Evaluation of Aerosolization by Spectrum Medical Heater-Cooler

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1 Background

In October 2015, *Mycobacterium chimaera* infections were reported in patients following cardiovascular surgery (Sax et al., 2015). Following that report, the U.S. Food and Drug Administration (FDA, 2015) and the European Centre for Disease Prevention and Control (ECDC, 2015) issued alerts. The *M. chimaera*-infections were traced to the presence of the infecting *M. chimaera* in the water reservoirs of one type of heater-cooler, the Sorin 3T, used to control blood temperature in patients during cardiac surgery. Infection occurred via aerosols from the *M. chimaera*-colonized Sorin 3T heater-coolers in the operating room during cardiac surgery (Sommerstein et al., 2016). Whole genome sequencing of *M. chimaera* isolates linked to Sorin 3T heater-coolers were shown to be closely related and clonal, indicating a single source of colonization (Hasan et al., 2019). The source of *M. chimaera* in the water reservoir of the Sorin 3T heater-coolers was the water used to test the operation of the instruments in the manufacturing facility in Munich, Germany (Haller et al., 2016) and the *M. chimaera* aerosols might have escaped from the instrument due to degradation of the holes and joints in the top of the reservoir (Chand et al., 2017). M. *chimaera* is a member of the group of environmental mycobacteria (nontuberculous mycobacteria, NTM) that are widely distributed in the human environment (Tortoli et al., 2004). Importantly for these outbreaks, the NTM habitats include drinking water distribution systems and premise plumbing, most notably hospitals and homes.

Mycobacterium abscessus infections have also been identified and linked to the Sorin 3T heater cooler and the source of those outbreaks was the hospital water supply (Baker et al., 2017).

NTM cells are quite hydrophobic, due to the presence of a lipid-rich outer membrane (Brennan and Nikaido, 1995). That hydrophobicity contributes to NTM: (1) disinfectant-resistance, (2) aerosol-concentration, and (3) surface attachment and biofilm-formation. NTM cells are resistant to the concentrations of disinfectants commonly used for drinking water treatment (e.g., chlorine, chloramine, and ozone). They are at least 1,000-times more resistant than the bacterium used as the industry standard for disinfection, *Escherichia coli* (Taylor et al., 2000). For example, at a concentration

of 1 ppm chlorine, it would take approximately 5 seconds to kill 99.9 % of *E. coli* cells, but 2 hrs to kill *Mycobacterium intracellulare*, a close relative of *M. chimaera* (Taylor et al., 2000). NTM cells are concentrated, up to 10,000-fold, in water droplets. Any NTM cells in water suspension preferentially attach to air bubbles rising in the water column. As the bubbles rise, their surface is coated with NTM cells, and when the bubble reaches the surface, the bubble forms a mycobacterial coated crater and bursts. The closing of the crater leads to the ejection of droplets of water to heights of 10 cm (Parker et al., 1983). These NTM-enriched droplets are carried by air currents to susceptible hosts. The hydrophobic mycobacterial cells preferentially attach to surfaces and grow, forming thick biofilms containing high (e.g., 10,000/cm^2) cell numbers (Mullis and Falkinham, 2013). The layers of cells and extracellular material in biofilms substantially increase disinfectant-resistance (Sneed and Falkinham, 2006). Thus, standard disinfection regimens fail to kill even a small proportion of NTM cells in biofilms and the surviving biofilm cells can recolonize the circulating water.

2 Objectives

(1) Inoculate Spectrum Medical heater-coolers with high numbers of *M. chimaera* to produce a heavily colonized instrument.

(2) Determine the potential for the *M. chimaera*-inoculated heater-cooler to aerosolize *M. chimaera* under conditions of normal operation at 37° C and 10° C.

(3) Measure the numbers of *M. chimaera* inoculated into the Spectrum Medical heater-coolers to estimate their persistence over time.

3 Experimental Methods

3.1 **Bacterial Strain.** *M. chimaera* strain NC-W-2-1 is a recent, un-transferred isolate recovered from the reservoir of a Sorin 3T heater-cooler.

3.2 Growth Medium and Water-Acclimation. *M. chimaera* strain NC-W-2-1 was grown to mid-exponential phase from a single isolated colony in Middlebrook 7H9 broth (BD, Sparks, MD)

containing 0.5 % (v/v) glycerol and 10 % (vol/vol) oleic acid albumin with aeration (60 rpm) at 37° C. High density suspensions for inoculation were prepared by collecting mycobacterial cells by centrifugation (5,000 x g), washed twice in sterile Blacksburg tap water, and suspending cells in 1/10 the volume of sterile Blacksburg tap water. The resulting suspensions were incubated at 25° C for 7 days with aeration (60 rpm) to acclimate cells to water. The water-acclimated suspension contained 2.1 x 10^{10} CFU *M. chimaera*/mL. Five (5) mL of that *M. chimaera* suspension was used to inoculate each Spectrum Medical heater-cooler.

