CULTURE-BASED METHODOLOGIES FOR THE ISOLATION OF NONTUBERCULOUS MYCOBACTERIA FROM WATER SAMPLES

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Introduction

Nontuberculous mycobacteria (NTM) are important environmental pathogens, and exposure from both natural and man-made sources has been associated with pulmonary, soft tissue and disseminated disease. Culture-based techniques are often used to identify the presence of NTM within these samples.

However, culturing from diverse water samples is associated with several unknown factors which impact on the growth and recovery of the NTM including

1. Variable growth rates and nutrient requirements of the different NTM species
2. Different levels of contamination with bacteria and fungi in the source water

To overcome these unknowns a variety of different culture techniques are used for optimal NTM recovery.

Research Question

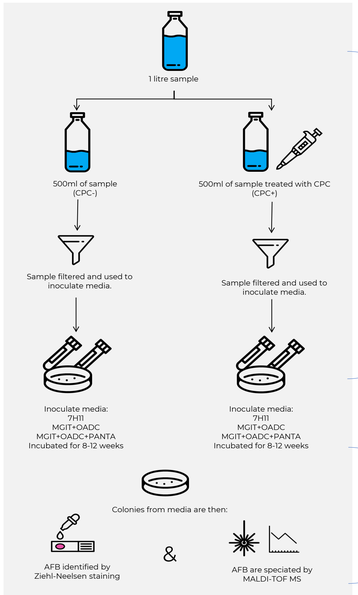
1. Which culture technique results in the greatest NTM recovery?
2. Which culture technique is the most successful at reducing fungal contamination?
3. Can we rationalise the media used?

Materials & Methods

Between July 2020 and February 2022 water samples were collected from 263 individual taps from homes, hospitals and the drinking water distribution system (DWDS) in South East 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 isolates undergo formal identification.



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

Results

NTM RECOVERY RATES: COMPARISON OF MEDIA

- From these water sites, 526 agar plates and 1052 MGITs have been inoculated
- 429 individual ZN positive colonies have been isolated, including 383 confirmed as NTM species using MALDI-TOF, and 46 awaiting identification
- When comparing recovery from the individual media types with the various supplements, the isolation of NTM was highest from the CPC treated compared to untreated water samples (CPC+ 29% vs CPC- 19%, p<0.00001)
- Table 1 describes the recovery rates for each of the water collection sites for each of the different media
  - A high level of variation was observed across sites, seasons and media types
- Recovery rates showed that the highest yield was from the 7H11 media regardless of water treatment and the lowest from the MGIT+OADC inoculated with CPC- water

Table 1: Recovery rates of nontuberculous mycobacterium from South East Queensland water sources

			Number of NTM isolated from the individual media					
			7H11 Middlebrook Agar		MGIT+OADC		MGIT+OADC+PANTA	
Collection Site	Season Collected	Number of water collections	CPC- n (%)*	CPC+ n (%)*	CPC- n (%)*	CPC+ n (%)*	CPC- n (%)*	CPC+ n (%)*
Hospital 1	Summer	32	18 (56.3)	10 (31.3)	1 (3.1)	4 (12.5)	3 (9.4)	4 (12.5)
	Winter	16	9 (56.3)	3 (18.8)	0 (0.0)	6 (37.5)	0 (0.0)	6 (37.5)
Hospital 2	Summer	36	7 (19.4)	7 (19.4)	1 (2.8)	6 (16.7)	2 (5.6)	4 (11.1)
	Winter	18	3 (16.7)	5 (27.8)	2 (11.1)	5 (27.8)	2 (11.1)	3 (16.7)
Hospital 3	Summer	16	7 (43.8)	6 (37.5)	0 (0.0)	8 (50.0)	2 (12.5)	4 (25.0)
	Winter	16	4 (25.0)	3 (18.8)	0 (0.0)	7 (43.8)	0 (0.0)	11 (68.8)
DWDS	Summer	28	6 (21.4)	2 (7.1)	2 (7.1)	5 (17.9)	9 (32.1)	6 (21.4)
	Winter	14	2 (14.3)	1 (7.1)	0 (0.0)	3 (21.4)	2 (14.3)	3 (21.4)
Participants (n=18)	N/A	87	39 (44.8)	40 (46.0)	13 (14.9)	32 (36.8)	17 (19.5)	33 (37.9)

