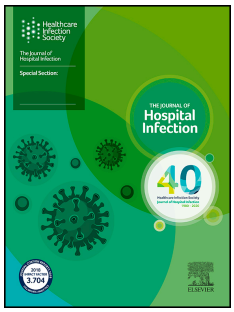

Longitudinal increase in the detection rate of *Mycobacterium chimaera* in heater-cooler device-derived water samples

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Longitudinal increase in the detection rate of *Mycobacterium chimaera* in heater-cooler device-derived water samples

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Abstract

Colonization with *Mycobacterium chimaera* and other nontuberculous mycobacteria (NTM) has been reported for heater-cooler devices (HCD) produced by several manufacturers. Up to now, exclusively LivaNova (London, UK) HCDs have been associated with *M. chimaera* infections after cardiac surgery. The vast majority of studies on HCD colonization were cross-sectional. We were interested in longitudinal dynamics of mycobacterial growth in HCD water samples and analyzed data of a prospective mycobacterial surveillance of five LivaNova 3T HCDs. Nontuberculous mycobacteria were isolated in 319 (48.0%, 21 water samples grew more than one mycobacterial species) of a total of 665 water samples. The most frequently detected species were *M. chimaera* (N= 247/319, 77.4%), *Mycobacterium gordonae* (46/319, 14.4%) and *Mycobacterium paragordonae* (34/319, 10.7%). Detection rates increased longitudinally for any NTM (odds ratio (OR) per year in use: 1.60, 95% CI 1.17-2.24, P<0.001) and for *M. chimaera* (OR per year in use: 1.67, 95% CI 1.11-2.57, P<0.01).

Introduction

The international *Mycobacterium chimaera* outbreak after cardiac surgery was traced back to colonized heater-cooler devices (HCD) (1). HCDs are critical components of cardiac surgery used in conjunction with heart-lung machines during cardioplegia, using water as heat transfer medium (2). *M. chimaera* was detected in both water of HCDs and the surrounding air (1, 3). During cardiac surgery, *M. chimaera*-containing aerosols can reach the operational field (4) and the pathogens can settle on the open surgical wound and implants, causing subsequent infections (1). Several reports, mostly derived from cross-sectional testing and few longitudinal studies (5, 6), indicated the frequent detection of nontuberculous mycobacteria (NTM) and most prominently *M. chimaera* in HCD-derived water samples. It is hypothesized that colonization of LivaNova HCDs with *M. chimaera* during production line is the predominant point source for the outbreak (7). Up to now, data on the frequency of mycobacterial growth in HCD-derived water samples over time is scarce. We addressed this research gap by analyzing data from our prospective HCD surveillance.

Material and Methods

Setting

This study was conducted at the University Hospital Zurich (USZ), Switzerland, a 941-bed tertiary care center with approximately 700 open-heart surgeries with extracorporeal circulation per year.

Heater-cooler devices and mycobacterial surveillance

In 2014, all five HCDs were replaced by new LivaNova 3T HCDs at USZ: two in January, one in April and two in September. As previously described, HCDs were maintained according to the manufacturer's recommendations before mid-April 2014 and with an intensified in-house protocol thereafter (5, 8). The intensified protocol included an increased frequency of water changes and disinfection cycles (6). All HCDs were subjected to prospective mycobacterial surveillance. Every month, 50 mL of the patient and cardioplegia circuit each were analysed for mycobacterial growth, as previously described (9). Briefly, water samples were concentrated and incubated in the BD BACTEC MGIT 960 automated mycobacterial detection system (Becton Dickinson, Sparks, MD, USA) and on Middlebrook 7H11 agar plates (BD Difco Mycobacteria 7H11 Agar; Becton Dickinson) at 37 °C for up to 7 weeks or until growth occurred. Species identification was performed with partial 16S rRNA gene sequence analysis. As additional measure for patient safety, custom-built housings for HCDs were constructed. These housings were directly connected to the operating room exhaust conduit and ensured strict separation between the air surrounding the HCD and the air in the operating room (5). In line with the manufacturer’s recommendations, all HCDs were modified with the Vacuum & Sealing Upgrade and Aerosol Collection Kit in July 2018. After this modification, HCDs had to be used without the custom-built housings, as the modified HCDs were not compatible with the housings.

