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Impact of artificial accelerated ageing of PVC surfaces and surface degradation on disinfectant efficacy

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SUMMARY

Background: Standardized efficacy surface tests for disinfectants are performed on pristine surfaces. There is a growing interest in understanding the impact of surface ageing on disinfectant activity, owing for example to the increased usage of ultraviolet (UV) radiation and oxidative chemistries for surface decontamination. This acknowledges that general surface 'wear and tear' following UV radiation and oxidative biocide exposure may impact biocidal product efficacy.

Methods: PVC surfaces were aged through thermal and UV-A radiation (340 nm wavelength) following the use of standard ageing surface protocols to simulate natural surface degradation. Surface roughness, contact angle and scanning electron microscopy were performed to evaluate physical changes in PVC surfaces before and after artificial ageing. The efficacy of five pre-impregnated disinfectant wipes were evaluated using the ASTM E2967-15 on stainless-steel (control) and PVC surfaces (aged and non-aged).

Results: The type of formulation and the organism tested remained the most significant factors impacting disinfectant efficacy, compared with surface type. Both thermal ageing and UV-A exposure of PVC surfaces clearly showed signs of surface degradation, notably an increase in surface roughness. Physical changes were observed in the roughness of PVC after artificial ageing. A difference in disinfectant efficacy dependent on aged PVC surfaces was observed for some, but not all formulations.

Conclusion: We showed that surface type and surface ageing can affect biocidal product efficacy, although in a non-predictable manner. More research is needed in this field to ascertain whether surface types and aged surfaces should be used in standardized efficacy testing.

Introduction

The ability to disinfect hospital surfaces effectively is paramount to community health and safe hospitals [1,2]. Surfaces play an important role in the transmission of pathogens [3,4].

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The risk of transmission throughout the hospital increases where airborne pathogens deposit on surfaces [5] and floors are contaminated through spillages [6].

There are a variety of environmental surfaces used in healthcare settings, the most commonly used are stainless steel and polyvinylchloride (PVC). Stainless steel is used in medical fixtures, bed rails and working surfaces, whilst PVC is used in medical devices, wall coverings, ceilings and floor coverings of operating theatres and wards. Each surface is unique in surface roughness, durability and longevity.

Natural ageing of surfaces occurs through environmental stressors such as exposure to sunlight, temperature fluctuation, wetting and abrasion and surface disinfection [7—9]. The type of damage imparted to the surface by UV-C or disinfectants depends on surface materials, with an increase in surface roughness often observed [7,10]. Hydrophobic surfaces decrease bacterial adhesion and can impact disinfection efficacy. Increasing surface roughness results in an increasing area available for bacterial adhesion whilst offering increased protection against shear stress and mechanical removal [11]. As such, increased surface roughness has been implicated in bacterial retention on surfaces, biofilm formation [12] and rapid surface recolonization post wiping [13].

The bactericidal efficacy of biocidal products, and pre-impregnated wipes in particular, is conducted with standard efficacy tests such as the ASTM E2967-15 [14] or the EN16615-15 in Europe [15] which inform product claims on label. These standard tests are performed on pristine surfaces that have not been exposed to the common aforementioned environmental surface stressors. The impact of aged surfaces on biocide efficacy remains poorly documented [7], despite the increased usage of disinfectants in combination with wiping [16] and UV-C disinfection in hospital settings [17].

Here we report the effect of surface types, but also aged PVC surfaces on the microbicidal efficacy of five formulations commonly used in healthcare settings. We also describe the use of two artificial ageing processes, thermal shock and UV-A exposure (in accordance with ASTM G154-16 [18]) on PVC surfaces.

Methods

Test products

Five commercially available pre-impregnated wipe products (Table I) were tested along with water combined with J-cloth (control). J-cloth is a non-antimicrobial reusable cloth comprising cellulosic fibres from wood pulp, this was used as a negative control material.

Test surfaces

Stainless-steel discs (0.7 ± 0.07 mm thickness; 10 ± 0.5 mm diameter and 20 ± 0.5 mm diameter; 20 × 50 cm; 2B finish; AISI; Goodfellow, UK) were used as control surfaces as stainless steel is commonly used in standard efficacy tests (e.g., ASTM E2967, 2015) [14]. PVC (polyvinylchloride with a polyurethane (PUR) coating; 2 mm thickness; 10 ± 0.5 mm diameter; 20 ± 0.5 mm diameter; 20 × 50 cm; DLW Flooring, Germany) was used for aged and non-aged surface testing.

Table I
Product active ingredient and material as listed on product packaging

Wipe	Active ingredients	Wipe material
A	Propan-1-ol <20%; didecyldimethylammonium chloride (DDAC) <0.025%	Polyethylene terephthalate (PET)
B	Hydrogen peroxide 0.36% w/w	Polypropylene
C	Benzalkonium chloride (BZC) <0.5%; DDAC <0.5%; polyhexamethylene biguanide (PHMB) <0.10%	60% PET; 40% viscose
D	DDAC 0.3%	100% viscose
E	25 g ethanol (94 %), 35 g propan-1-ol	Information not available

Artificial ageing of surfaces using thermal and UV ageing techniques

PVC surfaces were aged in the DURALAB facility (School of Engineering, Cardiff University). To mimic natural 'wear and tear' of surfaces we decided to use thermal ageing and UV irradiation. Temperature is an important environmental factor affecting surfaces [19]. To accelerate temperature-based surface ageing, we used cycle exposure to a very high temperature [20]. PVC surfaces were cut to 23 × 30 cm and subjected to thermal cycle exposure, comprising 1 h at 130 °C in a pre-heated oven, followed by 1 h at -10 °C, in order to trigger surface degradation and micro-cracking by thermal shock [20].

The impact of UV on surfaces reflected the increasing use of UV-C disinfection. However, there is overall little information on the impact of UV-C on surfaces and we decided to base UV irradiation on the established ASTM G154-16 standard, which explores the effect of UV-A on surfaces [18]. PVC specimens were cut to 15 ×7.5 cm and mounted into the sample holder of a UV weathering chamber (QUV Accelerated Weathering Tester; Q-LAB, UK) where a reduced area of 6 × 9 cm surface was exposed to a 200-h cycle; 1 h of UV-A light (irradiance 1.50 W/m^2 per nm control wavelength; 340 nm) followed by 1 h of dark at room temperature based on recommendations of the ASTM G154-16 standard operating procedure [18].

Artificially aged surfaces were analysed for surface roughness, hydrophobicity and visualized using scanning electron microscopy (SEM) and used in wipe efficacy testing.

Surface roughness of stainless steel and PVC using the stylus profilometer technique

A stylus profilometer (Mitutoyo; SURFTEST SV-2000; data analysed through SURFPAK-SV Version 1.300) was used to measure surface rugosity of a 1-cm disc. Five discs were analysed for each surface type. In order to avoid curved edges of the surfaces a 4-mm sample of each disc was measured.

Surface hydrophobicity

Surface hydrophobicity of a 1-cm disc was analysed using a One Attension Theta Lite and software provided by the instrument (Biolin Scientific). Five discs were analysed for each

surface type. A 4-μL drop of either water or extracted wipe formulation was dropped on to the surface and contact angle was measured over a 30-s interval.

Imaging of PVC surfaces before and after artificial ageing

SEM was used to obtain images of the surfaces before and after artificial ageing. The surfaces of 1-cm discs were coated in a thin layer (20 nm) of gold—palladium using a Bio-Rad Coater SC500 whilst in a vacuum chamber. Argon gas was used to purge the sputter chamber before coating. Both the top layer of the surfaces and a section through the surface were imaged using the secondary electron image (SEI) setting on a Philips XL30 field emission gun-scanning electron microscope (FEG-SEM). Images were taken at × 75 and × 1200 magnifications with a 10-mm ± 1-mm working distance.

