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Major Article

Impact of antimicrobial wipes compared with hypochlorite solution on environmental surface contamination in a health care setting: A double-crossover study

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Disinfection
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Objective: Antimicrobial wipes are increasingly used in health care settings. This study evaluates, in a clinical setting, the efficacy of sporicidal wipes versus a cloth soaked in a 1,000 ppm chlorine solution.

Intervention: A double-crossover study was performed on 2 different surgical and cardiovascular wards in a 1,000-bed teaching hospital over 29 weeks. The intervention period that consisted of surface decontamination with the preimpregnated wipe or cloth soaked in chlorine followed a 5-week baseline assessment of microbial bioburden on surfaces. Environmental samples from 11 surfaces were analyzed weekly for their microbial content.

Results: A total of 1,566 environmental samples and 1,591 ATP swabs were analyzed during the trial. Overall, there were significant differences in the recovery of total aerobic bacteria ($P < .001$), total anaerobic bacteria ($P < .001$), and ATP measurement ($P < .001$) between wards and between the different parts of the crossover study. Generally, the use of wipes produced the largest reduction in the total aerobic and anaerobic counts when compared with the baseline data or the use of 1,000 ppm chlorine. Collectively, the introduction of training plus daily wipe disinfection significantly reduced multidrug-resistant organisms recovered from surfaces. Reversion to using 1,000 ppm chlorine resulted in the number of sites positive for multidrug-resistant organisms rising again.

Conclusions: This double-crossover study is the first controlled field trial comparison of using preimpregnated wipes versus cotton cloth dipped into a bucket of hypochlorite to decrease surface microbial bioburden. The results demonstrate the superiority of the preimpregnated wipes in significantly decreasing microbial bioburden from high-touch surfaces.

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Multidrug-resistant organisms (MDROs) are commonly associated with health care-associated infections. MDROs have a significant influence on patient morbidity and mortality and represent a substantial financial burden.¹⁻³ Hospital surfaces can be persistent reservoirs for health care-associated infections.⁴⁻⁸ Patients admitted to a room previously occupied by a patient with MDROs have an increased risk of acquiring these pathogens.⁹⁻¹² The use of a wipe or cloth in association with liquid/spray/vaporized disinfectants is becoming a common method to apply disinfectants to hospital surfaces.¹³ Preimpregnated wipes are increasingly being used for hospital cleaning or disinfection because of their ease of use and

activity claims.¹³ Whilst the majority of studies investigating preimpregnated wipes have focused on in vitro studies,¹⁴⁻¹⁹ there is a limited number of studies that have assessed the efficacy of wipes for surface cleaning or disinfection in a clinical setting.²⁰⁻²³ To date, no study has evaluated the comparative effectiveness of preimpregnated wipes against a disinfectant solution.

Our primary objective was to evaluate whether daily use of a peracetic acid/hydrogen peroxide preimpregnated wipe in place of the existing standard practice (detergent cleaning with cloth soaked in a bucket containing 1,000 ppm chlorine) led to a significant reduction in surface microbial contaminants.

METHODS

Setting

This study was conducted on 2 identical surgical and cardiovascular wards in a 1,000-bed teaching hospital over a 29-week period

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between August 2013 and April 2014. Following a 5-week baseline period (using a combination of detergent cleaning with cloth soaked in a 1,000 ppm chlorine (baseline), a 24-week double-crossover study was conducted (phases 1 and 2) (Fig 1) to assess the efficacy of the standard practice of chlorine disinfection with a cloth versus the introduction of a peracetic acid/hydrogen peroxide wipe.

Cleaning and disinfection protocol

For the purpose of this study, 1,000 ppm chlorine solution in a bucket was used in combination with cotton cloths following a detergent cleaning step for all the surfaces sampled. The disinfectant wipe was a dry preimpregnated (sporicidal) wipe that generates peracetic acid/hydrogen peroxide when activated with water. The number of wipes required per surface was determined depending on the surface area according to the manufacturer's instructions. Procurement of wipes was calculated on expected use per ward per week. To ensure the correct product was used during the intervention period, all detergent and chlorine-containing agents were removed from the specified ward.

Training

Training (approved by the infection prevention and control [IPC] team) was delivered to nurses, health care assistants, and environmental services cleaning staff, including supervisors. Training was conducted over a 2-week period in groups of 1-5 staff members, for 30-45 minutes before both intervention periods (Fig 1).

Environment sampling

Surface samples were collected weekly from 11 sites (bed control, bed rails, tray table, call button, patient chair, drug locker, commode top, bathroom door handle, flush handle, toilet grab rail, and toilet seat) between 6 AM and 7 AM, before cleaning. Locations included ward, isolation rooms, 4-bed bays, single and shared bathrooms, and sluice room.

