

## Introduction

Nontuberculous mycobacteria (NTM) are common environmental organisms and some species of NTM are capable of causing opportunistic infections, such as the *Mycobacterium abscessus* group (Mabs)<sup>1</sup>.

The prevalence of Mabs infections is increasing globally, especially respiratory infections<sup>2</sup> but our understanding of how Mabs respiratory infections are acquired and spread is limited. Mabs have previously been detected in potable (drinking) water (3,4,5).

MABS are hardy organisms, resistant to multiple antibiotics (6). It is these properties which have likely assisted Mabs with its transition from environmental organism to human pathogen (1). While the environment may be a possible acquisition source, little is known about how this transmission pathway causes infection.

The **major aim** of the MAP study is to comprehensively investigate possible transmission pathways of Mabs using isolates derived from clinical and environmental sources to improve our understanding of how these infections are acquired. The information gathered will then be used to develop infection prevention strategies that are effective at reducing the risk of susceptible patients being exposed to these potentially contaminated environmental sources.

Presented here are the preliminary results of the MAP study, highlighting the rates of Mabs and other NTM present in the participant home water samples.

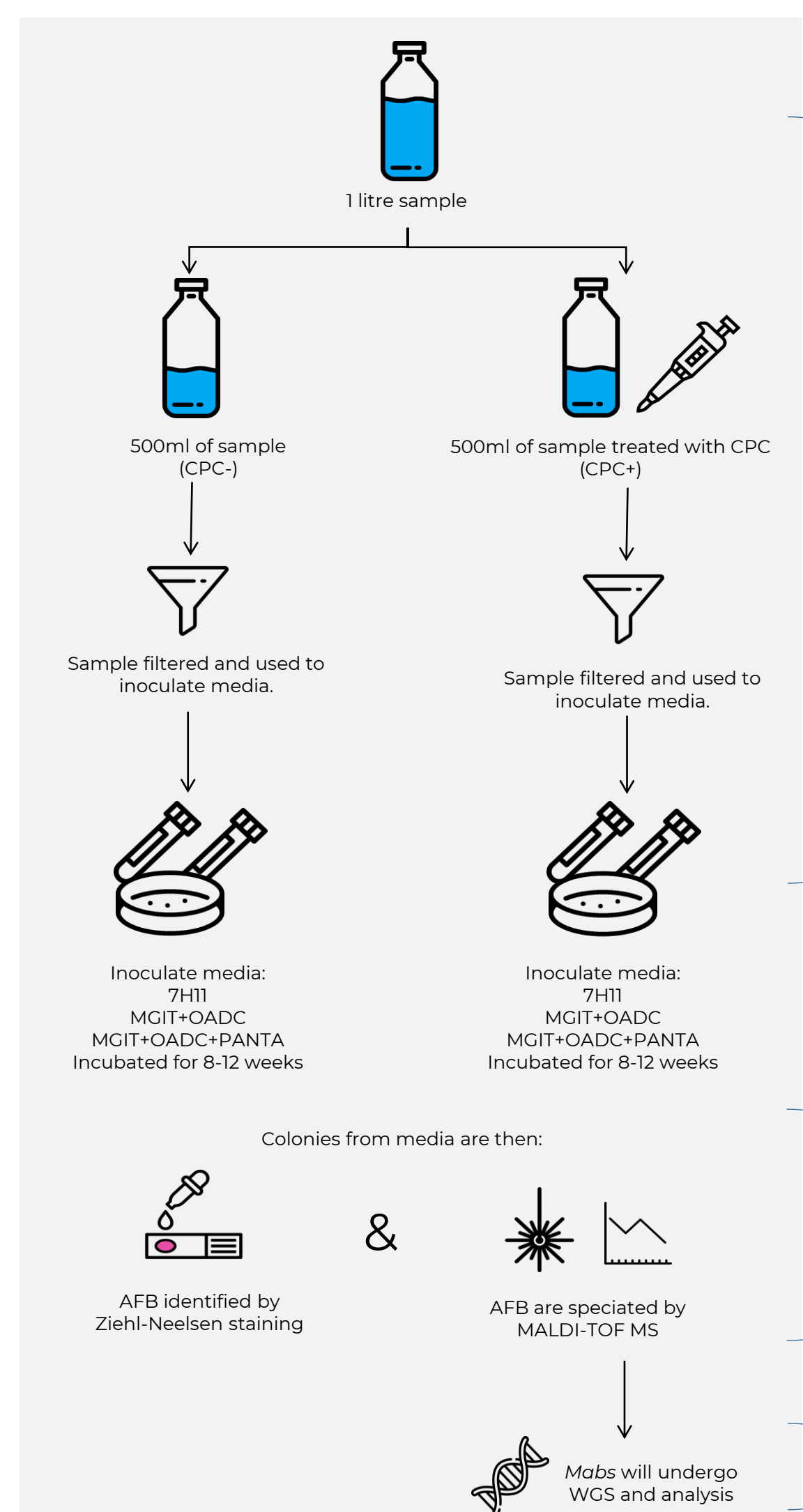
## Materials & Methods

Between July 2020 and August 2022 water samples have been collected from the homes of 30 Mabs infected participants, residing in Southeast Queensland.

