Title

Evaluating the environmental microbiota across four National Health Service hospitals within England.

Running Title

Evaluating the environmental microbiota across four National Health Service hospitals within England.

Author names and affiliations

Fergus Watson[1,2], Sandra Wilks[3], Bill Keevil[1], John Chewins[2]

[1]School of Biological Sciences, University of Southampton, Southampton, United Kingdom [2]Bioquell UK Ltd., Andover, United Kingdom

[3]School of Health Sciences, University of Southampton, Southampton, United Kingdom

Corresponding author

John Chewins
Phone Number: +44 (0)1264 835835
Email: john.chewins@ecolab.com
Address:
Bioquell, an Ecolab Solution
52 Royce Close,
Portway Ind. Est.,
Andover,
SP10 3TS,
United Kingdom

Present/permanent address

Bioquell, an Ecolab Solution
52 Royce Close,
Portway Ind. Est.,
Andover,
SP10 3TS,
United Kingdom

Summary

Hospital surfaces contaminated with microbial soiling such as dry surface biofilms (DSBs) can act as a reservoir for pathogenic microorganisms and inhibit their detection and removal during routine cleaning. Studies have recognised such increases in bioburden can hinder the impact of disinfectants and mask the detection of potential pathogens. Cleanliness within healthcare settings is often determined through routine culture-based analysis, whereby surfaces that exhibit > 2.5 colony forming units (CFU) per cm^2 pose a risk to patient health and therefore, any underestimation could have detrimental effects. In this study, we quantified the microbial growth on high-touch surfaces in four hospitals within England over 19 months. This was achieved using environmental swabs to sample a variety of surfaces within close proximity to the patient and plating onto non-specific low nutrient detection agar. The presence of DSBs were confirmed using real-time imaging through episcopic differential interference contrast microscopy combined with epifluorescence. Approximately two-thirds of surfaces tested exceeded the limit for cleanliness (median: 2230 CFU/cm^2) whilst 83% of surfaces imaged with *Bac*Light™ LIVE/DEAD™ staining confirmed traces of biofilm. Despite the differences in infection control methods and patient demographic at each hospital, this was not reflected in the microbial variation observed and resulting risk to patients. This highlights a potential limitation in the effectiveness of the current standards for all hospital cleaning and further development using representative clinical data is required to overcome this limitation.

Introduction

Despite the advances made in infection prevention and control (IPC), hospital-acquired infections (HAIs) remain a serious complication in hospitalised patients. Approximately 20% of patients in the National Health Service (NHS) are affected by HAIs and this results in yearly financial losses estimated at £1 billion[1-3]. Evidence suggests a considerable amount of HAI incidences can be prevented through stringent IPC measures such as barrier precautions for isolation and screening of patients, environmental disinfection, and hygiene compliance. The World Health Organisation and National Audit Office estimate that between 20 - 50% of cases are preventable[4-6]. The importance of environmental surfaces can be often underestimated in IPC measures and arguably play a significant role in the acquisition and transmission of HAI within healthcare with up to a quarter of HAI cases believed to originate from contaminated clinical surfaces[7,8]. Several studies have established links between the HAI rates or outbreaks in healthcare, and the bioburden in the built environment; the primary focus of these studies being to target specific pathogens referred to as indicator organisms, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecalis* or *Acinetobacter baumannii,* typically, by using selective agars[9-12]. Whilst the results may be useful in governing existing IPC performance the studies routinely overlook the levels of resident microbes and organic soiling present and their potential roles[13].

Contaminated surfaces, by microorganisms and/or organic matter, are proven to reduce the efficiency of biocidal products and inhibit removal when using chemical and physical cleaning. As a consequence, these can increase the transmission risk for nosocomial pathogens[14-17]. This risk is further increased by the recent discovery of dry surface biofilms (DSB) on hospital surfaces which impose a similar hindrance to hospital cleaning regimes. Biofilms are microbial communities attached to a substrate and embedded in self-produced extracellular polymeric substances (EPS) and in comparison to planktonic cells are significantly less susceptible to antimicrobial agents[18]. Ledwoch *et al*. (2018) and Vickery *et al*. (2012) isolated traces of DSB, containing both multi-drug resistance organisms and environmental flora, in high-risk areas such as intensive care units, and in spite of continuous exposure to hypochlorite-based cleaning agents[19,20]. The persistence seen in HAI rates may be explained by the high levels of resident microorganisms existing as DSBs; which in turn can feed into the explanation as to why common hospital disinfectants, with proven efficacy against test standards using planktonic organisms, are failing to achieve the desired effect. Current United Kingdom (UK) guidance states there is an increased risk to patient health if

total colony counts (TCC) for touch surfaces exceed 2.5 colony forming units (CFU) per cm^2 [21,22]. In this study, we investigate the degree of surface contamination on dry surfaces across four English NHS Trust sites. The surfaces chosen for sampling were considered high-touch and thus high-risk for microbe transmission within a healthcare environment as previously described[23]. We aimed to observe the abundance of microbes per surface (CFU/ cm^2) whilst also characterising biofilm presence in the UK to determine the potential risk within the healthcare environment.

Methods

Sample sites and strategy

The study was conducted at four NHS trust sites geographically separated throughout England (Southeast/ Southwest/ Midlands/ Northeast). Sampling was performed across a range of care facilities from non-critical care and general admission wards to high dependency childcare and intensive care units. A total of 12 wards were included in this study. The number of beds per ward ranged from 20 to 35 together with up to 7 side rooms. Each bed had an adjoining table, personal belongs cabinet and visitor's furniture. The patient and staff environments were sanitised by healthcare workers and all cleaning schedules were maintained and remained unchanged throughout the study. A variety of cleaning procedures and agents were employed by each of the NHS trust sites including the use of chlorine-based, polymer-based and dodecylamine-based solutions. This study was performed over a 19-month period between August 2018 and February 2020. All staff were made aware of the study but were not aware of the precise time or locality at which sampling took place. Sampling was performed across all surface types categorised according to contact audits as previously shown by Adams *et al*. (2017)[23]. These surface types comprised of: those near the patient such as bed rails, mattresses, patient chairs; those further away from the patient such as visitors' furniture, patient tables and personal belonging cabinets; and finally, clinical equipment within the confines of the patient wards.

Microbiota sample collection

Sampling for surface microbes was performed in accordance with Johani *et al*. (2018) with minor adaptations[24]. In brief, sterile premoistened foam swabs (Technical Service Consultants Ltd, Lancashire, UK) were vigorously wiped over approximately 100 cm^2 of each surface. The swab was transported to the University of Southampton and processed within two weeks. The swab tip was aseptically removed into 2 mL of phosphate buffer saline (PBS) containing sterile glass beads (2 mm diameter) and allowed to soak for up to 15 minutes at room temperature, after which each sample was vortexed twice for 10 - 15 seconds intervals. Vortexed samples were used for culture and microbial analysis.

