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

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ABSTRACT

In acute care hospitals, susceptible patients and large, legacy water systems contribute to increased risk of nosocomial infections associated with drinking water pathogens. This study aimed to evaluate the long-term (>1-year) impact of onsite monochloramine treatment on *Legionella pneumophila* (*Lp*), nontuberculous mycobacteria (NTMs), *Vermamoeba vermiformis* (*Vv*), and physico-chemical water quality in a hospital hot water system. Using an innovative sampling approach, the efficacy of treatment was assessed at 22 distal sites (faucets, showerheads, handwashing stations) and compared to 10 control points representing the main flowing distribution system (return loops, heaters, remote sites). Monochloramine nearly eliminated *Lp*, achieving up to 3-log reductions in culturability (<24 h) and gene copies (4-week). Mean *Vv* concentrations decreased by 2-log within 24 h, with no evidence of a shift towards increased NTMs. Optimal reductions in all organisms were observed at monochloramine concentrations of 2–3 mg/L combined with temperatures exceeding 55 °C. However, these conditions were only consistently maintained at control points, where post-treatment mean concentrations were systematically 1-log lower than those at distal sites. The interruption of dosage (5-day and 4-week) also revealed significant and rapid rebounds of *Legionella*, NTMs, and *Vv* (>1–2-log), highlighting their persistence in biofilms. Short-term increases in metal release were observed, with mean copper and lead concentrations rising 1.8- and 4.6-fold, respectively. Overall, results confirmed the high and rapid efficacy of onsite monochloramine to control *Lp* and other organisms. Analysis of water quality, temperature distribution, and usage patterns emphasize the importance of maintaining optimized hydraulic and thermal regimes to ensure effective pathogen control at points of exposure. This study provides actionable insights and practical evidence to support healthcare facilities in implementing robust long-term monitoring and control strategies.

1. Introduction

Drinking water-associated pathogens causing opportunist infections (DWPI) in vulnerable individuals, including *Legionella pneumophila* and nontuberculous mycobacteria (NTMs), represent a major public health concern (Collier et al., 2021) because of their propensity to grow and persist in building plumbing systems (Falkinham III et al., 2015). Nosocomial transmission of *Legionella* bacteria has been associated with elevated mortality rates compared to community-acquired infections, both in the United States (Soda et al., 2017) and Europe (Beauté et al., 2020), with *Legionella pneumophila* identified most frequently in reported Legionnaires' disease (LD) cases. Additionally, significant healthcare prevalence of NTMs investigations (Perkins et al., 2019) represents a growing concern, particularly given the wide diversity of infectious *Mycobacterium* species and infection pathways (Dowdell et al., 2019). As healthcare facilities (HCFs) treat patients with heightened susceptibility, implementing comprehensive monitoring and mitigation strategies to reduce exposure risks to DWPI is increasingly

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recommended by guidance and regulations (NASEM, 2019).

In response to more stringent regulations to minimize disinfection by-products, water utilities have converted to monochloramine as a secondary disinfectant in distribution systems due to its greater stability and lower reaction rate with natural organic matter (Bradley et al., 2020). Early findings demonstrated that community-wide conversion to monochloramine reduced the prevalence of culturable *Legionella* species (spp.) in the distribution system (Pryor et al., 2004), and drastically reversed prevalence in buildings (Moore et al., 2006), acting on all L. *pneumophila* serogroups (Flannery et al., 2006). A significantly lower incidence rate of nosocomial LD has been reported in hospitals supplied with monochloramine-treated water compared to those using other disinfectants (Heffelfinger et al., 2003). Lower rates of positive samples for culturable or quantitative PCR (qPCR) L. *pneumophila* have also been observed in distribution systems using monochloramine for secondary disinfection (LeChevallier, 2019). This impact is also shown by two extensive studies demonstrating lower L. *pneumophila* occurrence and abundance in buildings with residual monochloramine rather than with residual free chlorine (Donohue et al., 2019; Dowdell et al., 2023).

