Reduction of bacterial load with the addition of ultraviolet-C disinfection inside the hyperbaric chamber

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Abstract
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Introduction: Healthcare acquired infections (HAIs) are associated with increased mortality, morbidity and prolonged hospital stays. Microbiological contamination of the hospital environment directly contributes to HAIs. Optimising environmental cleaning reduces transmission of HAIs. The hyperbaric chamber poses a specific challenge for infection control as certain disinfectants and alcohol-based hand sanitisers are prohibited due to fire risk. Patients often possess multiple risk factors for HAIs. This study compared the bacteria remaining on a surface (bioburden) after a standard clean and after adjunctive disinfection with an ultraviolet-C (UV-C) robot.

Methods: Internal hyperbaric chamber surfaces were first manually cleaned with Clinell® universal wipes and the floor was mopped with Whiteley neutral detergent. Allocated surfaces were swabbed using sterile cotton swabs and processed using a standard microbial culture and a bacteria-specific rapid metabolic assay. Bacterial contamination was also measured by direct contact plating on flat surfaces. The plexiglass ports were covered to protect from potential UV-C mediated damage and used as a negative control. A UV-C disinfection robot was then used to disinfect the chamber for 30 min, whereafter surfaces were swabbed again.

Results: There was a significantly greater mean reduction in bioburden following adjunctive UV-C disinfection than with standard cleaning alone. The surfaces not routinely manually cleaned (e.g., bench, phone) showed greatest reduction in bacterial load following UV-C cleaning.

Conclusions: There was a significant reduction in the bacterial load in the chamber following an adjunctive UV-C clean compared with that of a standard clean. Adjunctive cleaning of the hyperbaric chamber environment with a non-touch UV-C device shows promise as a method to reduce HAIs.

Introduction

Healthcare-associated infections (HAIs) are an ongoing concern in hospital settings and are a well-recognised contributor to morbidity, mortality and hospital length of stay.[1] Environmental contamination (bacteria remaining on surfaces and equipment following patient use) has been shown to directly contribute to the transmission of HAIs.[2,3] Consequently, enhanced environmental cleaning has shown to be an effective strategy to reduce the transmission of infectious pathogens in hospital settings, especially that of multidrug-resistant organisms.[4–6]

The hyperbaric unit presents a specific challenge when it comes to infection control as alcohol and other potentially flammable cleaning products and hand sanitisers are prohibited in the chamber and its surrounds. Whilst we found only one record of an adverse event related to alcohol-based hand sanitiser use in the chamber,[7] strict adherence to guidelines from the National Fire Prevention Association and other similar bodies, prevents the use of flammable materials in the chamber.

There are no published studies monitoring infection rates in hyperbaric units. Routine screening of patients for multidrug-resistant organisms (MDROs) is not standard practice in most units, despite high turnover and the patient cohort often possessing at least one risk factor for infection.[8] These include, but are not limited to, frequent hospitalisations, intensive care stays, prior antibiotic treatment, chronic non-healing wounds and having invasive medical devices.[8]

An under appreciated route of disease transmission is the contamination of medical equipment and/or the healthcare

environment.[9] In all units there are environmental reservoirs of pathogens that remain viable.[10] Contact with the contaminated environment by healthcare workers or patients may result in the transmission of these pathogens throughout the healthcare facility.[11] While hand hygiene is currently the most effective measure to prevent cross-transmission of infectious pathogens, compliance is extremely low and only transiently effective.[12] Alcohol-based hand rubs have shown to improve compliance rates;[12] however, these products are prohibited in hyperbaric units due to the fire risk.

Previous studies have demonstrated that enhanced environmental cleaning protocols resulted in lower overall contamination;[13–15] and that a decreased bioburden resulted in reduced infection rates.[4,16,17] Therefore, it is essential to consider enhanced environmental cleaning to prevent the transmission of HAIs in the hyperbaric medicine unit.

Ultraviolet-C (UV-C) is a high-energy, low-wavelength form of light that has germicidal properties.[18] It is absorbed by nucleic acids in microorganisms, resulting in irreversible cell damage, and rapid cell death. UV-C can effectively kill a broad range of pathogens, including viruses, fungi, moulds, multi-drug resistant bacteria (methicillin-resistant *Staphylococcus aureus*) and bacterial spores (*Clostridium difficile*).[18] UV-C has been utilised for its germicidal properties for decades, particularly for food and beverage processing, as well as air, water and surface disinfection.[18] More recently, UV-C technologies have been developed to disinfect the clinical environment.