3.3 Medium for Isolation and Enumeration of *M. chimaera*. *M. chimaera* was grown on Middlebrook 7H10 agar medium (M7H10) for colony enumeration.

3.4 Ultrasonic Humidifier. An Ultrasonic 360° Humidifier (Dorel Juvenile Group, Columbus, Indiana) Model 49292 was used as a positive control to demonstrate the lab's ability to collect aerosolized *M. chimaera* cells by impaction on M7H10 agar plates in the Andersen 6-Stage Samplers. Aerosols were generated by the operation of an inoculated ultrasonic humidifier for 60 min at 25° C.

3.5 Aerosol Sampler. Two 6-Stage Andersen Samplers (Andersen, 1958) were employed and operated at the collection rate of 1 ft^3/min (1 cfu/min). The Andersen samplers were placed 1 and 2 meters from the heater-cooler at a height of 1 meter from the floor to mimic conditions in the operating theatre (Sax et al., 2015).

3.6 Aerosolization Collection Room. The Spectrum Medical heater-coolers and Andersen 6-Stage Cascade Samplers were placed in an unventilated and sealable room of 30 m^3 volume. Before and after all aerosol collections, microorganisms in the room's air were eradicated by 30 min exposure to ultraviolet irradiation. Preliminary experiments demonstrated that 30 min of UV-irradiation resulted in complete loss of any aerosolized microorganisms as colony-forming units on all the stages of the Andersen Sampler from a 30 min collection (30 cu ft.). Surfaces in the

room were sterilized by wiping with 3 % (vol/vol) Lysol disinfectant (Reckitt Benckiser, Inc., Parsippany, NJ).

3.7 Aerosol collection.

(1) The aerosol collection room was disinfected by 30 min exposure to UV-light in the dark.

(2) One Spectrum Medical heater-cooler with attached accessory tubes was moved into the aerosol collection room with the two Andersen 6-Stage Aerosol Samplers loaded with M7H10 agar plates.

(3) The Andersen 6-Stage Samplers were placed at a height of 1 meter from the floor at a distance of 1 and 2 meters from the Spectrum Medical heater-cooler.

(4) Before inoculation of the Spectrum Medical heater-cooler with *M. chimaera* cells, 60 min aerosols were collected to provide background (control) measurements of microorganisms in the aerosol collection room.

(5) The aerosol-exposed M7H10 agar plates were removed, sealed with Parafilm® to prevent drying, and incubated at 37° C.

(6) The aerosol collection room was disinfected by 30-exposure to UV-light in the dark.

(7) The Spectrum Medical heater-cooler was set to run at 37° C.

(8) Once the Spectrum Medical heater-cooler reached the set temperature, heater-cooler was inoculated with 5 mL of the water-acclimated suspension of *M. chimaera* [total = 1.3×10^{11} colony-forming units (CFU)] and the heater-cooler operated on "Prime" for 10 min to disperse the inoculum in the fluid/HTF circuit.

(9) During that period, the two Andersen 6-Stage Samplers were filled with M7H10 agar plates.

(10) Before any aerosol collection, 5 mL of reservoir fluid of the Spectrum Medical heater-cooler was collected, and 0.1 mL samples were spread in triplicate on M7H10 agar for identification and enumeration of *M. chimaera*.

(11) The Spectrum Medical heater-cooler was turned on (Operating) and the room's aerosols were collected for 60 min (1 hr.) at a rate of 1 cfm (total = 60 cu ft) by both Andersen Samplers.

(12) The aerosol-exposed M7H10 agar plates were removed, sealed with Parafilm® to prevent drying, and incubated at 37° C.

(13) Following each aerosol collection with an inoculated heater-cooler, the fluid in the reservoir of each Spectrum Medical heater-cooler was sampled, 0.1 mL spread in triplicate on M7H10 agar for identification and enumeration of *M. chimaera* and plates sealed with Parafilm® and incubated at 37° C.

(14) The aerosol collection room was disinfected by 30 min exposure to UV-light in the dark.

(15) The Spectrum Medical heater-cooler was set to run at 10° C.

(16) Once the Spectrum Medical heater-cooler reached the set temperature, aerosols were again collected and colony counted as described above.

(17) After 21-days incubation, colonies of *M. chimaera* were counted.

(18) The same protocol was followed for the other two Spectrum Medical heater-coolers.

