Journal of Infection and Public Health 11 (2018) 418–424

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: http://www.elsevier.com/locate/jiph

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

Khalid Johani[a,b,*], Danya Abualsaud[c], Dayane M. Costa[a,d], Honghua Hu[a], Greg Whiteley[e], Anand Deva[a], Karen Vickery[a]

[a] Surgical Infection Research Group, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, Australia
[b] Central Military Laboratories and Blood Bank, Prince Sultan Military Medical City, Riyadh, Saudi Arabia
[c] Molecular Laboratory, Medical Microbiology, King Fahad Armed Forces Hospital, Saudi Arabia
[d] Faculty of Nursing, Federal University of Goias, Goiania, Goias, Brazil
[e] Whiteley Corporation, North Sydney, Australia

ARTICLE INFO

Article history:
Received 13 July 2017
Received in revised form 7 October 2017
Accepted 12 October 2017

Keywords:
ICU
ATP
Biofilm
Microbial community
Antibiotic resistance

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),

* Corresponding author at: Macquarie University, NSW 2109, Australia.
E-mail address: KHALID.ALJOHANI@HDR.MQ.EDU.AU (K. Johani).

https://doi.org/10.1016/j.jiph.2017.10.005

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 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

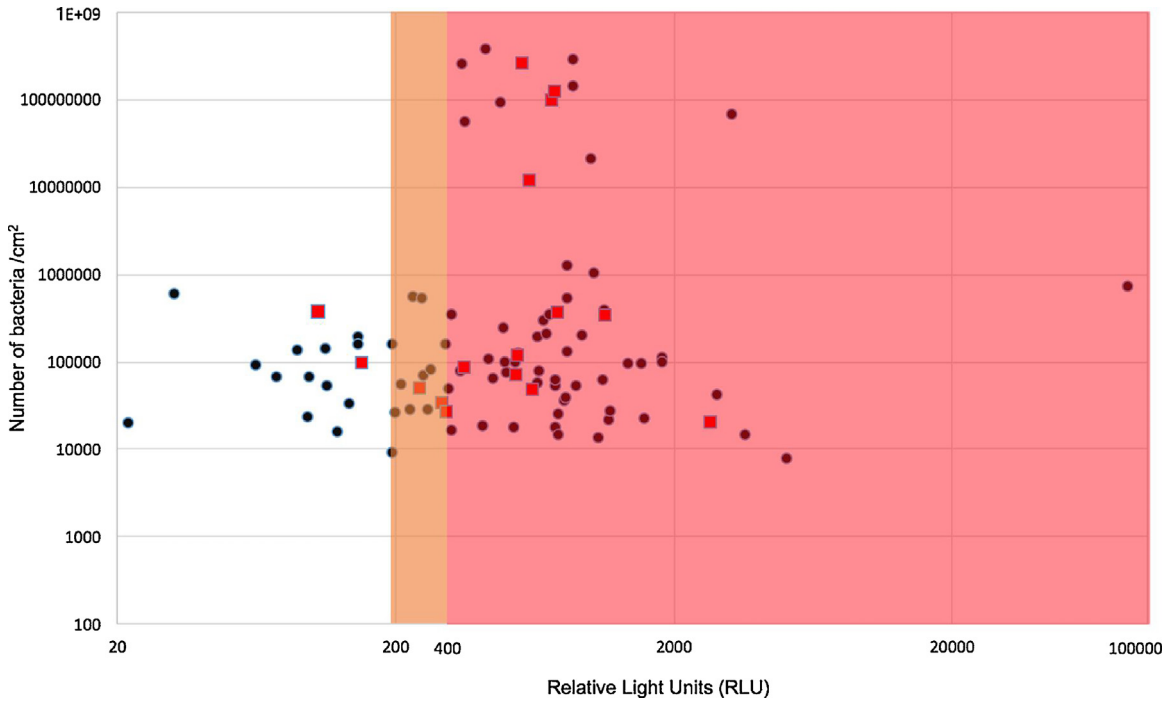

Fig. 1. Level of soiling on ICU surfaces n = 95. RLU readings and quantitative PCR values based on total soiling of 100 cm^2 of surface. Kikkoman® benchmarks RLU readings <200 are considered low contaminated, between 200–400 RLU medium contaminated, and >400 RLU are deemed to be highly contaminated. MDRO depicted by red squares.

