Global outbreak of severe Mycobacterium chimaera disease after cardiac surgery: a molecular epidemiological study


Summary

Background Since 2013, over 100 cases of Mycobacterium chimaera prosthetic valve endocarditis and disseminated disease were notified in Europe and the USA, linked to contaminated heater–cooler units (HCUs) used during cardiac surgery. We did a molecular epidemiological investigation to establish the source of these patients’ disease.

Methods We included 24 M chimaera isolates from 21 cardiac surgery-related patients in Switzerland, Germany, the Netherlands, and the UK, 218 M chimaera isolates from various types of HCUs in hospitals, from LivaNova (formerly Sorin; London, UK) and Maquet (Rastatt, Germany) brand HCU production sites, and unrelated environmental sources and patients, as well as eight Mycobacterium intracellulare isolates. Isolates were analysed by next-generation whole-genome sequencing using Illumina and Pacific Biosciences technologies, and compared with published M chimaera genomes.

Findings Phylogenetic analysis based on whole-genome sequencing of 250 isolates revealed two major M chimaera groups. Cardiac surgery-related patient isolates were all classified into group 1, in which all, except one, formed a distinct subgroup. This subgroup also comprised isolates from 11 cardiac surgery-related patients reported from the USA, most isolates from LivaNova HCUs, and one from their production site. Isolates from other HCUs and unrelated patients were more widely distributed in the phylogenetic tree.

Interpretation HCU contamination with M chimaera at the LivaNova factory seems a likely source for cardiothoracic surgery-related severe M chimaera infections diagnosed in Switzerland, Germany, the Netherlands, the UK, the USA, and Australia. Protective measures and heightened clinician awareness are essential to guarantee patient safety.

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Introduction Since 2013, over 100 severe cases of Mycobacterium chimaera infections, often fatal, have been notified in four European countries (Switzerland, Germany, the Netherlands, and the UK), the USA, and Australia, all among patients who had undergone cardiothoracic surgery.7 Initial epidemiological investigations suggested a link to the use of specific heater–cooler units (HCUs) that are used to control temperature within the extracorporeal circulation during cardiac surgery.7,8 The possibility of a global outbreak caused by HCUs sparked investigations by the European Centre for Disease Prevention and Control (ECDC) and the US Centers for Disease Control and Prevention (CDC).

Non-tuberculous mycobacteria, particularly M chimaera, are ubiquitous in the environment, are opportunistic human pathogens, and are intrinsically resistant to most classes of antibiotics and disinfectants. Partly as a result of biofilm formation and hydrophobicity favouring aerosolisation, non-tuberculous mycobacteria cause outbreaks of disease disseminated by contaminated medical devices.9

HCU water reservoirs provide favourable environmental conditions for growth of microorganisms such as non-tuberculous mycobacteria and have been considered potential sources of infection. As HCU water systems are not airtight and the cooling fans produce a far-reaching airflow, they potentially expose patients to aerosols containing non-tuberculous mycobacteria, during cardiac surgery.10

To explore the possibility of a multi-country outbreak of severe M chimaera disease, we did a molecular epidemiological investigation by applying whole-genome sequencing, the most powerful tool for tracing pathogen transmission,11 on clinical and environmental M chimaera isolates from the four European countries involved.

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Research in context

Evidence before this study
To identify publications on infections with non-tuberculous mycobacteria associated with extracorporeal assist devices including heater-cooler units (HCUs), we searched the PubMed database without language filters until Dec 15, 2016, with

("non-tuberculous mycobacteri*" OR Mycobacterium Infections[MeSH] OR "chimaera") AND (“heater-cooler” OR “thermoregulatory” OR “heart surgery” OR “card* surgery” OR “prosthesis” OR “extracor*” “implant surgery”, OR “cardiac surgery”, OR “heart valve surgery” OR “cardiothoracic surgery”).

16 peer-reviewed publications featured environmental HCU microbiological investigations (n=6) or patient case reports or series (n=5), or both (n=5). All except an earlier three were published in 2016 (ie, after the start of the current investigation). After the first report in 2013 of cases of invasive Mycobacterium chimaera infections with an identical strain after cardiac implant surgery, a paper by Sax and colleagues in 2015 established the likely transmission from contaminated HCUs to the surgical field via aerosolisation in the operating room based on RAPD-PCR typing. This pathway has since been also suggested by other investigators using culture, smoke, and particle counter experiments. Overall, we found 49 published patient cases from Switzerland (n=6), the EU (n=27), and the USA (n=16) with a maximum latency of 6 years and a mortality of about 50%, presenting with two exceptions as prosthetic valve endocarditis, vascular graft, or disseminated disease.

