Antimicrobial susceptibility of *M. abscessus* water isolates

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Introduction

Mycobacterium abscessus (Mabs) is an opportunistic pathogen, naturally occurring in the environment and incompletely inactivated by disinfection in drinking water distribution systems (DWDS). Mabs accumulates in distribution pipes and becomes abundant in home and hospital plumbing systems. Nosocomial outbreaks of *M. abscessus* have been reported with the source being hospital water. The frequency of isolation of Mabs in clinical samples has been increasing. This project aimed to complete antimicrobial susceptibility testing (AST) on Mabs isolates from Brisbane's municipal water at different time points to investigate if a decrease in susceptibility to antimicrobials could explain the increasing frequency of isolation.

Background

The Mabs group comprises three subspecies; subspecies *abscessus* (Maa), *bolletii* (Mab) and *massiliense* (Mam) and has been described as an "antibiotic nightmare" due to its innate resistance to many antimicrobials [1]. There have been several outbreaks of Mabs disease within hospitals including one linked with the hospital tap water [2]. Chlorine and chloramine are used for disinfection of tap water and Mabs susceptibility to these has not been previously quantified. In Brisbane's DWDS from June 2021-22 the maximum residual free chlorine concentration measured was 1.4 ppm with an average of <0.1 ppm [3]. Chloramine is targeted to 3.2-3.5 ppm at the water treatment plant and a residual concentration of 1.5 - 2 ppm [4].

Method

Water samples from Brisbane's municipal water supply were collected and cultured for Mabs in 2007, 2017-18 and 2021-22 as part of ongoing studies. Isolates were selected from each time point and AST performed by broth microdilution following the Clinical Laboratory Standards Institute guidelines.

Conclusions

- Antimicrobial susceptibility did not decrease across the three time points
- *M. abscessus* isolates had significantly lower MICs for chloramine than chlorine.
- *M. abscessus* subsp. *massiliense* had significantly lower MICs for chloramine than subsp. *abscessus* which could explain the fewer number of subsp. *massiliense* isolates from the 2007 and 2017-18 water samples.
- The MICs for chlorine and chloramine are much higher than the concentrations used in DWDS thus additional preventative measures should be used to protect against future nosocomial Mabs outbreaks

Results

Antibiotic susceptibility

There was no significant difference in antibiotic susceptibility between isolates from the different time points except for three Mam isolates with low minimum inhibitory concentrations (MIC) to doxycycline and minocycline. None of the Maa had inducible clarithromycin resistance which differs to the prevalence in clinical isolates.

Table 1: Antibiotic susceptibility of *M. abscessus* water isolates. Antibiotic susceptibility testing was performed by broth microdilution following the Clinical Laboratory Standards Institute guidelines.

		Minimum Inhibitory Concentration (PPM)								
Year	Subspecies	LZD	IMI	FOX	AMI	DOX	MIN	TGC	CLA (D3)	CLA (D14)
2007	*abscessus*	8	8	32	16	>16	>8	0.25	0.12	1
2007	*abscessus*	32	16	32	8	>16	>8	0.5	0.25	2
2007	*abscessus*	4	8	16	4	>16	>8	0.12	0.12	0.5
2018	*abscessus*	16	8	16	8	>16	>8	0.5	0.25	1
2017	*abscessus*	32	8	32	4	>16	>8	0.5	0.25	1
2017	*abscessus*	16	8	32	4	>16	>8	0.5	0.25	1
2017	*massiliense*	8	8	32	16	>16	>8	0.5	0.25	1
2021	*massiliense*	32	8	32	8	1	<1	0.12	0.12	0.5
2021	*massiliense*	16	8	32	8	0.5	<1	0.25	0.12	0.5
2021	*massiliense*	2	16	32	4	4	<1	0.5	0.25	0.25
2021	*abscessus*	32	16	64	8	>16	>8	0.25	0.25	1

LZD = Linezolid, IMI = Imipenem, FOX = Cefoxitin, AMI = Amikacin, DOX = Doxycycline, MIN = Minocycline, CLA (D3) = Clarithromycin at Day 3, CLA (D14) = Clarithromycin at Day 14 (inducible resistance)

Sensitive	Resistant
Intermediate	No interpretive criteria

Subspecies difference in chloramine susceptibility

Mam had a significantly lower chloramine MIC than Maa.

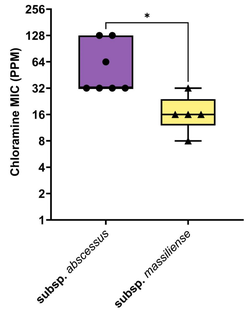

Figure 2: *M. abscessus* subsp. *massiliense* and subsp. *abscessus* chloramine susceptibility. Susceptibility testing was performed by broth microdilution modified from the Clinical Laboratory Standards Institute guidelines. * p<0.05

Disinfectant susceptibility across time

There was no significant difference in chlorine or chloramine susceptibility across the three time points. This suggests that decreased susceptibility to disinfectants is not responsible for the increasing frequency of isolation.

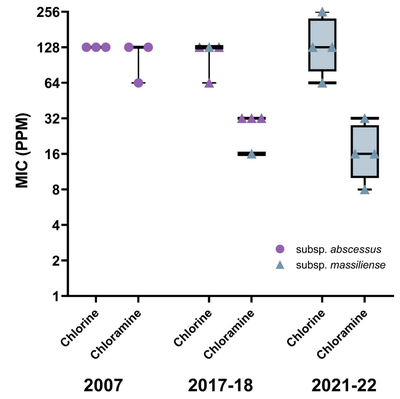

Figure 1: Chlorine and chloramine susceptibility of *M. abscessus* isolates across three time points. Susceptibility testing was performed by broth microdilution modified from the Clinical Laboratory Standards Institute guidelines.

Chlorine and chloramine susceptibility

The chloramine MIC was significantly lower than the chlorine MIC for both Maa and Mam.

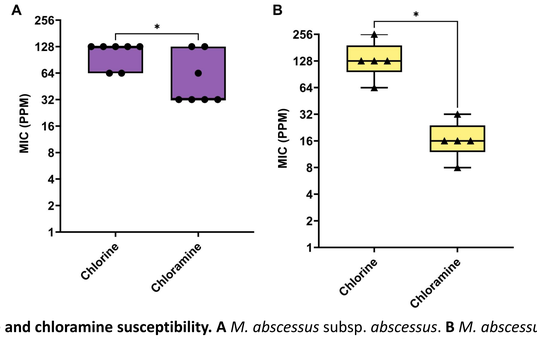

Figure 3: Chlorine and chloramine susceptibility. A *M. abscessus* subsp. *abscessus*. **B** *M. abscessus* subsp. *massiliense*. Susceptibility testing was performed by broth microdilution modified from the Clinical Laboratory Standards Institute guidelines. * p<0.05

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