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).

While only 71 of the items were culture positive, all samples had evidence of bacterial contamination as detected by qPCR of 16s rRNA gene. The number of bacteria contaminating ICU surfaces varied between 7821 to 370000000 bacteria/100 cm^2. However, the median value was 90 000 bacteria/100 cm^2, and in 57% of sites the bacterial load was below 100000 bacteria/100 cm^2 (Fig. 1). There was no difference in RLU or bacterial load between samples that were culture positive and culture negative (P = 0.25).

Multidrug-resistant bacteria detection (MDRO)

Swabs from 71/95 sites (75%) were culture-positive, of these 16/71 sites (22.5%) grew MDRO (Table 1). Twelve of the 95 samples grew *S. aureus*. There was no difference in RLU or bacterial load in samples that grew a MDRO and those that did not (P = 0.45). While 13/20 (65%) of the destructively sampled items were culture positive and grew ESKAPE organisms, including *S. aureus* and *Enterococcus* spp., no MDRO were isolated.

Composition of the microbial community

Direct analysis based on next generation DNA sequencing from 92 high-touch objects and 20 destructively sampled surfaces yielded 763 208 reads, ranging from 7 to 169 811 reads/sample,

Table 1
Location of MDRO positive environmental surfaces.

Location	Number of items sampled	Positive item	Isolate
Adult (unit 1)	14	Roller blind	MRSA
Adult (unit 2)	19	Bed railing Patient file table	*Proteus* (ESBL) *Enterococcus faecalis* (VRE)
Adult (unit 3)	23	IV pump face Bed controller	*Enterococcus faecium* (VRE) MRSA
Paediatric (bed 1) unoccupied bed	14	Glove box Chair Chair	*Enterococcus faecalis* (VRE) *Klebsiella* (ESBL) MRSA
Paediatric (bed 2) occupied by patient infected with *Pseudomonas*	8	Stethoscope IV device top Clipboard Chair	MRSA *Klebsiella* (ESBL) MRSA *Enterococcus faecium* (VRE)
Pediatric (two nurse stations)	17	Kidney Dish Tray Keyboard Calculator Clipboard	*Enterococcus faecium* (VRE) MRSA MRSA MRSA

MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococci; ESBL, extended-spectrum beta-lactamase Gram-negative bacilli.

