Impact of antimicrobial wipe compared with hypochlorite solution on environmental surface contamination in a healthcare setting: a double crossover study

Harsha Siani, BSc.; Rebecca Wesgate, BSc.; Jean-Yves Maillard, PhD.

[a]School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, UK

Address for correspondence

Jean-Yves Maillard

Cardiff School of Pharmacy and Pharmaceutical Sciences,

Cardiff University, King Edward VII Avenue

Cardiff CF10 3NB

United Kingdom

Tel: 44(0)2920879088

Email: maillardj@cardiff.ac.uk

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Abstract (250/250)

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

Intervention: A double crossover study was performed on two different surgical and cardiovascular wards in a 1000-bed teaching hospital over 29 weeks. The intervention period which consisted of surface decontamination with the pre-impregnated 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 1566 environmental samples and 1591 ATP swabs were analyzed during the trial. Overall, there were significant differences in the recovery of total aerobic bacteria ($p<0.001$), total anaerobic bacteria ($p<0.001$) and ATP measurement ($p<0.001$) 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 to 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 saw the number of sites positive for MDRO rise again.

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

Keywords: wipes, hypochlorite, double-crossover trial, disinfection

INTRODUCTION

Multidrug resistant organisms (MDRO) are commonly associated with healthcare-associated infections (HCAIs). MDRO have a significant impact on patient morbidity and mortality and represent a substantial financial burden.[1-3] Hospital surfaces can be persistent reservoirs for HCAIs.[4-8] Patients admitted to a room previously occupied by a patient with MDRO have an increased risk of acquiring these pathogens.[9-12] The use of wipe or cloth in association with liquid/spray/vaporised disinfectants is becoming a common method to apply disinfectants to hospital surfaces.[13] Pre-impregnated wipes are increasingly being used for hospital cleaning/disinfection because of their ease of use and activity claims.[13] Whilst the majority of studies investigating pre-impregnated 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/disinfection in a clinical setting.[20-23] To date, no study has evaluated the comparative effectiveness of pre-impregnated wipes against a disinfectant solution.

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

Methods:

Setting

This study was conducted on two identical surgical and cardiovascular wards in a 1000-bed teaching hospital over a 29 weeks period between August 2013 and April

2014. Following a 5-week baseline period (using a combination of detergent cleaning with cloth soaked in a 1,000ppm chlorine (Baseline), a 24 weeks double crossover study was conducted (Phases 1 and 2; Figure 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/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 pre-impregnated (sporicidal) wipe, which 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 usage per wards 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 IPC team) was delivered to nurses, healthcare assistants and environmental services cleaning staff, including supervisors. Training was conducted over a 2-week period, in groups of 1-5 staff, for 30-45 minutes before both intervention periods (Figure 1).

Environmental 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 and 7 am, prior to cleaning. Locations included ward, isolation rooms, four-bedded bays, single and shared bathrooms, and sluice room.

A 10 x 10 cm^2 sterile template (ThermoFisher, UK) was placed on surfaces where possible. Surfaces were wiped with a pre-moistened (neutralizing buffer) cellulose sponge (3M™ Sponge-Stick, UK) 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 h of sampling. Handles were aseptically removed, and sponges processed following the method of Dubberke et al.[24] with the following modifications: excess liquid was aseptically squeezed into the stomacher bag which were placed in a stomacher®400 (Seward Stomacher, UK) and homogenized for 15 min 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 µL sample was plated onto Brain Heart Infusion Agar (BHI; Oxoid Ltd, UK), incubated at 37°C for 72 h for aerobic colony counts. For anaerobic colony counts

pre-reduced BHI agar was inoculated and incubated in an anaerobic workstation (MG500; DW Scientific, UK) for 72 h. All the results were expressed as total aerobic/anaerobic count (in colony-forming units [CFUs]) per cm^2 of sampled surface.

Indicator Microorganisms

The presence of MRSA, VRE, ESBL, CRE and *C. difficile* on environmental surfaces was monitored by inoculating 10 µL 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, UK).

For *C. difficile* a two stage process was undertaken: i) direct inoculation onto pre-reduced Cefoxitin Cycloserine Fastidious Anaerobe Agar (CCFA) (LabM) supplemented with 5 mg/mL lysozyme (Sigma-Aldrich UK), 1% (w/v) sodium taurocholate (Sigma-Aldrich UK) and 1% (v/v) defibrinated sheep blood (VH Bio Ltd, UK) and ii) post-enrichment inoculation – following anaerobic incubation of samples for 72 h, tubes were centrifuged at 5,000 *g* for 5 mins at 4°C, re-suspended in 80 % (v/v) absolute ethanol and held for 1 h at room temperature. Following ethanol shock samples were centrifuged, re-suspended in 2 mL sterile de-ionised water and heat shocked for 20 min at 60°C. Samples were allowed to cool to room temperature and 10 µL plated onto CCFA supplemented with 5 mg/mL lysozyme, 1% (w/v) sodium taurocholate and 1% (v/v) defibrinated sheep blood and incubated anaerobically for 72 h. All isolates that were recovered from the chromogenic selective media were sub-cultured and identified as 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 sub-cultured and identified using the RapID ANA II system (Remel, Lenexa). All reactions were interpreted as described in the manufacturer's interpretation guide. A positive control of *C. difficile* NCTC 11209 (PHE, 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, 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, UK) as per the manufacturer's instructions and stored at -20°C until further use. DNA purity and concentration were measured using a NanoDrop™ (Thermo Scientific, UK). For *C. difficile*, samples were ribotyped at the Anaerobic Reference Unit, Cardiff.

