Mitigation of opportunistic drinking water pathogens by onsite monochloramine disinfection in a hospital water system

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Supplementary materials

1. Hospital setting configuration and sampling sites location

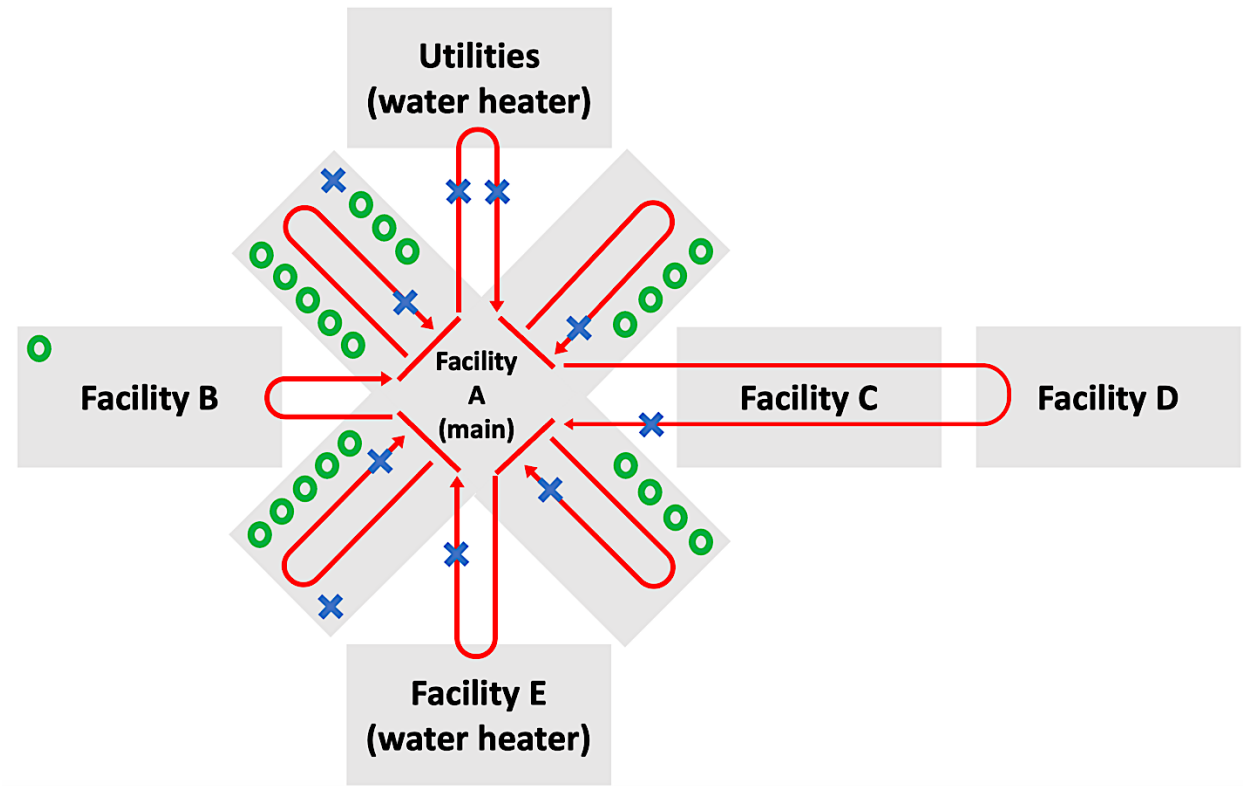

Fig. S1. Hospital setting layout and sampling site location (distal sites [n = 22]: green circles, system sites [n = 10]: blue crosses, horizontal hot water loops: red arrows).

