Pathogen Transfer and High Variability in Pathogen Removal by Detergent Wipes

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ABSTRACT

The rise in healthcare associated infections has placed a greater emphasis on cleaning and disinfection practices. The majority of policies advocate using detergent based products for routine cleaning, with detergent wipes increasingly being utilized; there is no information about their ability to remove and subsequently transfer pathogens in practice.

Seven detergent wipes were tested for their ability to remove and transfer S*. aureus, A. baumannii* and *C. difficile* spores using the 3-stage wipe protocol. The ability of the detergent wipes to remove S*. aureus, A. baumannii* and *C. difficile* spores from a stainless steel surface ranged from 1.50 $\log_{10}$ (range, 0.24-3.25), 3.51 $\log_{10}$ (range, 3.01-3.81) and 0.96 $\log_{10}$ (range, 0.26-1.44) respectively following a 10 s wiping time. All wipes repeatedly transferred significant amount of bacteria/spores over three consecutive surfaces, even though the percentage of total microorganisms transferred from the wipes after wiping was low for a number of products. Detergent based wipe products have two major drawbacks: their variability in removing microbial bioburden from inanimate surfaces and their propensity to transfer pathogens between surfaces. The use of additional complimentary measures such as combined detergent-disinfectant based product and/or antimicrobial surfaces need to be considered for appropriate infection control and prevention.

Keywords: surface cleaning, disinfection, detergent wipes, *Clostridium difficile, Acinetobacter baumannii, Staphylococcus aureus*

INTRODUCTION

A detergent is a group of chemical compounds (synthetic or organic) which are liquid or water soluble. Unlike soaps, detergents are not prepared from animal and vegetable fats and oils and are not inactivated by hard water. The major components in cleaning products are surfactants (surface-active agents); detergent surfactants are now commonly made from petrochemicals and/or oligochemicals. Surfactants can be classified into four groups depending on the polar head group; anionics, cationics, non-ionics and zwitterionics.[1] The majority of cleaning products will be formulated to contain one or more surfactants in combination with additional compounds, such as preservatives, enzymes and perfume.

The majority of current UK infection control policies advocate the use of detergent and water or microfiber and water for cleaning of soiled/contaminated surfaces.[2] Detergent wipes are increasingly being utilised, serving as a convenient, ready-to-use disposable product for environmental cleaning. The ability of microorganisms such as methicillin-resistant *Staphylococcus aureus*, vancomycin resistant *Enterococci* and *Clostridium difficile* to persist on inanimate surfaces for prolonged periods is well recognized,[3,4] with common healthcare associated pathogens frequently isolated from surfaces in close proximity to the patient (high touch points). There is a growing body of evidence demonstrating

the importance of environmental contamination in the transmission of clinically relevant pathogens.[5,6] Although multiple studies have investigated the efficacy of microfiber cloths[7,8 and 9] and antimicrobial wipes,[10,11,12,13,14,15 and 16] to the best of our knowledge no study has yet investigated the efficacy of detergent wipes. Although it has been suggested that a 'one wipe, one surface, one direction' approach be implemented, in practice a wipe (detergent or disinfectant based) is likely to be used on multiple surfaces. The purpose of any cleaning wipe is to firstly ensure the efficient removal of microorganisms from a surface and secondly to ensure the microorganisms are retained on the wipe, thus preventing the transfer of pathogenic microorganisms. The aim of this study was to test using a modified 3-stage protocol[13] the efficacy of a number of commercially available detergent wipes to remove S*. aureus, A. baumannii* and *C. difficile* spores from surfaces and prevent their transfer between surfaces.

MATERIALS AND METHODS

Detergent Wipes

Seven detergent wipes currently used in healthcare facilities in the UK were obtained from different manufacturers. Details of wipe ingredients and manufacturers are summarized in Table 1.