Presumptive *S. aureus* colonies were further characterized by the presence of the *spa* fragment (180-600 bp) and *mecC* (138 bp) following the PCR method of Stegger et al.,[25] *mecA* (533 bp) as outlined by Murakami et al.,[26] and typed by RAPD following the method by Cheeseman et al.[27] Gels were visualized under UV illumination using

the ChemiDoc™ XRS+ (Bio-Rad, UK). Digital files were standardized for band detection with the Image Lab (Bio-Rad, UK) software. All gels included DNA from control strains and a DNA ladder (GeneRuler 100 bp DNA Ladder, Thermo Scientific, UK). Control strains included *mecA* positive *S. aureus* NCTC 12493 (PHE, UK) and *mecC* positive *S. aureus* NCTC 13552 (PHE, UK).

ATP Sampling

Adenosine triphosphate sampling was performed with Ultrasnap swabs (SystemSure Plus system Hygiena® Int. Ltd, UK) following the manufacturer's instructions. Prior to sampling the system was calibrated on a weekly basis using ATP positive and negative controls as per manufacturer's instructions (Hygiena® Int. Ltd, UK). Where possible directly adjacent surfaces to microbiological 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 = 1505), Aerobic (n = 1438) or Anaerobic (n = 1438) 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. Step-wise model reduction was performed by comparing AIC 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 2.13.2 (R Development Core Team 2012).

Results

Environmental Sampling Results

In total, 1566 environmental samples and 1591 ATP swabs were taken from the two wards. Ward 1 closed halfway through phase 2 (Figure 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 pre-impregnated wipes produced the largest significant reduction in the total aerobic ($p<0.001$), anaerobic ($p<0.001$) counts and ATP RLU measurements ($p<0.001$) when compared to the baseline data (Figure 2). The overall reduction of aerobic counts (for all surfaces) was significantly higher ($p<0.001$) following the use of pre-impregnated wipes compared to the use of 1,000 ppm chlorine solution during the trial. The RLU count was significantly lower (LogATP $p<0.001$) following the use of pre-impregnated wipe rather the use of chlorine 1,000 ppm in ward 2 only (Figure 2B).

During the baseline study, a number of sites registered total aerobic and anaerobic count >2.5 cfu/cm^2, whereas during the intervention period all sites showed a <2.5 cfu/cm^2 regardless of the wards. The introduction of training plus daily disinfection reduced the number of sites with RLU values >250 to 21 sites (8% of sites, compared

to 18% in the baseline period) and 19 sites (7% of sites, compared to 21% in the baseline period) for wards 1 and 2, respectively.

In ward 1, the re-introduction of using detergent and chlorine (phase 2) following the use of pre-impregnated wipe (phase 1) so a significant increase ($p<0.001$) in aerobic count in some (toilet flush handle, tray table and locker) but not all sites sampled (figure 3A). This increase was not as pronounced with the total anaerobic count (figure 3B). For the call button the number of aerobic and anaerobic counts continued to decrease in phase 2 (Figure 3). The introduction of pre-impregnated wipes (phase 2) following the use detergent and chlorine 1,000 ppm decreased significantly the total aerobic ($p<0.001$) and anaerobic ($p<0.001$) counts (Figure 4). The impact of staff training is shown in Figure 4, where a significant decrease ($p<0.001$) 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 (Figure 4). Other surfaces show a non-significant decrease ($p>0.001$) in count between baseline and the use of detergent and chlorine

Isolation of specific bacteria

In the baseline period, 7% (35/522) of all sites sampled were positive for VRE, CRE or ESBL (Figure 5). The introduction of training and pre-impregnated wipes reduced this to 1% (5/522) (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% (14/522)(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 7/522 (Ward 2, phase 1) and this number decreased further to 3 sites following

the use of pre-impregnated wipes (Figure 5B). Overall VRE 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 (280/1566) were recovered, the majority of which were *S. 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 1566 environmental samples obtained, only 45 cultures (3%) were identified as *C. difficile* following the post-enrichment 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 however reported for the clinical samples submitted for ribotyping during the trial period. The predominant ribotype at the time was RT027 followed by RT020 (communication from Trefor Morris, UK anaerobe reference unit Public Health Wales). Other anaerobic bacteria were identified (data not shown).

Discussion

This double crossover study is the first controlled field trial comparison of the use of pre-impregnated wipes *vs.* cotton cloth dipped into a bucket of hypochlorite to decreasing surface microbial bioburden in two surgical and cardiovascular wards. Here we showed that pre-impregnated wipes contributed to significantly decreasing microbial bioburden from a number of high touch surfaces. The number of sites with