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Methods

Study design and strain selection
The M chimaera isolates included in this analysis were collected according to evolving strategies of the primary outbreak investigations in Switzerland, Germany, the Netherlands, the UK, and HCU production sites in Germany (appendix, p 8).

We included isolates from all patients with M chimaera disease associated with cardiothoracic surgery with extracorporeal circulation identified at the initiation of this international collaborative investigation on July 1, 2015 (related patients). For comparison, we added a convenience sample of isolates from patients with pulmonary or disseminated M chimaera diseases from all
four countries, for whom sufficient clinical data were available to exclude that they had ever undergone open-heart surgery (unrelated patients).

We included environmental isolates from water and associated air cultures from LivaNova model 3T (formerly Sorin; London, UK) and Maquet (Rastatt, Germany) HCU s, from other water-containing medical devices, from tap water, and from drinking water dispensers in participating hospitals. The LivaNova and Maquet companies contributed *M* chimaera isolates from their HCU production sites. Isolates from serial and simultaneous sampling of air and water of a single HCU were included where available, to test for multiple strain contamination and persistence over time. For further comparison, we included the *M* chimaera type strain DSM 44623, four *Mycobacterium intracellulare* clinical isolates, and the genomes of four additional *M* intracellulare strains (1956, ATCC13950, MOTT-02, MOTT-64; accession numbers in appendix) since this species is most closely related to *M* chimaera. 

All isolates from clinical specimens and environmental samples (HCU water, air, and tap water from all participating countries) were cultured and identified using methodologies now promoted by ECDC.12,13 In accordance with legislation in the four participating countries, ethical approval was not required for this investigation as detailed in the appendix (p 4).

**Procedures**

Whole-genome sequencing was done on the Illumina NextSeq 500 and Illumina HiSeq (for the isolates from the UK) next-generation sequencing platforms as instructed by the manufacturer (Illumina, San Diego, CA, USA). Reads were submitted to the EMBL ENA sequence read archive (accession numbers in appendix). We used PacBio long-read sequencing on an RSII instrument (Pacific Biosciences, Menlo Park, CA, USA) to generate fully closed reference genome sequences for *M* chimaera strains ZUERICH-1 (DSM 101591), ZUERICH-2 (DSM 101592), and the DSM 44623 type strain.12 Genome sequences were submitted to the NCBI GenBank database and assigned accession numbers CP015267 to CP015280.

For genotyping and phylogenetic analysis, the illumina reads were mapped to the genome of *M* chimaera (draft genome, National Center for Biotechnology Information [NCBI], GenBank coordinates CP015267 to CP015280).

**Figure 1:** Alignment of chromosome and plasmid sequences from *Mycobacterium chimaera* strains ZUERICH-1, ZUERICH-2, DSM 44623, and MC045. The figure was generated using BRIG software. (A) The similarity between a central reference chromosome (ZUERICH-1) against query sequences as indicated, and (B) the similarity between reference plasmids (ZUERICH-1, plasmids 1–5) and plasmids from strains ZUERICH-2, DSM 44623, and MC045, respectively. Plasmid 2 (154 kbp) from strain ZUERICH-2 and plasmid 2 (324 kbp) from strain MC045 displayed no homology to any of the plasmids from ZUERICH-1 (not shown). There is no plasmid 1 in MC045, according to NCBI annotation.
Articles

**M chimaera** DSM 44623, ZUERICH-1, or ZUERICH-2 (full genome sequences determined in this study) using the SARUMAN aligner. All datasets reached a mean genomic coverage depth of at least 30 fold. The combined set of detected single nucleotide polymorphisms (SNPs) positions was used to reconstruct phylogenetic trees. In parallel, we grouped isolates based on a maximum distance to the nearest group member of 1000 SNPs for group or ten SNPs for subgroup attribution. To analyse mixed populations, we extracted a set of signature SNPs characteristic for groups, subgroups, or branches of the constructed phylogenetic trees. For each isolate, we calculated the mean allele frequency for each group-specific set of SNP alleles, setting a threshold of at least 5% for detection of a strain from that group.

A detailed description of the whole-genome sequencing workflow and phylogenetic analysis methods is presented in the appendix (p 1).

