Evaluation of quantitative polymerase chain reaction assays targeting *Mycobacterium avium*, *M. intracellulare*, and *M. avium* subspecies *paratuberculosis* in drinking water biofilms

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ABSTRACT

Mycobacterium avium (MA), *Mycobacterium intracellulare* (MI), and *Mycobacterium avium* subsp. *paratuberculosis* (MAP) are difficult to culture due to their slow growing nature. A quantitative polymerase chain reaction (qPCR) method for the rapid detection of MA, MI, and MAP can be used to provide data supporting drinking water biofilms as potential sources of human exposure. The aim of this study was to characterize two qPCR assays targeting partial 16S rRNA gene sequences of MA and MI and use these assays, along with two previously reported MAP qPCR assays (IS*900* and Target 251), to investigate *Mycobacterium* occurrence in kitchen faucet biofilms. MA and MI qPCR assays demonstrated 100% specificity and sensitivity when evaluated against 18 non-MA complex, 76 MA, and 17 MI isolates. Both assays detected approximately 1,000 cells from a diluted cell stock inoculated on a sampling swab 100% of the time. DNA analysis by qPCR indicated that 35.3, 56.9 and 11.8% of the 51 kitchen faucet biofilm samples collected contained MA, MI, and MAP, respectively. This study introduces novel qPCR assays designed to specifically detect MA and MI in biofilm. Results support the use of qPCR as an alternative to culture for detection and enumeration of MA, MI, and MAP in microbiologically complex samples.

Key words | biofilm, *M. avium*, *M. avium* subsp. *paratuberculosis*, *M. intracellulare*, qPCR

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INTRODUCTION

Nontuberculous mycobacteria (NTM) cause a considerable amount of waterborne illness in the USA, costing nearly $500 M in hospitalizations per year (Collier *et al.* 2012). NTM are the causative agents of pulmonary, skin, soft tissue, and disseminated infections, among others. Pulmonary NTM infections account for almost half of all NTM hospitalizations and are typically caused by *Mycobacterium avium* (MA) and *Mycobacterium intracellulare* (MI) (Griffith *et al.* 2007; Kasperbauer & Daley 2008). Although information relating risk factors to mycobacteria infection is limited, a major source of human exposure is drinking water and several reports have described occurrence of MA and MI in drinking water distribution systems (von Reyn *et al.* 1994; Eaton *et al.* 1995; Falkinham 1996, 2002, 2009; Aronson *et al.* 1999; Covert *et al.* 1999; Falkinham *et al.* 2001; Vaerewijck *et al.* 2005; Reed *et al.* 2006; Marciano-Cabral *et al.* 2010).

Recent epidemiological investigations suggest rates of NTM infection are on the rise, elevating the importance of understanding the ecology of NTM in drinking water (Marras *et al.* 2007; Cassidy *et al.* 2009). The majority of occurrence studies to date have focused on the analysis of bulk water samples using culture methods. Few studies have attempted to determine the source of mycobacteria in bulk water, but sloughing biofilm from surfaces of drinking water pipes has been implicated (Falkinham *et al.* 2001;

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Feazel *et al.* 2009). Unique characteristics associated with MA and MI, such as their slow growth rate, ability to survive in nutrient-poor environments, resistance to disinfectants and high temperatures, and the hydrophobicity and impermeability of their outer membrane enable them to thrive in drinking water biofilms (Taylor *et al.* 2000; Falkinham *et al.* 2001; Vaerewijck *et al.* 2005; Torvinen *et al.* 2007). However, due to their slow growth rate, culturing mycobacteria requires days to months to produce results. Over-growth of non-target organisms results in frequent sample loss. Development of a rapid molecular method such as quantitative polymerase chain reaction (qPCR) would enable faster results and detection of even the most difficult-to-culture mycobacteria. The aim of this study was to evaluate two novel qPCR assays targeting the 16S rRNA genes of MA and MI while investigating their occurrence in drinking water biofilms obtained from kitchen faucets in homes. Biofilm samples were also analyzed for *Mycobacterium avium* subsp. *paratuberculosis* (MAP) using two previously reported qPCR assays, the IS*900* assay and the Target 251 assay, which is more specific but less sensitive than the former (Beumer *et al.* 2010). This research provides data supporting the use of qPCR for detection of MA, MI, and MAP in drinking water biofilm; an important tool that can increase our understanding of the ecology of these opportunistic pathogens and advance efforts to control them.

