Failure to eradicate non-tuberculous mycobacteria upon disinfection of heater–cooler units: results of a microbiological investigation in northwestern Italy

Failure to eradicate non-tuberculous mycobacteria upon disinfection of heater-cooler units: Results of a two-year investigation

Savina Ditommaso [#], Monica Giacomuzzi, Gabriele Memoli, Carla M. Zotti

Department of Public Health and Pediatrics, University of Turin, Italy.

[#]Corresponding author. Address: savina.ditommaso@unito.it; tel.: 0039-0116705841; fax: 0039-0116705881

Running Title: Disinfections of heater-cooler units

Keywords: mycobacterium chimaera, PCR, heater-cooler unit, cardiac surgery, microbiological monitoring, disinfection.

SUMMARY

Background: Heater-cooler units (HCUs) used during cardiopulmonary bypass often become contaminated with non-tuberculous mycobacteria (NTM), including *Mycobacterium chimaera.* Recently, a worldwide investigation conducted in hospitalized infected patients has detected *M. chimaera* in several Stockert 3T HCUs manufactured by LivaNova.

Aim: Microbiological surveillance on Stockert 3T (LivaNova) and HCU40 Maquet (Getinge) devices as well as an evaluation of the efficacy of their recommended decontamination protocols.

Methods: A total of 308 water samples collected from 29 HCUs (Stockert 3T LivaNova and HCU40 Maquet) were tested for total viable counts (TVCs) both 22° and 37°C, *P. aeruginosa,* coliform bacteria and NTM. The investigation Microbiological surveillance began in June 2017 by monthly water sampling.

Findings: We analyzed 308 HCU water samples, 65.5% of which were NTM^+. The most frequently contaminated device was the Stockert 3T (88.2%), with a frequency of positive samples of 59.5%. The HCU40 Maquet devices were less contaminated (33.3%), with a frequency of NTM^+ samples of 13.6%. Disinfection procedures were proven effective in TVCs of bacteria with the exception of NTM species. No significant association between disinfection and NTM positivity was found.

Conclusion: Since validated disinfection protocols are not yet available, as also supported by our findings, the most effective way to control the risk of NTM exposure is to monitor closely the water quality of the HCU and to clean periodically the device according to the manufacturer's instructions.

INTRODUCTION

Heater-cooler units (HCUs) are essential pieces of equipment in cardiothoracic surgical suites. In the US and Europe, non-tuberculous mycobacteria (NTM) infections have been occasionally identified in patients undergoing cardiothoracic surgeries. In this regard, epidemiologic analysis and laboratory investigations have revealed a positive association between patients with deep seeded, valvular and bloodstream NTM infections (i.e., sepsis) and prior HCU exposure due to cardiopulmonary bypass (CPB) [1–3]. Indeed, some types of bacteria, such as *Mycobacterium chimaera*, present in hospital water sources are capable of contaminating heater-cooler devices used for open-chest cardiac surgery.

HCUs are closed, non-sterile circuits placed outside the sterile field, where no contact with the patient's blood or body fluids may occur. Nonetheless, NTM species, chiefly *M. chimaera*, can spread throughout the operating room *via* HCU-mediated aerosolization (particle size < 1 μm) [1,4] of contaminated water [5]. The *M. chimaera*-containing aerosol then can fall onto the patient's open surgical wound, leading to surgical site infections. Upon exposure, the bacteria grow slowly and may not present for months or years, which makes epidemiological determination quite difficult.

In hospitals with confirmed *M. chimaera* infections, reported incidence rates among HCU-exposed patients range from 1/100 to 1/1,000 persons [6–8], whereas the case-fatality rate is of approximately 50% [4,9]. Of note, these infections are seen more frequently among patients undergoing valve or other implants replacement during surgery [3].

Although the presence of *M. chimaera* has been detected in several Stockert 3T HCUs manufactured by LivaNova PLC (Sorin Group Deutschland GmbH), this contamination does not seem to just be limited to this type of device as *M. chimera* has also been detected in HCUs manufactured by Maquet (Maquet Getinge Group, Rastatt, Germany) [9]. In this regard, the risk associated with exposure to HCUs from other manufacturers is currently unknown.

In order to minimize the risk of infection associated with HCU exposure, the Center for Disease Control and Prevention (CDC) [10], Food and Drug Administration (FDA) [11] and

European Center for Disease Prevention and Control (ECDC)[12] recommend to comply with the manufacturer's instructions, especially in terms of cleaning, disinfection and maintenance. In this regard, due to the international alert, in 2015, the Sorin Group issued new guidelines concerning the timelines for maintenance and disinfection of its Stockert 3T HCUs.