We compared our genomic data with publicly available datasets from Ireland, the USA, Denmark, and Australia, by incorporating the respective datasets in our analysis pipeline; the US and Australian sets included isolates from cardiac surgery-related patients. For the **M chimaera** DSM 44623 type strain, we obtained a fully closed sequence of 5,865,644 base pairs (bp; figure 1). Using this genome as a reference enabled a detailed phylogenetic analysis of the study collection. All isolates classified as **M chimaera** were affiliated to a distinct branch of the resulting phylogenetic tree (figure 2). All but eight **M chimaera** isolates were grouped into four groups both in the tree and by the distance-based grouping with 1000 distinct SNP positions as the threshold. Of these, groups 1 and 2 contained most of the isolates investigated (200 isolates in group 1 and 29 isolates in group 2; figure 2). All **M chimaera** isolates of the 21 related patients were classified into group 1, whereas unrelated patients were distributed across the tree (figure 3, appendix p 8).

To interrogate the two major **M chimaera** groups at the highest resolution, we obtained the full genome sequences of isolates ZUERICH-1 (related patient isolate) and ZUERICH-2 (environmental isolate), representatives of the major groups 1 and 2, for mapping of Illumina short-read data. The resulting phylogeny provided a high-resolution picture of the 200 group i isolates; cluster analysis using the threshold of ten SNPs as maximum distance between group members identified 11 distinct subgroups (figure 3, appendix p 27).

Of these, subgroup 1.1 contained all isolates of related patients apart from one patient (patient RP4; appendix p 8) who was part of subgroup 1.5 that also contained **M chimaera** from LivaNova HCU air cultures from the same hospital (figure 3, appendix p 28).

**Role of the funding source**

The funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author (HS) had full access to all the data in the study and had final responsibility for the decision to submit for publication.

**Results**

We included 250 whole-genome sequencing datasets in our study collection: 24 isolates from 21 related patients, 36 from 35 unrelated patients, 126 from LivaNova HCUs in use (85 water cultures, 41 air cultures), 13 from LivaNova HCUs returned to the production site in Germany for disinfection, four from the LivaNova production site (three from newly produced HCUs, one from a water source), two from Maquet extracorporeal membrane oxygenation (ECMO) devices in use, 14 from Maquet HCUs in use, 15 from new Maquet HCUs sampled at the production site, seven from hospital water supplies in Switzerland, Germany, and the Netherlands, the **M chimaera** DSM 44623 type strain, and eight **M intracellulare** strains (four unrelated patients from Germany and four published genomes; appendix).

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Likewise, we used the genome of strain ZUERICH-2 as a reference to resolve the phylogeny of the 29 isolates of group 2, consisting of environmental and unrelated patient isolates, in more detail (figure 4). All but three isolates of group 2 formed a tight subgroup clearly distinct from isolates of two unrelated patients.

Within group 1, subgroups 1.1 and 1.8 contained most isolates from water systems of LivaNova HCUs in clinical use, isolates from HCUs sampled at the LivaNova production site, and isolates from HCU exhaust air sampled in operating rooms in hospitals in the four participating countries (figures 3 and 5, appendix p 28). Strains from HCUs sampled at the Maquet production site formed a separate subgroup (1.11) and were different from M. chimaera strains found in Maquet HCUs in active clinical use (figures 3 and 5). Overall, the isolates within group 1 showed comparatively little diversity, with a median pairwise distance of 21 SNPs (range 0–232), and for subgroup 1.1 a median pairwise distance of only four SNPs (0–20).

Figure 3: Maximum likelihood tree built from 860 SNP positions of the 200 group 1 isolates mapped to the genome of Mycobacterium chimaera ZUERICH-1 shown as a circular phylogram. The maximum SNP distance-based classification, as well as isolate source, country of origin, year of isolation, and major groups or branches are indicated by subsequent coloured circles. Circles on nodes indicate resampling support of at least 90% (green circles) or at least 70% (black circles). Each leaf of the tree is labelled by sample ID and coloured according to its subgroup, with ungrouped samples in white. ECMO=extracorporeal membrane oxygenation. HCU=heater-cooler unit. SNP=single nucleotide polymorphism.
To screen for mixed bacterial populations, we determined specific SNP signatures for groups 1 and 2, for subgroups 1.1, 1.6, 1.8, 1.11, and 2.1, and for two branches (branch 1 and branch 2) of the phylogenetic tree of group 1 (figure 3, appendix p 9). Using the signature SNPs, we detected mixed populations in 73 whole-genome sequencing datasets (62 LivaNova HCUs, one LivaNova production site, four Maquet HCUs, three Maquet production site, two ECMO units, one tap water; no patients). The most frequent mix comprised subgroup 1.1 and subgroup 1.2 strains (n=29; 27 LivaNova HCU, one Maquet HCU, and one ECMO unit), followed by mixed populations of subgroup 1.1 and 1.8 strains (n=24; 23 LivaNova HCU and one LivaNova production site samples; appendix p 8). For 17 of 21 LivaNova HCUs repeatedly sampled over time, serial samples provided evidence of multiple strains. Most were mixed populations of subgroups 1.1 and 1.8 (n=10), or 1.1 and 2.1 (n=8), with four HCUs harbouring mixed populations of 1.1, 1.8, and 2.1. Of the two serially sampled Maquet HCUs, one contained a mixture of groups 4 and subgroup 1.6, and one a mixture of subgroups 1.6 and 1.8. All three serially sampled patients showed a single strain each (appendix p 8).