It is in this setting that we chose to compare cleaning protocols by looking at the environmental bioburden (bacterial load on surfaces) following a standard clean with Clinell® universal wipes and Whiteley neutral detergent surface cleaner, to that following additional treatment with an automated UV-C disinfection robot. This study aimed to assess the efficacy of an automated UV-C disinfection robot to reduce the environmental bioburden in the hyperbaric chamber beyond that achieved with a standard clean.

Methods

The study was conducted in the routine patient treatment compartment of a large multiplace hyperbaric chamber after a single busy treatment day.

ROUTINE CLEANING

The compartment was cleaned at the end of a treatment day. Visually soiled surfaces and patient chairs were cleaned with Clinell® universal wipes (benzalkonium chloride 0.45%, didecyl dimethyl ammonium chloride 0.4% and polyhexamethylene biguanide 0.1%). Additionally, the floor was mopped with Whiteley neutral detergent (ethoxylated nonylphenol and dipentene) surface cleaner.

UV-C DISINFECTION

An automated UV-C disinfection robot (ThorUVC®, Finsen Tech, London, UK) was used as an adjunct cleaning method in the chamber. Following routine chamber cleaning, the UV-C robot was placed in the centre of the room and operated remotely with an accompanying tablet. As UV-C is potentially damaging to acrylic surfaces, the plexiglass ports in the chamber were covered prior to activation of the robot. At 254 nm, UV-C light cannot penetrate past the superficial surface layer of materials, thus the plexiglass ports were adequately protected with thin rubber covers. Once activated, the robot extended up to 2.25 m (depending on ceiling height) and conducted a 3-dimensional room scan to detect parameters such as room size and surface area. Large objects that created shadows were depicted in the room scan generated. Once the disinfection cycle was initiated, UV-C light (254 nm) was used to irradiate the chamber for a treatment time of 30 min. This treatment time was calculated automatically using an algorithm based on the inverse square law: that disinfection time is inversely proportional to the square of the distance from the UV-C source.

SAMPLE COLLECTION

Samples from ten surfaces, nine of which could be regarded as high-touch clinical surfaces (the exception being the acrylic port) inside the chamber were taken at three times: prior to routine cleaning, post-routine cleaning and post-UV-C treatment. All equipment, including patient chairs, were kept in the chamber during disinfection. The designated surfaces, each with an approximate area of 10 cm x 10 cm (100 cm^2), were swabbed. If the clinical surface was less than 100 cm^2, the entire surface was swabbed (e.g., door handle). Surfaces were uniformly swabbed to avoid collection error. Swabs were then immersed in 500 µL sterile collection fluid.

In addition to surface swabbing, five of the ten clinical surfaces were suitable for direct-contact plating (flat sample surface). RODAC-style plates (surface area 21.5 cm^2) were prepared using enough trypticase-soy agar (TSA) to achieve a convex meniscus. Plates were then air-dried for 1 h and exposed to UV-C light for 10 min. Plates were then covered and stored at 4°C until required. Direct-contact plates were pressed against clinical surfaces with a uniform downward pressure to ensure the whole agar surface contacted the clinical surface.

BACTERIAL MEASUREMENTS

Conventional microbial culture

A 450 µL aliquot of each sample fluid was inoculated onto a TSA plate and aerobically cultured at 37°C. Viable bacterial colonies were enumerated after 24 h and recorded as colony forming units (CFU). If individual colonies could not be distinguished (entire surface area covered) the CFU

was recorded as 100 (100%). Quality control plates were included to ensure no bacterial contamination prior to surface inoculation. The acrylic port in the chamber was used as an additional negative control as it was covered and hence not exposed to UV-C light, or cleaning chemicals.

Bacteria-specific rapid metabolic assay

A 50 µL aliquot of sample fluid was processed through a bacteria-specific, rapid metabolic assay (BSRMA) (Profile 1, Q Biotechnologies, London, UK). This assay was used as a 'real-time' test to assess the bacterial contamination of surfaces. The assay was performed according to manufacturer's instructions. Briefly, the sample fluid was filtered (0.45 µm) to remove somatic cells and non-bacterial adenosine triphosphate (ATP). Bacterial cells were then lysed to release ATP and combined with 50 µL luciferin/luciferase to initiate an ATP-dependent, light-producing reaction. This reaction was quantified with a luminometer and the output was recorded in relative light units (RLU). The amount of fluorescence detected by the luminometer is proportional to the number of viable bacteria present, where 1 RLU is proportional a single bacterium in the sample.[19]

Direct contact plating

The plates obtained using direct contact plating were covered and aerobically incubated at 37°C. CFUs were enumerated following 24 h incubation.

