ECDC TECHNICAL DOCUMENT

EU protocol for case detection, laboratory diagnosis and environmental testing of *Mycobacterium chimaera* infections potentially associated with heater-cooler units: case definition and environmental testing methodology

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Introduction

Mycobacterium chimaera is a slow-growing non-tuberculous mycobacterium (NTM) distinguished as a species within the *Mycobacterium avium* complex (MAC) in 2004 [1]. A study in Germany in 2006 sequenced the 16–23S internal transcribed spacer region of 166 clinical isolates that had been assigned as *Mycobacterium intracellulare* by a tertiary hospital and a national reference laboratory for mycobacteria between 2002 and 2006, identifying that 86% were *M. chimaera* [2].

M. chimaera has been linked to lung infections in patients with underlying lung disease, such as chronic obstructive pulmonary disease [1,2]. Studies in the United States and Germany suggest that *M. chimaera* is less pathogenic than other species within MAC, e.g. *M. intracellulare* and *Mycobacterium avium* [2,3]**.** Its prevalence in the environment and subsequently among MAC respiratory infections and colonisations appears to vary geographically [3]. The natural reservoir of *M. chimaera* is not well known but studies have reported that it is prevalent in water networks and biofilms [3,4]. In the EU/EEA, its prevalence in the environment is mostly unknown. Several studies in the Netherlands, investigating the environmental sources of MAC infections, did not find *M. chimaera* in the drinking water by either culture or PCR [5-7].

Multi-country outbreak of *Mycobacterium chimaera* infections

Since 2011, cases of invasive cardiovascular infection caused by *M. chimaera* have been reported in patients having previously undergone cardiac surgery in Switzerland, the Netherlands, Germany and the UK [8-12]. The time from the operation to onset of disease has ranged from 1 to 4 years. The patients presented with prosthetic valve endocarditis, vascular graft infection and/or manifestations of disseminated mycobacterial infection, including embolic and immunologic manifestations (splenomegaly, arthritis, osteomyelitis, bone marrow involvement with cytopenia, chorioretinitis, lung involvement, hepatitis, nephritis and myocarditis).

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Environmental microbiology investigations

Following the outbreak alerts by Sax et al, environmental microbiology investigations into potential sources of peri-operative contamination of patients who underwent open chest cardiovascular surgery with cardiopulmonary bypass (CPB), and later developed surgical site or disseminated infection with *M. chimaera,* have been initiated in several cardiothoracic surgery centres across Europe [10,11,13,14].

In the teaching hospital in Switzerland where the first outbreak was investigated, contamination with *M. chimaera* and other waterborne opportunistic pathogens was documented in the water tanks and tubing within heater-cooler units used as part of CPB. In addition, *M. chimaera* was isolated from air samples collected in the operating room during the surgical procedures and in drinking water fountains of the hospital. Analysis of the results of molecular subtyping of isolates by randomly amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) did not show similar patterns between case isolates and environmental isolates [1-2].

Purpose and scope of this protocol

The aim of this protocol is to support the harmonised data collection on cases and environmental samples across Europe to facilitate the sharing of information, the investigation of the association between invasive infection by *M. chimaera* or MAC and heater-cooler units, the assessment of the burden of these infections, and ultimately the prevention of further cases.

The scope of this protocol is limited to *M. chimaera* and MAC. However, infections by other bacterial species (e.g. *Pseudomonas* spp., *Legionella* spp., fast-growing non-tuberculous mycobacteria) may also presumably be associated with the use of heater-cooler units.

Retrospective case identification

Case definition

Clinical criteria

Any of the following:

- Prosthetic valve endocarditisi
- Prosthetic vascular graft infection
- Sternotomy wound infection
- Mediastinitis
- Manifestations of disseminated infection including embolic and immunologic manifestations, e.g. splenomegaly, arthritis, osteomyelitis, bone marrow involvement with cytopenia, chorioretinitis, lung involvement, hepatitis, nephritis, myocarditis.

Exposure criteria

A patient having undergone surgery requiring cardiopulmonary bypass in the five years prior to the onset of symptoms of infection.

Confirmed case

A patient meeting the clinical and exposure criteria:

AND

M. chimaera detected by culture and identified by DNA sequencing in an invasive sample (blood, pus, tissue biopsy or implanted prosthetic material).

[i] As evidenced by echocardiography (vegetations, new or partial dehiscence of a prosthetic valve, pseudo-aneurysm or an abscess in the tissues surrounding a heart valve)

Probable case

A patient meeting the clinical and exposure criteria:

AND

M. chimaera detected by direct PCR and amplified DNA sequencing from an invasive sample (blood, pus, tissue biopsy or implanted prosthetic material)

OR

Mycobacterium avium complex (MAC) detected by culture or direct PCR from an invasive sample (blood, pus, tissue biopsy or implanted prosthetic material).

OR

Histopathological detection of non-caseating granuloma and foamy/swollen macrophages with acid fast bacilli in cardiac or vascular tissue in the proximity of the prosthetic material or in specimen from the sternotomy wound.

