Supplemental Information

Molecular Survey of Occurrence of *Legionella* spp., *Mycobacterium* spp., *Pseudomonas aeruginosa* and Amoeba Hosts in Two Chloraminated Drinking Water Distribution Systems

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Table S1 qPCR primers, probes, and assay conditions used in this study

Targeted organisms	**Targeted genes**	**Sequences (5’-3’)**	**Program**		**Amplicon (bp)**	**Reference**
			Initial denaturation and enzyme activation	Denaturing / Annealing / Extension		
***Legionella* spp.**	23S rRNA	Leg23SF: CCCATGAAGCCCGTTGAA Leg23SR:ACAATCAGCCAATTAGTACGAGTTAGC Probe: HEX-TCCACACCTCGCCTATCAACGTCGTAGT	95 ^{0}C for 2 min	40 cycles of 95 ^{0}C for 5 s and 58.5 ^{0}C for 10 s	92	(3)
L. pneumophila	*mip*	LmipF:AAAGGCATGCAAGACGCTATG LmipR: GAAACTTGTTAAGAACGTCTTTCATTTG Probe: FAM-TGGCGCTCAATTGGCTTTAACCGA	95 ^{0}C for 2 min	40 cycles of 95 ^{0}C for 5 s and 60 ^{0}C for 10 s	78	(3)
***Mycobacterium* spp.**	16S rRNA	110F: CCTGGGAAACTGGGTCTAAT I571R: CGCACGCTCACAGTTA H19R: FAM-TTTCACGAACAACGCGACAAACT	95 ^{0}C for 2 min	45 cycles of 95 ^{0}C for 5 s, 55 ^{0}C for 15 s and 72 ^{0}C for 10 s	462	(4)
M. avium	16S rRNA	MycavF: AGAGTTTGATCCTGGCTCAG MycavR: ACCAGAAGACATGCGTCTTG	98 ^{0}C for 2 min	40 cycles of 98 ^{0}C for 5 s and 68 ^{0}C for 18 s	180	(7)
H. vermiformis	18S rRNA	Hv1227F: TTACGAGGTCAGGACACTGT Hv1728R: GACCATCCGGAGTTCTCG	98 ^{0}C for 2 min	40 cycles of 98 ^{0}C for 5 s and 72 ^{0}C for 18 s	502	(2)
Acanthamoeba spp.	18S rRNA	TaqAcF1: CGACCAGCGATTAGGAGACG TaqAcR1: CCGACGCCAAGGACGAC Probe:	95 ^{0}C for 2 min	40 cycles of 95 ^{0}C for 5 s and 60 ^{0}C for 10 s	66	(5)

		FAM-TGAATACAAAACACCACCATCGGCGC				
P. aeruginosa	*ecf*X & *gyr*B	*ecf*X-F: CGCATGCCTATCAGGCGTT *ecf*X-R: GAACTGCCCAGGTGCTTGC Probe: HEX-ATGGCGAGTTGCTGCGCTTCCT *gyr*B-F: CCTGACCATCCGTCGCCACAAC *gyr*B-R: CGCAGCAGGATGCCGACGCC Probe: FAM-CCGTGGTGGTAGACCTGTTCCCAGACC	95 ^{0}C for 2 min	50 cycles of 95 ^{0}C for 5 s and 60 ^{0}C for 10 s	63 (*ecf*X) 220 (*gyr*B)	(1)
Total Bacteria	16S rRNA	BACT1369F: CGGTGAATACGTTCYCGG PROK: GGWTACCTTGTTACGACTT	98 ^{0}C for 2 min	40 cycles of 98 ^{0}C for 5 s and 55 ^{0}C for 5 s	124	(6)

q-PCR specificity validation using drinking water samples

DNA amplicons obtained from each q-PCR amplification were cloned to TOPO® TA cloning® kit for sequencing (Invitrogen, Carlsbad, CA) according to manufacturer's protocol. Inserts were amplified from each clone using vector-specific primer M13F and M13R. Only M13 PCR products with insert length >100 bp were visualized on a 2% agarose gel using a Molecular Image® ChemiDocTM XRS system (Bio-Rad, CA) to eliminate clones without inserts prior to sequencing. M13 PCR products with insert length ≤100 bp were sequenced without evaluating insert presence. Sequences of M13 amplicons were analyzed using the NCBI Basic Local Alignment Search Tool (BLAST).

