



Utilizzo del Whole Genome Sequencing per la caratterizzazione di *Mycobacterium chimaera*

10/11/2019

Arash Ghodousi, PhD

WHO Collaborating Centre in TB Laboratory Strengthening

TB Supranational Reference Laboratory

Emerging Bacterial Pathogens Unit

IRCCS San Raffaele Scientific Institute, Milan, Italy

ghodousi.arash@hsr.it



Disclosures

- no disclosures relative to this presentation



Objectives

- Discuss the global outbreak of *M. chimaera* infections following cardiac surgery
- Describe current knowledge about the heater-cooler units implicated as the cause of the outbreak
- The molecular epidemiological investigation by applying whole-genome sequencing



Background

“...a thing of immortal make, not human,
lion-fronted and snake behind, a goat in the
middle” Homer; Iliad 6 179-18

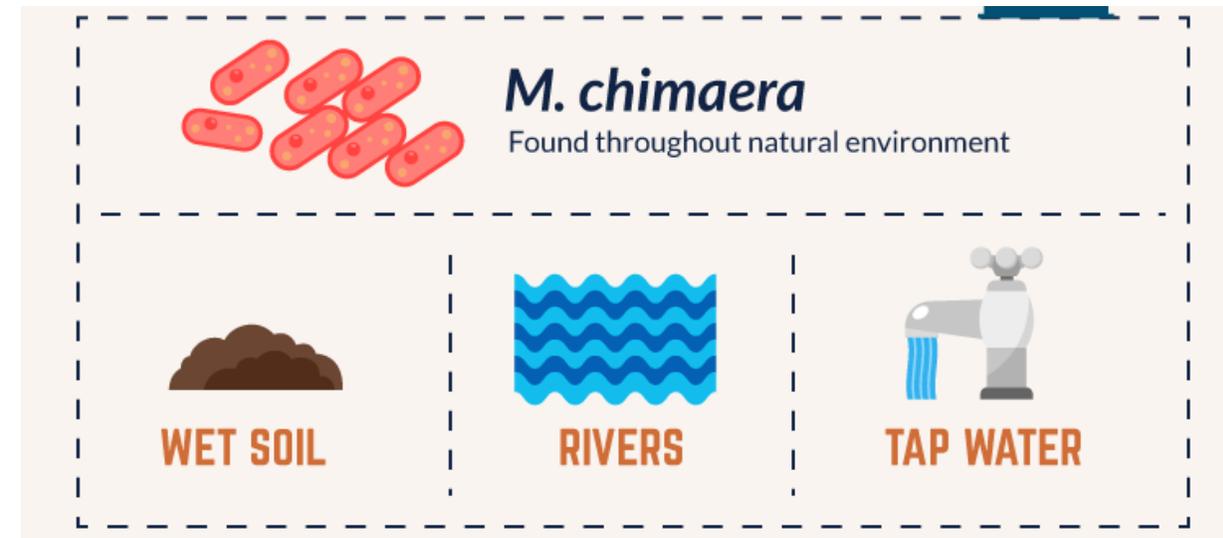
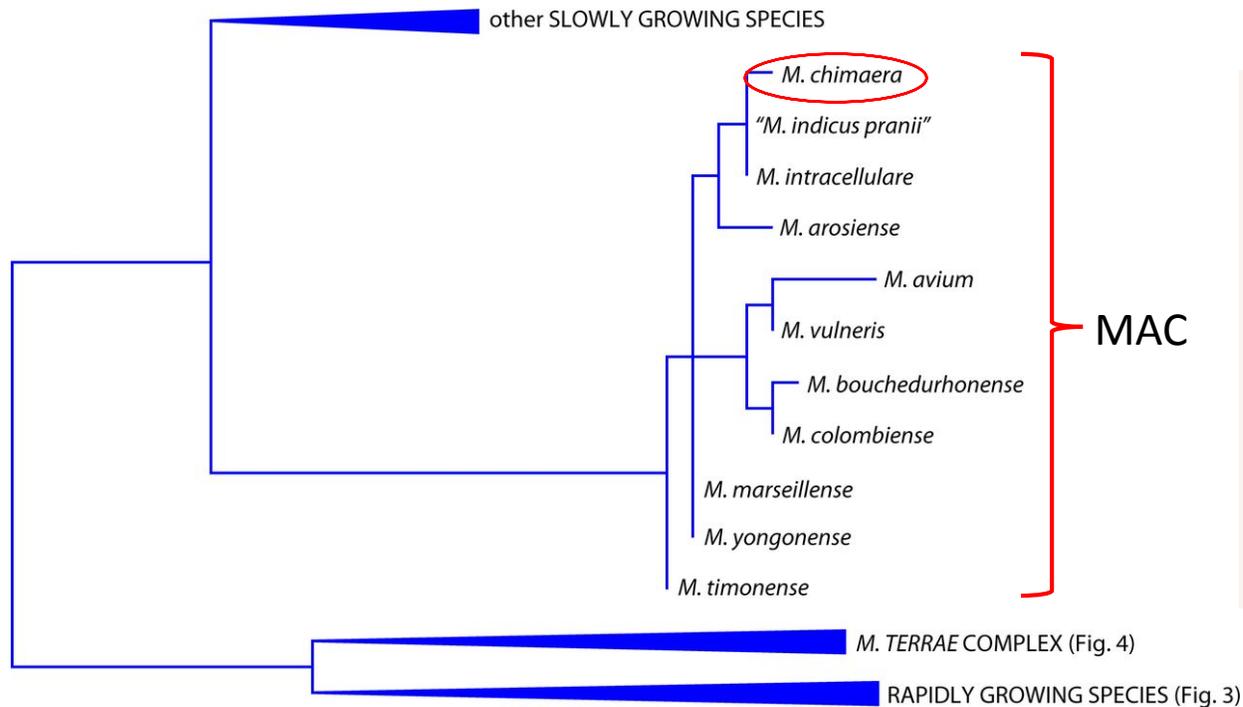




Mycobacterium chimaera

Slowly growing non-tuberculous mycobacterium (NTM)

Known as a genetic variant called “MAC-A” until Tortoli et al. described it as a separate species in 2004