In 2016, the same Group also released a safety warning, strongly recommending that both 1T and 3T devices should be microbiologically monitored. As a result, since June 2017, a program of HCU monitoring has been implemented in several open-heart surgery facilities across the Piedmont region of Italy.

Here, we report the results of an over two-year long microbiological surveillance on Stockert 3T (LivaNova) and HCU40 Maquet (Getinge) devices as well as an evaluation of the efficacy of their recommended decontamination protocols.

METHODS

Setting

The microbiological surveillance was carried out in 9 cardiac surgery facilities treating patients suffering from a wide range of cardiac diseases and 1 pediatric cardiac surgery suite, all located in the Piedmont region of northwestern Italy.

HCUs

The environmental investigation on the Stockert 3T HCUs began in 2017, while the HCU40 Maquet devices started being monitored in 2019. Testing was initiated after a safety alert issued by LivaNova in 2016 to all hospitals equipped with Stockert 3T HCUs. The surveillance continued according to the recommendations on the prevention of NTM infection in cardiac surgery settings from National Health Authorities [13].

1. *Disinfection procedure and microbiological monitoring recommended for Stockert 3T devices*

At the end of June 2015, an official update of HCU maintenance and disinfection procedures was released by the Stockert 3T manufacturer LivaNova PLC (Sorin Group Deutschland GmbH). These procedures recommended HCU disinfection with peracetic acid (Puristeril® 340, Fresenius Medical Care, Bad Homburg, Germany), at a final concentration of 3.3%, or with Clorox® Regular Bleach (i.e., sodium hypochlorite), at a final concentration of 1.3%, every 14 days, along with weekly changes of water in the presence of 100 mL 3% hydrogen peroxide (Table 1). All the cardiac surgery suites enrolled in this study adopted Puristeril® 340 for routine HCU disinfection. The water used to fill the HCU tanks was filtered with disposable water filters (Pall-Aquasafe™; 0.2 μm membrane).

In 2016, a safety alert issued by the Sorin Group recommended microbiological monitoring to assess the efficacy of the aforementioned maintenance and disinfection procedures for the Stockert 1T and 3T HCUs. In particular, it was recommended that all water samples obtained from the HCU water circuits be analyzed before the disinfection phase to determine total viable counts (TVCs), *Pseudomonas aeruginosa,* coliform bacteria and NTM biweekly. In case of positivity, the manufacturer suggested that the devices should be taken out of service to allow thorough disinfection of their water tanks and circuits, and that sampling should be repeated in order to evaluate the efficacy of the treatment.

To help reduce potential risk of infection during open-chest cardiac surgery, on October 18th 2018, LivaNova issued a Medical Device Correction letter to healthcare facilities providing updated instructions to ensure that a sufficient concentration of hydrogen peroxide (>100 ppm) would be used to limit microbial growth in the HCU water circuits. Furthermore, it announced the availability of a Stockert 3T design upgrade consisting of a vacuum canister and internal sealing that reduced the risk of potential emission of aerosols from the device.

2. *Disinfection procedure and microbiological monitoring recommended for HCU40 Maquet devices.*

For the HCU40 Maquet devices in use since January 2016, Getinge initially suggested a monthly disinfection with 2% tosylchloramide sodium (Clorina, Lysoform, Brugg, Switzerland) and water change biweekly. Since November 2016, this disinfection protocol has been strengthened by including a weekly treatment with 2% chloramine-T for the entire water volume of the device. A cleaning wizard guides you through this routine disinfection, which takes up to 150 to 200 min. If atypical mycobacteria are found in the water system, the treatment should be performed with 5% chloramine-T solution for 24 h. A terminal water filter with a pore size of 0.2 μm should be used to fill the HCU40 Maquet water tank.

Water sampling

Samples from HCUs were collected monthly, a procedure that was repeated after disinfection of contaminated HCUs. Water samples from both the patient and cardioplegia circuits were collected in sterile 2-lt plastic bottles, containing sodium thiosulphate (10% w/v). Samples were immediately sent to the laboratory or, if not possible, stored at a controlled temperature (5 ± 3 ° C for 12-15 h). All tests were performed within 24 h of collection.

Microbiological testing

All samples were tested for TVCs, *P. aeruginosa,* coliform bacteria and NTM. TVCs is a general indicator of water cleanliness and can be used as a surrogate indicator of decontamination effectiveness. Briefly, the enumeration of background bacteria in the water samples from the HCU circuits was investigated by determining aerobic heterotrophic bacteria TVCs according to the UNI EN ISO protocol 6222 [14]. The number of colony-forming units (CFUs) per mL of sample was calculated according to the number of colonies that had formed on the medium after 7 days of incubation at 22 °C and after 5 days of incubation at 36 °C, according to the US standard method [15].