A 10 × 10 cm² sterile template (Thermo Fisher Scientific, Waltham, MA) was placed on surfaces where possible. Surfaces were wiped with a premoistened (neutralizing buffer) cellulose sponge (Sponge-Stick; 3M Company, Maplewood, MN) under aseptic conditions. Sponge-Sticks were applied firmly 3 times horizontally and 3 times vertically on each side of the sponge so that the designated area was sampled. For the call button, the entire surface (front, back, and

sides) was sampled; for the toilet flush handle, the flush handle itself and area immediately surrounding the flush handle was sampled.

Sponge heads were placed in individually sealed bags and transported within 3 hours of sampling. Handles were aseptically removed, and sponges processed following the method of Dubberke et al²⁴ with the following modifications: Excess liquid was aseptically squeezed into a stomacher bag, which was placed in a Stomacher 400 (Seward, West Sussex, UK) and homogenized for 15 minutes at room temperature. The volume of homogenized liquid was measured to the nearest decimal point with a 10 mL stripette and placed into a 50 mL centrifuge tube.

Total aerobic and anaerobic counts

A 100- μ L sample was plated onto brain heart infusion agar (Oxoid Ltd, Cheshire, UK), incubated at 37°C for 72 hours for aerobic colony counts. For anaerobic colony counts, prerduced brain heart infusion agar (Oxoid Ltd) was inoculated and incubated in an anaerobic workstation (MG500; Don Whitley Scientific, West Yorkshire, UK) for 72 hours. All the results were expressed as total aerobic/anaerobic count (in colony forming units per centimeters²) of sampled surface.

Indicator microorganisms

The presence of methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), extended-spectrum beta-lactamases (ESBLs), carbapenem-resistant Enterobacteriaceae (CRE) and *C difficile* on environment surfaces was monitored by inoculating 10 μ L of each sample onto the appropriate selective culture media, including Brilliance MRSA 2 Agar, Brilliance VRE Agar, Brilliance ESBL Agar, and Brilliance CRE Agar (Oxoid Ltd).

For *C difficile*, a 2-stage process was undertaken: direct inoculation onto prerduced cefoxitin cycloserine fastidious anaerobe agar (LabM, Heywood, UK) supplemented with 5 mg/mL lysozyme (Sigma-Aldrich, St Louis, MO), 1% (w/v) sodium taurocholate (Sigma-Aldrich), and 1% (v/v) defibrinated sheep blood (VH Bio Ltd, Gateshead, UK) and postenrichment inoculation—following anaerobic incubation of samples for 72 hours, tubes were centrifuged at 5,000 g for 5 minutes at 4°C, resuspended in 80% (v/v) absolute ethanol, and held for 1 hour at room temperature. Following ethanol shock, samples were centrifuged, resuspended in 2 mL sterile deionized water, and heat shocked for 20 minutes at 60°C. Samples were allowed to cool to room temperature and 10 μ L plated onto cefoxitin cycloserine fastidious anaerobe agar supplemented with

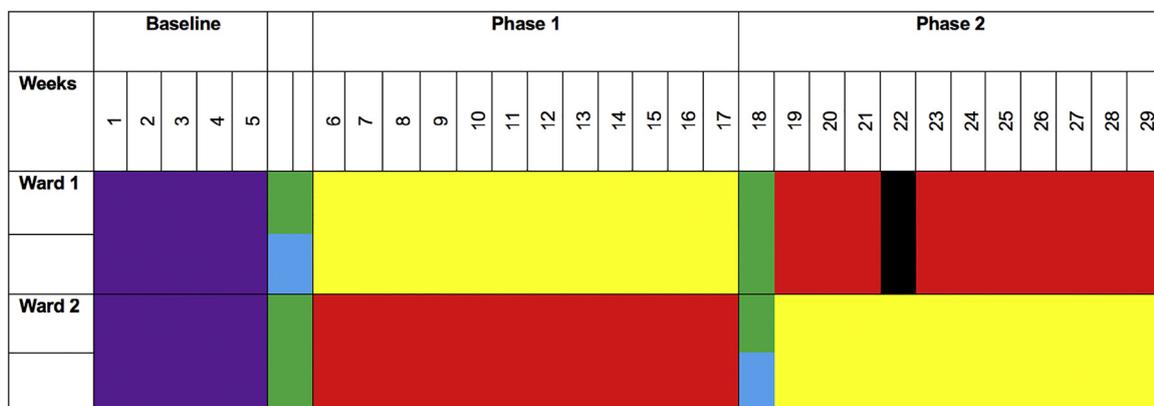


Fig 1. Schematic of double crossover field study. Purple shading indicates baseline date: Use of standard cleaning regimen. Red shading indicates use of detergent and chlorine 1,000 pm. Yellow shading indicates use of preimpregnated sporicidal wipes. Green shading indicates general training on disinfectant use, wiping, and infection prevention. Blue shading indicates specific training on the use of preformulated wipes. Black shading indicates wards closure.