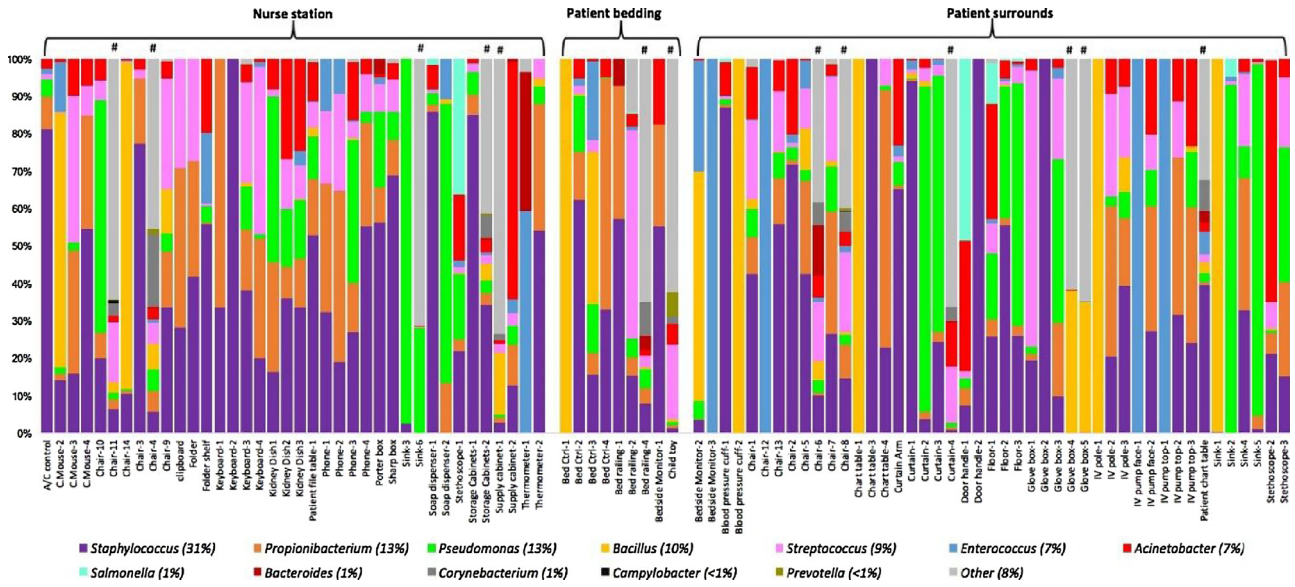

Fig. 2. Relative abundance of predominant genera in microbial communities contaminating individual intensive care unit surfaces. # Destructive sample.

with a richness range of 1–85 genera/sample and more than 408 OTU. Seven of the twenty destructively sampled items and 79/95 swab collected items had insufficient DNA for metagenomic analysis.

A comparison of the microbial communities at the genus level showed that *Staphylococcus, Propionibacterium, Pseudomonas, Bacillus, Enterococcus, Streptococcus* and *Acinetobacter* were the most abundant genera present in patients' surrounds, nurses' station and patient bedding. Analysis of pooled data revealed that 31% of the microbial community was composed of *Staphylococcus* followed by *Propionibacterium* (13%), *Pseudomonas* (13%), *Bacillus* (10%), *Streptococcus* (9%) *Enterococcus* (7%), *Acinetobacter* (7%) and *Salmonella* (1%) (Fig. 2). These genera formed 10% or more of individual microbiomes in 67 (*Staphylococcus*), 36 (*Propionibacterium*), 26 (*Pseudomonas*), 13 (*Bacillus*), 28 (*Streptococcus*), 12 (*Enterococcus*), 20 (*Acinetobacter*) and 3 (*Salmonella*) of the 92 samples. Individual results for destructively sampled items and swab items are found in Tables S2 and S3, respectively (Supplementary material).

At the species level, microorganisms classified as major causes of nosocomial infections were present. For instance, *S. aureus* was detected from 19 (50%) samples from nurses' stations, 5 (55%) patients' bedding and 15 (33%) patients' surroundings. Whereas *Salmonella enterica* was found among 13 samples (14%) and in three samples (door handle, stethoscope, floor) from 2 different rooms, it formed 49, 36 and 11% of the microbial communities respectively. For these three samples, *Acinetobacter* also contributed between 18 and 35% of the bacterial population. The potentially pathogenic *Acinetobacter schindleri* and *Acinetobacter johnsonii* were identified among 44 (48%) of samples.

