Combining detergent/disinfectant with microfibre material provides a better control of microbial contaminants on surfaces than the use of water alone.

Robertson A, Barrell M, Maillard J-Y*

School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, UK

* Corresponding author:

Prof Jean-Yves Maillard: School of Pharmacy and Pharmaceutical Sciences, Cardiff University Redwood Building, King Edward VII avenue, Cardiff CF10 3NB, UK

telephone: + 44(0)2920879088

e-mail: maillardj@cardiff.ac.uk

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Summary

The use of microfibre cloths with either water, detergent or disinfectant is currently recommended for hospital cleaning. We explore the efficacy of a microfibre cloth with either water or detergent/disinfectant or sporicidal products using the ASTM2967-15 standard against *Staphylococcus aureus, Acinetobacter baumannii* and spores *Clostridium difficile* spores. The use of detergent/disinfectant or sporicidal products had a significantly (ANOVA, $p<0.001$) better activity than water alone in reducing bacteria and spores' viability, and in reducing the transfer microorganisms between surfaces. The use of water alone with a microfibre cloth is less effective and should not replace the use of biocidal products.

Introduction

Efficient cleaning and disinfection are an integral part of infection-control regimens currently used in healthcare facilities [1] and can result in a reduction in healthcare associated infections (HCAIs) [2] and in the impact of infection outbreaks [3]. Such reduction in HCAIs will have in turn a significant financial benefit to healthcare systems [4]. For surface decontamination, formulated product or water are used in combination of various materials [5] although the use of formulated wipes might be more efficacious [6]. The type of materials used will impact on the concentration of formulation (notably quaternary ammonium compounds) delivered [7]. Microfibres, which are commonly used for surface decontamination [8], have a higher density of strands, when compared cotton cloths and nonwoven materials, increasing the surface area of the cloth [9]. The cleaning efficacy of microfibre cloths has been proven to be so effective that UK infection control polices advocate their use with water [10]. The term 'cleaning' describes the physical removal of soil, dirt or dust from surface [1], but in the process may also remove microorganisms from surfaces. Indeed, the use of materials in combination with various detergents including quaternary ammonium compounds for cleaning purpose has been shown to impact not only on the removal of pathogens from inanimate surfaces but on their transmission to other surfaces [11]. Thus, combining water of formulated solutions, whether detergent or disinfectant, with materials should be evaluates for their impact in removing and transferring microorganisms from and between surfaces. With this in mind, the impact of using water alone in combination with microfibre materials to remove or prevent pathogen transfer between surfaces has not been widely reported. Here, we tested the impact of using water *vs.* QAC based-detergent/disinfectant or sporicidal products in combination with a microfibre material using the ASTM2967-15 standard to measure wipe products' efficacy.

Methods

S. aureus (ATCC 6538) and *A. baumannii* (ATCC 19568) and spores of *C. difficile* (NCTC 11209) were used. Test bacteria inocula were resuspended in a buffer (tryptone 1 g/L; sodium chloride 8.5 g/L; TSC) following overnight propagation at 37°C in tryptone soya broth (TSB;

Oxoid) [11]. *C. difficile* spores were resuspended in sterile distilled water following propagation and purification based on the Clospore method [12]. Test bacteria/spores (1 x 10^9 cfu-spores/mL; final concentration) were added to bovine serum albumin (BSA) at a final concentration of 0.3 g/L (clean condition) or to BSA 3 g/L and sheep erythrocytes 3 ml/L (final concentration; dirty condition). The ASTM 2967-15 [13] was used to measure bacteria/spores removal from, and transfer between surfaces. A 10 sec wiping time with 300 g weight was used with the detergent/disinfectant and sporicidal products as it reflects condition of use in practice. With the detergent/disinfectant, surfaces were neutralised immediately after wiping. With the sporicidal product, surfaces were left 15 min before neutralisation occur in accordance with the manufacturer's instruction. For the transfer experiment, the used wipe was used to wipe a clean surface (10 sec, 300 g) immediately after the initial wiping. Bacteria deposited on the clean surface were enumerated after neutralisation as described below. In addition, considering the ability of spores to survive well in the environment, a 24 hour at 25°C and 40 % relative humidity after wiping following the use of the sporicidal product was also investigated. The conditions of use of water with the microfibre reflected the use of the detergent/disinfectant or sporicidal product. Two surfaces were used: stainless steel (AISI Type 430; 1 cm diameter and 0.7 mm thickness), and polyvinyl chloride (PVC with a PUR coating, 1 cm diameter and 0.7 mm thickness; Armstrong, Stuttgarter Str. 75, 74321 Bietigheim Bissingen). Stainless-steel disks were cleaned and sterilised by autoclave. The PVC disks were cleaned then disinfected in 3 % peracetic acid. Prior to wiping, surfaces were contaminated with 0.01 mL of test suspension (1 x 10^8 cfu/mL) and left to dry in a biological safety cabinet until the disks were visibly dry. Surfaces were then tested against a microfibre wipe (Decitex), soaked in sterile water and a wipe soaked in a solution of 0.25% detergent/disinfectant product [containing N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine (5.1%) and didecyldimethyl ammonium chloride (2.5%)] or 0.5% sporicidal product [containing peracetic acid (750 ppm) and N-alkyl(C12-14)-N-benzyl-N,N-dimethylammonium chloride (0.012%)]. The wipe microfibres were then wrung lightly until no longer dripping and used only once. The sporicidal product was only used against *C. difficile* spores. To quench the activity

