Decontamination of heater–cooler units associated with contamination by atypical mycobacteria

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SUMMARY

Background: Non-tuberculosis mycobacteria such as Mycobacterium chimaera are found widely in hospital water systems. Invasive M. chimaera infections have recently been attributed to heater–cooler units (HCUs) of cardiopulmonary bypass equipment.

Aim: To assess the extent of microbiological contamination within the HCUs and to inform decontamination strategies for reducing the microbial load.

Methods: Water samples taken from HCUs used at University Hospitals Birmingham for cardiopulmonary bypass surgery were sampled to determine the number of microorganisms by membrane filtration. Various decontamination processes were used throughout the study, all based on the manufacturer’s guidance.

Findings: Total viable counts >300 cfu per 100 mL containing a wide variety of microorganisms were obtained from water inside the HCUs. Working with the manufacturers, we significantly reduced the microbial load of the water within the HCUs by removing the internal tubing soiled with biofilm followed by a weekly decontamination regimen with peracetic acid.

Conclusion: A decontamination cycle including an initial replacement of internal tubing with weekly microbiological water samples is required to maintain the water quality within HCUs at an acceptable level.

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Introduction

Non-tuberculosis mycobacteria such as Mycobacterium chimaera are part of the Mycobacterium avium complex (MAC: M. avium and M. intracellulare). Non-tuberculosis mycobacteria are a recognized constituent of hospital water systems that form biofilms and may give rise to patient infections primarily via an aerosol route.1–4

Since 2011, teams in Switzerland, The Netherlands, and Germany have reported cases of sterile site M. chimaera infection in patients after cardiac surgery.5–7 These infections have been attributed to transmission of organisms from contaminated heater–cooler units (HCUs) used in theatre during cardiothoracic surgery, via production of an aerosol of contaminated water from the device.5–7 HCUs are used to regulate the temperature of the blood during extracorporeal...
circulation using filtered tap water as a heat exchanger. In the original Swiss outbreak, microbiological sampling of the air detected *M. chimaera* when HCUs were operating, and water cultures showed that the organism was present in the water reservoirs of outbreak-associated HCUs. A UK investigation found a similar potential risk and identified a small number of cases of *M. chimaera* and other similar mycobacteria in cardiothoracic surgery patients, including some deaths where heart valves had been replaced or repaired. The link with the HCU in causing infection has not been demonstrated in the UK; however, it is postulated that the mycobacteria may be transmitted to the surgical site by aerosolization of contaminated water from within the HCU. HCUs are used in cardiopulmonary bypass surgery at the University Hospitals Birmingham (UHB) NHS Foundation Trust. Here we report an investigation of both the microbial load within the HCU and a comparison of different decontamination strategies to try to reduce the microbial load within the HCU. We believe that this is the first report to investigate the decontamination of HCUs.

**Methods**

**Setting**

UHB is a tertiary referral teaching hospital in Birmingham, UK and provides clinical services to nearly one million patients every year. The UHB cardiac department treats adult patients with a wide range of cardiac disease, including adults with congenital heart disease and heart disease in pregnancy.

**Water sampling**

Water samples (100 mL) were collected from the incoming water in the HCUs used at UHB. All samples were tested for the enumeration of micro-organisms by membrane filtration. Tryptone soya agar and cysteine lactose electrolyte-deficient agar plates were incubated for three days at 30°C. Results were expressed as total viable count (TVC) per 100 mL. Water samples (100 mL) were also sent on the day of testing (at 4°C) to the Mycobacterium Reference Laboratory (MRL, Public Health England, Heart of England NHS Foundation Trust, Birmingham) for mycobacterium testing every two weeks.

**Isolation and characterization of coliforms**

Any suspected coliforms growing on the membrane filtration agar plates were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Vitek MS; bioMérieux, Marcy l’Etoile, France) and Vitek (bioMérieux).

**Heater—cooler unit**

The Sorin Stöckert 3T° Heater—Cooler System (LivaNova Milan, Italy) is a three-circuit-heating/cooling system with three independent tanks each with a volume of 6 L. There is a compressor-based interchangeable heating (15–41°C) and cooling (2–10°C) circuit system connected via tubing to the oxygenator or cardioplegia heat exchangers. UHB has four of these HCUs, which are used daily having been commissioned in 2012.