5 mg/mL lysozyme, 1% (w/v) sodium taurocholate, and 1% (v/v) defibrinated sheep blood and incubated anaerobically for 72 hours. All isolates that were recovered from the chromogenic selective media were subcultured and identified per the manufacturer's instructions. Growth or colonies with colors other than those specified in the manufacturer's instructions were reported as negative but were stored at -20°C for subsequent analysis. Colonies displaying the atypical morphology (large, irregular, ground glass appearance) and smell were recorded. All isolates were subcultured and identified using the RapID ANA II system (Remel Products, Lenexa, KS). All reactions were interpreted as described in the manufacturer's interpretation guide. A positive control of *C difficile* NCTC 11209 (Public Health England, London, UK) was included to aid in interpretation.

Presumptive staphylococci

Colonies recovered from the Brilliance MRSA 2 Agar were identified using the API-Staph identification kit according to the manufacturer's instruction (BioMerieux, Marcy l'Etoile, France).

DNA extraction

Colonies recovered from the Brilliance MRSA 2 Agar and those identified as *C difficile* with the RapID ANA II system were subjected to further molecular testing. DNA was isolated using the GeneJET Genomic DNA purification kit (ThermoFisher) per the manufacturer's instructions and stored at -20°C until further use. DNA purity and concentration were measured using a NanoDrop (Thermo Scientific). For *C difficile*, samples were ribotyped at the Anaerobic Reference Unit, Cardiff, UK.

Presumptive *S aureus* colonies were further characterized by the presence of the *spa* fragment (180–600 bp) and *mecC* (138 bp) following the polymerase chain reaction method of Stegger et al,²⁵ *mecA* (533 bp) as outlined by Murakami et al,²⁶ and typed by RAPD (Random Amplification of Polymorphic DNA) following the method by Cheeseman et al.²⁷ Gels were visualized under ultraviolet illumination using the ChemiDoc XRS+ (Bio-Rad, Hercules, CA). Digital files were standardized for band detection with the Image Lab (Bio-Rad) software. All gels included DNA from control strains and a DNA ladder (GeneRuler 100 bp DNA Ladder; Thermo Scientific). Control strains included *mecA*-positive *S aureus* NCTC 12493 (Public Health England) and *mecC* positive *S aureus* NCTC 13552 (Public Health England).

ATP sampling

ATP sampling was performed with Ultrasnap swabs (SystemSure Plus system; Hygiene International Ltd, Hertfordshire, UK) following the manufacturer's instructions. Before sampling, the system was calibrated on a weekly basis using ATP positive and negative controls per the manufacturer's instructions. Where possible, directly adjacent surfaces to microbiologic sampling were sampled. For the flush handle, call button, and bed control ATP samples were obtained before sampling with the Sponge-Sticks was conducted.

Statistical analysis

Data were analyzed with a mixed effect model utilizing \log_{10} (+1) transformed data of ATP ($n = 1,505$), aerobic ($n = 1,438$), or anaerobic ($n = 1,438$) count as dependent variable. Ward, baseline, and intervention periods, as well as an interaction term thereof, were used as independent variables. Repeated measures across weeks were accounted for in the random model. Stepwise model reduction was performed by comparing Akaike information criterion

values between full and reduced models. Standardized residuals from each model were first checked visually for normality and homogeneity of variance using a histogram, Q-Q plots, and fitted values. To test for correlation between ATP values and bacterial counts, a Spearman rank correlation test was performed. All analyses were performed utilizing the nlme library in R version 2.13.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Environment sampling results

In total, 1,566 environment samples and 1,591 ATP swabs were taken from the 2 wards. Ward 1 closed halfway through phase 2 (Fig 1) following a norovirus outbreak and underwent enhanced disinfection with sodium hypochlorite 5,000 ppm and twice-daily cleaning. No samples were collected during this period.

Overall, the use of preimpregnated wipes produced the largest significant reduction in the total aerobic ($P < .001$), anaerobic counts ($P < .001$), and ATP RLU measurements ($P < .001$) when compared with the baseline data (Fig 2). The overall reduction of aerobic counts (for all surfaces) was significantly higher ($P < .001$) following the use of preimpregnated wipes compared with the use of 1,000 ppm chlorine solution during the trial. The relative light unit count was significantly lower (LogATP $P < .001$) following the use of preimpregnated wipe rather the use of chlorine 1,000 ppm in ward 2 only (Fig 2B).