METHODS

QPCR assay development

MA- and MI-specific primers and probes were designed from an alignment of 16S rDNA sequences (>1,300 bp) including four MA strains (GenBank Accession numbers AJ536037, X52934, NR_102855.1, M61672.1), seven MI strains (AJ536036, GQ153276.1, X529271.1, X88916, X88919, X88920, X88921), 19 non-MAC *Mycobacterium* species (X52923, X55589, X55603, AF480598, AF058712, X52932, AF480601, X52930, M29571, AF480603, X55594, U92090, EU379247, HM011256, U57632, U84502, X82459, AY208857, DQ536403.2), and *Nocardia abscessus* (AF430018) using Lasergene (DNASTAR, Madison, WI) and Primer Express (Applied Biosystems, Foster City, CA). The MA assay was designed to detect all subspecies of *M. avium*. Primers and probes were evaluated *in silico* for specificity using the PrimerBlast tool at NCBI (www.ncbi.nlm.nih.gov) prior to *in vitro* testing (Ye *et al.* 2012). The final MA and MI qPCR assays utilize the same forward primer and probe, but MA- and MI-specific reverse primers differed by 6 bp (Table 1).

Specificity and sensitivity analyses

Eighteen non-MAC *Mycobacterium* reference strains, and 76 MA and 17 MI isolates obtained from humans and

Table 1 | Real-time PCR assays for detection of MA, MI, and MAP

Target	Sequence (5′ to 3′)	Product size (bp)	Calibration model parameter	Coefficient of determination, R^2	Amplification efficiency[a]	Reference
M. avium (16S rRNA)	F: GGGTGAGTAACACGTGTGCAA; R: CCAGAAGACATGCGTCGTGA; P: TGCACTTCGGGATAAGCCTGGGAAA	97	$Y = -3.01x + 40.6$	0.97	1.1	This study
MI (16S rRNA)	F: GGGTGAGTAACACGTGTGCAA; R: CCACCTAAAGACATGCGACTAAA; P: TGCACTTCGGGATAAGCCTGGGAAA	100	$Y = -3.05x + 38.6$	0.98	1.1	This study
MAP (IS*900*)	F: CCGCTAATTGAGAGATGCGATTGG; R: ATTCAACTCCAGCAGCGCGGCCTCG; P: TCCACGCCCGCCCAGACAGG	230				Kim *et al.* (2002)
MAP (Target 251)	F: GCAAGACGTTCATGGGAA; R: GCGTAACTCAGCGAACAACA; P: CTGACTTCACGATGCGGTTCTTC	200				Rajeev *et al.* (2005)

F: forward primer; R: reverse primer; P: probe (5′-FAM and 3′-TAMRA).
[a]US Environmental Protection Agency (2009).

environmental sources were used to test specificity and sensitivity of the MA and MI qPCR assays (Tables 2, 3(a) and 3(b)). Reference strains were obtained from ATCC, Los Angeles County Hospital, and the Centers for Disease Control and Prevention. Human and environmental MAC isolates were described previously (Aronson *et al.* 1999; Yoder *et al.* 1999). Strains were grown on Middlebrook 7H10 agar supplemented with oleic albumin dextrose catalase (OADC) enrichment (Becton-Dickinson, Germany) and incubated at 37 °C for a minimum of 2 weeks prior to DNA extraction using the Wizard® Genomic DNA Purification Kit (Promega Corporation, Madison, WI.).