Bacterial strains

The following organisms were used in this study: S. *aureus* NCIMB 9518 (PHE, UK)*, A. baumannii* NCTC 10788 (NCIMB Ltd, UK) and *C. difficile* NCTC 11209 (PHE, UK). *S. aureus* and *A. baumannii* were grown overnight in Tryptone Soya Broth (Oxoid, UK), centrifuged at 5,000 *g* for 20 min at 4°C and the pellet resuspended in phosphate buffered saline (PBS)+0.1% Tween-80 (PBST) (Fisher Scientific) before use. For the preparation of the *C. difficile* spores, the method by Perez *et al*.,[17] was followed with the following modifications; multiple colonies of *C. difficile* 11209 were inoculated into 20 mL of reduced Brain Heart Infusion (BHI) broth (Oxoid, UK) and cultured overnight at 37°C under anaerobic conditions (5% H_2: 10% CO_2: 85% N_2) in a Whitley MG500 workstation (DW Scientific, UK). The overnight culture was gently vortexed and 1% was added to 500 mL of reduced Clospore and incubated for 7 days. The spore preparation was centrifuged at 10,000 *g* for 20 min at 4°C. Spores were purified as described by Perez *et al*.,[17] assessed by phase contrast microscopy and heat shock at 60°C for 20 min. Spores were enumerated by diluting in PBST and plated onto Brain Heart Infusion (BHI) agar supplemented with 0.1% (w/v) sodium taurocholate (BHIS) (Fisher Scientific). Purified spores were stored at 4°C until use.

Bactericidal and Sporicidal Activity

Bactericidal and sporicidal activity was determined using a protocol based on the European standard method for chemical disinfectants EN 13727.[18] All testing

was conducted on fluid expressed from wipes; a single wipe was placed in a sterile 20 mL syringe; solution from the wipe was collected by applying pressure for 30-60 seconds. The process was repeated until sufficient fluid had been collected and used within 5 minutes. For bactericidal activity, the test organism was cultured in 10 mL of TSB, after 24 h of incubation at 37°C the cell suspension was centrifuged and re-suspended in PBST and combined with bovine serum albumin so that the organic load in the test was 3 g/L ('dirty conditions'). The average number of cells/spores in the test was 7.91 ± 0.12 Log_{10}, 8.14 ± 0.20 Log_{10} and 5.43 ± 0.54 Log_{10} CFU/mL, for S*. aureus*, *A. baumannii* and *C. difficile*, respectively. The test suspension was held at 20°C for 1 min and enumerated. To conduct the test 0.1 mL of bacterial or spore inoculum was added to 0.9 mL wipe solution. After a contact time of 1 min 0.1 mL of the test solution was transferred to 0.9 mL of a neutralizing solution consisting of saponin (Sigma) 30 g/L, polysorbate 80 (Sigma) 30 g/L, azolectin from soybean (Sigma) 3 g/L, L-Histidine (Sigma) 1 g/L and sodium dodecyl sulphate (Sigma) 5 g/L, 5 g/L sodium thiosulphate prepared in de-ionised water. Neutraliser toxicity and neutraliser efficacy were determined in suspension using the protocol described by Knapp *et al*.[19]

Efficacy test protocol – 3-stages protocol

The 3-stage protocol described in Williams *et al.*[13] was adapted, utilizing the 'Wiperator®' system (http://www.filtaflex.ca/wiperator.htm; accessed 9 January 2014). Wipes were cut aseptically in squares of 2 x 2 cm for testing.

Measurement-1 - efficacy of wipes to remove microorganisms from surfaces: microorganisms (10 µL) were inoculated onto clean magnetized, brush stainless sterile steel discs (AISI Type 430 (European equivalent X6Cr17 and number 1.4016); Group 2; No. 4 finish (EN 10088-2 1J/2J)) and dried for 30 min at 37°C. A detergent wipe was attached to a plastic boss to allow an elliptical mechanical rotation for 10 s exerting a weight of 150 g. Steel discs were transferred into bottles containing neutralizer (1 mL) and glass beads (1 g; 3 mm diameter; Sigma). After horizontal shaking (150 rpm for 1 min) and neutralization for 5 min, the suspension was serially diluted and used to inoculate appropriate agar. *S. aureus* and *A. baumannii* were counted after 24 h incubation at 37°C and *C. difficile* after 48 h anaerobic incubation. The $\log_{10}$ cell removal from the disk surfaces was calculated by subtracting the mean $\log_{10}$ number of cells recovered from the disc after using the wipes from the number of cells recovered from the dry control.