During the baseline study, a number of sites registered total aerobic and anaerobic count >2.5 CFU/cm², whereas during the intervention period all sites showed a <2.5 CFU/cm² regardless of the wards. The introduction of training plus daily disinfection reduced the number of sites with relative light unit values >250 to 21 sites (8% of sites, compared with 18% in the baseline period) and 19 sites (7% of sites, compared with 21% in the baseline period) for wards 1 and 2, respectively.

In ward 1, the reintroduction of using detergent and chlorine (phase 2) following the use of preimpregnated wipe (phase 1) saw a significant increase ($P < .001$) in aerobic count in some (toilet flush handle, tray table, and locker) but not in all sites sampled (Fig 3A). This increase was not as pronounced with the total anaerobic count (Fig 3B). For the call button the number of aerobic and anaerobic counts continued to decrease in phase 2 (Fig 3). The introduction of preimpregnated wipes (phase 2) following the use detergent and chlorine 1,000 ppm decreased significantly the total aerobic ($P < .001$) and anaerobic ($P < .001$) counts (Fig 4). The influence of staff training is shown in Figure 4, where a significant decrease ($P < .001$) in total aerobic count or anaerobic count can be observed for the toilet seat and tray table between the baseline period and the use of detergent and chlorine (Fig 4). Other surfaces showed a nonsignificant decrease ($P > .001$) in count between baseline and the use of detergent and chlorine.

Isolation of specific bacteria

During the baseline period, 7% (35 out of 522) of all sites sampled were positive for VRE, CRE, or ESBL (Fig 5). The introduction of training and preimpregnated wipes reduced this to 1% (5 out of 522) (phase 1 ward 1). Reversion to the use of detergent and 1,000 ppm chlorine saw the number of sites positive for VRE, CRE, or ESBL rise to 3% (14 out of 522) (ward 1 phase 2), although this number was below that of the baseline for ward 1. For ward 2, training was effective in reducing the number of positive sites from 13 to 7 out of 522 (ward 2 phase 1) and this number decreased further to 3 sites following the use of preimpregnated wipes (Fig 5B). Overall, VRE