P. aeruginosa was detected and quantified by culture according to the ISO 16266 method[16], filtering 250 ml of the water sample with a sterile cellulose ester membrane filter with

a rated pore diameter equivalent to 0.45 μm (Millipore, Billerica, MA, USA). The membrane was aseptically placed on a petri dish containing Cetrimide Agar™ (Thermo Scientific) and incubated at 36±2 °C for 44±4 h. All colonies that produced a blue/green (i.e., pyocyanin) color were confirmed to be *P. aeruginosa.*

According to ISO 9308 [17], total coliform count was performed by filtering 100 ml of the water sample through a 0.45 μm membrane filter. The membrane was then placed on the surface of E. coli-Coliforms Chromogenic Agar (CCA). Successively, the petri dish with the membrane was incubated for 18-24 h at 36 ± 2ºC. All colonies with a salmon-rose to red color were counted as coliform bacteria, whereas all colonies colored from deep blue to violet were counted as *E. coli*. Total coliform count was obtained by summing the numbers of the aforementioned colonies.

For detection of NTM by propidium monoazide (PMA)-PCR assay, 1 liter of each water sample was concentrated by filtration through a 0.45 μm polycarbonate filter (Millipore, Billerica, MA, USA). The filter was then treated with PMA and, after irradiation, soaked in lysis solution for DNA as described previously [18]. For amplification, the extracted genomic DNA was analyzed for the presence of amplifiable sequences using a qualitative multiplex PCR assay (Anyplex™ plus MTB/NTM MDR-TB Real-Time Detection, V2.0, Seegene) [19]. Oligonucleotides were designed to amplify a 366 or 491 bp DNA sequence in *M. tuberculosis* (MTB) (IS6110 & MPB64) or NTM (16S rRNA), respectively. Since *M. chimaera* was not included among the 21 strains listed in the kit instructions, we tested a reference strain of *M. chimaera* (DSM No. 44623) by Anyplex™, with positive results [20].

On July 2019, we participated in the first proficiency test on *Mycobacterium* spp. scheme organized by Public Health England. This scheme provides samples to laboratories monitoring *Mycobacterium* spp in HCU water samples. We were given two samples: the first sample was contaminated with *M. chimaera* (47 CFU) and *Pseudomonas fluorescens* (15 CFU), while the second one contained *Mycobacterium chelonae* (55 CFU) and *Burkholderia multivorans* (7 CFU/). Our laboratory detected correctly mycobacteria in both samples.

Data analysis

Differences between pre-disinfection and post-disinfection water samples were analyzed using chi-square test and Fisher's exact test. A P value < 0.05 was considered statistically significant. The Mann-Whitney U test was adopted to evaluate the between-sample (pre-disinfection and post-disinfection water samples) differences in TVCs.

RESULTS

The environmental investigation on HCUs was conducted from June 2017 to October 2019 in 10 cardiac surgery facilities across the Piedmont region in northwestern Italy. We analyzed 308 water samples obtained from 29 HCUs, of which 17 were Stockert 3T by LivaNova and 12 were HCU40 Maquet by Getinge. The age of medical devices is shown in Table 3.

Out of 308 water samples, 163 (53.3%) were NTM positive and the 65.5% of the devices were contamined by NTM. The most frequently contaminated devices were the Stockert 3T (88.2%) and 59.5% (157/264) of water samples obtained from 3T yielded NTM. Noteworthy, the only two Stockert 3T devices negative for NTM had both been manufactured in 2019. In comparison with the Stockert 3T, the HCU40 Maquet devices were less frequently contaminated (33.3%), with a frequency of positive samples of 13.6% (6/44). The results obtained from our microbiological surveillance are summarized in Tables 4 and 5.

Complete water tests (i.e., NTM, coliform bacteria, *P. aeruginosa* and TVCs) were performed on 299/308 water samples. In all HCU samples, total coliform counts were consistently < 1 CFU/100 mL. Although *P. aeruginosa* was isolated initially from 3 LivaNova HCUs, after the three devices were disinfected, it was no longer detectable. Of note, the bacterial load of the post-disinfection samples was significantly lower than that of the pre-disinfection ones, as judged by CFU/mL (Table 6).

A significant association between disinfection and TVCs was found for both Stockert 3T (P = 0.001495) and HCU40 (P = 0.000285) devices (Table 7).

NTM were detected in both pre-disinfection (50.1%) and post-disinfection (55.7%) samples, and no significant association between disinfection and NTM results was found (Table 8).

Figure 1 shows the results of one-year monitoring of one Stockert 3T. The graph shows changes in microbial load of TVCs and mycobacteria. Despite the bi-monthly disinfection cycles with Puristeril, mycobacteria were still present. Figure 2 shows the data relative to a 10-month microbiological monitoring of an HCU40. After a brief period of contamination with NTM, no contamination was detected in the samples analyzed from May to November. Figure 3 shows the results obtained from the analysis of samples taken from an HCU by LivaNova always negative for NTM.