Table S2 Blast results for *Legionella* spp. q-PCR assay

Sample	bp	%	gap	Acc #	Highest similarity
C1	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)
C2	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)
C3	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)
C4	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)
C5	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)
C6	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)
C7	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)
C8	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)
C9	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)
C10	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)

C11	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)
C12	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)
C13	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)
C15	83/92	90	4	Z30458.1	*L.rubrilucens* WA-270A-C2 genes for 23S ribosomal RNA and 5S ribosomal RNA
C16	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)
C17	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)
C18	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)
C19	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)
C20	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)
C21	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)
C23	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)
C24	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)
C25	90/91	99	0	CP003192.1	*Legionella pneumophila* subsp. *pneumophila* ATCC 43290 (rRNA-23S ribosomal RNA)

Note: Sequencing of C14 and C22 failed.

Table S3 Blast results for *L. pneumophila* q-PCR assay

Sample	bp	%	gap	Acc #	Highest similarity
C26	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds
C27	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds
C28	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds
C29	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds
C30	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds
C31	76/78	97	1	JN697588.1	*Legionella pneumophila* culture-collection DMST:17221 macrophage infectivity potentiator (*mip*) gene, complete cds
C32	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds
C33	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds
C34	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds
C35	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds

C36	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds
C37	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds
C38	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds
C39	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds
C40	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds
C41	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds
C42	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds
C43	76/78	97	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds
C44	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds
C45	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds
C46	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds

C47	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds
C48	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds
C49	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds
C50	77/78	99	1	JN697590.1	*Legionella pneumophila* culture-collection DMST:24600 macrophage infectivity potentiator (*mip*) gene, complete cds

Table S4 Blast results for *Acanthamoeba* spp. q-PCR assay

Sample	bp	%	gap	Acc #	Highest similarity
C51	63/63	100	0	FJ042636.1	*Acanthamoeba* sp. Had_008 18S ribosomal RNA gene, partial sequence Length=425
C52	64/64	100	0	FJ042636.1	*Acanthamoeba* sp. Had_008 18S ribosomal RNA gene, partial sequence Length=425
C53	229/239	96	0	BA000040.2	*Bradyrhizobium japonicum* USDA 110 DNA, complete genome
C54					No match
C55	63/63	100	0	FJ042636.1	*Acanthamoeba* sp. Had_008 18S ribosomal RNA gene, partial sequence Length=425
C56	63/63	100	0	FJ042636.1	*Acanthamoeba* sp. Had_008 18S ribosomal RNA gene, partial sequence Length=425
C57	64/64	100	0	FJ042636.1	*Acanthamoeba* sp. Had_008 18S ribosomal RNA gene, partial sequence Length=425
C58	63/63	100	0	FJ042636.1	*Acanthamoeba* sp. Had_008 18S ribosomal RNA gene, partial sequence Length=425
C59	63/63	100	0	FJ042636.1	*Acanthamoeba* sp. Had_008 18S ribosomal RNA gene, partial sequence Length=425
C60	63/63	100	0	FJ042636.1	*Acanthamoeba* sp. Had_008 18S ribosomal RNA gene, partial sequence Length=425
C61	63/63	100	0	FJ042636.1	*Acanthamoeba* sp. Had_008 18S ribosomal RNA gene, partial sequence Length=425
C62	62/63	98	0	FJ042636.1	*Acanthamoeba* sp. Had_008 18S ribosomal RNA gene, partial sequence Length=425
C63	65/65	100	0	JQ408990.1	*Acanthamoeba* sp. A-T4-3-146 18S ribosomal RNA gene, partial sequence
C64	64/65	98	1	JQ408990.1	*Acanthamoeba* sp. A-T4-3-146 18S ribosomal RNA gene, partial sequence
C65	60/64	94	1	FJ042636.1	*Acanthamoeba* sp. Had_008 18S ribosomal RNA gene, partial sequence Length=425