Analytical sensitivity was evaluated using reference strains MA ATCC 76102 and MI ATCC 13950. Strains were propagated in 20 mL Middlebrook 7H9 broth containing albumin dextrose catalase (ADC) and 2 mg·L^{-1} mycobactin J (Allied Monitor, Fayette, MO), pelleted and washed three times with sterile phosphate-buffered saline (PBS) pH 7.0, and resuspended in 5 mL sterile PBS. The

Table 2 | List of non-MAC strains used in specificity analysis

Non-MAC isolates	ATCC No.
M. septicum	700,731
M. parvum	49,939
M. abscessus	[a]
M. abscessus	[a]
M. abscessus	[a]
M. abscessus	[a]
M. peregrinum	[a]
Nocardia cyriacigeorgica	[a]
M. chelonae	19,977
M. bovis	35,743
M. kansasii	12,478
M. fortuitum	6841
M. fortuitum	6842
M. fortuitum	[a]
M. senegalense	35,796
M. margentense	700,351
M. gordonei	[a]
M. simiae	25,275

[a]Isolates donated by CDC, Los Angeles County Hospital.

Table 3 | (a) Mean C_q of *M. avium* isolates analyzed by *M. avium* and MI qPCR assays

MA isolate ID	MA mean C_q	MI mean C_q	MA isolate ID	MA mean C_q	MI mean C_q
D41CO1	20.26	–	D20BNIN	21.14	–
D10CN4W	21.93	–	DIDCC5	16.31	–
CA3	20.34	–	H08FC8N	20.47	–
W292	20.20	–	H14FC8N	18.14	–
H10B01	20.66	–	H13AC8p	20.45	–
D20DN8N	20.85	–	H01B01	20.05	–
W209	19.05	–	H08FC8N	27.08	–
B07B01	23.57	–	H07AC8N	17.58	–
H13AC8P	20.79	–	H05DN5	20.63	–
CA2	21.87	–	D55AC8N	19.47	–
H07AC8N	21.02	–	D20DN8N	24.23	–
H05BN5	23.24	–	D20CO5N	19.40	–
W290	19.81	–	D10CC5tlsp	21.37	–
CA7	19.42	–	CW5	20.20	–
W289	19.74	–	D41EC8N	18.65	–
W205	20.29	–	D41BC8N	18.06	–
W274	19.18	–	D41E08N	18.59	–
H13AC8P	20.70	–	D55A01	19.12	–
H14FC8N	18.68	–	F86	17.59	–
D20DN8N	19.87	–	CA7	19.34	–
H14FC8N	20.53	–	W354	19.26	–
W291	22.64	–	CA2	25.54	–
Bla	19.26	–	CA3	25.20	–
W347	18.28	–	CW68	17.55	–
W351	19.76	–	W359	17.82	–
H08AC8N	19.28	–	W352-1	19.90	–
CA4	19.36	–	F72B	19.67	–
CA5	20.10	–	W348	22.26	–
W46	20.45	–	W317	22.77	–
W88	28.23	–	W129	19.05	–
F72C	21.79	–	W125	19.06	–
F100	17.51	–	W80	20.30	–
H05BN5be	20.02	–	W41	18.99	–
F72b	19.93	–	W17	20.02	–
F103B	21.54	–	W10	18.22	–
CW4	19.64	–	CW26	23.25	–
W359	23.77	–	CW12	17.76	–
F7A	20.04	–	CW36	19.88	–