DISCUSSION

Being a well established environmental source of NTM, drinking water has been extensively studied to characterize the risk of human exposure to such bacteria[21–23]. In particular, the water contained in HCUs has been shown to harbor various pathogens, including NTM, commonly found in hospital drinking water, some of which can pose serious health risks for immunocompromised individuals [24–26].

In 2014, airborne transmission of *M. chimaera* from HCUs was reported for the first time in Switzerland [1]. Since then, several cases of *M. chimaera* infection associated with contamination of HCUs have been reported worldwide [2,4,10,27–29]. In Italy, between December 2108 and October 2019, 36 cases of severe infection with *M. chimaera* were reported, all of them in patients who had undergone open-chest cardiothoracic surgery and extracorporeal circulation, with an extremely high rate of case-fatality (58.3%)[30]. In Piedmont, case finding started on December 2018: hospital clinicians were alerted to be aware of the disease and to notify all *M. chimaera* cases to the Regional Health Authorities and to date 3 cases of *M. chimaera* infection have been reported

to health authorities. The three patients diagnosed in 2019 were detected by passive case finding for the clinical awareness of their clinicians

According to recent investigations, the risk of *M. chimaera* contamination of HCUs, which has now reached global proportions [3], can be mitigated by implementing practices of good monitoring, cleaning and disinfection and careful positioning of the HCUs [31]. Thus, in June 2017, our lab initiated a microbiological surveillance program for *M. chimaera* on three Stockert 3T devices used in one cardio surgery facility in Piedmont, Italy, which was later on expanded to nine other cardiac surgery suites, reaching a total of number of ten. All samples were analyzed in our laboratory because we routinely perform real-time PCR testing.

Overall, our findings indicate that LivaNova HCUs are twice more likely to be contaminated by NTM than HCU40 Maquet devices (88.2 % *vs.* 40%, respectively). High TVCs values were initially encountered in all HCUs monitored. The high concentration of heterotrophic bacterial count was not associated with the presence of *P. aeruginosa* and coliform bacteria in the water samples analyzed. Moreover, we found a poor correlation between the presence of NTM and TVCs in the water samples from the HCUs analyzed, supporting the notion that TVCs is not a suitable surrogate for the presence or absence of NTM [32].

The implementation of cleaning and disinfection procedures led to high colony counts (> 100 CFU/mL) in only 8.7% (12/138) of post-disinfection samples, where the bacterial loads ranged from 3.5 x 10^1 CFU/mL (22°C) to 3.6 x 10^1 CFU/mL (37°C). Nevertheless, the disinfection procedure recommended by the manufacturer did not seem to inhibit significantly NTM growth as NTM contamination persisted in Stockert 3T HCUs (Figure 1), in good agreement with previous reports [33,34].

The resistance to disinfectants could be explained by the ability of NTM species, such as *M. avium* and *M. intracellulare*, to adhere to surfaces made of stainless steel, glass, zinc-galvanized steel or PVC and copper plumbing surfaces and to form biofilms [35], a process that is favored by the hydrophobic surface of mycobacteria [36]. Furthermore, the water used in the HCU circuits

offers optimal conditions for NTM growth and biofilm formation—the operating temperature ranges from +15°C to +35°C. Once this biofilm has formed, it becomes highly resistant to standard disinfectants [37], making NTM extremely difficult to eradicate. The most effective way to completely remove these pathogens is through HCU disassembly, replacement of the contaminated tubing and completion of a thorough disinfection [38]. For instance, a study carried out in Greece [21] reported that the occurrence of environmental mycobacteria in drinking water was significantly decreased after replacing the old water distribution pipes coated with biofilm with new ones.

In order to reduce the risk of aerosol transmission, LivaNova has recently upgraded its 3T devices manufactured before 2016 with a new internal sealing. In addition, it has equipped them with a vacuum pump aspirating liquids into the central suction system of the hospital, which reduces—albeit not fully eliminating—the exhaust emissions from the rear of the machine. Furthermore, LivaNova recommends that any HCUs with *M. chimaera* positive water samples should be subjected to thorough deep cleaning and disinfection (i.e., disassembly and brushing of all circuits and internal surfaces), and that this procedure should be performed by LivaNova itself.

Given the technical challenges of HCU disinfection, some hospitals have decided to replace the contaminated devices with new ones produced by different manufacturers. However, it should be noted that the potential risks associated with other models has been previously reported [29,39] but epidemiological investigations have not described an association between patients and HCUs from other manufacturers.