2. Physico-chemical analysis

Temperature, pH, conductivity, dissolved oxygen, free and total chlorine were measured as described by Grimard-Conea and Prévost, 2023. Total organic carbon (TOC) was measured in duplicates (2 X 20 mL) according to the standard method 5310C (APHA et al., 2005) by conductivity on the Sievers M5310C Laboratory TOC Analyzer (Veolia, Boulder, CO, USA). Metals concentrations were analyzed in triplicates (3 X 5 mL, with nitric acid 0.15% v:v) by inductively coupled plasma mass spectrometry (ICP-MS) on the Perkin Elmer NexION 5000 instrument (PerkinElmer Inc., Woodbridge, ON, Canada) according to the manufacturer's instructions. ICP-MS detection limits (µg/L) were of 0.0009, 0.0150, 0.0060, and 0.0003 for manganese, iron, copper, and lead, respectively. Ammonium ions were assessed by colorimetry with sodium salicylate in compliance with the published analysis method MA. 300-N 2.0 (CEAEQ, 2014), whereas nitrite and nitrate concentrations were assessed by ion chromatography according to the published analysis method MA. 300 – Ions 1.3 (CEAEQ, 2020). Reported limits of detection for ammonium, nitrite and nitrate were of 0.020 mg/L.

3. Microbiological analysis

Culturable *L. pneumophila* concentrations were determined as specified in Grimard-Conea and Prévost (2023), through an enzymatic culture-based method. Gene copies (gc) quantification of *Legionella* species, *L. pneumophila*, *L. pneumophila* serogroup 1, *Mycobacterium* species, and *V. vermiformis* was conducted in triplicates by real-time quantitative polymerase chain reaction (qPCR) according to specifications provided in **Table S1**. For all qPCR assays, template DNA and master mix were assembled in hard-shell 96-well PCR plates (Bio-Rad, catalog number HSR9905), and fluorescence curves were retrieved on the Bio-Rad CFX Opus 96 Real-Time PCR instrument. Amplification efficiencies between 75% and 125%, and correlation coefficients (R^2) greater than 0.99 recovered from standard curves for DNA quantification were systematically ensured for each targeted organism and qPCR assay.

Table S1. Overview of qPCR protocols and standard curves specifications for each targeted organism.

Organism	Targeted gene	Manufactured kit or referenced protocol	Amplification efficiencies	Standard curve R^2
Legionella species	Not specified	Triplex protocol as described in the commercial laboratory microproof® *Legionella* Quantification LyoKit (Biotecon Diagnostics, R 602 45)	84.1-98.1%	0.995-0.999
Legionella pneumophila	Not specified		92.0-100.8%	0.994-0.999
Legionella pneumophila sg 1	Not specified		95.6-104.5%	0.994-1.000
Mycobacterium species	*atpE*	Adapted laboratory protocol from Haig et al. (2018)	84.9-123.2%	0.993-1.000
Vermamoeba vermiformis	18S rRNA	Laboratory protocol from Kuiper et al. (2006)	75.0-106.3%	0.990-0.999

Legionella DNA quantification, including *Legionella* species, *L. pneumophila* and *L. pneumophila* serogroup 1, was performed as a multiplex assay using the commercial microproof® *Legionella* Quantification LyoKit based on recommendations from the experience of the Ontario Public Health (Canada) environmental microbiology laboratory. The manufacturer's kit procedure was thoroughly followed for the program setup (cycling conditions, fluorescence channels), the preparation of the PCR mix, the data interpretation and quantification, in addition to the verification for the presence of inhibiting agents.

Nontuberculous mycobacteria (NTM) were quantified with an adapted protocol from Haig et al. (2018) which targets with a high specificity the *atpE* gene in the *Mycobacterium* species genomes from environmental samples (Radomski et al., 2013). NTM standards were prepared with genomic DNA from *Mycobacterium avium* subspecies *paratuberculosis* strain K-10 (ATCC BAA-968D™) and sterile PCR water in order to get a series of dilutions ranging