Measurement-2 - bacterial transfer from wipes: Following the application of wipes to the contaminated surfaces as described above, the subsequent transfer of contamination onto three consecutive stainless steel discs was measured together with the effect of the mechanical action (10 s wipe, 150 g pressure). Steel discs were placed in neutraliser and bacterial colonies enumerated.

Dry control: Prior to the use of wipes, cell deposited and dried on the surface of the disk were recovered into bottles containing neutralizer and glass beads as described above. After horizontal shaking (150 rpm for 1 min) for 5 min, the suspension was serially diluted and used to inoculate appropriate agar.

Biological Replicates and Statistical Analysis

All data presented in this manuscript represent the results of three independent experiments. Data were checked visually for normality and homogeneity of variance using a histogram, Q-Q plots and fitted values. A one-way ANOVA at the 95% confidence interval with a post hoc Tukey's test was performed or a paired-sample t-test. All analyses were completed in SPSS Statistics 20.

RESULTS

In this study, S*. aureus*, *A. baumannii* and *C. difficile* spores were used to firstly assess the microbicidal activity of seven detergent wipes and secondly the ability of the wipes to remove and transfer microorganisms onto three consecutive surfaces. Prior to use a modified EN13727 suspension test, the neutralizer toxicity and neutralizer efficacy to quench the active contained in the wipe were assessed. The neutralizer did not display any toxicity and was found to be efficacious in quenching the activity of the wipe with <1 $\log_{10}$ reduction reported for all organisms tested (data not shown). Unsurprisingly expressed solution

177 from the seven wipes tested displayed no bactericidal or sporicidal activity (data
178 not shown).
179 In order to test the impact of drying on the organisms tested, a paired-samples t-
180 test was conducted. No statistically significant difference was found between the
181 viable counts pre and post drying for *S. aureus* ($p = 0.418$, two-tailed) and *C.*
182 *difficile* ($p = 0.419$, two-tailed). A statistically significant decrease was found for
183 *A. baumannii* pre (7.13 ± 0.40 $\log_{10}$) and post (6.00 ± 0.33 $\log_{10}$) drying, with the
184 eta squared statistic (0.91) indicating a large effect size. For this reason all
185 calculations for removal utilized the dry control values. Initial analysis by means
186 of a two-way ANOVA between groups assessed the impact of wipes and bacteria
187 on removal. The interaction effect between wipes and bacteria was found to be
188 significant ($F(12, 42) = 10.34$, $p < 0.001$), thus all subsequent analysis was
189 undertaken with a one-way analysis of variance. The detergent wipes tested in
190 this study showed marked differences in their ability to remove microbial
191 bioburden from surfaces following a 10 second wipe, as shown in Figure 1. The
192 average removal of *S. aureus* from a stainless steel surface by the wipes tested
193 was 1.45 $\log_{10}$ (range: 0.24-3.25). Wipe D removed significantly more (ANOVA,
194 *post hoc* Tukey's test, $p < 0.05$) *S. aureus* from the stainless steel disk than the
195 other wipes. All the wipes repeatedly transferred large number of *S. aureus* onto
196 three consecutive surfaces except wipe G for which transfer of bacteria was
197 below the limit of detection for this test (<17 CFU; recorded as 0.00% transfer;
198 Table 2). The average removal of *A. baumannii* by the wipes tested was 3.51

