3	320.75	282.67	141.34	4	0.00	770.55	
	Postactivation #4	25.00	50.00	25.00	4	0.00	104.56	
Kentucky floor MRSA burden	Baseline	1,382.75	903.11	451.55	4	0.00	2,819.80	<i>P</i> = .167
	Postactivation #1	964.50	1,087.48	543.74	4	0.00	2,694.92	
	Postactivation #2	166.00	119.49	59.74	4	0.00	356.13	
	Postactivation #3	129.75	182.44	91.22	4	0.00	420.05	
	Postactivation #4	61.75	123.50	61.75	4	0.00	258.27	

ANOVA, analysis of variance; CFU, colony-forming unit; MRSA, methicillin-resistant *Staphylococcus aureus*.

Table 1E
Louisiana air ANOVA statistics

Variable	Time point	Mean (CFU)	Std. deviation	Std. error	N	95% Confidence interval		Sig. of within-subjects (Greenhouse-Geisser)
						Lower bound	Upper bound	
Louisiana air bacterial burden	Baseline	27.30	22.78	7.20	10	11.00	43.60	<i>P</i> = .025
	Postactivation #1	10.80	10.84	3.43	10	3.05	18.55	
	Postactivation #2	14.90	8.61	2.72	10	8.74	21.06	
	Postactivation #3	4.10	2.77	0.87	10	2.12	6.08	
	Postactivation #4	7.70	5.40	1.71	10	3.84	11.56	
Louisiana air fungal burden	Baseline	10.60	14.73	4.66	10	0.06	21.14	<i>P</i> = .083
	Postactivation #1	2.00	1.41	0.45	10	0.99	3.01	
	Postactivation #2	2.20	1.48	0.47	10	1.14	3.26	
	Postactivation #3	0.70	0.82	0.26	10	0.11	1.33	
	Postactivation #4	1.20	1.23	0.39	10	0.32	2.08	

ANOVA, analysis of variance; CFU, colony-forming unit.

the Louisiana site from baseline to postactivation #4. Mean fungal CFUs were reduced by 98% (1,299.50 CFUs to 25.0 CFUs) and mean MRSA CFUs were reduced by 96% (1,382.75 CFUs to 61.75 CFUs) at the Kentucky site.

A one-way repeated measures ANOVA determined mean floor CFUs were statistically significant at different time points during the study (Tables 1C and 1D). Post-hoc simple contrasts showed a statistically significant reduction from baseline to final postactivation #4 at both sites for mean fungal (Louisiana *P* = .026; Kentucky *P* = .021) and MRSA CFUs (Louisiana *P* = .017; Kentucky *P* = .05) (Tables 2C and 2D).

Environmental air

Mean aerobic bacterial and fungal CFUs were reduced by 72% (27.3 CFUs to 7.7 CFUs) and 89% (10.6 CFUs to 1.18 CFUs), respectively, from baseline to postactivation #4 at the Louisiana site. At the Kentucky site, mean aerobic bacterial CFUs increased by 6% (9.29 CFUs to 9.86 CFUs), and mean fungal CFUs were reduced by 27% (1.57 CFUs to 1.14 CFUs).

A one-way repeated measures ANOVA determined that mean airborne aerobic bacterial CFUs were statistically significant at

Table 1F
Kentucky air ANOVA statistics

Variable	Time point	Mean (CFU)	Std. deviation	Std. error	N	95% Confidence interval		Sig. of within-subjects (Greenhouse-Geisser)
						Lower bound	Upper bound	
Kentucky air bacterial burden	Baseline	9.29	2.50	0.94	7	6.98	11.60	<i>P</i> = .017
	Postactivation #1	7.86	9.08	3.43	7	0.00	16.26	
	Postactivation #2	9.57	6.40	2.42	7	3.65	15.49	
	Postactivation #3	26.14	14.19	5.36	7	13.02	39.27	
	Postactivation #4	9.86	5.30	2.01	7	4.96	14.76	
Kentucky air fungal burden	Baseline	1.57	1.13	0.43	7	0.52	2.62	<i>P</i> = .097
	Postactivation #1	1.57	1.27	0.48	7	0.39	2.75	
	Postactivation #2	0.00	0.00	0.00	7	0.00	0.00	
	Postactivation #3	0.00	0.00	0.00	7	0.00	0.00	
	Postactivation #4	1.14	2.27	0.86	7	0.00	3.24	

ANOVA, analysis of variance; CFU, colony-forming unit.

different time points during the study (Tables 1E and 1F). Post-hoc simple contrasts showed a statistically significant reduction in mean CFUs at the Louisiana site from baseline to final postactivation #4 for airborne aerobic bacteria (*P* = .028); the reduction in airborne fungi did not achieve statistical significance (*P* = .078); (Table 2E). At the Kentucky site, mean CFUs for aerobic bacteria (*P* = .806) and fungi (*P* = .510) did not achieve statistical significance (Table 2F).

