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Effect of disinfectant formulation and organic soil on the efficacy of oxidizing disinfectants against biofilms

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SUMMARY

Background: Biofilms that develop on dry surfaces in the healthcare environment have increased tolerance to disinfectants. This study compared the activity of formulated oxidizing disinfectants with products containing active ingredients against *Staphylococcus aureus* dry-surface biofilm (DSB) alone.

Methods: DSB was grown in the CDC bioreactor with alternating cycles of hydration and dehydration. Disinfectant efficacy was tested before and after treatment with neutral detergent for 30 s, and in the presence or absence of standardized soil. Biofilms were treated for 5 min with peracetic acid (Surfex and Proxitane), hydrogen peroxide (Oxivir and 6% H_2O_2 solution) and chlorine (Chlorclean and sodium dichloroisocyanurate tablets). Residual biofilm viability and mass were determined by plate culture and protein assay, respectively.

Findings: Biofilm viability was reduced by 2.8 $\log_{10}$ for the chlorine-based products and by 2 $\log_{10}$ for Proxitane, but these products failed to kill any biofilm in the presence of soil. In contrast, Surfex completely inactivated biofilm (6.3 $\log_{10}$ reduction in titre) in the presence of soil. H_2O_2 products had little effect against DSB. Biofilm mass removed in the presence and absence of soil was <30% by chlorine and approximately 65% by Surfex. Detergent treatment prior to disinfection had no effect.

Conclusion: The additives in fully formulated disinfectants can act synergistically with active ingredients, and thus increase biofilm killing whilst decreasing the adverse effect of soil. It is suggested that purchasing officers should seek efficacy testing results, and consider whether efficacy testing has been conducted in the presence of biological soil and/or biofilm.

Introduction

Hospital-acquired infections (HAIs), particularly with multi-drug-resistant organisms (MDROs), are significant contributors to morbidity and a major risk factor for mortality [1]. Multiple predisposing factors contribute to the emergence and spread

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of MDROs, such as unjustified or incorrect use of antibiotics, improper hospital cleaning and lack of hand hygiene compliance. An estimated 20–40% of HAIs are caused by infectious agent transmission via the hands of healthcare personnel [2]. As hands are just as likely to become contaminated from the environment as from touching the patient [3], proper implementation of environmental cleaning and disinfection is of utmost importance [4]. For some organisms, the healthcare environment plays a key role in facilitating their transmission [5]. The risk of acquiring meticillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, *Acinetobacter* spp. and *Clostridium difficile* infections is increased over two-fold if the previous occupant of that room had the infection [6].

Under suitable hospital settings, organisms can proliferate and survive for prolonged periods of time on environmental surfaces, increasing the probability of transmission to patients. The presence of biofilms on dry hospital environmental surfaces has been confirmed [7–9]. These dry-surface biofilms (DSBs) have been shown to be composed of multiple species normally found in both environmental and pathogenic niches, and include MDROs such as MRSA, VRE, *Acinetobacter* spp. and ESBL-producing Gram-negative bacteria [7]. Within DSB, bacteria are highly protected from desiccation, with approximately 50% surviving for over 12 months without nutrition or hydration [7]. Bacteria incorporated into hydrated biofilms have increased tolerance to removal by cleaning agents [10] and disinfectants [11,12]. However, Almatroudi *et al.* [11] have shown *S. aureus* DSB to have more tolerance to chlorine disinfection than biofilms, and may, therefore, act as a constant source of pathogenic bacteria.

Typically, disinfectants used in a healthcare environment in Australia are classified as hospital grade disinfectants. These disinfectants may be used for the disinfection of environmental surfaces such as walls, floors, benchtops etc. Hospital grade disinfectants are not, however, intended for use on medical devices such as non-critical or semi-critical devices. These medical devices require disinfection using instrument grade disinfectants. These are classified as low-level, intermediate-level and high-level instrument grade disinfectants. The choice of instrument grade disinfectant is typically governed by the Spaulding classification (Table I) [13].

In order to be approved and registered by the Australian Therapeutic Goods Administration (TGA), a hospital grade is required to pass the TGA disinfectant test, and a bactericidal carrier test such as the AOAC hard surface carrier test (Table I) [14]. The TGA test requires challenging diluted disinfectant with a planktonic bacterial inoculum (2×10^8–2×10^9 organisms) and measuring viability after a given time. Following this, a second challenge inoculum is added and viability is determined after a given time. The bacteria tested include *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli* and *S. aureus* [14]. Depending on the product label, the test is conducted under either Option A (no organic soil) or Option B (addition of organic soil), with Option B being more reflective of clinical conditions than Option A.

Despite the recommendations of the Australian and other jurisdictional regulators, to date, there is little or no guidance on disinfectants capable of disrupting biofilm. The International Organization for Standardization (ISO) standard for automated endoscope reprocessors (ISO 15883-4: 2008) mandates a cleaning efficacy test against a hydrated model biofilm soil, and several detergent systems with claims against the Annex F biofilm soil in ISO TS 15883-5: 2006 are available on the market [15].