**Decontamination**

The following decontamination methods for the HCUs were carried out at UHB, with water samples taken at each stage of the decontamination process. Decontamination was based on guidance from Sorin with minor modifications.

**Decontamination regimen 1**

Before national notification of the problem (Medicines and Healthcare Products Regulatory Agency, medical device alert), UHB followed the manufacturer’s guidance which included the following: HCUs were decanted of water every two weeks and refilled with filtered (PALL filter 0.2μm) tap water with addition of 100 mL of medical grade 3% hydrogen peroxide (Care Plus, UK) to the HCU tanks. Every five days a further 50 mL of 3% hydrogen peroxide was added. A full system decontamination using Maranon (Ecolab, Northwich, UK; >30% sodium hypochlorite) was undertaken on the HCU every three months following the manufacturer’s instructions. A full system decontamination included the following: water tanks were drained and refilled with filtered tap water; Maranon (420 mL) was added to the tanks and run through the HCU circuits; the HCU was then drained of Maranon and refilled with filtered tap water.

**Decontamination regimen 2**

Following notification of the problem nationally, UHB decided to carry out two full system decontamination cycles consecutively on the HCU with Maranon, to reduce the bio-burden. We then adapted decontamination regimen 1 as follows: the HCUs were decanted of water weekly and refilled with filtered tap water, with a daily addition of medical grade 3% hydrogen peroxide (100 mL) to the HCU tanks, and a weekly full system decontamination using Maranon.

**Decontamination regimen 3**

A change in the manufacturer’s guidance from using a chlorine-releasing agent in the full system decontamination to peracetic acid led to an additional modification of the HCU decontamination regimen. Regimen 3 included two consecutive full system decontamination cycles using Puristeril 340 (peracetic acid; Fresenius Medical Care, Germany). Following this initial decontamination, the HCUs were decanted of water daily and refilled with filtered tap water, with a daily addition of medical grade 3% hydrogen peroxide (100 mL) to the HCU tanks, and a weekly full system decontamination using peracetic acid. A full system decontamination included the following: water tanks were drained and refilled with filtered tap water; peracetic acid (450 mL) was added to the tanks and run through the HCU circuits; the HCU was then drained of peracetic acid and refilled with filtered tap water.

**Biofilm formation**

During the study period, the machines were stripped down to the components and all internal pipe work was replaced. Tubing from within the HCU was removed by LivaNova and cut up to assess any biofilm formation. Biofilms from the tubing
within the HCU were visualized only through staining with 200 μL of 1% Crystal Violet (CV) (Sigma Aldrich, Poole, UK), further rinsed with distilled water to remove unbound CV.

Results

Water sampling

Water sampling was undertaken on all four Sorin 3T HCU systems undergoing decontamination regimen 1. Water counts from all four HCUs yielded TVCs >300 cfu per 100 mL including a range of environmental Gram-negatives, e.g. *Pseudomonas aeruginosa* as well as non-aspergillus moulds (*Paecilomyces* spp.) (Table I). *Mycobacterium chimaera* was cultured from water samples sent to the MRL (Table I).

Decontamination with chlorine-releasing agent

In decontamination regimen 2, two consecutive full system decontamination cycles were performed with a chlorine-releasing agent to reduce the bioburden in the HCU. After the first decontamination procedure, water samples yielded 100 cfu per 100 mL including a range of environmental Gram-negatives only (Table I). Water samples taken after a second decontamination of the HCU, done immediately after the first decontamination, yielded 1 cfu per 100 mL. During decontamination regimen 3, weekly water samples were taken before and after the weekly full system decontamination. Before the decontamination procedure the microbial load within the HCU had increased, ranging between 1 and 100 cfu per 100 mL, including a range of environmental Gram-negatives, *Pseudomonas aeruginosa*, and non-aspergillus moulds (*Paecilomyces* spp.). In addition, *M. chimaera* was cultured from weekly water samples sent to the MRL. After decontamination, the counts were reduced to ~10–100 cfu per 100 mL.