The closed full genome sequence of M. chimaera DSM 44623, ZUERICH-1, and ZUERICH-2, as well as the recently published MC045 strain from Australia,7 had similar sizes and were largely in synteny (figure 1). However, we noted several differences with potential biological consequences. The ZUERICH-1 chromosome harboured several mobile genetic elements that were either very different or lacking in ZUERICH-2; these amounted to 276 kb of chromosomal DNA and included two large genomic islands, one prophage, and several complex transposons on the bacterial chromosome. Additionally, each strain carried a unique assortment of plasmids. ZUERICH-1 carried five plasmids (14–95 kbp), which were only partly represented in ZUERICH-2, MC045, and DSM 44623 (figure 1). Strains ZUERICH-2 and MC045 each carried large plasmids (154 kbp and 324 kbp in size, respectively) that displayed no homology to any plasmids from the other strains (figure 1).

The 20 isolates from the USA (from 11 patients and five LivaNova 3T HCUs from hospitals in Pennsylvania and Iowa, USA),7 and one out of five patient isolates from Australia7 were classified into subgroup 1.1 based on group-specific SNPs (appendix pp 24, 28). The three Irish
patient isolates (isolated from respiratory cases)\textsuperscript{15} and the four other Australian patient isolates were also classified as group 1 \textit{M. chimaera}, but were not attributed to the outbreak subgroups 1.1 or 1.8 (appendix pp 24, 28), constituting non-related patients.

**Discussion**

The remarkable clonality of isolates of almost all patients with \textit{M. chimaera} disease associated with cardiac surgery strongly points to a common source of infection. Based on the high degree of similarity between these patients' isolates and \textit{M. chimaera} isolates recovered from most LivaNova HCUs in clinical use and isolates from the LivaNova production site, it is most probable that LivaNova HCUs represent the common source of infection and that contamination of most of these HCUs occurred during production at this production site. The comparison with strains from recent investigations in the USA\textsuperscript{5} and Australia\textsuperscript{7} underline the global dimension of the outbreak by adding another 12 epidemiologically linked patients infected with the outbreak strain (appendix pp 24, 28).

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**Figure 5:** Relative frequency of isolated strains (n=342; multiple entries for mixed populations) per source and whole-genome sequence group and subgroup attribution

The relative strain frequency is depicted as bubble size, showing subgroups 1.1, 1.8, 1.11, and 2.1 in detail, and 1.x (other strains in group 1), 2.x (other strains in group 2), and x (ungrouped strains). Within isolates found to contain mixed populations, each detected strain was included individually. HCU=heater–cooler unit. ECMO=extracorporeal membrane oxygenation.
Within the HCU outbreak group 1.1, the median pairwise genetic distance was only four SNPs, with 98% of all pairwise distances within 12 SNPs, a value used as a limit for detecting clonal M. tuberculosis transmission, and 100% of pairwise distances within the threshold of 25 SNPs found for clustered isolates of Mycobacterium abscessus.\textsuperscript{10,11}

Considering the genome-based phylogenies, three distinct strains of M. chimaera appear to have contaminated the water systems of LivaNova HCUs at the production site, belonging to subgroups 1.1, 1.8, and 2.1. However, most M. chimaera isolates from air samples taken near operating LivaNova HCUs and those of all but one of the related patients belong to subgroup 1.1. This finding further supports the presumed airborne transmission pathway leading to endocarditis, aortic root infection, disseminated disease, and surgical site infections in the affected patients.\textsuperscript{1,2,4,10}

In the one cardiac surgery-associated patient with a strain belonging to subgroup 1.5, we hypothesise that HCUs, also yielding a subgroup 1.5 isolate, became contaminated with M. chimaera at the hospital level and that this was responsible for the infection. The risk of hospital-level HCU contamination is underlined by the M. chimaera isolates obtained from hospital tap water, drinking water dispensers, hospital-built HCU systems, and in-use Maquet HCUs. Finding M. chimaera in production sites of two companies underlines the general risk of non-tuberculous mycobacteria contamination of medical devices during production.