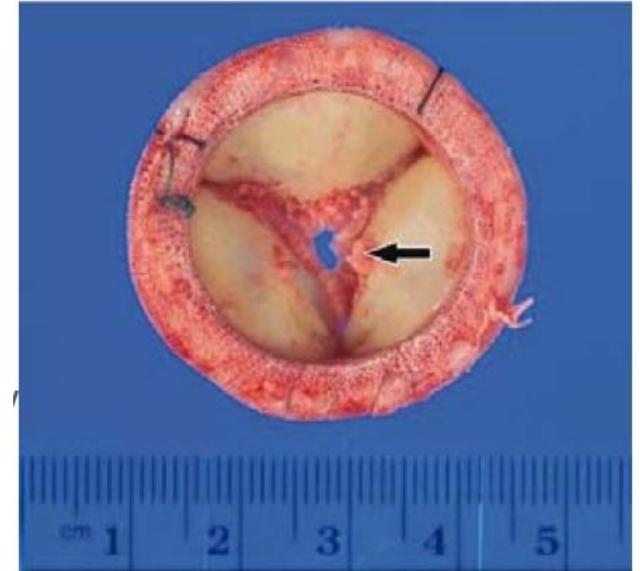


«Virulence greater than other MAC organisms»



First Report of Cases

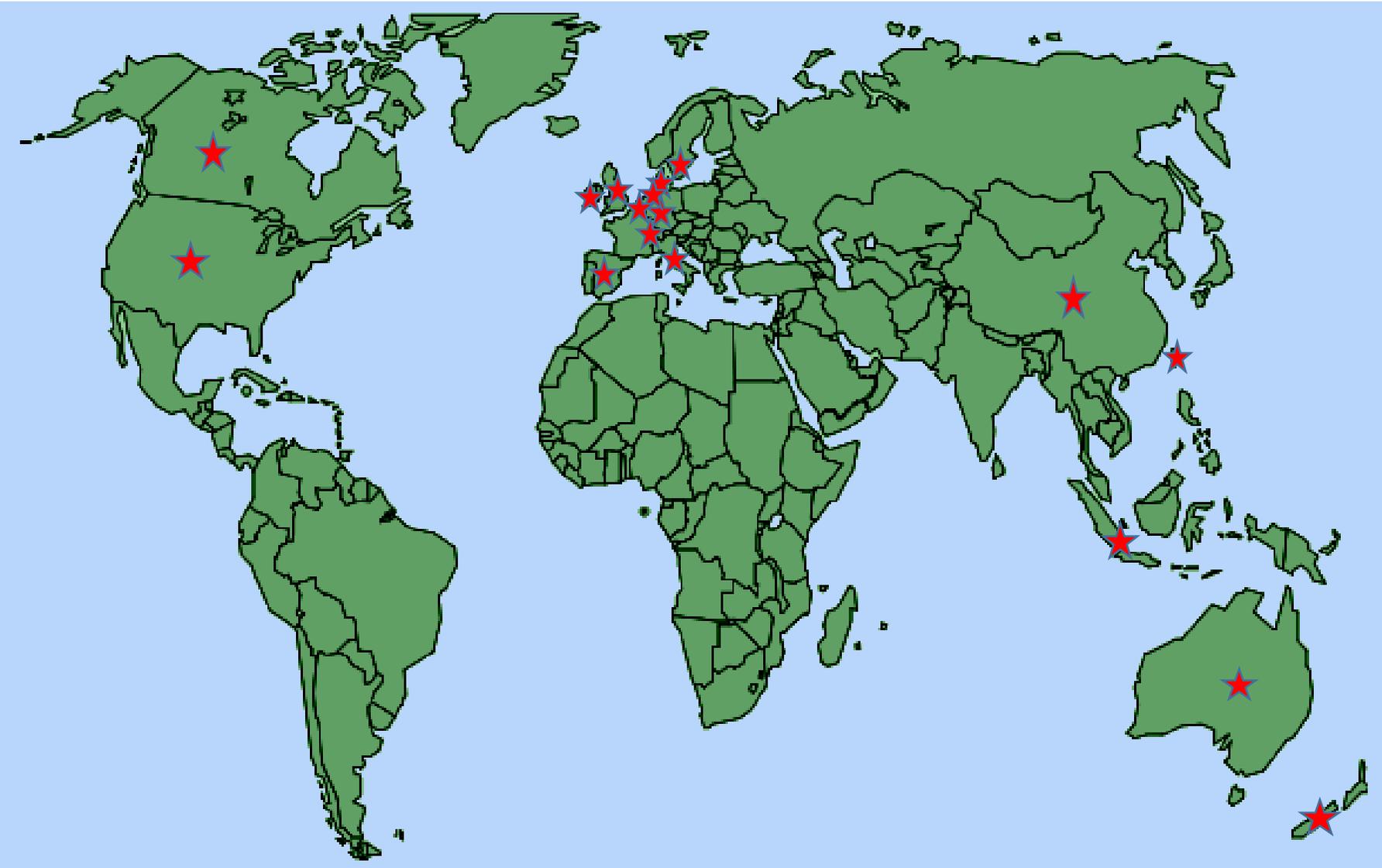
- In 2015, the infection control team at University Hospital Zurich reported that multiple patients had infections due to *M. chimaera* following open-heart surgery
- First case dated back to surgery performed in 2012; reported latency of infection was 18-43 months after surgery
- *M. chimaera* was cultured from blood, cardiac tissue and other surgical specimens for all cases
- *M. chimaera* was also cultured from heater-cooler units used for cardiac bypass during surgery and from air samples in the surgical suite



<http://creativecommons.org/licenses/by-nc/3.0/>;
Sattar A, Yu S, Koirala J - *Infect Dis Rep* (2015)



A Global Outbreak



- Reports from >20 countries in four continents
- More than 150 confirmed cases worldwide

Sources: PubMed indexed publications and Government reports until July 2019



OUTBREAK INVESTIGATION

- **Thousands** of cardiothoracic surgical procedures annually in the world raising concern that the cases detected so far are only the beginning a larger problem
- *M. chimaera* Infections are **extremely difficult to treat** and patients with such infections have a mortality rate of **approximately 50%**¹⁻³



Creative Commons: <https://pixabay.com/p-471549/>

1. Kohler P, 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
2. Marra AR, et al. *Mycobacterium chimaera* infections associated with contaminated heater-cooler devices for cardiac surgery: Outbreak Management. *Clin Infect Dis*. 2017;64:335–42
3. Chand M, et al. Insidious Risk of Severe *Mycobacterium chimaera* Infection in Cardiac Surgery Patients. *Clin Infect Dis*. 2017;64:335–42