**WATER PROCESSING:** Each one litre water sample was split into two samples, filtered, treated with decontamination reagents and growth supplements and inoculated into growth media.

**ISOLATION OF NTM:** Staining and microscopy is used as a screening tool, before the NTM are identified.

**BIOINFORMATIC ANALYSIS:** Genetic relatedness of water to clinical Mabs isolates will be undertaken using whole genome sequencing (WGS).



### Water Processing:

**Decontamination solution:**  
CPC = 0.005% cetylpyridinium chloride

**Solid Agar:**  
7H11 Middlebrook agar

**Liquid Growth Media:**  
MGIT = Mycobacteria Growth Indicator Tube

**Growth Supplement:**  
OADC = Oleic Albumin Dextrose Catalase

**Antimicrobial Cocktail:**  
PANTA = polymyxin-B, amphotericin-B, nalidixic acid, trimethoprim, azlocillin

### Isolation of NTM:

**Staining:**  
ZN = Ziehl-Neelsen

**Initial Identification:**  
AFB: Acid Fast Bacilli

**Identification:**  
MALDI-TOF MS: Matrix-assisted laser desorption/ionization time of flight mass spectrometry

### Bioinformatic Analysis:

**DNA sequencing:**  
WGS = Whole Genome Sequencing

## Results

### PRELIMINARY ANALYSIS

- 30 participants have been enrolled to date, 23 adults and 7 children
- Recruited from the:
  - FORMaT trial (n=26), National NTM study (n=1) and non-trial participants (n=3).
- One participant had samples collected from two independent residential addresses and one had repeat sampling of their home.
- A total of 143 water samples were collected (Table 1)
- From each home, water is sampled from the kitchen sink, showerhead and the bathroom handbasin.
- Additional sites from within and around the house also sampled include filtered water sources, bathtubs, plumbed-in fridges and outdoor water sources

Table 1: Participant water collection sites and preliminary results to date

Participant	Shower	Handbasin	Kitchen sink	Water Filter*	Outdoor sources^	Plumbed-in fridge	Bathtubs	Laundry	Culture Progress
1	1	1	1	0	1	0	0	1	NTM Identified; complete
2	1	1	1	0	0	1	0	0	NTM Identified; complete
3	1	1	1	5	0	1	0	0	NTM Identified; complete
4	1	1	1	0	0	1	0	0	NTM Identified; complete
5	2	4	2	0	0	0	0	0	NTM Identified; complete
6	1	1	1	1	0	0	0	0	NTM Identified; complete
7	1	1	1	1	0	0	0	0	NTM Identified; complete
8	1	1	1	1	0	0	0	0	NTM Identified; complete
9	1	1	1	0	1	0	1	0	NTM Identified; AFB identified; in-progress
10	1	1	1	0	0	0	1	0	NTM Identified; complete
11	1	1	1	1	0	0	0	0	NTM Identified; complete
12	1	1	1	1	2	0	0	0	NTM Identified; complete
13	1	1	1	2	0	0	0	0	NTM Identified; complete
14	1	1	1	1	0	0	0	0	NTM Identified; complete
15	1	1	1	0	2	0	0	0	NTM Identified; complete
16	1	1	1	1	0	0	0	0	NTM Identified; complete
17	0	1	1	1	1	0	1	0	NTM Identified; complete
18	2	2	2	0	0	1	0	0	NTM Identified; complete
19	1	1	1	0	0	1	0	0	AFB identified; in-progress
20	1	1	1	0	2	0	0	0	AFB identified; in-progress
21	1	2	1	1	0	0	0	0	AFB identified; in-progress
22	1	1	1	0	0	0	0	0	in-progress
23	1	1	1	2	1	0	0	0	AFB identified; in-progress
24	1	1	1	1	0	0	0	0	AFB identified; in-progress
25	1	1	1	0	0	0	0	0	in-progress
26	1	1	1	0	0	0	0	0	in-progress
27	1	1	1	1	1	0	0	0	AFB identified; in-progress
28	1	1	1	0	0	0	0	0	AFB identified; in-progress
29	2	1	1	0	0	0	0	0	AFB identified; in-progress
30	1	1	1	13	0	0	1	0	AFB identified; in-progress
<b>Total</b>	<b>32</b>	<b>35</b>	<b>32</b>	<b>33</b>	<b>11</b>	<b>5</b>	<b>4</b>	<b>1</b>	