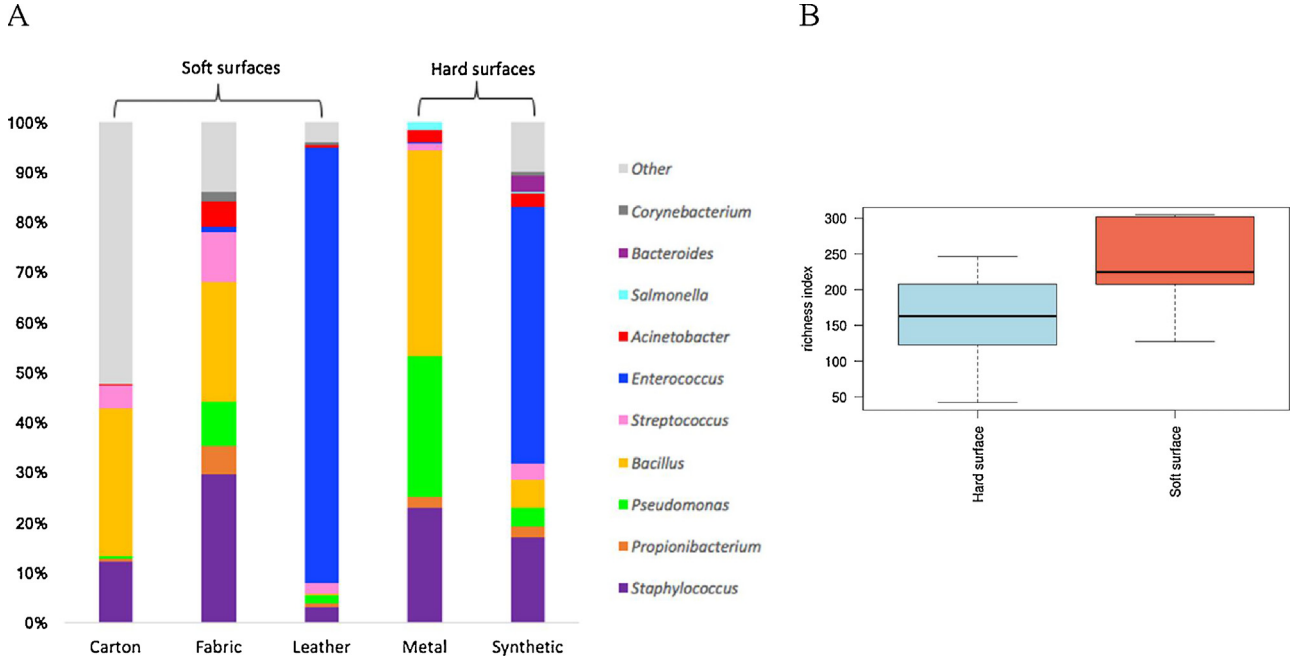

Fig. 3. (A) Genus analysis indicated the relationships between sample composition and bacterial diversity. (B) Observed OTUs richness for hard (n = 72) and soft (n = 20) surfaces (P = 0.048).

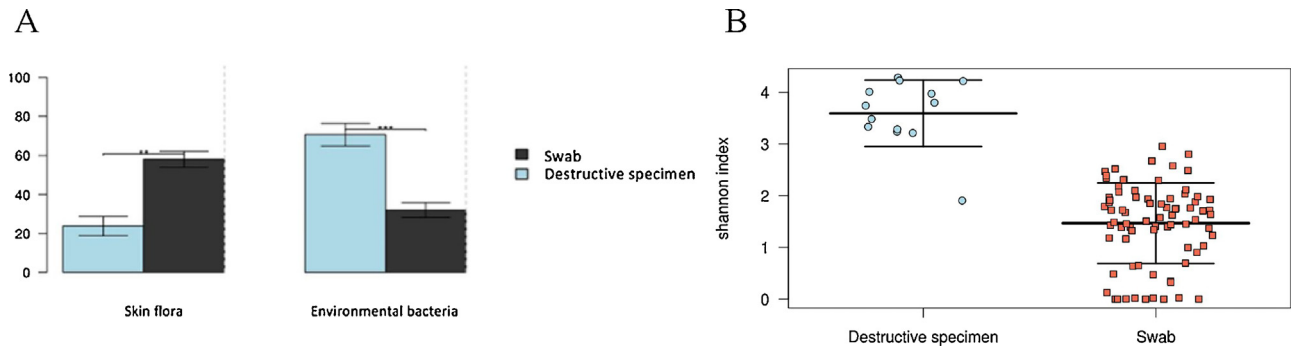

Fig. 4. (A) Comparison of the microbial communities between swab and destructive samples. (B) Community diversity of swab and destructive samples measured by the Shannon weaver index identified significantly more diversity in destructively sampled items ($P < 0.05$, ANOVA).