Finally, even though ECDC recommends relocating HCUs outside of the operating room or placing them at a maximum distance from the operating table, with the vent exhaust directed and channeled away from the patient and, when possible, close to the room air suction exhaust, this solution may not be feasible in every setting. Thus, centers using HCUs should strictly follow the guidelines issued by the manufacturer, in particular those pertaining to cleaning and decontamination. It is also advisable to establish a quality control process with written procedures, including traceability of the HCU used in each operation.

In conclusion, until effective disinfection protocols become available, the only effective way to minimize the risk of NTM contamination is to closely monitor the water quality in the cooler-heater, keep it as clean as possible, and treat it like any other biohazardous material. Regularly changing the water in the HCU, and periodically cleaning the device with a chemical disinfectant according the manufacturer's instructions, should help maintain good water quality and reduce the risk of secondary environmental contamination by aerosols. In this regard, engineering solutions able to prevent bioaerosols from spreading throughout the operating theatre are urgently needed.

However, evaluation of environmental cleaning should be performed by measuring the microbial load in the HCU after the cleaning procedure. According to FDA guidelines, the manufacturer should follow the validation process using criteria published for Class II medical washing devices[40]. Specifically, for high-level disinfection the cleaning process should lead to a minimum of a 6-log kill of vegetative organisms, such as *P. aeruginosa*, *S. aureus*, *E. coli*, *Klebsiella* and NTM species.

ACKNOWLEDGMENTS

Acknowledgments

The authors would like to thank the Infections Prevention and Control Teams of Piedmont Hospital for collaboration.

Conflict of interest statement

The authors declare no conflict of interest.

REFERENCES

[1] Sax H, Bloemberg G, Hasse B, Sommerstein R, Kohler P, Achermann Y, et al. Prolonged Outbreak of Mycobacterium chimaera Infection After Open-Chest Heart Surgery. Clin Infect Dis Off Publ Infect Dis Soc Am 2015;61:67–75. https://doi.org/10.1093/cid/civ198.

[2] van Ingen J, Kohl TA, Kranzer K, Hasse B, Keller PM, Katarzyna Szafrańska A, et al. Global outbreak of severe Mycobacterium chimaera disease after cardiac surgery: a molecular epidemiological study. Lancet Infect Dis 2017;17:1033–41. https://doi.org/10.1016/S1473-3099(17)30324-9.

[3] Sommerstein R, Hasse B, Marschall J, Sax H, Genoni M, Schlegel M, et al. Global Health Estimate of Invasive *Mycobacterium chimaera* Infections Associated with Heater–Cooler Devices in Cardiac Surgery. Emerg Infect Dis 2018;24:576–8. https://doi.org/10.3201/eid2403.171554.

[4] Sommerstein R, Schreiber PW, Diekema DJ, Edmond MB, Hasse B, Marschall J, et al. Mycobacterium chimaera Outbreak Associated With Heater-Cooler Devices: Piecing the Puzzle Together. Infect Control Hosp Epidemiol 2017;38:103–8. https://doi.org/10.1017/ice.2016.283.

[5] Götting T, Klassen S, Jonas D, Benk C, Serr A, Wagner D, et al. Heater-cooler units: contamination of crucial devices in cardiothoracic surgery. J Hosp Infect 2016;93:223–8. https://doi.org/10.1016/j.jhin.2016.02.006.

[6] Lyman MM, Grigg C, Kinsey CB, Keckler MS, Moulton-Meissner H, Cooper E, et al. Invasive Nontuberculous Mycobacterial Infections among Cardiothoracic Surgical Patients Exposed to Heater-Cooler Devices1. Emerg Infect Dis 2017;23:796–805. https://doi.org/10.3201/eid2305.161899.

[7] Schreiber PW, Sax H. Mycobacterium chimaera infections associated with heater-cooler units in cardiac surgery. Curr Opin Infect Dis 2017;30:388–94. https://doi.org/10.1097/QCO.0000000000000385.

[8] Cappabianca G, Paparella D, D'Onofrio A, Caprili L, Minniti G, Lanzafame M, et al. Mycobacterium chimaera infections following cardiac surgery in Italy: results from a National Survey Endorsed by the Italian Society of Cardiac Surgery. J Cardiovasc Med Hagerstown Md 2018;19:748–55. https://doi.org/10.2459/JCM.0000000000000717.

[9] Walker J, Moore G, Collins S, Parks S, Garvey MI, Lamagni T, et al. Microbiological problems and biofilms associated with Mycobacterium chimaera in heater-cooler units used for cardiopulmonary bypass. J Hosp Infect 2017;96:209–20. https://doi.org/10.1016/j.jhin.2017.04.014.

