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Presence of biofilm containing viable multiresistant organisms despite terminal cleaning on clinical surfaces in an intensive care unit

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SUMMARY

Despite recent attention to surface cleaning and hand hygiene programmes, multiresistant organisms (MROs) continue to be isolated from the hospital environment. We hypothesize that reservoirs of MROs exist in the environment as biofilms (bacteria embedded in exopolymeric substances, EPS). Biofilms are difficult to remove due to their increased resistance to detergents and disinfectants. These biofilms periodically release free-swimming planktonic bacteria back into the environment which then may act as an infection source. Following terminal cleaning, equipment and furnishings were removed aseptically from an intensive care unit (ICU) and subjected to culture and scanning electron microscopy (SEM). Samples were placed in 5 mL of tryptone soya broth, sonicated for 5 min before plate culture on horse blood agar, Brilliance MRSA and Brilliance VRE agar plates. Samples for SEM (mattress, sterile supply reagent bucket, opaque plastic door, venetian blind cord, sink rubber, curtain) were fixed in 3% glutaraldehyde and hexamethyldisilazane (HMDS) prior to sputter-coating with gold and examination in an electron microscope. Biofilm was demonstrated visually on the sterile supply bucket, the opaque plastic door, the venetian blind cord, and the sink rubber, whereas EPS alone was seen on the curtain. Viable bacteria were grown from three samples, including MRSA from the venetian blind cord and the curtain. We have demonstrated the presence of biofilm and biofilm containing MROs on clinical surfaces from an ICU despite terminal cleaning, suggesting that current cleaning practices are inadequate to control biofilm development. The presence of MROs being protected within these biofilms may be the mechanism by which MROs persist within the hospital environment.

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Introduction

Healthcare-associated infections (HAIs) are a widespread problem, affecting 5–10% of all patients.¹ In the intensive care unit (ICU), the presence of very sick, elderly and immunocompromised patients results in a disproportionate percentage (20%) of patients developing HAI.² This problem is compounded by the spread of multiresistant organisms (MROs), making treatment difficult or

ineffective.³ HAIs add considerable morbidity, increase hospital stay times, increase mortality, and add costs to patient care.^{1,2,4}

Contamination of the inanimate environment around patients constitutes an important reservoir of MRO with the risk of HAI increased by an average of 73% if the patient previously occupying the room had MRSA, vancomycin-resistant enterococcus (VRE), acinetobacter, *Clostridium difficile* or other pathogens.^{3,5,6} Numerous studies have shown persistence of these organisms in the environment even in the face of enhanced terminal cleaning.^{7–9}

Biofilms are generally found in moist environments, causing infection on implantable medical devices such as catheters and breast implants or on instruments routinely immersed in fluid.^{10–12} We hypothesize that, despite the decreased moisture availability on dry surfaces, bacteria within the ICU environment also reside in

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biofilms, and that within these biofilms, MROs are protected from physical removal and chemical disinfection.

A biofilm is a structured community of organisms encased and attached to a surface by exopolymeric substances (EPS). The EPS makes up to 90% of the biofilm providing protection from environmental desiccation and this EPS is extremely difficult to remove using detergents.^{13–15} Additionally, bacteria within biofilms are up to 1500 times (typically 100–250 times) more resistant to biocides than the same 'planktonic' bacteria growing in liquid culture.¹³ These properties of biofilms result in decreased efficacy of cleaning and disinfection, thereby promoting the persistence of bacteria, including MROs, in the environment.

In this study we investigated whether biofilms can be found on furnishings in the ICU.

Methods

Following terminal cleaning in a 16-bed ICU, i.e. initial cleaning with neutral detergent, followed by disinfection with 500 ppm chlorine (Diversol5000, Johnson Diversey, Smithfield, Australia), equipment and furnishings were aseptically removed from patient and common-use areas.

Sample collection

Items were destructively sampled using sterile gloves, forceps, pliers, scissors, or scalpel blades, depending on the material being sampled. Gloves and instruments were changed between each sample. Samples were then placed into sterile containers for transport to the laboratory. Small items, such as a sterile supply reagent box, were transported intact to the laboratory; larger items, such as the mattress and door, had sections removed (up to 8 × 10 cm in size) into sterile containers. Following transport to the laboratory, these large pieces were further sectioned into smaller pieces, using a sterile technique.