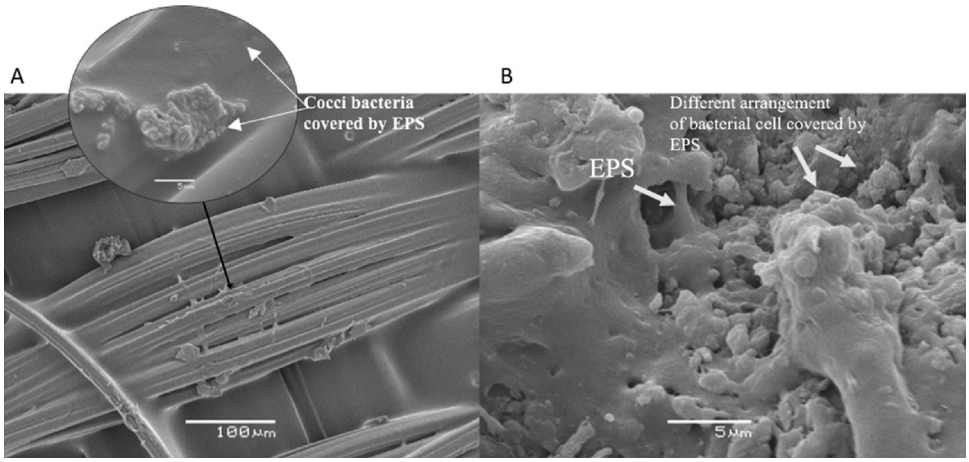

Fig. 5. Scanning electron micrograph of (A) curtain showing coccoid microbial cells encased in extracellular matrix (EPS); (B) nurses' station sink, showing micro-colonies predominantly composed of coccoid microbial cells encased in thick EPS.

Sample material composition in general seemed to have little effect on the genus of bacteria persisting on their surface, although, leather was colonized with high numbers of *Enterococcus,* and metal with more *Pseudomonas* and *Salmonella* (Fig. 3A). However, the texture of the surface had a significant effect with 20 soft surface samples (carton, fabric, leather) having more abundant OTU (mean total = 210/sample) than the 72 hard surface samples (metal and synthetic, mean total = 160/sample) ($P = 0.048$) (Fig. 3B). This was not related to degree of soiling as determined by measuring residual ATP ($P > 0.05$).

The microbiome of destructively sampled items had significantly more environmental bacteria and significantly less organisms normally found inhabiting the skin ($P < 0.05$) (Fig. 4A). The community richness and evenness (Shannon index) of the swabs and destructive samples showed greater diversity on the destructive samples ($P < 0.05$) suggesting that surface swabbing fails to sample the entire microbial contamination (Fig. 4B).

Presence of biofilms in intensive care unit

The presence of biofilm was visually confirmed, using SEM, in 70% (14/20) of items destructively sampled from high-touch objects. The biofilm was composed of bacteria with various morphological characteristics embedded in thick amorous extracellular polymeric substances (EPS) (Fig. 5). Biofilm positive samples were chairs (5/5), nurses' station sink (1/1), child's toys (2/2), curtains (2/4), glove boxes (2/2), bedside table (1/1) and storage cabinet (1/1). Bacteria failed to be isolated from 5 positive biofilm samples, however, bacterial viability staining and CLSM, confirmed the presence of live bacteria in all these culture negative samples (Fig. 6).

Discussion

Advances in sequencing technologies have allowed researchers to sequence entire microbial communities in a high-throughput manner. The relevance of the present study lies in the environments analysed, including adult ICU isolation wards and paediatric ICU. Understanding hospital environmental microbial community composition, is critical for improving decontamination procedures, which directly impact patient health. The sequencing revealed a large microbial diversity, contaminating high-touch objects, with over 408 distinct OTUs belonging to 115 different genera identified. The predominant genera belonged to microorganisms associated with the human microbiome e.g. *Staphylococcus*, *Propionibacterium, Bacillus, Enterococcus, Streptococcus* as well as environmental microorganisms such as *Pseudomonas, Bacillus,* and *Acinetobacter* and included known ESKAPE pathogens.

Due to extensive use of broad spectrum antibiotics, selective pressure for antibiotic resistance is high in the ICU environment and we cultured 16 MDRO from 115 samples. Notably, MRSA was found in all five rooms and at both of the nurses' stations, demonstrating its persistence in the environment. VRE was also present in four of the five rooms and at the nurses' station. We also found three different MDROs (MRSA, VRE and an ESBL producing *Klebsiella*) in both the paediatric ICU bed bays, one of which was vacant and sampled after terminal cleaning and the other bed inhabited by an infant colonised with *Pseudomonas* spp. This is concerning given that these rooms had been serviced and appeared clean.

Although ICU wards and ICU paediatric bays are frequently and strictly sanitised with disinfectants, biofilm was still visually confirmed to contaminate 14 of the 20 destructively sampled surfaces.