from 2.0E+01 gc/L to 2.0E+05 gc/L. The qPCR master mix for one 96-well PCR plate (25 samples in triplicates) was composed of 605 µl of sterile PCR water, 1100 µl of the SsoFast™ EvaGreen® Supermix (Bio-Rad, catalog number 1725201), 55 µl of bovine serum albumin (20 mg/mL) (Thermo Scientific™, catalog number B14), 110 µl of a 100-fold diluted forward *atpE* primer (5'-CGG YGC CGG TAT CGG YGA-3'), and 110 µl of a 100-fold diluted reverse *atpE* primer (5'-CGA AGA CGA ACA RSG CCA T-3'), with both sets of primers synthesized with custom IDT oligos. PCR plates (Bio-Rad, catalog number HSR9905) were filled with a total reaction volume of 20 µl (18 µl of the qPCR master mix, 2 µl of sampled DNA extract or NTM standard or sterile PCR water [negative controls]), using triplicates of each content. Cycling conditions (40 cycles) included an initial denaturation (5 min, 95 ˚C), followed by a denaturation (20s, 95 ˚C), and an annealing (30s, 57 ˚C) and elongation phases (30 sec, 72˚C).

Finally, targeting the 18S rRNA gene in *Vermamoeba vermiformis*, the DNA of this protozoan was quantified following the qPCR procedure briefly outlined in Cazals and colleagues (2023), which was previously developed by Kuiper and colleagues (2006). Concentrations were expressed as cell equivalents (CE), considering that 1,330 copies of the targeted 18S rRNA gene were approximatively present in each *V. vermiformis* cell (Kuiper et al., 2006).

4. Data analysis

For both statistical and graphical curation, samples with culturable concentrations of *L. pneumophila* below the detection limit (10 MPN/L) were assigned a value of 5 MPN/L. Contrastingly, the sole sample with a concentration exceeding the upper detection limit (22,726 MPN/L) was capped at 30,000 MPN/L. For each qPCR assay (*Legionella*, NTM, *Vermamoeba*), concentrations that were undetectable (< 20 gc/L) were set at 10 gc/L. For concentrations falling between the limit of detection and the lower limit of quantification, or for concentrations above the upper limit of quantification, values were kept at their estimated value or capped at the upper limit of quantification, respectively.

5. Results and discussion

Table S2. Mean total chlorine concentrations (mg/L) per sampling site location.

Sampling site location	Before monochloramine	Before dosage increase	After dosage increase	Dosage interruption periods
Target value (mg/L)	N/A	1.5	2.5	N/A
System sites [2-min flush] (*range*)	0.09 (*0.05 – 0.15*)	1.10 (*0.40 – 1.60*)	2.09 (*0.11 – 3.40*)	0.35 (*0.08 – 1.20*)
Heater outlet	0.11	1.30	2.22	0.19
Combined return	0.11	1.10	1.99	0.13
Main cross-shape building 1	0.09	1.20	2.21	0.59*
Main cross-shape building 2	0.08	1.20	2.08	0.47*
Main cross-shape building 3	0.09	1.10	2.10	0.59*
Main cross-shape building 4	0.09	1.10	2.04	0.57*
Facility C/D	0.09	1.10	2.05	0.68*
Facility E	0.08	0.80	1.57	0.12
Remote hot water faucets	0.09	1.30	2.32	0.11
Distal sites [first draws] (*range*)	0.12 (*0.05 – 0.58*)	0.70 (*0.10 – 1.50*)	1.62 (*0.21 – 3.60*)	0.17 (*0.06 – 0.75*)
Hot water faucets	0.09	0.90	1.91	0.17
Showerheads [tepid]	0.23	0.30	0.90	0.17
Hand washing stations [tepid]	0.19	0.20	0.77	0.23

* Monochloramine dosage was restarted right before sampling during the first prolonged 4-week stop.