The microbial loading of each sample was determined by serial dilution through to 10^{-4} and spreading 30 µL aliquots of each dilution onto Reasoner's 2 Agar (R2A). The plates were incubated at room temperature for up to 120 hours. The number of colonies on each plate were recorded and reported as CFU per cm^2 using the following calculation:

$$CFU/cm^2 = \left[\left(\frac{(mean\ cfu/plate)}{volume\ of\ sample\ plated}\right) \times \left(\frac{volume\ scraped\ into}{surface\ area\ scraped}\right) \times (dilution)\right]$$

Volume scraped into = 2 mL

Surface area scraped = 100 cm^2

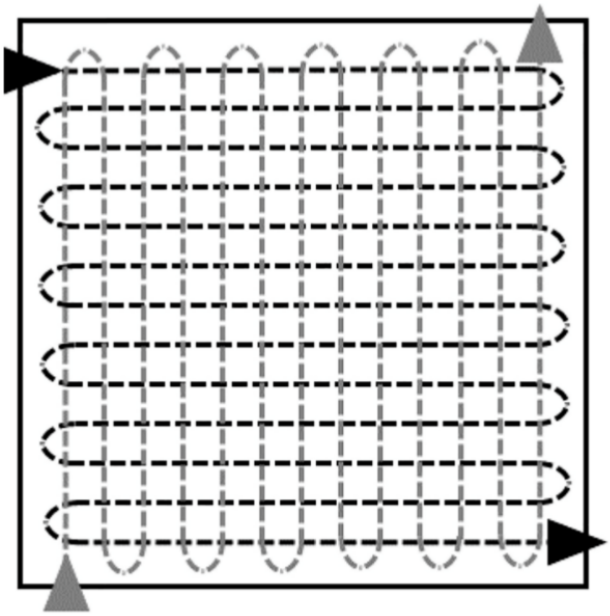

Figure 1 Diagram of the sampling procedure as shown by Jansson *et al*. (2020)[25]. The black arrow heads depict the first sweeping motion whilst the grey arrow heads the second time.

Detection and visualisation of microbiota from hospital surfaces

Material samples from high touch surfaces of each hospital were physically removed, post swabbing, from the environment and stained with *Bac*Light™ LIVE/DEAD™ Bacterial Viability Kit (Invitrogen™, UK) to identify viable and non-viable cell populations. Episcopic differential interference contrast (EDIC) microscopy combined with Epifluorescence (EF) using an NikonEclipseLV100D microscope (Best Scientific, UK) was used to examine the surfaces[26].

Additionally, culture-negative vortexed samples were subjected to similar viability staining in accordance to Wilks *et al*. (2021)[27]. In brief, 1 mL of sample was stained with Bacterial Viability Kit, filtered onto black polycarbonate nucleopore filters (0.2 µm) (Whatman, UK) and placed onto glass slides for EF microscopy. An estimated number of stained bacteria were counted across 10 randomly selected fields of view using ImageJ version 1.52a (National Institute of Health).

Statistical analysis

The statistical significance of our data was evaluated with GraphPad PRISM® (ver. 7.04) using one-way analysis of variance (ANOVA).

Results

Microbial loading on hospital surfaces

Collectively more than 1500 swabs and samples of high-touch surfaces were processed across the four clinical sites and results show 68% (1080/1589) of these exhibited signs of growth during incubation on R2A. The levels of microbial loading for culture-positive results varied from 1 to 2.01 x 10^8 CFU per cm^2, with a median of 8.34 x 10^5 CFU/cm^2. According to the distribution frequency for these counts approximately half of swab samples taken were ≥10^3 CFU/cm^2 (Figure 2).

Despite prolonged incubation, 32% of the surfaces recorded no microbial growth. A random selection (20/509) of these culture-negative samples were filtered out of solution and subjected to LIVE/DEAD™ staining, with SYTO-9 and propidium iodide, to detect traces of cell viability using episcopic differential interference contrast (EDIC) microscopy combined with epifluorescence (EF). We were able to show the number of intact bacterial (live) cells per swab ranged from 3.80 x 10^1 – 3.88 x 10^3 bacteria (median = 1.32 x 10^3 bacteria) (Figure 3).

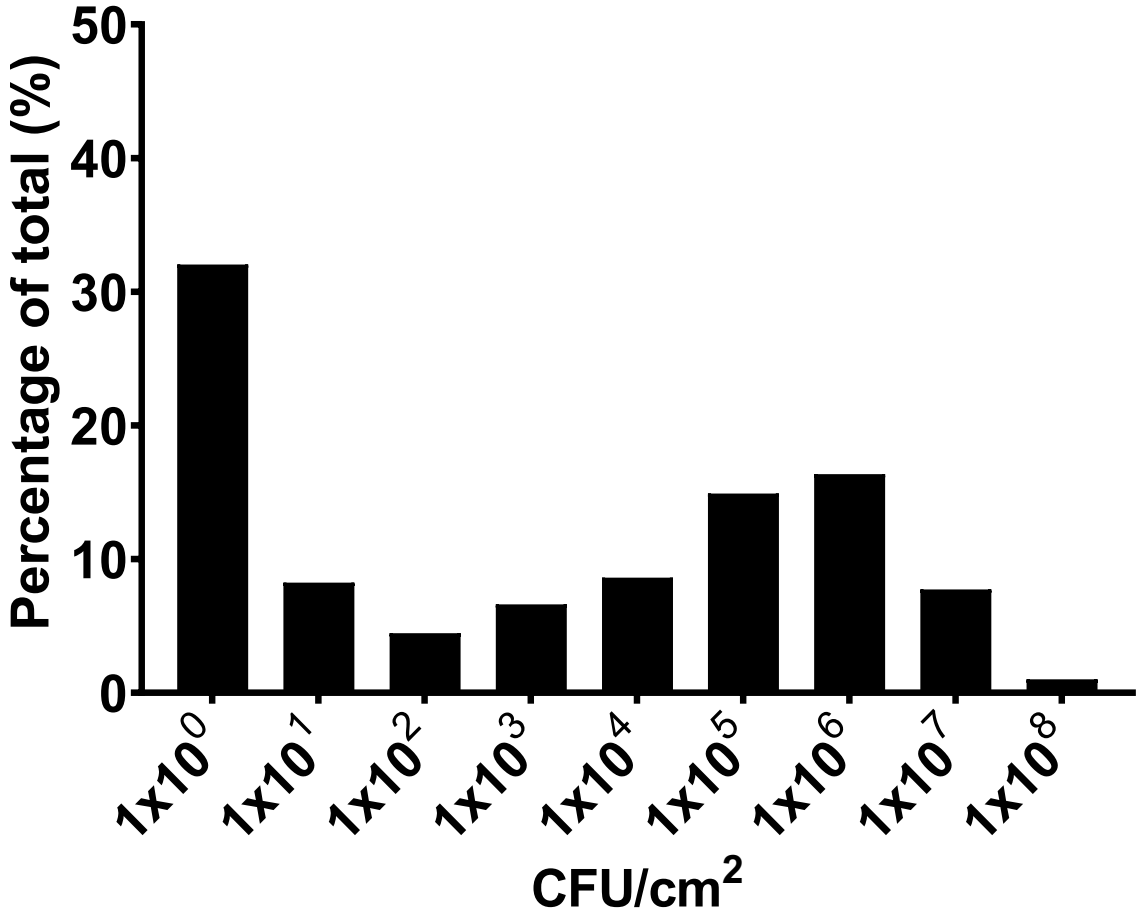

Figure 2. The collective distribution frequency for microbial loadings of each swab as a percentage of the total (n=1589) taken across the four study sites over a 19-month period. The average concentration of microbes across all the surfaces samples was 2.03 x 10^6 CFU/cm^2 with a standard error of the mean of 2.64 x 10^5 CFU/cm^2.