Heater-Cooler Unit (HCU)

During HCU operation, the organism has been demonstrated to aerosolize via the exhaust of contaminated devices, resulting in airborne inoculation of the surgical site

No direct contact with the patient or with patient blood

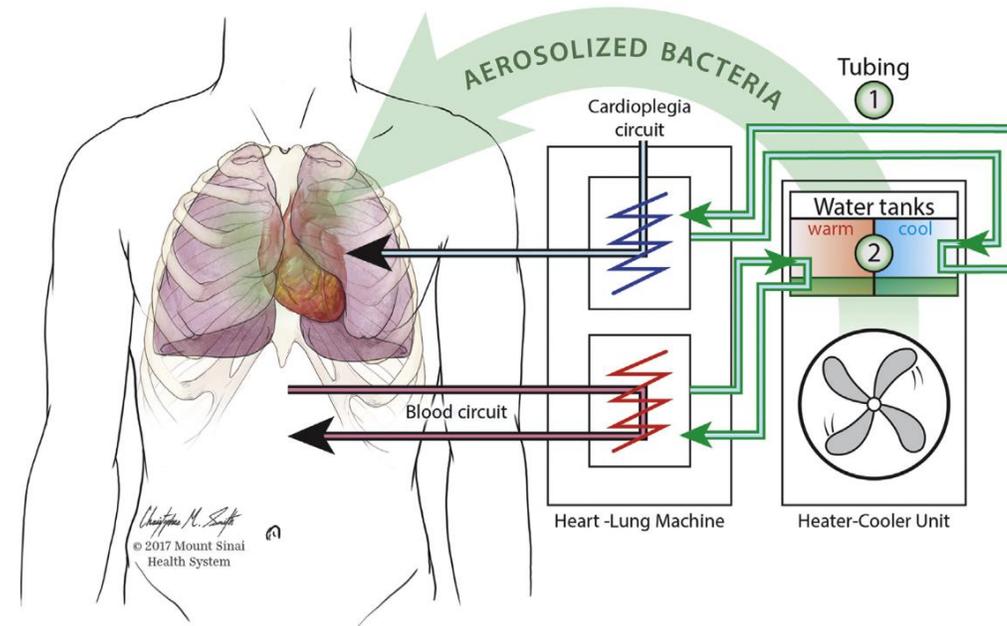
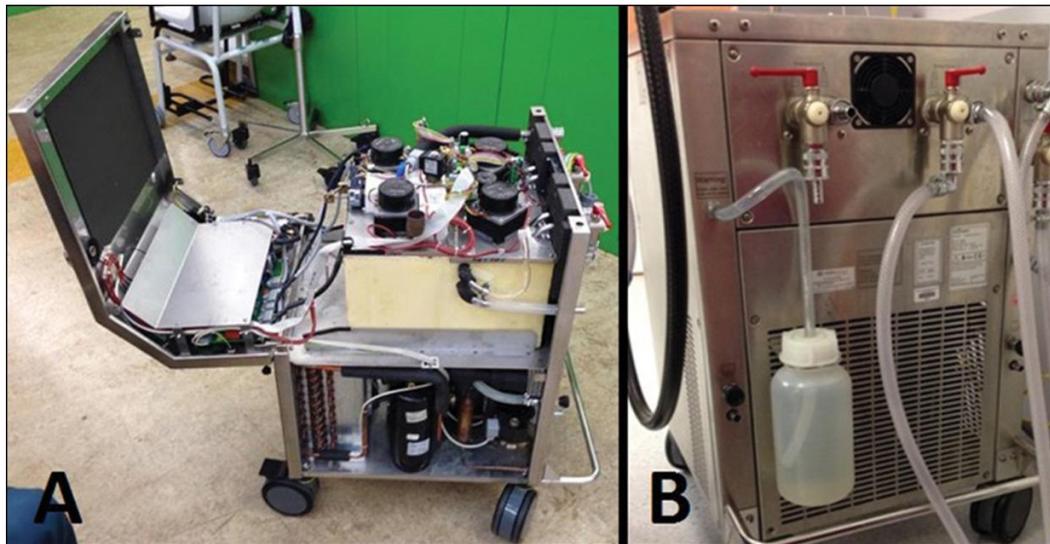


Fig 1. Heater-cooler devices used during cardiac surgery aerosolize *Mycobacterium chimaera*, subsequently subjecting the operating field to possible infection. Main sites of heater-cooler device contamination include tubing (1) and water tanks (2).



OUTBREAK RESPONSE

The two immediate priorities were:

1) Raising awareness to improve case finding

Challenges :

- a) Symptom onset is delayed from time of surgery
- b) There is no screening test to assess asymptomatic patients for exposure or infection

2) Mitigating the exposure risk by advising hospitals regarding disinfection and use of their HCUs

Challenges :

- a) LivaNova 3T has the largest share of the HCU market (**60% to 80% worldwide**)
- b) Impossible to recall all 3T HCUs without disrupting the availability of lifesaving cardiac surgery
- c) ***M. chimaera* is virtually ineradicable from HCUs once it has colonized the water circuit:**
 - The lipid-rich cell wall of organisms within MAC complex
 - high concentration of such organisms within biofilms render them highly resistant to standard disinfectants



