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Pathogen Transfer and High Variability in Pathogen Removal by Detergent 1 2 **Wipes** 3 Ramm Lauren, MPharm, Harsha Siani, MPhil, Rebecca Wesgate, BSc and Prof. 4 Jean-Yves Maillard, PhD 5 School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, 6 United Kingdom 7 8 Address for correspondence 9 Jean-Yves Maillard School of Pharmacy and Pharmaceutical Sciences, 10 11 Cardiff University, 12 King Edward VII Avenue Cardiff CF10 3NB 13 14 United Kingdom 15 Tel: 44(0)2920879088 16 Email: maillardj@cardiff.ac.uk 17 18 Running title: Efficacy of detergent wipes 19 20 21 22

ABSTRACT

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24 The rise in healthcare associated infections has placed a greater emphasis on 25 cleaning and disinfection practices. The majority of policies advocate using 26 detergent based products for routine cleaning, with detergent wipes increasingly 27 being utilized; there is no information about their ability to remove and 28 subsequently transfer pathogens in practice. 29 Seven detergent wipes were tested for their ability to remove and transfer S. 30 aureus, A. baumannii and C. difficile spores using the 3-stage wipe protocol. 31 The ability of the detergent wipes to remove S. aureus, A. baumannii and C. 32 difficile spores from a stainless steel surface ranged from 1.50 log₁₀ (range, 33 0.24-3.25), 3.51 log₁₀ (range, 3.01-3.81) and 0.96 log₁₀ (range, 0.26-1.44) 34 respectively following a 10 s wiping time. All wipes repeatedly transferred 35 significant amount of bacteria/spores over three consecutive surfaces, even 36 though the percentage of total microorganisms transferred from the wipes after 37 wiping was low for a number of products. Detergent based wipe products have 38 two major drawbacks: their variability in removing microbial bioburden from 39 inanimate surfaces and their propensity to transfer pathogens between surfaces. The use of additional complimentary measures such as combined detergent-40 41 disinfectant based product and/or antimicrobial surfaces need to be considered 42 for appropriate infection control and prevention.

- 44 Keywords: surface cleaning, disinfection, detergent wipes, Clostridium difficile,
- 45 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. 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% H₂: 10% CO₂: 85% N₂) 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.

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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

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Efficacy test protocol – 3-stages protocol

134	The 3-stage protocol described in Williams et al. 13 was adapted, utilizing the
135	'Wiperator®' system (http://www.filtaflex.ca/wiperator.htm; accessed 9 January
136	2014). Wipes were cut aseptically in squares of 2 x 2 cm for testing.
137	Measurement-1 - efficacy of wipes to remove microorganisms from surfaces:
138	microorganisms (10 μ L) were inoculated onto clean magnetized, brush stainless
139	sterile steel discs (AISI Type 430 (European equivalent X6Cr17 and number
140	1.4016); Group 2; No. 4 finish (EN 10088-2 1J/2J)) and dried for 30 min at 37°C.
141	A detergent wipe was attached to a plastic boss to allow an elliptical mechanical
142	rotation for 10 s exerting a weight of 150 g. Steel discs were transferred into
143	bottles containing neutralizer (1 mL) and glass beads (1 g; 3 mm diameter;
144	Sigma). After horizontal shaking (150 rpm for 1 min) and neutralization for 5 min,
145	the suspension was serially diluted and used to inoculate appropriate agar. S.
146	aureus and A. baumannii were counted after 24 h incubation at 37°C and C.
147	difficile after 48 h anaerobic incubation. The log ₁₀ cell removal from the disk
148	surfaces was calculated by subtracting the mean log10 number of cells recovered
149	from the disc after using the wipes from the number of cells recovered from the
150	dry control.
151	Measurement-2 - bacterial transfer from wipes: Following the application of wipes
152	to the contaminated surfaces as described above, the subsequent transfer of
153	contamination onto three consecutive stainless steel discs was measured
154	together with the effect of the mechanical action (10 s wipe, 150 g pressure).
155	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 log₁₀ reduction reported for all organisms tested (data not shown). Unsurprisingly expressed solution

from the seven wipes tested displayed no bactericidal or sporicidal activity (data not shown). In order to test the impact of drying on the organisms tested, a paired-samples ttest was conducted. No statistically significant difference was found between the viable counts pre and post drying for S. aureus (p = 0.418, two-tailed) and C. difficile (p = 0.419, two-tailed). A statistically significant decrease was found for A. baumannii pre $(7.13 \pm 0.40 \log_{10})$ and post $(6.00 \pm 0.33 \log_{10})$ drying, with the eta squared statistic (0.91) indicating a large effect size. For this reason all calculations for removal utilized the dry control values. Initial analysis by means of a two-way ANOVA between groups assessed the impact of wipes and bacteria on removal. The interaction effect between wipes and bacteria was found to be significant (F(12, 42) = 10.34, p < 0.001), thus all subsequent analysis was undertaken with a one-way analysis of variance. The detergent wipes tested in this study showed marked differences in their ability to remove microbial bioburden from surfaces following a 10 second wipe, as shown in Figure 1. The average removal of S. aureus from a stainless steel surface by the wipes tested was 1.45 log₁₀ (range: 0.24-3.25). Wipe D removed significantly more (ANOVA, post hoc Tukey's test, p < 0.05) S. aureus from the stainless steel disk than the other wipes. All the wipes repeatedly transferred large number of S. aureus onto three consecutive surfaces except wipe G for which transfer of bacteria was below the limit of detection for this test (<17 CFU; recorded as 0.00% transfer; Table 2). The average removal of A. baumannii by the wipes tested was 3.51