Table 3 | (b) Mean C_q of MI isolates analyzed by *M. avium* and MI qPCR assays

MI isolate ID	MA mean C_q	MI mean C_q	MI isolate ID	MA mean C_q	MI mean C_q
D09FCLC	–	16.63	H15DC8N	–	16.33
H07EN81	–	34.90	D01ACFB	–	16.16
H03AN5st	–	22.42	D09FCLC	–	16.46
W173	–	15.92	H15CC1	–	15.88
CW6-8	–	17.23	D09CC5be	–	18.51
W2	–	17.53	H03AC8a	–	39.75
R10N6	–	17.71	D01AC5	–	32.87
B03CC8N	–	18.76	B07FC5N	–	36.80
D09F03beap	–	18.88			

concentration of cells was determined in triplicate by enumerating colonies obtained from spread plating serial dilutions on Middlebrook 7H10 agar supplemented with OADC and mycobactin J. Analytical sensitivity, defined as the lowest approximate number of *M. avium* and MI cells per sample that could be detected 100% of the time by qPCR (Beumer *et al.* 2010), was determined by spiking 10-fold serial dilutions of cells from frozen cell stocks ranging from 10^3 to a theoretical single cell onto five sterile swabs which were processed identically to biofilm samples.

Sample collection

Biofilm samples were collected from kitchen faucets in 51 different homes in the midwest region of the United States from June through August, 2010. Residents of homes providing samples in this study received water from one of five different public water systems that were either small (serving 500–3,300 people), large (serving 10,001–100,000 people), or very large (serving >100,000 people) systems. These systems used either groundwater, surface water, or a mixture of both as source water, and a variety of different treatments, but all five systems used chlorine as the primary disinfectant. Two sterile cotton swab samplers, each containing 10 mL Butterfield's phosphate buffer (Weber Scientific, Hamilton, NJ), were given to each resident. Residents swabbed the interior of their kitchen faucet, or the exterior surface of their faucet aerator if it could not be removed, first thing in the morning before faucets were used. Swabs were transported to the laboratory on ice and biofilm was resuspended by vortexing swabs for 2 min. The resulting biofilm slurry was immediately processed for protein estimation and DNA isolation (described below).

DNA isolation and protein analysis

DNA from biofilm was purified using a bead beating method described previously (Beumer *et al.* 2010). Briefly, 10 mL biofilm slurry was vacuum filtered through a 47.0 mm, 0.45 μm polycarbonate membrane (GE Water & Process Technologies, Trevose, PA) and the membrane transferred to a sterile 2 mL conical microcentrifuge tube containing 0.3 g of 0.1 mm glass beads (Biospec Products Inc., Bartlesville, OK). Microorganisms were lysed by adding 500 μL WaterMaster Lysis Solution (EpiCentre, Madison, WI) and bead-milled in a Mini-beadbeater (BioSpec Products Inc.) for 3 min. DNA was purified from the crude lysate using selected WaterMaster DNA Purification Kit reagents (Epicentre) and modified WaterMaster protocol (Beumer *et al.* 2010).

The size, shape, and construction materials of kitchen faucets are highly variable, as is the manner in which individuals swab them. To normalize for sample-to-sample variation, protein concentrations were determined using 100 μL aliquots of biofilm slurry and the Micro BCA™ Protein Assay Kit (Thermo Scientific, Inc., Rockford, IL) and a Spectramox M2 spectrophotometer (absorbance = 562 nm; Molecular Devices, Sunnyvale, CA). Estimates of mycobacteria were normalized to amount of protein (μg) in each sample.

Amplification by qPCR

The TaqMan Exogenous internal positive control (IPC) (Applied Biosystems) was included to detect inhibition. Amplification was performed using 5 μL DNA extract, 12.5 μL TaqMan Environmental Master Mix 2.0 (Applied Biosystems, Foster City, CA), 2.5 μL Exogenous IPC mix, 0.5 μL Exogenous IPC DNA, 1.0 μL 0.5 mg·mL^{-1} bovine serum albumin, 900 nM each primer, and 200 nM probe in a total volume of 25 μL. All reactions were carried out in an ABI Model 7900 HT sequence detection system (Applied Biosystems, Foster City, CA) in triplicate. Thermocycler conditions consisted of 50 °C for 2 min, 95 °C for 10 min, 40 cycles of 95 °C for 15 sec, and 60 °C for 1 min. Data were analyzed with cycle quantification (C_q) threshold set at 0.15.