199 log_{10} (range: 3.01-3.81). No statistically significant difference was observed in
200 the efficacy of the wipes to remove *A. baumannii* from a stainless steel surface
201 (Fig. 1). The wipes tested were particularly poor at preventing the transfer of *S.*
202 *aureus* but much better in preventing the transfer of *A. baumannii* with the
203 exception of wipe C, which performed poorly with both bacteria. Of the three
204 microorganisms tested, the wipes removed the least number of spores from the
205 surface (0.96 log_{10}, range: 0.26-1.44). Wipes A, D, E, and G removed
206 significantly more spores than Wipes B and C (ANOVA, *post hoc* Tukey's test, p
207 < 0.05). As with the vegetative bacteria, all wipes tested failed to retain the
208 spores. Between 117 and 34377 spores were transferred onto surfaces
209 (corresponding to 1.29% transfer, wipe G and 114.95% transfer, wipe C; Table
210 2). Wipes A and C performed particularly poorly and wipe G performed better
211 than the others. The percentage of bacteria (CFU) transferred was estimated
212 based on the assumption that the difference in the number of CFU on the
213 stainless steel disk before and after wiping ended up into the wipe (Table 2). On
214 three occasions the percentage exceeded 100%, which would indicate that the
215 number of CFU picked up by the wipes were underestimated. The percentages of
216 bacteria/spores transferred onto 3 surfaces were at times very low, particularly
217 with *A. baumannii*, indicating that this microorganism is retained better regardless
218 of the wipe material and formulation (Table 2). It can also be noted that the
219 percentage of *C. difficile* spores transferred is high despite the calculated low
220 spore number on the wipes.

DISCUSSION

The lack of microbicidal activity demonstrated by the wipes was unsurprising given the wipes composition (Table 1). The lack of activity needed to be evaluated to ensure that the propensity of the wipes to remove and/or transfer microbial bioburden from surfaces was not affected by any intrinsic wipe microbicidal activity. The Gram-positive *S. aureus* and spores of *C. difficile* were not affected by drying, however the Gram-negative *A. baumannii* was. These results support findings of other studies, which have demonstrated Gram-positive organisms are more tolerant of desiccation than Gram-negative organisms.[20,21,22 and 23] It is important to take into consideration the impact a dry inoculum can have when assessing the efficacy of a product, it would be misleading to associate a mean difference of 1.4 $\log_{10}$ between pre and post drying of *A. baumannii* to the product being tested. In order to overcome such issues a higher stating inoculum can be used, the inoculum can be combined with proteins in order to stabilize the organism[20,21 and 22] or the impact of drying can be stated and taken into consideration during analysis.

The efficacy of the detergent wipes to remove microbes from a surface varied considerably; for example Wipe A removed the greatest amount of *A. baumannii* 3.81 $\log_{10}$, 1.23 $\log_{10}$ *C. difficile* but only 0.25 $\log_{10}$ *S. aureus*, demonstrating the ability of the wipe to remove bioburden from a stainless steel surface is dependent on the microorganism tested. This interaction effect has also been

observed when assessing the efficacy of microfiber cloths.[24] In the aforementioned study methicillin-resistant *Staphylococcus aureus* (MRSA) was consistently more difficult to remove than *C. difficile* spores and *E. coli*; these findings are somewhat akin to our findings in that the Gram-negative organism (*A. baumannii*) was consistently removed by all detergent wipes tested, whereas *C. difficile* spores and *S. aureus* (with the exception of Wipe D) proved to be more difficult to remove. Although it should be noted that in the study by Smith *et al.,*[24] a wet inoculum was utilized and although an automated cleaning rig was utilized the pressure employed in the study was not specified. In a study performed by Tuladhar *et al.,*[25] the $\log_{10}$ reduction of *S. aureus* was ~2.30 $\log_{10}$ with liquid soap applied to a viscose cleaning cloth, this is 1 $\log_{10}$ higher than the median value obtain in this study (1.45 $\log_{10}$). This difference may be due to the material tested, the strain used or the method of wiping the surface (hand *vs.* automated system). In a previous study comparing the efficacy of a detergent wipe to a disinfectant wipe using the 3-stage protocol, both wipes were found to remove on average ~1.72 (± 0.32) and 1.74 (± 0.96) *S. aureus* respectively, in dirty conditions.[14] Here, among the seven wipes tested an average of 1.45 (± 1.15) was observed. This suggests that disinfectant wipes may outperform detergent wipes in removing *S. aureus*, although the protocol used in most of these studies were different, which makes comparison difficult. The variability in results reflects the differences in the ability of the detergent wipes tested to remove this bacterium.