Test micro-organisms

Staphylococcus aureus ATCC6538, *Acinetobacter baumannii* ATCC19568, *Enterococcus hirae* ATCC10541, *Enterococcus faecium* 18-373, *Enterococcus faecium* 22-006 and *Enterococcus faecium* AUS0004 were used as test bacteria. Bacteria were grown in tryptone soya broth (TSB) (Oxoid, Basingstoke, UK) incubated at 37 °C ± 1 °C for 16—24 h. Test inocula were prepared from harvesting an overnight TSB culture centrifuged at 5000 *g* for 10 min and resuspended in deionized water (diH_20) at a final test inoculum of 1—5 × 10^6 cfu/mL. Strains were stored on Protect Beads Blue (Fisher Scientific, Loughborough, UK) at −80 °C ± 1 °C and restricted to a maximum of two subcultures from the original freezer stock prior to exposure to testing conditions.

Virus test strains were adenovirus type 5 (Ad5; ATCC# VR-5); vaccinia virus (VV; ATCC# VR-1354); murine norovirus (MNV; ATCC# VR-1937). A549 (ATCC # CRM-CCL-185), Vero E6 (ATCC # CRL-1586), and RAW 264.7 (ATCC# TIB-71) cells were maintained at 37 °C, 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM; Merck) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Merck). Virus stocks were grown on A549 (Ad5), Vero E6 (VV), or RAW 264.7 (MNV) cells in the presence of 2% heat-inactivated fetal bovine serum (FBS; Sigma) and titrated as below to determine test inoculum concentrations of 9.4 × 10^8 pfu/mL (Ad5), 8.1 × 10^8 pfu/mL (VV), and 1.5 × 10^8 pfu/mL (MNV).

Virus enumeration with plaque assay

Serially diluted virus samples were used to infect duplicate monolayers of RAW 264.7 (for MNV), A549 (for Ad5), or Vero E6 (for VV) cells seeded 18 h prior in 12-well plates with 1 × 10^6 (MNV), or 1 × 10^5 (Ad5, VV) cells. After 1 h incubation, inocula were removed and cells overlayed with a 1:1 mix of 2.4% Avicel® and 2 × MEM (20% 10 × MEM; 2% L-Glutamine; 4% FBS; 5.4% sodium bicarbonate (7.5% solution)). Cells were incubated for 48 (MNV) or 72 h (VV), or six days (Ad5) at 37 °C, 5% CO_2 at which point overlay was removed, cells were washed once with PBS and fixed with 1 mL/well methanol for at least 5 min. Following removal of methanol, cells were stained with 0.1% (w/v) crystal violet, and plaques counted.

Efficacy of wipes to remove/kill micro-organisms from surfaces

A method based on the ASTM E2967-15 standard [14] was used. A bacterial test suspension of 10 μL (1—5 × 10^6 cfu/mL) was inoculated on to sterile stainless-steel magnetized 1-cm discs and dried for 30 min at 37 °C. Pre-impregnated wipes or J-cloths, wetted with 2 mL water (control) were loaded on to a plastic boss. The inoculated discs were wiped with an elliptical mechanical rotation for 30, 10 or 5 s, exerting a weight of 650 g (150 g intrinsic Wiperator force and 500 g additional weight) or 300 g (150 g intrinsic Wiperator force and 150 g additional weight). Two different weights were used to ascertain the impact of pressure on wipe efficacy. Wiped discs were left for a contact time of 30 s or 5 min or neutralized immediately after wiping, before being transferred into tubes containing 1 mL neutralizer and 1 g glass beads. Suspensions were vortexed for 1 min at 150 rpm and left for 5 min. Suspensions were serially diluted, incubated (24 h at 37 °C) and enumerated using the drop counting method. The $\log_{10}$ bacterial cell removal from the disc surfaces was determined using the following equation:

$$\text{cfu/mL} = \text{average number of colonies}/(D \times V)$$

where D is the dilution factor and V is the volume of diluted bacterial suspension.

Bacterial transfer from wipes

Following wiping of contaminated surfaces (30 s wiping, 500 g pressure), bacterial transfer on to a new sterile stainless-steel disc was measured [14]. The wiped steel disc was placed in the neutralizer and bacterial colonies enumerated as described above.

Neutralization of active ingredients

A universal neutralizer containing 8.5 g/L sodium chloride; 1 g/L tryptone; 1 g/L L-histidine/3 g/L lecithin; 5 g/L sodium thiosulphate; 30 g/L polysorbate80 and 30 g/L saponin [15] was prepared using deionized water and autoclaved at 125 °C for 15 min. The neutralizer was used to quench the activity of active ingredients in test products and its efficacy against test products was validated.

Prior to testing, a bacterial suspension of 10 μL (1—5 × 10^6 cfu/mL) was deposited on the surface of a sterile stainless-steel disc and dried for 30 min at 37 °C. The disc was deposited into 1 mL neutralizer and 1 g glass beads as described above. After shaking (150 rpm for 1 min) for 5 min, the suspension was serially diluted, incubated (24 h at 37 °C) and enumerated.

Efficacy of wipes to remove virus from surfaces

The test protocol was also based on the ASTM E2967-15 standard [14]. Ten microlitres of virus inoculum (see above for concentrations) was inoculated on to sterile magnetized stainless-steel discs or PVC, and dried at room temperature for 60 min in a Biosafety level-2 cabinet. Pre-impregnated wipes were loaded on to a plastic boss. The inoculated discs were wiped with an elliptical mechanical rotation for 5 s exerting a weight of 300 g. Discs were neutralized immediately after wiping by transfer into tubes containing 1 mL DMEM.

Suspensions were vortexed for 1 min at 150 rpm and left for 5 min. Samples were 10-fold serially diluted in DMEM and residual virus titrated by plaque assay. The $\log_{10}$ reduction in infectious virus on disc surfaces was calculated by subtracting the mean $\log_{10}$ virus titre recovered from the disc after wiping, from the mean $\log_{10}$ virus titre recovered from an un-wiped control.

Virus transfer from wipes

Following wiping of contaminated surfaces as described above, virus transfer on to new sterile stainless-steel or PVC discs was evaluated according to Ref. [14] as described above. Viruses were neutralized and titrated as above.

Statistical analysis

Statistical analysis was performed with Graphpad Prism Version 9.5.1. Two-way ANOVA was used to compare surface data and multiple linear regression was used to identify most significant factors affecting efficacy data.

Results

Artificial ageing of PVC surfaces

Surface roughness of stainless steel and PVC using the stylus profilometer technique

PVC surfaces were rougher than stainless steel with higher average Ra (μm) and Ry (μm) values (Figure 1). Ra values represent a calculated average of every peak and every trough of the surface profile, whereas Ry values represent the calculated average of the highest and lowest peaks of the surface profile. The lower the Ra and Ry values, the smoother a surface, and *vice versa*. The average Ra value for stainless steel was 0.301 (±0.11) and Ry was 2.85 (±1.04). The average Ra value for PVC was 2.13 (±0.2361) and Ry was 12.21 (±1.77). Before artificial ageing, PVC surface was significantly rougher than that of stainless steel (two-way ANOVA; P=0.0054). When the PVC surface was thermally aged, Ra and Ry values significantly increased by 3.23-fold (two-way ANOVA; P<0.0001) for Ra and 2.50-fold Ry (two-way ANOVA; P<0.0001) (Figure 1). UV-A treatment resulted in a small and statistically not significant (two-way ANOVA; P>0.05) decrease in PVC surface roughness (Figure 1).

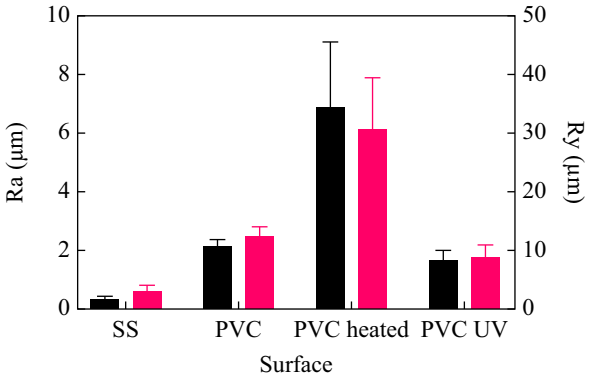

Figure 1. Calculated average Ra and Ry values for stainless steel (SS), polyvinylchloride (PVC), thermally aged PVC (PVC heated) and UV-A-aged PVC (PVC UV) surfaces (N = 5). Black, Ra (μm); red, Ry (μm). Ra, calculated average of every peak and every trough of the surface profile; Ry, calculated average of the highest and lowest peaks of the surface profile.