*One media type inoculated for each water collected
Abbreviations: 0.005% cetylpyridinium chloride, CPC; decontaminated water sample, CPC+; neat water sample, CPC-; drinking water distribution system, DWDS; mycobacteria growth indicator tube, MGIT; nontuberculous mycobacteria, NTM; oleic, albumin, dextrose, catalase, OADC; polymyxin-B, amphotericin-B, nalidixic acid, trimethoprim, azlocillin, PANTA

DIVERSITY OF NTM SPECIES

- 12 species have been identified from the water collected from South East Queensland (Table 2)
- *M. gordonae* and *M. mucogenicum* were the most frequently cultured species
- Species associated with pulmonary infections, such as *M. abscessus*, *M. chelonae*, *M. kansasii* and *M. intracellulare* have been cultured from these water samples
  - These species can be found in water collected from homes, hospitals and the DWDS
- The lowest diversity of recovered species was from the neat water (CPC-) cultured in MGITs
- Of the 263 sites sampled, NTM were isolated from 146 (55%).
  - For 66% sites, a single species was recovered, 24% had two species, and 10% had 3-5 different species.

Table 2: The diversity of NTM cultured from South East Queensland water sources

			Diversity of NTM species isolated from the individual media					
			7H11 Middlebrook Agar		MGIT+OADC		MGIT+OADC+PANTA	
Collection Site	Season Collected	Number of water collections	CPC- n (%)*	CPC+ n (%)*	CPC- n (%)*	CPC+ n (%)*	CPC- n (%)*	CPC+ n (%)*
Hospital 1	Summer	32	m	a, co, g, m	m	g, k, m	a	co, m
	Winter	16	g, m	m	-	g, k, m	-	g, k, m
Hospital 2	Summer	36	g, m	g, k	g	g, k, m	g	g, k
	Winter	18	g, m	g, k	g	g, k, m	g, k	g, k
Hospital 3	Summer	16	ch, g, m	ch, g	-	g, l	g	g
	Winter	16	m	ch, g	-	g, k, l	-	ch, g, l, m
DWDS	Summer	28	a, co, m, n	k, l	m	g, k, l, m	a, co, m	a, co, l
	Winter	14	a, m	a	-	k, l	k, m	k, l
Participants (n=18)	N/A	87	a, ch, g, l, m	a, ch, g, im, in, k, l, m, s	a, ch, g, l, m	a, ch, f, g, k, l, m	a, g, in, l, m	a, ch, g, k, l, m

Abbreviations: 0.005% cetylpyridinium chloride, CPC; decontaminated water sample, CPC+; neat water sample, CPC-; drinking water distribution system, DWDS; mycobacteria growth indicator tube, MGIT; nontuberculous mycobacteria, NTM ; oleic, albumin, dextrose, catalase, OADC; polymyxin-B, amphotericin-B, nalidixic acid, trimethoprim, azlocillin, PANTA
Mycobacterium species: *M. abscessus*, a; *M. chelonae*, ch; *M. cosmeticum*, co; *M. fortuitum* complex, f; *M. gordonae*, g; *M. immunogenum*, im; *M. intracellulare*, in; *M. kansasii*, k; *M. lentiflavum*, l; *M. mucogenicum*, m; *M. neoaurum*, n; *M. szulgai*, s

Results

FUNGAL CONTAMINATION

- By far the biggest limitation in successfully isolating NTM from these samples has been fungal overgrowth
- Fungi was present in 136 of the 247 sites where contamination rates were recorded (55.1%)
- The 7H11 agar had the highest contamination rates
- Media inoculated with CPC+ water had significantly higher rates of fungal growth compared to CPC- water (CPC+ n=196 vs CPC- n=142, p=0.0008).
- Water collected in summer and spring had the highest rates of fungal contamination, 74.3% and 69.8%, respectively, compared to autumn (64%) and winter (34.4%).
- Despite this, all sites had at least one or more uncontaminated media allowing the recovery of NTM.