The wipes tested in this study are generally composed of non-ionic surfactants, preservatives and perfume, therefore they would be expected to perform on par with each other (Table 1). However, from the data presented above this is not the case, the performance of the detergent wipes may be influenced by the type of nonwoven, quality of the raw materials and non-woven, the liquid to wipe ratio and the packaging of the product.[26] Indeed the difference in performance between wipe B and wipe G might be explained by the use of viscose in wipe G. The other factors were not investigated in this study but the differences in efficacy of the wipes tested suggests there is scope for further development of these products, which are increasingly being utilized in the healthcare setting. Furthermore the formulation of the detergent and its compatibility with the non-woven may impact the efficacy of the wipe as seen with cotton towels and disinfectant based cleaners.[27]

Although all detergent wipes tested removed microbial bioburden from a stainless steel surface, they repeatedly transferred a large amount of bacteria/spores on three consecutive transfers. Only wipe G performed better than the others with the vegetative bacteria, where no transfer was detected. On the other hand wipe C caused the highest release of bacteria and spores. On three occasions the number of bacteria/spores transferred were higher than the calculated number of bacteria/spores on the wipe. It is possible that bacteria/spores are in the form of dense aggregates given the high concentration of the starting inoculum used in this study (~8 and 5 $\log_{10}$ for bacteria and spores, respectively) combined with

287 the desiccation process when the inoculum is deposited on the surfaces. Despite
288 using saponin, polysorbate 80 and sodium dodecyl sulfate in the neutralizer and
289 glass beads, the presence of aggregates cannot be ruled out. The presence of *C.*
290 *difficile* spores aggregates during wipe efficacy testing has been reported
291 previously.[10] It is conceivable that the surfactant-based formulation of the wipe
292 tested breaks up releases aggregates,[10] although it is interesting to note that the
293 Gram-negative *A. baumannii* was not concerned with these observations. These
294 results highlight the need to assess the efficacy of wipes to both remove and
295 transfer microbes. This is particularly pertinent with the release of *C. difficile*
296 spores, since the infectious dose was estimated to be as low as < 5
297 spores/cm^2.[28] Although the calculated spores number in the wipes was relatively
298 low (when compared to the vegetative bacteria) from 5,000 and 90,000 spores,
299 the lowest number of spores transferred was 117 (corresponding to 1.29%
300 transfer; wipe G, table 3). While this is not the first study to demonstrate the
301 transfer of microbes to clean surfaces by wipes,[10,11,13,14 and 16] it is the first
302 instance where the transfer of microorganisms onto multiple surfaces has been
303 quantified in this way and the percentage transfer estimated. The potential
304 repeated seeding of the healthcare environment by wipes is of concern and
305 raises questions as to how best to use wipes in practice; should a 'one wipe, one
306 surface, one direction' approach be universally and strictly implemented as
307 already recommended? Although infection control teams provide some guidance
308 on product use, surely a standard policy document is required. Currently the

closest guidance document available on wipes was issued by the Royal College of Nursing.[29] Manufacturers are also providing comprehensive guidance documents and training packages for their products, but could do more to educate the end users on the appropriate use of their products.[4] In view of the findings from our study, additional complimentary ways to decrease surface microburden should be explored including the use of combined detergent-disinfectant wipes and antimicrobial surfaces. [10,11,13,14 and 16] The later is showing promising results in significantly reducing microorganisms from environmental surfaces in healthcare settings.[30]

CONCLUSION

In conclusion the efficacy of commercially available detergent wipes to remove microbial bioburden from surfaces was found to be variable between products. The efficacy of the wipes to remove *A. baumannii* from surfaces was appropriate, but far to be satisfactory with *S. aureus* and spores of *C. difficile*. Worryingly all of the wipes repeatedly transferred bacteria and spores onto multiple surfaces. Given that detergent cleaning is advocated in many national guidance documents it is imperative that such recommendations and guidance take into account the wipe limitations found in this study. The issue of potential transfer onto multiple surfaces needs to be addressed to avoid the potential spread of microbial pathogens.