[10] CDC. Contaminated Heater-Cooler Devices | HAI | CDC 2019. https://www.cdc.gov/hai/outbreaks/heater-cooler.html (accessed October 17, 2019).

[11] FDA. Nontubercolous Mycobacterium (NTM) infections Associated with Heater Cooler Devices(HCD) durinng Cardiororacic Surgery. 2016.

[12] ECDC. EU protocol for case detection, laboratory diagnosis and environmental testing of Mycobacterium chimaerainfections potentially associated with heater-cooler units: case definition and environmental testing methodology. 2015.

[13] Ministero della Salute. Indicazioni operative riguardanti gli aspetti di laboratorio in merito ai casi di infezione da Mycobacterium chimaera in Italia e aggiornamento delle informazioni disponibili. 2019.

[14] UNI EN ISO 6222. Qualità dell'acqua. Valutazione quantitativa dei microrganismi vitali. 2001.

[15] Eaton A.D.;Clesceri L.S.; Greenberg A.E.; American Public Health Association; American Water Works Association. Standard methods for the examination of water and wastewater. Washington,D.C. : American Public Health Association, 1995; 1995.

[16] International Standard Organization. ISO 16266:2006 Water quality - Detection and enumeration of Pseudomonas aeruginosa - Method by membrane filtration 2006.
[17] ISO. ISO 9308-1:2014(en), Water quality — Enumeration of Escherichia coli and coliform bacteria — Part 1: Membrane filtration method for waters with low bacterial background flora 2014. https://www.iso.org/obp/ui/#iso:std:55832:en (accessed December 17, 2019).
[18] Ditommaso S, Ricciardi E, Giacomuzzi M, Arauco Rivera SR, Zotti CM. Legionella in water samples: how can you interpret the results obtained by quantitative PCR? Mol Cell Probes 2015;29:7–12. https://doi.org/10.1016/j.mcp.2014.09.002.
[19] Sali M, Maio FD, Caccuri F, Campilongo F, Sanguinetti M, Fiorentini S, et al. Multicenter Evaluation of Anyplex Plus MTB/NTM MDR-TB Assay for Rapid Detection of Mycobacterium tuberculosis Complex and Multidrug-Resistant Isolates in Pulmonary and Extrapulmonary Specimens. J Clin Microbiol 2016;54:59–63. https://doi.org/10.1128/JCM.01904-15.
[20] Ditommaso S, Giacomuzzi M, Memoli G, Cavallo R, Curtoni A, Avolio M, et al. Reduction of turnaround time for non-tuberculous mycobacteria detection in heater-cooler units by propidium monoazide-real-time polymerase chain reaction. J Hosp Infect 2019. https://doi.org/10.1016/j.jhin.2019.10.010.
[21] Tsintzou A, Vantarakis A, Pagonopoulou O, Athanassiadou A, Papapetropoulou M. Environmental Mycobacteria in Drinking Water Before and After Replacement of the Water Distribution Network. Water Air Soil Pollut 2000;120:273–82. https://doi.org/10.1023/A:1005266827726.
[22] Sood A, Sreedhar R, Kulkarni P, Nawoor AR. Hypersensitivity pneumonitis-like granulomatous lung disease with nontuberculous mycobacteria from exposure to hot water aerosols. Environ Health Perspect 2007;115:262–6. https://doi.org/10.1289/ehp.9542.
[23] Falkinham JO. Environmental sources of nontuberculous mycobacteria. Clin Chest Med 2015;36:35–41. https://doi.org/10.1016/j.ccm.2014.10.003.
[24] Iroh Tam P-Y, Kline S, Wagner JE, Guspiel A, Streifel A, Ward G, et al. Rapidly growing mycobacteria among pediatric hematopoietic cell transplant patients traced to the hospital water supply. Pediatr Infect Dis J 2014;33:1043–6. https://doi.org/10.1097/INF.0000000000000391.
[25] Sivagnanam S, Podczervinski S, Butler-Wu SM, Hawkins V, Stednick Z, Helbert LA, et al. Legionnaires' disease in transplant recipients: A 15-year retrospective study in a tertiary referral center. Transpl Infect Dis Off J Transplant Soc 2017;19. https://doi.org/10.1111/tid.12745.
[26] Gamage SD, Ambrose M, Kralovic SM, Simbartl LA, Roselle GA. Legionnaires Disease Surveillance in US Department of Veterans Affairs Medical Facilities and Assessment of Health Care Facility Association. JAMA Netw Open 2018;1:e180230. https://doi.org/10.1001/jamanetworkopen.2018.0230.
[27] Kohler P, Kuster SP, Bloemberg G, Schulthess B, Frank M, Tanner FC, et al. Healthcare-associated prosthetic heart valve, aortic vascular graft, and disseminated Mycobacterium chimaera infections subsequent to open heart surgery. Eur Heart J 2015;36:2745–53. https://doi.org/10.1093/eurheartj/ehv342.
[28] Haller S, Höller C, Jacobshagen A, Hamouda O, Abu Sin M, Monnet DL, et al. Contamination during production of heater-cooler units by Mycobacterium chimaera potential cause for invasive cardiovascular infections: results of an outbreak investigation in Germany, April 2015 to February 2016. Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull 2016;21. https://doi.org/10.2807/1560-7917.ES.2016.21.17.30215.
[29] Chand M, Lamagni T, Kranzer K, Hedge J, Moore G, Parks S, et al. Insidious Risk of Severe Mycobacterium chimaera Infection in Cardiac Surgery Patients. Clin Infect Dis Off Publ Infect Dis Soc Am 2017;64:335–42. https://doi.org/10.1093/cid/ciw754.

