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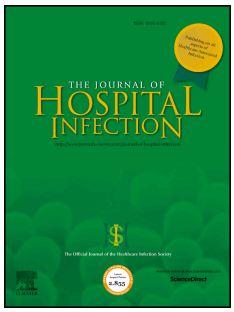

The effect of disinfectant formulation and organic soil on the efficacy of oxidising disinfectants against biofilms

Durdana Chowdhury, MBBS, M. Phil, Arifur Rahman, BSc, MSc, Honghua Hu, BSc, Grad DipSc, PhD, Slade O. Jensen, Anand Kumar Deva, BSc (Med), MBBS, MS, FRACS, Karen Vickery, BVSc (Hons), MVSc, PhD, MASM

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Durdana Chowdhury**,** MBBS, M. Phil [a]
Arifur Rahman BSc, MSc [a]
Honghua Hu, BSc, Grad DipSc, PhD[a]
Slade O. Jensen [b,c,]
Anand Kumar Deva, BSc (Med), MBBS, MS, FRACS[a]
*Karen Vickery, BVSc (Hons), MVSc, PhD, MASM[a]

Authors' Affiliation

[a] Surgical Infection Research Group, Faculty of Medicine and Health Sciences, Macquarie University, New South Wales, Australia

[b] Antibiotic Resistance and Mobile Elements Group, Ingham Institute for Applied Medical Research, Liverpool, New South Wales,
Australia

[c] Medical Sciences Research Group, School of Medicine, Western Sydney University, New South Wales, Australia

Corresponding author

* Corresponding author. Address: Faculty of Medicine and Health Sciences, Macquarie University, New South Wales, Australia.
Tel.: +61 2 9850 2773
E-mail address: karen.vickery@mq.edu.au

Summary

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

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 seconds and in the presence or absence of standardized soil. Biofilms were treated for 5 minutes with peracetic acid (Surfex and Proxitane), hydrogen peroxide (Oxivir and 6% H_2O_2 solution) and chlorine (Chlorclean and sodium dichloroisocyanurate [SDIC] 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 the soil. In contrast, the formulated Surfex completely inactivated biofilm (6.3log_{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. We suggest that purchasing officers seek efficacy testing results and consider whether efficacy testing has been conducted in the presence of biological soil and/or biofilm.

Key Words

Oxidising disinfectants

Disinfectant efficacy

Biofilms

Disinfection

Dry hospital surfaces

Removing biofilms

Introduction

Hospital acquired infections (HAI), particularly with multidrug-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 of MDROs such as unjustified or incorrect use of antibiotics, improper hospital cleaning and lack of hand hygiene compliance. An estimated 20-40% of HAI are caused by infectious agent transmission via the hands of health care personnel [2]. As hands are just as likely to become contaminated from the environment as touching the patient [3] proper implementation of environmental cleaning and disinfection is of upmost importance [4]. For some organisms, the healthcare environment plays a key role in facilitating their transmission [5]. The risk of acquiring methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *enterococci* (VRE), extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, *Acinetobacter* spp. and *Clostridium difficile* infections is increased over 2-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 (DSB) 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 and ESBL-producing Gram-negative bacteria [7]. Within DSB, bacteria are highly protected from desiccation with approximately 50% surviving 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. (2016) [11] have shown *S. aureus* DSB to have more tolerance to chlorine disinfection than biofilm, 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, see Table I [13].

In order to be approved and registered by the Australian Therapeutic Goods Administration, a hospital grade is required to pass the TGA Disinfectant Test, and a bactericidal carrier test such as the AOAC Hard Surface Carrier test (see Table I) [14]. The TGA test requires challenging diluted disinfectant with a planktonic bacterial inoculum ($2x10^8$ – $2x10^9$ organisms) and measuring viability after a given time. Following this a second challenge inoculum is added and the viability 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 (ie 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 ISO standard for automated endoscope reprocessors (ISO 15883-4: 2008) does mandate 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 hospital such as, alcohol, quaternary ammonium compounds (QAC), 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 dry surface biofilm 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 oxidising agents (actives), peracetic acid, hydrogen peroxide and chlorine against *S. aureus* DSB and to determine if non-active additives, added to disinfectant formulations, affected efficacy of actives.