Standards and controls

Five milliliters of biofilm sample was spiked with approximately 10^3 *M. avium* ATCC 76102, MI ATCC 13950, and MAP ATCC 49164 cells and processed in a similar way to non-spiked samples to determine the recovery efficiency of the method. Standard curves, filter blanks, sterile swabs, no template controls and IPCs were run alongside all samples. Master standard curves were generated for each assay from six independent sets of serially diluted purified genomic DNA from MA, MI, and MAP ranging from approximately 1 to 10^5 target copies analyzed in triplicate. Absolute quantification from master standard curves was performed to estimate MA, MI 16S rRNA and MAP IS*900* target copies in each biofilm sample (Sivaganesan *et al.* 2010).

Biofilm samples positive for the MAP-specific IS*900* qPCR assay were also analyzed with the Target 251 qPCR assay in duplicate as a presence/absence confirmation assay. IS*900* is a common target used to identify MAP and has an average of 14–18 copies per genome. Target 251 corresponds to a portion of the MAP-specific 2765c gene which has one copy per genome (Kim *et al.* 2002; David Alexander, personal communication). Only samples positive for both assays were considered positive for MAP.

Data analyses

Concentration estimates were calculated as mean target copies μg^{-1} of protein. Associations in the concentrations of protein and MA, MI, and MAP target copies were evaluated using Pearson's correlation test. A one-way ANOVA was used to determine if protein or target copy concentrations were influenced by sampling inside or outside the kitchen faucet grate or by location of sample sites to water treatment facilities. Statistical analyses were performed using SPSS, version 16 (SPSS, Inc., Chicago, IL).

RESULTS

Specificity and sensitivity analyses

Experimental analysis of the MA and MI qPCR assays with 18 non-MAC strains confirmed that both assays were 100% specific. Furthermore, 17 MI isolates correctly tested negative with the MA qPCR assay and 76 MA isolates correctly tested negative with the MI qPCR assay (Tables 2, 3(a) and 3(b)). Sensitivity analysis of the qPCR assays on DNA extracts from 76 MA and 17 MI culture isolates showed that both the MA and MI assays were 100% sensitive. Evaluation of the analytical sensitivity indicated that the MA and MI qPCR assays were able to detect dilutions containing theoretically 1,000 cells spiked on a sampling swab 100% (5/5) of the time. When >100 cells were spiked onto the sampling swab, MA and MI were detected 93 and 74% of the time, respectively. The specificity and sensitivity of MAP IS*900* and Target 251 qPCR assays were previously evaluated (Kim *et al.* 2002; Rajeev *et al.* 2005; Beumer *et al.* 2010).

Biofilm samples spiked with MA, MI, and MAP cells showed no inhibition (C_q values were ±2.5 standard deviations of the means of the C_q values of the corresponding control samples) and all method blanks ($n = 96$) and no template controls were negative. C_q values from IPC and spike analyses indicated that inhibition or interference did not impact the efficiency of DNA recovery or amplification. None of the spiked biofilm samples had C_q values outside the acceptable range.

Occurrence in biofilm samples

MA, MI, and MAP (IS*900* assay) genetic targets were found in 35.3, 56.9, and 47.1% of the drinking water biofilm samples analyzed (Figure 1). Target copy estimates ranged from 15 to 11,959 copies μg^{-1} protein, 2 to 15,626 copies

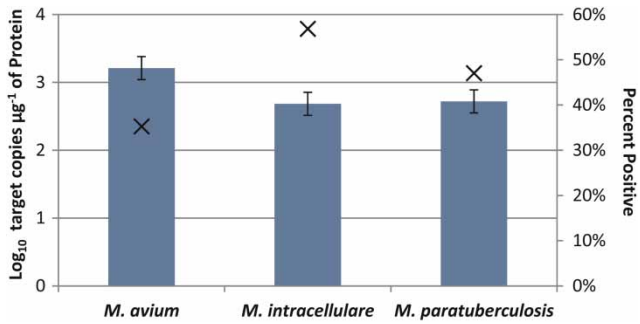

Figure 1 | Mean $\log_{10}$ estimates of MA, MI, and IS*900* MAP target copies (bars) and percent positive (crosses) in kitchen faucet biofilm samples analyzed. The proportion of samples positive for the MAP confirmatory Target 251 qPCR assay was 11.8% and is not shown.

