Real-time PCR, the best approaches for rapid testing for Mycobacterium chimaera detection in heater cooler units and extracorporeal membrane oxygenation

Abstract

According to recent investigations, the risk of *M. chimaera* contamination of HCUs has reached global proportions. Our aim was to field evaluate a protocol for early detection of *M. chimaera* contamination.

We assessed the presence of viable *M. chimaera* in 395 water samples obtained from 48 devices (heater cooler unit and extracorporeal membrane oxygenation) by Real Time PCR. Thirty devices were NTM positive, of which 14 were contaminated with *M. chimaera*. The most frequently contaminated devices were the Stockert 3T. Noteworthy, Stockert 3T devices were positive for *M. chimaera.*

In conclusion, this study introduces novel PMA-PCR designed to specifically detect *M. chimaera* in HCUs and ECMO devices; this method can replace the culture method for continuous microbiological surveillance. The timely detection of *M. chimaera* contamination can then be used to improve effective management of the devices.

Introduction

Heater cooler units (HCUs) used during cardiopulmonary bypass and extracorporeal membrane oxygenation (ECMO) can generate potentially infectious aerosols containing a range of bacteria commonly found in tap water, especially *Mycobacterium chimaera.* In particular, Stockert 3T heater cooler units (HCUs) (LivaNova, Sorin Group Deutschland GmbH, Norderstedt, Germany) employed in approximately 60% to 80% of cardiac surgeries[1], have been recently involved in the transmission of bacteria to surgical theaters through aerosolization of contaminated water sources.[2,3] Due to the international alert[4], in 2015, the Sorin Group issued new guidelines concerning the timelines for maintenance and disinfection of its Stockert 3T HCUs and in 2016, released a safety warning, strongly recommending that both 1T and 3T devices should be microbiologically monitored (detection of *Pseudomonas aeruginosa*, coliform bacteria, total viable counts, and NTM). 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 at no additional cost.

In addition, Maquet (Getinge, Rastatt, Germany), another HCU manufacturer, has issued a warning of the risk of *M. chimaera* contamination of its HCU water tanks, along with a set of guidelines to manage such risk.

In 2017, a regional microbiological investigation conducted by our lab revealed that 67.8% of HCUs (19/28) were contaminated by non-tuberculous mycobacteria (NTM). These results were obtained by analyzing HCU water samples through a combined approach of propidium monoazide (PMA)-qPCR (Anyplex™ plus MTB/NTM MDR-TB Real-Time Detection, V2.0, Seegene) and traditional culture techniques.[5] Specifically, we found a good agreement (80.0%) between conventional culture testing that require long incubation time (around six-eight weeks) due to the slow growth rate of mycobacteria. In addition, culture are unable to detect viable but non-culturable bacteria under stressful conditions.

Consequently this previous validation of PMA-PCR, we tried to assess the presence of viable *M. chimaera* by PMA-PCR, in order to adopt a fast and easy-to use procedure to monitor viability of *M. chimaera* in HCUs. For this purpose, we amplified the *M. chimaera* SR1 sequence previously found to be 100% conserved across multiple *M. chimaera* strains.[6]

METHODS

Settings

From June 2017 to February 2020, water samples were collected from 9 adult and 1 pediatric cardiac surgery facilities, all located in Piedmont (Italy). Surveillance and microbiological investigations of those surgical suites are currently ongoing. For this study we analyzed the frozen DNA samples collected and analyzed in our previous investigation (281 water samples) [5] and new samples (114 water samples) collected from April 2019 to February 2020.

Sampling

One liter of circulating water from each HCU (from the patient circuit and the cardioplegia circuit) and ECMO (from the water tanks of thermoregulatory devices used for ECMO treatment), was collected in a sterile plastic bottle containing sodium thiosulphate (10% w/v).

Analysis

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 DNA was extracted as described previously.[5] DNA samples were stored in buffer (Aquadien; Bio-Rad, Marnes-la-Coquette, France) at -20° C.

PCR was performed using a customized kit "On Demand Advanced DNA Kit for *M. chimaera* Detection" by Genesig, Primer Design Ltd, Southampton, UK. The kit, based on TaqMan chemistry, contains reagents to amplify a sequence within the SR1 region of the *M. chimaera*[6] genome. Results can be acquired in less than 2 h with a limit of detection (LOD) < 100 genomic units (GU) *per* well.