Disinfectants used in hospitals, such as alcohol, quaternary ammonium compounds and oxidizing agents, are expected to be effective against organisms in the hospital environment. However, to date, there are no cleaning and/or disinfecting products demonstrated to remove DSB from hospital environmental surfaces. Failure to eradicate biofilm and thus pathogens from environmental surfaces is a great challenge to HAI. Therefore, the aim of this study was to assess the efficacy of three commonly used oxidizing agents (active ingredients) – peracetic acid, hydrogen peroxide and chlorine – against *S. aureus* DSB and to determine if non-active additives, added to disinfectant formulations, affect the efficacy of active ingredients.

Table I
Microbiological soil testing required for disinfectant registration by the Australian Therapeutic Goods Administration (TGA)

Disinfectant grade	Data required
Hospital grade	Option A or B of the TGA disinfectant test Bactericidal carrier test
Low-level instrument grade	Option B of the TGA disinfectant test Bactericidal carrier test Virucidal test data (minimum carrier test with enveloped/lipid virus)
Intermediate-level instrument grade	Option B of the TGA disinfectant test Bactericidal carrier test Fungicidal test Tuberculocidal carrier and enumerated test Virucidal test data (minimum of polio/parvo virus, adenovirus and herpes virus)
High-level instrument grade	As for intermediate-level instrument grade plus: Sporicidal tests (carrier) Sporicidal D value tests Simulated in-use tests

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Materials and methods

Bacterial culture preparation

S. aureus (ATCC 25923) DSB was grown *in vitro* on polycarbonate coupons (Bio Surface Technologies Corporation, Bozeman, MT, USA) in the CDC bioreactor (Bio Surface Technologies Corporation) over a period of 12 days, as detailed previously [16]. Briefly, growth was initiated by adding 10^8 *S. aureus* to 500 mL of 5% tryptone soya broth (TSB) and grown under sheer (provided by baffle rotation at 130 revolutions per min) for 48-h batch phase at 35 °C, after which the media was drained, and the biofilm was dehydrated for 48 h at room temperature (22–25 °C) with filter-sterilized air-conditioned air (average relative humidity 66%) pumped into the bioreactor at 3 L/min. An additional three cycles of batch growth (5%TSB, shear, 35°C for 6 h) alternated with prolonged dehydration phases of 66, 42 and 66 h at room temperature resulted in an average of 2.078×10^6 ($\log_{10}$ 6.30 ± 0.127) colony-forming units (cfu) of *S. aureus* per control coupon ($N = 29$).

An overnight culture of *S. aureus* (ATCC 25923) in TSB was used for planktonic challenges.

Test disinfectants

The products used in this study were of two types: fully formulated products and close generic equivalents (Table II). Formulated products were Surfex (Whiteley Medical, North Sydney, Australia), Chlorclean (Guest Medical, Aylesford, UK) and Oxivir Tb (Diversey Australia Pty Ltd, Smithfield, NSW, Australia).

Surfex, a low-level instrument grade disinfectant, comprises a powder blend consisting of a hydrogen peroxide source (sodium percarbonate), an acetyl source (tetraacetylethylenediamine), chelating agents and sodium dodecyl sulphate, which on initial dissolution in water releases a mixture of approximately 1000 mg/L hydrogen peroxide and 2100 mg/L peracetic acid. The product also has specific claims against a range of organisms, and is indicated for the disinfection of environmental surfaces.

Chlorclean is a tableted hospital grade disinfectant comprising sodium dischoroisocyanurate [17] formulated with a foaming anionic surfactant (sodium toluenesulfonate) and binders (adipic acid), which on dissolution in water releases 1000 mg/L chlorine. The product is a listed hospital grade disinfectant, meaning the product does not have specific claims.

Oxivir Tb is a ready-to-use hospital grade disinfectant solution comprising 0.5% hydrogen peroxide, formulated with other proprietary ingredients to give 5000 mg/L hydrogen peroxide. This product is an example of the 'Accelerated Hydrogen Peroxide' technology licensed from Virox Inc. (Oakville, ON, Canada) [18], and has specific claims against a range of organisms.

Generic equivalents of these three disinfectants were: Proxitane (Solvay Interox, Botany, NSW, Australia), an equilibrium solution of hydrogen peroxide (27% w/w), acetic acid (7.5% w/w) and peracetic acid (5.0% w/w), which on dilution in water gives a 4% v/v mixture of 10,000 mg/L hydrogen peroxide and 2200 mg/L peracetic acid; an unformulated sodium dichloroisocyanurate (SDIC) tablet (Redox Chemicals, Minto, NSW, Australia) containing sodium diisocyanurate alone that, on dissolution in water, releases 1000 mg/L; and a 6% solution of hydrogen peroxide (Gold Cross, Biotech Pharmaceuticals Pty Ltd, Laverton North, Victoria, Australia) to give 6000 mg/L hydrogen peroxide.