C68	62/65	95	2	JQ408985.1	*Acanthamoeba* sp. A-T4-1-89 18S ribosomal RNA gene, partial sequence
C69	63/64	98	1	JQ031557.1	*Acanthamoeba* sp. AcaKM01 18S ribosomal RNA gene, partial sequence
C70	121/125	97	0	AP012280.1	*Pseudomonas aeruginosa* NCGM2.S1 DNA, two-component sensor
C71	63/64	98	1	JQ031557.1	*Acanthamoeba* sp. AcaKM01 18S ribosomal RNA gene, partial sequence
C72	64/65	98	1	JQ408990.1	*Acanthamoeba* sp. A-T4-3-146 18S ribosomal RNA gene, partial sequence
C73					No match
C74	63/64	98	1	JQ268236.1	Acanthamoeba sp. P4 18S ribosomal RNA gene, partial sequence
C75	63/64	98	1	JQ031557.1	*Acanthamoeba* sp. AcaKM01 18S ribosomal RNA gene, partial sequence
C76	63/64	98	1	JQ031557.1	*Acanthamoeba* sp. AcaKM01 18S ribosomal RNA gene, partial sequence
C77	64/65	98	1	JQ031557.1	*Acanthamoeba* sp. AcaKM01 18S ribosomal RNA gene, partial sequence
C78	62/64	97	1	JQ268236.1	*Acanthamoeba* sp. P4 18S ribosomal RNA gene, partial sequence

Note: Sequencing of C66 and C67 failed. M13 PCR products of C53, C54, C70, C73 can’t be amplified using *Acanthamoeba* spp. q-PCR (with a probe).

Table S5 Blast results for *Mycobacterium* spp. q-PCR

Sample	bp	%	gap	Acc #	Highest similarity
C91	460/464	99	2	NR_042919.1	*Mycobacterium mucogenicum* strain ATCC 49650 16S ribosomal RNA
C92	452/462	98	2	NR_043002.1	*Mycobacterium massiliense* strain CCUG 48898 16S ribosomal RNA
C93	460/464	99	2	NR_042919.1	*Mycobacterium mucogenicum* strain ATCC 49650 16S ribosomal RNA
C94	467/477	98	0	NR_043588.1	*Mycobacterium arupense* strain AR30097 16S ribosomal RNA
C95	460/464	99	2	NR_042919.1	*Mycobacterium mucogenicum* strain ATCC 49650 16S ribosomal RNA
C96	455/462	98	1	NR_042280.1	*Mycobacterium llatzerense* strain : MG13 16S ribosomal RNA
C97	460/463	99	1	NR_042919.1	*Mycobacterium mucogenicum* strain ATCC 49650 16S ribosomal RNA
C98	458/462	99	1	NR_042919.1	*Mycobacterium mucogenicum* strain ATCC 49650 16S ribosomal RNA
C99	456/462	99	1	NR_042280.1	*Mycobacterium llatzerense* strain : MG13 16S ribosomal RNA
C100	470/477	99	1	NR_043588.1	*Mycobacterium arupense* strain AR30097 16S ribosomal RNA
C101	461/463	99	0	NR_042919.1	*Mycobacterium mucogenicum* strain ATCC 49650 16S ribosomal RNA
C102	457/463	99	0	NR_042923.1	*Mycobacterium farcinogenes* strain NCTC 10955 16S ribosomal RNA
C103	459/463	99	0	NR_042919.1	*Mycobacterium mucogenicum* strain ATCC 49650 16S ribosomal RNA
C104	463/470	99	2	NR_042919.1	*Mycobacterium mucogenicum* strain ATCC 49650 16S ribosomal RNA
C105	465/470	99	2	NR_042919.1	*Mycobacterium mucogenicum* strain ATCC 49650 16S ribosomal RNA
C106	459/463	99	0	NR_042919.1	*Mycobacterium mucogenicum* strain ATCC 49650 16S ribosomal RNA
C107	457/462	99	1	NR_042919.1	*Mycobacterium mucogenicum* strain ATCC 49650 16S ribosomal RNA
C108	470/476	99	1	NR_043588.1	*Mycobacterium arupense* strain AR30097 16S ribosomal RNA
C109	460/463	99	1	NR_042919.1	*Mycobacterium mucogenicum* strain ATCC 49650 16S ribosomal RNA
C111	454/462	98	1	NR_042919.1	*Mycobacterium mucogenicum* strain ATCC 49650 16S ribosomal RNA