μg^{-1} protein, and 5 to 12,621 copies μg^{-1} protein, respectively. Of the 21 samples positive with the MAP IS*900* assay, only six (11.8%) were confirmed positive with the Target 251 assay. Protein concentrations ranged from 3.3 to 39.5 μg protein mL^{-1} of biofilm sample. No significant associations between the levels of protein and estimates of MI or MAP target copies in biofilm samples were observed. However, protein concentrations were found to be positively associated with MA target copy estimates ($r = 0.57$; $p < 0.05$). No significant differences were detected between the location of sample collection (interior of faucet or exterior surface of aerator) with the occurrence of MA or MI target copies or protein concentrations except with MAP where higher target copy estimates were detected inside the faucet compared to the exterior surface ($p < 0.05$).

DISCUSSION

While MAP was detected in 47.1% of biofilm samples with the IS*900* qPCR assay, only 11.8% of biofilm samples were confirmed positive with the Target 251 assay. The IS*900* assay is more sensitive than the Target 251 assay, having a limit of detection (LOD) ten-fold lower than the LOD of the Target 251 assay (Beumer *et al.* 2010). This observation may be due to a difference in the number of IS*900* and Target 251 gene copies in the MAP genome: there are an estimated 14 to 18 copies of IS*900* and one copy of Target 251 per genome (Imirzalioglu *et al.* 2011). The qPCR products from biofilm samples testing positive by both IS*900* and Target 251 assays contained single bands of the correct size (230 and 200 bp, respectively) by gel electrophoresis. Of the six biofilm samples confirmed positive for MAP by the Target 251 assay, only four were positive with the MA qPCR assay. Sampling variability associated with the low concentrations of Target 251 and 16S target sequences which both occur as single copies in the MA and MAP genomes, as well as differences in amplification efficiency, may explain the differences in detection.

MI occurred more frequently than MA in kitchen faucet biofilm samples (Figure 1). These findings support those of Falkinham *et al.* (2001) where MI was more frequently recovered from biofilm samples and MA from bulk water samples using a culture method. However, the total estimated concentration (mean $\log_{10}$ target copy number from all samples) of MA was greater than MI. Investigations on the diversity of opportunistic pathogens in biofilms from showerheads detected MA in 78% of biofilm samples by qPCR, but concentrations were not reported (Feazel *et al.* 2009). A recent finding by Wallace *et al.* (2013) suggests that the MI qPCR assay may be amplifying *Mycobacterium chimaera*; however, more studies may be needed to confirm its presence in these biofilm samples. Although the frequency of occurrence of MAP (IS*900* qPCR assay) in biofilm samples reported by Beumer *et al.* (2010) was 6.4 times higher than in this study, target copy concentrations were similar: <100 target copies in 68 and 100% in the previous and current studies, respectively.

The qPCR assays described here are not the first to target mycobacteria. Torvinen *et al.* (2010) and Radomski *et al.* (2010) described genus-specific *Mycobacterium* qPCR assays targeting the 16S rDNA for estimating mycobacteria occurrence in surface water and household dust samples, respectively. QPCR assays have been developed to detect MA (Feazel *et al.* 2009) and other clinically relevant species of mycobacteria (Richardson *et al.* 2009) using 16S species-specific primers and SYBR Green detection. This approach may be sufficient for certain applications, such as identification of cultured isolates, but may be problematic for quantification from biofilm due to non-specific binding of SYBR Green to dsDNA and lack of sequence-specific probes. Restrepo *et al.* (2006) developed a qPCR assay targeting MA 16S rDNA for identification of this pathogen in paucibacillary disease. Specificity of the assay was evaluated on one clinical MA isolate. Leung *et al.* (2009) developed qPCR assays targeting MA and MI 16S rDNA using universal bacterial primers and MA or MI-specific probes for purposes of identifying *Mycobacterium* isolates in a high-throughput public health laboratory. In that study, approximately 8% of clinical MA isolates were positive with the MI assay and sensitivity of the assays on environmental samples was not evaluated. Since most mycobacteriosis is acquired from human exposure to contaminated drinking water and nearly 50% of hospitalizations in the USA are caused by MAC (Collier *et al.* 2012), accurate estimates of MA and MI occurrence in drinking water and biofilm is essential and necessitated development of new MA- and MI-specific qPCR assays for this purpose. As we obtain