identified MDRO also decreased significantly following the use of the pre-impregnated 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 re-introduction of cotton cloth dipped into a bucket of 1,000 ppm chlorine solution (phase 2). In ward 2, the use of the pre-impregnated wipes in phase 2 contributed to a further reduction (statistically significant 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 as the type of cloth used for cleaning and disinfection ranged from re-usable microfiber to disposable cotton cloths. Given the estimated wipe usage, the ward layout out and the number of surfaces on the 38-bed wards it would appear that a 'one-wipe-one-direction-one-surface' recommendation was not strictly adhered to. In spite of this, a significant reduction in total microbial counts was observed when the intervention product was used. The efficacy of the pre-impregnated 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 pre-formulated wipe with an oxidizing chemistry against the use of a quaternary ammonium compound-based wipe in significantly reducing surface contamination.[23] It has been suggested that aerobic colony count on hand touch surfaces should not exceed 5 CFU/cm^2, although a "clean' cut-off point of <2.5 CFU/cm^2 has been proposed.[28-30] With this in mind, a number of surfaces in the baseline period would not be considered as "clean". The intervention resulted in all surfaces passing a <2.5 CFU/cm^2. Boyce and Havill reported that the use of a new

hydrogen peroxide wipe lead to 99% of surfaces treated with <2.5 cfu/cm^2 following surface cleaning.[20] In our study, it is encouraging that the use of pre-impregnated 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* spore (genotype RT001) were recovered overall, while all the clinical *C. difficile* were of the RT027 and RT020 ribotypes at the time of study. Introducing sporicidal wipes to control *C. difficile* outbreaks has been reported in one study, in which replacing the use of hypochlorite with a pre-impregnated 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 pre-impregnated wipes. The use of hypochlorite-formulated wipes has however been showed to contribute significantly to the decrease of *C. difficile* infection,[22] although pre-formulated hypochlorite wipe can potentially transferred microorganisms between surfaces.[18] These studies and ours highlight that pre-formulated 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 usage auditing are essential.[2,31] Although staff awareness of the trial might have contributed to the observed improved performance,[23,32] the introduction of specific training has undoubtedly had an impact. Here, training saw an average 17% reduction in the mean total aerobic count in both wards, although the introduction of wipe saw a 34 and 40% reduction in the mean total aerobic count in wards 1 and 2 respectively. Measurement of RLU using an ATP sampler to indicate surface

cleanliness in healthcare setting is not new.[28,33,34] In our study, a Spearman's rank correlation test identified total aerobic count (cfu/cm^2 $\log_{10}$+1) and ATP (RLU) to be highly correlated (p-value < 0.0000). By log transforming the ATP data, a more even distribution was achieved although the data was still not normally distributed. Our results however support the data presented by Boyce and Havill who observed a good correlation between total aerobic count and ATP measurement.[20]

The cost effectiveness in using formulated wipe products need to be justified. The use of formulated wipes offers many advantages compare to the practice of using hypochlorite solution in a bucket. These include i) a better control of microbial bioburden, ii) ease of use, avoiding the use of highly concentrated biocidal solutions to be diluted down, iii) increasing efficacy by optimizing the combination between the disinfectant solution and the wipe material, iv) compatibility with, and decreasing damages to, the surfaces to be wiped, v) decreasing time required to disinfect the patient room/ward, vi) avoiding contaminating the disinfecting solution / product following repeated use and vii) the provision of clear instructions on label, including support instructions/posters, training packages by the manufacturer. Of these advantages, eliminating the risk of human error during product preparation / dilution is attractive since a decrease in biocidal product concentration can affect bacterial survival, resistance and cross-resistance to antimicrobials.[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 impact of other hygiene measures, such as hand washing. The diversities of patients and patient length of stay on the two wards, the inability to measure the antimicrobials used on the wards on the daily/weekly basis (there was no significant difference ($p>0.05$) in the monthly antimicrobial stock data between phases and/or wards for both systemic and topical antimicrobials), 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 pre-impregnated wipes.

In conclusion, this crossover trial demonstrated that the use of a pre-impregnated wipe product provided a 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.

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The authors have no conflict of interest to declare.

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Figure 1 Schematic of double crossover field study. ■ baseline date: use of standard cleaning regimen; ■ Use of detergent and chlorine 1,000pm; ■ Use of pre-impregnated sporicidal wipes; ■ General training on disinfectant usage, wiping and infection prevention; ■ Specific training on the use of pre-formulated wipes; ■ wards closure.