Several factors underscore the need for alternative mitigation solutions for *Legionella* control. These include (1) the limited long-term benefits of hyperchlorination (Orsi et al., 2014; Grimard-Conea et Prévost, 2023) and heat shocks (Chen et al., 2005; Bédard et al., 2016), especially when elevated temperatures (>70 °C) are not reached (Ji et al., 2018; Cazals et al., 2022), to limit *Legionella* regrowth in response to colonization or nosocomial infection, (2) the limitations of *in situ* copper-silver ionization (Loret et al., 2005; Bédard et al., 2016) or chlorine dioxide (Marchesi et al., 2020; Lee-Masi et al., 2023) to fully prevent *Legionella* positivity, (3) the cost- and time-consuming installation of points of use (PoU) filter in high-risk areas of HCFs (Casini et al., 2014), (4) the limited long-term impact of device (Hozalski et al., 2020; Grimard-Conea et al., 2022) or building-wide flushing (Rhoads et al., 2022; Angert et al., 2023) on reducing *Legionella* loads, and (5) the poor persistence of incoming free chlorine residuals with stagnation and increased water temperatures (Grimard-Conea et al., 2024).

The introduction of monochloramine in the hot water system (HWS) of several large HCFs generally led to sharp reductions, and in some cases complete eradication of *Legionella* culture-positive sites (Marchesi et al., 2012; Casini et al., 2014; Duda et al., 2014; Mancini et al., 2015; Casini et al., 2018; Coniglio et al., 2018; Marchesi et al., 2020; Lytle et al., 2021). It is regarded as a highly effective building-level mitigation strategy to significantly reduce the risk of *Legionella* exposure, especially in areas with vulnerable populations. A major concern regarding the increased use of monochloramine is the potential for microbial shifts, particularly an increased prevalence of NTMs. However, studies investigating this possibility have produced contradictory results. Simultaneous increases in the positivity and mean concentrations (<2-log) of culturable NTMs were measured in one study (Casini et al., 2014). Consistent observations were also reported in two other investigations, including Baron and colleagues (2014), who documented a relative abundance enrichment of *Mycobacterium* spp. in a full-scale hospital hot water system, and Busch and colleagues (2024), who recorded significant increases in *Mycobacterium* spp. gene copies in a pilot-scale plumbing rig after monochloramine introduction. In contrast, Duda and colleagues (2014) reported no significant changes in culture-positive occurrence rates of NTMs, whilst considerable decreases in both culturable (<1-log) and qPCR (up to 2-log) *Mycobacterium* spp. were documented by Lytle and colleagues (2021). When reviewing these studies, important inconsistencies between experimental approaches become evident, including the use of culture and qPCR-based detection methods, disparate sampling timeframes, pooling of samples for analysis, collection of first-flush or semi-flushed samples, types of PoU tested, and the limited characterization of the HWS. Additionally, as L. *pneumophila* and some species of NTMs rely on intracellular replication in hosts, the efficacity of monochloramine to inactivate trophozoites and cysts in water and in the biofilm needs additional research (Loret et al., 2005; Xi et al., 2024).

This study aims to comprehensively evaluate the longitudinal (1-year) effectiveness of *in situ* disinfection of a large hospital's HWS with monochloramine on the abundance of DWPI (*Legionella*, NTMs) and *Vermamoeba vermiformis*, a thermotolerant amoeba species frequently isolated from hospital water systems (Delafont et al., 2018), while also evaluating changes in water quality resulting from this intervention. Sampling was conducted at 22 distal sites under semi-controlled stagnation periods and different water temperature operation, including faucets, showerheads, and hand washing stations, and 10 points representative of the main flowing HWS. The effect of two dosage interruptions – one prolonged (4-week) and another shorter one (5-day) – was further assessed.

2. Materials and methods

2.1. Hospital setting and rationale

The facility is a 540-bed (10 floors) acute care academic hospital built in 1954 (Quebec, Canada). Most rooms have en-suite bathrooms (sink faucet and toilet) and accommodate overnight patients. Some rooms include a shower whereas in other sectors of the hospital, a shared shower is available for all rooms in the same hallway. The hospital complex features an extensive plumbing system centered around a main cross-shape building, which houses nearly all inpatient rooms. Additional facilities, including laboratories, outpatient care, radio-oncology, offices, and utility areas, extend from this central structure (**Fig. S1**). The HWS consists of two centralized 1500-liter pre-heated electrical water heaters, supplying hot water to all connected facilities. In the main cross-shape building, one hot water riser serves two adjacent rooms, with a shared horizontal hot water return loop located in each wing. Additionally, a small 500-liter electrical water heater provides reheated water for Facility E. The hospital receives chlorinated water (0.37–0.64 mg/L during summertime) from the municipal system, and no culturable L. *pneumophila* has ever been detected at the hospital's point of entry in prior testing.