Scanning electron microscopy (SEM)

Samples up to 1 cm² were fixed in 3% glutaraldehyde, dehydrated through ethanol, immersed in hexamethyldisilazane (HMDS; Polysciences Inc., Warrington, PA, USA) for 3 min before sputter-coating with 20 nm gold film and examined in an SEM microscope as previously described.¹² An item was classified as being biofilm positive if bacteria attached to a surface and surrounded by EPS could be visualized.

Microbiology

Sections of equipment or furnishings up to 2 cm² were placed in 4 mL of tryptone soya broth, sonicated for 5 min and 100 µL spread over horse blood agar plates (HBA), Brilliance MRSA agar plates for the detection of multiresistant *Staphylococcus aureus* (MRSA) and Brilliance VRE agar plates for the detection of vancomycin-resistant enterococcus (Oxoid, Adelaide, Australia). MRSA plates were incubated for 18–24 h and VRE and HBA plates up to 48 h.

Results

Six samples were examined by SEM (Table 1). We failed to demonstrate biofilm on only one sample. Four samples had principally coccoid-shaped bacteria encased in large amounts of EPS and the sample from the curtain had 'strings' of dehydrated EPS evident (Figure 1).

Bacteria grew on HBA from four of the six samples, demonstrating the presence of culturable organisms. The venetian blind

Table 1
Scanning electron microscopy (SEM) and culture results for environmental surfaces

Sample	SEM	Culture plates		
		HBA	MRSA	VRE
Curtain	Positive EPS	Growth	Positive	Negative
Venetian blind cord	Positive biofilm	Growth	Positive	Negative
Mattress bay	Negative	Growth	Positive	<i>E. faecium</i>
See-through plastic door	Positive biofilm	Negative	Negative	Negative
Wash basin rubber	Positive biofilm	Negative	Negative	Negative
Sterile supply reagent bucket	Positive biofilm	Growth	Negative	Negative

HBA, horse blood agar; MRSA, multiresistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococcus.

cord and curtain, positive for biofilm by SEM, also grew MRSA. The mattress grew MRSA and *E. faecium* but we were unable to demonstrate biofilm visually on this sample (Table 1). Two samples positive for biofilm were culture negative, using the procedure described above.

Discussion

Many studies have shown that contamination of the environment makes an important contribution to HAI and that enhanced cleaning protocols reduce environmental contamination, which translates into decreased incidence of HAI.^{5,6} In Dancer *et al.*'s study, the addition of one extra member of cleaning staff, five days a week, resulted in a 32.5% reduction in microbial contamination of hand-touch sites and a 26.6% reduction in new MRSA infections, saving the hospital an estimated £30,000 to £70,000.⁷ Termination of the extra cleaner resulted in new clusters of MRSA infection within two to four weeks. However, even with enhanced cleaning, MROs can still be isolated from the environment.^{7–9}

We hypothesize that surface condensation occurs, producing a thin film of water, or that the relative humidity in the ICU is high enough to allow biofilms to develop on ICU surfaces. Once formed, the EPS would protect the bacteria from desiccation and make them harder to remove.

We further hypothesize that MROs persist in the environment, in the face of enhanced cleaning, as biofilms. Although detergents are good at removing patient soil and planktonic bacteria, they are less effective at removing biofilm, rendering current cleaning protocols less efficient.^{14,15} In industry, extreme measures including physical scraping and use of concentrated biocides are often required to remove biofilm, such as when removing legionella from water-cooling towers.

Of the six furnishings sampled bacteria were demonstrated to be embedded in EPS on four samples and residual EPS on one, whereas only the mattress sample was negative for biofilm by SEM. SEM of the non-porous covering of the hospital mattress shows that the surface is not completely level but has many microscopic dips. This is similar to the dips and imperfections that have been observed on new Teflon endoscope tubing.¹² With use, many of these dips or imperfections in endoscope tubing became contaminated with biofilm.¹² A similar situation may exist with the hospital mattresses and, if a larger area were to be inspected, biofilm may be found.