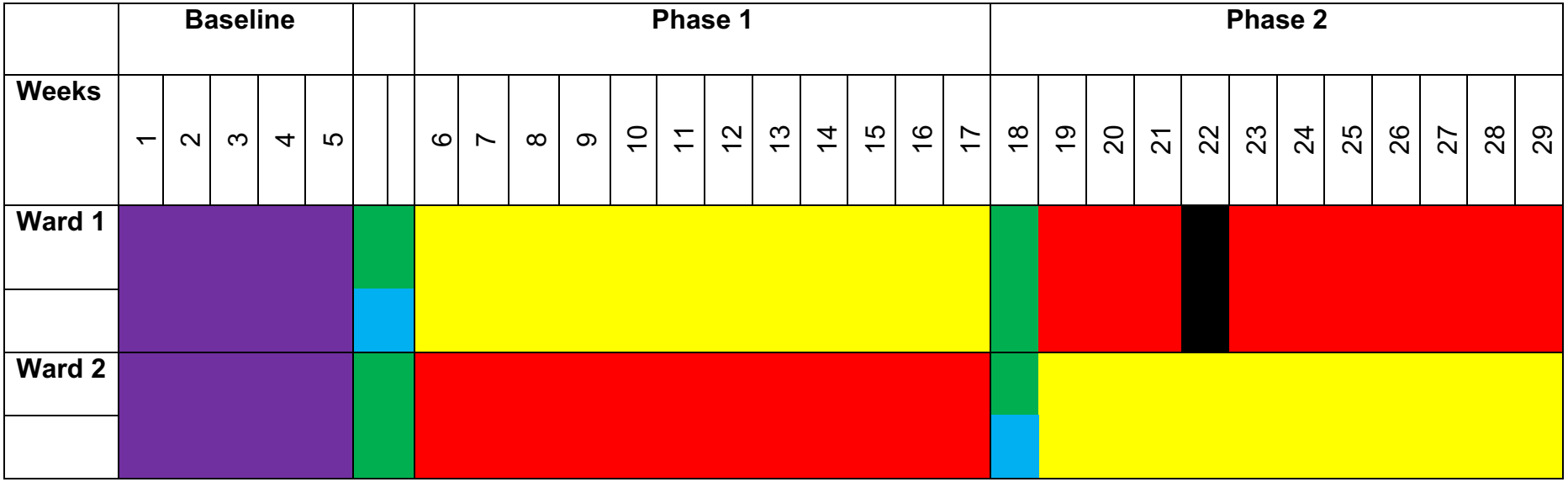
Figure 2 Overall total aerobic and anaerobic counts (Log_{10} +1/cm^2) and ATP count (RLU Log_{10} +1/cm^2). ■ Baseline; ■ Intervention with sporicidal wipe and ■ Cleaning and use of chlorine 1,000 ppm. A) ward 1; B) ward 2

Figure 3 Total counts (Log_{10}/cm^2) per individual sites for ward 1. ■ Baseline; ■ Intervention with sporicidal wipe (phase 1) and ■ Cleaning and use of chlorine 1,000 ppm (phase 2). A) Total aerobic count; B) total anaerobic count.

Figure 4 Total counts (Log_{10}/cm^2) per individual sites for ward 2. ■ Baseline; ■ Intervention with sporicidal wipe (phase 2) and ■ Cleaning and use of chlorine 1,000 ppm (phase 1). A) Total aerobic count; B) total anaerobic count.

Figure 5 Number of sites positive for MDRO. ■ ESBL; ■ CRE and ■ VRE. A) ward 1 B) ward 2.

Fig. 1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Ward 2

Fig. 2 A)

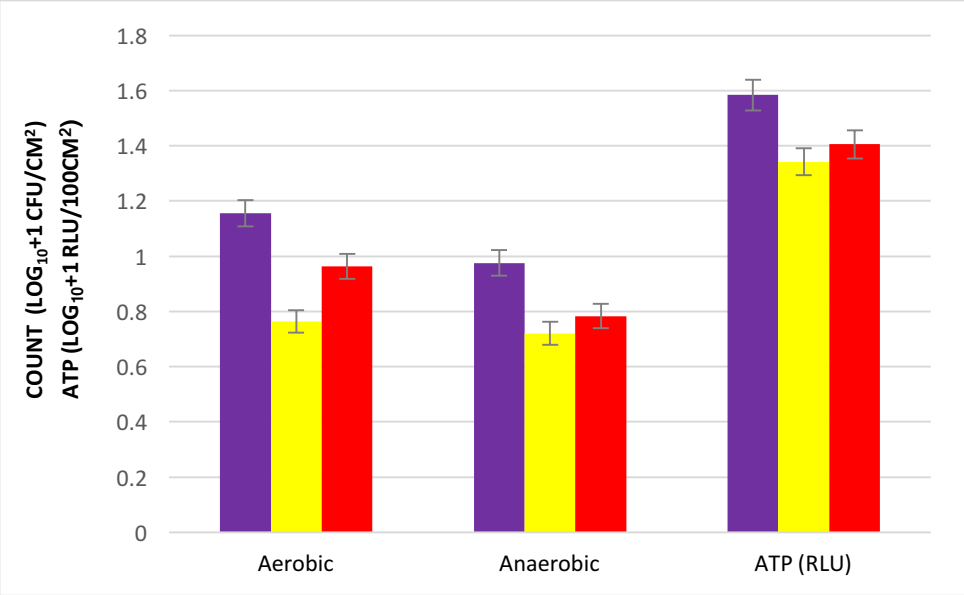

1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
COUNT (LOG10+1 CFU/CM2)
ATP (LOG10+1 RLU/100CM2)
Aerobic
Anaerobic
ATP (RLU)

Fig. 2 B)