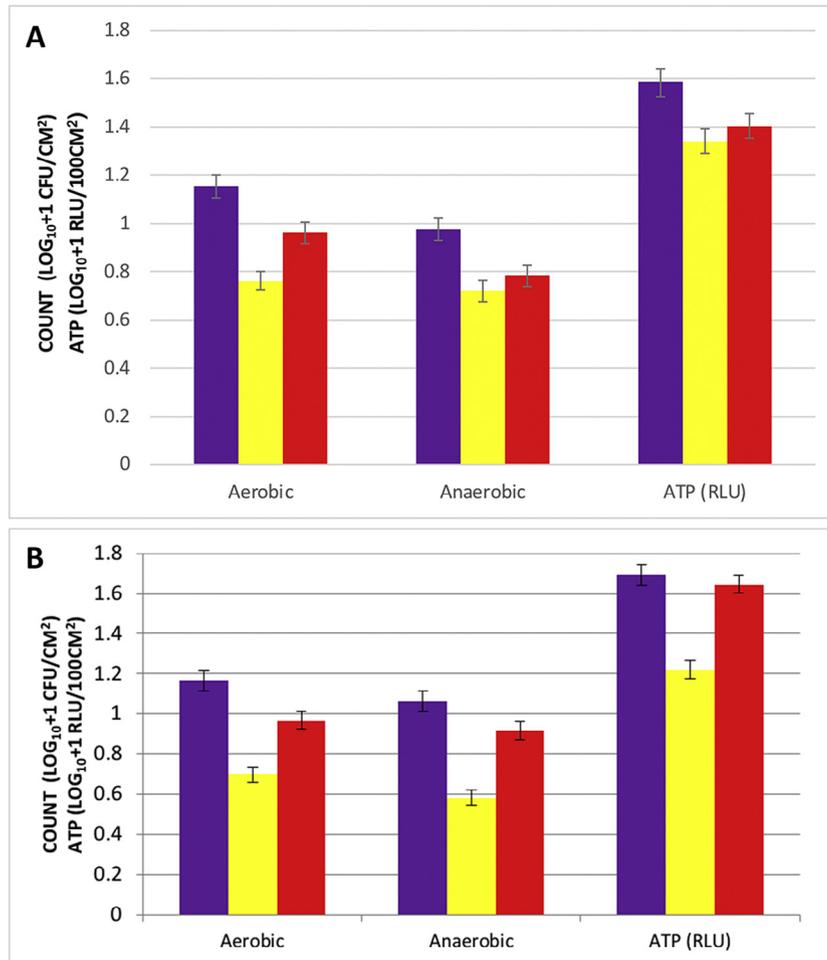


Fig 2. Overall total aerobic and anaerobic counts ($\text{Log}_{10} +1/\text{cm}^2$) and ATP count (relative light unit $\text{Log}_{10} +1/\text{cm}^2$). Purple shading indicates baseline. Yellow shading indicates intervention with sporicidal wipe. Red shading indicates cleaning and use of chlorine 1,000 ppm. (A) Ward 1. (B) Ward 2.

was the most common isolated MDRO (6% of samples), primarily from toilet seat and toilet grab rail (data not shown).

Collectively, a large number of confirmed staphylococci (280 out of 1,566) were recovered, the majority of which were *Staphylococcus haemolyticus* (45%) and *S aureus* (25%). For *S aureus*, 58% of isolates were positive for *mecA*, 30% for *mecC*, and 40% for *spaA*. For *S haemolyticus*, 66% were positive for *mecA*, 11% for *mecC*, and 29% for *spaA* (data not shown).

Of the 1,566 environment samples obtained, only 45 cultures (3%) were identified as *C difficile* following the postenrichment step. *C difficile* counts increased in both wards during phase 2 regardless of the intervention (data not shown). All isolates were confirmed to be typed as RT001 (data not shown). No RT001 was reported for the clinical samples submitted for ribotyping during the trial period. The predominant ribotype at the time was RT027 followed by RT020 (personal communication from Trefor Morris, UK anaerobe reference unit, Public Health Wales, November, 2014). Other anaerobic bacteria were identified (data not shown).

DISCUSSION

This double-crossover study is the first controlled field trial comparison of the use of preimpregnated wipes versus cotton cloth dipped into a bucket of hypochlorite to decreasing surface microbial bioburden in 2 surgical and cardiovascular wards. We showed that preimpregnated wipes contributed to significantly decreasing

microbial bioburden from a number of high-touch surfaces. The number of sites with identified MDROs also decreased significantly following the use of the preimpregnated wipes. In ward 1, results showed an initial significant decrease in microbial bioburden where the wipes were used immediately after the baseline (phase 1), followed by an increase in microbial count following the reintroduction of cotton cloth dipped into a bucket of 1,000 ppm chlorine solution (phase 2). In ward 2, the use of the preimpregnated wipes in phase 2 contributed to a further reduction (statistically significant ($P < .001$) for a number of surfaces) of microbial count on surfaces. During the intervention period, an average of 150 and 175 wipes were used per day on wards 1 and 2, respectively. It was not possible to collect data on the average number of cleaning cloths used during the baseline period because the type of cloth used for cleaning and disinfection ranged from reusable microfiber to disposable cotton. Given the estimated wipe use, the ward layout and the number of surfaces on the 38-bed wards, it appears that a 1-wipe-1-direction-1-surface recommendation was not strictly adhered to. Despite this, a significant reduction in total microbial counts was observed when the intervention product was used. The efficacy of the preimpregnated wipe may be due to its ability to retain and not transfer microbial burden to multiple surfaces, as demonstrated in an earlier laboratory study.¹⁶ A recent crossover trial highlighted the superiority of using preformulated wipe with an oxidizing chemistry against the use of a quaternary ammonium compound-based wipe in significantly reducing surface