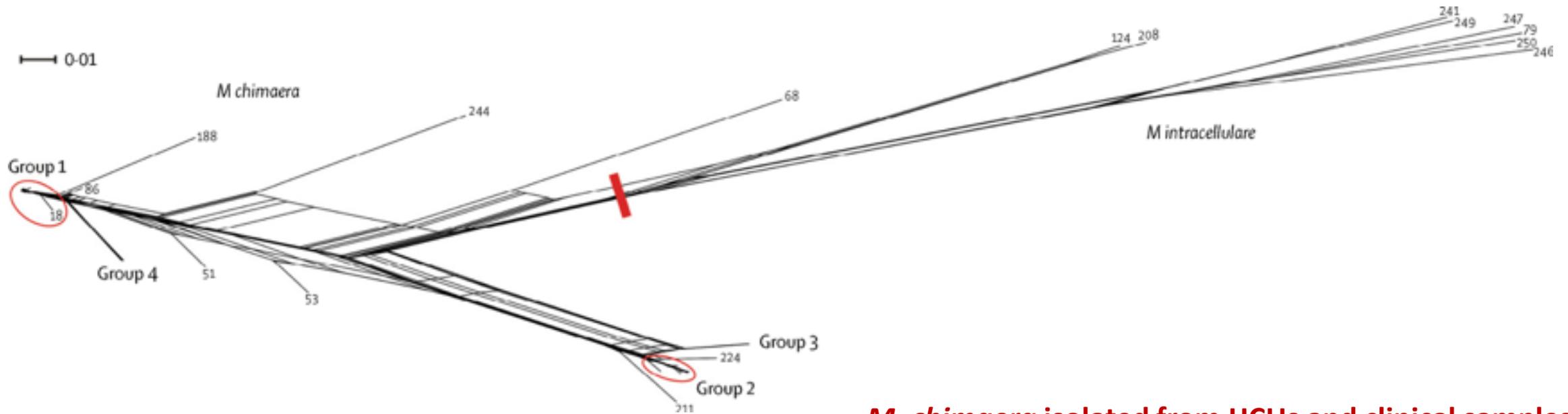
Situation in Italy

- More than 40,000 patients undergo cardiac surgery every year in Italy
- A national survey endorsed by the Italian Society of Cardiac Surgery (ISCS) in 2017 found only three patients affected by *M. chimaera* infection ¹
- By 2019 the total number of published cases of *M. chimaera* infections after cardiac surgery in Italy increased to 12 cases²
- According to ISCS the estimated national risk of *M. chimaera* infections following cardiac surgery in Italy in 2017: 0.3 in 10,000 operations

1. Cappabianca G , 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* 2018
2. Inojosa WO, et al. *Mycobacterium chimaera* infections following cardiac surgery in Treviso Hospital, Italy, from 2016 to 2019: Cases report. *World J Clin Cases*. 2019



M. chimaera Genotyping

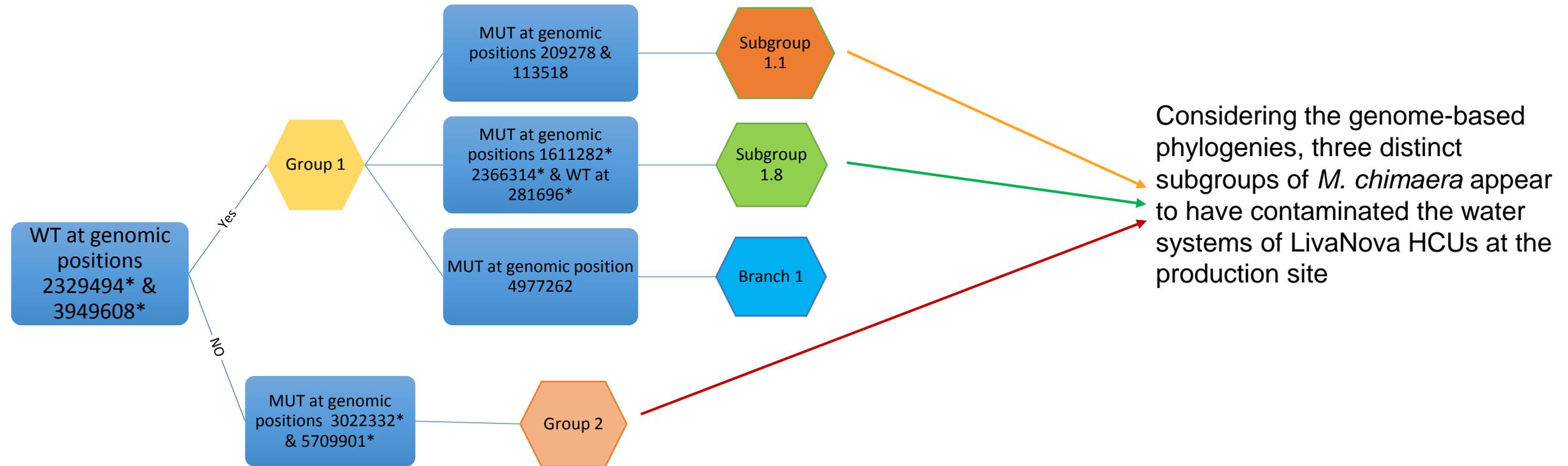


***M. chimaera* isolated from HCUs and clinical samples are mainly from Group 1 and Group 2**

Neighbor Net splits-tree to show different *M. chimaera* groups



WGS based Genotyping of *M. chimaera*

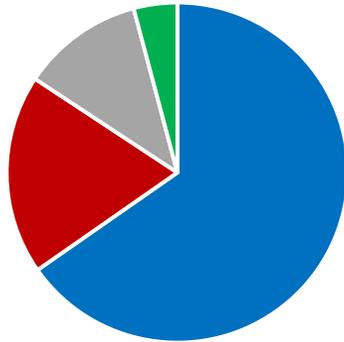


- **Most *M. chimaera* isolates from air samples taken near operating LivaNova HCUs and related patients belong to subgroup 1.1**