Materials and Methods

Bacterial culture preparation

S. aureus (ATCC 25923) dry surface biofilm (DSB) was grown in vitro on polycarbonate coupons (Bio Surface Technologies Corporation) 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 500mL of 5% tryptone soya broth (TSB) and grown under sheer (provided by baffle rotation at 130 revolutions per min) for 48-hour batch phase at 35^{o}C, after which the media was drained, and the biofilm dehydrated for 48 hours at room temperature (22-25 ^{o}C) with filter-sterilised air-conditioned air (average relative humidity 66%) pumped into the bioreactor at 3L/min. An additional three cycles of batch growth (5%TSB, shear, 35^{o}C for 6 hours) alternated with prolonged dehydration phases of 66,

42 and 66 hours at room temperature and resulted in an average of $2.078x10^6$ (Log_{10} 6.30 ± 0.127) 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, Kent, United Kingdom), 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, or TAED), chelating agents, and sodium dodecyl sulphate (SDS), which on initial dissolution in water releases a mixture of approximately 1000mg/L hydrogen peroxide and 2100mg/L peracetic acid. The product also has specific claims against a range of organisms and is also indicated for the disinfection of environmental surfaces.

Chlorclean is a tabletted 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 1000mg/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 5000mg/L hydrogen peroxide. This product is an example of the "Accelerated® Hydrogen Peroxide" technology licensed from Virox Inc. [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 (7.5% w/w) and peracetic acid (5.0% w/w), which on dilution in water to give a 4% v/v solution contained mixture of 10,000mg/L hydrogen peroxide and 2200mg/L peracetic acid on dilution to give a 4% v/v solution in water; an unformulated SDIC tablet (Redox Chemicals, Minto, NSW Australia) containing only sodium diisocyanurate that, on dissolution in water released 1000mg/L; and a 6% solution of hydrogen peroxide (Gold Cross, Biotech Pharmaceuticals Pty Ltd, Laverton North, Victoria, Australia) to give 6000mg/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 one litre [16].

Experimental protocol for testing disinfectant efficacy against planktonic and DSB

The efficacy of test disinfectants to kill control planktonic bacteria and biofilm 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 to manufacturer's instruction (Speedy Clean, Whiteley Medical, North Sydney, Australia) on disinfectant efficacy was also tested (Figure 1). Each condition was tested with five replicates for determining residual bacterial number (colony forming units – CFU) and five replicates for determining residual protein contamination.