C112	460/462	99	1	NR_042919.1	*Mycobacterium mucogenicum* strain ATCC 49650 16S ribosomal RNA
C113	456/462	99	1	NR_042280.1	*Mycobacterium llatzerense* strain : MG13 16S ribosomal RNA
C114	459/463	99	1	NR_042923.1	*Mycobacterium farcinogenes* strain NCTC 10955 16S ribosomal RNA
C115	468/476	98	1	NR_043588.1	*Mycobacterium arupense* strain AR30097 16S ribosomal RNA
C116	466/477	98	1	NR_043588.1	*Mycobacterium arupense* strain AR30097 16S ribosomal RNA
C117	466/477	98	1	NR_043588.1	*Mycobacterium arupense* strain AR30097 16S ribosomal RNA

Note: Sequencing of C110 and C118 failed

Table S6 Blast results for *H. vermiformis* q-PCR

Sample	bp	%	gap	Acc #	Highest similarity
C119	497/502	99	0	DQ407609.1	*Hartmannella vermiformis* isolate CT8.4 18S ribosomal RNA gene gene, partial sequence
C124	500/502	99	0	DQ407609.1	*Hartmannella vermiformis* isolate CT8.4 18S ribosomal RNA gene gene, partial sequence
C125	501/502	99	0	DQ407609.1	*Hartmannella vermiformis* isolate CT8.4 18S ribosomal RNA gene gene, partial sequence
C128	500/502	99	0	DQ407609.1	*Hartmannella vermiformis* isolate CT8.4 18S ribosomal RNA gene gene, partial sequence
C129	500/502	99	0	DQ407609.1	*Hartmannella vermiformis* isolate CT8.4 18S ribosomal RNA gene gene, partial sequence
C130	500/502	99	0	DQ407609.1	*Hartmannella vermiformis* isolate CT8.4 18S ribosomal RNA gene gene, partial sequence
C131	498/502	99	0	DQ407609.1	*Hartmannella vermiformis* isolate CT8.4 18S ribosomal RNA gene gene, partial sequence
C132	498/502	99	0	DQ407609.1	*Hartmannella vermiformis* isolate CT8.4 18S ribosomal RNA gene gene, partial sequence
C133	501/502	99	0	DQ407609.1	*Hartmannella vermiformis* isolate CT8.4 18S ribosomal RNA gene gene, partial sequence
C134	501/502	99	0	DQ407609.1	*Hartmannella vermiformis* isolate CT8.4 18S ribosomal RNA gene gene, partial sequence
C135	498/499	99	0	DQ407609.1	*Hartmannella vermiformis* isolate CT8.4 18S ribosomal RNA gene gene, partial sequence
C136	497/502	99	0	DQ407609.1	*Hartmannella vermiformis* isolate CT8.4 18S ribosomal RNA gene gene, partial sequence
C137	496/502	99	0	DQ407609.1	*Hartmannella vermiformis* isolate CT8.4 18S ribosomal RNA gene gene, partial sequence
C138	496/502	99	0	DQ407609.1	*Hartmannella vermiformis* isolate CT8.4 18S ribosomal RNA gene gene, partial

					sequence
C139	498/499	99	0	DQ407609.1	*Hartmannella vermiformis* isolate CT8.4 18S ribosomal RNA gene gene, partial sequence
C140	501/502	99	0	DQ407609.1	*Hartmannella vermiformis* isolate CT8.4 18S ribosomal RNA gene gene, partial sequence
C141	497/499	99	0	DQ407609.1	*Hartmannella vermiformis* isolate CT8.4 18S ribosomal RNA gene gene, partial sequence
C142	501/502	99	0	DQ407609.1	*Hartmannella vermiformis* isolate CT8.4 18S ribosomal RNA gene gene, partial sequence
C143	500/502	99	0	DQ407609.1	*Hartmannella vermiformis* isolate CT8.4 18S ribosomal RNA gene gene, partial sequence
C144	492/499	99	0	DQ407609.1	*Hartmannella vermiformis* isolate CT8.4 18S ribosomal RNA gene gene, partial sequence

Note: Sequencing of C120, C121, C122, C123, C126, C127 failed.