Two nosocomial cases of LD caused by the same L. *pneumophila* strain (including one mixed infection) were confirmed at the hospital in the two years prior to this study (2020–2021). These spatio-temporally linked cases prompted an extensive sampling campaign of the hospital's cold and HWSs, revealing high positivity rates for culturable (29 %) and qPCR (81 %) L. *pneumophila* in hot water (data not shown). Typing results from these water samples matched the strain isolated from clinical samples (Najeeb et al., 2025). In response, short-term preventative and corrective actions were rapidly implemented, including sterile water protocols, PoU filters in targeted high-risk units, restrictions on showers and baths use to reduce water aerosolization and exposure, and two serial superheat-and-flush procedures at 65 °C, spaced three weeks apart. However, the poor efficacy of these heat shocks, as indicated by minimal changes in the occurrence rates and concentrations of culturable L. *pneumophila*, led to the installation of a monochloramine generator in 2022 as a long-term mitigation measure.

2.2. Monochloramine generator system and study timeline

In mid-June 2022, the monochloramine generator system (Sanipur Sanikill, PA, USA) was integrated at the hot water outlet of the water heater. This onsite system combines two monochloramine precursors: a solution of sodium hypochlorite (HOCl, Enoxin) to a solution of ammonium salts (NH_3, Zebion), which ratio is modulated by two metered pumps as a function of the cold make-up water flowrate supplied to the water heater and the redox potential of the hot water return. Sampling events were conducted at three monthly time-point before the introduction of monochloramine into the hospital's HWS, shortly (24 h) after, then on a weekly (for four weeks on), monthly and bimonthly basis in the following year for a total of 17 sampling events. Due to unresolved alerts, dosage was periodically interrupted throughout the study, including for short (5-day) and prolonged (4-week) periods after which sampling was also carried out.

2.3. Sample collection and site location

For each sampling campaign, water samples were collected from 32 sites across the hospital water system, including 22 first draws from 16 manual faucets, four showerheads, and two electronic hand washing stations, and 10 two-min flushed samples from sites across the main flowing HWS, including two manual faucets remotely located from the water heaters, all six individual hot water return loops from the cross-shape main building, and the inlet and outlet of the water heaters (**Fig. S1**). First draws are herein defined as distal sites and flushed samples are designated as system sites, which are representative of the flowing (hot water recirculation) system. Distal sites were selected based on the presence of high-risk patients (hematology-oncology, kidney transplant, pneumology, neonatology, intensive care and COVID-19 units) and previous confirmed nosocomial LD cases and positive L. *pneumophila* measurements. Hot water was solely collected from manual faucets, whereas tepid water was collected from showerheads and hand washing stations. For all 544 samples gathered in this study, two sequential one liter of water were drawn in autoclaved HDPE bottles.

2.4. Water use monitoring

After the onset of treatment, 16 flushing monitors with hand-held shower adaptors and connectors for under-sink pipe connections (TapSnap™, Trusted Water LLC, Fort Collins, CO, USA) were installed at distal sites. Sensing vibrations imputable to water use, monitors showed a green light if at least one 30-second or more of continuous use was detected within the last seven days or a red light, otherwise reflecting no recent water use.

2.5. Physico-chemical and microbiological measurements

Field measurements included water temperature, pH, conductivity, dissolved oxygen, free and total chlorine, using approximatively 150 mL of water for onsite analysis. An additional 75 mL was reserved for laboratory measurements of nitrite, nitrate, ammonium, total organic carbon (TOC) and metals (manganese, iron, copper, lead), before adding one mL of sterile sodium thiosulfate (10 % v:v) in all samples with chlorine concentrations above 0.05 mg/L. The remaining volume of water (1500–2200 mL) was processed in the laboratory within 12 h of sampling for culturable L. *pneumophila* (culture-based enzymatic test), and further vacuum-filtered on sterile 0.2 µm (Ø 47 mm) Supor® PES membranes (PALL Corp., Mississauga, ON, Canada) for a triplex qPCR assay simultaneously targeting *Legionella* spp., L. *pneumophila*, and L. *pneumophila* serogroup 1. Additional qPCR assays individually targeting *Mycobacterium* species and *V. vermiformis* were also performed. All qPCR analysis were processed in triplicates and DNA was extracted from membranes using an adapted protocol from the FastDNA® SPIN kit (MP Biomedicals, Solon, OH, USA) and diluted in 150 µl of sterile PCR water, as described in Grimard-Conea and Prévost (2023). Filtered membranes and DNA extracts were kept at −80 °C and −25 °C, respectively. Detailed descriptions of physico-chemical and microbiological analysis are provided as supplementary materials (Sections 2, 3, and 4).