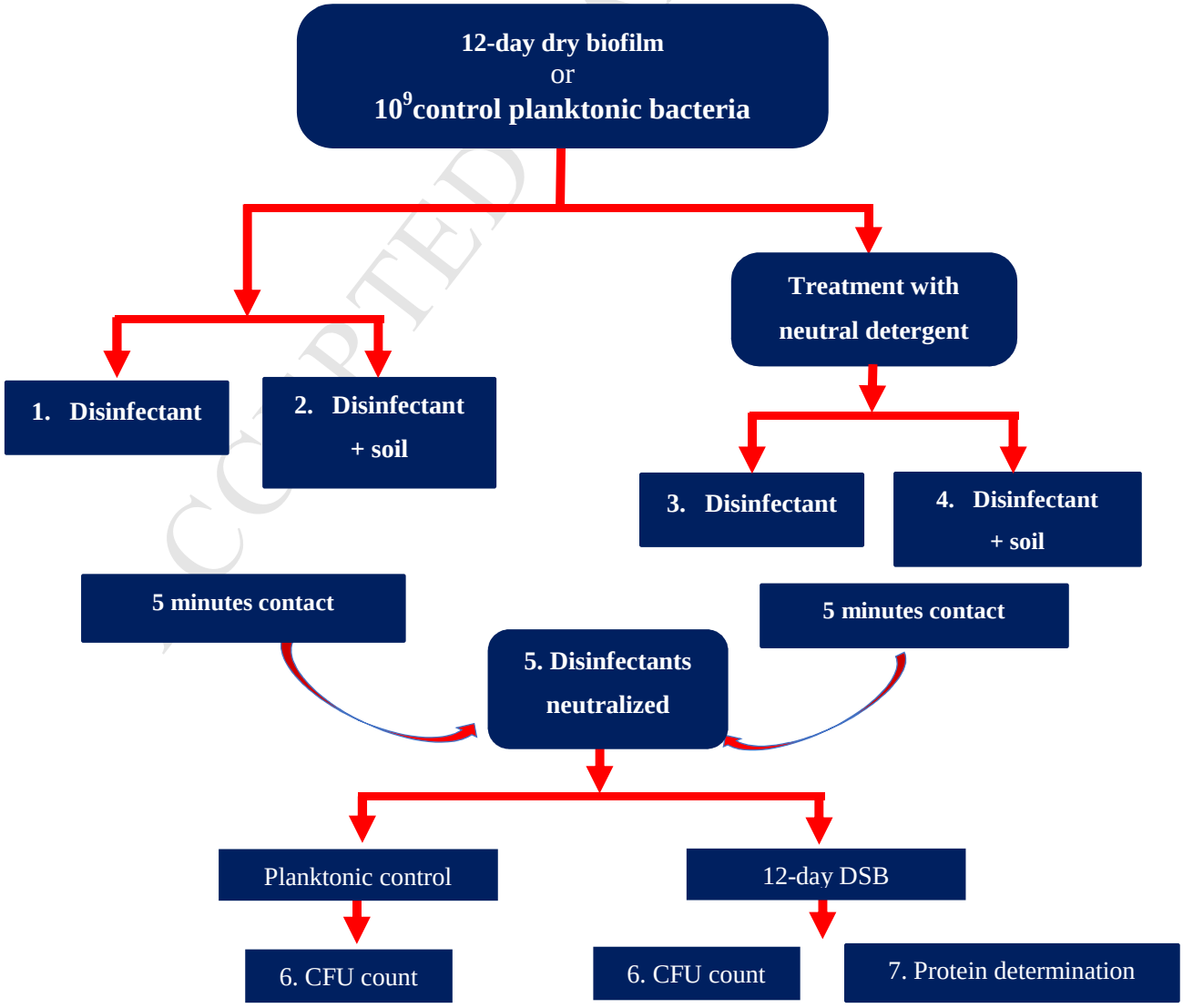

Figure 1: Experimental protocol for disinfection testing

Protocol for efficacy testing against planktonic and biofilm bacteria

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

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 10µl of TSB containing approximately 10^9 planktonic bacteria for the planktonic challenge or a biofilm coated coupon for the DSB challenge, for 5 minutes contact time (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 organic soil and immediately adding 10µl of TSB containing approximately 10^9 planktonic bacteria for the planktonic challenge or a biofilm coated coupon for the DSB challenge, for 5 minutes contact time (n=5/disinfectant) (Figure 1, box 2).

c) We confirmed that the neutral detergent had no biocide action by mixing 10µl of TSB containing approximately 10^9 bacteria with either 1 ml of Speedy Clean for 30 seconds 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 seconds, the coupon was removed from the detergent and immediately added 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 five minutes.

d) For parts a-c, at the end of the 5 minutes contact time, disinfectant activity was completely inactivated by the addition of 1 ml of neutraliser containing 1% sodium thiosulphate, 6 % Tween 80, 5% Bovine Calf Serum (BCS) and 10% Bovine Serum Albumin (BSA) in Phosphate Buffered Saline (PBS) (Figure 1, box 5).

e) Residual bacterial viability for planktonic control was determined by serial 10-fold dilution and overnight plate culture at 37^{o}C and CFU determination (Figure 1, box 6).

Biofilm viability for DSB was determined by subjecting control and test coupons to sonication at 80 kHz for 20 minutes prior to serial 10-fold dilution and overnight plate culture at 37^{o}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 (Micro BCA) Assay (Figure 1, box 7).

Controls:

The positive controls for the planktonic challenge (5 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; 3 for each disinfectant) controls were subjected to the same treatments as described above but biocides were replaced with hard water.