Observations of HAIs

A decline in total aggregate counts of HAIs (ie, CAUTI, CLABSI, *C difficile*, MRSA bacteremia, and pneumonia) at both ICUs was observed when compared to a historical count 21 months prior to AP

activation for the Louisiana site (71% reduction; 24 HAIs to 7 HAIs) (Fig 3A) and 12 months prior at the Kentucky site (70% reduction; 10 HAIs to 3 HAIs) (Fig 3B).

DISCUSSION

Principal findings

Reducing the bioburden in an enclosed hospital environment is a monumental task, and even when tasks are performed correctly, the cleaning is still only intermittent. This study clearly demonstrates that despite intensive efforts by EVS, serious microbes remain on surfaces, floors, and in the air. The study environmental surface and floor results demonstrated a statistically significant reduction in

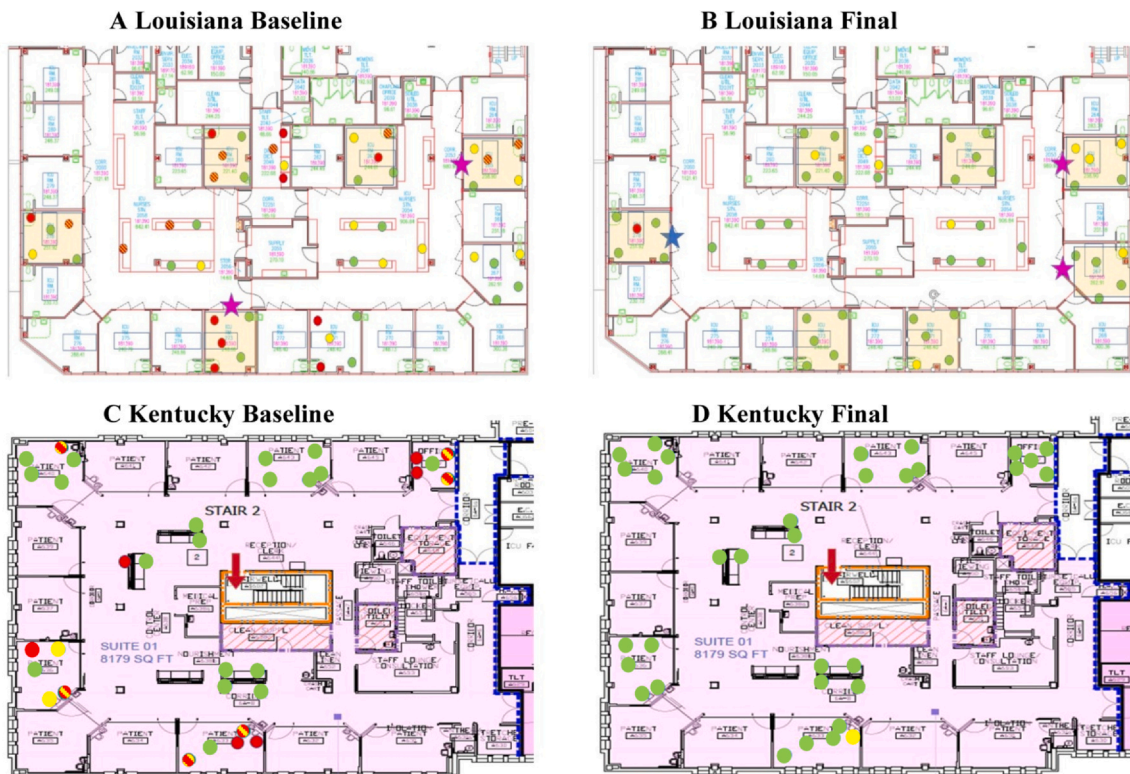


Fig. 2. Heat maps showing the swab locations in the Kentucky and Louisiana ICUs and color-coded to denote CFUs of MRSA found at baseline and final testing at Louisiana (A, B) and Kentucky (C, D). CFUs, colony-forming units; ICU, intensive care unit; MRSA, methicillin-resistant *Staphylococcus aureus*.