Results

Conventional microbiological culture data show that prior to manual cleaning, all nine high touch surface samples were positive for bacterial growth (Table 1). Following routine cleaning, the mean reduction in bioburden was 47% (SD 61). Following an additional UV-C disinfection, the mean reduction in bioburden was 62%. Statistical analysis, using a Wilcoxon signed rank test with continuity correction, demonstrates there was a significant difference between post-clean and post-UV-C bioburdens (P = 0.049). No change was seen in the acrylic port negative control.

Similarly, the BSRMA data shows that prior to routine cleaning, all nine surfaces were positive for bacterial growth. Following routine cleaning, the mean reduction in bioburden was 42% (SD 36). Following an additional UV-C disinfection, the mean reduction in bioburden was 92% (SD 9). Statistical analysis, using a Wilcoxon signed rank test with continuity correction, demonstrates there was a significant difference between post-clean and post-UV-C bioburdens (P = 0.001). No significant difference was seen in the acrylic port negative control.

Again, direct-contact plate sampling demonstrated that prior to routine cleaning, all five suitable surfaces were positive for bacterial growth. Following routine cleaning, the mean reduction in bioburden was 47% (SD 43). Following an additional UV-C disinfection, the mean reduction in bioburden was 95% (SD 11). Statistical analysis, using a Wilcoxon signed rank test with continuity correction, demonstrates there was a significant difference between post-clean and post-UV-C bioburdens (P = 0.001).

Discussion

This study assessed the ability of a UV-C disinfection robot to decrease the bioburden of clinical surfaces in the multiplace hyperbaric chamber. Following routine manual cleaning, viable bacteria were detected on all surfaces (Tables 1–3). Adjunctive UV-C disinfection significantly reduced this bioburden. Additionally, we compared three

Table 1

Conventional microbiological cultures. The bioburden (colony forming units) on clinical surfaces is reduced when exposed to UV-C treatment, compared to manual room cleaning only*. P-value derived from Wilcoxon signed rank test with continuity correction

Clinical Surface	Pre-clean	Post-clean	Post-UVC*
Bench	8	11	1
Chair (arm rest)	100	2	0
Chair (seat)	2	0	0
Door handle	100	1	1
Floor (doorway)	20	3	1
Floor (shadow)	9	13	1
Phone	3	2	3
Station oxygen supply	12	1	0
Tap	1	1	0
Acrylic port (negative control)	2	2	2
P-value		0.049*	

Table 2

Bacteria-specific rapid metabolic assay. The bioburden (relative light units detected by the luminometer, proportional to the number of individual bacteria) on clinical surfaces is reduced when exposed to UV-C treatment, compared to manual room cleaning only*. *P*-value derived from Wilcoxon signed rank test with continuity correction

Surface	Pre-clean	Post-clean	Post-UVC*
Bench	170	192	4
Chair (arm rest)	322	107	3
Chair (seat)	627	214	13
Door handle	333	102	4
Floor (doorway)	93	29	9
Floor (shadow)	61	58	5
Phone	269	240	27
Station oxygen supply	158	130	4
Tap	886	136	7
Acrylic port (negative control)	278	297	354
P-value		0.001*	

Table 3

Direct-contact plating for surface contamination. The bioburden (colony forming units) on clinical surfaces is reduced when exposed to UV-C treatment, compared to manual room cleaning only*. *P*-value derived from Wilcoxon signed rank test with continuity correction

Surface	Pre-clean	Post-clean	Post-UVC*
Chair (arm rest)	4	1	0
Chair (seat)	1	1	0
Door handle	30	4	1
Floor	100	100	0
Phone	4	1	0
P-value		0.001*	

different surface-testing methods to quantify the bioburden. As each testing method varies greatly in its methodology, sensitivity and specificity, the three methods cannot be compared within each other. Instead, we used the post-manual clean and post-UV-C data in each method to assess the efficacy of adjunctive UV-C disinfection. The most sensitive detection method was the BSRMA (Table 2), with an overall mean reduction in bioburden of 92%.

The sensitivity of surface-testing methods is particularly important when considering infectious pathogens with low minimum infectious doses (e.g., *Escherichia coli*). Detectable CFUs ranged between 1–100, whereas RLUs ranged between 3–886. The BSRMA detected viable but non-culturable bacteria on the chair (arm rest and seat), oxygen inlet, tap and phone (Table 2), where bacterial growth on agar was negative. Conventional microbial testing relies on aerobic culture on agar plates, yet the sensitivity of this method is extremely low. Less than 1% of bacteria found in water and soil grow into visibly detectable colonies (CFU) on 2-dimentional surfaces such as agar.[20] Bacteria present in clinical surface samples often have slower metabolic turnover and may require different growth nutrients and/or conditions in order to replicate.[20]

Real-time metabolic testing has shown to be more sensitive and reliable than microbial culture. Due to the evolutionarily conserved ATP production in bacteria, the BSRMA is highly sensitive to the viable but non-culturable bacteria present in the sample. In this study the floor was mopped with a detergent solution and allowed to dry before surface testing. After routine cleaning, direct-contact plates showed 100 CFUs on the floor (Table 3). After UV-C disinfection, no CFUs were detected. While a reduction was still seen using the BSRMA, viable bacteria were still detected. This is why the study included the BSRMA as an additional method, as it is more sensitive to enumerating individual bacteria present in the sample.