Neutralisation control: Confirmation that disinfectant activity was completely inactivated by the neutraliser was achieved by the addition of 1 ml of the neutraliser to the disinfectant test mixture prior to adding a DSB covered coupon and reacting for 5 minutes 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 (Micro BCA^{TM} Assay; Thermo Scientific). Briefly, each coupon was rinsed in 10 ml of PBS three times and transferred to individual McCartney bottles containing 1mL of ice-cold 20 mM 2M-Morpholino-ethane sulfonic acid 0.9% saline. A 120µL aliquot of 30% NaOH was added, the samples sonicated at 60^{o}C for 1 hour, vortexed and then incubated at 30^{o}C for 30 minutes followed by incubation in a boiling water bath for 15 minutes. The samples were cooled and 86µL of 32%HCl added prior to centrifuging at 13,000 rpms in a bench top centrifuge for 5 minutes. An aliquot (1 mL) of the supernatant was used for protein determination. Residual protein contaminating samples was determined by measuring sample absorbance at 562nm wavelength, subtracting the absorbance of negative control coupons (n=3) and calculating protein concentration (µg/mL) using a standard curve prepared using the kits' standard, according to manufacturer's instructions.

Statistical Analysis

A one way analysis of variance (Anova) combined with the Holm-Sidak all pairwise multiple comparison procedure was used to test for significant differences in Log_{10} reduction in titre utilising the SigmaPlot 13 (Systat Software, San Jose, Ca) statistical package. 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 five minutes contact time all the disinfectants, used in this study, killed 7 Log_{10} of planktonic organisms. The formulated peracetic acid disinfectant Surfex was unaffected by organic soil, whereas the generic disinfectant Proxitane's efficacy was greatly reduced. The efficacy of hydrogen peroxide and chlorine based disinfectants were also highly affected by the presence of organic soil (see Figure 2).

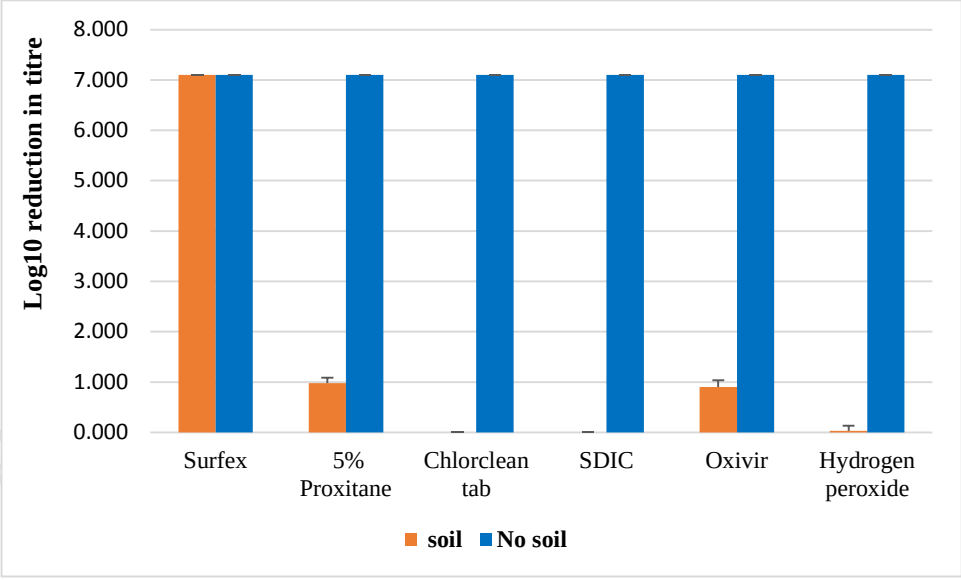

Figure 2. Log_{10} reduction in planktonic *S. aureus* titre following 5 min contact with disinfectants containing peracetic acid and hydrogen peroxide (Surfex, Proxitane), chlorine (Chlorclean, SDIC) and hydrogen peroxide (Oxivir, hydrogen peroxide) as active ingredients. Disinfectant efficacy was determined in hard water with and without added biological soil.

S. aureus DSB

Positive control DSB coupons had a mean of $2.08x10^6$ (Log_{10} 6.32 ± 0.127) CFU of *S. aureus* per coupon (n=29). In the absence of organic soil and with a five minutes 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 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 products 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).