[30] Ministero della salute. Indicazioni operative riguardanti gli aspetti di follow-up e notifica dei casi di infezione da Mycobacterium chimaera in Italia e aggiornamento delle informazioni disponibili 2019. http://www.trovanorme.salute.gov.it/norme/renderNormsanPdf?anno=2019&codLeg=71653&parte=1%20&serie=null.
[31] Chan T, Ling ML, Teng SY, Chiu KY, James EM. Microbiological monitoring of heater-cooler unit to keep free of Mycobacterium chimaera infection. Perfusion 2019;34:9–14. https://doi.org/10.1177/0267659118787152.
[32] Zozaya-Valdés E, Porter JL, Coventry J, Fyfe JAM, Carter GP, Johnson PDR, et al. Target-Specific Assay for Rapid and Quantitative Detection of Mycobacterium chimaera DNA. J Clin Microbiol 2017;55:10.
[33] Schreiber PW, Kuster SP, Hasse B, Bayard C, Rüegg C, Kohler P, et al. Reemergence of Mycobacterium chimaera in Heater-Cooler Units despite Intensified Cleaning and Disinfection Protocol. Emerg Infect Dis 2016;22:1830–3. https://doi.org/10.3201/eid2210.160925.
[34] Kuehl R, Banderet F, Egli A, Keller PM, Frei R, Döbele T, et al. Different Types of Heater-Cooler Units and Their Risk of Transmission of Mycobacterium chimaera During Open-Heart Surgery: Clues From Device Design. Infect Control Hosp Epidemiol 2018;39:834–40. https://doi.org/10.1017/ice.2018.102.
[35] Steed KA, Falkinham JO. Effect of growth in biofilms on chlorine susceptibility of Mycobacterium avium and Mycobacterium intracellulare. Appl Environ Microbiol 2006;72:4007–11. https://doi.org/10.1128/AEM.02573-05.
[36] Brennan PJ, Nikaido H. The envelope of mycobacteria. Annu Rev Biochem 1995;64:29–63. https://doi.org/10.1146/annurev.bi.64.070195.000333.
[37] Walker JT, Marsh PD. Microbial biofilm formation in DUWS and their control using disinfectants. J Dent 2007;35:721–30. https://doi.org/10.1016/j.jdent.2007.07.005.
[38] Garvey MI, Ashford R, Bradley CW, Bradley CR, Martin TA, Walker J, et al. Decontamination of heater-cooler units associated with contamination by atypical mycobacteria. J Hosp Infect 2016;93:229–34. https://doi.org/10.1016/j.jhin.2016.02.007.
[39] Allen KB, Yuh DD, Schwartz SB, Lange RA, Hopkins R, Bauer K, et al. Nontuberculous Mycobacterium Infections Associated With Heater-Cooler Devices. Ann Thorac Surg 2017;104:1237–42. https://doi.org/10.1016/j.athoracsur.2017.04.067.
[40] FDA. Class II Special Controls Guidance Document: Medical Washers and Medical Washer-Disinfectors; Guidance for the Medical Device Industry and FDA Review Staff. US Food Drug Adm 2019. http://www.fda.gov/regulatory-information/search-fda-guidance-documents/class-ii-special-controls-guidance-document-medical-washers-and-medical-washer-disinfectors-guidance (accessed November 27, 2019).

Table 1. Disinfection protocol for the Stockert 3T by Sorin

Time	Task
• Prior to initial operation; • Prior to storing the heater-cooler;	• Disinfect all surfaces and water circuits;
• After every operation;	• Disinfect all surfaces;
• Every day;	• Check hydrogen peroxide concentration; • Add hydrogen peroxide when necessary;
• Every 7 days;	• Change the water and add hydrogen peroxide to the tank; • Disinfect the overflow bottle;
• Every 14 days;	• Disinfect the water circuit;
• Annually;	• Change the tubing used with the system;
• When needed (e.g., *M. chimaera* contamination);	• Perform a deep disinfection.