Decontamination with peracetic acid

On the recommendation of the HCU manufacturers, the full decontamination was changed from using a chlorine-releasing agent to peracetic acid. With TVCs ranging from 1 to 300 cfu per 100 mL from the weekly water sampling in decontamination regimen 2, it was decided again to undertake two consecutive decontamination cycles, this time with peracetic acid, to reduce the bioburden in the HCU. After the first decontamination procedure, water samples yielded 10 cfu per 100 mL including a range of environmental Gram-negatives only. Water samples taken after a second decontamination of the HCU, immediately after the first decontamination, yielded 0 cfu per 100 mL. During decontamination regimen 3, weekly water samples were taken before and after the weekly full system decontamination. Before the decontamination procedure the microbial load within the HCU had increased, ranging between 1 and 100 cfu per 100 mL, including a range of environmental Gram-negatives, *Pseudomonas aeruginosa*, and non-aspergillus moulds (*Paecilomyces* spp.). No atypical mycobacteria were isolated from the weekly water samples.

Removal of plastic tubing

Various decontamination regimens were implemented at UHB to reduce the microbial load in the HCUs, yielding reductions in TVCs but without totally eradicating the bioburden. UHB worked with LivaNova to investigate the internal working of the HCUs and replace potentially biofouled tubing within the machine. Opening the HCUs revealed tubing that had visible biofilm inside (Figures 1 and 2). Figure 1 shows two pieces of tubing: one which is plugged and is a visible dead leg, and the other which leads to an overflow container. Both pieces of tubing contained visible biofilms. The water tanks themselves appeared clean when opened, with no visible biofilm

<table>
<thead>
<tr>
<th>Decontamination regime</th>
<th>TVC</th>
<th>Environmental Gram negatives</th>
<th>Pseudomonas sp.</th>
<th>Fungi</th>
<th>M. chimaera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decontamination regime 1</td>
<td>&gt;300 CFU</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Decontamination regime 2 (chlorine releasing agent)</td>
<td>&gt;300 CFU</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>First decontamination*</td>
<td>100 CFU</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Second decontamination*¹</td>
<td>1 CFU</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Weekly Counts</td>
<td>~1-300 CFU</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Decontamination regime 3 (peracetic acid)</td>
<td>&gt;300 CFU</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>First decontamination*</td>
<td>1-10 CFU</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Second decontamination*¹</td>
<td>0 CFU</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Weekly Counts</td>
<td>~1-100 CFU</td>
<td>+</td>
<td>(0-5 CFU)</td>
<td>+ (0-30 CFU)</td>
<td>-</td>
</tr>
<tr>
<td>Current regime – decontamination regime 3 (tubing replaced)</td>
<td>0 CFU</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>First decontamination*</td>
<td>0 CFU</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Second decontamination*¹</td>
<td>0 CFU</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>Weekly Counts</td>
<td>0 CFU</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Key.** + = organisms present, - = no organisms present

Two consecutive decontamination cycles* first decontamination, *¹ second decontamination.
There were visible green particles in the HCU tank (likely copper particles; personal communication with the University of Birmingham), the significance of which is unclear at present (Figure 3B). After all the tubing within the HCU had been replaced, decontamination regimen 3 was undertaken. Two consecutive decontamination cycles with peracetic acid were undertaken, as before, on the first decontamination.

Following the double decontamination regimens and replacement of the HCU tubing, microbial enumeration has shown zero organisms. This effect has been observed for three months to date. Sampling is continuing.

Discussion

Invasive *M. chimaera* infections associated with HCUs have been reported throughout Europe.\textsuperscript{5–8} Data from this study show that the HCUs used at UHB in cardiopulmonary surgery were contaminated with Gram-negative bacteria, e.g. coliforms and *Pseudomonas* spp., atypical mycobacteria, and fungi, which may have posed a serious infection risk in cardiac patients. This study indicates that decontamination procedures are essential to reduce the microbial load in the HCUs and that weekly microbiological sampling is required to monitor the bioburden in the HCU.