Surface hydrophobicity (contact angle) following wipe treatment

A significant difference was found between average contact angles of stainless steel and PVC when water was applied to the surface (two-way ANOVA; P<0.0001) suggesting that PVC surfaces are more hydrophobic than stainless steel (Figure 2a). All formulations reduced the average contact angle (Figure 2a) which was significant for formulations A, B, C and E (two-way ANOVA; P<0.0001) but not for formulation D (two-way ANOVA; P=0.0004). When PVC surfaces were thermally or UV-A aged, contact angles were not significantly different (two-way ANOVA; P>0.05) to that of the non-aged PVC (Figure 2b).

Imaging of PVC surfaces before and after artificial ageing

Thermal ageing of the surface produced a decrease in the layer depth of the polyurethane coating on the PVC (Figure 3). SEM imaging of a top-down view of the surface showed some distinguishable differences in physical characteristics (Figure 4). The top layer of the surface appeared noticeably more textured after thermal ageing (Figure 4b) at a low magnification (×75), but not at a higher one (×1200). SEM imaging showed no distinguishable change in surface characteristics in UVA-aged PVC (Figure 4c, f).

Parameters affecting pre-impregnated wipe efficacy

Although the ASTM E2967-15 [14] does not stipulate requirements for a pass or fail of the test, for the purpose of this study a 4-$\log_{10}$ reduction was considered a 'pass' for removal/killing and 1.70 $\log_{10}$ was considered a 'pass' for transfer of micro-organisms where possible. This would be comparable to the EN16615 [15] for which a 'pass' is the transfer of <50 colonies per surface, although this arbitrary criterion is close to our limit of detection for bacterial transfer.

In the first instance, we tested a number of parameters that could impact disinfectant wipe performance [16], notably wiping time, pressure (weight), residual time post-wiping and types of bacteria, notably impact of clinical isolates. Then the impact of aged surfaces on disinfectant efficacy was investigated against both bacteria but also viral pathogens.

The efficacy of the pre-impregnated wipes against *S. aureus* was affected by the lack of residual time post-wiping, wiping time and weight imparted on the wipe during wiping (Figure 5). The absence of residual contact time decreased product efficacy below the arbitrary 4-$\log_{10}$ reduction pass criterion (Figure 5e, g) and increased microbial transfer (Figure 5h, f). Without residual time post-wiping, pre-impregnated wipes performed better on stainless-steel surfaces compared with PVC (wipe A: two-way ANOVA; P=0.003; wipe B: two-way ANOVA; P=0.0092; wipe C: two-way ANOVA; P<0.0001; Figure 5f). Shorter wiping time (5 instead of 10 s) with no residual time post-wiping resulted in poorer performance for some products (Figure 5e, g). A combination of 30 s wiping with additional residual time post-wiping resulted in attaining the 4-$\log_{10}$ reduction threshold (Figure 5a, c), although wipe D did not perform well on PVC with only a 300 g weight (Figure 5b)

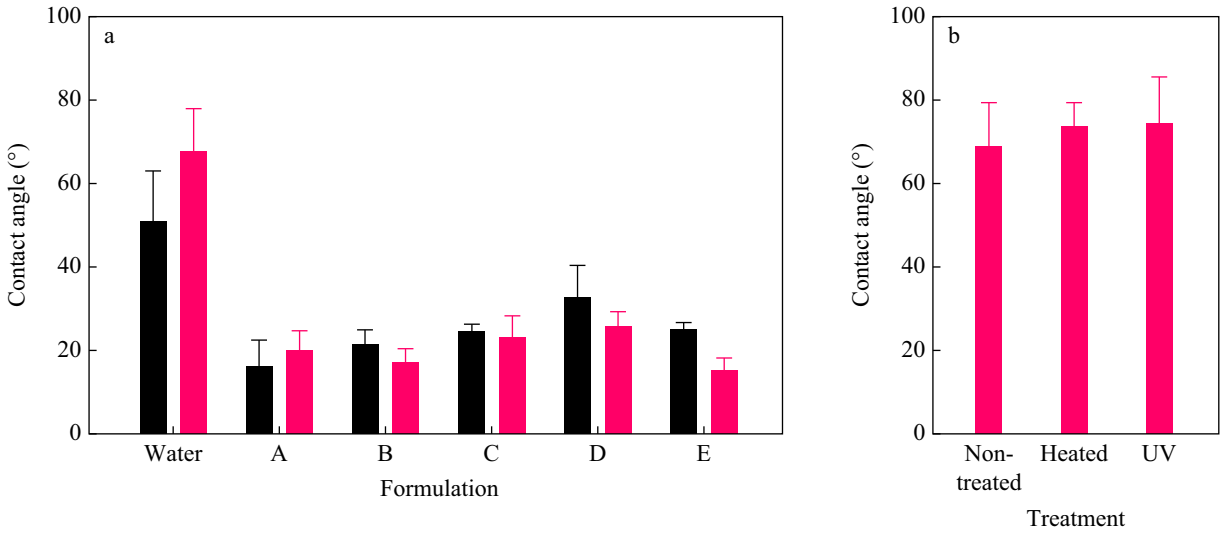

Figure 2. Average contact angle measurement of polyvinylchloride (PVC) and stainless-steel (SS) surfaces ageing. (a) Average contact angle on SS and PVC surfaces following wipe treatment. (b) Average contact angle of non-aged (non-treated), thermally aged (heated) or UV-A-aged PVC (UV) ($N = 5$). Black, SS; red, PVC.

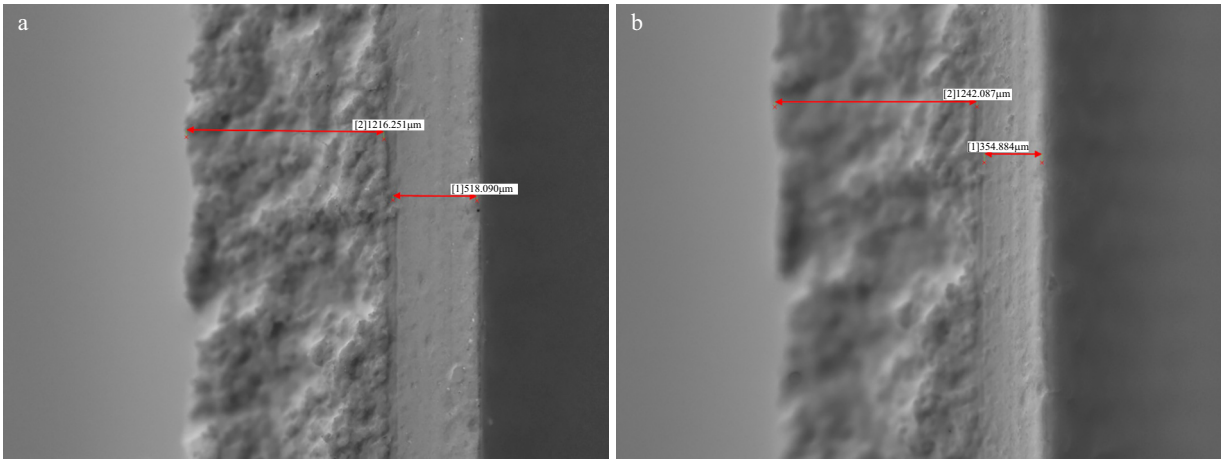

Figure 3. Polyvinylchloride (PVC) surface before and after thermal ageing. (a) Before heating: layer depth of polyurethane (PUR) coating measuring 518 µm. (b) After heating: layer depth of PUR coating measuring 355 µm.

despite longer residual time. With a shorter residual time, but increased wiping pressure (Figure 5c), all wipes failed to decrease bacterial transfer on the stainless-steel surface (Figure 5d). Overall, the type of surface impacted on *S. aureus* $\log_{10}$ reduction (multiple linear regression; P=0.0048) but not on bacterial transfer (multiple linear regression; P>0.05). The type of pre-impregnated wipe products was the variable that impacted most significantly on $\log_{10}$ reduction in *S. aureus* (multiple linear regression; P=0.0009).