Table S7 Blast results for *P. aeruginosa* q-PCR

Sample	bp	%	gap	Acc #	Highest similarity
ecf1	62/63	98	1	AP012280.1	*Pseudomonas aeruginosa* NCGM2.S1 DNA, RNA polymerase ECF-subfamily sigma-70 factor
ecf2	63/63	100	0	AP012280.1	*Pseudomonas aeruginosa* NCGM2.S1 DNA, RNA polymerase ECF-subfamily sigma-70 factor
ecf3	56/56	100	0	AP012280.1	*Pseudomonas aeruginosa* NCGM2.S1 DNA, RNA polymerase ECF-subfamily sigma-70 factor
ecf4	65/65	100	0	AP012280.1	*Pseudomonas aeruginosa* NCGM2.S1 DNA, RNA polymerase ECF-subfamily sigma-70 factor
ecf5	63/63	100	0	AP012280.1	*Pseudomonas aeruginosa* NCGM2.S1 DNA, RNA polymerase ECF-subfamily sigma-70 factor
ecf6	61/64	95	1	AP012280.1	*Pseudomonas aeruginosa* NCGM2.S1 DNA, RNA polymerase ECF-subfamily sigma-70 factor
ecf7	62/63	98	1	AP012280.1	*Pseudomonas aeruginosa* NCGM2.S1 DNA, RNA polymerase ECF-subfamily sigma-70 factor
ecf9	62/63	98	1	AP012280.1	*Pseudomonas aeruginosa* NCGM2.S1 DNA, RNA polymerase ECF-subfamily sigma-70 factor
ecf10	65/65	100	0	AP012280.1	*Pseudomonas aeruginosa* NCGM2.S1 DNA, RNA polymerase ECF-subfamily sigma-70 factor
ecf11	64/65	98	0	AP012280.1	*Pseudomonas aeruginosa* NCGM2.S1 DNA, RNA polymerase ECF-subfamily sigma-70 factor
ecf12	63/63	100	0	AP012280.1	*Pseudomonas aeruginosa* NCGM2.S1 DNA, RNA polymerase ECF-subfamily sigma-70 factor
ecf13	65/65	100	0	AP012280.1	*Pseudomonas aeruginosa* NCGM2.S1 DNA, RNA polymerase ECF-subfamily sigma-70 factor
ecf14	65/65	100	0	AP012280.1	*Pseudomonas aeruginosa* NCGM2.S1 DNA, RNA polymerase ECF-subfamily sigma-70 factor
ecf15	69/70	99	1	AP012280.1	*Pseudomonas aeruginosa* NCGM2.S1 DNA, RNA polymerase ECF-subfamily

					sigma-70 factor
ecf16	63/63	100	0	AP012280.1	*Pseudomonas aeruginosa* NCGM2.S1 DNA, RNA polymerase ECF-subfamily sigma-70 factor
ecf17	63/63	100	0	AP012280.1	*Pseudomonas aeruginosa* NCGM2.S1 DNA, RNA polymerase ECF-subfamily sigma-70 factor
ecf18	62/63	99	1	AP012280.1	*Pseudomonas aeruginosa* NCGM2.S1 DNA, RNA polymerase ECF-subfamily sigma-70 factor
ecf19	63/63	100	0	AP012280.1	*Pseudomonas aeruginosa* NCGM2.S1 DNA, RNA polymerase ECF-subfamily sigma-70 factor
ecf20	65/65	100	0	AP012280.1	*Pseudomonas aeruginosa* NCGM2.S1 DNA, RNA polymerase ECF-subfamily sigma-70 factor
gyr1	70/79	89	0	AP012224.1	*Pseudogulbenkiania* sp. NH8B DNA, DNA protecting protein DprA
gyr2					No match
gyr3					No match
gyr4					No match
gyr5	70/79	89	0	AP012224.1	*Pseudogulbenkiania* sp. NH8B DNA, DNA protecting protein DprA
gyr6	222/222	100	0	CP002496.1	*Pseudomonas aeruginosa* M18, complete genome, DNA gyrase subunit B
gyr7	291/328	89	0	CP000356.1	*Sphingopyxis alaskensis* RB2256, phosphoribosylformylglycinamidine synthase subunit II
gyr8	70/79	89	0	AP012224.1	*Pseudogulbenkiania* sp. NH8B DNA, DNA protecting protein DprA
gyr9					No match
gyr10	133/156	85	0	CP002417.1	*Variovorax paradoxus* EPS, major facilitator superfamily MFS_1
gyr11	141/166	85	1	CP002417.1	*Variovorax paradoxus* EPS, major facilitator superfamily MFS_1
gyr13					No match
gyr14	139/163	85	1	CP002417.1	*Variovorax paradoxus* EPS, major facilitator superfamily MFS_1