All disinfectants were dissolved or diluted in artificial hard water which was prepared by dissolving 0.304g anhydrous $CaCl_2$ and 0.065g anhydrous $MgCl_2$ in distilled water to make 1 L [16].

Experimental protocol for testing disinfectant efficacy against planktonic and DSB bacteria

The efficacy of test disinfectants to kill control planktonic and biofilm bacteria was measured in the presence and absence of organic soil [5% bovine calf serum (BCS) and 10% bovine serum albumin (BSA) in phosphate-buffered saline (PBS)]. The effect of prior treatment of biofilm with a neutral detergent reconstituted in accordance with the manufacturer's instructions (Speedy Clean, Whiteley Medical, North

Table II
Test disinfectants and their components

Product	Composition	Concentration of active ingredients (at use)
Formulated products		
Surfex powder	Sodium percarbonate 49% Tetraacetylethylenediamine 27% Sodium dodecyl sulphate 0.65% Chelating agents 7.9%	1100 mg/L hydrogen peroxide 2200 mg/L peracetic acid
Chlorclean tablet	Sodium dischoroisocyanurate >30% Sodium toluenesulfonate 5–10% Adipic acid <12%	1000 mg/L chlorine
Oxivir Tb ready-to-use solution	0.5% (5000 mg/L) accelerated hydrogen peroxide + surfactants	5000 mg/L hydrogen peroxide
Generic equivalents		
Proxitane solution	Hydrogen peroxide 27% Acetic acid 7.5% Peracetic acid 5%	10,080 mg/L hydrogen peroxide 2200 mg/L peracetic acid
20 g SDIC tablets	Sodium diisocyanurate	1000 mg/L chlorine
6% hydrogen peroxide solution	6% hydrogen peroxide	0.6% (6000 mg/L) hydrogen peroxide

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Sydney, Australia) on disinfectant efficacy was also tested (Figure 1). Each condition was tested with five replicates to determine residual bacterial number (cfu) and five replicates to determine residual protein contamination.

Protocol for efficacy testing against planktonic and biofilm bacteria

The following protocols were followed for efficacy testing of disinfectants against planktonic and DSB bacteria:

(a) Disinfectant efficacy in the absence of organic soil was tested by mixing 1 mL of test disinfectant (all disinfectants) with 1 mL of hard water, and immediately adding 10 μL of TSB containing approximately 10^9 planktonic bacteria for the planktonic challenge or a biofilm-coated coupon for the DSB challenge, for a contact time of 5 min ($N = 5$/disinfectant) (Figure 1, Box 1).

(b) Disinfectant efficacy in the presence of organic soil was tested by mixing 1 mL of test disinfectant (all disinfectants) with 1 mL of organic soil, and immediately adding 10 μL of TSB containing approximately 10^9 planktonic bacteria for the planktonic challenge or a biofilm-coated coupon for the DSB challenge, for a contact time of 5 min ($N = 5$/disinfectant) (Figure 1, Box 2).

(c) It was confirmed that the neutral detergent had no biocide action by mixing 10 μL of TSB containing approximately 10^9 bacteria with either 1 mL of Speedy Clean for 30 s or hard water (positive control), followed by serial dilution and plate culture (results not shown). The effect of prior biofilm contact with neutral detergent on disinfectant efficacy was tested by soaking a DSB-covered coupon in 1 mL of Speedy Clean for 30 s, removing the coupon from the detergent, and adding it immediately to the disinfectant test mixes (Chlorclean, SDIC and Surfex) in the absence of organic soil ($N = 5$/disinfectant) (Figure 1, Box 3) or in the presence of organic soil ($N = 5$/disinfectant) (Figure 1, Box 4). The DSB-coated coupons were left in contact with the disinfectant for 5 min.

(d) For Parts a–c, at the end of the 5-min contact time, disinfectant activity was inactivated completely by the addition of 1 mL of neutralizer containing 1% sodium thiosulphate, 6% Tween 80, 5% BCS and 10% BSA in PBS (Figure 1, Box 5).

(e) Residual bacterial viability for planktonic control was determined by serial 10-fold dilution and overnight plate