more genome sequence information from clinical and environmental isolates, we hope to improve these assays by incorporating additional unique targets for MA and MI to verify their detection in biofilm samples.

A major challenge of detecting and quantifying mycobacteria by culture in environmental samples, especially biofilm, is the rapid over-growth of non-target microorganisms that often prevent their isolation. Culturing of mycobacteria requires days to months to produce results. Interference and/or contamination by non-target microorganisms often occur resulting in frequent loss of samples. We attempted to culture MA and MI in this study, using the method described in Covert *et al.* (1999), to confirm our qPCR results (data not shown). However, due to the microbiological complexity of the kitchen tap biofilm, the majority of culture samples had to be discarded prior to the 8-week minimum incubation period because of overgrowth of non-target organisms. We recovered 113 isolates from biofilm, none of which were MA or MI by 16S rDNA and *hsp*65 sequence analysis. Rapid qPCR methods enable detection of even the most difficult-to-culture mycobacteria while avoiding sample loss. Results from this study demonstrate that qPCR analysis of mycobacteria from biofilm samples can be more easily performed without interference being a major limitation.

To date, little information is known about the occurrence of mycobacteria in drinking water biofilms or the abiotic and biotic factors that influence their ecology. Geographic distribution and proximity of sample sites to water treatment facilities were examined in this study but the limited sample size ($n = 51$) provided insufficient statistical power to determine significant trends. Falkinham *et al.* (2001) found mycobacteria more frequently in distribution system samples than at points farther from the treatment facility. For the majority of samples collected in this study, age of the kitchen plumbing was not known. At least three of the biofilm samples were from newly replaced faucets and MA, MI, and MAP were absent in those samples. Temperature or seasonal variability, treatment processes, nutrient levels in source water, and water flow in distribution systems have been found to contribute to the occurrence of mycobacteria in biofilm (Kubalek & Komenda 1995; Falkinham *et al.* 2001; Torvinen *et al.* 2007).

Although investigation of mycobacteria occurrence in the environment by qPCR has shown promise (Khan & Yadav 2004; Ravva & Stanker 2005; Feazel *et al.* 2009; Beumer *et al.* 2010; Radomski *et al.* 2010; Torvinen *et al.* 2010), a disadvantage of qPCR is its inability to distinguish between live and dead cells. Detection of live MAP cells with the use of qPCR coupled with propidium monoazide has been investigated although further research with MA and MI is needed (Kralik *et al.* 2010). Nevertheless, the advantages of qPCR for detection of mycobacteria are greater than the culture method: rapid time-to-results and minimal sample loss.

CONCLUSIONS

The use of qPCR for environmental mycobacteria detection enables quantification of difficult-to-culture mycobacteria, overcoming a major limitation of the culture method. To our knowledge, this is the first report on the use of MA and MI qPCR assays targeting 16S rRNA genes to investigate occurrence in biofilm samples from kitchen faucets, a site of human exposure to water and aerosols. Further research should be conducted to better understand the significance of qPCR-estimated concentrations of mycobacteria in biofilm, as well as the concentrations and mechanisms with which the organisms are transported from pipe-attached biofilm to drinking water. Information from this study supports the use of a rapid molecular method to better assess risk of exposure to mycobacteria in drinking water biofilm.

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