Table 2A
Louisiana surface contrast statistics

Baseline vs postactivation #4						
Variable	Contrast	F	Sig.	Partial Eta squared	95% Confidence interval	
					Lower bound	Upper bound
Louisiana surface fungal burden	485.49	13.55	$P < .001$	0.22	220.34	750.64
Louisiana surface MRSA burden	423.98	8.81	$P = .005$	0.16	136.72	711.24

MRSA, methicillin-resistant *Staphylococcus aureus*.

Table 2B
Kentucky surface contrast statistics

Baseline vs postactivation #4						
Variable	Contrast	F	Sig.	Partial Eta squared	95% Confidence interval	
					Lower bound	Upper bound
Kentucky surface fungal burden	487.16	8.06	$P = .008$	0.20	137.69	836.62
Kentucky surface MRSA burden	172.97	9.57	$P = .004$	0.23	59.08	286.86

MRSA, methicillin-resistant *Staphylococcus aureus*.

Table 2C
Louisiana floor contrast statistics

Baseline vs postactivation #4						
Variable	Contrast	F	Sig.	Partial Eta squared	95% Confidence interval	
					Lower bound	Upper bound
Louisiana floor fungal burden	11,437.40	7.08	$P = .026$	0.44	1,716.24	21,158.56
Louisiana floor MRSA burden	2,512.00	8.53	$P = .017$	0.49	566.14	4,457.87

MRSA, methicillin-resistant *Staphylococcus aureus*.

Table 2D
Kentucky floor contrast statistics

Baseline vs postactivation #4						
Variable	Contrast	F	Sig.	Partial Eta squared	95% Confidence interval	
					Lower bound	Upper bound
Kentucky floor fungal burden	1,274.50	19.69	$P = .021$	0.87	360.32	2,188.68
Kentucky floor MRSA burden	1,321.00	9.57	$P = .05$	0.76	0.00	2,679.81

MRSA, methicillin-resistant *Staphylococcus aureus*.

Table 2E
Louisiana air contrast statistics

Baseline vs postactivation #4						
Variable	Contrast	F	Sig.	Partial Eta squared	95% Confidence interval	
					Lower bound	Upper bound
Louisiana air bacterial burden	19.60	6.81	$P = .028$	0.43	2.60	36.60
Louisiana air fungal burden	9.40	3.94	$P = .078$	0.31	0.00	20.11

mean fungi and MRSA CFUs from baseline to final postactivation #4 at both sites. Surface locations with more than 500 CFU/100 cm² of MRSA were reduced by 100% at the Kentucky site and 90% at the Louisiana site, with the only location of more than 500 CFU/100 cm² remaining on the bedrail of a patient with an active MRSA infection.

A statistically significant reduction in mean aerobic bacterial air CFUs was found at the Louisiana site, but the reduction in mean fungal CFUs did not achieve statistical significance. At the Kentucky site, mean air aerobic bacterial and fungal CFUs did not achieve statistical significance, which is likely due to insufficient sample size ($n = 7$). Mean aerobic bacterial air CFUs at Kentucky were the only