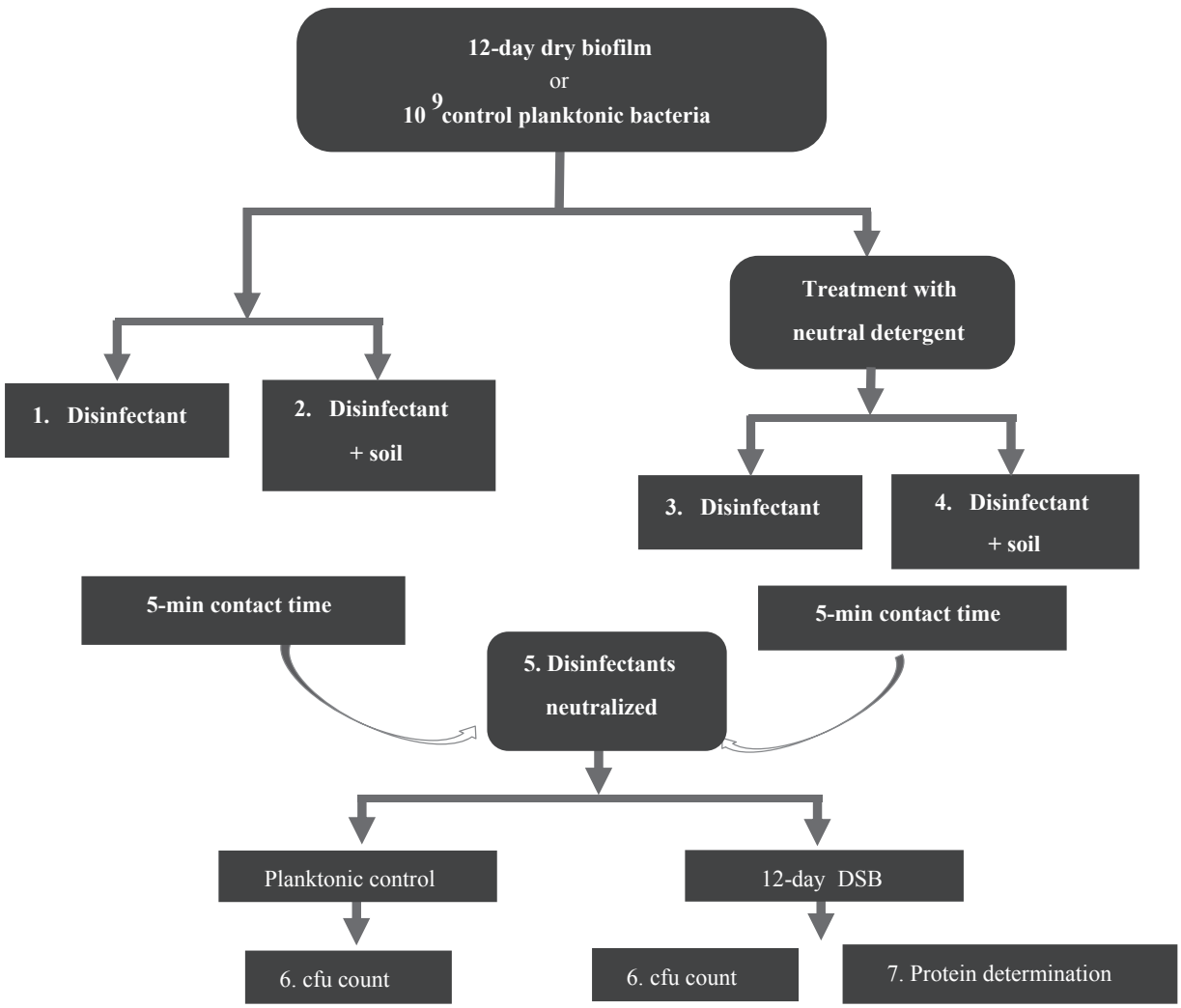

Figure 1. Experimental protocol for disinfection testing. cfu, colony-forming units; DSB, dry-surface biofilm.

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culture at 37°C and cfu determination (Figure 1, Box 6). Biofilm viability for DSB was determined by subjecting control and test coupons to sonication at 43 ± 2 kHz for 20 min prior to serial 10-fold dilution and overnight plate culture at 37°C and cfu determination (Figure 1, Box 6).

(f) The experiment was repeated and the amount of residual protein contaminating disinfected coupons was determined using a bicinchoninic acid assay (Micro BCA assay; Thermo Scientific, Waltham, MA, USA) (Figure 1, Box 7).

Controls

The positive controls for the planktonic challenge (five replicates for each disinfectant) were subjected to the same treatments as described above, but biocides were replaced with hard water.

Positive (DSB-covered coupons) and negative (clean sterile coupons; three for each disinfectant) controls were subjected to the same treatments as described above, but biocides were replaced with hard water.

For the neutralization control, confirmation that disinfectant activity was completely inactivated by the neutralizer was achieved by the addition of 1 mL of the neutralizer to the disinfectant test mixture prior to adding a DSB-covered coupon and reacting for 5 min prior to cfu determination ($N = 10$/test disinfectant) (results not shown).

The amount of residual protein contaminating coupons was determined by alkaline hydrolysis of the biofilm as described by Li *et al.* (2006), followed by the Micro BCA assay. Briefly, each coupon was rinsed three times in 10 mL of PBS and transferred to individual McCartney bottles containing 1 mL of ice-cold 20 mM 2M-Morpholino-ethane sulfonic acid 0.9% saline. A 120-μL aliquot of 30% NaOH was added, the samples were sonicated at 60°C for 1 h, vortexed and then incubated at 30°C for 30 min, followed by incubation in a boiling water bath for 15 min. The samples were cooled and 86 μL of 32% HCl was added prior to centrifuging at 13,000 rpm in a bench top centrifuge for 5 min. An aliquot (1 mL) of the supernatant was used for protein determination. Residual protein contaminating samples was determined by measuring sample absorbance at 562 nm wavelength, subtracting the absorbance of negative control coupons ($N = 3$) and calculating the protein concentration (μg/mL) using a standard curve prepared using the kit's standard, according to the manufacturer's instructions.