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432 rate of healthcare-acquired infections in the intensive care unit. Infect
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435

436 **Table 1.** Detergent wipes' ingredients

Wipe	Composition[a]	Product	Manufacturer
Wipe A	Amongst other ingredients; <5% non-ionic surfactants, parfum, DMDM hydantoin, iodopropynyl butylcarbamate.	Azodet™ Detergent Wipe	Synergyhealth, Derby, UK
Wipe B	<5% non-ionic surfactants and preservatives (old formulation). [b]	Clinell® Detergent Wipe	GAMA Healthcare, London, UK
Wipe C	Dimethyl oxazolidine, parfum.	Sani Cloth Detergent Wipe	PDI Europe, Flint, UK
Wipe D	<5% non-ionic surfactant, DMDM hydantoin, iodopropynyl butylcarbamate.	Aquamed MA Detergent Wipe	Marshal Curtis, Didcot, UK
Wipe E	<5% non-ionic surfactant, DMDM hydantoin, iodopropynyl butylcarbamate.	Clinitex® Detergent Wipe	Techtex®, Manchester, UK
Wipe F	Amongst other ingredients; Parfum, DMDM hydantoin, iodopropynyl butylcarbamate.	Tuffie Detergent Wipe	Vernacare, Bolton, UK
Wipe G	<5% non-ionic surfactants and preservatives (new formulation). [b]	Clinell® Detergent Wipe	GAMA Healthcare, London, UK

437 [a] Composition noted from packaging

438 [b] Difference between wipe B and G is the material used (viscose) wipe G

Table 2: CFU and % transfer in *S. aureus, A. baumannii* and *C. difficile* onto three consecutive surfaces. Mean values from 3 biological repeats.

Wipes	CFU/spores on wipes*	Transfer 1st surface	Transfer 2nd surface	Transfer 3rd surface	Total % transferred
		% microbial/spore transfer			
S. aureus					
A	66890	66.43	82.28	64.74	213.45
B	3633282	11.01	9.75	13.14	33.90
C	5078282	8.58	66.05	44.83	119.46
D	4941786	0.04	0.03	0.04	0.11
E	14537759	0.43	0.39	0.37	1.20
F	13388894	0.09	0.07	0.21	0.37
G	16705056	0.00	0.00	0.00	0.00
A. baumannii					
A	13388894	0.02	0.01	0.01	0.04
B	1505426	0.02	0.01	0.02	0.05
C	3442779	8.00	0.03	0.02	8.05
D	1505426	0.01	0.01	0.01	0.03
E	507976	0.03	0.02	0.03	0.08
F	507804	0.02	0.02	0.02	0.06
G	777048	0.00	0.00	0.00	0.00
C. difficile					
A	92684	2.88	13.10	11.68	27.66
B	24111	2.89	7.18	2.69	12.76
C	29907	114.95	71.78	36.52	223.25
D	25275	8.16	20.88	1.76	30.80
E	5928	5.34	3.09	2.53	10.96
F	5360	16.61	20.42	31.10	68.13
G	9070	5.33	6.43	1.29	13.05

* Average number of bacteria/spore on the wipe following wiping – calculated from the difference between bacteria left on surface before and after wiping.

Figure 1: Mean log_{10} bacterial removal from disks using the 3-step method examining the efficacy of detergent wipes against *S. aureus* (■), *A. baumannii* (■) and *C. difficile* (spores) (■). Data is a mean of 3 biological repeats, bars represent SD of replicates.