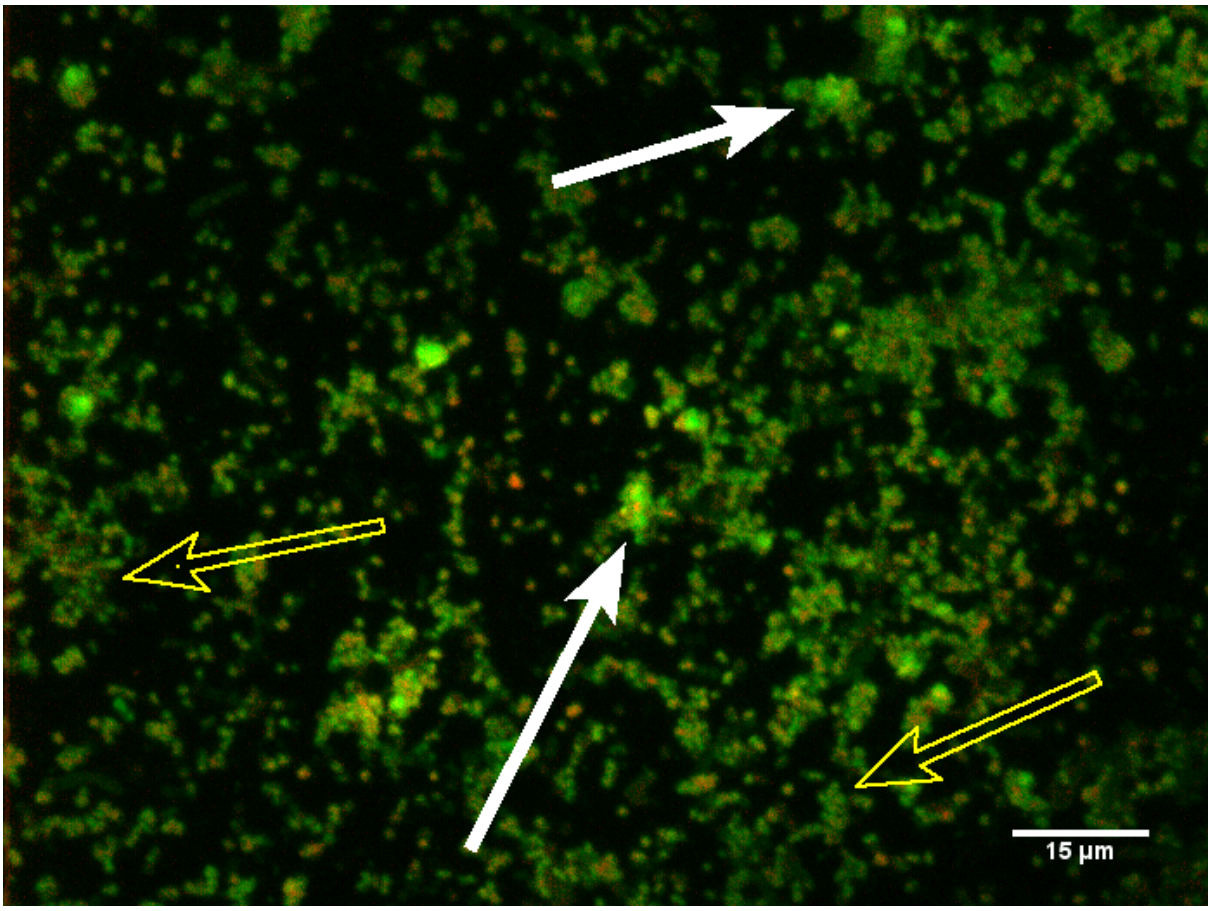

Figure 3. EDIC/EF micrographs of microorganisms from culture-negative samples filtered onto nucleopore filter paper stained with bacterial viability stains SYTO-9 (green) and propidium iodide (red) used to detect live and dead bacteria, respectively. The outlined yellow arrows indicate an abundance of viable cells whilst the solid white arrows highlight suspected traces of EPS within the culture-negative samples.

Variability across study sites

A comparison of the NHS trust sites showed only a 10% deviation in incidence rates for culture-positive results with the medians ranging from 7.67 x 10^4 to 2.20 x 10^5 CFU/cm^2 (Figure 4). There was a significant difference in median microbial loadings for only two of the four sites (P = 0.0031).