Statistical analysis

One-way analysis of variance combined with the Holm–Sidak all pairwise multiple comparison procedure was used to test for significant differences in $\log_{10}$ reduction in titre using SigmaPlot 13 (Systat Software, San Jose, CA, USA). A Mann–Whitney rank sum test was used to test for significant differences in the $\log_{10}$ reduction in microbial titre between coupons subjected to prior detergent treatment and no detergent treatment.

Results

Disinfectant efficacy in the presence and absence of soil

S. aureus planktonic

In the absence of organic soil and with a 5-min contact time, all the disinfectants used in this study killed 7 $\log_{10}$ of planktonic organisms. The efficacy of the formulated peracetic acid disinfectant Surfex was unaffected by organic soil, whereas the efficacy of the generic disinfectant Proxitane was greatly reduced. The efficacies of hydrogen-peroxide- and chlorine-based disinfectants were also highly affected by the presence of organic soil (see Figure 2).

S. aureus DSB

Positive control DSB coupons had a mean of 2.08×10^6 ($\log_{10}$ 6.32 ± 0.127) cfu of *S. aureus* per coupon ($N = 29$). In the

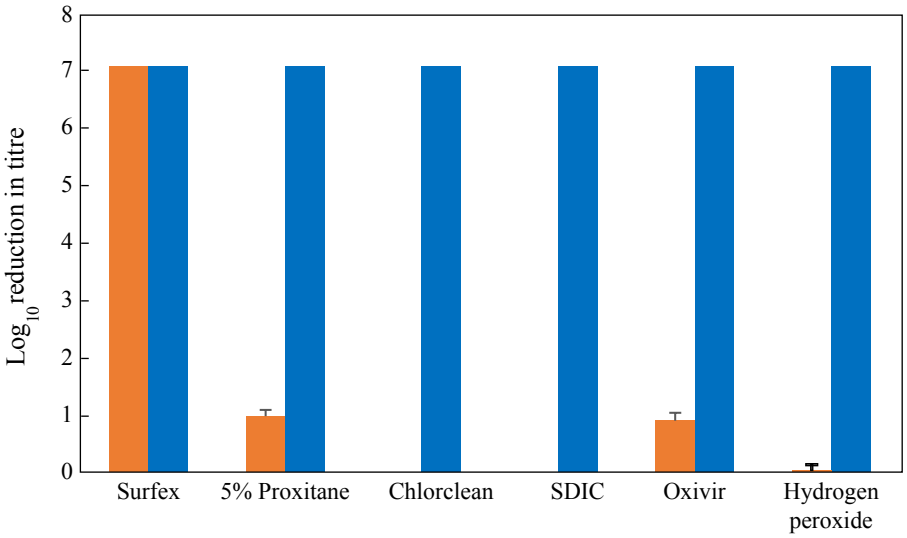

Figure 2. $\log_{10}$ reduction in planktonic *Staphylococcus aureus* titre following a 5-min contact with disinfectants containing peracetic acid and hydrogen peroxide (Surfex, Proxitane), chlorine [Chlorclean, sodium dichloroisocyanurate (SDIC)] and hydrogen peroxide (Oxivir, hydrogen peroxide) as active ingredients. Disinfectant efficacy was determined in hard water with (orange bars) and without (blue bars) added biological soil.

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absence of organic soil and with a 5-min contact time, the chlorine-based disinfectants, SDIC and Chlorclean, reduced biofilm viability by 2.8 $\log_{10}$ ($P < 0.001$). For both SDIC and Chlorclean, disinfectant efficacy was significantly decreased in the presence of soil, resulting in no reduction in titre ($P < 0.001$). In contrast, the addition of the organic soil had no effect on the efficacy of Surfex, completely inactivating DSB resulting in >6 $\log_{10}$ reduction in titre ($P < 0.001$) (Figure 3). Whilst the generic equivalent to Surfex, Proxitane, significantly reduced cfu 4.15 $\log_{10}$ ($P < 0.002$) in the absence of soil, it failed to kill DSB in the presence of soil. Chemistries based solely on hydrogen peroxide performed poorly against DSB, with only Oxivir Tb reducing biofilm counts by approximately 1 $\log_{10}$ ($P = 0.01$) in the absence of soil, and the presence of soil inactivated Oxivir Tb. Generic hydrogen peroxide had no activity. In the absence of soil, Surfex killed 3.5 $\log_{10}$ (>3000)-fold more biofilm bacteria than the next best product, and >6 $\log_{10}$ more in the presence of soil ($P < 0.001$). In the absence of soil, chlorine-based products, Chlorclean and SDIC, killed significantly more DSB than Proxitane ($P < 0.001$), which killed significantly more bacteria than Oxivir Tb ($P < 0.001$), which in turn had greater efficacy than generic hydrogen peroxide ($P < 0.001$) (Figure 3).