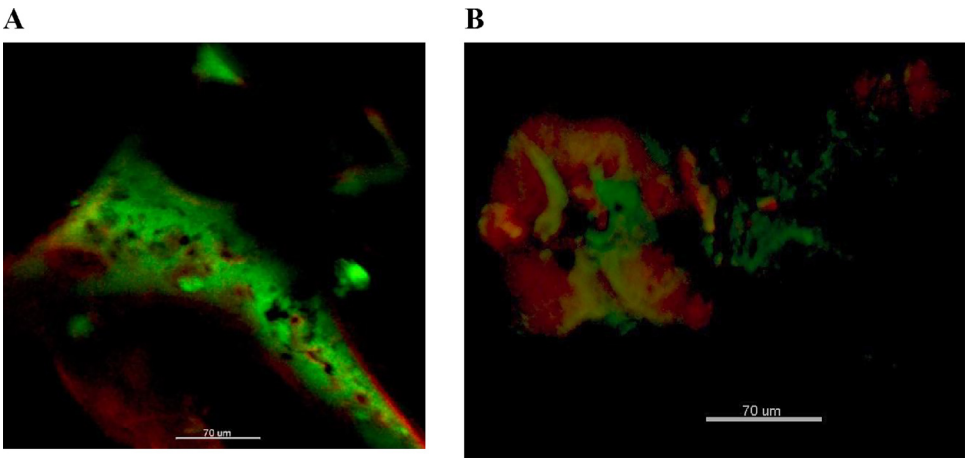

Fig. 6. Confocal laser scanning micrograph of biofilm stained with BacLight Live/Dead stain showing the presence of live bacteria (green) and dead bacteria (red). (A) Sample from a chair at a nurse station which was culture positive for a mix growth of Gram positive bacteria (B) Sample from a patient chart table showing live bacteria although the sample was culture negative.

Of the confirmed biofilm-positive samples, 9 were culture positive, and grew ESKAPE organisms such as *E. faecium, S. aureus, Klebsiella pneumoniae, A. baumannii, P. aeruginosa, and Enterobacter* species, and 5 samples were culture negative. Close contact with other microorganisms in a biofilm equates with possible exchange of antibiotic resistance genes. Ability to form biofilms has been positively correlated with antibiotic resistance, in clinical isolates of *K. pneumoniae* ($P < 0.01$) [15] and it is accepted that biofilm plays a crucial role in establishing resistant strains of bacteria in the environment [16]. This is because the high cell density of antibiotic resistant and sensitive bacteria coexisting in mixed biofilm, combined with accumulated mobile genetic elements results in efficient horizontal gene transfer of antibiotic resistance genes; conjugation rates have been estimated to be 1000-fold higher in biofilms as compared to planktonic cultures as reviewed by Balcázar et al. [17]. Moreover, Gram-negative bacteria are of particular concern as these organisms are highly efficient at up-regulating or acquiring genes that code for mechanisms of antibiotic drug resistance, especially in the presence of antibiotic selection pressure [18]. Therefore, the detection of MDRO and biofilm in the two ICUs sampled using microbial community profile analysis highlights the need for more stringent and thorough cleaning and disinfection strategies.

In this study, we collected either surface swab samples of high-touch objects or destructively sampled high-touch objects. We found that pathogens associated with the normal skin flora were significantly more pronounced in swab samples compared to destructively sampled objects, $P < 0.05$ (Fig. 4A). In contrast, we detected significantly more environmental bacteria in destructively sampled objects compared to swab samples $P < 0.05$ (Fig. 4A). Moreover, we found a significantly more diverse microbial community in destructively sampled objects compared to swab samples, $P < 0.001$ (Fig. 4B). The disparity between the two sampling approaches could be due to swabs only collecting surface bacteria, possibly only planktonic bacteria, whilst destructive sampling ensured that the whole biofilm structure was sampled.

Bacterial attachment is increased in proportion to surface roughness [19,20]. Uneven surfaces have a greater surface area compared to smooth surfaces, providing more bacterial attachment sites. In addition, bacteria are subjected to reduced shear forces when being wiped as happens during cleaning [21,22]. This was shown in the present study where the microbial diversity was significantly higher on soft surfaces (carton, fabric, leather) with 210 OTUs compared to hard surfaces (metal and synthetic) with 160 OTUs. These findings can be attributed to the fact that cleaning and disinfecting procedures are often easier on hard surface finishes so more bacteria are removed.