Statistical analyses

We analyzed data of the prospectively collected results between January 2014 and June 2021. For each HCD and the total of HCDs, the number of positive samples over the entire study period and by species were calculated, and reported by

detection rate per 3 months in service. For determination of the detection rate, we calculated the rate of cultures growing either any NTM or *M. chimaera* by the number of samples tested per time period. For each time period and corresponding detection rate, 95% confidence intervals (CI) were calculated. These results were plotted as time series for visualization of longitudinal variations in the detection rate. The association between growth of any NTM or *M. chimaera* and duration of HCD use (elapsed time since introduction of each HCD to our hospital) was modelled using logistic regression analysis with time as a fixed effect, and allowing for a random intercept/slope for each HCD. All statistical analyses were performed with R (version 3.5.0; R Foundation for Statistical Computing, Vienna, Austria).

Results

A total of 665 water samples were analyzed for mycobacterial growth, of which 346 (52.0%) were negative and 319 (48.0%, 21 water samples with growth of more than one mycobacterial species) were positive for NTM. The most common NTM were *M. chimaera* (247/319, 77.4%), followed by *Mycobacterium gordonae* (46/319, 14.4%) and *Mycobacterium paragordonae* (34/319, 10.7%). In all five HCDs, *M. chimaera* was the most frequently detected species (**Table 1).** Detection of any NTM (**Figure 1 A**), and detection of *M. chimaera* (**Figure 1 B**) became more frequent with increasing duration of HCD use. The duration of HCD-use was significantly associated with both growth of any NTM [odds ratio (OR) per year in use: 1.60, 95% CI 1.17-2.24, P<0.001] and growth of *M. chimaera* (OR per year in use OR 1.67, 95% CI 1.11-2.57, P<0.01).

Discussion

Prospective mycobacterial surveillance of HCD-derived water samples identified an increase in the detection rate of NTM overall and *M. chimaera* over time. In all NTM-positive water samples, *M. chimaera* was the most frequent species.

A recent joint analysis on whole genome sequencing studies of cardiac surgery-associated *M. chimaera* isolates supported HCD colonization with *M. chimaera* during the manufacturing process (7). Early water samples might have tested culture-negative due to concentrations of *M. chimaera* below the detection limit (9). One hypothesis for increasing detection rates is biofilm formation within HCDs. Species of the *Mycobacterium avium* complex (MAC) including *M. chimaera* are capable of building biofilms (10). Biofilm formation is believed to be a time-dependent process. *M. chimaera* is categorized as a slowly growing NTM. The slow growth rate might explain the observation that the detection rate was low at delivery but increased slowly over time. A recent study suggests that biofilm due to *M. chimaera* within HCDs is a major challenge for HCD decontamination with successful decontamination requiring replacement of hardware (11). During our study, none of the HCDs was disassembled for biofilm removal, but all HCDs were maintained with an intensified disinfection protocol after mid-April 2014. The longitudinal increase in the detection rate of NTM and especially *M. chimaera* suggests insufficient efficacy of the disinfection protocol, even if intensified. A promising protocol to reduce biofilm burden within HCDs might be maintenance with a combination of an enzyme detergent cleaning agent and Clorox®, as a recent report indicated delayed reappearance of *M. chimaera* in water samples gathered from a Hemotherm model 400 CE Dual Reservoir Cooler/Heater (Cincinnati Sub-Zero Products, Inc., Cincinnati, OH, USA) (12).

Notably, no *M. chimaera* was recovered from monthly gathered air samples (Supplement) and no cases of cardiac surgery-associated *M. chimaera* infections were detected (data not shown).

A strength of the present study is the prospective design with samples encompassing a time period of seven years. Furthermore, HCD maintenance was standardized and the study started with factory-new HCDs.