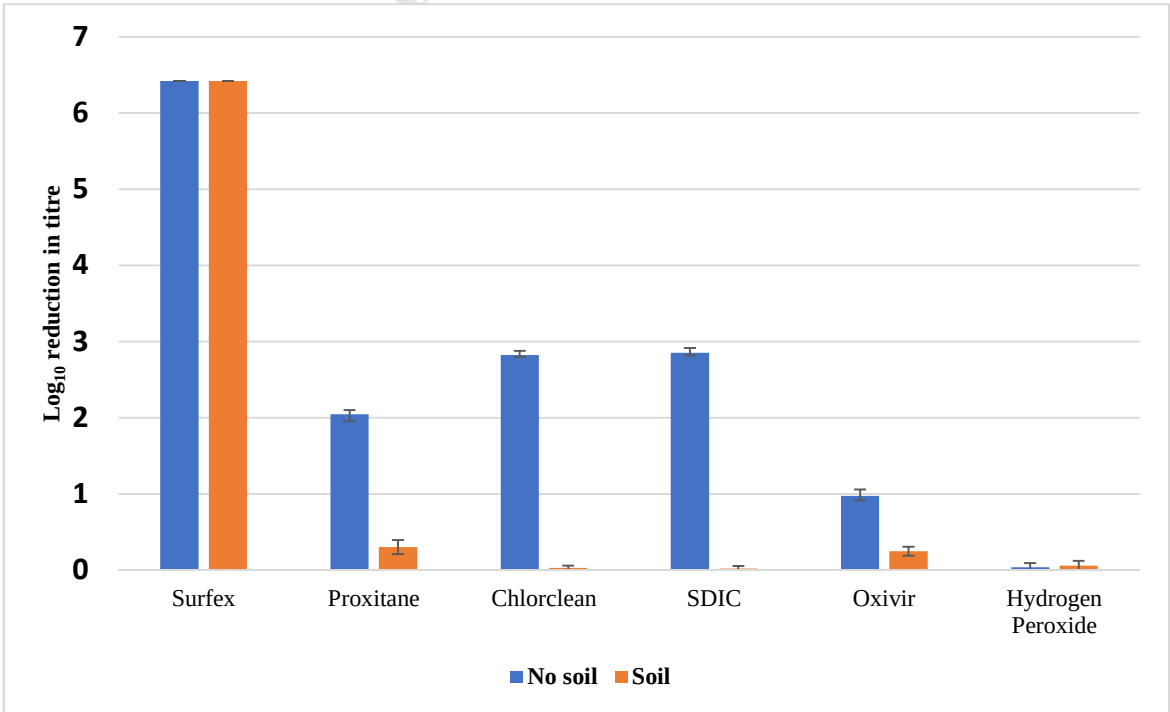

Figure 3. Log_{10} reduction in biofilm titre following 5 min contact with disinfectants containing peracetic acid and hydrogen peroxide (Surfex, Proxitane), chlorine (Chlorclean, SDIC) and hydrogen peroxide (Oxivir, hydrogen peroxide) as active ingredients. Disinfectant efficacy against Log_{10} 6.32 *S. aureus* DSB was determined in hard water with and without added biological soil.

Disinfectant efficacy following detergent treatment in the presence of 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 (>6Log_{10} reduction in titre) under all conditions tested, the effect of prior biofilm contact with detergent was unable to be measured.

