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Short report

Difficulty in removing biofilm from dry surfaces

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SUMMARY

Cleaning is fundamental to infection control. This report demonstrates that a *Staphylococcus aureus* biofilm is significantly more difficult to remove than dried planktonic bacteria. A single wiping action removed >99.9% (>3 $\log_{10}$) of dried planktonic bacteria, whereas only 1.4 $\log_{10}$ of biofilm (96.66%) was removed by 50 wiping actions with a standardized wiping process.

Introduction

Biofilms have been demonstrated on >90% of dry healthcare surfaces in intensive care units (ICU) [1–3]. These dry surface biofilms (DSB) have been shown to contain multi-drug-resistant organisms (MDRO), including meticillin-resistant *Staphylococcus aureus* which is responsible for many healthcare-associated infections (HAI) [1,2]. Bacteria within mature biofilm are heterogeneic with respect to phenotype, with some cells having a very low metabolic rate allowing for extended survival times. Bacteria in DSB can remain viable in the absence of nutrition for >12 months [2].

Growing bacteria from DSB is a challenging pursuit, and more aggressive microbial recovery methods are normally required [4]. Methods to replicate the behaviour of micro-organisms commonly present in DSB have been developed with apparently similar morphology and characteristics similar to biofilms found *in situ* within an ICU [5]. Laboratory-produced DSB have demonstrated enhanced resistance profiles to disinfection and sterilization [6,7]. The objective of this study was to compare the removal of *S. aureus* (ATCC 25923) DSB with removal of dried planktonic bacteria from a coupon surface by wiping with a moistened cloth using a standardized wiping process.

Methods

DSB were grown on non-porous polycarbonate coupons as per Almatroudi *et al.* using a CDC Biofilm reactor (BioSurface Technologies Corporation, Bozeman, MT, USA) with alternating cycles of nutrition and dehydration [5]. An 10-μL aliquot of overnight planktonic culture was spread on the polycarbonate coupon and airdried for 40 min at 37°C. As the production of DSB incorporates cycles of dehydration, DSB were not subjected to additional drying. The test DSB and dried planktonic coupons were fitted into a template on a flat surface for wiping using a 70% viscose/

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30% polyester blend material from American Hygienics (Shenzen, China), moistened in sterile water. To ensure that the wiping process was repeatable, a scrub testing device with a mechanical arm, set at 1000 g downward pressure (equivalent to 28 g/cm^2), was used to provide a linear two-way wiping process (Elcometer 1720 Abrasion Tester, Phillro Industries, Moorabin, Australia). Based on preliminary experiments demonstrating that dried planktonic organisms were easy to remove and DSB were difficult to remove (results not shown), the mechanical arm was set to 0, 1, 5, 10 and 20 wipes for planktonic-covered coupons and 0, 10, 25 and 50 wipes for DSB coupons. The arm moved at a speed of 60 cm/s in a linear motion.

DSB control coupons and dried planktonic control coupons were not wiped. The control and wiped DSB coupons were rinsed three times in 5 mL water to remove detached biofilm. Coupons with dried planktonic cultures were not rinsed as this could result in removal of some of the bacteria. All coupons were placed in 1 mL isotonic solution of phosphate buffer solution and sonicated for 5 min (Soniclean, JMR Australia), using a frequency of 42–47 kHz, followed by vortexing for 2 min. The supernatant was subjected to serial 10-fold dilution and standard plate culture. All experiments were conducted in triplicate.

Statistical analysis was conducted using Sigma Plot 13 (Systat Software, Inc., San Jose, CA, USA). One-way analysis of variance followed by the Holm–Sidak method of all pairwise comparisons was used to test for significant differences between control and wiped coupons. Transformation of data was necessary for both DSB and dried planktonic cultures to ensure equal variance and normality.

Results

The number of bacteria on DSB-covered coupons was $\log_{10}$ 6.16±0.48 colony-forming units (cfu)/coupon, whilst the number of dried planktonic bacteria on coupons was $\log_{10}$ 6.44±0.05 cfu/coupon.

The density of organisms, both on the DSB coupons and the coupons with dried planktonic bacteria, was sufficient to replicate worst-case conditions and replicate the in-use conditions for cleanliness monitoring tools such as fluorescent markers [8].