Our study also has limitations. Given the single center design, the number of HCDs was limited to five and included a single HCD model. Although this does not allow conclusions to the use of HCDs in general, LivaNova 3T HCD represents the most relevant HCD type based on its association with *M. chimaera* infections after cardiac surgery and big market share. Finally, we exclusively used a cultural approach for the detection of *M. chimaera* and other NTM while recent data support a higher sensitivity of PCR-based detection methods (13). However, cultivation based methods enable superior opportunities for subsequent studies, e.g. physiological and phylogenetic characterization of strains and drug-susceptibility testing.

For LivaNova 3T HCDs, maintenance protocols based on regular application of chemical disinfectants are insufficient to stop mycobacterial replication. New maintenance and disinfection protocols need to be defined and tested for efficacy.

Conflict of Interest statement

PWS received travel grants from Pfizer and Gilead, honorary as speaker and advisory board member from Pfizer and, honorary from Gilead as Advisory Board member outside of the submitted work. VS received travel grants from Gilead outside of the submitted work.

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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. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2015;61(1):67-75.

2. Schreiber PW, Sax H. Mycobacterium chimaera infections associated with heater-cooler units in cardiac surgery. Current opinion in infectious diseases. 2017;30(4):388-94.

3. 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. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2017;64(3):335-42.

4. Sommerstein R, Ruegg C, Kohler P, Bloemberg G, Kuster SP, Sax H. Transmission of Mycobacterium chimaera from Heater-Cooler Units during Cardiac Surgery despite an Ultraclean Air Ventilation System. Emerging infectious diseases. 2016;22(6):1008-13.

5. Schreiber PW, Kuster SP, Hasse B, Bayard C, Ruegg C, Kohler P, et al. Reemergence of Mycobacterium chimaera in Heater-Cooler Units despite Intensified

Cleaning and Disinfection Protocol. Emerging infectious diseases. 2016;22(10):1830-3.

6. Kaelin MB, Kuster SP, Hasse B, Schulthess B, Imkamp F, Halbe M, et al. Diversity of nontuberculous mycobacteria in Heater-Cooler Devices - results from prospective surveillance. The Journal of hospital infection. 2020.

7. Schreiber PW, Kohl TA, Kuster SP, Niemann S, Sax H. The global outbreak of Mycobacterium chimaera infections in cardiac surgery - A systematic review of whole genome sequencing studies and joint analysis. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2021.

8. Sorin Deutschland GmbH. Heater-cooler system 3T operating instructions. Version 09/2012

9. Schreiber PW, Köhler N, Cervera R, Hasse B, Sax H, Keller PM. Detection limit of Mycobacterium chimaera in water samples for monitoring medical device safety: insights from a pilot experimental series. The Journal of hospital infection. 2018;99(3):284-9.

10. Siddam AD, Zaslow SJ, Wang Y, Phillips KS, Silverman MD, Regan PM, et al. Characterization of Biofilm Formation by Mycobacterium chimaera on Medical Device Materials. Frontiers in microbiology. 2020;11:586657.

11. 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. The Journal of hospital infection. 2016;93(3):229-34.

12. Falkinham JO, 3rd. Disinfection and cleaning of heater-cooler units: suspension- and biofilm-killing. The Journal of hospital infection. 2020.

13. Zozaya-Valdés E, Porter JL, Coventry J, Fyfe JAM, Carter GP, Gonçalves da Silva A, et al. Target-Specific Assay for Rapid and Quantitative Detection of Mycobacterium chimaera DNA. Journal of clinical microbiology. 2017;55(6):1847-56.

Table 1: Results of prospective mycobacterial surveillance cultures by heater-cooler device

HCD	Number of tests	No growth	Growth	*M. chimaera*	*M. gordonae*	*M. paragordonae*	*M. chelonae*	*M. abscessus*	Other NTM
1	158	65 (41.14%)	93 (58.86%)	79 (50.00%)	11 (6.96%)	6 (3.80%)	1 (0.63%)	0 (0.00%)	1 (0.63%)
2	116	63 (54.31%)	53 (45.69%)	32 (27.59%)	18 (15.52%)	6 (5.17%)	5 (4.31%)	1 (0.86%)	3 (2.59%)
3	149	84 (56.38%)	65 (43.62%)	42 (28.19%)	9 (6.04%)	14 (9.40%)	1 (0.67%)	0 (0.00%)	2 (1.34%)
4	113	70 (61.95%)	43 (38.05%)	36 (31.86%)	5 (4.42%)	3 (2.65%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
5	129	64 (49.61%)	65 (50.39%)	58 (44.96%)	3 (2.33%)	5 (3.88%)	0 (0.00%)	0 (0.00%)	1 (0.78%)
Total	665	346 (52.03%)	319 (47.97%)	247 (37.14%)	46 (6.92%)	34 (5.11%)	7 (1.05%)	1 (0.15%)	7 (1.05%)