Residual time duration post-wiping and weight imparted during wiping did not affect the efficacy of the products against *A. baumannii* to the same extent as they did against *S. aureus*. All wipe products passed the arbitrary efficacy criteria (Figure 6a—d). However, the absence of residual time post-wiping dramatically affected product performance (Figure 6e, f). When wiping for 5 s only with a 300-g weight, wipes B and wipe D did not achieve a 4-$\log_{10}$ reduction in *A. baumannii* on either stainless-steel or PVC surfaces (Figure 6e) and wipe D did not prevent bacterial transfer on either surface (Figure 6f). Overall, the type of surface did not have a significant impact on $\log_{10}$ reduction in *A. baumannii* (multiple linear regression; P=0.2029) or transfer of (multiple linear regression; P=0.6818). As with *S. aureus*, the type of pre-impregnated wipe product had the most significant impact on the efficacy against *A. baumannii* reduction (multiple linear regression; P=0.0181) and transfer post-wiping (multiple linear regression; P<0.0001).

Whilst standard efficacy tests are performed on standard bacterial strains only, we were interested to investigate the impact of clinical strains on efficacy on different surface types. The clinical enterococci isolates selected for testing were epidemic strains of interest and have been profiled for antibiotic resistance. The standard *E. hirae* ATCC10541 strain is used as a reference strain in EN standardized efficacy test

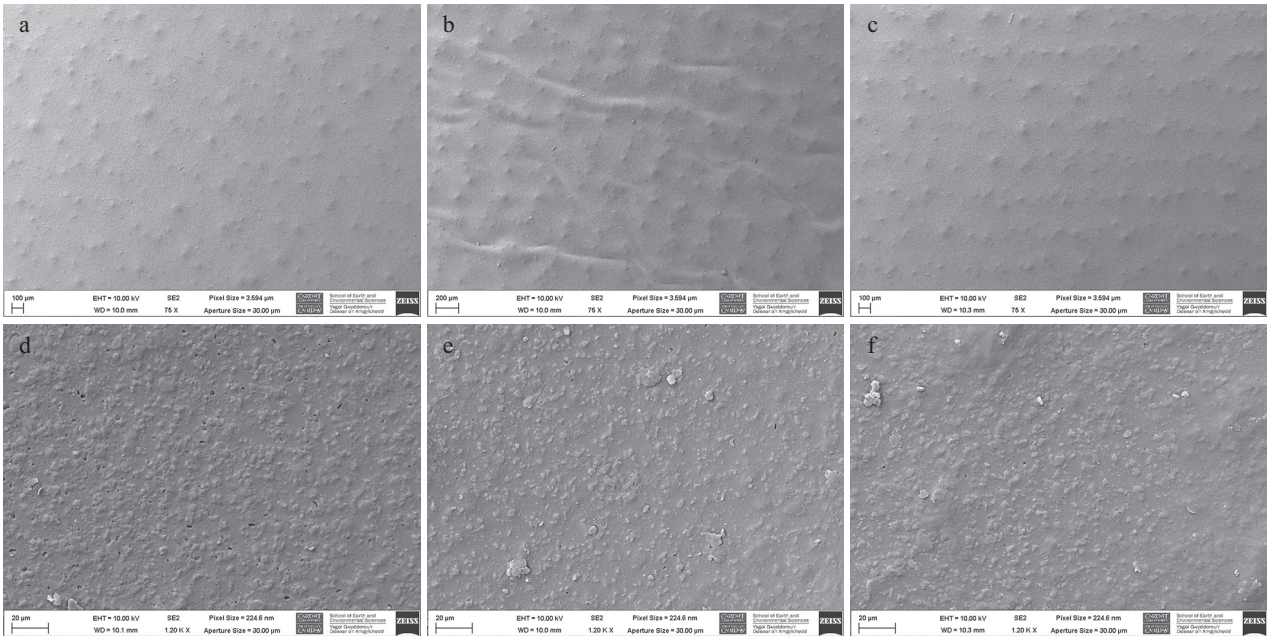

Figure 4. Scanning electron microscope images of polyvinylchloride (PVC) surface, top-down view, before and after thermal and UV-A ageing. (a) Untreated PVC (×75 magnification); (b) thermally aged PVC (×75 magnification); (c) UV-A-aged PVC (×75 magnification); (d) untreated PVC (×1200 magnification); (e) thermally aged PVC (×1200 magnification); (f) UV-A-aged PVC (×1200 magnification).

methods. As with the other bacteria tested, the type of pre-impregnated wipe product was the most significant factor effecting product efficacy against, and transfer of, enterococci (multiple linear regression; $P<0.0001$). This was particularly evident with wipe D that performed poorly against any of the isolates tested (Figure 7), although it produced a significantly greater reduction in *E. faecium* AUS004 (two-way ANOVA; $P=0.0057$) on stainless steel compared with PVC (Figure 7g). Wipe B was significantly more efficacious against *E. faecium* 18-373 on the stainless-steel surface compared with PVC (two-way ANOVA; $P=0.0018$) (Figure 7c). Conversely wipe B transferred significantly (two-way ANOVA; $P<0.0001$) more bacteria (3.97 $\log_{10}$) on to the PVC surface, failing to pass the arbitrary <1.70-$\log_{10}$ transfer (Figure 7d). Wipe A produced a 4.53-$\log_{10}$ reduction in *E. faecium* 22-006 on stainless steel but a statistically significant decrease in efficacy (two-way ANOVA; $P=0.0009$) on PVC (Figure 7e). The difference in wipe efficacy between surfaces was statistically significant for *E. hirae* (two-way ANOVA; $P=0.0025$), *E. faecium* 18-373 (two-way ANOVA; $P=0.0001$) and *E. faecium* 22-006 (two-way ANOVA; $P=0.0012$). In addition, significantly more bacteria were transferred on to PVC for *E. faecium* 18-373 (two-way ANOVA; $P=0.0120$), *E. faecium* 22-006 (two-way ANOVA; $P<0.0001$) and *E. faecium* AUS004 (two-way ANOVA; $P<0.0001$) (Figure 7d, f, h). Wipe E produced a >4-$\log_{10}$ reduction of *E. faecium* 18-373 on stainless steel but not on PVC (two-way ANOVA; $P<0.0189$) (Figure 7d).

Finally, we explored the impact of surface type on virucidal efficacy of pre-impregnated wipes. A 4-$\log_{10}$ reduction in viral titre was considered a 'pass' for removal of virus from surfaces, while ≤1.70 $\log_{10}$ (50 virus particles) was considered a 'pass' for transfer of viruses. No wipes were able to achieve the required 4-$\log_{10}$ reduction in viral titre against MNV or Ad5, with the exception of wipe A against MNV on the PVC surface. Wipe D also failed to perform against VV, in contrast to wipes A, B, C, and E (Figure 8). The type of surfaces did not seem to impact wipe performance when looking at removal from surfaces (Figure 8).

Wipe D resulted in transfer of >1.7 $\log_{10}$ of all three viruses on PVC surfaces. In contrast, wipe A succeeded in preventing transfer of viruses on both surfaces, except Ad5 where the transfer was <1.45 $\log_{10}$. The greatest transfer of virus was observed for Ad5, with wipes B, C, and D all resulting in virus transfer above the pass mark. More virus was observed transferring onto PVC than stainless steel (six of seven failures), suggesting this surface is more susceptible to virus transfer.

Impact of aged surface on wipe efficacy

The most notable and statistically significant difference between aged and non-aged surfaces was observed with the efficacy of wipe C against *S. aureus* for both thermally aged (two-way ANOVA; $P<0.0001$) and UV-A -aged PVC (two-way ANOVA; $P<0.0001$) surfaces (Figure 9a). There was also a significant difference (two-way ANOVA; $P<0.0001$) in $\log_{10}$ reduction in *A. baumannii* by wipe C between non-aged and UV-A-aged surfaces (Figure 9c).