Disinfectant efficacy following detergent treatment in the presence or absence of soil

Treatment of biofilm-covered coupons with detergent prior to disinfection in the absence of soil marginally increased the number of biofilm bacteria killed by chlorine-based products, Chlorclean and SDIC, but this was not significant (Figure 4). There was no improvement in kill by prior detergent treatment in the presence of soil. As Surfex resulted in complete kill (>6 $\log_{10}$ reduction in titre) under all conditions tested, it was not possible to measure the effect of prior biofilm contact with detergent.

Disinfectant efficacy in removing biofilm mass

The ability of the disinfectants to remove DSB was evaluated by determining the amount of biofilm protein remaining on the coupons following treatment. Percentage biofilm removal for Surfex in the presence and absence of soil was 64.7% and 65.3%, respectively, whereas the reduction in biofilm mass by chlorine-based disinfectants was 17.6% and 22.14% for Chlorclean and 13.12% and 29.71% for SDIC in the presence and absence of soil, respectively (Figure 5). As the bacterial viability reduction rate was very low for Proxitane and hydrogen-peroxide-based disinfectants, it was assumed that these disinfectants would have no significant effect on biofilm mass, and thus residual protein determination was not conducted for these disinfectants.

Discussion

In this study, *S. aureus* DSB [16] was chosen for testing hospital surface disinfectants as 50% of clinical biofilms incorporate *S. aureus* [7] which commonly causes HAI [19]. The efficacy of three formulated disinfectants, based on three differing active ingredients (chlorine, hydrogen peroxide and peracetic acid), along with generic (unformulated) solutions containing these three active ingredients were evaluated. In this manner, the excipient (non-active) ingredients, as well as the active ingredients themselves, could be evaluated. Tests were undertaken in the presence of organic soil, as combined cleaning/disinfecting systems are becoming more popular as clinical surfaces are often not precleaned prior to disinfection. Thus, efficacy testing in the presence of large amounts of organic soil is more reflective of worse-case clinical conditions.

This study evaluated three formulated, commercially available disinfectant systems, each of which contained an oxidizing biocide, along with other ingredients such as surfactants. The effect of the addition of the proprietary ingredients

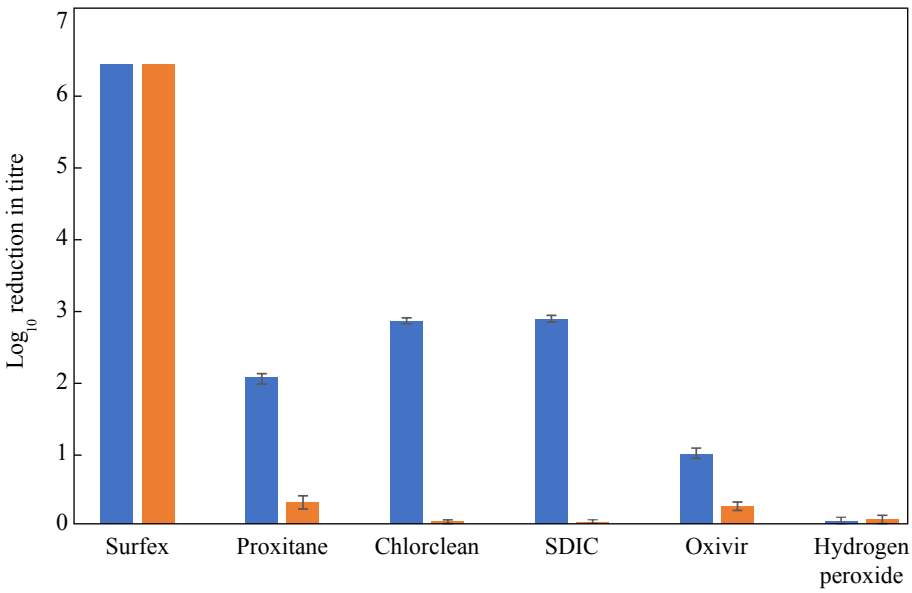

Figure 3. $\log_{10}$ reduction in biofilm titre following a 5-min contact with disinfectants containing peracetic acid and hydrogen peroxide (Surfex, Proxitane), chlorine [Chlorclean, sodium dichloroisocyanurate (SDIC)] and hydrogen peroxide (Oxivir, hydrogen peroxide) as active ingredients. Disinfectant efficacy against $\log_{10}$ 6.32 *Staphylococcus aureus* dry-surface biofilm was determined in hard water with (orange bars) and without (blue bars) added biological soil.