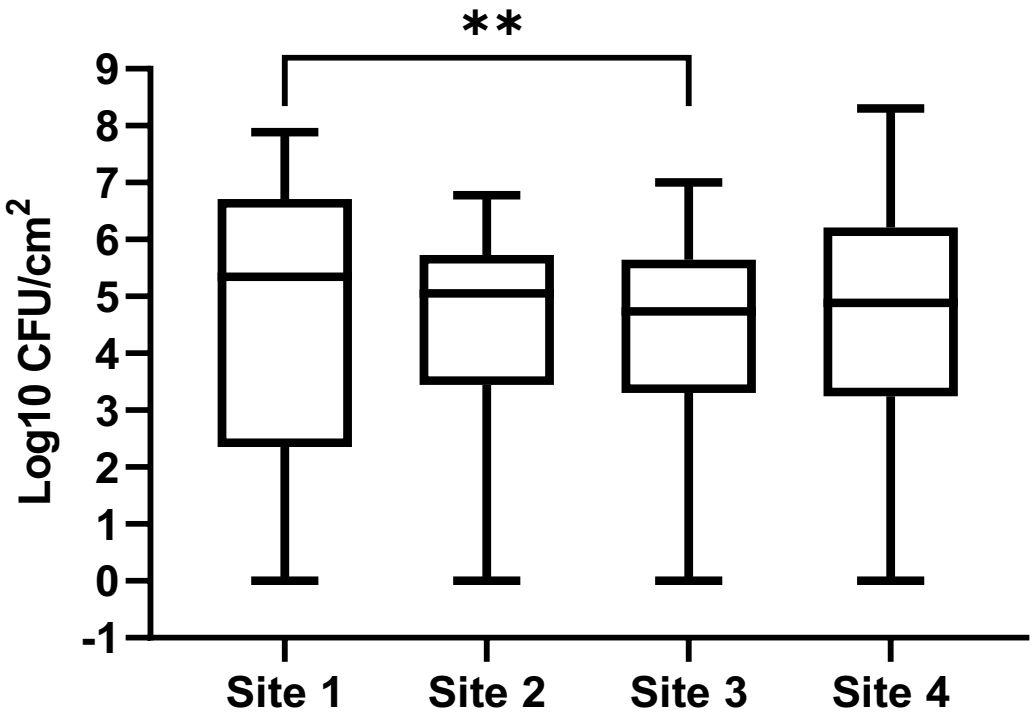

Figure 4. Comparison of microbial loadings Log_{10} CFU per cm^2 for the culture-positive swabs recorded across four study sites. The average counts for each site are as follows: Site 1 (n=329) 4.87 x 10^6 ±5.01 x 10^5 CFU/cm^2 (median = 2.20 x 10^5 CFU/cm^2); Site 2 (n=259) 6.04 x 10^5 ±7.00 x 10^4 CFU/cm^2 (median = 1.13 x 10^5 CFU/cm^2); Site 3 (n=398) 4.68 x 10^5 ±5.66 x 10^4 CFU/cm^2 (median = 5.47 x 10^4 CFU/cm^2); Site 4 (n=94) 1.36 x 10^7 ±3.85 x 10^6 CFU/cm^2 (median = 7.67 x 10^4 CFU/cm^2).

Biofilm presence on hospital surfaces

The presence of bacterial biofilms was visually confirmed using EDIC and EF microscopy on 83% (24/29) of items retrieved from the hospital sites. These included bathroom flooring (3/5), privacy curtains (2/3), personal belongs cabinets (1/2), staff notice board (1/1), computer keyboards (5/6) and mattress covers (12/12). Clusters of viable bacterial cells can be seen to aggregate along the cracks and undulations of the substrate surface, as well as potential traces of extracellular nucleic acids signified by the undefined staining surrounding the microcolonies indicative of biofilm formation and maturation (Figure 5).

A discrepancy was observed between the microscopy and culture results for selected materials whereby two curtain samples with visual traces of viable bacteria failed to grow during culture analysis. The inverse was true for four additional samples, bathroom flooring (2/5), bedside cabinet (1/2) and keyboards (1/6), which all presented growth during incubation but lacked signs of viable bacteria on the surface.

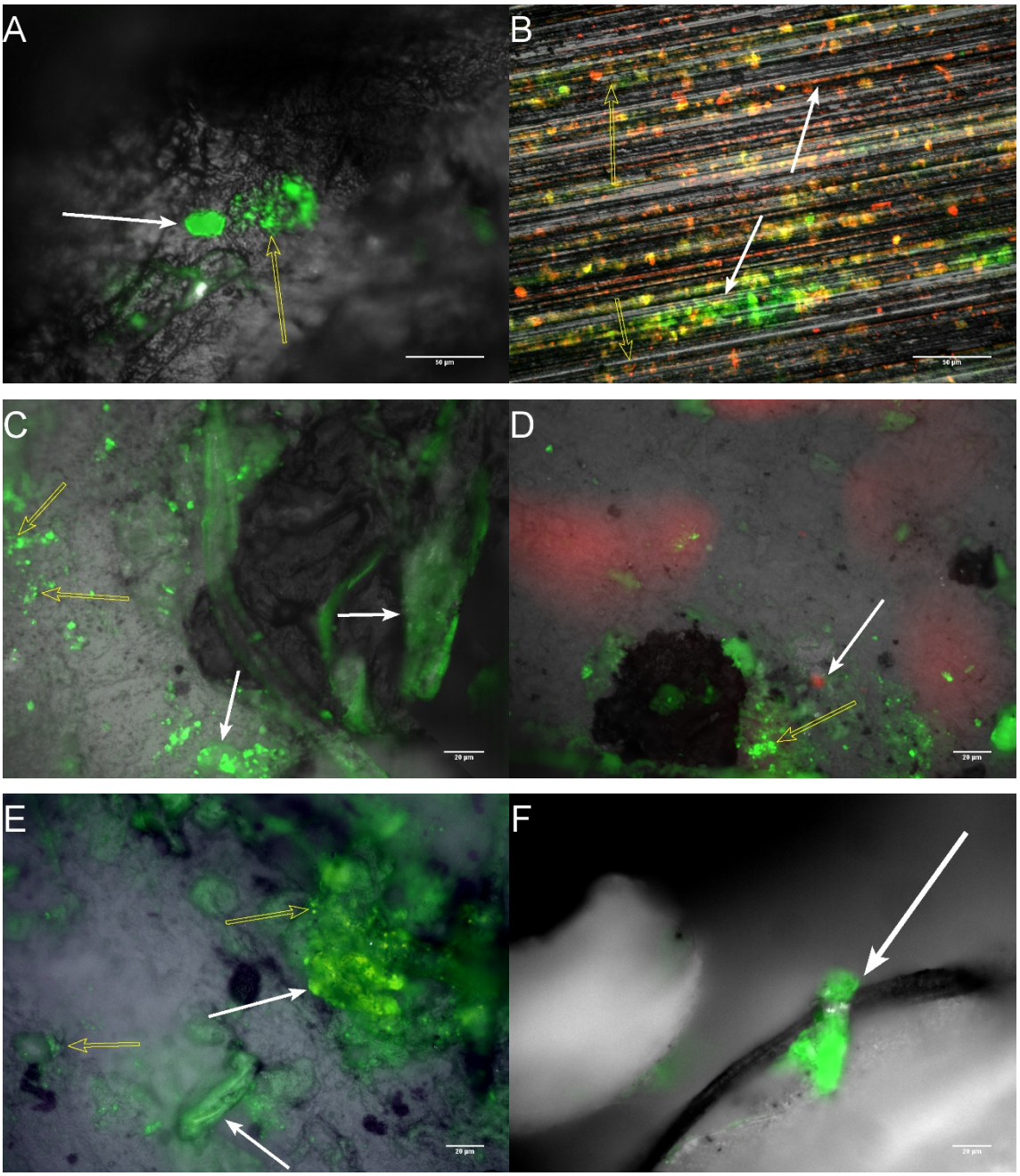

Figure 5. EDIC/EF microscopy of clinical surfaces (A – bathroom flooring, B – personal belongs cabinet, C – staff notice board, D – mattress cover, E – computer keyboard, F – privacy curtains) stained with *Bac*Light™ LIVE/DEAD™ demonstrating clusters of microcolonies embedded within the undulating topography indicated by the solid yellow arrows. The outlined white arrows indicated suspected traces of extracellular nucleic acid indicated by undefined edges and soft fluorescent staining seen to surround some of the microcolony formations. The micrographs highlight signs of viable bacteria in samples that failed to exhibit growth during culture analysis.