gyr15	139/163	85	1	CP002417.1	*Variovorax paradoxus* EPS, major facilitator superfamily MFS_1
gyr17	187/218	86	16	AP012206.1	*Bradyrhizobium j*, aponicum USDA 6 DNA, glucarate dehydratase 5-dehydro-4-deoxyglucarate dehydratase
gyr18	70/79	89	0	AP012224.1	*Pseudogulbenkiania* sp. NH8B DNA, DNA protecting protein DprA
gyr20	70/79	89	0	AP012224.1	*Pseudogulbenkiania* sp. NH8B DNA, DNA protecting protein DprA

Note: Sequencing of ecf8, gyr16 and gyr19 failed. gyr12 is an empty vector without insert. q-PCR results of the M13 PCR products of gyr clones demonstrated that only gyr6 has amplification, indicating that the specificity of gyrB q-PCR assay strongly relied on the probe.

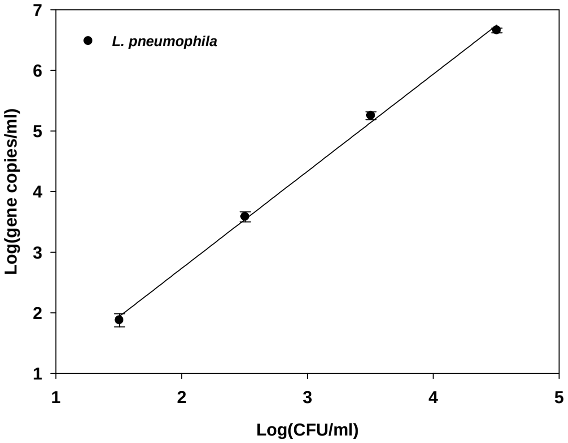
Table S8 Blast results for *M. avium* q-PCR

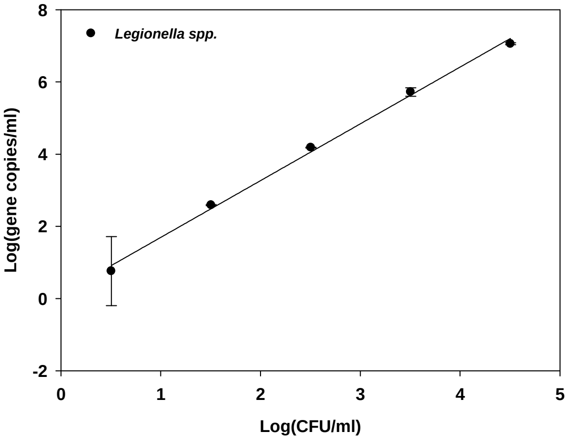
Sample	bp	%	gap	Acc #	Highest similarity
MAC1	189/191	99	0	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC2	189/191	99	0	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC3	191/191	100	0	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC4	186/187	99	0	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC5	190/193	98	1	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC6	187/187	100	1	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC7	190/193	98	1	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC8	186/187	98	1	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC9	192/193	99	1	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC10	189/191	99	1	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC11	187/187	100	0	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC12	191/191	100	0	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC13	191/191	100	0	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC14	190/193	98	1	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC15	193/193	100	0	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC16	192/194	99	1	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC17	185/187	99	0	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC18	190/191	99	0	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC19	191/191	100	0	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC20	189/191	99	0	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence

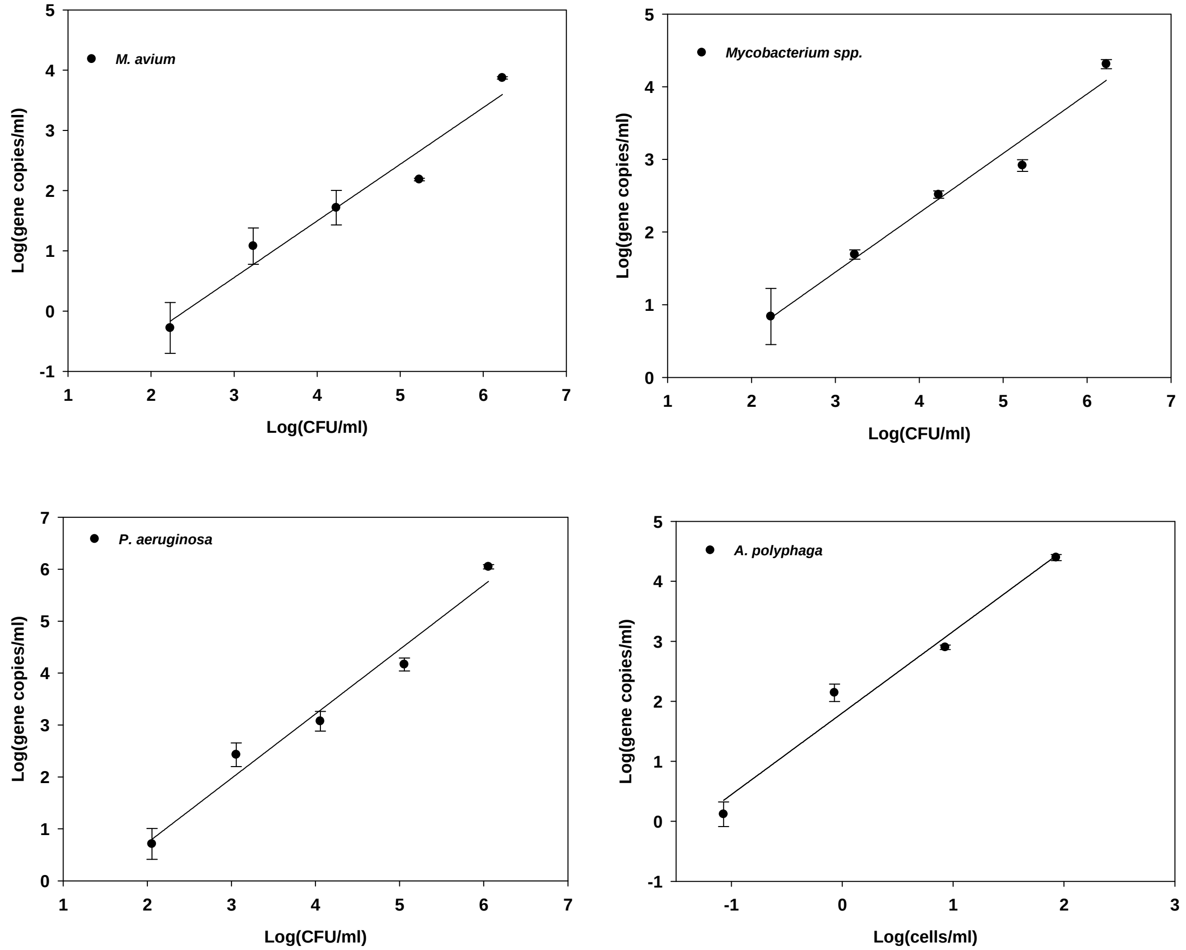
MAC21	190/191	99	0	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC22	189/191	99	0	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC23	193/193	100	0	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC24	192/193	99	1	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence
MAC25	193/193	100	0	JN899580.1	*Mycobacterium avium* strain E6 16S ribosomal RNA gene, partial sequence

Comparison of Recovery Efficiency and Limit of Quantification Using Culture and qPCR