of the detergent/disinfectant and sporicidal products the following neutraliser was used: 30 g/L polysorbate 80; 30 g/L saponin; 5 g/L sodium thiosulphate; 3 g/L azolectin; 1 g/L histidine; dissolved in TSC. Neutralised suspensions were diluted in TSC, plated on TSA and incubated 24 h at 37°C for bacteria. Recovered spores were plated on BHI agar containing 0.1% sodium taurocholate for spores and incubated for 48 hours, anaerobically (MG500 anaerobic workstation, Don Whitley) at 37°C. Statistical analyses (ANOVA) was performed using the R-program [14].

Results and Discussion

There was a significant difference (ANOVA, $p<0.001$) in the number of bacteria removed from surfaces following wiping between the use of water alone and the detergent/disinfectant product, regardless of the type of surface (Table 1). Although the performance of combining the microfibre cloth with water reduced bacterial counts mostly by 1-2 $\log_{10}$ (Table 1), bacterial transfer from the microfibre to a different surface following wiping was significant (3-4 $\log_{10}$ bacterial transfer)(Table 2). In comparison the use of detergent/disinfectant significantly (ANOVA, $p<0.001$) prevented the transfer of bacteria. The level of organic load did not affect the efficacy of the test product and material performance.

The use of the sporicidal product significantly (ANOVA, $p<0.001$) reduced the concentration of *C. difficile* spores comparing to the use of water regardless of the type of surfaces and organic load (Table 1). Following a 24h recovery period post-wiping, the sporicidal product performed significantly better (ANOVA, $p<0.001$) than the use of water. Of practical significance, the use of the sporicidal product prevented the transfer of *C. difficile* spores between surfaces, regardless of the type of surfaces or level of organic load (Table 2). The use of water was associated with significant spore transfer 15 min post wiping or 24 h after wiping.

Although it has been previously suggested that microfibre cloths can reduce the transfer of spores [15], our results clearly indicate that the water-damp microfibre cloth was able to transfer high levels of spores. This suggests that the spores are not retained within the material

and are at risk to being re-deposited on to clean surfaces during wiping/moping. The sporicidal product, maybe not surprisingly, was sporicidal following 10 sec wiping time and 15 min surface contact time as recommended by the manufacturer.

Overall the type of surface used did not have a significant effect of the removal of bacteria/spores (ANOVA, p=0.754), or the transfer of bacteria/spores (ANOVA, p=0.642).

Likewise soiling had no significant effect of the removal of bacteria/spores (ANOVA, p=0.915) or the transfer of bacteria/ spores (ANOVA, P=0.424). Our results also highlighted that there were no significant differences in removal (ANOVA with Tukey post-hoc test; p=0.959), or transfer (p=0. 815) between vegetative bacteria.