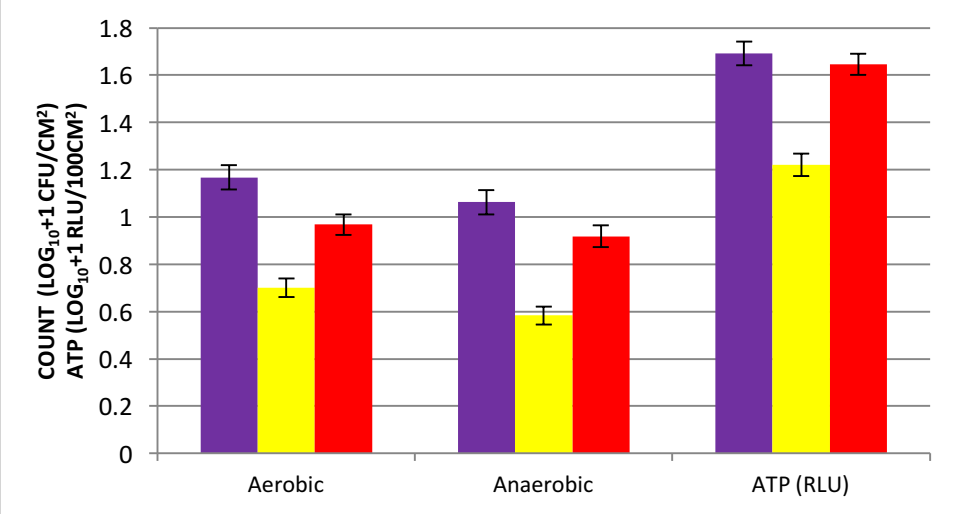

1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
COUNT (LOG10+1 CFU/CM2)
ATP (LOG10+1 RLU/100CM2)
Aerobic
Anaerobic
ATP (RLU)

Fig. 3 A)

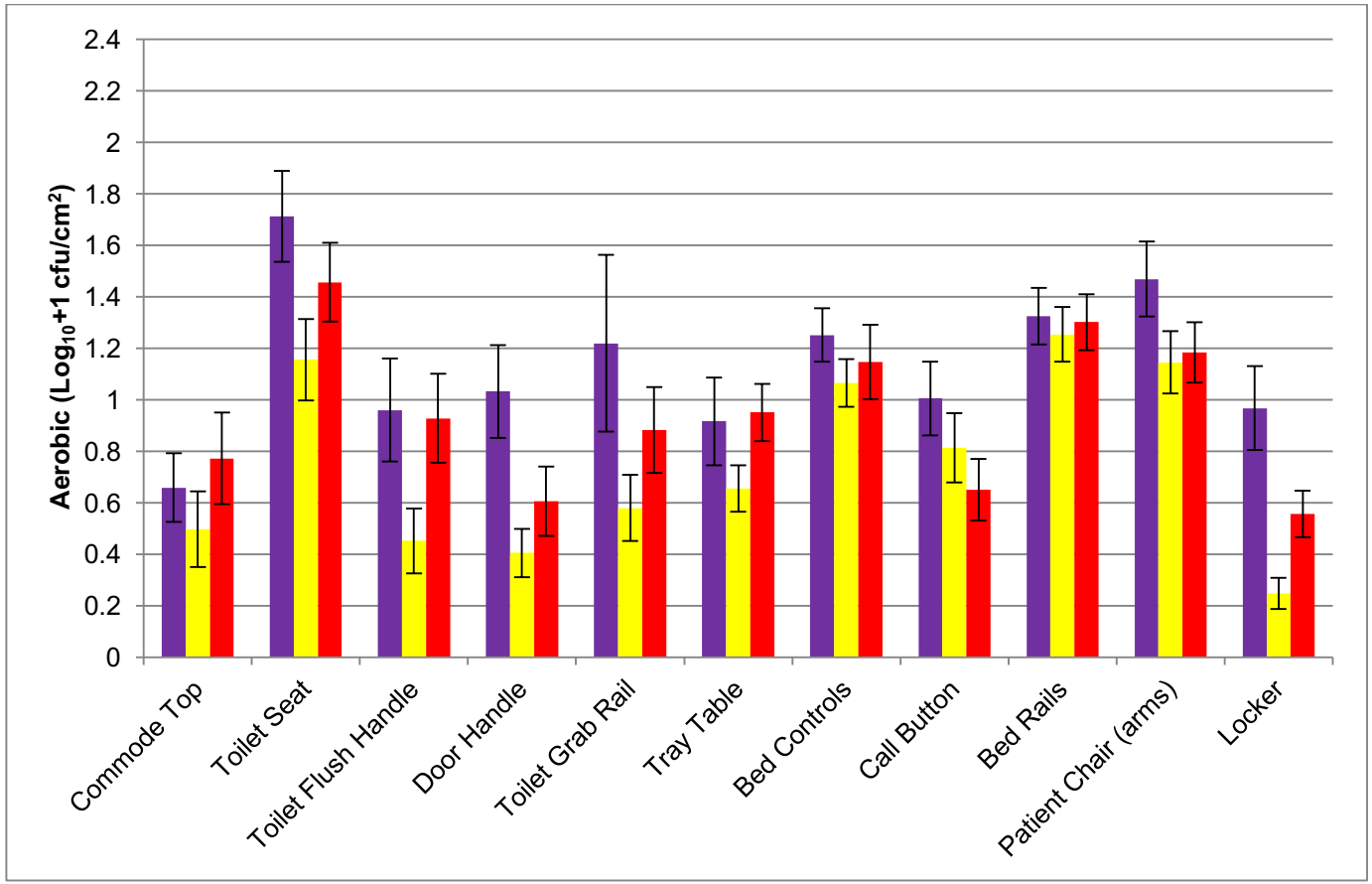

Aerobic (Log10+1 cfu/cm2)
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Commode Top
Toilet Seat
Toilet Flush Handle
Door Handle
Toilet Grab Rail
Tray Table
Bed Controls
Call Button
Bed Rails
Patient Chair (arms)
Locker

Fig. 3 B)