Overall, the artificial ageing of surfaces did not significantly impact the efficacy of the wipe products (multiple linear regression; $P>0.05$) but the reduction in viability was significantly impacted by organism type (multiple linear regression; $P=0.0003$) and type of wipe (multiple linear regression; $P<0.0001$).

The impact of surface ageing on virucidal efficacy of pre-impregnated wipes was also explored against vaccinia virus (Figure 10). Wipe D failed to reach the arbitrary 4-$\log_{10}$ reduction on either treated surface. Wipe B on the UV-A-aged surface, and wipe E on the thermally aged surface also failed to reach a 4-$\log_{10}$ reduction in pfu/mL (Figure 10). Only the use of wipe D resulted in the transfer of VV compared with the

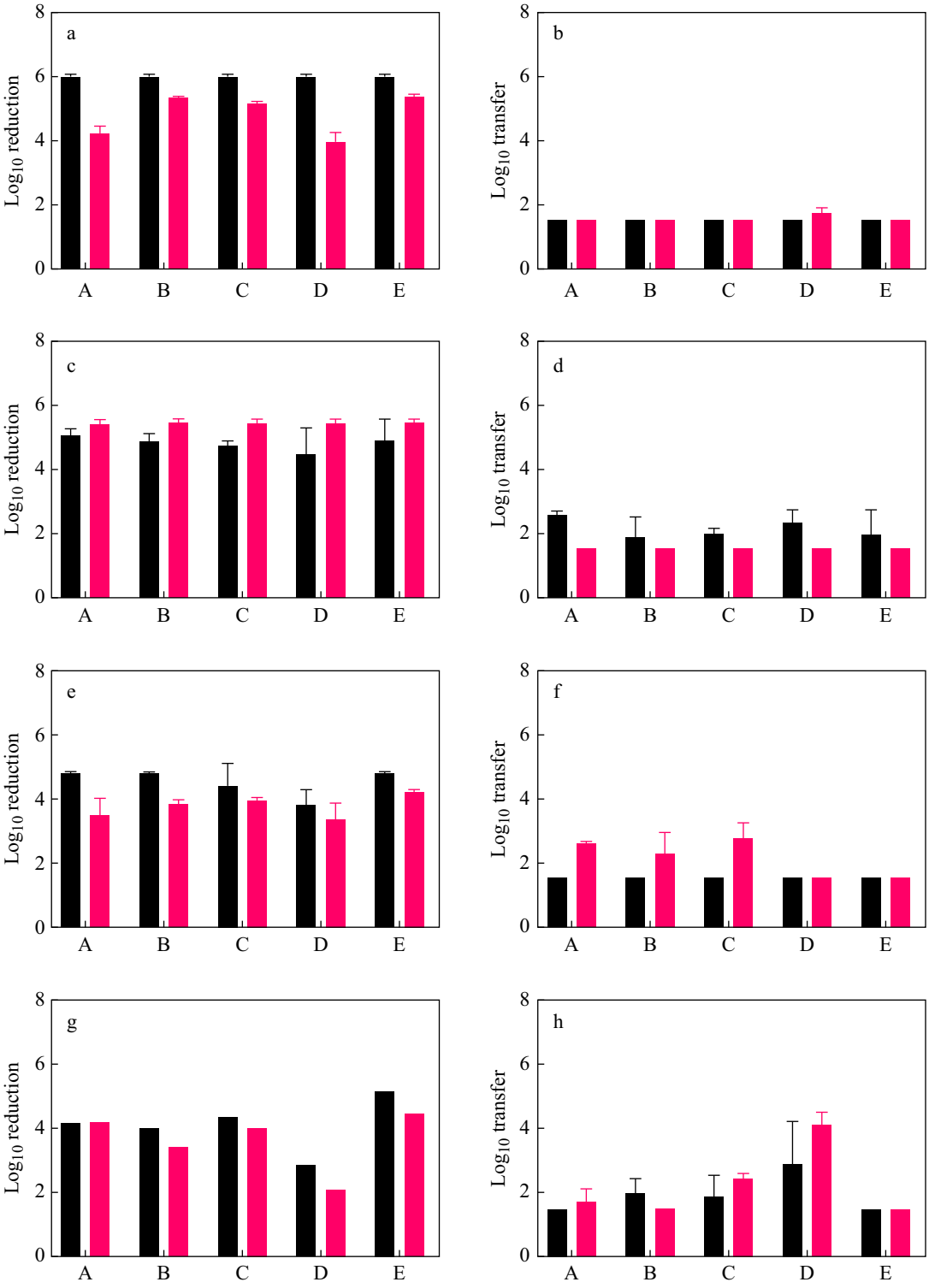

Figure 5. Log_{10} reduction in, and log_{10} transfer of, *Staphylococcus aureus* after wiping on stainless-steel or polyvinylchloride (PVC) surfaces. Log_{10} reduction is shown in (a) 30 s wiping + 5 min contact (300 g weight); (c) 30 s wiping + 30 s contact (650 g weight); (e) 10 s wiping only (650 g weight); (g) 5 s wiping only (300 g weight). Log_{10} transfer is shown in (b), (d), (f) and (h). ($N = 3$). Black, stainless steel; red, PVC.