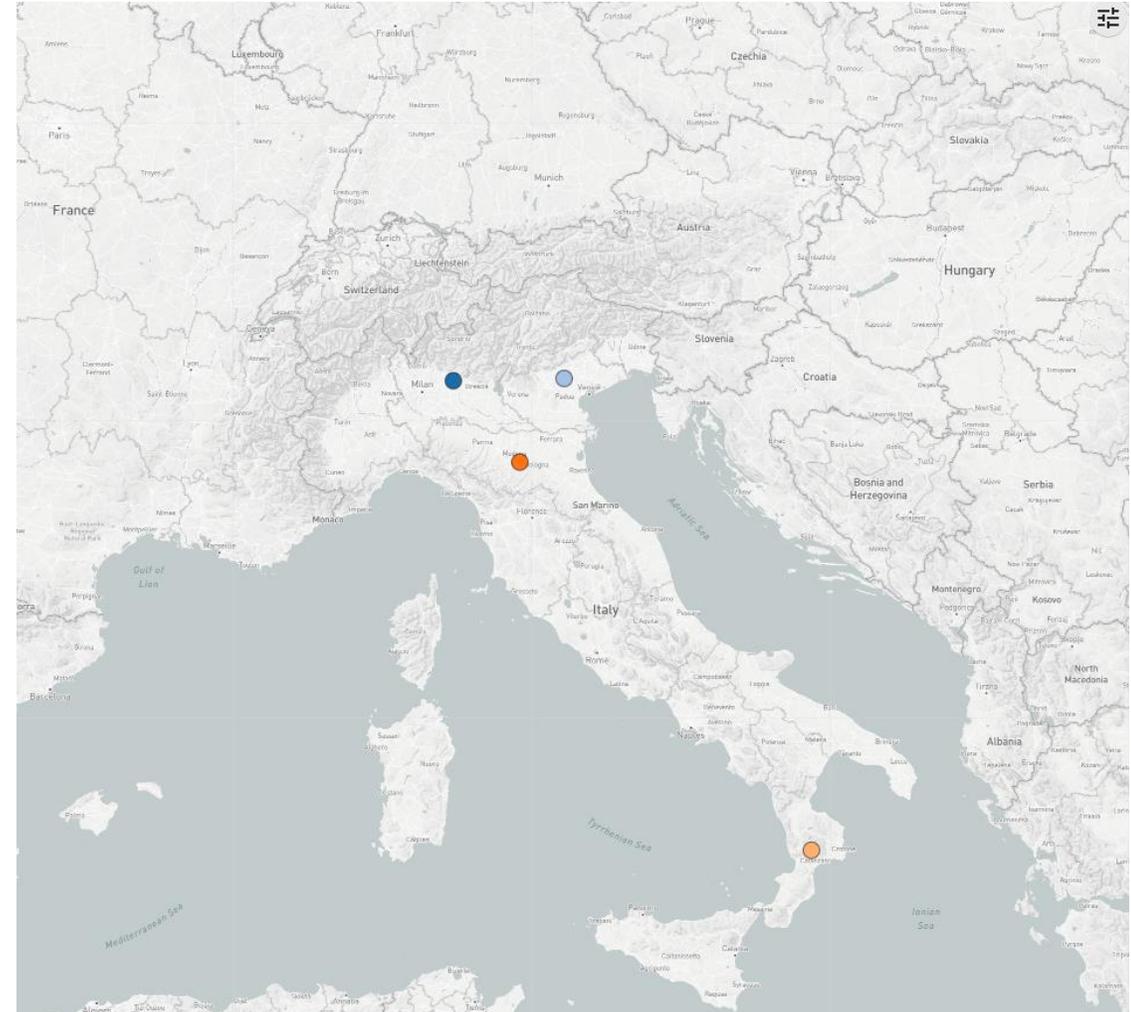
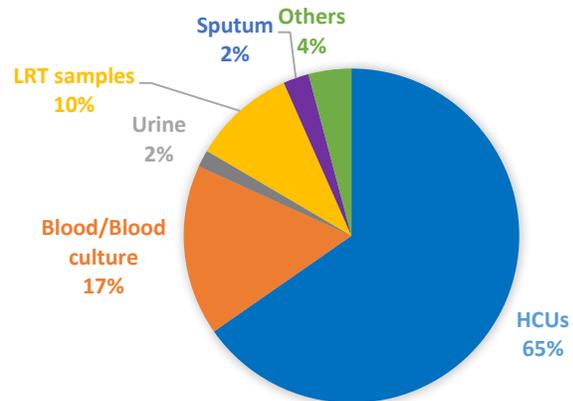
Genomic analysis of *M. chimaera* at OSR Milano

121 *M. chimaera* isolates from four Regions in Italy



- HCU
- Related patients
- Unrelated patients
- Patients with missed data

Source of Strains





WGS Workflow to analyze *M. chimaera* genome at OSR Milano

Reference Genome:
M. chimaera DSM-44623 type strain
M. chimaera strains ZUERICH-1

➤ Alignment tool:

Burrows-Wheeler Aligner (BWA)

➤ Mappings refined:

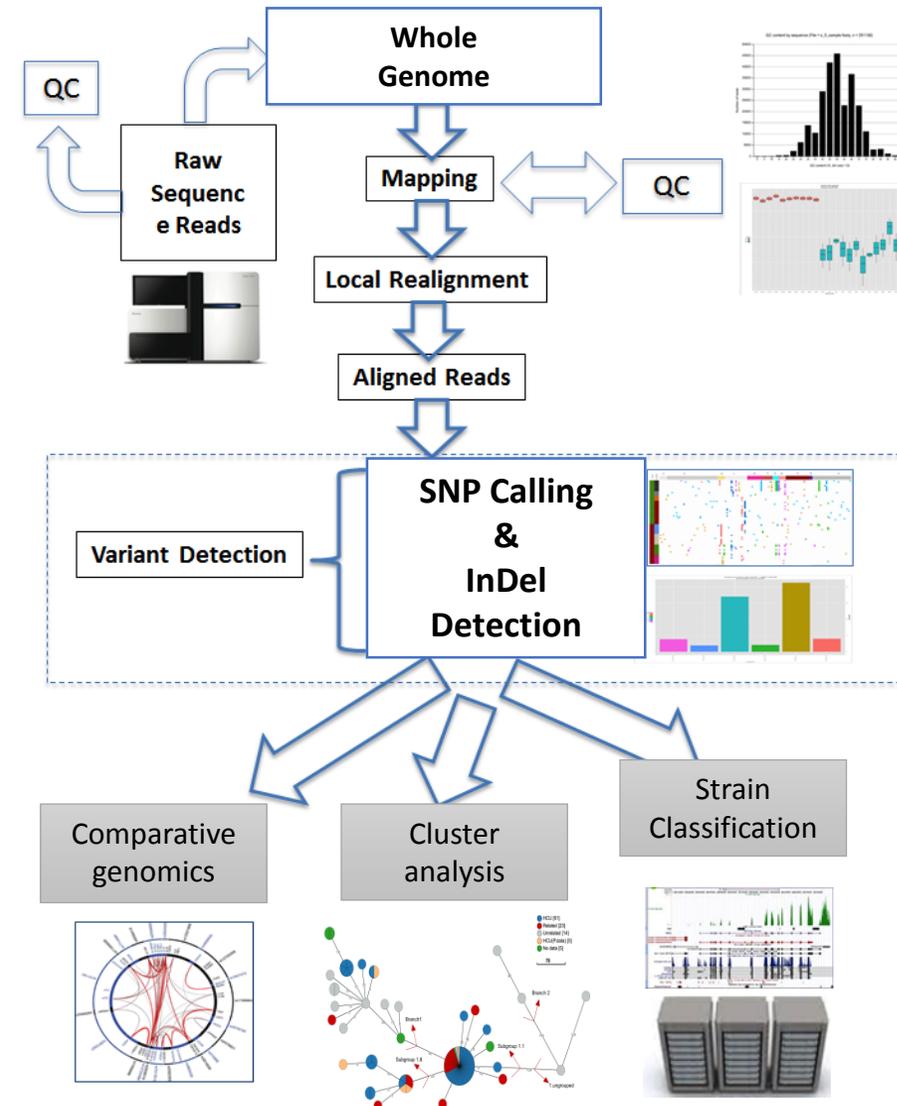
GATK and Samtools

➤ Variant detection:

Samtools

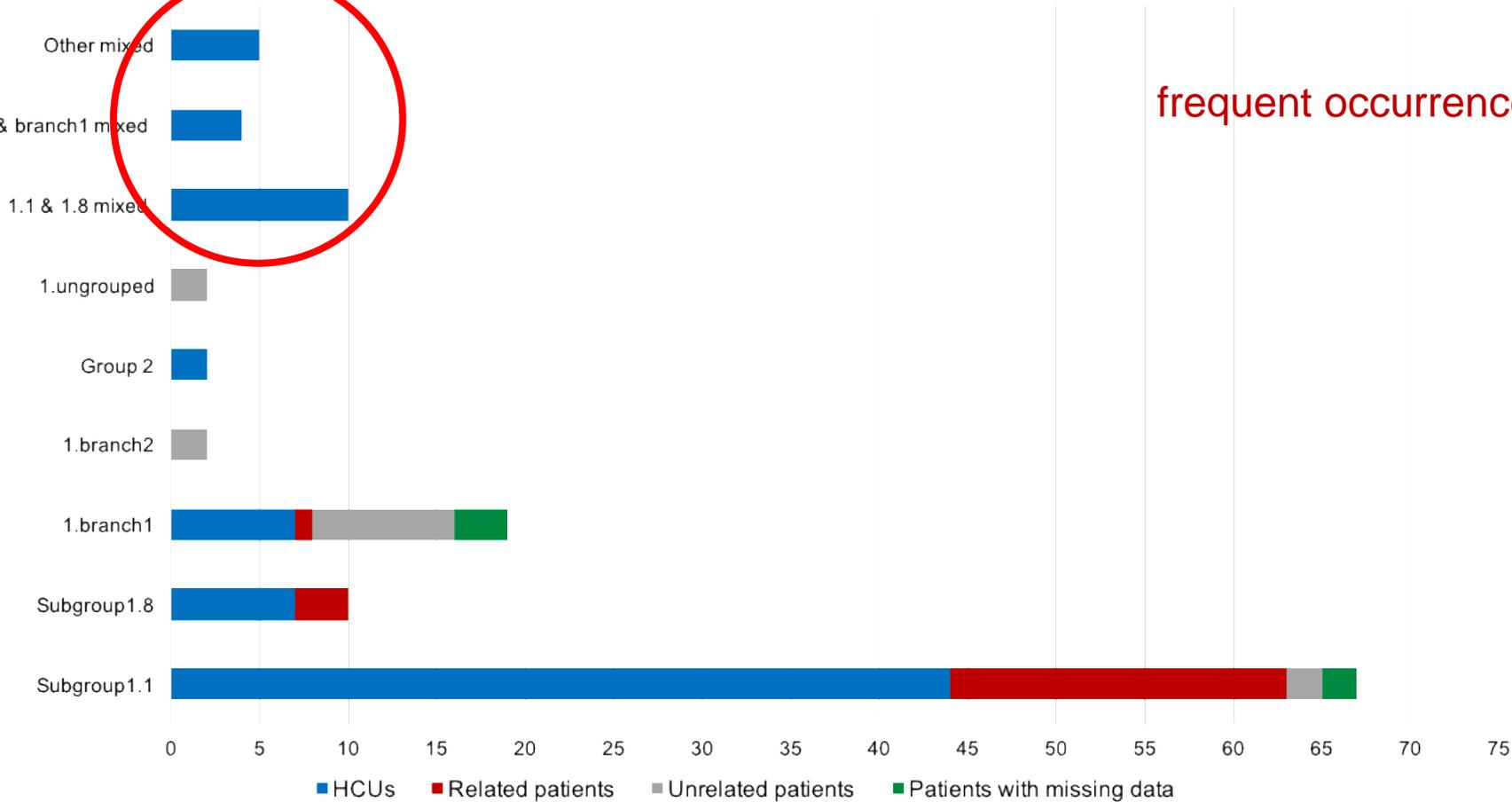
➤ To calculate maximum likelihood phylogenetic tree:

RAxML Version 8





Distribution of different groups/sub-groups among *M. chimaera* isolates in this study



frequent occurrence of mixed cultures from HCUs

Based on WGS phylogenies, three distinct strains of *M. chimaera* appear to have contaminated the HCUs at the production site, belonging to subgroups 1.1, 1.8, and 2.1

