Legionella spp., *L. pneumophila* and Mycobacterium avium complex (MAC) in potable and reuse water distribution pipelines

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Statement of Authenticity

I certify that this thesis does not incorporate without acknowledgment and material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Harriet Whiley

20th August 2014

Statement of Co-Authorship

The following people contributed to the publication of the work undertaken as part of this thesis. The co-authors are listed in the order that the co-authored publications appears in the thesis.

Associate Professor Richard Bentham

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Dr Alexandra Keegan

Dr Michael Taylor

Professor Howard Fallowfield

Dr Kirstin Ross

All above listed contributions equated to no more that 25% of the work necessitated for publication of research manuscripts.

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Publications

Whiley, H., Keegan, A., Giglio, S. & Bentham, R. (2012), Mycobacterium avium complex – the role of potable water in disease transmission, *Journal of Applied Microbiology*, 113**,** 223-232.

Whiley, H., and Taylor, M., (2014), *Legionella* detection by culture and qPCR: comparing apples and oranges, *Critical Reviews in Microbiology*, doi:10.3109/1040841X.2014.885930.

Whiley, H., Keegan, A., Fallowfield, H. and Bentham, R (2014), *Legionella , L. pneumophila* and Mycobacterium avium complex (MAC) detected in South Australian potable water distribution systems, *International Journal of Environmental Research and Public Health*, 11(7), 7393-7405; doi:10.3390/ijerph110707393.

Whiley, H., Keegan, A., Fallowfield, H., and Bentham, R., (2014), The presence of opportunistic pathogens, *Legionella* spp., *L. pneumophila* and Mycobacterium avium complex (MAC), in South Australian reuse water distribution pipelines, *Journal of Water and Health*, in press, doi:10.2166/wh.2014.317.

Whiley, H., Keegan, A., Fallowfield, H., and Ross, K., (2014), Uncertainties associated with assessing the public health risk from *Legionella*, *Frontiers in Microbiology*, 5, doi: 10.3389/fmicb.2014.00501.

Abstract

Legionella spp. and Mycobacterium avium complex (MAC) are opportunistic human pathogens of public health concern. The clinical manifestations of *Legionella* include Pontiac fever, an acute febrile illness, and Legionnaires' disease, a severe atypical pneumonia. *L. pneumophila* is the most common causative agent of Legionellosis. In Australia, MAC is not a nationally notifiable disease, but it is responsible for a wide spectrum of illness dependent on subspecies, route of infection and a patient's pre-existing conditions. This includes, but is not limited to, a range of respiratory, gastrointestinal and cutaneous infections. Evidence also suggests that MAC is a causative agent of Crohn's disease.

This study investigated the presence of *Legionella* spp., *L. pneumophila* and MAC along South Australian potable and reuse water distribution pipelines using qPCR. Two potable water distribution systems were chosen (one chlorine disinfected and the other chloramine disinfected) and two reuse water distribution systems (one utilising recycled wastewater treated with chlorine and UV disinfection and the other recycled wastewater combined with reclaimed stormwater treated with chlorine disinfection only). Samples were collected along each of the pipelines throughout the year, to determine any seasonal variation. Relationships between temperature, chlorine or chloramine residual, indicator bacteria, distance from treatment plant and concentration of *Legionella* and MAC was explored.

Legionella spp., *L. pneumophila* and MAC were detected in both potable water distribution systems throughout the year. Maximum concentrations detected were 10^3, 10^3 and 10^3 copies/mL respectively in the chlorine disinfected system and 10^6, 10^3 and 10^4 copies/mL respectively in the chloramine disinfected system. The

concentrations of these opportunistic pathogens were primarily controlled throughout the distribution network through the maintenance of disinfection residuals. At a dead-end where the disinfection residual was not maintained significant ($P<0.05$) increased numbers of *Legionella* spp., *L. pneumophila* and MAC were observed when compared to the concentration measured closest to the processing plant in the same pipeline and sampling period.

In the reuse water distribution systems *Legionella* spp., *L. pneumophila* and MAC were detected using qPCR at maximum concentrations of 10^5, 10^3 and 10^5 copies/mL respectively. During the summer period of sampling the concentration of all three organisms significantly ($P<0.05$) increased along the pipeline, suggesting multiplication and hence viability. No seasonality in the decrease in chlorine residual along the pipelines was observed. This suggests that the combination of reduced chlorine residual and increased water temperature promoted the presence of these opportunistic pathogens.

This study demonstrates the ability of *Legionella* spp., *L. pneumophila* and MAC to survive the potable and reuse water disinfection process and highlights the need for greater understanding of *Legionella* ecology related to risk associated with point of use. Determining the potential public health risk presented by the presence of *Legionella* spp., *L. pneumophila* and MAC is difficult to quantify due to the uncertainties regarding *Legionella* and MAC epidemiology and detection. A comprehensive review comparing culture and qPCR method of *Legionella* detection from environmental samples was conducted. The uncertainties associated with *Legionella* risk assessment were also collated and discussed providing a useful tool for considering risk assessment data and important areas for future *Legionella* research were also identified.

Aims

The aims of this investigate were:

- To determine whether opportunistic pathogens *Legionella* spp., *L. pneumophila* and Mycobacterium avium complex (MAC) are present in South Australian potable and reuse water distribution pipelines.
- To identify factors that may influence the growth of these opportunistic pathogens along the potable and reuse water pipelines.

Objectives

In order to achieve these aims, the objectives of this study were:

- To examine the literature and determine the potential role of potable water as a source of MAC infection.
- To evaluate the optimum method for *Legionella* spp., *L. pneumophila* and MAC enumeration in environmental sources.
- To examine the relationship between pipeline length, seasonality, water quality, disinfectant residual, indicator organisms and *Legionella* spp., *L. pneumophila* and MAC concentrations in the potable and reuse water distribution pipelines.
- To use information from this study to create a risk assessment for *Legionella* spp., *L. pneumophila* and Mycobacterium avium complex (MAC) exposure from South Australian potable water.

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