We found no correlation between bioluminescence-based ATP testing and microbial load as determined by qPCR of high-touch objects at both ICUs. In this study, we found that 58 of the 95 (61%) of the readings were considered heavily soiled based on Kikkoman's benchmark of 400 RLU [23] suggesting a failure of cleaning. The ATP bioluminescence method of "rapid hygiene monitoring" has previously been shown to give accurate readings for medium to high levels of contamination, however, at low levels of ATP, detection occurs below the lower level of quantification resulting in relatively higher levels of error [24]. ATP readings can also be affected by disinfectant chemistry used on the surfaces where samples were collected [25]. The main component of PRESEPT is a chlorine releasing agent and hence its use to disinfect surfaces in the hospital where the samples were collected may have affected ATP results. As ATP reflects the amount of organic residues, including food and bacteria, present on a surface, it should be used as a guide for microbiological testing, given that other studies have shown that moderate to highly soiled surfaces are more likely to yield pathogens [26] rather than assuming low ATP readings equates with being microbiologically safe.

A shortcoming of the present study is that microbial load was determined using qPCR which amplifies DNA from viable, fastidious, non-culturable and dead bacteria. Usually such analyses are performed using culture based methods often utilising simple media. Our more sensitive method could account for why all the ICU surfaces sampled were found to be highly contaminated with bacterial DNA. However, as depicted in Fig. 6 all culture-negative destructively sampled items were contaminated with an average of 68% live bacteria, demonstrating that the culture based method grossly underestimated microbial load and that the qPCR results probably provide a truer reflection of microbial contamination. If these high levels of live but non-culturable bacteria contribute to protecting environmental pathogens is unknown. However, we do know that any soil, including bacterial protein, decreases cleaning and disinfectant efficacy which may impact on pathogen persistence.

In conclusion, we employed extensive molecular analyses techniques including qPCR, microbiome analysis and live/dead staining combined with traditional culture for MDRO detection, ATP assessment and visual confirmation of biofilm presence using SEM. The two ICUs were cleaned and disinfected regularly, appeared clean, yet we found biofilms incorporating ESKAPE species on 70% of ICU surfaces. The physiological relevance of this is that biofilms

are tolerant to detergent removal, disinfectant action and display increased transfer of genetic material between incorporated bacteria. All these properties play a crucial role in establishing and maintaining MDROs in the environment. Therefore, we emphasise the importance of performing regular cleaning assessments. However, as we have demonstrated, the use of a single method of assessment, such as qPCR or culture is not sufficient to determine if the surface is microbiologically safe or even clean.

Funding

No funding sources.

Competing interests

None declared.

Ethical approval

Not required.

Acknowledgements

Khalid Johani was academically sponsored by the Medical Services Department (MSD) of the Ministry of Defence and Aviation, Saudi Arabia. Dayane Costa was in receipt of a Cotutelle International Macquarie University Research Excellence iMQRES scholarship, and Coordination for the Improvement of Higher Education Personnel Scholarship (CAPES — process no. 99999.006361/2014-05). Karen Vickery was in receipt of a Macquarie University Vice Chancellor Innovation Fellowship. Whiteley Corporation provided the ATP swabs.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jiph.2017.10.005.