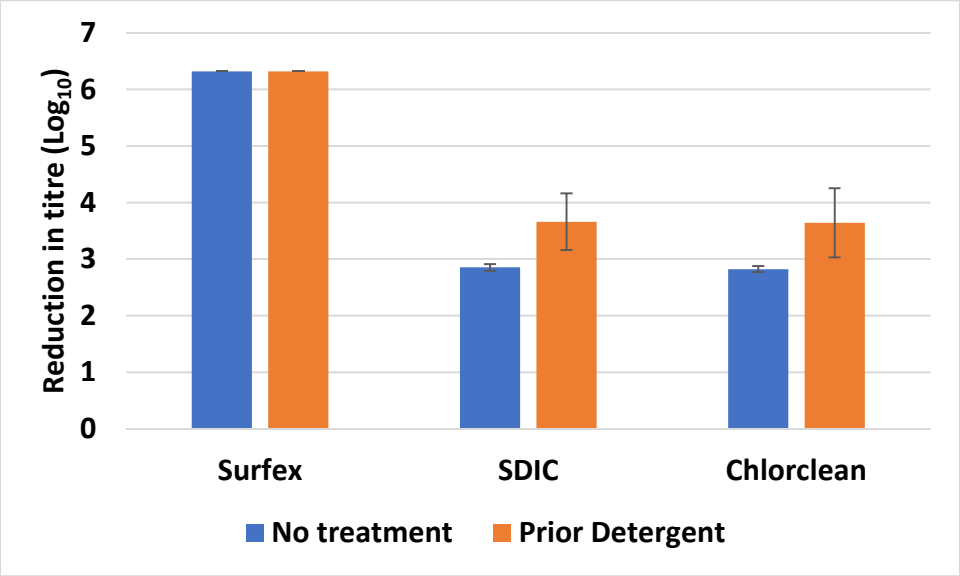

Figure 4: Reduction in DSB titre (Log_{10}), in the absence of biological soil, obtained with (orange boxes) and without (blue boxes) prior biofilm contact with detergent (Speedy clean for 30 seconds) followed by Surfex, SDIC and Chlorclean disinfection for five minutes contact time.

Disinfectant efficacy in removing biofilm mass

The ability of the disinfectants to remove DSB biofilm 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 tablet 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 H_2O_2 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.

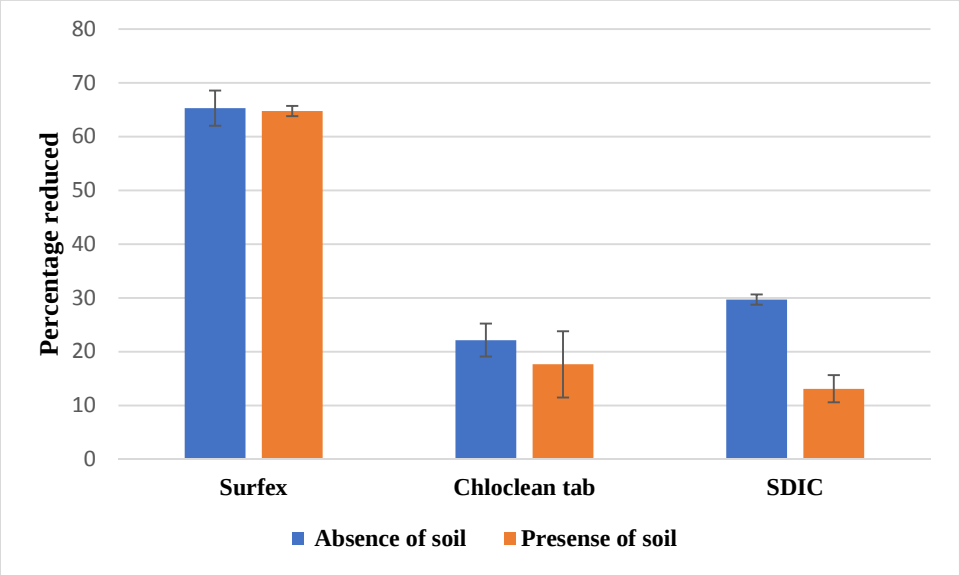

Figure 5: Percentage reduction of biofilm mass (protein) after disinfection with Surfex Chlorclean tablet and SDIC for five minutes in the presence and absence of soil.

Discussion

In this study, *S. aureus* DSB model[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 actives were evaluated. In this manner, the excipient (non-active)

ingredients, as well as the active ingredients themselves could be evaluated. We tested in the presence of organic soil as combined cleaning/disinfecting systems are becoming more popular, in that clinical surfaces are often not precleaned prior to disinfection. Thus, the efficacy testing in the presence of large amounts of organic soil is more reflective of worse-case clinical conditions.