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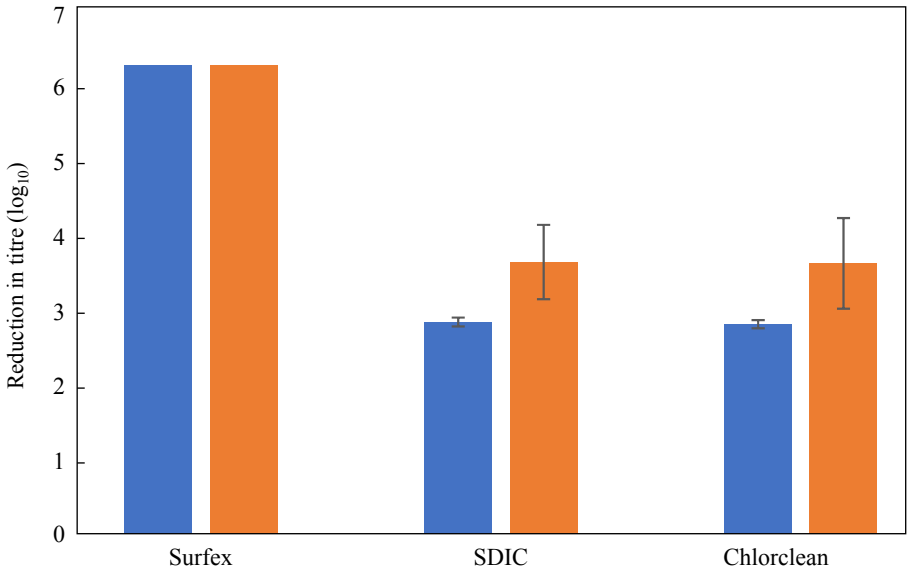

Figure 4. Reduction in dry-surface biofilm titre (log_{10}), in the absence of biological soil, obtained with (orange bars) and without (blue bars) prior biofilm contact with detergent (Speedy Clean for 30 s) followed by Surfex, sodium dichloroisocyanurate (SDIC) and Chlorclean disinfection for a contact time of 5 min.

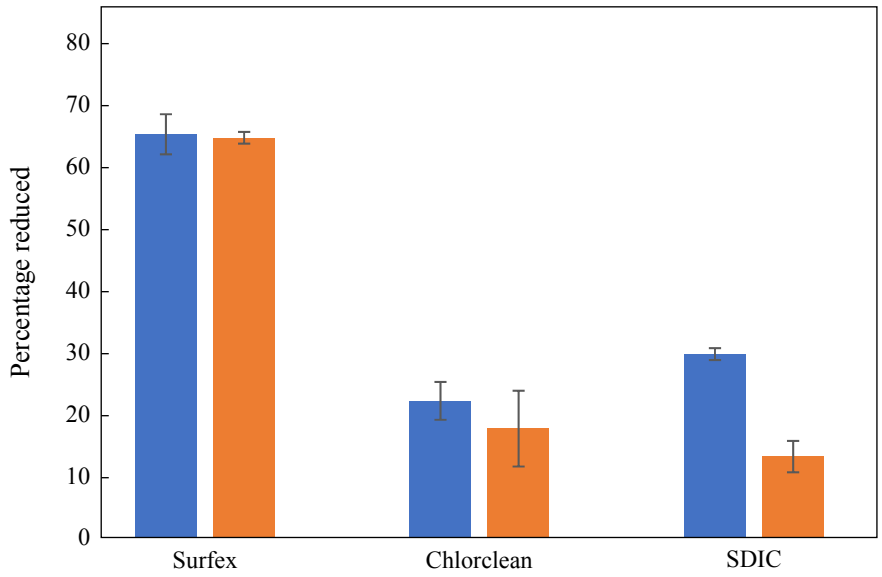

Figure 5. Percentage reduction of biofilm mass (protein) after disinfection with Surfex, Chlorclean and sodium dichloroisocyanurate (SDIC) for 5 min in the presence (orange bars) and absence (blue bars) of soil.

to disinfectant efficacy was evaluated by comparing the formulated disinfectants with generic equivalents in a bid to determine if biofilm removal is due to the active ingredient alone or if the proprietary ingredients act in synergy with the active ingredient. The outstanding performer in this study was Surfex, which completely inactivated the DSB in the presence or absence of soil. The formulated chlorine-based product Chlorclean, and unformulated SIDC tablets, were the next best performers, although they killed significantly fewer biofilm bacteria (3 log_{10}) than Surfex ($P < 0.001$) and only in the absence of soil. Previous studies have demonstrated that chemicals such as hypochlorite are consumed by the surface layers of the biofilm neutralizing the disinfectant before it can penetrate into deeper layers [20], making hydrated biofilm more tolerant than planktonic cells to these disinfectants [12]. However, a study on the efficacy of hypochlorite against DSB found that this semi-dehydrated biofilm was more tolerant to hypochlorite than hydrated biofilm [11]. The water content of hydrated *S. aureus* biofilm grown in the CDC bioreactor is 90%, whilst that of DSB is 61% [21]. This lower water content, in combination with the thicker extracellular polymeric substances (EPS), may result in lower diffusion of biocides and hence contribute to biocide tolerance.