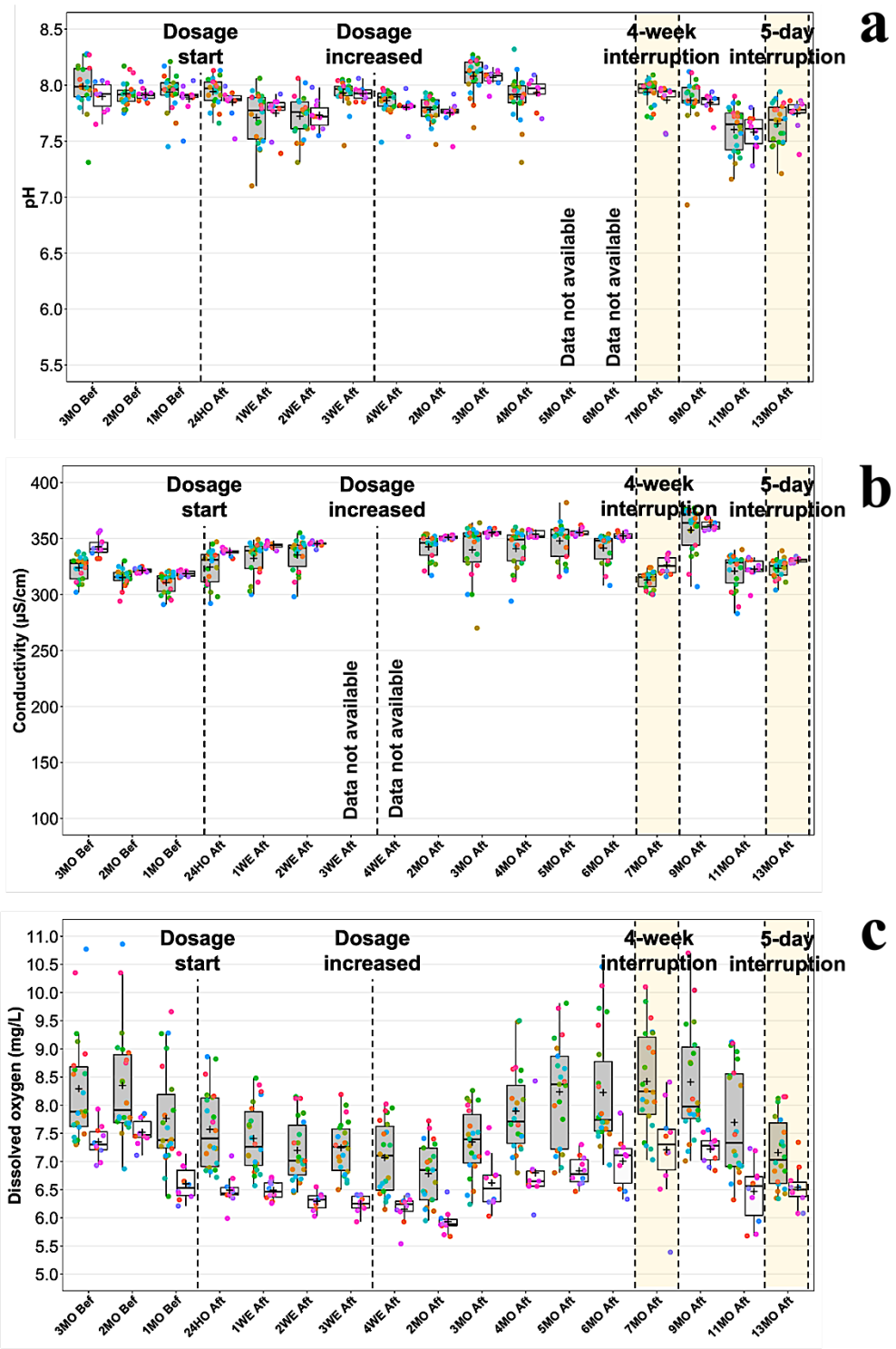

Fig. S2. Box plots of (**a**) pH, (**b**) Conductivity, and (**c**) Dissolved oxygen per samplings (x-axis) at distal sites (grey boxes, n = 22) and system sites (white boxes, n = 10). Legend: Black cross – Mean, Horizontal black line – Median, Boxes – 25th and 75th percentiles, Colored dots – Raw data per sampling site location, MO – Month, WE – Week, Bef – Before, Aft – After.