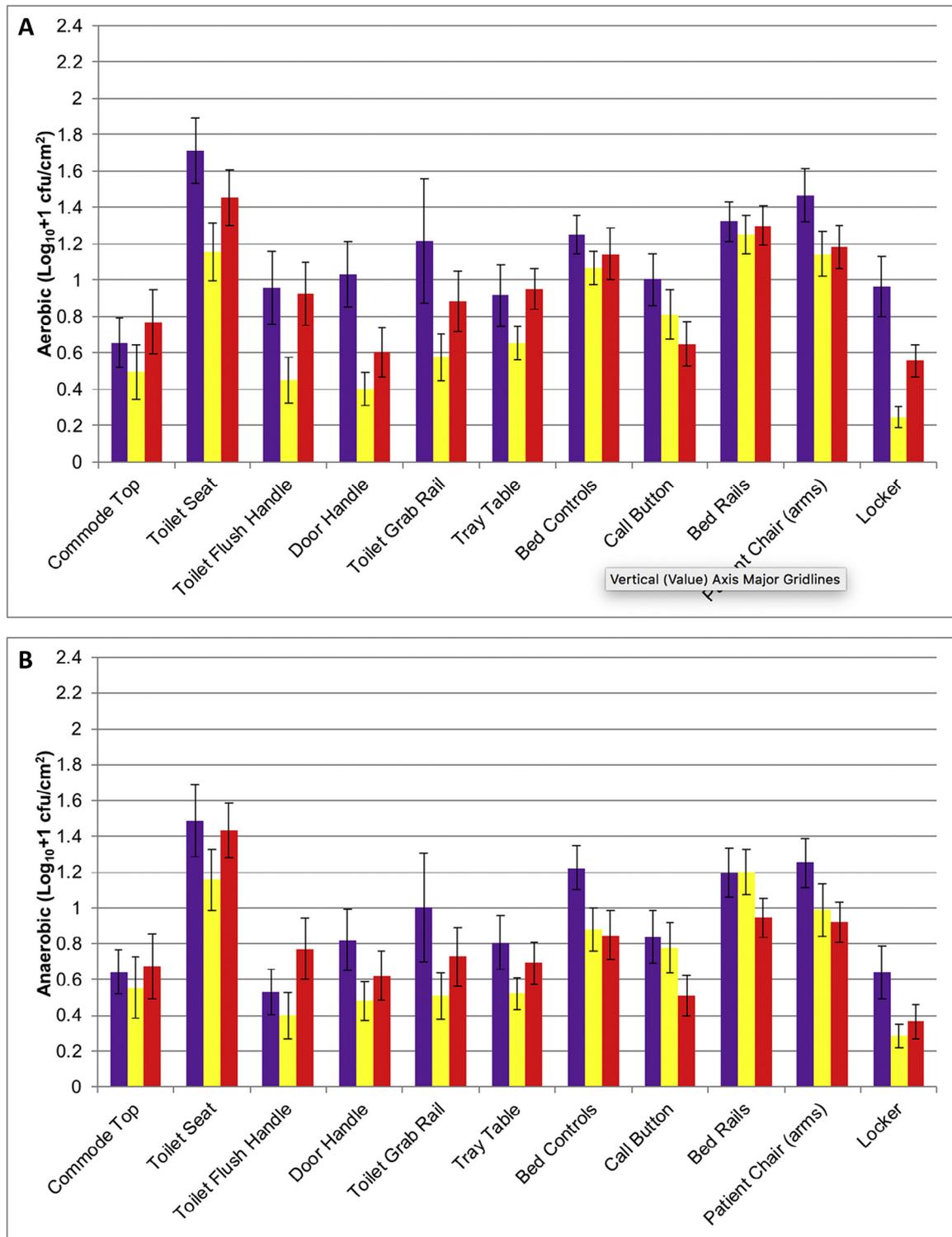


Fig 3. Total counts ($\text{Log}_{10}/\text{cm}^2$) per individual sites for ward 1. Purple shading indicates baseline. Yellow shading indicates intervention with sporicidal wipe (phase 1). Red shading indicates cleaning and use of chlorine 1,000 ppm (phase 2). (A) Total aerobic count. (B) Total anaerobic count.

contamination.²³ It has been suggested that aerobic colony count on hand-touch surfaces should not exceed 5 CFU/cm², although a clean cut-off point of <2.5 CFU/cm² has been proposed.²⁸⁻³⁰ With this in mind, a number of surfaces in the baseline period would not be considered clean. The intervention resulted in all surfaces passing a <2.5 CFU/cm² standard. Boyce and Havill²⁰ reported that the use of a new hydrogen peroxide wipe led to 99% of surfaces treated with

<2.5 CFU/cm² following surface cleaning. In our study, it is encouraging that the use of preimpregnated wipes achieved the cut-off points, considering that sampling was performed once a week and before cleaning.

Sporicidal wipes are designed to eliminate spores of *C difficile* on surfaces. Here, very few *C difficile* spores (genotype RT001) were recovered overall, whereas all the clinical *C difficile* samples were