Overall, this in vitro study justified the use of detergent/disinfectant or sporicidal products in the control of microorganisms or spores on surfaces and it does not favour the use of water only. Hamilton and colleagues [17] reported on the performance of ultra- microfibre cloths and mops moistened with water or a copper-based biocide in a cross-over trial over a 7 weeks period in an in situ study. Although this trial did not investigate microbial transfer, the authors reported that the use of the biocide significantly enhanced the efficacy of the microfibre in reducing total viable count. Some hospitals have advocated the use of microfibre materials with water alone with no other interventions. Such practice might need to be reconsidered since the use of a detergent/disinfectant or sporicidal based product in combination of the microfibre cloth provide the assurance that the potentially harmful bacteria or spore are not only eliminated from surfaces but that they cannot be transmitted to any other surfaces during wiping/moping. As published 10 years ago [18], wipes should be used on “one surface, one direction” before being disposed of; this however may be more difficult to apply to mops. Staff training is essential to minimise the spread of pathogens when using such materials [19].

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Conflict of interest

None

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Table 1 Bacteria/spores removal from surfaces following wiping in a) clean and b) dirty conditions.

a) Clean condition		**Log_{10} removal (±SD) from surfaces**		
Stainless steel	**Sampling time[1]**	**Water**	**Detergent/ Disinfectant**	**Sporicide**
S. aureus	0	2.10 (0.19)	4.23 (0.25)	
A. baumannii	0	2.53 (0.27)	5.21 (1.20)	
C. difficile[2]	0	1.38 (0.39)		5.67 (0.06)
	24	1.24 (0.35)		5.99 (0.21)
PVC				
S. aureus	0	2.14 (0.51)	3.19 (0.40)	
A. baumannii	0	2.72 (0.68)	3.86 (0.97)	
C. difficile[2]	0	1.88 (0.23)		4.16 (0.18)
	24	1.63 (0.22)		6.14 (0.12)
b) Dirty condition		**Log_{10} removal from surfaces**		
Stainless steel	**Sampling time[1]**	**Water**	**Detergent/ Disinfectant**	**Sporicide**
S. aureus	0	2.23 (0.18)	4.67 (0.58)	
A. baumannii	0	2.05 (0.39)	4.50 (0.97)	
C. difficile[2]	0	1.55 (0.56)		5.89 (0.04)
	24	1.72 (0.21)		6.07 (0.52)
PVC				
S. aureus	0	2.60 (0.79)	4.12 (0.77)	
A. baumannii	0	2.82 (0.36)	5.01 (0.83)	
C. difficile[2]	0	1.12 (0.50)		4.28 (0.19)
	24	1.84 (0.34)		5.90 (0.30)

[1] sampling time: 0: surfaces were neutralised immediately after wiping; 24: surfaces were left 24h at 25°C and 40 % relative humidity before neutralisation and processing

[2] spores of *C. difficile*

Table 2 Bacteria/spores transfer between surfaces following wiping in a) clean and b) dirty conditions.

a) Clean condition		**Log_{10} transfer (±SD) between surfaces**		
Stainless steel	**Sampling time[1]**	**Water**	**Detergent/ Disinfectant**	**Sporicide**
S. aureus	0	4.66 (0.53)	0.89 (0.43)	
A. baumannii	0	3.52 (1.58)	0.40 (0.00)	
C. difficile[2]	0	4.73 (0.44)		0.40 (0.00)
	24	3.69 (0.37)		0.40 (0.00)
PVC				
S. aureus	0	5.09 (0.67)	1.15 (1.05)	
A. baumannii	0	4.55 (0.74)	0.66 (0.45)	
C. difficile[2]	0	4.46 (0.46)		0.76 (0.32)
	24	2.52 (0.51)		0.40 (0.00)
b) Dirty condition		Log_{10} transfer (±SD) between surfaces		
Stainless steel	**Sampling time[1]**	Water	Detergent	**Disinfectant**
S. aureus	0	2.92 (0.14)	0.40 (0.00)	
A. baumannii	0	2.39 (0.26)	0.40 (0.00)	
C. difficile[2]	0	4.18 (0.55)		0.40 (0.00)
	24	3.12 (0.27)		0.50 (0.17)
PVC				
S. aureus	0	4.64 (0.76)	0.40 (0.00)	
A. baumannii	0	4.30 (0.36)	0.40 (0.00)	
C. difficile[2]	0	4.72 (0.59)		0.56 (0.28)
	24	2.61 (0.30)		0.40 (0.00)

[1] sampling time: 0: surfaces were neutralised immediately after wiping; 24: surfaces were left 24h at 25°C and 40 % relative humidity before neutralisation and processing

[2] spores of *C. difficile*