RESULTS

We analyzed 395 water samples obtained from 48 devices, of which 21 were Stockert 3T, 12 HCU40 and 15 HCU 35. Thirty devices (62.5%) were NTM positive, of which 14 (29.2%) were contaminated with *M. chimaera*. The most frequently contaminated devices were the Stockert 3T (81.2%). In comparison with the Stockert 3T, the HCU40 and HU35 devices were less frequently contaminated by NTM (41.7% and 53.3%, respectively). Noteworthy, 13 (61.9%) Stockert 3T devices were positive for *M. chimaera*, whereas this bacterium could only be detected in one HU35. The results obtained from our microbiological surveillance are summarized in Table 1.

DICUSSION

According to recent investigations, the risk of *M. chimaera* contamination of HCUs has reached global proportions.[7] In this regard, epidemiologic analysis and laboratory investigations have

revealed a positive association between isolates from clinical cases and contaminated HCUs and whole genome sequencing data support a common-source outbreak originating at an HCUs manufacturing facility .[8,9]

We have successfully developed a PMA-PCR protocol to rapidly assess the risk of *M. chimaera* infection from contaminated HCUs among open-chest surgery patients.[5] This microbiological investigation confirms previous findings indicating that *M. chimaera* contamination occurs predominantly in water samples obtained from Stockert 3T LivaNova devices.[10] Specifically, our results show that despite the two amendments of Sorin Group, 13 Stockert 3T were positive for *M. chimaera* while one HU35 were positive for *M. chimaera* and all HCU40 were negative. This suggests that the disinfection procedure recommended by the manufacturer (peracetic acid at a final concentration of 3.3%, or 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) did not seem to inhibit significantly *M. chimaera* colonization that persisted in Stockert 3T HCUs, consistently with previous reports.[4,11] As regards the Maquet devices, the monochloramine adopted for disinfection (weekly treatment with 2% chloramine-T or 5% chloramine-T solution when atypical mycobacteria are found in the water system) seems to offer greater efficacy against mycobacteria.

Our protocol allows timely detection of *M. chimaera* contamination in HCUs, which can then be used to request LivaNova's no-charge deep disinfection services for 3T Heater-Coolers (the most effective way to completely remove these pathogens is through HCU disassembly, replacement of the contaminated tubing and completion of a thorough disinfection).[12] In this regard, all devices undergoing deep disinfection are certified as "*M. chimaera* free" before being placed back in use.[13] Compared with culture-based methods, the gold standard methods, the PMA-PCR assay offers several advantages such as a faster diagnosis, the need for lower bacterial DNA concentrations, and the ability to detect viable but non-culturable cells under stressful conditions. Nevertheless, it must be highlighted that some studies have found that the use of these viability dyes was not fully effective to remove the signal from membrane-compromised dead cells.[14–17] The ability of PMA-PCR to detect

dead cells of *Mycobacterium paratuberculosis* was tested on artificial samples with different amounts of killed NTM cells by several authors. These studies confirmed that difference between completely live and dead NTM organisms following PMA treatment is approximately 2 $\log_{10}$.[16,18,19] However, the exclusion of 99% of dead NTM cells already represents a significant advantage over regular qPCR.

In conclusion, this study introduces novel PMA-PCR designed to specifically detect *M. chimaera* in HCUs and ECMO devices; this method can replace the culture method for continuous microbiological surveillance and we hopes that this fast technology can be applied worldwide to better assess risk exposure to *M. chimaera* in HCUs. Effective contamination monitoring of HCUs is crucial to ensure the safety of patients undergoing open-chest cardiac surgery.

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Table 1. Summary of NTM contamination by device

	N° devices	NTM positive n (%)	*M. chimaera* positive (%)
Stockert 3T LivaNova	21	17 (81.0)	13 (61.9)
HCU40 Maquet	12	5 (41.7)	0 (0.0)
HU35 Maquet	15	8 (53.3)	1 (6.6)
Total	48	30 (62.5)	14 (29.2)