Discussion

Surface contamination of the built environment by nosocomial pathogens plays a significant role in the transmission of HAIs across nearly all healthcare platforms[28]. Studies have repeatedly shown the ease at which these pathogens can be transferred between patients, inanimate objects, and hospital staff[29-31]. Cleaning practices remain a basic but necessary component within IPC and inadequacies have been linked to enhanced risks of HAI acquisition in patients[32,33]. In the past decade, studies have shown a distinct lack of *in situ* data supporting the impact of hospital cleaning on HAIs; and there is a clear discrepancy in the correlation between visual cleanliness and microbe presence and soiling on surfaces[34-36]. Our findings revealed 63% of the surfaces tested were contaminated with a culturable microbial community exceeding the current guidance for acceptable levels of aerobic microbes (< 2.5 CFU/cm^2) and thus cleanliness; notably greater than previously published (25-50%)[23,37,38].

In the UK, clinical studies have used < 2.5 CFU/cm^2 and < 1.0 CFU/cm^2 as guidance for monitoring hospital cleaning efficacy on high touch or high-risk surfaces when quantifying TCC and pathogen counts, respectively. Internationally this increases to < 5.0 CFU/cm^2 and is used frequently for food contact surfaces in the food industry[39]. Depending on the hospital and IPC policies, isolating a specific nosocomial pathogen will often result in enhanced or targeted hospital cleaning[40]. Nonetheless, irrespective of the microbe speciation the guidance suggests a breech in the counts can result in an increased risk of patients acquiring or transmitting infection within that environment [41,42]. In this instance, our results share a similarity to studies by Johani *et al*. (2018) where comparable methodologies were used for swabbing[24]. The group reported 75% positivity in culture growth versus our 68% and using quantitative polymerase chain reaction of RNA reporting a range in bacterial loads (78 to 3.70 x 10^6 bacteria/cm^2) comparable to our own (median: 8.34 x 10^5 CFU/cm^2). Although the results support those cited within the biofilm studies, the use of polymerase chain reaction methods, albeit fast and sensitive, can amplify the DNA of both dead and viable bacteria indistinguishably. This should be considered upon review as the DNA can remain stable for extended periods after cell death[43]. Generalised environmental monitoring of clinical surfaces is not frequently practised in hospitals and the majority of studies cited here, and thus IPC practices are focused towards isolating nosocomial pathogens in patient samples to dictate subsequent cleaning approaches[44,45]. The incident rates shown here greatly exceed those shown by other clinical studies, for example Shams *et al*. (2016) and Widmer *et al*. (2019) described high TCC recoveries for contamination on frequently touched surfaces ranging

from 1 to 300 CFU /cm^2 [46]. Widmer *et al*. (2019) were able to show that at these levels TCC is a poor indicator for the presence of pathogens of high clinical relevance; however, it is not clear whether this applies to the significant levels of resident microbes shown in this study and the threat to patient health [38]. The difference between the levels of microbiota detection is likely to stem from differences in sampling and culturing techniques, the latter of which has been shown to greatly influence results [34,47]. High presence of bioburden on surfaces would suggest the following suppositions: firstly, it indicates current cleaning practices are insufficient and lack the appropriate level of efficacy for the microbial challenge presented, therefore improving the chance of pathogen survival and persistence. Secondly, this may hinder the detection of clinically relevant nosocomial pathogens residing within the microbiota and mask their true abundance, a common trait of DSB [23,24]. Thirdly, through increased levels of surface bioburden there may be increased contact-based transmission of microorganisms amongst patients and hospital staff [48].

Our understanding of the nutrient availability on dry hospital surfaces is limited and it is assumed that a film of organic and inorganic matter meets the needs of resident flora to facilitate growth and subsequent transmission [48-51]. The choice of sampling equipment and culture media used in this study aimed to cover a range of surface material types and scenarios whilst detecting a variety of microorganisms. The macro-foam swabs shown here exhibit superior flexibility and possess an open structure to allow efficient detection and release of difficult to reach microorganisms. We postulate our use of R2A had the greatest influence on results achieving almost 30% more microbe detection in our samples when compared to routine culture media. R2A is a non-selective, low nutrient media developed originally for use in the water industry where it was known to yield considerably higher microbial counts for difficult to culture heterotrophic bacteria [52,53]. Studies in comparably low nutrient environments have illustrated the choice of recovery media can significantly influence both the TCC and diversity recorded [54]. Such low nutrient environments, like those seen here, can often induce a state of dormancy or suppressed metabolism and as a result some species of bacteria remain viable but non-culturable (VBNC) on routine culture media like those seen in the clinical studies cited herein [55,56]. A state of VBNC can also be stimulated through exposure to chemical stresses such as antimicrobial agents or surface disinfectants commonly found in hospitals [57,58]. This VBNC state is often seen as a survival mechanism and can reduce the organism's susceptibility to antimicrobials such as cleaning agents [59]. Our investigation highlights the potential underestimation of overall levels of

microbiota on hospital surfaces and thus overlooking the impact this may be having on patient health or HAI transmission.

About 65% of nosocomial infections are believed to stem from biofilms, however this figure does not account for DSB on hospital surfaces whose impact is largely unknown [60]. DSBs comprised of multi-drug resistance organisms are becoming widely recognised across high-risk surfaces and have been shown to survive extended periods of time in spite of extensive cleaning [19,20,50]. We have been able to contribute further to this evidence by demonstrating clusters of bacterial microcolonies forming within the fabric of hospital surfaces. The extracellular nucleic acids identified by the fluorescent haze within the matrix plays a crucial role in the attachment and maturation as well as the structural stability of a biofilm [61]. These inherent characteristics of biofilms contribute to their tolerance to eradication either by physical removal as a result of the enhanced attachment forces to the substrate or the quenching effect had on antimicrobials within the matrix thus reducing the biocidal concentration to suboptimal levels [16,62]. The presence of biofilm contamination for this study falls within the reported values of Johani *et al*. (2018) (70%) and Ledwoch *et al*. (2018) (95%) [19,24]. Furthermore, as shown by the groups, biofilm was detected on two samples despite a negative culture result. The results highlight a necessity for multifaceted approaches to biofilm detection on hospital surfaces; most notably since swabbing has limited detection capabilities [63]. The presence of DSB provides a possible explanation for the difficulties encountered when tackling persistent HAI outbreaks as well as highlighting the need for better biofilm efficacy claims on hospital disinfectants [64,65].