Table 3: The prevalence of fungal contaminations from South East Queensland water sources

			Prevalence of fungal contamination on the individual media					
			7H11 Middlebrook Agar		MGIT+OADC		MGIT+OADC+PANTA	
Collection Site	Season Collected	Number of water collections	CPC- n (%)*	CPC+ n (%)*	CPC- n (%)*	CPC+ n (%)*	CPC- n (%)*	CPC+ n (%)*
Hospital 1	Summer	16	7 (43.8)	4 (25.0)	5 (31.3)	4 (25.0)	4 (25.0)	1 (6.3)
	Winter	16	1 (6.3)	2 (12.5)	0 (0.0)	3 (18.8)	1 (6.3)	0 (0.0)
Hospital 2	Summer	36	22 (61.1)	19 (52.8)	2 (5.6)	19 (52.8)	2 (5.6)	12 (33.3)
	Winter	18	5 (27.8)	2 (11.1)	0 (0.0)	2 (11.1)	0 (0.0)	1 (5.6)
Hospital 3	Summer	16	12 (75.0)	10 (62.5)	0 (0.0)	8 (50.0)	1 (6.3)	4 (25.0)
	Winter	16	6 (37.5)	8 (50.0)	0 (0.0)	3 (18.8)	0 (0.0)	1 (6.3)
DWDS	Summer	28	22 (78.6)	15 (53.6)	11 (39.3)	13 (46.4)	11 (39.3)	20 (71.4)
	Winter	14	2 (14.3)	2 (14.3)	0 (0.0)	2 (14.3)	0 (0.0)	1 (7.1)
Participants (n=18)	N/A	87	16 (18.4)	22 (25.3)	5 (5.7)	6 (6.9)	7 (8.0)	12 (13.8)

*One media type inoculated for each water collected
Abbreviations: 0.005% cetylpyridinium chloride, CPC; decontaminated water sample, CPC+; neat water sample, CPC-; drinking water distribution system, DWDS; mycobacteria growth indicator tube, MGIT; nontuberculous mycobacteria, NTM ; oleic, albumin, dextrose, catalase, OADC; polymyxin-B, amphotericin-B, nalidixic acid, trimethoprim, azlocillin, PANTA

Conclusion

1. **Which culture technique results in the greatest NTM recovery?**
   - CPC+ water had the highest rates of NTM recovery and the greatest species diversity
   - 7H11 agar had the highest rates of NTM recovery and the greatest species diversity
2. **Which culture technique is the most successful at reducing fungal contamination?**
   - Collections during the winter months had the lowest rates of fungal contamination
   - CPC- water had significantly lower rates of fungal contamination compared to CPC+ water
     - Is this due to CPC- media being discarded during the initial weeks of culturing due to bacterial overgrowth and therefore not being incubated for extended periods to see the fungal growth come up?
     - More work is needed to fully understand the cause of this
3. **Can we rationalise the media used?**
   - Of the six different media inoculated for the isolation of NTM, culturing CPC+ water samples in MGIT+OADC+PANTA is not required for optimal NTM recovery
   - NTM species cultured on MGIT+OADC+PANTA are also found on one or more of the other media types

Using a variety of different media with and without decontamination results in a greater yield that using a single method only. Furthermore, this approach allows for the different growth requirements of the individual NTM species and the unknown contamination burden of the water samples.

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