Planktonic organisms were relatively easy to remove by wiping with a moistened cloth; a single wiping action significantly reduced the number of contaminating bacteria by 3.33 $\log_{10}$ or 99.95% ($P<0.001$). Five wiping actions removed significantly more bacteria (4.22 $\log_{10}$ or 99.99%; $P<0.001$). Additional wiping actions resulted in further removal of bacteria, albeit at a non-significant rate (Figure 1). In contrast, wiping DSB 10 or 20 times with a moistened cloth failed to remove a significant amount of the biofilm. Fifty wiping actions were required to remove a significant amount of DSB, which equated to removing just 1.48 $\log_{10}$ or 96.66% of the contaminating bacteria ($P<0.008$).

Discussion

This experiment only assessed removal or dislodgement from the initial disc surface on which the biofilm was grown or the planktonic bacteria were dried. The method for this experiment did not attempt to measure recovery from the wiping cloth, as no biocidal action was anticipated. Many factors impact the efficacy of surface decontamination, including – but not limited to – wiping efficacy (pressure/frequency), ratio of detergent/disinfectant to towelette, surface properties and target organisms [9]. A limitation of the current study is that commercial wipes contain detergent/disinfectant but this study only investigated the physical removal of bacteria. Ledwoch *et al.* used water-moistened wipes as a control in their study, using a circular wiping action with a much heavier per cm^2 weight [10]. Similarly, the effect of smearing the biofilm on to the immediately adjacent surfaces either side of the coupon was not measured in this experiment, as the primary intention was to assess the tenacity of the biofilm during a normal wiping action, similar to that used during hospital cleaning processes. The efficacy of different wipes and transfer of bacteria to adjacent surfaces has been shown to vary between brands of wipes [10].

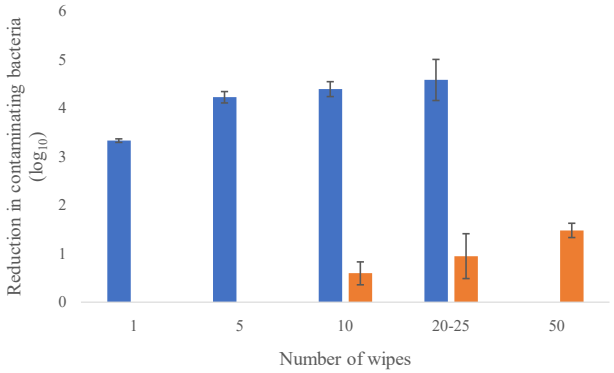

Figure 1. The impact of a linear wiping action on different growth cultures of *Staphylococcus aureus*. Blue bars, planktonic; orange bars, biofilm.

Wiping surfaces has been shown to have a beneficial effect on HAI rates [11]. The importance of validating the process of wiping/cleaning has also been shown to have a beneficial impact on the risks of HAI within healthcare settings [8]. Where wiping is not performed, the risk of bacterial survival and transmission is enhanced dramatically [12].

To the authors' knowledge, this is the first experiment to subject a DSB to a standardized linear wiping process in an effort to understand the dynamics of cleaning hospital surfaces, particularly inside an ICU. The importance of a more aggressive surface sampling approach when conducting field-based recovery is underscored by the results demonstrated.

Ten wiping actions only removed 75% of the DSB, leaving 25% of bacteria on the surface. It took 50 separate wiping actions to remove more than 95% of the DSB bacteria. This falls well below an expected cleaning level demonstrated by the dried planktonic culture. This work highlights the unexpected difficulty in removing DSB in normal cleaning conducted within healthcare environments such as the ICU. Despite the poor removal of DSB by wiping, DSB is easily transferred by both gloved and ungloved hands [13]. The revelation that many of the bacteria, including pathogens, present within an ICU are resident on surfaces and embedded within biofilms may explain, in part, why so much cleaning activity is ineffective.

These results highlight the importance of revisiting the role of effective cleaning as a meaningful intervention for infection control practice. The results also highlight the underlying complexity of succeeding with any healthcare cleaning process. Further work is underway to expand this experimental series into various specific areas of healthcare cleaning using a

commercial array of methods, cleaning materials and products intended for use within healthcare settings.

Conflict of interest statement

GSW and TG are employees of Whiteley Corporation, although there are no conflicts of interest arising from this study. None of the other authors report any conflicts of interest relevant to this paper.

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