The efficacious properties of hospital disinfectants, and cleaning regimes, are dependent upon numerous factors including the antimicrobial agents used, and the initial levels, distribution and presentation of surface bioburden within the target environment [21]. Whilst comparing the microbiota of each study site we found only sites 1 and 3 differed with any significance. Ashokan *et al*. (2021) demonstrated a similar lack of significant difference, in terms of hospital microbiota, between two hospitals despite a variation in the building design and age [66]. Although not reported here patient admissions and hospital activity during the study period may explain the statistical difference seen, as for example, increased bed occupancy can be linked to high microbial contamination on surfaces, and therefore this study would benefit from a retrospective cross comparison[67]. Broadly speaking, NHS sites will tailor their IPC approach in accordance with governmental guidance and their patient demographic [68]. Therefore, cleaning will vary considerably amongst trusts and even departments depending upon the resources, managerial support and training available [69,70]. In this instance the

primary antimicrobial agents used at each hospital included: sodium hypochlorite (NaOCl), a widely used disinfectant which elicits kill through chlorine oxidizing agents, hypochlorous acid and hypochlorite, which reacts to numerous biological molecules such as amino acids and lipids [71]; sodium dichloroisocyanurate (NaDCC), similar to NaOCl uses reactive chlorine agents for oxidative degradation of cellular components [72]; didecyldimethyl ammonium chloride (DDAC), a biocidal quaternary ammonium compound with an alkyl group capable of causing cell membrane distortion and disruption to cell wall functions; and 2-bromo-2-nitropropionamide (known as bronopol), a halo-nitro compound which exhibits antibacterial activity through the oxidation of thiol groups such as cysteine [73]. Despite the variety of mechanisms, unsurprisingly, all these cleaning agents make near-identical efficacy claims for bactericidal, sporicidal and virucidal properties against European Norm. Standards (e.g., EN 1276). All the study sites shown here exhibited a similar detection rate for surface contamination (63 – 73%), and we postulate this would demonstrate a universal level of background flora within hospitals. If these levels of contamination are deemed harmful to patients and the formation of biofilms on dry surface are suspected of hindering disinfectant efficacy, testing standards must incorporate a more representative microbial challenge that depicts those found *in situ,* as shown here, to provide clinicians a more realistic expectation when used in hospitals. There are currently no standardised efficacy tests for biofilms within Europe and the only available tests, from the United States, fail to accurately represent dry surfaces, often using hydrated biofilms grown under nutrient-enriched conditions [64,74].

From this study, it is clear the levels of resident microbes (in terms of TCC) frequently exceeded the acceptable limits and posed a potential risk to patient health. Due to limitations within the data collection method we were unable to correlate the occurrence of cleaning with environmental sampling. However, as shown by Saka *et al*. (2017), surfaces within the patient environment become rapidly re-contaminated post cleaning, taking as little as 6 hours to achieve levels equivalent to those prior [75]. Initial re-contamination in most incidents is influenced greatest by the patient's own flora [76]. Although we believe the work presented here is representative of the hospital microbiota a more comprehensive study would benefit from incorporating the monitoring of IPC practices such as cleaning.

From our results, we acknowledge the pathogenicity of the microorganisms cultured within this study cannot be determined unlike other clinical studies which are able to identify antimicrobial resistance and thus understand the potential risk to patients[77,78]. Incorporating the use of 16S RNA sequencing into this study would support our culture data and enhance this study further[24].

For IPC to effectively combat the threat of HAIs and their transmission amongst contaminated surfaces; healthcare workers and patients alike, we must be able to define the microbial challenge present more clearly, particularly when choosing biocidal products. The data shown here supports claims that biofilms regularly form on high-risk surfaces around patients irrespective of cleaning, and more importantly in areas exposed to desiccation and infrequent moisture availability [20]. Moreover, the findings raise questions as to the suitability of current standards for testing the biocidal properties of hospital disinfectants and the requirement for a robust biofilm efficacy model beyond the existing protocols for hydrated biofilms, such as ASTM E2871 the 'standard test method for evaluating disinfectant efficacy against *P. aeruginosa* biofilms grown in CDC Biofilm Reactor' [79]. The data shown here should be used to develop biofilm models capable of more accurately representing the microbial challenges found *in situ* for *in vitro* efficacy testing. Buckingham-Meyer *et al*. (2007) have previously underlined the importance of emulating the environmental growth conditions of the scenario in question and the pitfalls of models which fail to do so; and future dry surface biofilm models should look to include desiccation, nutrients source and availability, and shear forces that resemble the clinical environment [80]. Our study demonstrates how current surveillance techniques are significantly underestimating the microbial burden on high-touch and high-risk surfaces in close proximity to the patient. Using a more comprehensive approach to monitoring surface cleanliness, IPC teams can be more informative decisions when implementing new antimicrobial strategies

References

1. Public Health England. English Surveillance Programme for Antimicrobial Utilisation and Resistance, https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/921039/Ted_Final_version__1318703-v45-One_Health_Report_2019_FINAL-accessible.pdf; 2017. [accessed May 2021]
2. Naylor, Nichola R., et al. "Estimating the burden of antimicrobial resistance: a systematic literature review." Antimicrobial Resistance & Infection Control 7.1 (2018): 1-17.
3. Graves, Nicholas. "Economics and preventing hospital-acquired infection." Emerging infectious diseases 10.4 (2004): 561.
4. National Audit Office. Reducing healthcare associated infections in hospitals in England, https://www.nao.org.uk/report/reducing-healthcare-associated-infections-in-hospitals-in england/. (2009) [accessed May 2021].
5. World Health Organization. "Report on the burden of endemic health care-associated infection worldwide." (2011).
6. Moro, Maria Luisa, et al. "Rates of surgical-site infection: an international comparison." Infection Control & Hospital Epidemiology 26.5 (2005): 442-448.
7. Weinstein, Robert A. "Nosocomial infection update." Emerging infectious diseases 4.3 (1998): 416.
8. Stiefel, Usha, et al. "Contamination of hands with methicillin-resistant Staphylococcus aureus after contact with environmental surfaces and after contact with the skin of colonized patients." Infection control and hospital epidemiology 32.2 (2011): 185-187.
9. World Health Organization. "Report on the Burden of Endemic Health Care-Associated Infection Worldwide. WHO Libr Cat Data 2011: 40." (2018).
10. Edgeworth, Jonathan D. "Has decolonization played a central role in the decline in UK methicillin-resistant Staphylococcus aureus transmission? A focus on evidence from intensive care." Journal of antimicrobial chemotherapy 66.suppl_2 (2011): ii41-ii47.
11. Pearson, Andrew, Andrew Chronias, and Miranda Murray. "Voluntary and mandatory surveillance for methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-susceptible S. aureus (MSSA) bacteraemia in England." Journal of Antimicrobial Chemotherapy 64.suppl_1 (2009): i11-i17.
12. Denton, M., et al. "Role of environmental cleaning in controlling an outbreak of Acinetobacter baumannii on a neurosurgical intensive care unit." Journal of Hospital Infection 56.2 (2004): 106-110.
13. Ioan, Barliba, Andrei Stefan Nestian, and Silviu-Mihail Tita. "Relevance of key performance indicators (KPIs) in a hospital performance management model." Journal of Eastern Europe Research in Business & Economics 2012 (2012): 1.
14. Ali, I. A. A., et al. "The influence of substrate surface conditioning and biofilm age on the composition of Enterococcus faecalis biofilms." International endodontic journal (2019).
15. Murga, R., J. M. Miller, and R. M. Donlan. "Biofilm formation by gram-negative bacteria on central venous catheter connectors: effect of conditioning films in a laboratory model." Journal of clinical microbiology 39.6 (2001): 2294-2297.
16. Chowdhury, Durdana, et al. "Effect of disinfectant formulation and organic soil on the efficacy of oxidizing disinfectants against biofilms." Journal of Hospital Infection 103.1 (2019): e33-e41.
17. Maillard, Jean-Yves. "Factors affecting the activities of microbicides." Russell, Hugo & Ayliffe's Principles and Pratice of Disinfection, Preservation and Sterlization, 5th edition. West Sussex, Wiley-Blackwell (2013): 71-86.
18. Akinbobola, A. B., et al. "Tolerance of Pseudomonas aeruginosa in in-vitro biofilms to high-level peracetic acid disinfection." Journal of Hospital Infection 97.2 (2017): 162-168.