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log₁₀ (range: 3.01-3.81). No statistically significant difference was observed in the efficacy of the wipes to remove A. baumannii from a stainless steel surface (Fig. 1). The wipes tested were particularly poor at preventing the transfer of S. aureus but much better in preventing the transfer of A. baumannii with the exception of wipe C, which performed poorly with both bacteria. Of the three microorganisms tested, the wipes removed the least number of spores from the surface (0.96 log₁₀, range: 0.26-1.44). Wipes A, D, E, and G removed significantly more spores than Wipes B and C (ANOVA, post hoc Tukey's test, p < 0.05). As with the vegetative bacteria, all wipes tested failed to retain the spores. Between 117 and 34377 spores were transferred onto surfaces (corresponding to 1.29% transfer, wipe G and 114.95% transfer, wipe C; Table 2). Wipes A and C performed particularly poorly and wipe G performed better than the others. The percentage of bacteria (CFU) transferred was estimated based on the assumption that the difference in the number of CFU on the stainless steel disk before and after wiping ended up into the wipe (Table 2). On three occasions the percentage exceeded 100%, which would indicate that the number of CFU picked up by the wipes were underestimated. The percentages of bacteria/spores transferred onto 3 surfaces were at times very low, particularly with A. baumannii, indicating that this microorganism is retained better regardless of the wipe material and formulation (Table 2). It can also be noted that the percentage of *C. difficile* spores transferred is high despite the calculated low spore number on the wipes.

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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 log₁₀ 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 log₁₀, 1.23 log₁₀ *C. difficile* but only 0.25 log₁₀ *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 log₁₀ reduction of S. aureus was ~2.30 log₁₀ with liquid soap applied to a viscose cleaning cloth, this is 1 log₁₀ higher than the median value obtain in this study (1.45 log₁₀). 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.

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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 nonwoven 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 log₁₀ for bacteria and spores, respectively) combined with

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

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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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337	wipe manufacturers.							
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Tuladhar E, Hazeleger WC, Koopmans M, Zwietering MH, et al. Residual

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

Wipe	Composition ^a	Product	Manufacturer	
	Amongst other ingredients; <5% non-ionic	Azodet™	Synergyhealth, Derby,	
Wipe A	surfactants, parfum, DMDM hydantoin,	Detergent Wipe	UK	
	iodopropynyl butylcarbamate.			
Wipe B	<5% non-ionic surfactants and	Clinell® Detergent	GAMA Healthcare,	
vvipe D	preservatives (old formulation). b	Wipe	London, UK	
Wipe C	Dimethyl oxazolidine, parfum.	Sani Cloth	PDI Europe, Flint, UK	
wipe C	Dimetryi oxazolidine, partum.	Detergent Wipe		
Wina D	<5% non-ionic surfactant, DMDM	Aquamed MA	Marshal Curtis, Didcot,	
Wipe D	hydantoin, iodopropynyl butylcarbamate.	Detergent Wipe	UK	
Wipe E	<5% non-ionic surfactant, DMDM	Clinitex®	Techtex®, Manchester,	
vvipe L	hydantoin, iodopropynyl butylcarbamate.	Detergent Wipe	UK	
Wino E	Amongst other ingredients; Parfum, DMDM	Tuffie Detergent	Vernacare, Bolton, UK	
Wipe F	hydantoin, iodopropynyl butylcarbamate.	Wipe		
Wino G	<5% non-ionic surfactants and	Clinell® Detergent	GAMA Healthcare,	
Wipe G	preservatives (new formulation). b	Wipe	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	Transfer	Transfer	Transfer	Total %
	on wipes*	1 st surface	2 nd surface	3 rd surface	transferred
		% microbial/spore transfer			
S. aureus					
А	66890	66.43	82.28	64.74	213.45
В	3633282	11.01	9.75	13.14	33.90
С	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					
А	13388894	0.02	0.01	0.01	0.04
В	1505426	0.02	0.01	0.02	0.05
С	3442779	8.00	0.03	0.02	8.05
D	1505426	0.01	0.01	0.01	0.03
Е	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					
А	92684	2.88	13.10	11.68	27.66
В	24111	2.89	7.18	2.69	12.76
С	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 log₁₀ 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.