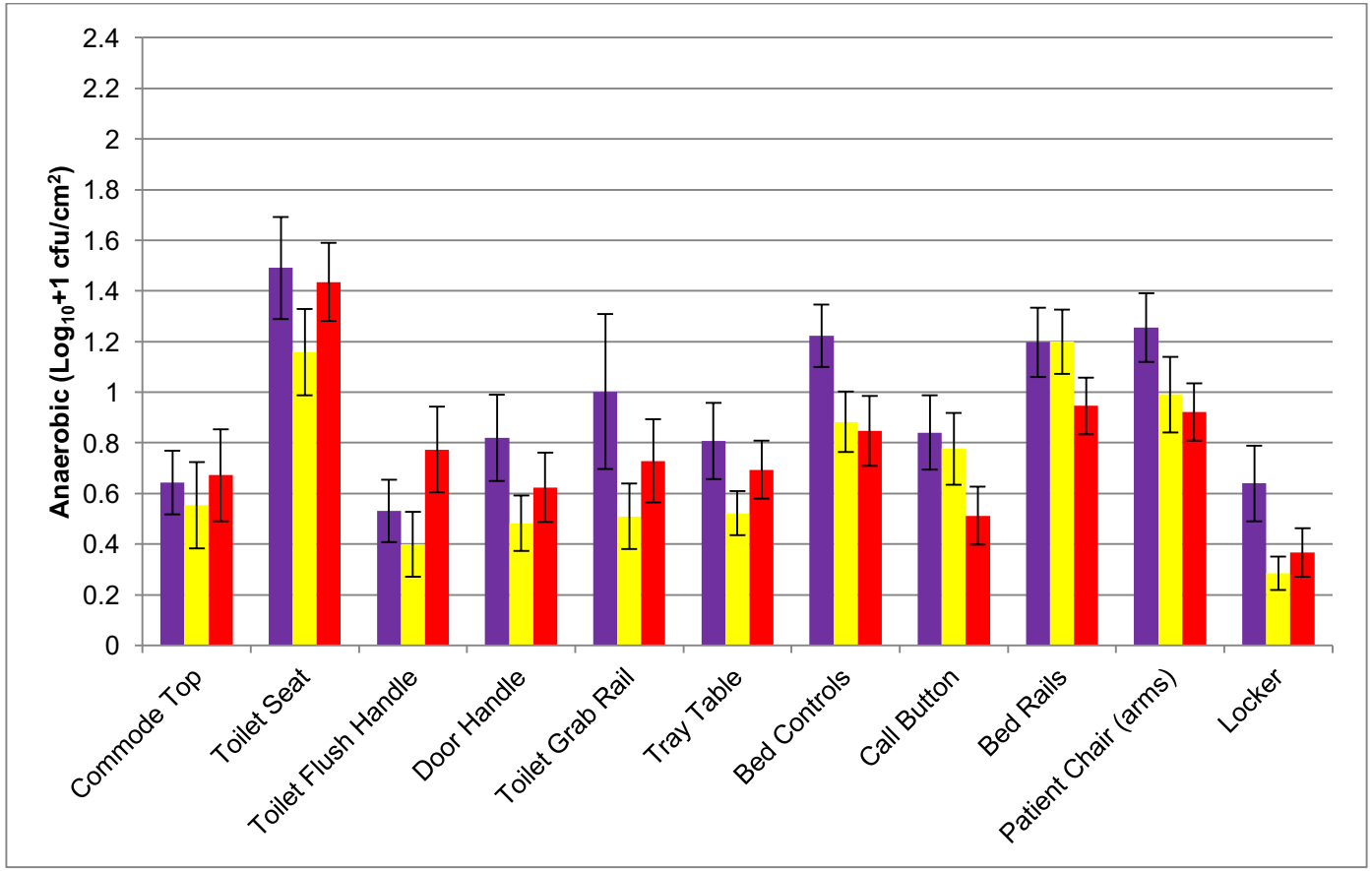

Anaerobic (Log10+1 cfu/cm2)
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Commode Top
Toilet Seat
Toilet Flush Handle
Door Handle
Toilet Grab Rail
Tray Table
Bed Controls
Call Button
Bed Rails
Patient Chair (arms)
Locker

Fig. 4 A)

