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Characterization of microbial community composition, antimicrobial resistance and biofilm on intensive care surfaces

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ABSTRACT

Background: Organisms causing healthcare associated infections can be sourced from the inanimate environment around patients. Residing in a biofilm increases the chances of these organisms persisting in the environment. We aimed to characterise bacterial environmental contamination, genetically and physiologically, and relate this to general intensive care unit (ICU) cleanliness.
Methods: Cleanliness was determined by adenosine triphosphate (ATP) measurements of 95 high-touch objects. Bacteriological samples were obtained from the same sites (n = 95) and from aseptically removed sections (destructive samples, n = 20). Bacterial enrichment culture was conducted using tryptone soya broth prior to plating on horse blood agar, MacConkey agar, and screening chromogenic agar for identification of multidrug resistance organism (MDRO). Bacterial load and microbial diversity were determined using quantitative PCR (qPCR) and next generation DNA sequencing respectively. Confocal laser scanning microscopy and scanning electron microscopy were used to visually confirm the biofilm presence.
Results: Many intensive care surfaces (61%) were highly contaminated by biological soil as determined by ATP bioluminescence testing. The degree of biological soiling was not associated with bacterial contamination as detected by qPCR. Bacterial load ranged from 78.21 to 3.71×10^8 (median = 900) bacteria/100 cm^2. Surface swabs from 71/95 sites (75%) were culture-positive; of these 16 (22.5%) contained MDRO. The most abundant genera were *Staphylococcus, Propionibacterium, Pseudomonas, Bacillus, Enterococcus, Streptococcus* and *Acinetobacter*. Biofilm was visually confirmed by microscopy on 70% (14/20) of items.
Conclusion: Bacterial biofilms and MDROs were found on ICU surfaces despite regular cleaning in Saudi Arabia, suggesting that biofilm development is not controlled by current cleaning practices.

Introduction

Each year millions of patients worldwide are admitted to intensive care units (ICUs). The ICU is often called the epicentre of opportunistic infections with 25% of all healthcare associated infections (HAI) occurring in ICU patients, resulting in increased morbidity, mortality and healthcare costs [1,2]. ICU patients have an increased risk of HAI due to their underlying conditions, impaired immunity, exposure to multiple invasive devices that bypass and disrupt patients' protective barriers (for example, urinary catheters), and the administration of drugs that can predispose patients to infection [3].

The ICU has been considered a bio-factory for the dissemination and propagation of superbugs or multidrug-resistant organisms (MDRO), due largely to the extensive antimicrobial use in ICUs, which imposes a selection pressure promoting the emergence of MDRO [4,5]. Infection with a MDRO has been shown to increase the mortality of ICUs patients by up to 500% even after adjusting for confounding factors [1,2,6].

Transmission of infectious agents can occur between patients, healthcare workers hands, from medical equipment and the clinical environment [7]. Carling and Bartley [8] showed that room occupation by a patient with vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile* or *Acinetobacter baumannii* infection, increased the risk for subsequent patients developing a nosocomial infection with these organisms (73%), suggesting that the bacteria remained

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in the environment despite room cleaning between patients [8]. A more recent review showed the odds ratio for pathogen acquisition was 2.14 (95% confidence interval, 1.65–2.77), if the previous patient was infected [9].

A contributing factor for persistence of environmental contamination may be the presence of biofilms which can protect incorporated microorganisms from both desiccation and the action of cleaning and disinfecting agents. We have previously shown that biofilms are present on majority of hospital intensive care surfaces in Australia [10,11]. In this study, we characterized microbial community composition, antibiotic resistance and biofilm presence on hospital surfaces in Saudi Arabia.

Materials and methods

ATP monitoring

Adenosine triphosphate (ATP) was used for detection of organic matter on high-touch objects at two ICUs, including isolation wards in adult ICUs, and in two bays of the paediatric ICU in a tertiary hospital in Jeddah, Saudi Arabia. The patients' room, including the floor and surfaces, was sanitised by the cleaners and the equipment by the nurses using chlorine solution (PRESEPT 5.0 g tablet, Johnson & Johnson, Irvine, California), everyday according to the manufacturer's instructions. ATP was collected from 95 high touch surfaces using LuciPac Pen™ swabs (Kikkoman®) moistened in sterile water. Samples were collected by rubbing the swab over approximately 100 cm^2 area prior to processing in the Lumitester PD-20 Bioluminometer (Kikkoman®, Chiba, Japan) according to the manufacturer's instructions.

Microbial sample collection

A total of 115 samples were collected for microbiological analysis at different ICUs.