19. Ledwoch, Katarzyna, et al. "Beware biofilm! Dry biofilms containing bacterial pathogens on multiple healthcare surfaces; a multi-centre study." Journal of Hospital Infection 100.3 (2018): e47-e56.
20. Vickery, Karen, et al. "Presence of biofilm containing viable multiresistant organisms despite terminal cleaning on clinical surfaces in an intensive care unit." Journal of Hospital Infection 80.1 (2012): 52-55.
21. Dancer, Stephanie J. "How do we assess hospital cleaning? A proposal for microbiological standards for surface hygiene in hospitals." Journal of Hospital Infection 56.1 (2004): 10-15.
22. Malik, Rifhat E., Rose A. Cooper, and Chris J. Griffith. "Use of audit tools to evaluate the efficacy of cleaning systems in hospitals." American journal of infection control 31.3 (2003): 181-187.
23. Adams, C. E., et al. "Examining the association between surface bioburden and frequently touched sites in intensive care." Journal of Hospital Infection 95.1 (2017): 76-80.
24. Johani, Khalid, et al. "Mapping the 'hospital microbiome'and the spread of antimicrobial resistance and biofilm on the intensive care units from different regions." Infection, Disease & Health 22 (2017): S12-S13.
25. Jansson, Linda, et al. "Impact of swab material on microbial surface sampling." Journal of Microbiological Methods 176 (2020): 106006.
26. Keevil, C. W. "Rapid detection of biofilms and adherent pathogens using scanning confocal laser microscopy and episcopic differential interference contrast microscopy." Water Science and Technology 47.5 (2003): 105-116.
27. Wilks, Sandra A., et al. "Biofilm Development on Urinary Catheters Promotes the Appearance of Viable but Nonculturable Bacteria." Mbio 12.2 (2021).
28. Otter, Jonathan A., Saber Yezli, and Gary L. French. "The role of contaminated surfaces in the transmission of nosocomial pathogens." Use of biocidal surfaces for reduction of healthcare acquired infections. Springer, Cham, 2014. 27-58.
29. Boyce, John M., et al. "Widespread environmental contamination associated with patients with diarrhea and methicillin-resistant Staphylococcus aureus colonization of the gastrointestinal tract." Infection Control & Hospital Epidemiology 28.10 (2007): 1142-1147.
30. Boyce, John M., et al. "Environmental contamination due to methicillin-resistant Staphylococcus aureus possible infection control implications." Infection Control & Hospital Epidemiology 18.9 (1997): 622-627.
31. Hayden, Mary K., et al. "Risk of hand or glove contamination after contact with patients colonized with vancomycin-resistant enterococcus or the colonized patients' environment." Infection Control & Hospital Epidemiology 29.2 (2008): 149-154.
32. Passaretti, Catherine L., et al. "An evaluation of environmental decontamination with hydrogen peroxide vapor for reducing the risk of patient acquisition of multidrug-resistant organisms." Clinical infectious diseases 56.1 (2012): 27-35.
33. Wu, Henry M., et al. "A norovirus outbreak at a long-term-care facility: the role of environmental surface contamination." Infection control and hospital epidemiology 26.10 (2005): 802-810.
34. Attaway III, Hubert H., et al. "Intrinsic bacterial burden associated with intensive care unit hospital beds: effects of disinfection on population recovery and mitigation of potential infection risk." American journal of infection control 40.10 (2012): 907-912.6 (2012): 475-481.
35. Dancer, Stephanie J., and Axel Kramer. "Four Steps to Clean Hospitals: Look; Plan; Clean; and Dry." Journal of Hospital Infection (2018).
36. Caselli, Elisabetta, et al. "Reducing healthcare-associated infections incidence by a probiotic-based sanitation system: A multicentre, prospective, intervention study." PLoS One 13.7 (2018): e0199616.

37. Dancer, Stephanie J., Liza White, and Chris Robertson. "Monitoring environmental cleanliness on two surgical wards." International journal of environmental health research 18.5 (2008): 357-364.
38. Widmer, F. C., et al. "Overall bioburden by total colony count does not predict the presence of pathogens with high clinical relevance in hospital and community environments." Journal of Hospital Infection 101.2 (2019): 240-244.
39. US Department of Agriculture. Guidelines for reviewing microbiological control and monitoring programs. Part 8. (1994).
40. National Standards of Healthcare Cleanliness, https://www.england.nhs.uk/wp-content/uploads/2021/04/B0271-national-standards-of-healthcare-cleanliness-2021.pdf. (2021) [Accessed May 2021].
41. Collins, B. J. "The hospital environment: how clean should a hospital be?." Journal of Hospital Infection 11 (1988): 53-56.
42. Bartram, Jamie, Lorna Fewtrell, and Thor-Axel Stenström. Harmonised assessment of risk and risk management for water-related infectious disease: an overview. IWA Publishing, London, (2001).
43. Barbau-Piednoir, Elodie, et al. "Evaluation of viability-qPCR detection system on viable and dead Salmonella serovar Enteritidis." Journal of microbiological methods 103 (2014): 131-137.
44. Rampling, A., et al. "Evidence that hospital hygiene is important in the control of methicillin-resistant Staphylococcus aureus." Journal of Hospital Infection 49.2 (2001): 109-116.
45. Ray, Amy J., et al. "A multicenter randomized trial to determine the effect of an environmental disinfection intervention on the incidence of healthcare-associated Clostridium difficile infection." infection control & hospital epidemiology 38.7 (2017): 777-783.
46. Shams, Alicia M., et al. "Assessment of the overall and multidrug-resistant organism bioburden on environmental surfaces in healthcare facilities." Infection control and hospital epidemiology 37.12 (2016): 1426.
47. Aycicek, Hasan, Utku Oguz, and Koray Karci. "Comparison of results of ATP bioluminescence and traditional hygiene swabbing methods for the determination of surface cleanliness at a hospital kitchen." International Journal of Hygiene and Environmental Health 209.2 (2006): 203-206.
48. Dancer, Stephanie J. "Importance of the environment in meticillin-resistant Staphylococcus aureus acquisition: the case for hospital cleaning." The Lancet infectious diseases 8.2 (2008): 101-113.
49. Almatroudi, Ahmad, et al. "A new dry-surface biofilm model: an essential tool for efficacy testing of hospital surface decontamination procedures." Journal of microbiological methods 117 (2015): 171-176.
50. Almatroudi, A., et al. Staphylococcus aureus dry-surface biofilms are not killed by sodium hypochlorite: implications for infection control. J Hosp Infect, 93, 263-270 (2016).
51. Otter, Jonathan A., Saber Yezli, and Gary L. French. "The role played by contaminated surfaces in the transmission of nosocomial pathogens." Infection Control & Hospital Epidemiology 32.7 (2011): 687-699.
52. Reasoner, D. J, and E. E. Geldreich. "A new medium for the enumeration and subculture of bacteria from potable water." Appl. Environ. Microbiol. 49.1 (1985): 1-7.
53. Azevedo, Nuno F., et al. "Proposal for a method to estimate nutrient shock effects in bacteria." BMC research notes 5.1 (2012): 422.
54. Oliver, James D. "The viable but nonculturable state in bacteria." The Journal of Microbiology 43.1 (2005): 93-100.
55. Rose, Laura, et al. "Swab materials and Bacillus anthracis spore recovery from nonporous surfaces." Emerging infectious diseases 10.6 (2004): 1023.