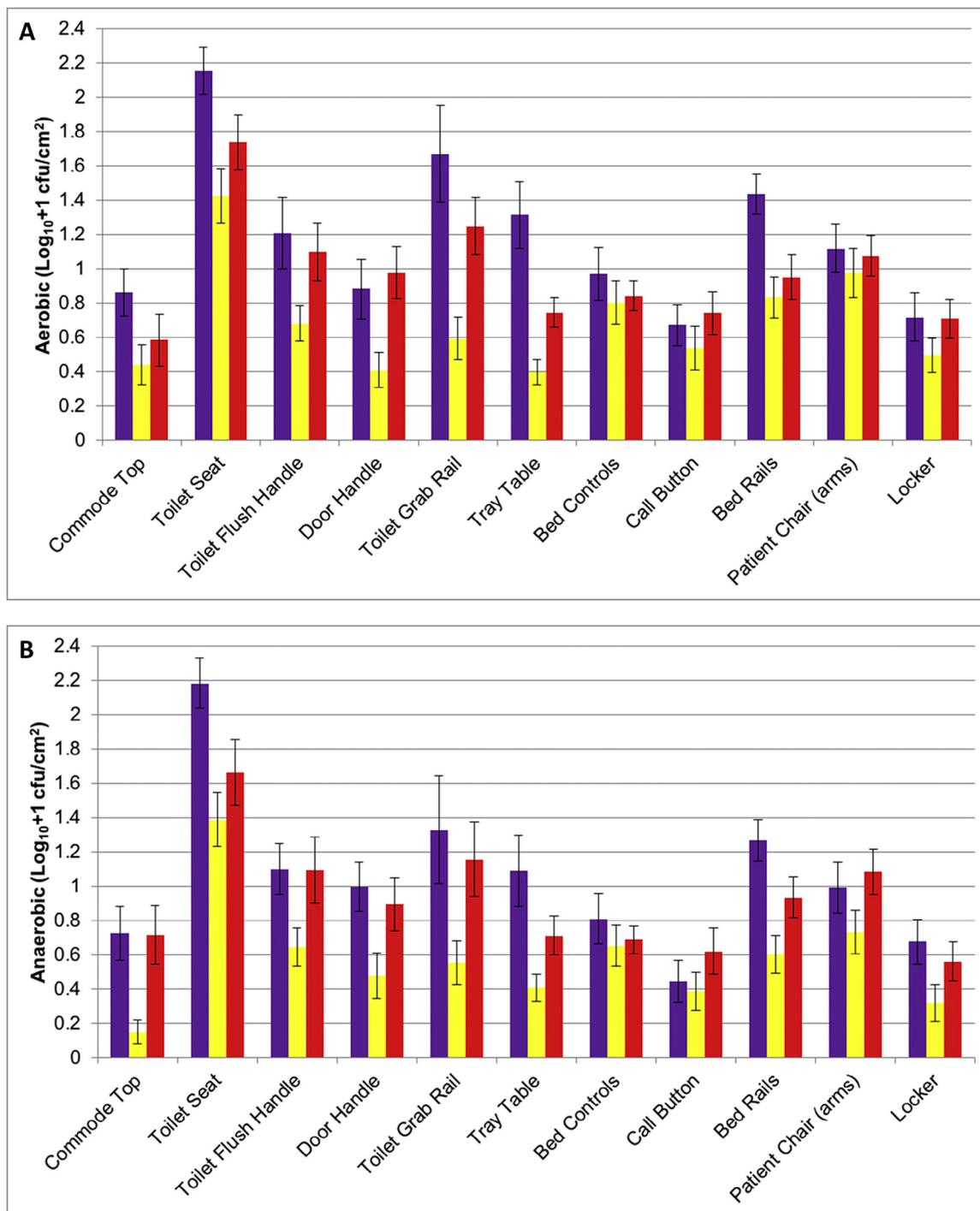


Fig 4. Total counts ($\text{Log}_{10}/\text{cm}^2$) per individual sites for ward 2. Purple shading indicates baseline. Yellow shading indicates intervention with sporicidal wipe (phase 2). Red shading indicates cleaning and use of chlorine 1,000 ppm (phase 1). (A) Total aerobic count. (B) Total anaerobic count.

of the RT027 and RT020 ribotypes at the time of study. Introducing sporicidal wipes to control *C. difficile* outbreaks has been reported in 1 study, in which replacing the use of hypochlorite with a preimpregnated sporicidal wipe led to a significant reduction in *C. difficile* infection rate over time.²¹

Here, the efficacy in reducing surface bioburden from combining a hypochlorite solution and cotton cloths was inferior to the preimpregnated wipes. The use of hypochlorite-formulated wipes has been shown to contribute significantly to the decrease of *C. difficile* infection,²² although preformulated hypochlorite wipes can

potentially transfer microorganisms between surfaces.¹⁸ These studies and ours highlight that preformulated wipes for which the disinfectant solution and the wipe material are optimized for activity have a better efficacy.