Cultures of *L. pneumophila*, *M. avium*, *A. polyphage*, and *H. vermiformis* of known concentrations were serially diluted into 500 ml autoclaved drinking water, which were subjected to membrane filtering concentration using 0.22 µm-pore-size mixed cellulose ester filters (Millipore, Billerica, MA). The membranes were subsequently fragmented and subjected to DNA extraction using a FastDNA® SPIN Kit (MP Biomedicals, Solon, OH) according to manufacturer protocol. The relationship between q-PCR and culturing are provided in Figure S1. The limit of quantitation was defined as the lowest detectable concentration with three positive threshold cycle (Ct) reading. The detection limit in 500 ml water samples for *Legionella* spp., *L. pneumophila*, *Mycobacterium* spp., *M. avium*, *P. aeruginosa*, *Acanthamoeba* spp. and *H. vermiformis* were reported to be 32, 32, 170, 170, 114, 0.85, 2.4 CFU or cells/ml. Regression analysis were performed between log (gene copies/ml) and log (CFU or cells/ml). The model equations are summarized in Table S9.







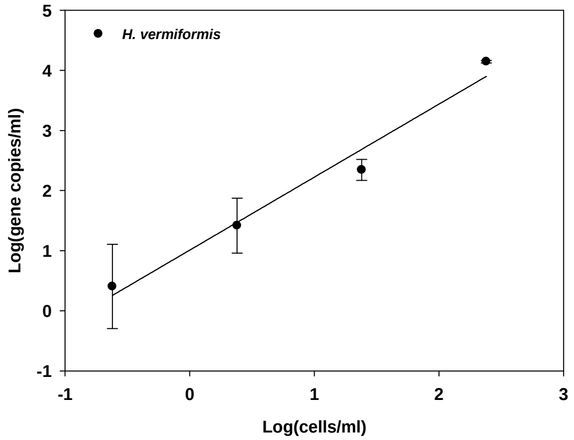


Table S9 Regression analysis of q-PCR and culturing data

Targeted organisms	Model Equation	R^2
Legionella spp.	Y=1.4X+0.6	0.9968
L. pneumophila	Y=1.6X+0.1	0.9981
Mycobacterium spp.	Y=0.8X-1.0	0.9734
M. avium	Y=0.9X-2.3	0.9565
P. aeruginosa	Y=1.2X-1.8	0.9740
Acanthamoeba spp.	Y=1.4X+1.8	0.9715
H. vermiformis	Y=1.2X+1.0	0.9734

Note: Y=log (gene copies/ml)
X=log (CFU or cells/ml)

Figure S1 Enumeration of microbial targets in serial dilutions via culturing versus qPCR.

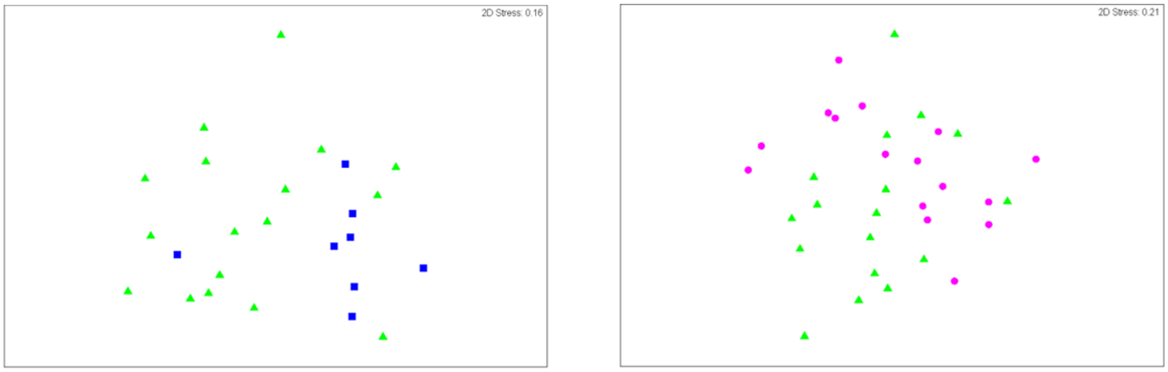


Figure S2 Multi-dimensional scaling analysis of bacterial community composition (T-RFLP profiles) for samples from BCV with different water age. Green triangle symbols represent samples with water age of 10-12 d. Blue square symbols represent samples with water age of 3-6d. Pink round symbols represent sample with water age of 8-10d. Note: samples without T-RFLP peaks were excluded from analysis.

Supplemental References

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