Sterile swabs (Puritan Foam Swab, Guilford, Maine, USA) were vigorously wiped over approximately 100 cm^2 of 95 high touch items, adjacent to the area used for ATP testing. The swab was transported to Australia and processed within two weeks. The swab tip was aseptically removed and placed in 2 ml phosphate buffer saline (PBS) and subjected to ultrasonication in an ultrasonic bath with a sweeping frequency of 42–47 kHz (Soniclean, JMR Australia) for five minutes, followed by vortexing for 5 s. An additional, 20 high touch items were aseptically, destructively sampled by cutting out sections of the item. A 1 cm^2 section was placed in 2 ml PBS and subjected to the same ultrasonication and vortexing process. Sonicated and vortexed samples were processed for multidrug-resistant organism isolation and for molecular analysis of microbial contamination.

Multidrug-resistant organism isolation (n = 115)

A 100 μl aliquot of sonicated/vortexed sample was transferred to 1 ml tryptone soya broth (TSB) and incubated aerobically for 18 h at 37 °C prior to plating on horse blood agar (HBA), MacConkey agar and the following chromogenic plates; Brilliance MRSA agar plates for the detection of MRSA, Brilliance VRE Agar Plates for the detection of VRE and Brilliance extended spectrum beta-lactamase (ESBL) agar plates (Oxoid, Adelaide, Australia) for the detection of ESBL producing Gram-negative bacteria. Plates were incubated aerobically at 37 °C for 24 h (HBA, MacConkey) and chromogenic plates were incubated at 37 °C as per manufacturer's instructions.

Molecular analysis of microbial contamination (n = 115)

DNA extraction and bacterial load

Genomic DNA was extracted from 200 μl of sonicated and vortexed samples using the High Pure Polymerase Chain Reaction (PCR) Template Preparation Kit (Roche Diagnostics, North Ryde, Australia) as per manufacturer's instructions. The number of bacteria in each sample, was determined by real-time quantitative PCR (qPCR) of the 16S rRNA gene using eubacterial universal primers as previously reported [12].

Composition of microbial community: metagenomics sequencing and data analysis

The V3-V4 region of 16S small subunit (SSU) ribosomal RNA was amplified using universal primer Bac_SSU_341F-806wR. Metagenomics sequencing was performed using the workflow outlined by Illumina (#15044223 Rev.B) by a commercial laboratory (Australian Centre for Ecogenomics, Brisbane, Australia). Briefly, PCR products of ~466 bp were amplified in standard PCR conditions, purified using Agencourt AMPure XP beads (Beckman Coulter, Australia) and indexed with unique 8 bp barcodes using the Illumina Nextera XT 384 sample Index Kit A-D (Illumina FC-131-1002) prior to sequencing on the MiSeq Sequencing System (Illumina).

Sequencing files were processed using QIIME's pick_open_reference_otus.py workflow with default parameters (97% similarity) and taxonomy assignment and alignment features suppressed [13]. Representative Operational Taxonomic Units (OTUs) sequences were then BLASTed against the reference database Greengenes version 2013/05 [14]. Statistical analyses and data mining were performed using Calypso software 7.6 (http://cgenome.net).

Detection and visualisation biofilm on dry hospital surfaces

Destructive sampled item fragments (3–5 cm^2) were stained with Live/Dead BacLight Bacterial Viability Kit (Life Technologies, Thermofisher Scientific, Boston USA) as described previously for biofilm covered surfaces [10] to distinguish viable from dead bacteria. The biofilm structure was examined under confocal laser scanning microscopy (CLSM) (Zeiss Axio Imager Microscope and/or ZEISS LSM 880, Carl Zeiss Ltd., Herefordshire, UK). Images were processed using ZEISS ZEN Imaging Software (Black Edition) and Imaris v 8.4, ImarisXT, Bitplane.

Following CLSM, samples were fixed in 3% glutaraldehyde, dehydrated though increasing concentrations of ethanol, coated in 20 nm of gold and examined in a scanning electron microscope (JEOL JSM- 6480), to determine biofilm presence, as described previously [10].

Statistical analysis

Linear regression was used to test for relationships between total bacterial load as determined by PCR and ATP relative light units (RLU) readings using SigmaPlot 13 statistical program (Systat Software, San Jose, United States of America). A Mann–Whitney Rank Sum test was used to compare 2 non-normally distributed groups such as ATP RLU readings and bacterial load for samples that were positive and negative for culture and MDRO isolation.

Results

ATP monitoring and bacterial load

The level of biological soiling as detected by bioluminescence-based ATP levels varied across the ICU surfaces. Based on the manufacturer's benchmark, 17 sites (18%) were considered low level soiling (<200 RLU), 20 sites (21%) were soiled to a medium level (between 200 to 400 RLU), but the majority of sites (61%) were deemed to be highly soiled (>400 RLU) (Fig. 1). There was no relationship between RLU readings and bacterial load of the sample as determined by qPCR ($P = 0.7$) (Fig. 1).

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