It is clear that product efficacy and the appropriate use of wipes as well as staff training and product use auditing are essential.^{23,31} Although staff awareness of the trial might have contributed to the observed improved performance,^{23,32} the introduction of specific training undoubtedly had an influence. Here, training saw an average 17% reduction in the mean total aerobic count in both wards, al-

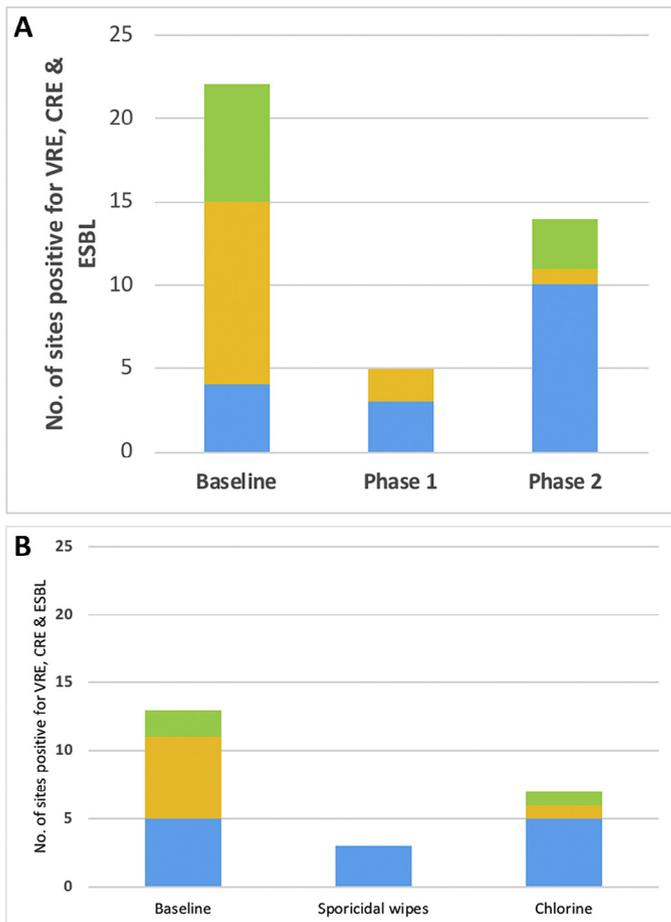


Fig 5. Number of sites positive for multidrug-resistant organisms (MDROs). Green shading indicates extended-spectrum beta lactamases (ESBL). Yellow shading indicates carbapenem-resistant Enterobacteriaceae (CRE). Blue shading indicates vancomycin-resistant enterococci (VRE). (A) Ward 1. (B) Ward 2.

though the introduction of wipes saw a 34% and 40% reduction in the mean total aerobic count in wards 1 and 2, respectively. Measurement of relative light units using an ATP sampler to indicate surface cleanliness in health care setting is not new.^{28,33,34} In our study, a Spearman rank correlation test identified total aerobic count (colony forming units / centimeters² Log₁₀ + 1) and ATP (relative light units) to be highly correlated (P value < .0000). By log transforming the ATP data, a more even distribution was achieved although the data were still not normally distributed. Our results support the data presented by Boyce and Havill,²⁰ who observed a good correlation between total aerobic count and ATP measurement.

The cost-effectiveness in using formulated wipe products needs to be justified. The use of formulated wipes offers many advantages compared with the practice of using hypochlorite solution in a bucket. These include better control of microbial bioburden; ease of use; avoiding the use of highly concentrated biocidal solutions to be diluted down; increasing efficacy by optimizing the combination between the disinfectant solution and the wipe material; compatibility with, and decreasing damages to, the surfaces to be wiped; decreasing time required to disinfect the patient room/ward; avoiding contaminating the disinfecting solution or product following repeated use; and the provision of clear instructions on labels, including support instructions or posters and training packages by the manufacturer. Of these advantages, eliminating the risk of human error during product preparation or dilution is attractive because a decrease in biocidal product concentration can affect

bacterial survival, resistance, and cross-resistance to antimicrobial agents.^{35,36} In addition, optimizing the disinfectant solution with the appropriate wipe materials not only increases the efficacy in removing microbial burden from surfaces, but also decreases microbial transfer if a wipe is misused on multiple surfaces.^{13,14,18}

There were several limitations in our study. We did not measure the influence of other hygiene measures, such as handwashing. The diversities of patients and patient length of stay on the 2 wards, the inability to measure the antimicrobial agents used on the wards on a daily or weekly basis (there was no significant difference [$P > .05$] in the monthly antimicrobial stock data between phases and/or wards for both systemic and topical antimicrobial agents), and the inability to get accurate figure of patient infection rate for just the trial period, impinged on demonstrating further benefits from the use of preimpregnated wipes.

CONCLUSIONS

This crossover trial demonstrated that the use of a preimpregnated wipe product provided better control of microbial burden on surfaces, simplified disinfection procedures, and questioned the practice of using hypochlorite diluted solution in a bucket in combination with some cloth materials.

Acknowledgments

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