Even in the absence of soil, the hydrogen-peroxide-based disinfectants killed significantly less biofilm bacteria than disinfectants based on chlorine or a combination of peracetic acid

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and hydrogen peroxide ($P < 0.001$). Oxivir killed approximated 1 $\log_{10}$ of the biofilm bacteria, while hydrogen peroxide solution had no effect; however, the manufacturer-recommended contact time for Oxivir for killing bacteria is 10 min, not 5 min as used in the study, and this could explain its lower performance. However, even a contact time of 5 min is probably excessive given the way in which dry hospital surfaces are cleaned. The majority of disinfectants have no residual effect and are only active when wet.

The difference in kill rates between Surfex (formulated additives) and Proxitane (no additives) suggests that the activity of Surfex against DSB may be governed not only by the active ingredients (hydrogen peroxide and peracetic acid), but also by other factors such as the added surfactants or excipients, chelating agents or its solution pH. Surfactants may increase diffusion of the active ingredients into the biofilm (due to a lowering of the solution surface tension, and hence improved wetting of the biofilm surface). Increased diffusion is likely to result in increased biofilm kill as all of the tested disinfectants, in the absence of organic soil, can kill 7 $\log_{10}$ of planktonic organisms. Chelating agents complex any calcium and magnesium ions present in the hard water, plus any other interfering metals often present in tap water such as iron and manganese, and thus increase disinfectant performance in hard water. Additionally, the source of peracetic acid in the two disinfectants is different, which under certain circumstances (e.g. disruption of Proxitane equilibrium) may affect levels of active ingredients. Proxitane is an equilibrium mixture formed by the reaction between hydrogen peroxide and acetic acid according to the following formula: $H_2O_2 + CH_3CO_2H \rightleftharpoons CH_3CO_3H + H_2O$ [18]. However, in Surfex, the peracetic acid is generated by the reaction of hydrogen peroxide with tetraacetylethylenediamine [22]. The source of hydrogen peroxide in Surfex is sodium peroxycarbonate, a 2:3 complex of hydrogen peroxide and sodium carbonate, that releases hydrogen peroxide on dissolution in water.

Except for Surfex, the efficacy of disinfectants was significantly decreased by the addition of soil, with little or no reduction in the viable bacteria load. This result is in agreement with most reports of chlorine disinfectants, where serious loss of efficacy has been demonstrated by the presence of organic matter [23] and hard water [24,25]. Both hydrogen peroxide and peracetic acid are effective oxidizing biocides. This study showed that the addition of organic soil had no effect on the efficacy of Surfex, whilst the generic equivalent, diluted Proxitane, was inactivated. This is most likely due to the other ingredients within the formulation, such as chelating agents, or perhaps due to the differences in pH (8.10 for Surfex vs 2.6 for a 4% solution of Proxitane). Compared with hydrogen peroxide, peracetic acid has the disadvantage that it is less stable when diluted, dissociating into acetic acid and hydrogen peroxide over a matter of hours due to the shift in equilibrium conditions brought on by dilution in water.

The very short detergent treatment used in this study was to simulate someone gently wiping over a surface with a damp cloth, thus wetting the surface of the DSB with surfactants to increase biocide activity. This detergent treatment had no significant effect on the efficacy of the three biocides tested (Chlorclean, SDIC and Surfex). However, even if hospital surfaces are precleaned, the likelihood of DSB being present is high [7–9].

Almatroudi *et al.* [16] demonstrated that protein was a principal component (56%) of both the in-vitro DSB model and biofilms contaminating dry clinical surfaces in hospitals with protein contents varying from 42% to 95%. Therefore, the present study measured residual protein on the treated coupons to determine the proportion of biofilm mass removed by the oxidizing action of the disinfectants. None of the disinfectants were able to completely remove all biofilm protein with a 5-min contact time; however, a higher percentage reduction of biofilm protein was observed in 5 min with Surfex (65%) than the other tested disinfectants (<30%), both in the presence and absence of soil.

In conclusion, disinfectant efficacy against biofilm can vary significantly, despite containing similar levels of biocides, due to their formulation/additives. The disinfectant formulation also affects disinfectant action in the presence of soil. Therefore, it is crucial to select clinically efficient disinfectant agents with the potential of effectively eradicating dry biofilm from hospital environments. It is suggested that purchasing officers should ask disinfectant manufacturers for efficacy testing results, and consider whether efficacy testing has been conducted in the presence of biological soil and/or dry biofilm.

Conflict of interest statement

Whiteley Corporation was the industrial partner associated with the Australian Research Council Linkage Project. They are a manufacturer of disinfectants and detergents for use in health care and one of their products was tested in this study. Their role did not lead to any bias in formulating, executing, analysing or writing up the research.

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