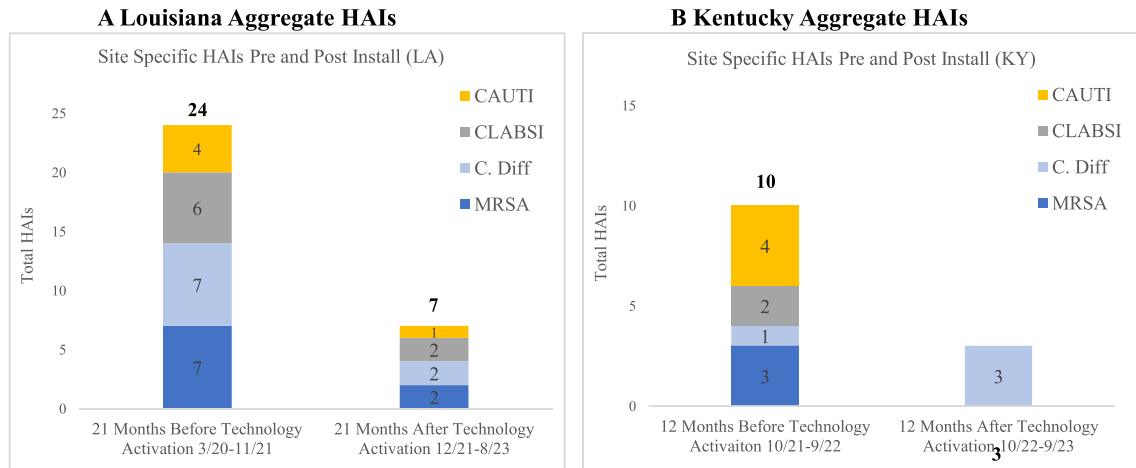
result to show an increase compared to baseline, which is likely due to the turbulent nature of airflow and ensuing variability of airborne microbes.³⁴ HAI data from the sites were used to evaluate the potential impact of microbial reductions on patient outcomes and observed a greater than 70% decline.

Strengths

MRSA was selected for evaluation because as a common environmental contaminant with about 1/3 of humans being colonized,³⁵ it plays a critical role in the acquisition of pathogens and

Table 2F
Kentucky air contrast statistics

Baseline vs postactivation #4						
Variable	Contrast	F	Sig.	Partial Eta squared	95% Confidence interval	
					Lower bound	Upper bound
Kentucky air bacterial burden	-0.57	0.07	$P = .806$	0.01	0.00	4.87
Kentucky air fungal burden	0.43	0.49	$P = .510$	0.08	0.00	1.93

**Fig. 3.** Aggregate counts of surveilled HAIs comparing matched time periods before and after ActivePure technology activation for Louisiana (A) and Kentucky (B). CAUTI, catheter-associated urinary tract infection; *C. difficile*, *Clostridioides difficile*; CLABSI, central line-associated bloodstream infection; MRSA, methicillin-resistant *Staphylococcus aureus*.

subsequent development of HAIs.³⁶ Patterns of air dispersal of *S. aureus* is not well understood, therefore, removal of bacteria from the air remains an attractive adjunct to studying reductions in airborne pathogens. With the strong impact the AP system had on MRSA CFUs along with its mechanism of action (ie, removes electrons to deactivate microbe), it is plausible to correlate these effects to other multi-drug resistant organisms.

Fungi, another epidemiologically important organism and ubiquitous in nature, can cause severe morbidity and mortality, particularly for those who are medically compromised. This study did show a significant impact on fungal contamination on all surfaces and floors reducing the overall fungal bioburden.

Limitations

This study was an experimental design without a concurrent ICU control due to the unique location and staff of each ICU. Both sites used the baseline to intervention comparison, making it difficult to rule out the impact of other varying factors. However, the depth and breadth of the durability of the outcomes over months and through full seasonally high respiratory infection time periods are reassuring. The study also limited itself to evaluating only one specific organism, MRSA. Future studies may consider studying more species since all organisms do not behave the same in the environment.³⁷

This study was not specifically designed for statistical correlation between microbial burden reductions and its impact on HAIs. However, these observations are important since the study time periods were at the same time as the repeated severe acute respiratory syndrome coronavirus 2 (Sars-CoV-2) surges and subsequent evaluations of HAIs since Sars-CoV-2 showed an increase nationwide (except *C. difficile* infections).^{38,39} Yet uniquely, the study's data showed a significant decline in 2 geographically distinct

ICUs when others declined less significantly.⁴⁰ Future studies with controls and statistical power can support these findings.

CONCLUSIONS

This study is on the pioneering edge of demonstrating that continuous decontamination of environmental air, surfaces, and floors can interrupt the “air-surface-air” nexuses and subsequent acquisition of pathogens that can lead to HAIs³³, all without the need for additional skilled labor, increases in cleaning and disinfection practices, or supplemental training.

The AP system provided a continual and persistent decline in microbial burden. A continuously clean and disinfected environment is needed to combat the continuous onslaught of contamination that the environment is subject to, and current technologies and practices only provide episodic effects. The study can be applied for use in a larger patient care setting, since similar environmental contamination occurs in all enclosed health care settings that serve the public.

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