56. Wille, I., et al. "Cross-sectional point prevalence survey to study the environmental contamination of nosocomial pathogens in intensive care units under real-life conditions." Journal of Hospital Infection 98.1 (2018): 90-95.
57. Kim, Jun-Seob, and Thomas K. Wood. 'Tolerant, growing cells from nutrient shifts are not persister cells.' MBio 8.2 (2017): e00354-17.
58. Ayrapetyan, M., et al. "Viable but nonculturable and persister cells coexist stochastically and are induced by human serum." Infection and immunity 83.11 (2015): 4194-4203.
59. Kim, Jun-Seob, et al. "Viable but non-culturable and persistence describe the same bacterial stress state." Environmental microbiology 20.6 (2018): 2038-2048.
60. Yezli, S., and J. A. Otter. "Does the discovery of biofilms on dry hospital environmental surfaces change the way we think about hospital disinfection?." Journal of Hospital Infection 81.4 (2012): 293-294.
61. Yu, Mi-Kyung, et al. "Role of extracellular DNA in Enterococcus faecalis biofilm formation and its susceptibility to sodium hypochlorite." Journal of Applied Oral Science 27 (2019).
62. Parvin, F., Hu, H., Whiteley, G.S., Glasbey, T. and Vickery, K., 2019. Difficulty in removing biofilm from dry surfaces. Journal of Hospital Infection, 103(4), pp.465-467.
63. Jones, Sarah L., et al. "Swabbing the surface: critical factors in environmental monitoring and a path towards standardization and improvement." Critical reviews in food science and nutrition 60.2 (2020): 225-243.
64. Watson, Fergus, et al. "Modelling vaporised hydrogen peroxide efficacy against mono-species biofilms." Scientific reports 8.1 (2018): 12257.
65. Espinal, P., S. Marti, and J. Vila. "Effect of biofilm formation on the survival of Acinetobacter baumannii on dry surfaces." Journal of Hospital Infection 80.1 (2012): 56-60.
66. Ashokan, Anushia, et al. "Environmental dynamics of hospital microbiome upon transfer from a major hospital to a new facility." Journal of Infection 83.6 (2021): 637-643.
67. White, Liza F., et al. "Are hygiene standards useful in assessing infection risk?." American journal of infection control 36.5 (2008): 381-384.
68. Chegra, Sylvia, and Martina Cummins. "Sterilization and decontamination." Tutorial Topics in Infection for the Combined Infection Training Programme (2019): 177.
69. Dancer, Stephanie J. "Hospital cleaning in the 21st century." European Journal of Clinical microbiology & infectious diseases 30.12 (2011): 1473-1481.
70. Kenters, N., et al. "An international survey of cleaning and disinfection practices in the healthcare environment." Journal of Hospital Infection 100.2 (2018): 236-241.
71. Fukuzaki, Satoshi. "Mechanisms of actions of sodium hypochlorite in cleaning and disinfection processes." Biocontrol science 11.4 (2006): 147-157.
72. Hiramine, Hiroko, et al. "Evaluation of Antimicrobial Effects on Dental Impression Materials and Biofilm Removal by Sodium Dichloroisocyanurate." Biocontrol Science 26.1 (2021): 17-25.
73. Yoshimatsu, Takashi, and Kei-Ichiro Hiyama. "Mechanism of the action of didecyldimethylammonium chloride (DDAC) against *Escherichia coli* and morphological changes of the cells." Biocontrol science 12.3 (2007): 93-99.
74. Ledwoch, K., et al. "Artificial dry surface biofilm models for testing the efficacy of cleaning and disinfection." Letters in Applied Microbiology 68.4 (2019): 329-336.
75. Saka, K. H., et al. "Bacterial Contamination of Hospital Surfaces According to Material Make, Last Time of Contact and Last Time of Cleaning/Disinfection." J Bacteriol Parasitol 8.312 (2017): 2.
76. Lax, Simon, et al. "Bacterial colonization and succession in a newly opened hospital." Science translational medicine 9.391 (2017): eaah6500.
77. Dolan, Anthony, et al. "Evaluation of different methods to recover meticillin-resistant *Staphylococcus aureus* from hospital environmental surfaces." Journal of Hospital Infection79.3 (2011): 227-230.

78. Galvin, S., et al. "Microbial monitoring of the hospital environment: why and how?." Journal of Hospital Infection82.3 (2012): 143-151.
79. ASTM E2871. Standard Test Method for Evaluating Disinfectant Efficacy Against *Pseudomonas aeruginosa* Biofilm Grown in CDC Biofilm Reactor Using Single Tube Method. ASTM International. (2019).
80. Buckingham-Meyer, Kelli, Darla M. Goeres, and Martin A. Hamilton. "Comparative evaluation of biofilm disinfectant efficacy tests." Journal of Microbiological Methods 70.2 (2007): 236-244.

Author Contributions

Fergus Watson conceived and coordinated the study, analysed the data, and prepared the paper. Professor Bill Keevil, Dr Sandra Wilks and John Chewins provided technical assistance. All authors reviewed the results and approved the final version of the manuscript.

Conflict of interest statement

Professor Bill Keevil and Dr Sandra Wilks do not have any conflicts to disclose. Fergus Watson and John Chewins are disclosed as employees of BIOQUELL UK Ltd a provider of hydrogen peroxide decontamination systems at the time of this study being conducted.

Funding

This study was funded by the Royal Commission for the Exhibition of 1851.

Ethical approval

Granted exempted status. This evaluation was undertaken as a quality improvement and infection control initiative in conjunction with the infection control team at each site.