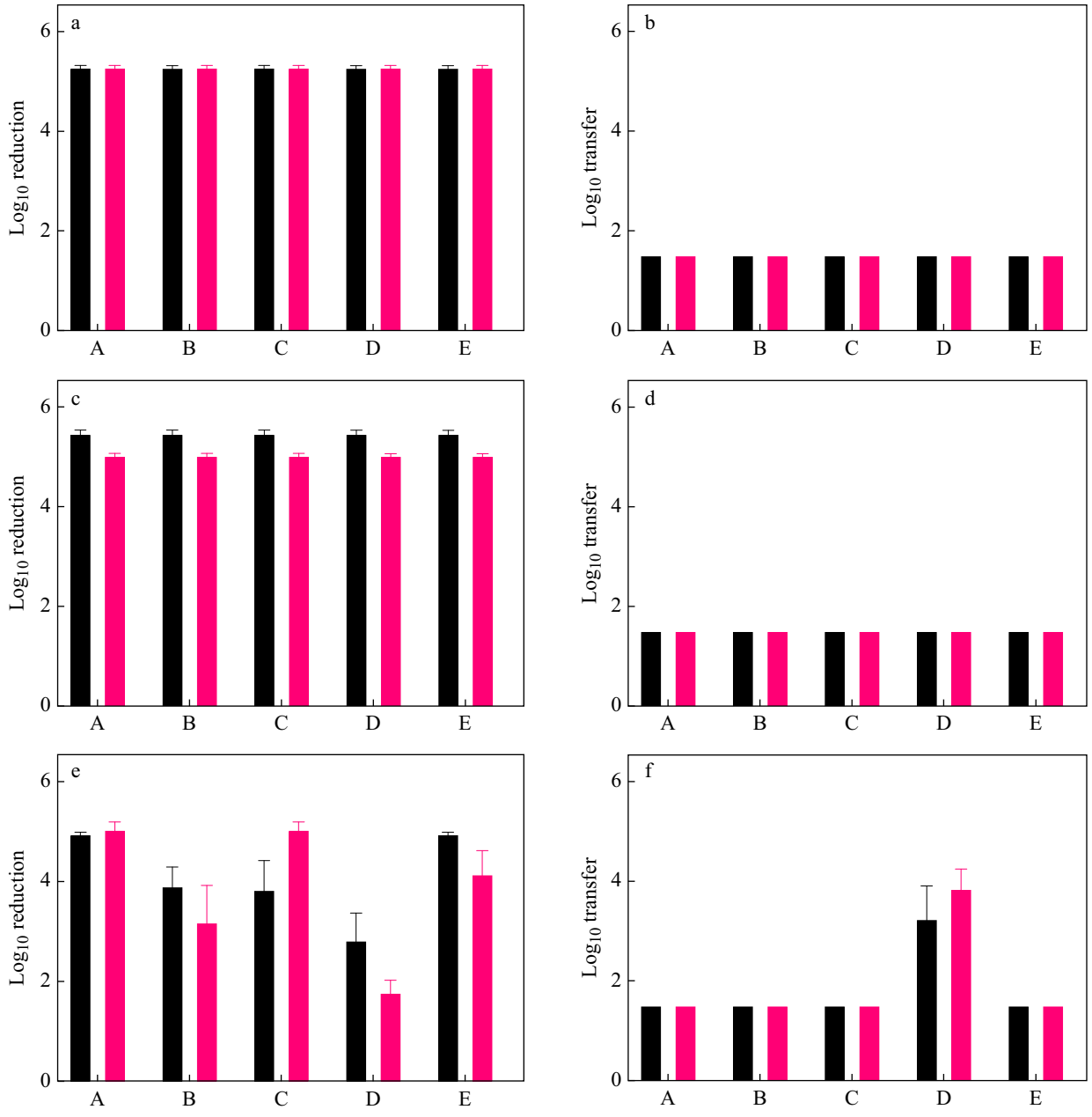

Figure 6. Log_{10} reduction in, and log_{10} transfer of *Acinetobacter baumannii* after wiping on stainless-steel (SS) or polyvinylchloride (PVC) surfaces. Log_{10} reduction is shown in (a) 30 s wiping + 5 min contact (300 g weight; (c) 30 s wiping + 30 s contact (650 g weight); (e) 5 s wiping only (300 g weight). Log_{10} transfer is shown in (b), (d) and (f). ($N = 3$). Black, SS; red, PVC.

other products (Figure 10). Overall, the efficacy of the products did not seem to be affected by surface ageing.

Discussion

Surfaces in healthcare settings play a major role in the transmission and dissemination pathogens [3—6]. Cleaning and disinfection of surfaces are essential measures to control viral and bacterial pathogens [21]. Yet, there are many factors that can compromise the efficacy of disinfectants, some concerning the product, some the target organism, and some related to product application, which includes the type of surface the product would be applied to [22]. When pre-impregnated wipe products are considered, additional factors related to the wipe usage including weight imparted during wiping, wiping time, and contact time post-wiping are important to consider among others [16]. Whilst the type of surface may play an important role, the impact of surface ageing on product efficacy has been rarely considered to date [7]. Disinfectant delivered by wipe can affect surface properties, including hydrophobicity and roughness, but these changes did not affect disinfectant efficacy [7]. The objective of this study was to ascertain the impact of surface type and surface ageing on pre-impregnated wipe product efficacy. Stainless-steel and PVC surfaces were chosen as these are widely incorporated into clinical settings to cover a variety of different furniture and flooring.

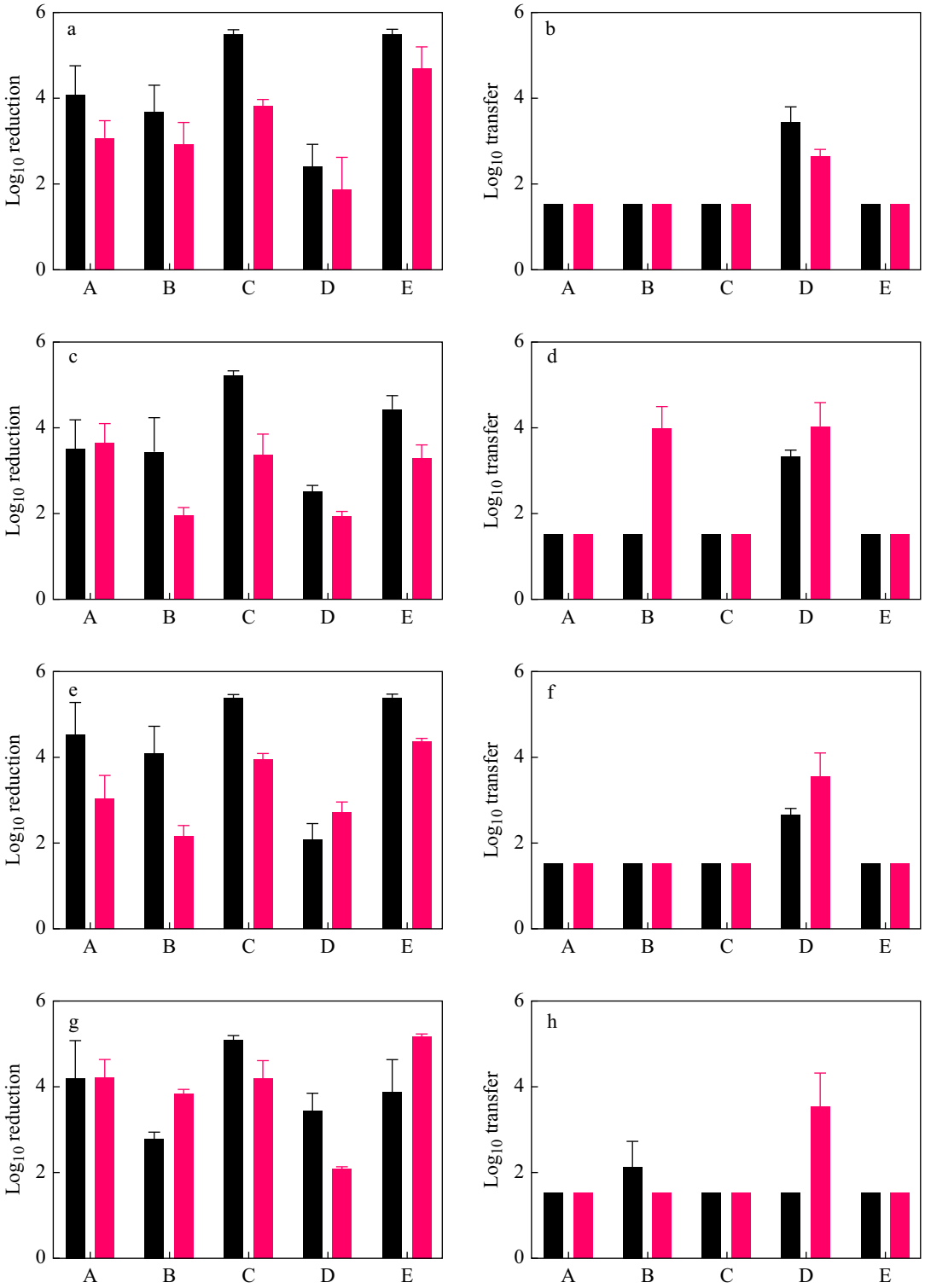

Figure 7. Log_{10} reduction and transfer of enterococci after 5 s wiping, 300 g weight on stainless-steel (SS) or polyvinylchloride (PVC) surfaces. Log_{10} reduction is shown in (a) *Enterococcus hirae* ATCC 10541, (c) *Enterococcus faecium* 18-373, (e) *E. faecium* 22-006 and (g) *E. faecium* AUS0004. Log_{10} transfer is shown in (b), (d), (f) and (h). ($N = 3$). Black, SS; red, PVC.

Surface type impacted significantly on the pre-impregnated wipe efficacy against *S. aureus* but not for the other organisms, although it affected the transfer of bacteria and viruses from PVC in many instances. It has previously been demonstrated with *S. aureus* that for efficacious disinfection, materials must have a low surface roughness (Ra) and be free of microscopic irregularities as these properties decrease cleanability and encourage the retention of micro-organisms on the surface [23].

