# Occurrence of non-tuberculous mycobacteria in households and urbanised environments

Paulina J. Wójtowicz(1), Jan Komarow(1), Alicja Okrasińska(1)(2), Hanna Irzyk(1), Emilia Bednarska(1), Klaudia Strus(1), Tomasz Jagielski(1)\*, Zofia Bakuła(1)\*\*

(1)Department of Medical Microbiology, Institute of Microbiology, Faculty of Biology, University of Warsaw, Warsaw, Poland; (2)Institute of Evolutionary Biology, Faculty of Biology, Biological and Chemical Research Centre, University of Warsaw, Warsaw, Poland;

\*t.jagielski@uw.edu.pl, \*\*zofiabakula@uw.edu.pl

Of the approximately 240 non-tuberculous mycobacteria (NTM) species identified to date, majority are free-living saprophytes that may, under certain conditions, act as opportunistic pathogens in humans and animals. However, the exact sources of NTM infections are still ill-defined.

# The aim of this study was to examine the occurrence of NTM in households and urbanised environments in Poland.

#### **MATERIAL**

Out of 109 samples (Fig. 1), 75 (68.8%) were collected from 20 households and 34 (31.2%) were originating from 16 urbanised or natural environments in two major cities in Poland, i.e. Warsaw and Cracow, between April and July 2023.

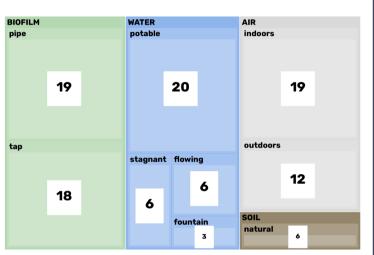


Fig.1. Number of samples of each type.

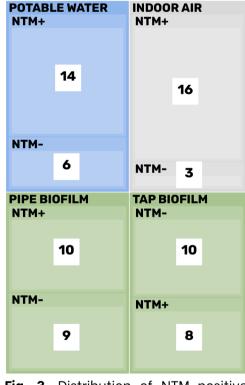
### **METHODS** 100 mL Samples were decontaminated and cultivated\* on two types of media, NATURAL WATER i.e. NTM ELite Agar (bioMerieux, Poland) and modified Middlebrook 7H11 (BD, Poland) agar, as shortly described on **Fig. 2**. The incubation was carried out at 30°C for 6 weeks in the dark. Colonies suspected of being mycobacteria, upon macromorphology observations, were subjected to Ziehl-Neelsen staining. Acid-fast bacilli cultures were POTABLE WATER selected and identified to the species level by PCR-sequencing of the hsp65 gene. Ziehl-Neelsen 🖋 staining SOIL (4.5-5 q) hsp65 BIOFILM (4.5-5 g) Middlebrook 7H11 Fig.2. Methodology: preparation of water, soil and air samples, + glycerol cultivation, selection and species identification using PCR-sequensing. malachite green

#### **RESULTS**

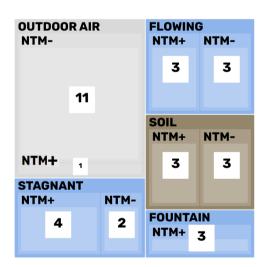
Of all **109** samples, **61 (56.0%)** yielded myco-bacterial growth. This number included **47/75 (62.7%)** household (Fig. 3) and **14/34 (41.2%)** urbanised and natural (Fig. 4) samples.

Among **household** sources, the most abundant in NTM was **air (84.7%)**, followed by **tap water (68.4%)**, **biofilm of pipe sludge (52.6%)** and **tap (44.4%)**. In all households NTM were present.

As for the urbanised and natural environments, the highest recovery of NTM was observed for municipal fountain water (100%), followed by flowing water (50.0%) and soil (50.0%), stagnant water (33.3%) and air (8.3%). All drinking water fountains (included in the potable water sources) were NTM-positive.