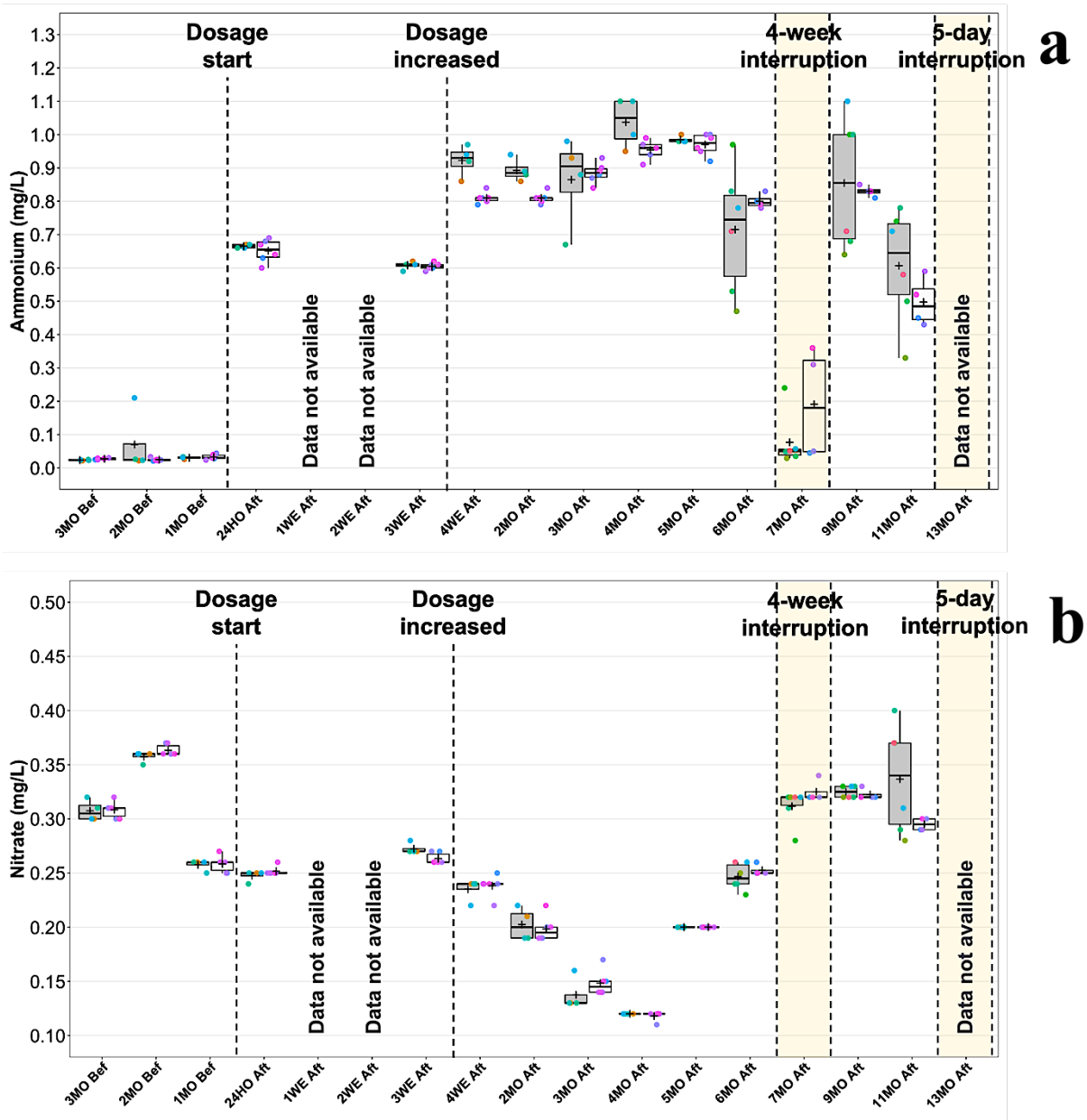

Fig. S3. Box plots of (**a**) Ammonium and (**b**) Nitrate concentrations per samplings (x-axis) at distal sites (grey boxes, n = 22) and system sites (white boxes, n = 10). Legend: Black cross – Mean, Horizontal black line – Median, Boxes – 25th and 75th percentiles, Colored dots – Raw data per sampling site location, MO – Month, WE – Week, Bef – Before, Aft – After.