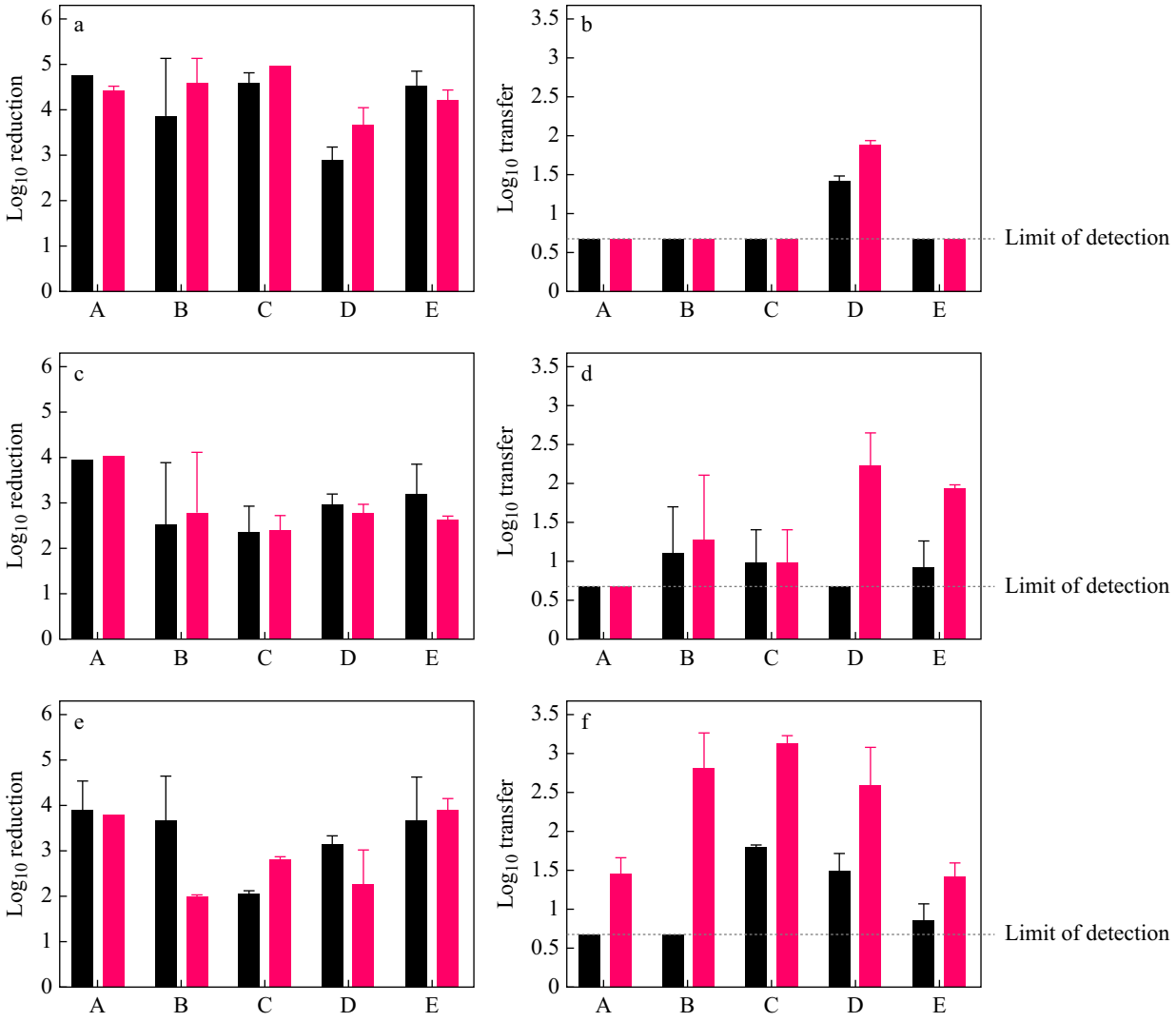

Figure 8. Log_{10} reduction in virus titre is shown for (a) vaccinia virus, (c) mouse norovirus, and (e) adenovirus type 5, and log_{10} transfer for (b) Vaccinia virus, (d) mouse norovirus, and (f) adenovirus type 5 after 5 s wiping, 300 g weight on stainless steel (SS) or polyvinyl-chloride (PVC) surfaces using the Wiperator. The limit of detection was 1.52, where no viable virus was recovered is denoted as a grey dotted line ($N = 2$). Black, SS; red, PVC.

Overall, the effect of surface type on both the reduction in, and transfer of, micro-organisms, either bacteria or viruses, was not predictable, although fewer products achieved a 4-log_{10} reduction with murine norovirus or adenovirus compared to bacteria. We made the same observation with aged surfaces despite the thermally and UV-A irradiation induced surface alterations. Before ageing, PVC surfaces were rougher than stainless-steel surfaces (Figure 1) and were less hydrophobic (Figure 2). Thermal ageing (four cycles of 130 °C and -10 °C exposure) increased PVC roughness compared with an unaged surface as was observed through contact profilometer and SEM imaging. Furthermore, imaging showed that the depth of the layer of polyurethane coating of the surface decreased. These observations are consistent with other studies on thermally aged surfaces. Ito and Nagai [24] showed that thermal exposure (110—120 °C) increases roughness of PVC surfaces and forms voids in the surface due to the proposed rearrangement and aggregation of the molecular chain through the annealing effect. Although the topography of the surface was altered, thermal ageing did not affect the hydrophobicity of PVC when exposed to water (Figure 2). There is no agreement on a correlation between artificial thermal ageing and years of surface exposure under natural conditions. Using controlled ageing conditions, we produced a change in PVC surface roughness. The rougher a surface, the more likely it is for bacteria to reside in pits and pores of a surface, making it more difficult to eliminate as biocide may not reach these areas [7]. However, the change in surface roughness did not always correlate with a decrease in disinfectant efficacy, which depended mainly on product type, wiping conditions and the type of micro-organism.

UV-A exposure (alternating 1-h light (Irradiance 1.50 W/m^2 per nm) and 1-h dark cycles for 200 h) did not result in a significant change in PVC surface roughness (Figure 1) and did not alter surface contact angle with water (Figure 2). SEM imaging showed no distinguishable visible change in PVC surface