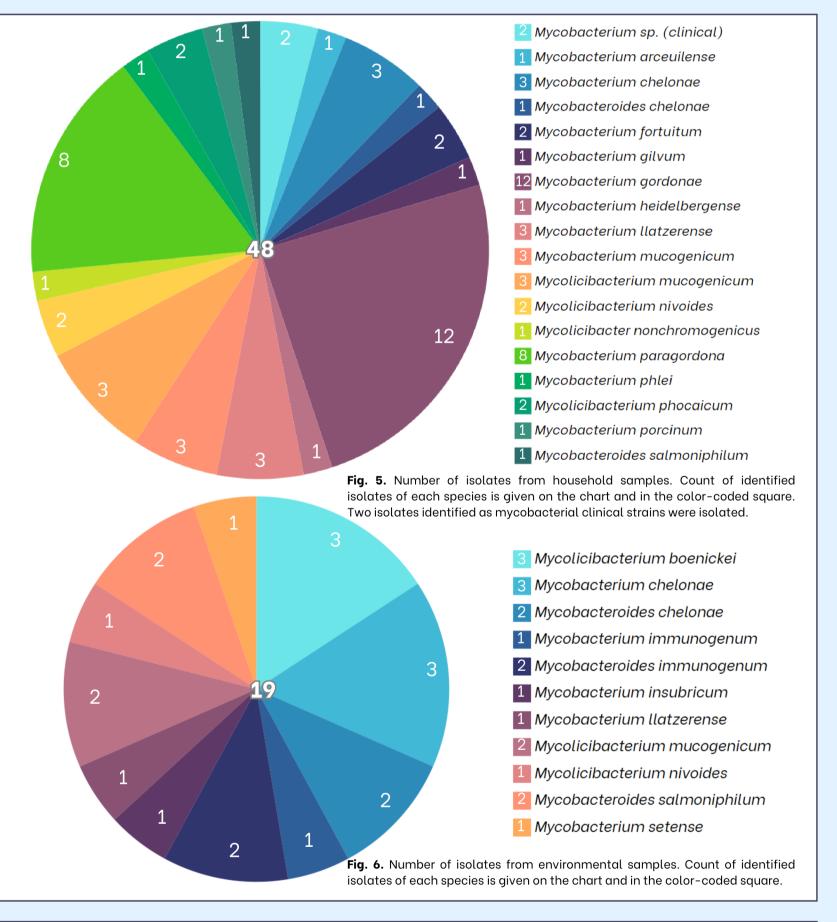


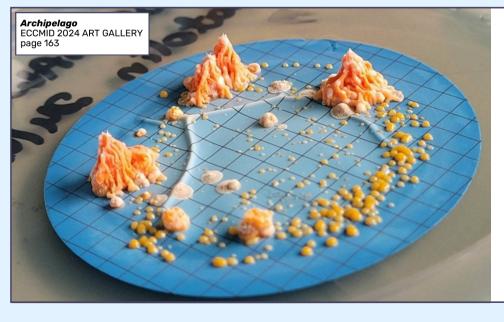
**Fig. 3.** Distribution of NTM-positive and NTM-negative household samples.



**Fig. 4.** Distribution of NTM-positive and NTM-negative environmental samples

In total, **67 isolates** were recovered - **48** (**71.6%**) originating from **households** and **19** (**28.4%**) of **environmental** sources. Species distribution varied between **household** (**Fig. 5**) and **environmental** (**Fig. 6**) samples. The most frequently occurring household species were **Mycobacterium gordonae** (**25.0%**) and **M. paragordonae** (**16.7%**). Environmental samples were dominated by **Mycolicibacterium boenickei** (**15.8%**) and **Mycobacterium chelonae** (**15.8%**).





## CONCLUSIONS

- This study provides the first investigation into the occurrence of NTM in household and environmental sources in Poland.
- Overall, **NTM were highly abundant** in both natural and anthropogenic environments.
- Importantly, various pathogenic species, such as *M. arceuilense*, *M. boenickei*, *M. chelonae*, *M. fortuitum* and *M. porcinum*, were identified in the analysed samples, including soil, drinking fountain and tap water.
- Interestingly, multiple NTM species were isolated from each fountain and flowing water samples
- Further WGS-based investigations are required to establish the potential sources of NTM infections.