Using destructive sampling followed by sonication and broth culture, bacteria were grown from three of these biofilm-positive samples. Both the venetian blind curtain cord and the curtain grew MRSA. Even the mattress, the sole sample for which we failed to visually demonstrate biofilm, grew MRSA and VRE. It is worrying that we demonstrated biofilm on the reagent bucket that was used to contain sterile supplies, such as catheters and bandages. Although we did not detect MRSA or VRE, we were able to show

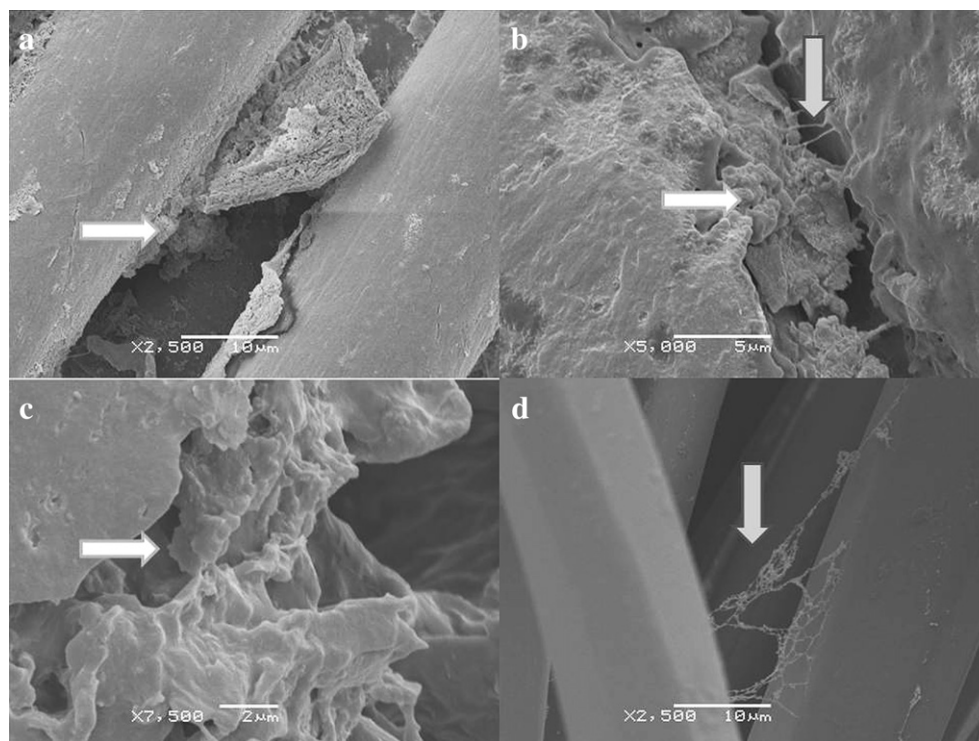


Figure 1. Scanning electron micrographs of: (a) blind cord (original magnification $\times 2500$); (b) see-through ward door (original magnification $\times 5000$); (c) red reagent box (original magnification $\times 7500$); (d) curtain (original magnification $\times 2500$). Horizontal arrows indicate coccoid bacteria embedded in exopolymeric substance (EPS). Vertical arrows indicate residual strings of EPS dehydrated during processing.

that viable bacteria were present in the biofilm. Additionally the rate of acquisition of new resistant determinants is increased in bacteria residing in biofilm.¹⁶ A significant correlation has been shown to exist between class 1 integron resistance genes, biocide resistance and biofilm formation in clinical strains of *Acinetobacter baumannii*.¹⁷ Whether this occurs when water is limited is unknown.

Despite visual confirmation of biofilm, neither the wash basin nor the plastic door grew bacteria when aerobic culture and HBA were used. These bacteria could have been dead, or not culturable using the conditions used, or unculturable due to their state of growth in the biofilm. Bacteria growing as biofilm are notoriously difficult to culture, although sonication of the sample in broth increases the rate of recovery.¹³

Dancer *et al.* found that antibiotic-resistant environmental bacteria were more prevalent in wards with a high level of antibiotic prescribing.¹⁸ The combination of high antibiotic use and environmental biofilms in the ICU may be the mechanism whereby increased genetic exchange occurs between bacteria residing in biofilms, leading to persistence of antibiotic-resistant environmental bacteria, despite enhanced cleaning.

Using destructive sampling, followed by SEM and culture, we have demonstrated the presence of biofilm and biofilm containing MROs on clinical surfaces from an ICU despite terminal cleaning, suggesting that current cleaning practices are inadequate to control biofilm development. The presence of MROs being protected within these biofilms may be the mechanism by which MROs persist within the hospital environment.

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Conflict of interest statements

None declared.

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