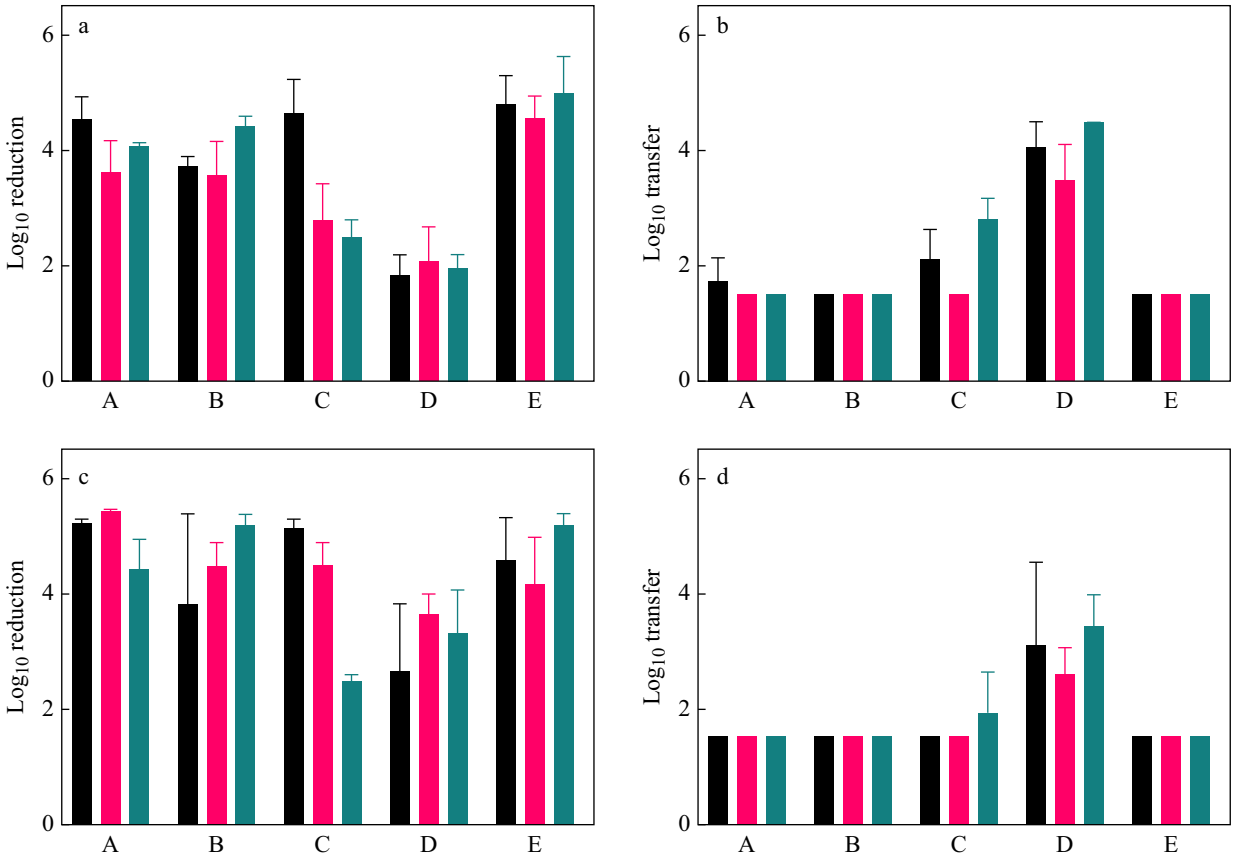

Figure 9. Log_{10} reduction in, and log_{10} transfer of, *S. aureus* (a, b) and *Acinetobacter baumannii* (c, d) after 5 s wiping, 300 g weight on non-aged ($N = 5$), thermally aged ($N = 3$) and UV-A-aged ($N = 3$) surfaces. Limit of detection (1.52 log_{10} transfer) is shown with the grey dotted line. ($N = 3$). Black, non-aged polyvinylchloride (PVC); red, thermally aged PVC; green, UVA-aged PVC.

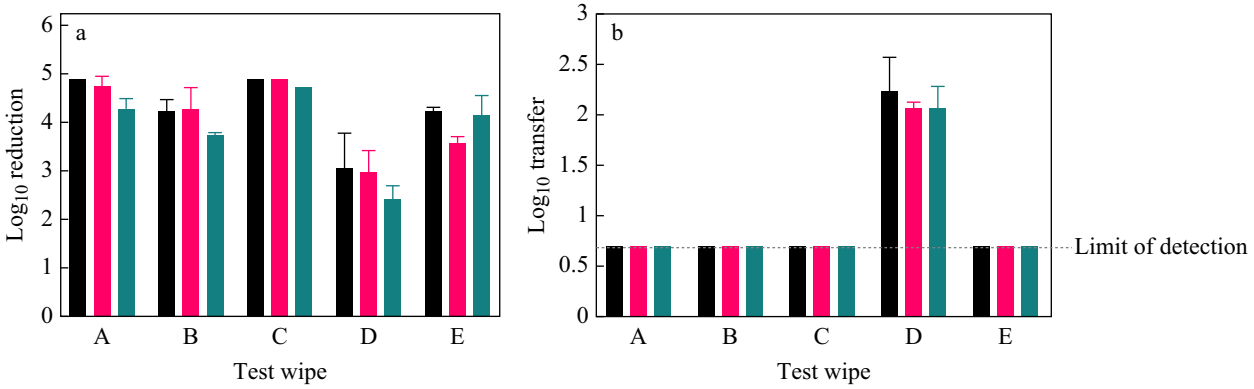

Figure 10. Log_{10} reduction in virus titre (a) and log_{10} transfer of virus (b) were measured for vaccinia virus on polyvinylchloride (PVC) aged via thermal or UV treatment and after 5 s wiping, 300 g weight, using the Wiperator. The limit of detection, where no viable virus was recovered is denoted as a grey dotted line ($N = 2$). Black, non-aged PVC; red, thermally aged PVC; green, UVA-aged PVC.

(Figure 4). Other studies observed that UV-A (wavelength 315—400 nm), UV-B (wavelength 280—315 nm) and UV-C (wavelength 100—280 nm) exposure results in coarser PVC surfaces due to micro-cracks induced by total organic carbon (TOC) leaching, a flow out of inorganic components or plasticizer and photolysis [24—29]. Because surface roughness increases with a longer duration of exposure [24], the 200 h UV exposure might not have been sufficient to produce physical differences in PVC surfaces [29]. The ASTM G154-16 [18] recommends at least 1000 h of UV exposure but such long exposure was not possible to attain for our study. In terms of relevance to surface exposure in practice, Azawa *et al.* [30] found that artificial accelerated weathering of polymer materials for 400—2000 h produced similar degradation to that of one year of

exposure under natural conditions. Due to environmental stressors being unpredictable, accelerated weathering does not replicate natural ageing unequivocally, there are similarities identified in artificially aged surfaces and those aged through natural environmental exposure [19,31]. Accelerated ageing procedures are usually carried out with the worst-case scenario in mind and to maximize the damage caused by the degradation mechanism. Therefore, materials are exposed to much higher or extreme values of key parameters (e.g., temperature, humidity, UV irradiance) in shorter time periods than would occur in real-life scenarios and it is difficult to identify correlation with artificial and natural ageing outcomes [19,20]. Thermal shock is usually employed as an accelerator of material ageing and does not represent true to life temperature conditions that a PVC flooring would endure [19]. Here, only the UV-A spectrum of light was employed, which represents the main component of solar terrestrial radiation. Going forward UV-C wavelengths should be tested to assess surface degradation equivalent to the UV-C disinfection techniques employed in hospital settings.

Despite the lack of observable PVC surface change, UV-A-aged surfaces affected the efficacy of some products against specific micro-organisms in a non-predictable manner.

Other parameters linked to the product itself or product usage had more of an impact on pre-impregnated wipe efficacy. The use of the standardized ASTM E2967-15 wiping test [14] enabled the control of parameters impacting product efficacy [16,32]. Perhaps not surprisingly, the one factor that consistently affected efficacy, including both $\log_{10}$ reduction and preventing bacterial transfer, was the product itself. Whilst the formulation, and the type of active ingredient in particular, dictates the spectrum of microbicidal activity, the wipe material contributes to the overall efficacy of the product, especially the prevention of microbial transfer post-wiping [33]. In addition, wipe material can affect the release of some actives such as quaternary ammonium [33,34]. Wipe D, which contains DDAC with a 100% viscose material did not perform as well as wipe A or C, which contained a more complex formulation and contain PET or PET/viscose as material (Table I). Pascoe *et al*. [35] observed extensive adsorption by viscose material of DDAC solution, in one instance resulting in 89% decrease in DDAC concentration released.

Other factors, particularly the pressure (weight) imparted during wiping and the residual time post-wiping affected product efficacy. Increased weight resulted in better efficacy. Wesgate *et al*. [34] showed that the use of the EN16615-15, another product test (2.3—2.5 kg weight), consistently produced better results than the ASTM E2967-15 test (300 g weight) using a number of different antimicrobial wipe products. Finally, we showed that having a residual time post-wiping was essential to increase product efficacy, although increasing residual time from 30 s to 5 min only benefited *S. aureus*, by decreasing bacterial transfer on stainless steel under $<$50 cfu/mL.

In conclusion, several parameters affect the efficacy of pre-impregnated microbicidal wipe products. Perhaps not surprisingly, the product itself is a major one, but also parameters related to product application such as residual time post-wiping and pressure (here weight) imparted to the surface. The type of surface has been considered as one of the factors that could impact efficacy, but our results showed that, although product efficacy on PVC or stainless steel at times differed, the impact of surface type on product efficacy did not seem to be predictable. Measuring both $\log_{10}$ reduction and transfer were essential to seeing differences in efficacy when they occurred. Product efficacy was also affected by surface ageing, but in a non-predictable manner. Here we used physical ageing protocols based on extreme temperature and UV-A exposure, but we did not explore the impact of physical mechanical damages such as scratching. Whilst standard efficacy tests use pristine materials, often stainless steel, it would be wise for manufacturers to document the efficacy of their products on different types of surfaces, including aged surfaces, to ensure product efficacy under a diverse range of environmental conditions during product usage. This would reassure end users and regulators of the robustness in product efficacy across a range of environmental conditions found at the point of use.

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Conflict of interest statement
C.K., P.T. and G.B. are Diversey employees. All other authors have no potential conflicts of interest to disclose.

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