We evaluated three formulated, commercially available disinfectant systems, each of which contained an oxidising biocide, along with other ingredients such as surfactants. The effect of addition of the proprietary ingredients 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 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, as well as unformulated SIDC tablets were the next best performers, although they killed significantly less 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 (reviewed by Otter)[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 biofilm reactor is 90% whilst that of DSB is 61% [21]. This lower water content in combination with the thicker 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 and hydrogen peroxide ($P<0.001$). Oxivir killed approximated 1 Log_{10} of the biofilm bacteria while hydrogen peroxide solution had no effect, however Oxivir's manufacturer's recommended contact time for killing bacteria is 10 not five minutes as used in the study and this could explain its lower performance. However, even a contact time of 5 minutes is probably excessive given the way that dry hospital surfaces are cleaned. The majority of disinfectants have no residual effect and remain active only when wet.

The differences in kill rate 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 these 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, 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 eg 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: $H_2O_2 + CH_3CO_2H \rightleftharpoons CH_3CO_3H + H_2O$ (Ramirez and Omidbakhsh, 2003). However, in Surfex, the PAA is generated by the reaction of hydrogen peroxide with tetraacetylethylenediamine (TAED) [22]. The source of hydrogen peroxide in Surfex is sodium peroxycarbonate, a 2:3 complex of hydrogen peroxide and sodium carbonate that releases the 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 of viable bacteria load observed. 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 oxidising biocides. This study showed that the addition of the organic soil had no effect on the efficacy of Surfex whilst the generic equivalent, diluted Proxitane was inactivated. This is most likely due the other ingredients within the formulation, such as chelating agents, or perhaps due to the differences in pH (8.10 for Surfex as compared to 2.6 for a 4% solution of Proxitane). Compared with hydrogen peroxide, peracetic acid does however, have the disadvantage that it is less stable when diluted: disassociating 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 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 (2015) demonstrated that protein was a principal component (56%) of both his *in vitro* DSB model and biofilms contaminating dry clinical surfaces in hospitals with protein contents varying from 42 to 95%. Therefore, in this study we measured residual protein on the treated coupons to determine the proportion of biofilm mass removed by the oxidising action of the disinfectants. None of the disinfectants were able to completely remove all biofilm protein with 5 min contact time, however a higher percentage reduction of biofilm protein was observed in five minutes with Surfex (65%) than the other tested disinfectants (<30%), both in the presence and absence of soil.

Conclusion

We conclude that disinfectant efficacy against biofilm can vary significantly, despite containing similar levels of biocides due to their formulation/additives. The disinfectant formulation also affected 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. We suggest that purchasing officers 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.

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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 healthcare 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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Table I. Microbiological soil testing required for disinfectant registration by the Australian Therapeutic Goods Administration

Disinfectant grade	Data required
Hospital grade	Option A or B of the TGA test Bactericidal carrier test
Low level instrument grade	Option B of the TGA test Bactericidal carrier test Virucidal test data (minimum carrier test with enveloped/lipid virus)
Intermediate Level instrument grade	Option B of the TGA 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 plus: Sporicidal tests (carrier) Sporicidal D value tests Simulated in-use tests

Table II: Test disinfectants and their components

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

Figure 1: Experimental protocol for disinfection testing.

Figure 2. Log_{10} reduction in planktonic *S. aureus* titre following 5 min contact with disinfectants containing peracetic acid and hydrogen peroxide (Surfex, Proxitane), chlorine (Chlorclean, SDIC) and hydrogen peroxide (Oxivir, hydrogen peroxide) as active ingredients. Disinfectant efficacy was determined in hard water with and without added biological soil.

Figure 3. Log_{10} reduction in biofilm titre following 5 min contact with disinfectants containing peracetic acid and hydrogen peroxide (Surfex, Proxitane), chlorine (Chlorclean, SDIC) and hydrogen peroxide (Oxivir, hydrogen peroxide) as active ingredients. Disinfectant efficacy against Log_{10} 6.32 *S. aureus* DSB was determined in hard water with and without added biological soil.

Figure 4: Reduction in DSB titre (Log_{10}), in the absence of biological soil, obtained with (orange boxes) and without (blue boxes) prior biofilm contact with detergent (Speedy clean for 30 seconds) followed by Surfex, SDIC and Chlorclean disinfection for five minutes contact time.

Figure 5: Percentage reduction of biofilm mass (protein) after disinfection with Surfex Chlorclean tablet and SDIC for five minutes in the presence and absence of soil.