The non-random distribution of isolates in the phylogenetic tree warrants further investigation of the relative capacity of M. chimaera group 1 and 2 strains to colonise HCU water reservoirs and, for subgroup 1.1 strains, to spread from the HCU and cause human disease. Group 1 strains have a very low diversity with a pairwise distance of maximally 232 SNPs. Such a high clonality of isolates related to health care obtained over 4 years and from different geographical regions is rather unexpected for environmental mycobacteria such as M. chimaera and underscores the need for use of whole-genome sequencing for accurate outbreak investigation.

During the outbreak investigation, cultures from the Maquet production site and in-use HCUs yielded M. chimaera, but without being associated with disseminated M. chimaera infections; in-hospital cross-contamination from LivaNova HCUs to in-use Maquet HCUs might have occurred through shared water tubes. Specific design features of LivaNova model 3T HCU such as the strong airflow produced by the cooling fan with aerosolisation from the water reservoir might favour mycobacterial aerosol transmission (figure 6).\textsuperscript{8} Given the low number of Maquet HCUs and related patients in this investigation, the risk attributable to these potential sources cannot be substantiated. To minimise the risk of infections, operating rooms and other hospital settings, especially those with immunocompromised patients, should be devoid of uncontrolled water sources and airflow-producing devices.\textsuperscript{9,10}

Endocarditis of prosthetic valves caused by non-tuberculous mycobacteria, although very rare, has been previously recorded,\textsuperscript{4} including one outbreak of Mycobacterium fortuitum endocarditis after cardiac surgery, affecting three children.\textsuperscript{20} The slow disease course and need for a specific diagnostic test to isolate and identify M. chimaera can delay diagnosis for up to 5 years.\textsuperscript{2} Thus, the true extent of the current outbreak remains unknown. Even if effective public health interventions are implemented now, we should expect more cases to emerge.

The strength of the current investigation is the large sample size originating from different countries with the use of whole-genome sequencing in the context of the outbreak investigation. Furthermore, the rich sampling scheme was able to show both long-time persistence of the same strain and co-existence of multiple strains in the same HCU, important findings for further epidemiological analysis.

Our investigation had several limitations. First, our data rely on a non-systematic selection of clinical and environmental isolates; typical in outbreak situations, the sampling strategy changed over time with accumulating information. Second, we observed limited genetic diversity between unrelated patients and the HCU isolates. Third, we were not able to link individual patients to individual HCUs and we had few coupled tap water, HCU water, and air samples to reliably pinpoint transmission events. As with all studies applying next-generation sequencing techniques, the pipeline used for data analysis is crucial. Although the pipeline used here had been validated before,\textsuperscript{4} and phylogenies remained intact with bootstrap analyses (figure 3), we have redone the full analysis using an alternative workflow similar to a recently published pipeline.\textsuperscript{9} Both analyses yielded identical results, supporting our hypothesis as to the origin of this outbreak (appendix pp 5, 30).

In conclusion, an extensive molecular epidemiological investigation including a large series of affected patients, the two market-leading brands of HCUs and their
production sites, as well as unrelated patients and environmental sources, suggests the possibility that the vast majority of cases of cardiothoracic surgery-related severe \textit{M. chimaera} infections diagnosed in Switzerland, Germany, the Netherlands, the UK, the USA, and Australia resulted from a single common source of infection, LivaNova HCs that were most likely contaminated during production in Germany. Local contamination of HCs with \textit{M. chimaera} also occurred and at least one patient could have been infected through this route. Operating rooms and other hospital settings with patients at increased risk of infection should be devoid of such uncontrolled water sources. Clinicians should be aware of this disease, its origin, and its likely global occurrence.

**Contributors**

GVB, ECB, MC, PMK, TAK, KK, TL, EG5, RS, Jv1, AB, AW, SN, and HS were responsible for the conception and design of the study. All authors collected the material and data. SLB, GB, ECB, MH, DH, PMK, KK, EG5, RS, CS, AT, and Jv1 were responsible for the microbiological laboratory procedures. BB, TAK, UN, CS, CU, and AKS did the whole-genome bioinformatic analysis. GVB, ECB, BB, MG, BH, CH, PMK, KK, TAK, TL, UN, PWS, RS, CS, AKS, Jv1, AB, AW, SN, and HS interpreted the data. Jv1, TAK, UN, UN, and HS wrote the main part of the paper. All authors drafted or revised the manuscript, and approved the final submitted manuscript. All authors are accountable for all aspects of the work ensuring an appropriately accurate investigation.

**Declaration of interests**

We declare no competing interests.

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