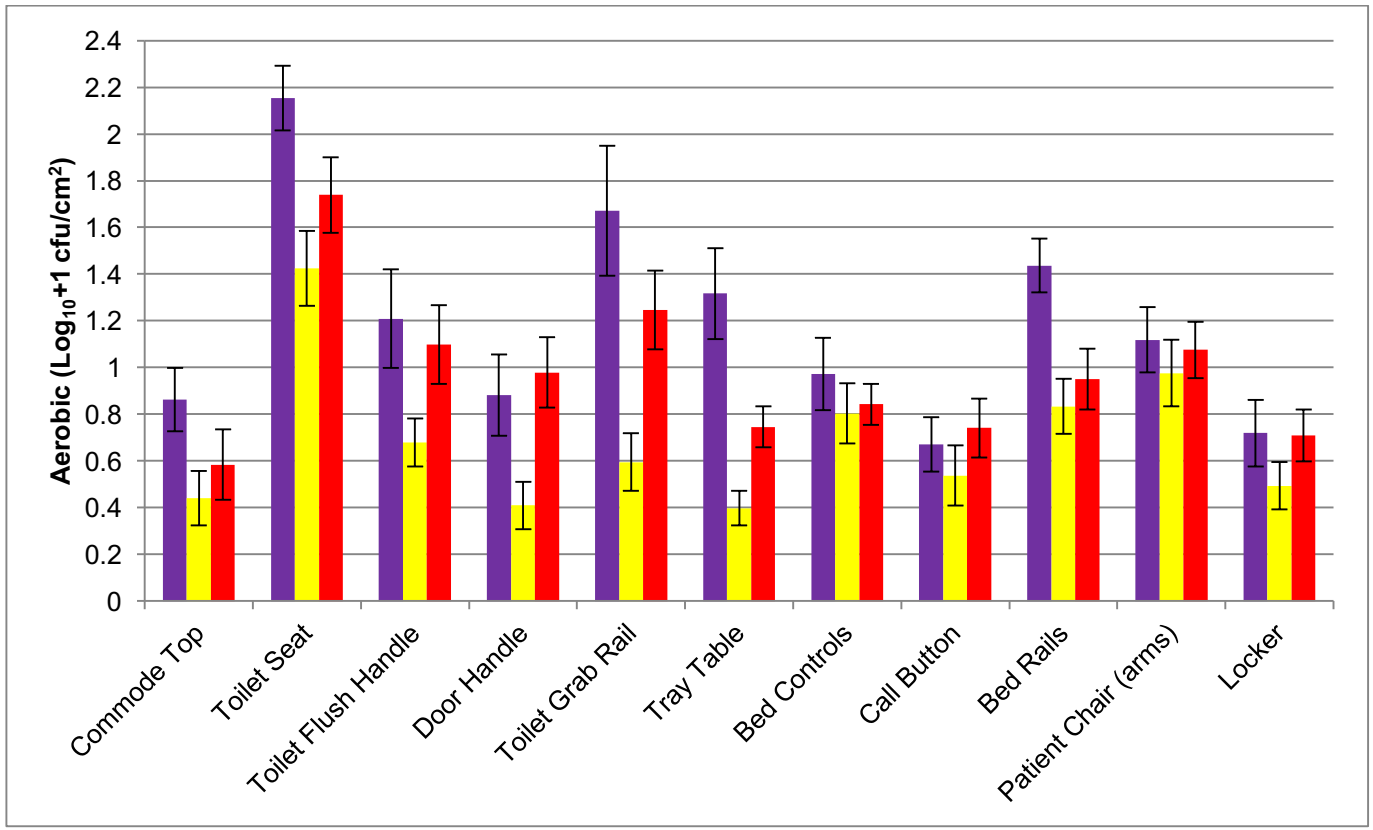

Aerobic (Log10+1 cfu/cm2)
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Commode Top
Toilet Seat
Toilet Flush Handle
Door Handle
Toilet Grab Rail
Tray Table
Bed Controls
Call Button
Bed Rails
Patient Chair (arms)
Locker

Fig. 4 B)