Environmental testing methodology

Samples

- Water (1L sample) from the ventilator tank, the patient blanket tank, as well as the heater-cooler units used for CPB.
- Sterile medicated fluids (50 ml) used in cardiothoracic surgical procedures: valve preserving solution, cardioplegic solution, skin antiseptic solution, heparin solution and other sterile medicated fluids.
- Water (1L sample) collected from water supply system outlets, if any, in the operating rooms. Samples should preferably be obtained with first opening of the day. Time of sample (date, time) should be recorded.
- Air (250-500 L samples) collected in the operating room during cardiovascular surgical interventions with CPB and during operation of the heater-cooling units. Two sampling positions at 0.3 m from the front and at 0.3 m behind the heater-cooler unit are recommended.

Culture methods

The above environmental water, aerosol and solution specimens should be processed in a biosafety containment level 2 mycobacteriology laboratory as described previously [11,15,16]. Whilst NTMs are not classified as Biosafety containment level 3 pathogens, given that mycobacteriology laboratories are of this standard (required for working with *M. tuberculosis*), further culturing and characterisation of samples may be processed in such a laboratory, as this is where the laboratory resources and expertise is located.

Concentration of water samples can be performed by filtration or centrifugation as described previously [15,17]. The filtration method has provided a more sensitive detection of *M. chimaera* than centrifugation (Jakko van Ingen, personal communication).

In brief, environmental water samples can be processed as schematically described in the Annex. If available, specimen decontamination of other environmental micro-organisms by use of cetylpyridinium chloride (0.005%) solution is to be preferred to the use of the more widely available standard solution of N-acetyl-L-cysteine sodium hydroxide (NALC-NaOH) or NaOH, as the latter decontamination solutions have been shown to decrease the recovery of MAC from environmental samples [17]. Water samples can be filtered and plated on selective (antimicrobial agent-containing) Middlebrook 7H11 or 7H10 agar or egg-based medium such as Löwenstein-Jensen agar. In addition, culture in liquid medium can be performed in parallel, using 50 ml samples centrifuged and then inoculated in the MGIT 960 system. Solid media should be preferred for culture if resources are limited.

Water samples should be collected preferably before the device is undergoing disinfection. Sterile sodium thiosulfate (20 mg/L) can be added to the water collection vials to neutralise any remaining hypochlorite in the water sample prior to further processing. However, this neutralisation step was not found to be necessary for recovery of MAC from potable water [16].

Processing of environmental air samples of bioaerosol can be performed as described previously [11,18-20].

Bioaerosol can be cultured from air samples collected with a surface impaction sampler. The sampler should be calibrated to sample a certain volume of air in a given period of time and utilise standard-sized selective (antimicrobial agent-containing) Middlebrook 7H11 or 7H10 agar plates. Samples of at least 250-500 L of air are recommended.

Identification

Incubation at 35°C for up to eight weeks is recommended for solid media. Weekly inspection should be performed for NTM growth and identification. When possible, estimates of the number of colony forming units (cfu) of mycobacterial growth should be counted and reported as cfu/L.

Species identification of NTM isolates should be performed in a reference laboratory for mycobacteria or in a laboratory with a reference function in molecular diagnostics based on sequencing of two of the following DNA regions: ITS, 16S rRNA, *rpo*β and *hsp*65 [21,22]. A pragmatic approach that has been used is by testing with the INNO-LiPA Mycobacteria v2 line probe assay, which includes a probe specific for *M. chimaera* (the MIN-2 probe) [23,24].

Genomic typing

There are several methods described for the molecular typing of MAC [18]. However, no standardised and validated subtyping system has been reported for molecular typing of *M. chimaera*. The use of whole-genome sequencing (WGS) for phylogenetic comparison is considered the most appropriate.Centralised *in silico* bioinformatic analysis can be based on sharing WGS raw reads produced in different laboratories following inter-laboratory agreement to harmonise sequencing protocols on the Illumina platforms. This should be supported by sharing a genomic DNA internal control of a reference strain for a reproducibility check between the laboratories. In addition, participating countries are invited to share a national subset of blind-coded, unrelated isolates of *M. chimaera* for inclusion in the comparative WGS analysis, to help estimate the background genomic diversity across Europe.

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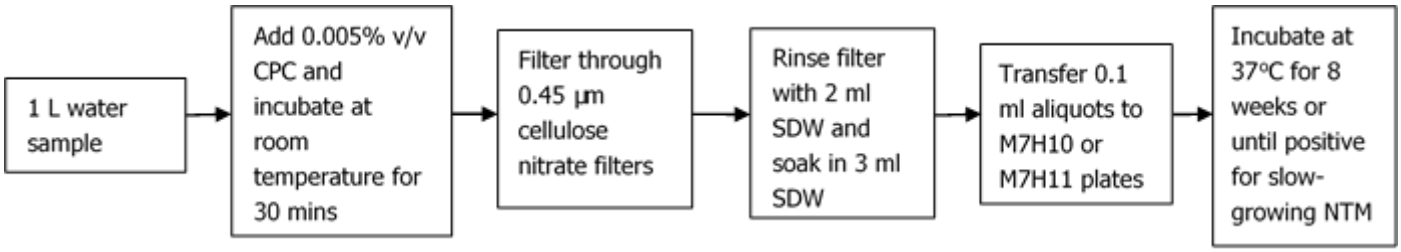
Annex

Methodology for environmental water sample processing and culture for MAC/*Mycobacterium chimaera*



Abbreviations:

CPC: Cetylpyridinium chloride

SDW Sterile distilled water

M7H10 Selective Middlebrook 7H10 agar

M7H11 Selective Middlebrook 7H11 agar

NTM Non-tuberculous mycobacteria