On initial examination, the HCUs had a high microbial load with TVC >300 cfu per 100 mL. The TVC results following decontamination protocols supplied by the manufacturer
showed that these decontamination methods were inadequate. The culture results were not surprising, as the presence of *M. chimaera* in Sorin HCUs has been demonstrated.\(^5\) The manufacturer’s decontamination guidance to reduce the microbial load in the HCUs was modified in this study. Two successive cycles of decontamination were undertaken to try to improve the decontamination. After the first decontamination the counts decreased from \(>300\) to \(100\) cfu per \(100\) mL, and on the second decontamination the counts decreased to \(10\) cfu per \(100\) mL. However, weekly water samples revealed an increase in counts, suggesting that the microbial load in the HCU had not been removed and that recalcitrant biofilms may have been present in the HCU. On discussions with the HCU manufacturer it was decided to change the decontamination agent to peracetic acid, as this has been shown to be efficacious at reducing the microbial loads in biofilms.\(^13\) We modified the decontamination regimen again. In decontamination regimen 3, successive decontamination cycles were undertaken on the first decontamination. We found that after the first decontamination, the counts decreased from \(100\) to \(10\) cfu per \(100\) mL, and on the second decontamination the counts decreased to \(~1\) cfu per \(100\) mL. However, weekly water samples again revealed an increase in counts, suggesting that the microbial load in the HCU had not been totally removed, although the weekly water counts were lower with peracetic acid compared with those using a chlorine-releasing agent.

It was suggested that the HCUs were harbouring biofilms internally and that a decontamination procedure alone would be insufficient to reduce the microbial load. Working with Public Health England and LivaNova, the HCUs were opened up and visible biofilm was identified in all of the tubing within the unit. Aquatic biofilms constitute a problem in many environmental, industrial, and medical settings.\(^5,4\) Bacteria isolated from biofilms may include environmental bacteria, and opportunistic and true pathogens such as *Pseudomonas* spp. and *Mycobacterium* spp. Our results showed that the HCUs had microbiologically contaminated water, primarily due to the presence of biofilms, and that chemical decontamination alone would be ineffective in reducing the bioburden in the HCU. Like the Swiss study, we found *M. chimaera* in our HCUs. Biofilms appear to support mycobacterial growth and protect the organism, which makes reliable disinfection of colonized water systems difficult to achieve.\(^15\) Removal of biofilm is essential for effective decontamination of HCUs.

Most of the literature on HCUs thus far has focused on *M. chimaera* infections.\(^5,7\) As most atypical mycobacteria are not sensitive to routine anti-tuberculous therapy, it is important to identify the causative mycobacterium.\(^2,14\) Diagnosis of *M. chimaera* infections is difficult and slow.\(^2,14,15\) The prevention of nosocomial infections and pseudo-infections due to non-tuberculous mycobacteria is challenging.\(^14,15\) We have never identified *M. chimaera* infections in UHB patients. However, our work has revealed that a variety of other potential pathogens may be isolated from these units. The possible transmission of other micro-organisms, such as *Pseudomonas* spp. in cardiac surgery surgical site infection or invasive infections from HCUs, has not been investigated.

We are replacing all the tubing in the HCUs at UHB with assistance from the manufacturer and implementing a decontamination regimen consisting of: two consecutive decontamination cycles with peracetic acid following replacement of the tubing. Following this initial decontamination, the water in the HCUs is decanted daily and refilled with filtered tap water, and 100 mL of medical grade 3% hydrogen peroxide is added to the HCU tanks. In addition, a weekly full system decontamination using peracetic acid is performed. Although water sampling has yielded no micro-organisms following this decontamination regimen, previous reports suggest that subsequent contamination is likely.\(^5\) We suggest that weekly water sampling for TVC and mycobacteria will be required indefinitely to monitor the water quality in these units as well as regular servicing to replace the tubing. The green discoloration noted in Figure 3B is likely to be copper particles. The significance of this is unclear. It may represent damage to copper components from the decontamination regimens used. Further work is needed to investigate this observation.

The decontamination regimen we have implemented has been effective in reducing the bioburden. However, the ongoing maintenance to eliminate biofilm requires additional resources. In this study we did not attempt to demonstrate that the organisms detected in the HCU are aerosolized. Since the postulated route of transmission is via aerosolization, further investigation of HCUs is needed to better understand the routes of transmission. In some European countries a different approach has been taken to address the potential infection risks. The HCUs are placed outside theatres or sometimes enclosed in a specially designed housing unit to minimize the risk of transmission.\(^2,7\) The possible risk to healthcare workers from micro-organisms aerosolized from HCUs has not been investigated. We believe that this is the first report to focus on the decontamination of HCUs. More work is needed to establish the link between infections associated with water from the HCUs and to identify the most effective way to deal with the problem.

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Conflict of interest statement

None declared.

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