Journal of Hospital Infection 91 (2015) 35–44

Available online at www.sciencedirect.com

Journal of Hospital Infection

journal homepage: www.elsevierhealth.com/journals/jhin

Intensive care unit environmental surfaces are contaminated by multidrug-resistant bacteria in biofilms: combined results of conventional culture, pyrosequencing, scanning electron microscopy, and confocal laser microscopy

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ARTICLE INFO

Article history:
Received 17 March 2015
Accepted 26 May 2015
Available online 25 June 2015

Keywords:
Biofilm
Intensive care unit
Healthcare-associated infections
Microbiome
Multidrug-resistant organisms
Staphylococcus aureus
Enterococci

SUMMARY

Background: Hospital-associated infections cause considerable morbidity and mortality, and are expensive to treat. Organisms causing these infections can be sourced from the inanimate environment around a patient. Could the difficulty in eradicating these organisms from the environment be because they reside in dry surface biofilms?
Aim: The intensive care unit (ICU) of a tertiary referral hospital was decommissioned and the opportunity to destructively sample clinical surfaces was taken in order to investigate whether multidrug-resistant organisms (MDROs) had survived the decommissioning process and whether they were present in biofilms.
Methods: The ICU had two 'terminal cleans' with 500 ppm free chlorine solution; items from bedding, surrounds, and furnishings were then sampled with cutting implements. Sections were sonicated in tryptone soya broth and inoculated on to chromogenic plates to demonstrate MDROs, which were confirmed with the Vitek2 system. Genomic DNA was extracted directly from ICU samples, and subjected to polymerase chain reaction (PCR) for *femA* to detect *Staphylococcus aureus* and the microbiome by bacterial tag-encoded FLX amplicon pyrosequencing. Confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) were performed on environmental samples.

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http://dx.doi.org/10.1016/j.jhin.2015.05.016

Findings: Multidrug-resistant bacteria were cultured from 52% (23/44) of samples cultured. *S. aureus* PCR was positive in 50%. Biofilm was demonstrated in 93% (41/44) of samples by CLSM and/or SEM. Pyrosequencing demonstrated that the biofilms were polymicrobial and contained species that had multidrug-resistant strains.
Conclusion: Dry surface biofilms containing MDROs are found on ICU surfaces despite terminal cleaning with chlorine solution. How these arise and how they might be removed requires further study.

Introduction

Hospital-acquired infections (HAIs) are a major problem. A recent study estimated that 648,000 patients have 721,800 HAIs annually in acute care hospitals in the USA.[1] This has been estimated to cost US hospitals US$28–34 billion annually.[2] 'ESKAPE' organisms (*Enterococcus* spp., *Staphylococcus aureus*, *Klebsiella* spp., *Acinetobacter* spp., *Pseudomonas aeruginosa*, and Enterobacteriaceae) continue to dominate, and *Clostridium difficile* is now the micro-organism most frequently causing HAIs.[1]

The cost-effectiveness of infection prevention and control programmes has been demonstrated, with hand hygiene being the most critical activity for controlling infection transmission.[3,4] However, sustained improvements in compliance rates are difficult to maintain, and infection control programmes targeting only hand hygiene are not necessarily associated with declining HAI rates.[5,6] By contrast, multiple strategies or bundles including active surveillance, patient isolation/cohort and improved hand hygiene have been shown to be successful in reducing meticillin-resistant *S. aureus* (MRSA) rates, even in hyperendemic regions.[7,8]

The Healthcare Infection Control Practices Advisory Committee (HICPAC) recommends a strategy to control multidrug-resistant organisms (MDROs) that consists of seven elements: administrative support, education, judicious use of antibiotics, MDRO surveillance, infection control precautions, environmental measures, and, where possible, decolonization.[9] An integrated approach to infection prevention should address environmental contamination. HAIs increase length of hospital stay, during which time patients contaminate their surrounding inanimate environment.[10,11] The risk of a patient developing an HAI increased by 73% if the patient previously occupying the room had a vancomycin-resistant enterococcus (VRE), MRSA, *C. difficile* or *Acinetobacter baumannii* infection.[12] Investigations focusing on the recovery of planktonic organisms from patient records and computer keyboards has helped to emphasize the importance of 'hand touch surfaces'.[11,13–15] Enhanced cleaning decreases, but does not eliminate, MRSA and other MDRO environmental isolation rates.[11] However, decreased environmental contamination rates have been associated with decreased MRSA acquisition rates.[16]

We recently showed the presence of dry surface biofilms containing viable MDROs on five out of six furnishings from an ICU, including a sterile supply box, privacy curtain, venetian blind cord, see-through ward entrance door, and rubber from around a sink.[17] As bacteria within biofilm are many more times resistant to desiccation, removal by detergents, and inactivation by disinfectants, we suggested that the presence of biofilms may contribute to the maintenance of environmental contamination in the face of cleaning.[17–20]

In this study we investigated the prevalence of biofilms in the environment immediately surrounding the patient and the frequency with which *S. aureus* was incorporated into these biofilms. In addition 15 samples were subjected to next-generation sequencing to determine the mix and ratio of microbial species present in biofilms contaminating dry surfaces.

Methods

Sample collection

Samples were obtained from an intensive care unit in a fully air-conditioned hospital and stored in a fully air-conditioned laboratory (temperature range 22–25°C, humidity 57–72%). Following a two-step terminal cleaning protocol using neutral detergent followed by disinfection with 500 ppm chlorine (sodium dichloroisocyanurate dehydrate, Diversol5000, Johnson Diversey, Smithfield, NSW, Australia), items from the patient bedding ($N = 11$), patient surrounds ($N = 19$), and fixed furnishings ($N = 14$) were aseptically sampled by cutting out a segment of the furnishing using sterile gloves, forceps, pliers, scissors, or scalpel blades, depending on the material being sampled. Samples were stored in sterile containers and gloves and instruments were changed between each sample.

Aerobic culture

Sample sections, up to 2 cm^2, were sonicated in 4 mL of tryptone soya broth for 5 min, prior to 100 μL being spread over horse blood agar plates (HBA) as a general non-selective medium, Brilliance MRSA agar plates for the detection of MRSA, Brilliance VRE Agar Plates for the detection of VRE, and Brilliance ESBL agar plates for the detection of extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria (Oxoid Adelaide, Australia).[17] MRSA plates were incubated for 18–24 h, and VRE, ESBL and HBA plates up to 48 h, aerobically at 37°C. Positive MDROs were confirmed using a combination of Vitek2 GPS-IX or Vitek2 AST-N149 cards (for Gram-positive or -negative isolates respectively) (bioMérieux-Vitek, Hazelwood, MO, USA) and partial sequencing of the 16S rRNA universal eubacterial gene according to the method described by Kidd *et al.*[21]

Staphylococcus aureus-specific PCR

Samples were sonicated in 300 μL digestion buffer (50 nM Tris/HCl pH 7.5, 150 nM NaCl, 2 mM ethylenediamine tetra-