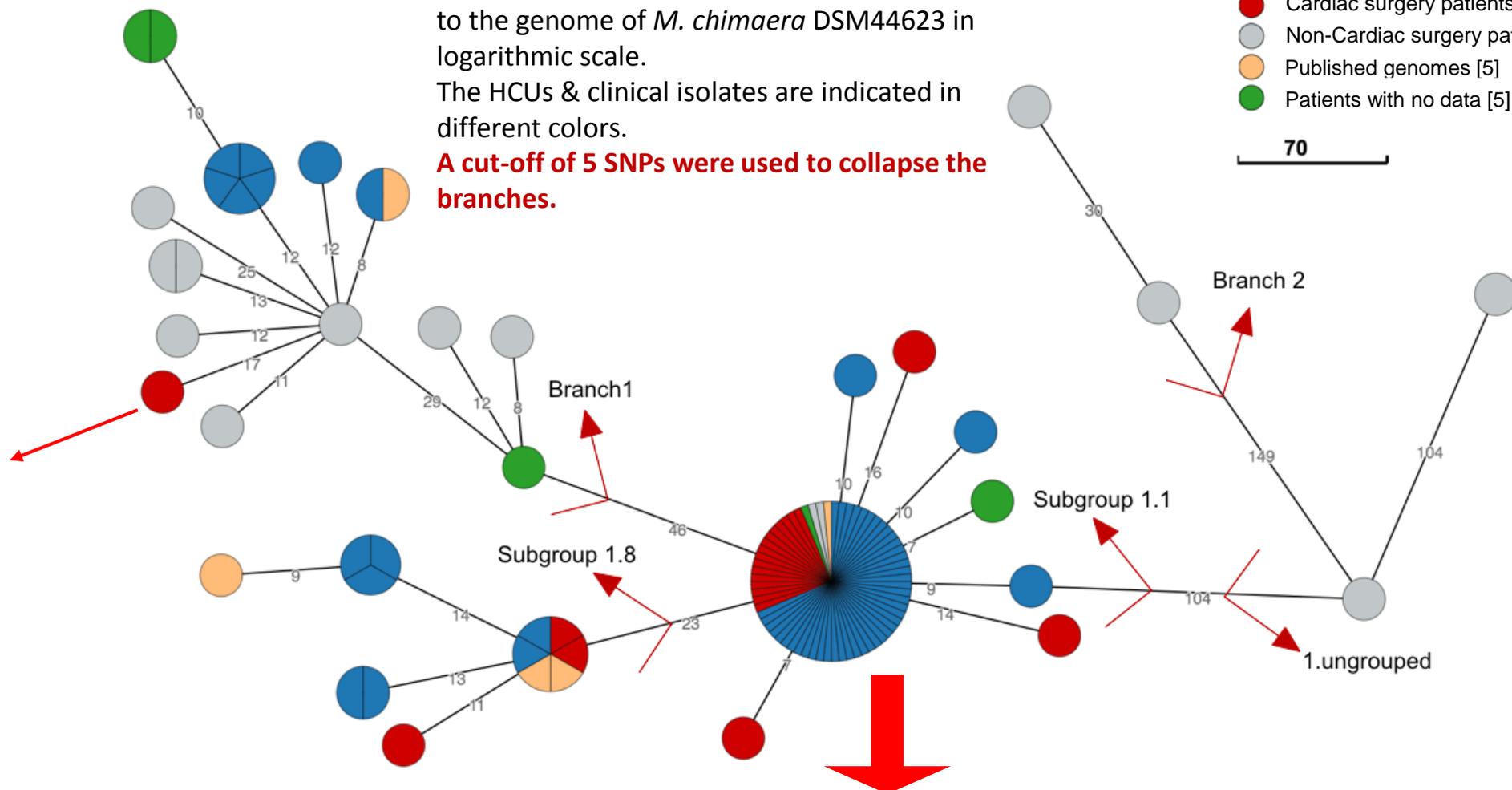
Maximum parsimony tree built from 348 SNP positions of the 108 group1 isolates mapped to the genome of *M. chimaera* DSM44623 in logarithmic scale.

The HCUs & clinical isolates are indicated in different colors.

A cut-off of 5 SNPs were used to collapse the branches.

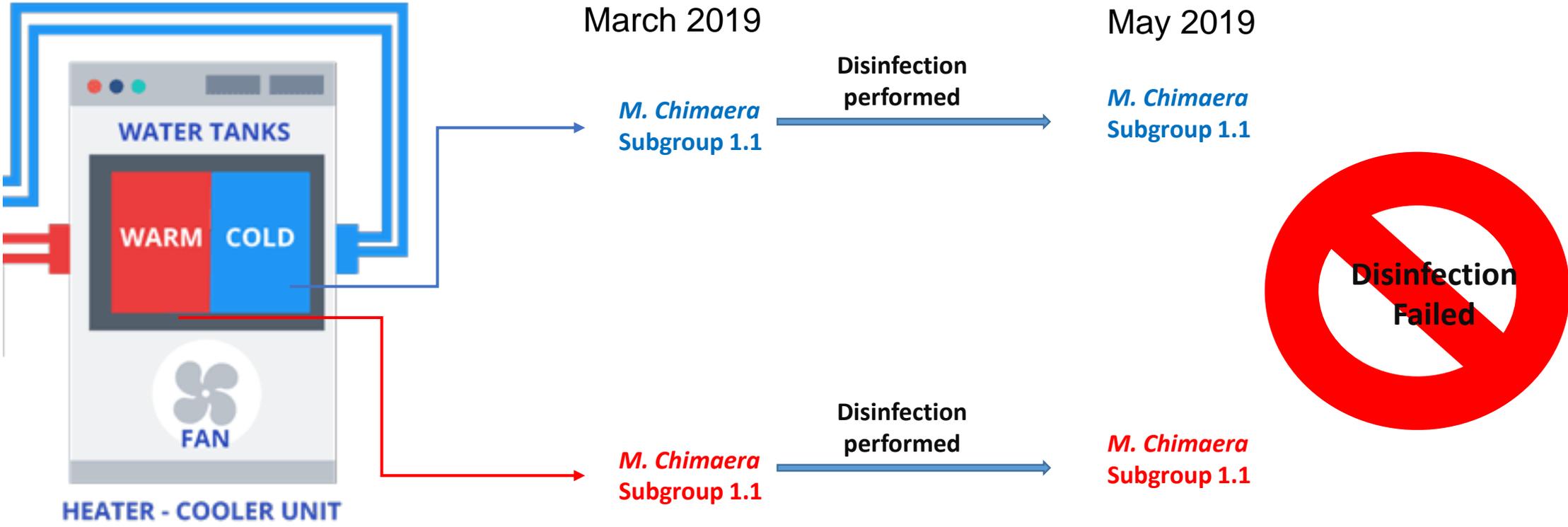
- HCU isolates[61]
- Cardiac surgery patients [23]
- Non-Cardiac surgery patients [14]
- Published genomes [5]
- Patients with no data [5]

70



In this case we hypothesize that HCU, also yielding an isolate from the same subgroup, became contaminated with *M. chimaera* at the hospital level and that this was responsible for the infection