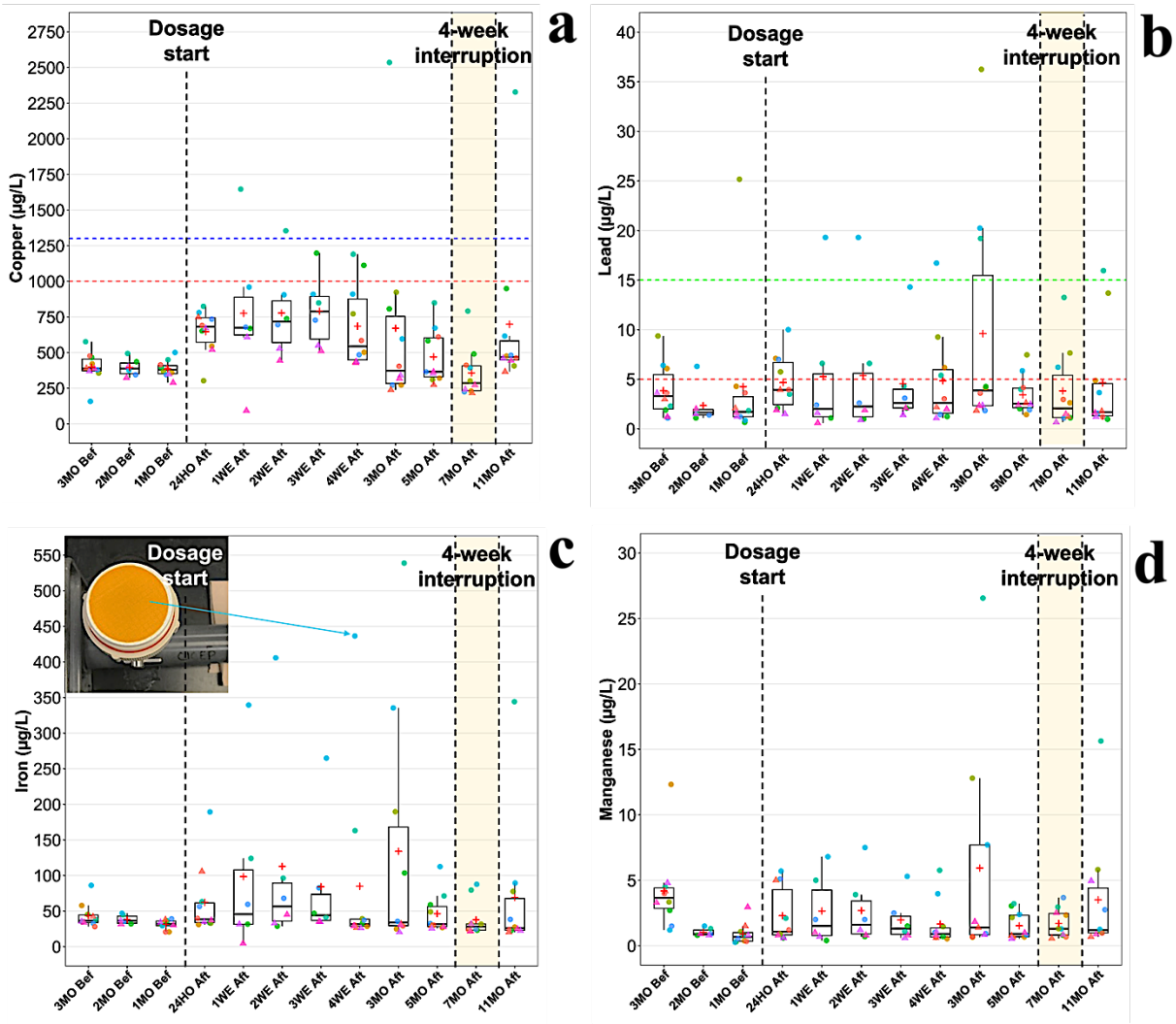

Fig. S4. Box plots of (**a**) Copper, (**b**) Lead, (**c**) Iron, and (**d**) Manganese per samplings (x-axis) at a subset of ten sampling sites. Legend: Red cross – Mean, Horizontal black line – Median, Boxes – 25th and 75th percentiles, Colored dots – Raw data per sampling site location, Circle points – Distal sites (n = 7), Triangle-shaped points – System sites (n = 3), Red lines – regulatory concentration in Quebec water quality guidelines, Blue line – U.S. EPA regulatory concentration for copper, Green line – U.S. EPA regulatory concentration for lead, MO – Month, WE – Week, Bef – Before, Aft – After.