HCD: heater-cooler device, NTM: nontuberculous mycobacteria

Nineteen and two samples yielded growth of two and three different NTM species, respectively.

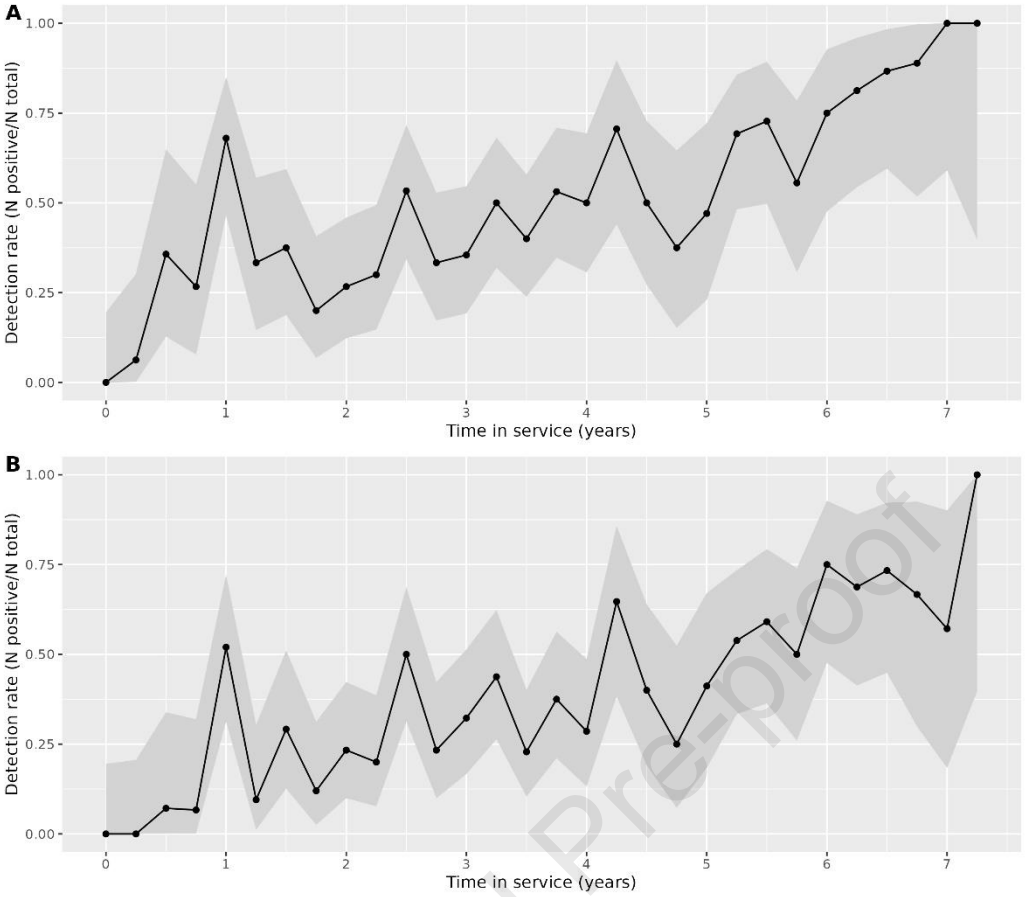

Figure 1: Results of prospective mycobacterial surveillance cultures over time in service

The x-axis indicates the duration of use for heater-cooler devices, the y-axis the detection rate of any nontuberculous mycobacteria (NTM) (A) or *Mycobacterium chimaera* (B) aggregated over all five HCDs.
Dots indicate the detection rate calculated for time intervals of 3 months in service, grey shaded areas correspond to 95% confidence intervals.