Median pairwise distance of **only 4 SNPs (range 0-20)** within subgroup 1.1₁₇

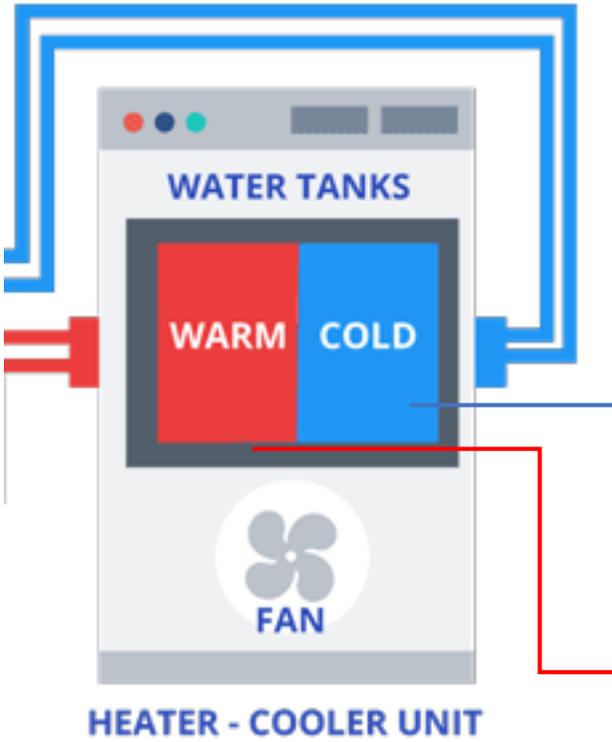


SNP difference between isolates was < 5
The same Strain



Pre-Disinfection

Post-Disinfection



Positive for *M. Chimaera* { ~ 60% subgroup 1.1
~ 20% subgroup 1.8
~15% Branch 1

Positive for *M. Chimaera* { ~60% subgroup 1.1
~ 40% subgroup 1.8

Disinfection performed

Positive for *M. Chimaera* { ~ 15% subgroup 1.1
~ 85% subgroup 1.8

Disinfection performed

Positive for *M. Chimaera* { ~90% subgroup 1.1
~ 10% subgroup 1.8



High resolution of WGS to detect more than one *M. chimaera* strains



LESSONS LEARNED

- The infection risk from a medical device can go unrecognized for long periods
- HCUs have been used during cardiopulmonary bypass **for decades**:
- ✓ The risk of bioaerosol formation has not been widely recognized until this outbreak
- **It's not known** how many **sporadic cases** of prosthetic valve endocarditis, or other infections may have resulted from HCU bioaerosol exposure
- Need for improved review of the safety of medical devices both by regulatory agencies & by individual hospital infection prevention programs.
- ✓ e.g., any device that includes a water source and a fan should clearly undergo careful review, or perhaps not be allowed in critical areas such as ORs.



FUTURE DIRECTIONS

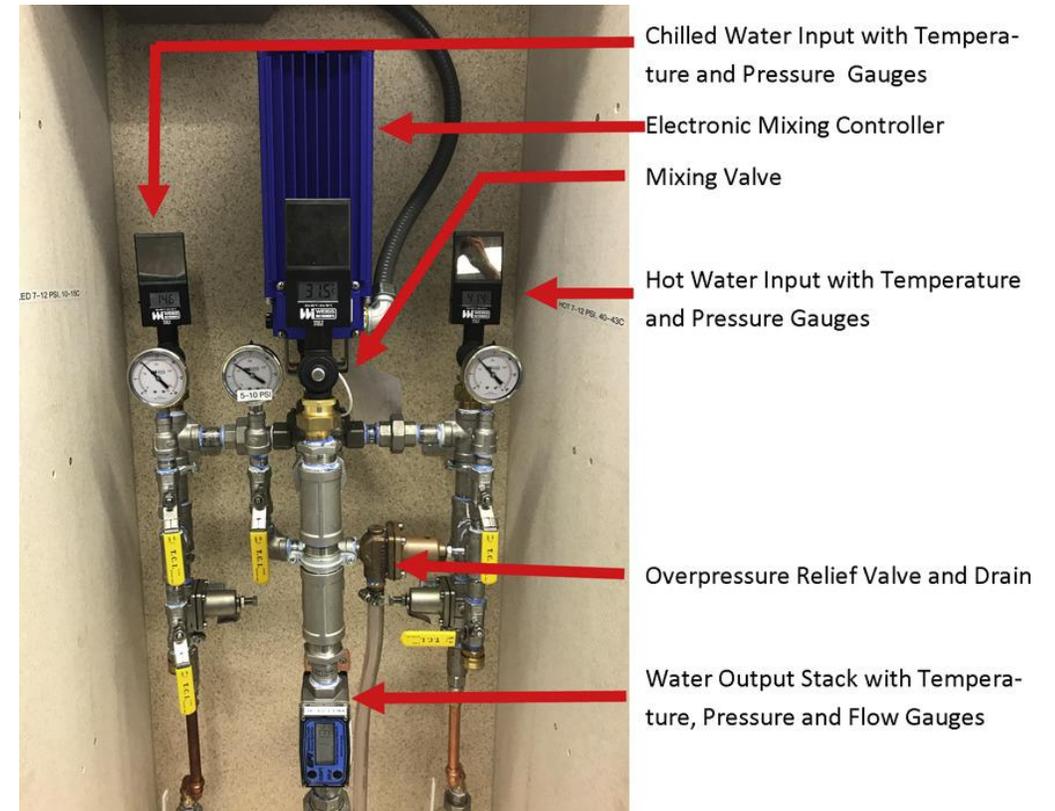
- HCUs must be designed in such a way as not to be able to produce bioaerosols



Tubing
Modular plate
Tubing connectors

1.Walker J, et al. J of Hospit Infect. 2017

- Other options for heating and cooling during bypass that do not require HCUs at all as recently described by Matte et al²



1.Matte GS, et al. J Thorac Cardiovasc Surg. 2018



Conclusion

- The power of advanced molecular diagnostics in outbreak recognition, Investigation & response
- Whole-genomic sequencing allows for sequences to be quickly compared across the globe to facilitate the investigation of transmission pathways and potential point sources, as occurred in this outbreak
- Samples obtained from HCUs can be mixes of two to three *M. chimaera* strains, which only one was found in our patient samples
- The isolates from Cardiac surgery patients are mostly from subgroup 1.1 & 1.8
- The isolates from NON- Cardiac surgery patients were heterogeneous in compare to Cardiac surgery patients and were distributed across the phylogenetic tree



Thank you for your attention

Acknowledgements

Riccardo Manganeli
Marta Peracchi
Giorgio Palù
Loredana Fallico
Mario Rassa
Vinicio Manfrin
Vincenzina Monzillo
Salvatore Nisticò



Special thanks:

Daniela Maria Cirillo
Enrico Tortoli
Emanuele Borroni
Virginia Quaresima
Matteo Moro
Nicola Colangelo



Sistema Socio Sanitario



UNIVERSITÀ
DEGLI STUDI
DI PADOVA