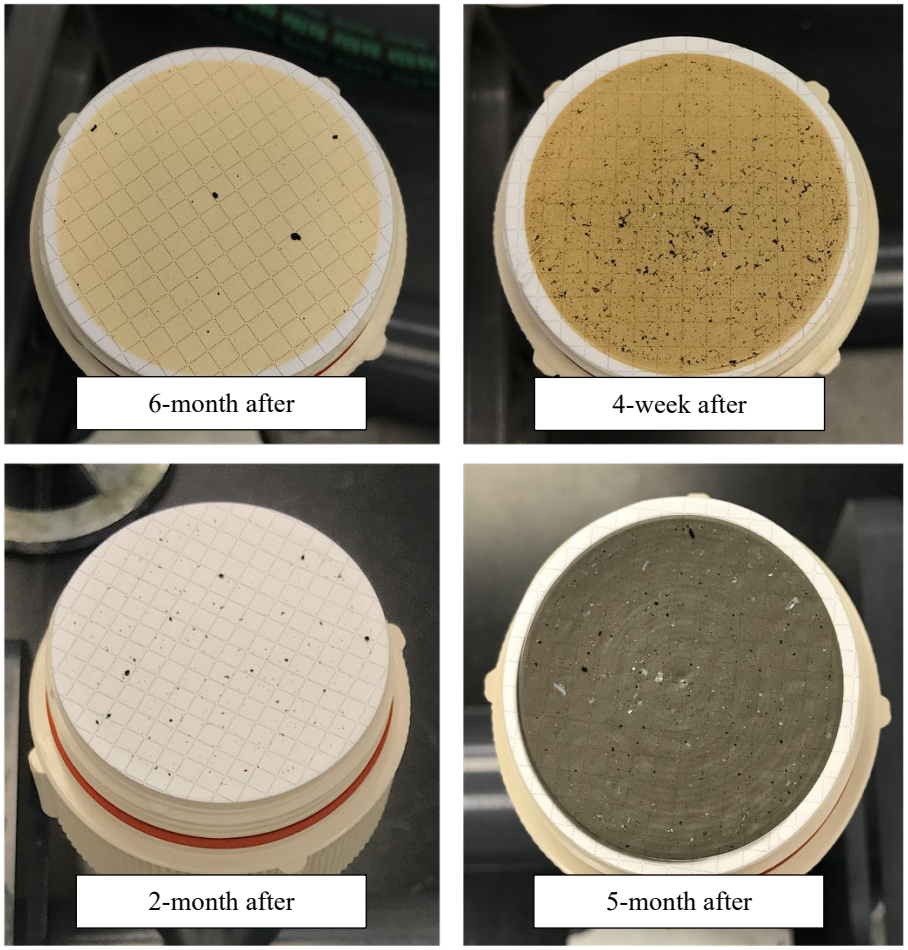

Fig. S5. Examples of plumbing metals in particulate and dissolved forms collected on 0.2 µm membranes during vacuum-filtration after the onset of monochloramine treatment in the hospital's hot water system.