One limitation of the BSRMA is that while it is highly sensitive, there is no specificity towards the species of bacteria and microbial culture is required to identify individual pathogens. The agar-cultured samples were only quantitively analysed for bacteria and individual species were not identified. While the characterisation of environmental pathogens is of interest to infection prevention personnel, it is not essential given the aim of this study. It is known that while some MDROs are less sensitive to detergents and disinfectants than their sensitive counterparts,[21] both populations are equally susceptible to UV-C disinfection.[18] The sporicidal setting of the ThorUVC® device calculates treatment time to ensure correct exposure time in order to kill the most persistent pathogens (e.g., *C. difficile* spores).[18]

Unlike other cleaning methods, bacteria are not known to acquire resistance to UV-C light. As UV-C causes molecular lesions in DNA, it is thought that neighbouring bacteria cannot acquire resistance genes via horizontal gene transfer if the genetic material is damaged by UV-C.[22] Further research into the benefit of UV-C disinfection on the reduction of MDRO-related HAIs would provide an excellent case for integration of this technology into the terminal cleaning protocol.

Measuring any reduction in HAI rates and associated costs was beyond the scope of this study. Yet it was clearly demonstrated that standard manual cleaning does not efficiently disinfect all surfaces (door handles, phones, bench tops), and surfaces that were manually cleaned demonstrated large variability in their cleaning efficacy (floors, chairs). There is an inherent limitation with manual cleaning in that not all surfaces may be reached (e.g., ceilings, walls) and other surfaces may simply be missed due to human error or time constraints. Paradoxically, surfaces may become contaminated during the cleaning process.[23] Dirty cleaning cloths, mops and contaminated cleaning chemicals have been shown to cross-contaminate surfaces.[24]

It is important to consider the benefits of automated disinfection technology, compared to enhanced manual cleaning protocols. It is repeatedly presented in the literature that the efficacy of manual cleaning is highly variable. For example, only 50% of high-touch hospital surfaces were appropriately disinfected by cleaning staff.[14] As healthcare facilities are increasingly looking for cost saving measures, the high cost of manual labour is an important consideration. Additionally, it should be acknowledged that adjunct UV-C disinfection resulted in an overall reduction in bioburden, of ~90%, in 30 min. The duration of manual cleaning required to achieve the same result is unknown.

These data do highlight one acknowledged limitation of UV-C disinfection; that shadowed surfaces demonstrate less efficient disinfection than direct line-of-sight surfaces. The lowest reductions were seen on partially shadowed surfaces such as the door handle (Table 1, Table 3) and the phone (Table 1, Table 2). Additionally, this study was conducted in a large rectangular multiplace chamber where the acrylic ports were covered from UV-C light. Given the potential for ultraviolet light to degrade acrylic, we would not recommend UV-C cleaning in monoplace chambers or in chambers where acrylic components could not be covered. In addition, as the 'shadowed' areas are dependent on light reflection, the results would be likely to be different in a cylindrical chamber.

While this study did not directly measure the effect of bioburden reduction on the rate of HAI, several other papers have assessed the clinical impact of enhanced environmental disinfection on HAI. Hayden et al. studied the role of environmental contamination in the cross-transmission of antibiotic-resistant bacteria.[4] They determined that enhanced cleaning resulted in a decreased bioburden, which resulted in reduced acquisition of vancomycin-resistant *Enterococcus*. McMullen et al. showed that enhanced cleaning protocols reduced the *Clostridium difficile* infection rate, which was maintained for two years following the enhanced cleaning regimen.[17] Furthermore, Dancer et al. suggested that employing one extra, full time cleaner had a profound impact on HAI rates.[16] In their study, enhanced cleaning of high-touch areas significantly lowered the overall bioburden and resulted in reduced methicillin-resistant *Staphylococcus aureus* infection rates over a 12-month study.[16]

Conclusions

This study suggests that adjunctive UV-C disinfection technology can significantly reduce the environmental bioburden in the multiplace hyperbaric oxygen chamber. Further studies on the baseline rates of HAI in the hyperbaric unit would be required to understand the clinical relevance of the bioburden reduction, especially with respect to reduction of multidrug-resistant organisms.

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