Table 2. Disinfection protocol for the HCU40 Maquet by Getinge

Time	Task
• After every operation;	• Clean and disinfect all surfaces;
• Every 7 days;	• Change the water; • Disinfect the water circuit;
• Every 4 weeks;	• Clean the air filter externally with a vacuum cleaner;
• Every 3 months;	• Decalcify the system with citric acid (2%);
• Annually;	• Perform a manufacturer's inspection.

Table 3. Summary of devices' age

	Livanova (n°)	Maquet (n°)
2000	0	1
2008	2	0
2015	6	0
2016	3	1
2017	2	2
2018	2	5
2019	2	3
Total	17	12

Table 4. Summary of NTM contamination by device

	Livanova n (%)	Maquet n (%)	Total n (%)
NTM^+	15 (88.2)	4 (33.3)	19 (65.5)
NTM^-	2 (11.8)	8 (66.7)	8 (34.5)
Total	17	12	29

Fisher exact test = 0.0045

Table 5. Summary of NTM contamination by setting and device

Facility/HCU type	HCU n	NTM^+ HCU n	Sampling rate	water samples n	NTM^+ water samples n (%)
Hospital A (Stockert 3T)	3	3	Monthly Before disinfection	75	26 (34.7)
Hospital A (HCU40)	3	1	Biweekly post disinfection	25	3 (12.0)
Hospital B (Stockert 3T)	2	2	Biweekly Before and post disinfection	126	99 (78.6)
Hospital C (Stockert 3T)	4	4	Monthly post disinfection	35	16 (45.7)
Hospital D (Stockert 3T)	3	2	Monthly post disinfection	15	10 (66.6)
Hospital E (Stockert 3T)	2	1	Monthly post disinfection	10	3 (30,0)
Hospital F (Stockert 3T)	1	1	Monthly post disinfection	1	1 (100)
Hospital F (HCU40)	2	1	Monthly post disinfection	2	1 (50.0)
Hospital G (HCU40)	3	1	Monthly post disinfection	3	1 (33.3)
Hospital H (Stockert 3T)	2	2	Monthly post disinfection	2	2 (100)
Hospital I (HCU40)	2	1	Monthly post disinfection	12	1 (8.3)
Hospital L (HCU40)	2	0	Monthly post disinfection	2	0 (0)
Total	29	19 (65.5%)		308	163 (53.3)

Table 6. Quantitative results of TVCs

Type of sample	*TVCs*	
	22°C	**37°C**
Pre-disinfection samples (geometric mean CFU/mL ± SD)	$2.3 \times 10^2 \pm 1.5 \times 10^3$	$1.3 \times 10^2 \pm 1.4 \times 10^3$
Post-disinfection samples (geometric mean CFU/mL ± SD)	$3.5 \times 10^1 \pm 8.3 \times 10^2$	$3.6 \times 10^1 \pm 9.4 \times 10^2$

22°C, U-value=7649.5; *P*-value=0.00018; 36°C, U-value=7407.5; *P*-value=0.00001 (Mann-Whitney)

Table 7. Relationship between devices, disinfection and TVCs results

	Stockert 3T LivaNova			**HCU40 Maquet**		
	<1 CFU/mL[a]	**1<CFU/mL<100[b]**	**>100 CFU/mL[b]**	**<1 CFU/mL[a]**	**1<CFU/mL<100[b]**	**>100 CFU/mL[b]**
Pre-disinfection samples	68	37	39	5	1	9
Post-disinfection samples	72	31	11	22	3	1
Total	140	68	50	27	4	10
	chi-square test = 13.0112, *P* = 0.001495			chi-square test = 16.3278, *P* = 0.000285		

[a] both TVCs (22°C and 37°C)
[b] at least one of the two TVCs (22°C and 37°C) or both within the limit assigned

Table 8. Relationship between device, disinfection and NTM results

	Livanova*		**Maquet§**		**Total**
	Positive n	**Negative n**	**Positive n**	**Negative n**	
Pre-disinfection samples	80	64	1	14	159
Post-disinfection samples	74	40	4	22	140
					299

*chi-square test = 2.3152, *P* = 0.128118; § Fisher exact test = 0.63571, *P* > 0.05

Figure 1

Results of heater cooler unit (HCU) water surveillance TVCs and NTM PCR by month: examples of a persistent contamination by NTM.

Figure 2

Results of heater cooler unit (HCU) water surveillance TVCs and NTM PCR by month: examples of a eradication of contamination by NTM.

Figure 3

Results of heater cooler unit (HCU) water surveillance TVCs and NTM PCR by month: examples of a device always negative by NTM during six month of surveillance.