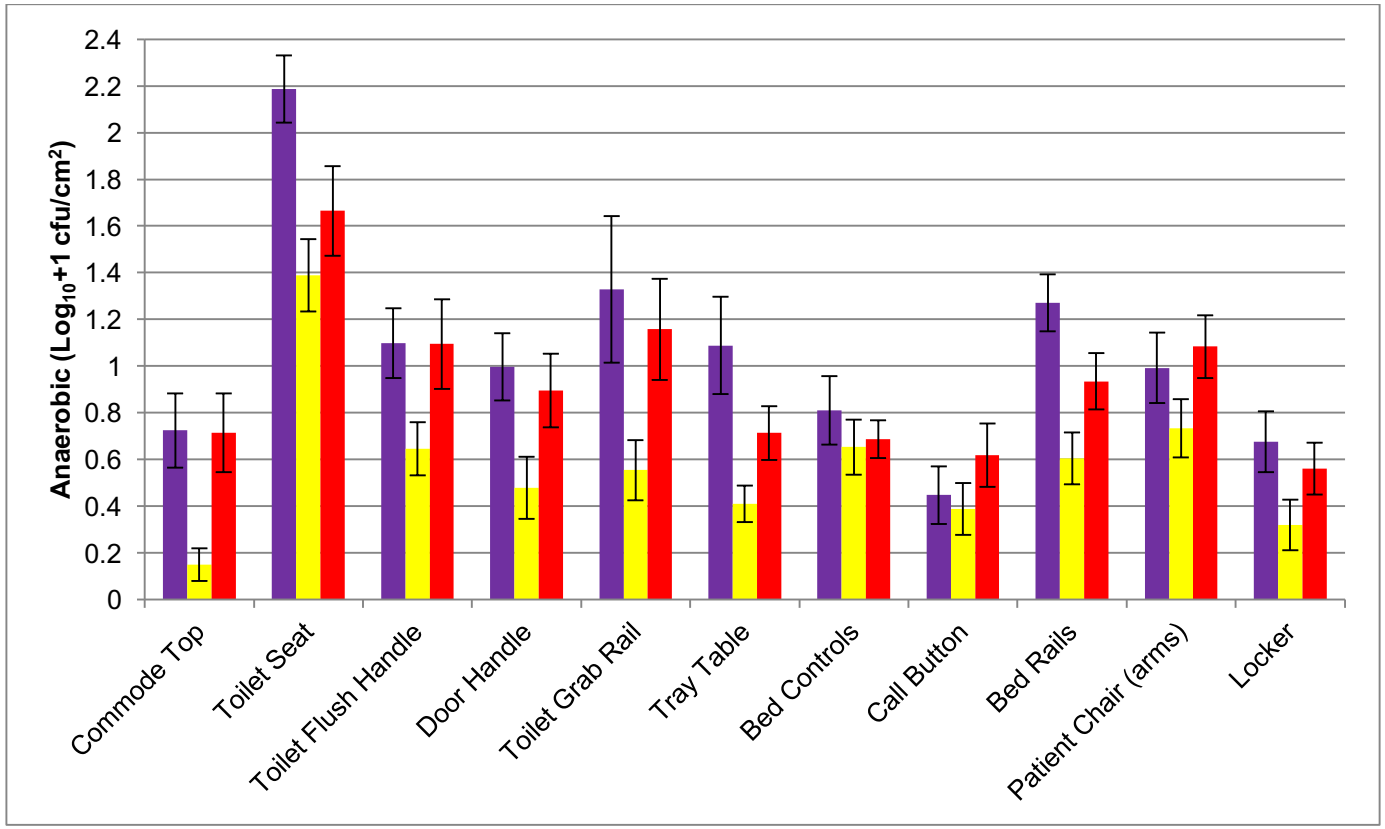

Anaerobic (Log10+1 cfu/cm2)
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Commode Top
Toilet Seat
Toilet Flush Handle
Door Handle
Toilet Grab Rail
Tray Table
Bed Controls
Call Button
Bed Rails
Patient Chair (arms)
Locker

Fig. 5 A)

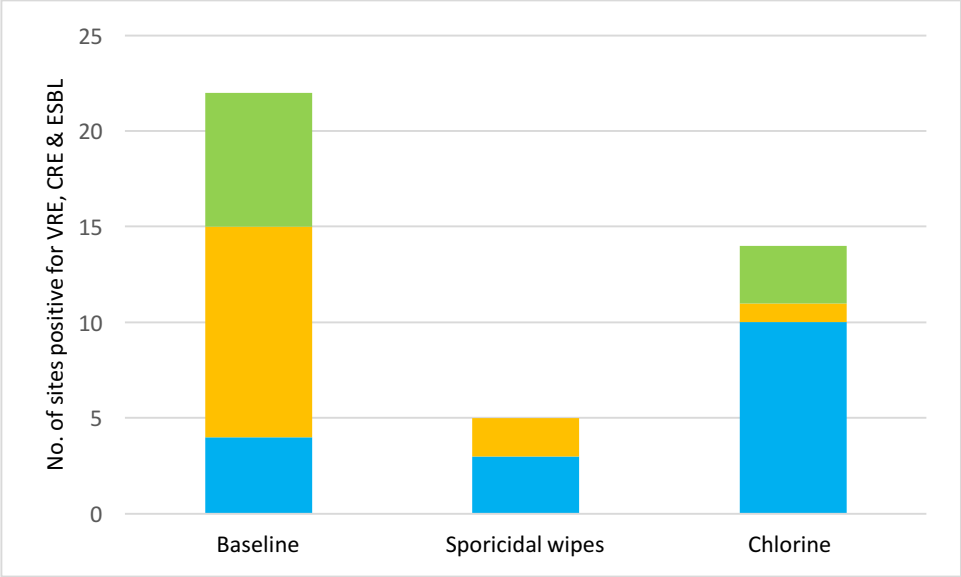
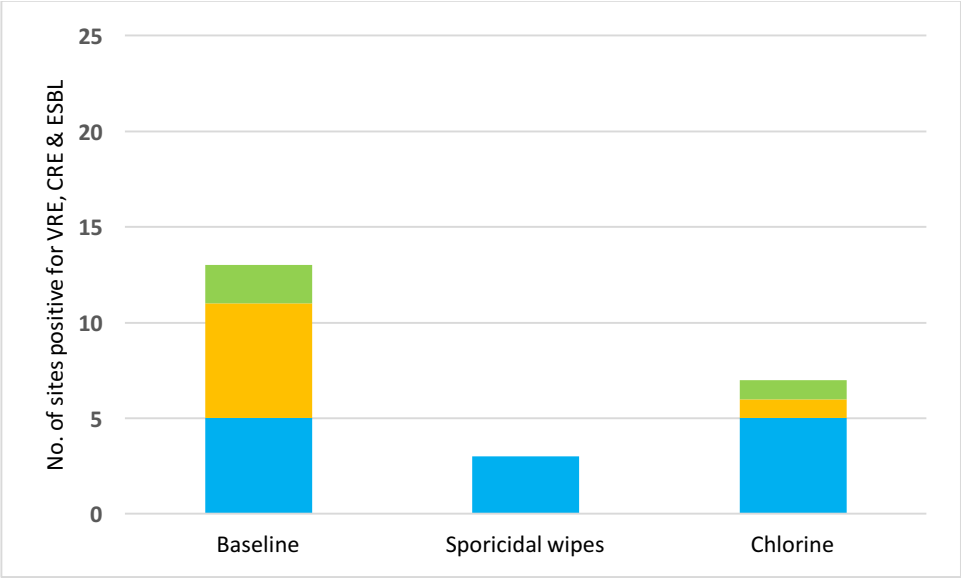

25
20
15
10
5
0
No. of sites positive for VRE, CRE & ESBL
Baseline
Sporicidal wipes
Chlorine

Fig. 5 B)


25
20
15
10
5
0
No. of sites positive for VRE, CRE & ESBL
Baseline
Sporicidal wipes
Chlorine