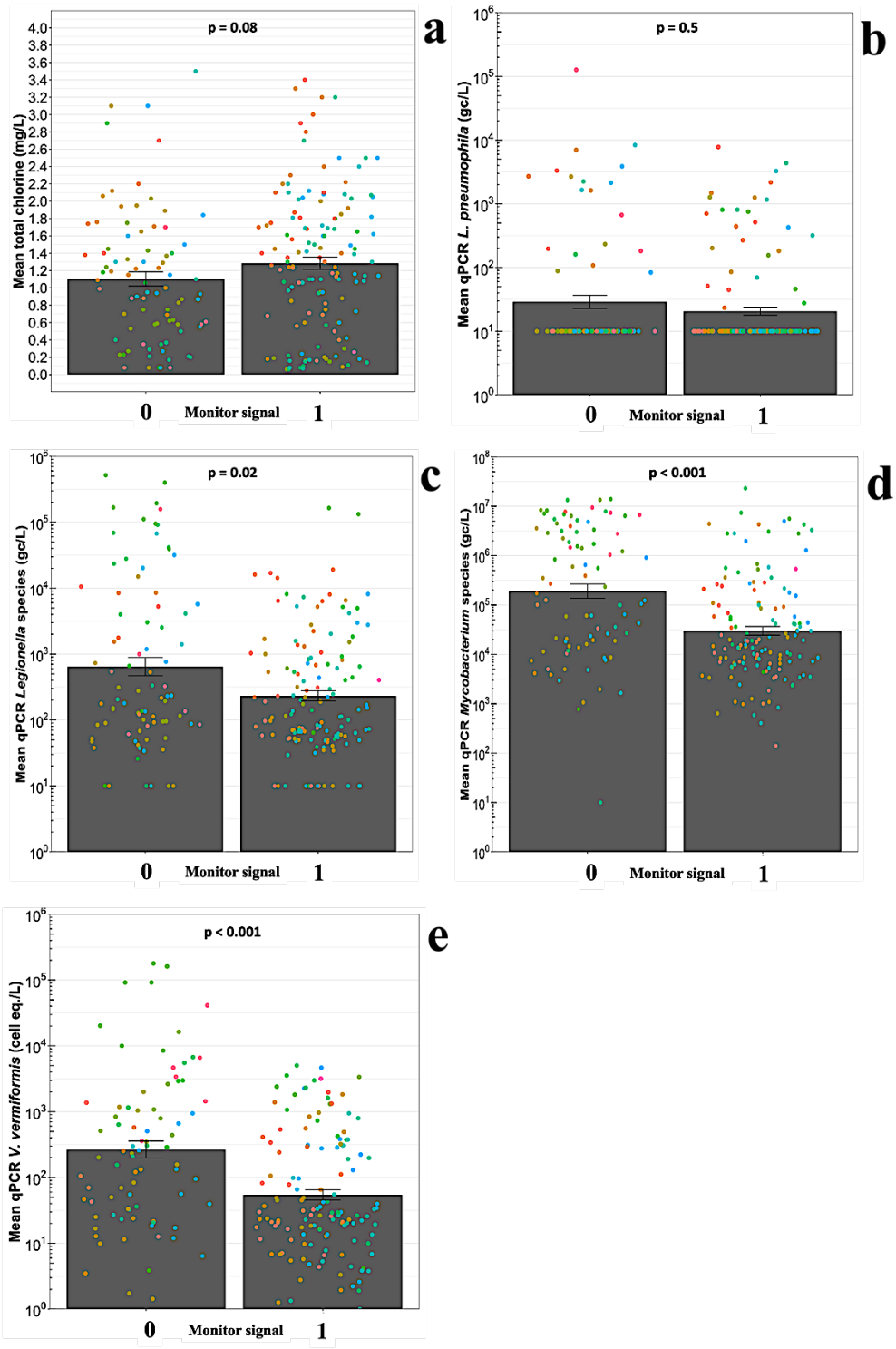

Fig. S6. Bar plots of mean (**a**) Total chlorine, (**b**) qPCR *Legionella pneumophila*, (**c**) qPCR *Legionella* species, (**d**) qPCR *Mycobacterium* species, (**e**) qPCR *Vermamoeba vermiformis* according to the flushing monitor signal installed following onset of monochloramine treatment (0: less than 30-sec use within the last week; 1: at least one 30-sec of continuous use within the last week). Legend: Bar plot – Mean values, Bracket – Error bars, Colored dots – Raw data per sampling site location.

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