4 Results

4.1 Aerosolization Controls

(1) The fluid in each of the un-inoculated Spectrum Medical heater-coolers was sampled. No colonies of *M. chimaera* or *Mycobacterium* spp. were recovered from the triplicate plates, each spread with 0.1 mL of the undiluted fluid. Thus, the number of *M. chimaera* or *Mycobacterium* spp. cells was less than 3.3 colony-forming units (CFU) per mL.

(2) A 60 min aerosol collection (60 cu ft) was performed in the collection room with one uninoculated Spectrum Medical heater-cooler (Serial Number ER 1000020) and the 2 Andersen Samplers. No *M. chimaera* or *Mycobacterium* spp. colonies were found on the plates from the two Andersen 6-Stage Samplers (Table 1). As the Andersen Samplers were operated at 1 cubic feet per minute and no colonies were isolated, the number of *M. chimaera* or *Mycobacterium* spp. CFU was less than 1 CFU/120 cubic feet of air.

Table 1. Absence of *M. chimaera* or *Mycobacterium* spp. in 1 hr Aerosols Collected in a Room with an Un-Inoculated, non-Operating Spectrum Medical Heater-Cooler Serial Number SN: ER1000020.

	***M. chimaera* or *Mycobacterium* spp.**	
	Colony-forming Units (CFU)/60 cu ft air	
Stage [a]	**1 meter [a]**	**2 meters [a]**
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0

[a] Samplers placed 1 meter from floor and at 1 and 2 meters from heater-cooler

4.2 Aerosol Collection from Spectrum Medical Heater-Cooler Serial Number: ER1000018.

(1) In spite of the 60 min duration of aerosol collection from the *M. chimaera*-inoculated Spectrum Medical heater-cooler SN: ER1000018, no *M. chimaera* were recovered from the aerosols (Table 2).

(2) The initial density of *M. chimaera* cells before aerosol collection was $9.3 \pm 3.7 \times 10^7$ CFU/mL and the density after aerosol collection was $2.2 \pm 4.9 \times 10^7$ CFU/mL. This difference (i.e., standard deviation) is within the typical range of *M. chimaera* colony counts due to the aggregation and clumping of the hydrophobic cells in suspension and adherence to the interior surfaces of the circulating liquid circuit.

Table 2. Absence of *M. chimaera* in 1 hr Aerosols from Spectrum Medical Heater-Cooler Serial Number SN: ER1000018.

	***M. chimaera* Colony-forming Units (CFU)/60 cu ft air**			
Andersen	**Operating @ 10° C**		**Operating @ 37° C**	
Stage	**1 meter**	**2 meters**	**1 meter**	**2 meters**
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0

[a] Samplers placed 1 meter from floor and at 1 and 2 meters from heater-cooler

4.3 Aerosol Collection from Spectrum Medical Heater-Cooler Serial Number: ER1000019.

(1) In spite of the 60 min duration of aerosol collection from the inoculated Spectrum Medical heater-cooler SN: ER1000019, no *M. chimaera* were recovered (Table 3).

(2) The initial density of *M. chimaera* cells before aerosol collection was $8.9 \pm 3.8 \times 10^6$ CFU/mL and the density after aerosol collection was $1.4 \pm 3.1 \times 10^7$ CFU/mL. This variation is within the typical range (i.e., standard deviation) of *M. chimaera* colony counts due to the aggregation and clumping of the hydrophobic cells in suspension and adherence to the interior surfaces of the circulating liquid circuit.

Table 3. Absence of *M. chimaera* in 1 hr Aerosols from Spectrum Medical Heater-Cooler Serial Number SN: ER1000019.

	***M. chimaera* Colony-forming Units (CFU)/60 cu ft air**			
Andersen	**Operating @ 10° C**		**Operating @ 37° C**	
Stage	**1 meter**	**2 meters**	**1 meter**	**2 meters**
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0

[a] Samplers placed 1 meter from floor and at 1 and 2 meters from heater-cooler

4.4 Aerosol Collection from Spectrum Medical Heater-Cooler Serial Number: ER1000020.

(1) One *Mycobacterium* spp. colony from one stage of the Andersen 6-Stage Sampler placed 1 meter from the Spectrum Medical heater-cooler serial number ER1000020 was recovered (Table 4). DNA sequence analysis showed that it was *M. chimaera*. As that heater-cooler was the first to be tested and was inoculated within the aerosol collection room, its presence in the aerosol collection is likely due to escape during the inoculation of the heater-cooler.