\*Water Filter: Under bench filters, zip taps, filter jugs, reverse osmosis, filter cartridge components  
^Outdoor sources: pool, garden tanks, pond, outdoor taps, caravan

### IDENTIFICATION OF *M. abscessus*

- Mabs has been isolated from the water of 11 of the 17 (64.7%) homes for which NTM has been identified using MALDI-TOF MS (Table 2)
- 39 individual Mabs isolates were cultured from 20 water sources
  - water filters and kitchen sinks (5)
  - shower (4)
  - handbasins and pools (2)
  - garden tanks and plumbed-in fridge (1)
- For one participant Mabs was isolated from each of the water samples collected (Participant 8)

Table 2: Distribution of Mabs from participant homes

Participant	# of Mabs isolates	Source of Mabs
2	1	plumbed-in fridge
3	12	shower/handbasin/filter/kitchen
4	1	shower
6	1	kitchen
8	12	shower/handbasin/filter/kitchen
11	1	filter
12	3	filter/pool
13	2	kitchen/shower
15	4	pool/tank
16	1	filter
18	1	kitchen

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## Results

### DIVERSITY OF NTM SPECIES

- 10 species have been identified from 17 participant homes for which NTM has been identified using MALDI-TOF MS (Table 3)
- There was a high degree of species diversity from across the water sources
- Samples from water filters had 7 different species identified
- Plumbed-in fridges had the lowest species diversity
- NTM has not been recovered from bathtub or laundry water to date
- Of the 86 sites sampled, NTM were isolated from 55 (64%)
  - For 47% sites, a single species was recovered, 35% had two species, and 16% had three and 2% had five different species.

Table 3: Diversity of NTM species from different collection sites from participant homes

Collection site	# of NTM cultured	Species Identified
Shower	31	<i>M. abscessus</i> , <i>M. chelonae</i> , <i>M. gordonae</i> , <i>M. kansasii</i> , <i>M. lentiflavum</i> , <i>M. mucogenicum</i>
Handbasin	35	<i>M. abscessus</i> , <i>M. gordonae</i> , <i>M. kansasii</i> , <i>M. lentiflavum</i> , <i>M. mucogenicum</i>
Kitchen sink	37	<i>M. abscessus</i> , <i>M. chelonae</i> , <i>M. gordonae</i> , <i>M. kansasii</i> , <i>M. lentiflavum</i> , <i>M. mucogenicum</i>
Water Filter*	47	<i>M. abscessus</i> , <i>M. chelonae</i> , <i>M. fortuitum</i> complex, <i>M. gordonae</i> , <i>M. lentiflavum</i> , <i>M. mucogenicum</i> , <i>M. szulgai</i>
Outdoor sources^	21	<i>M. abscessus</i> , <i>M. gordonae</i> , <i>M. lentiflavum</i> , <i>M. immunogenum</i> , <i>M. intracellulare</i> , <i>M. mucogenicum</i>
Plumbed-in fridge	5	<i>M. abscessus</i> , <i>M. gordonae</i> , <i>M. lentiflavum</i>
Bathtubs	0	
Laundry	0	

\*Water Filter: Under bench filters, filter jugs  
^Outdoor water sources: pool, garden tanks

## Conclusion

- While the SARS-CoV-2 pandemic slowed study recruitment, participant enrollment has steadily increased during 2022
- The MAP study aims to recruit 75 participant for home water sampling
- Mabs has been isolated from a significant number of patient homes (64.7%) to date
- This rate is significantly higher than homes sampled in 2010 (35%) and 2017 (11%), also from Southeast Queensland
- Mabs can be readily cultured from a range of different water sources
- Predominately only one water source from the home is positive for Mabs, however one home had Mabs in all samples collected for testing
- Other NTM species are commonly cultured from these home water samples
  - M. mucogenicum* (25.6%) is the most prevalent, followed by Mabs (22.2%), *M. lentiflavum* (20.5%) and *M. gordonae* (17.6%)
  - The remaining 6 species account for <15% of all NTM isolated
- Comparison of the water derived Mabs with the clinical samples using WGS will enhance understanding of acquisition routes.
- Currently, mitigation techniques to reduce NTM exposure within the home should be explored as a means of infection/reinfection prevention.

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