References

[1] Salgado CD, O'Grady N, Farr BM. Prevention and control of antimicrobial-resistant infections in intensive care patients. Crit Care Med 2005;33:2373–82.
[2] De Kraker M, Wolkewitz M, Davey P, Koller W, Berger J, Nagler J, et al. Burden of antimicrobial resistance in European hospitals: excess mortality and length of hospital stay associated with bloodstream infections due to *Escherichia coli* resistant to third-generation cephalosporins. J Antimicrob Chemother 2011;66:398–407.
[3] Marwick C, Davey P. Care bundles: the holy grail of infectious risk management in hospital? Curr Opin Infect Dis 2009;22:364–9.
[4] Carlet J, Ben Ali A, Tabah A, Willems V, Philippart F, Chafine A, et al. Multidrug resistant infections in the ICU: mechanisms, prevention and treatment. In: Kuhlen R, Moreno R, Ranieri VM, Rhodes A, editors. 25 years of progress and innovation in intensive care medicine. Berlin, Germany: Medizinisch Wissenschaftliche Verlagsgesellschaft; 2007.
[5] Kuhlen R, Moreno R, Ranieri M, Rhodes A. 25 years of progress and innovation in intensive care medicine. Medizinisch Wissenschaftliche verlagsgesellschaft; 2007.
[6] de Kraker ME, Wolkewitz M, Davey PG, Grundmann H, BURDEN Study Group. The clinical impact of antimicrobial resistance in European hospitals: excess mortality and length of hospital stay related to methicillin resistant *Staphylococcus aureus* bloodstream infections. Antimicrob Agents Chemother 2011;55:1598–605.
[7] Otter JA, Yezli S, French GL. The role played by contaminated surfaces in the transmission of nosocomial pathogens. Infect Control Hosp Epidemiol 2011;32:687–99.
[8] Carling PC, Bartley JM. Evaluating hygienic cleaning in health care settings: what you do not know can harm your patients. Am J Infect Control 2010;38:S41–50.
[9] Mitchell BG, Dancer SJ, Anderson M, Dehn E. Risk of organism acquisition from prior room occupants: a systematic review and meta-analysis. J Hosp Infect 2015;91:211–7.
[10] Hu H, Johani K, Gosbell IB, Jacombs AS, Almatroudi A, Whiteley GS, et al. 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. J Hosp Infect 2015;91:35–44.
[11] Vickery K, Deva A, Jacombs A, Allan J, Valente P, Gosbell I. Presence of biofilm containing viable multiresistant organisms despite terminal cleaning on clinical surfaces in an intensive care unit. J Hosp Infect 2012;80:52–5.
[12] Jacombs A, Allan J, Hu H, Valente PM, Wessels WL, Deva AK, et al. Prevention of biofilm-induced capsular contracture with antibiotic-impregnated mesh in a porcine model. Aesthe Surg J 2012;32:886–91.
[13] Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 2010;7:335–6.
[14] DeSantis TZ, Hugenholtz P, Larsen N, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 2006;72:5069–72.
[15] Yang D, Zhang Z. Biofilm-forming *Klebsiella pneumoniae* strains have greater likelihood of producing extended-spectrum β-lactamases. J Hosp Infect 2008;68:369–71.
[16] Brooks B, Firek BA, Miller CS, Sharon I, Thomas BC, Baker R, et al. Microbes in the neonatal intensive care unit resemble those found in the gut of premature infants. Microbiome 2014;2:1.
[17] Balcázar JL, Subirats J, Borrego CM. The role of biofilms as environmental reservoirs of antibiotic resistance. Front Microbiol 2015:6.
[18] Bonten MJ, Mascini EM. The hidden faces of the epidemiology of antibiotic resistance. Intensive Care Med 2003;29:1–2.
[19] Tebbs S, Sawyer A, Elliott T. Influence of surface morphology on in vitro bacterial adherence to central venous catheters. Br J Anaesth 1994;72:587–91.
[20] Bryers JD, Characklis WG. Processes governing primary biofilm formation. Biotechnol Bioeng 1982;24:2451–76.
[21] Prakash B, Veeregowda B, Krishnappa G. Biofilms: a survival strategy of bacteria. Curr Sci 2003;85:1299–307.
[22] Oliveira R, Azeredo J, Teixeira P, Fonseca A. The role of hydrophobicity in bacterial adhesion. Cardiff: BioLine; 2001.
[23] ATP+AMP Rapid Hygiene Monitoring, 2011.
[24] Whiteley GS, Derry C, Glasbey T. The comparative performance of three brands of portable ATP-bioluminometer intended for use in hospital infection control. Healthc Infect 2012;17:91–7.
[25] Omidbakhsh N, Ahmadpour F, Kenny N. How reliable are ATP bioluminescence meters in assessing decontamination of environmental surfaces in healthcare settings? PLoS One 2014;9:e99951.
[26] Whiteley GS, Knight JL, Derry CW, Jensen SO, Vickery K, Gosbell IB. A pilot study into locating the bad bugs in a busy intensive care unit. Am J Infect Control 2015;43:1270–5.