(2) The initial density of *M. chimaera* cells before aerosol collection was $7.1 \pm 2.8 \times 10^7$ CFU/mL (71 million) and the density after aerosol collection was $5.2 \pm 1.0 \times 10^6$ CFU/mL (5.2 million). This difference is within the typical range (i.e., standard deviation) of *M. chimaera* colony counts due to the aggregation and clumping of the hydrophobic cells in suspension and the adherence of the mycobacterial cells to the interior surfaces of the fluid system.

Table 4. Recovery of *M. chimaera* in 1 hr Aerosols from Spectrum Medical Heater-Cooler Serial Number SN: ER1000020.

	***M. chimaera* Colony-forming Units (CFU)/60 cu ft air**			
Andersen	**Operating @ 10° C**		**Operating @ 37° C**	
Stage	**1 meter**	**2 meters**	**1 meter**	**2 meters**
1	1	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0

[a] Samplers placed 1 meter from floor and at 1 and 2 meters from heater-cooler

4.5 Absence of *M. chimaera* in Fluids of Spectrum Medical Heater-Cooler.

(1) To determine the persistence of *M. chimaera* in the Spectrum Medical heater-cooler S/N ER1000020, a 5 mL sample of the reservoir fluid was collected on 9 April 2021 from one inoculated heater-cooler whose aerosols were collected on 17 March 2021, 23 days after inoculation and aerosol collection.

(2) Samples (0.1 mL) were spread in triplicate on M7H10 agar, sealed with Parafilm® and incubated at 37° C for 21 days.

(3) No *M. chimaera* colonies were recovered (density < 3.3 CFU/mL) from an initial density of 7.1 x 10^7 CFU/mL immediately following inoculation and priming to ensure the inoculum was distributed throughout the liquid circuit.

(4) The same sampling was repeated with all three heater-coolers on 11 May 2021, 8 weeks after inoculation and aerosol collections. No *M. chimaera* colonies were recovered (< 3.3 CFU/mL) from any of the three heater-coolers.

4.6 Presence of *M. chimaera* in a Biofilm Sample from Spectrum Medical Heater–Cooler S/N ER1000020.

(1) A biofilm sample was collected from one Spectrum Medical heater-cooler by swabbing a 2 x 2 cm square (4 cm^2) on the wall of the reservoir with a sterile swab. Access to the wall was through the access point on the top of the reservoir, through which the inoculum was also introduced.

(2) The biofilm sample was collected on 9 April 2021 from the inoculated heater-cooler whose aerosols were collected on 17 March 2021; 23 days after inoculation and aerosol collection.

(3) The swab was placed in 5 mL of sterile tap water and vortexed 60 sec at the highest setting, left on the bench for 30 min. and again vortexed 60 sec at the highest setting. Samples of the suspension (0.1 mL) were spread on M7H10 agar in duplicate, plates sealed with Parafilm®, and incubated at 37° C for 21 days.

(4) *Mycobacterium* spp.-like colonies appeared on the two plates; one colony on one plate and 3 colonies on the other. Following isolation of the four (4) isolates, 3 proved to be *M. chimaera*, one was not *Mycobacterium* spp. Based on that data, it can be estimated that 23 days following

inoculation with a high-density suspension of *M. chimaera*, the interior surfaces of the Spectrum Medical heater-cooler could have 18.75 CFU *M. chimaera*/cm^2 of surface (i.e., 3 isolates in 0.2 mL of a 5 mL suspension of 4 cm^2).

(5) Biofilms were sampled from all three Spectrum Medical heater-coolers as described above on 11 May 2021, 8-weeks after *M. chimaera* inoculation. No *Mycobacterium* spp. or *M. chimaera* colonies were recovered (< 6.25 CFU/cm^2).

4.7 Liquid and Biofilm Samples from Heat Exchangers and Tubing Fail to Yield *M. chimaera*.

(1) Liquid and biofilm (swab) samples were collected from the tubing used for all three Spectrum Medical heater-coolers on 13 May 2021. Thus, the tubing had been in contact with the inoculated fluid in the three different heater-coolers for 8 weeks.

(2) The tubing was aseptically sliced open and a small volume of liquid removed (1-2 mL) and the inside surface of the tubing swabbed (2 cm^2). The swab was placed in 2 mL of sterile water and vortexed 60 sec before 0.1 mL samples of the suspension were spread on M7H10 agar medium in duplicate. Likewise, 0.1 mL samples of the liquid removed from the tubing was directly spread on M7H10 agar.

(3) Liquid and biofilm (swab) samples were collected from both the large and small heat exchangers used for all three heater-coolers. Thus, the heat exchangers had been in contact with the inoculated fluid for 8-weeks. The tubing was separated from the heat exchangers and a small volume of liquid removed (1-2 mL) and the inside of the heat exchangers swabbed (2 cm^2). The swab was placed in 2 mL of sterile water and vortexed 60 sec before 0.1 mL samples were spread on M7H10 agar medium in duplicate. Likewise, 0.1 mL samples of the liquid removed from the tubing was directly spread on M7H10 agar.

(4) No colonies of *M. chimaera* were recovered from either the tubing or heat exchangers liquid (< 5 CFU/mL) and none were recovered from the swab samples of either the tubing or heat exchangers (< 5 CFU/cm^2).

4.8 *M. chimaera* in Filters from Spectrum Medical Heater Coolers.

(1) The filter was removed from Spectrum Medical heater-cooler S/N ER1000020 on 9 April 2021 whose aerosols were collected on 17 March 2021 (23 days after inoculation and aerosol collection) and the filter material inside the filter housing aseptically removed.

(2) The filter material was aseptically transferred to 5 mL of sterile tap water and vortexed 60 sec at the highest setting. The suspension was left on the bench for 30 min, vortexed again for 60 sec, and 0.1 mL samples of the resulting suspension spread on M7H10 agar plates in duplicate, sealed with Parafilm®, and incubated at 37° C for 21 days.

(3) No colonies of *Mycobacterium* spp. or *M. chimaera* were detected.

(4) Filters from the other two heater-coolers were removed on 13 May 2021 (8-weeksafter the inoculation and aerosol collection) and processed as described above. No colonies of *Mycobacterium* spp. or *M. chimaera* were detected.

4.9 Aerosol Collection from Ultrasonic 360° Humidifier.

(1) A positive control was used to demonstrate the lab's ability to collect aerosolized *M. chimaera* cells by impaction on M7H10 agar plates in the Andersen 6-Stage Samplers. Aerosols were generated by the operation of an inoculated Ultrasonic 360° Humidifier (Dorel Juvenile Group, Columbus, Indiana). The *M. chimaera* cells suspension used to inoculate the humidifier was the same as used for the heater-coolers.

(2) High numbers of *M. chimaera* colonies were recovered from all 6 stages of both Andersen Samplers (1 and 2 meters from the humidifier) (Table 5).

(3) The initial density of *M. chimaera* cells before aerosol collection was 2.1×10^7 CFU/mL and the density after aerosol collection was 7.5×10^6 CFU/mL.

(4) This confirms an earlier publication documenting the aerosolization of *Mycobacterium* spp. cells (i.e., *Mycobacterium avium* and *Mycobacterium abscessus*) from the same ultrasonic humidifier (Hamilton and Falkinham, 2018).

Table 5. Aerosolization of *M. chimaera* in 1 hr Aerosols from Ultrasonic Humidifier

M. chimaera Colony-forming Units (CFU)/60 cu ft air		
Andersen	Operating @ 25° C	
Stage	1 meter	2 meters
1	1,616	1,300
2	756	634
3	293	481
4	2,160	1,488
5	2,940	4,340
6	4,960	3,312

[a] Samplers placed 1 meter from floor and at 1 and 2 meters from humidifier

5 Conclusions.

(1) In spite of the very high numbers of *M. chimaera* cells inoculated into all three Spectrum Medical heater-coolers, there was no evidence of aerosolization. The source of the one *M. chimaera* isolate from an aerosol collection was likely due to contamination during inoculation of that specific instrument.

(2) The absence of recovery of *M. chimaera* from aerosols collected in a room with operating heater-coolers was not due to a failure to recover aerosolized *M. chimaera* cells as shown by successful recovery of *M. chimaera* from aerosols generated by an ultrasonic humidifier (Table 5).

(3) Although high numbers of *M. chimaera* were measured in the liquid in the inoculated Spectrum Medical heater-coolers immediately after inoculation and distribution, and coincident with aerosol collection, the *M. chimaera* cells disappeared within 1-month from the

liquid (HTF) of one instrument and were absent in all three, 8-weeks after inoculation. Evidently, the environment in the liquid circuit of the Spectrum Medical heater-coolers leads to the extermination of viable *M. chimaera* cells in liquid suspension.

(4) *M. chimaera* cells were recovered from one biofilm sample collected from one instrument 23-days after inoculation. Biofilm samples collected 8-weeks after inoculation did not yield any *M. chimaera* cells. Although biofilm-survival would be expected of *M. chimaera* cells in an inhibitory environment, eventually all *M. chimaera* cells in biofilms disappeared, reinforcing the conclusion that the liquid circuit and the surfaces lead to the extermination of *M. chimaera*; even those in biofilms on the walls of the liquid circuit.

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