2.6. Data analysis

Statistical analysis and graphic viewing were conducted on RStudio version 2024.04.2 + 764. Correlations among parameters were evaluated through the Spearman's rank test and statistical differences between two conditions with the Wilcoxon test, using significance levels set at a p-value of 0.05.

3. Results and discussion

3.1. Physico-chemical parameters monitoring

During the study, temperature remained generally stable across the HWS (Fig. 1a). Samples collected from the water heater outlet had a mean temperature of 58 °C, while those taken from the pipe consolidating all hot water return lines averaged 54 °C. Surprisingly, the return line from facility E (**Fig. S1**), equipped with an additional water heater, regularly failed to comply with the minimum temperature requirement of 55 °C mandated by the Construction Code of Quebec (Building Act, chapter B-1.1, r. 2), rather fluctuating between 47 and 52 °C. At the two faucets remotely located from the water heater, a 2-min flush was sufficient to reach temperatures exceeding 55 °C, indicating good hydraulic efficiency to inpatient rooms. Temperatures in first draws at distal sites (Fig. 1a) reflected the impact of mitigators at hand washing stations and showers, as well as the inter-use stagnation leading to water cooling in non-recirculated sections at faucets. Mean temperatures of 45 °C, 28 °C, and 27 °C were measured in manual faucets, showerheads, and hand washing stations, respectively. Free chlorine concentrations were consistently below 0.1 mg/L before onset of treatment, as expected with rapid decay at elevated temperatures and with stagnation (Grimard-Conea et al., 2024). PoU supplying tepid water (showers, hand washing stations) and those with low-water demand generally had concentrations below 0.2 mg/L, a threshold under which microbial growth is insufficiently controlled and greater occurrence of DWPIs is more likely (Donohue et al., 2019; Grimard-Conea et al., 2024). These levels contrasted with incoming free chlorine residuals entering the hospital water system (0.37 – 0.64 mg/L).

In the first three weeks of treatment, monochloramine was targeted at 1.5 mg/L, increasing to 2.5 mg/L thereafter. At the heater outlet injection point, total chlorine averaged 1.3 and 2.2 mg/L in the weeks leading to the dosage increase and in the subsequent months (excluding interruption periods), respectively (**Table S2**). Mean total chlorine concentrations across system sites were consistently lower than at the heater outlet, and even more so at distal sites (Fig. 1b), reflecting chlorine demand influenced by abiotic factors such as plumbing materials and scales, including pipe wall decay from corroded copper surfaces and from stagnation events (Nguyen et al., 2012), increasing surface-to-volume ratio as hot water moves away from the heater, and temperatures (Cullom et al., 2020), as well as biotic factors like microbial growth (Grimard-Conea et al., 2024). During interruption periods, total chlorine was barely detectable at distal sites. Additionally, lower total chlorine concentrations were measured in Facility E's return loop (**Table S2**), likely due to a lower capacity pump given the reduced water demand in this building.

Monochloramine did not alter pH (**Fig. S2**a) and conductivity (**Fig. S2**b), while ammonium concentrations varied with monochloramine dosage (**Fig. S3**a). Nitrite levels remained below detection limits (<0.02 mg/L), and variations in dissolved oxygen (**Fig. S2**c), nitrate (**Fig. S3**b) and TOC reflected seasonal changes in water quality from raw water. Nitrification is a major concern associated with the use of excess ammonia, leading to undesired microbial growth and disinfectant decay (Bradley et al., 2020). In this study, no concurrent substantial pH decreases, nitrate increases, or dissolved oxygen decreases, common indicators of nitrification (Hossain et al., 2022), were observed.

3.2. Plumbing metals

Monochloramine treatment notably increased metal concentrations, with mean levels of copper, lead, iron, and manganese rising from 393 µg/L, 4 µg/L, 38 µg/L, and 2 µg/L, to 633 µg/L, 5 µg/L, 78 µg/L, and 4 µg/L (**Fig. S4**a-d). Particulate and dissolved metals visibly accumulated on membranes, highlighting the clear disruption of plumbing scales post-treatment (**Fig. S5**). Non-compliance rates to Canadian water quality guidelines – a maximum acceptable concentration (MAC) of 5

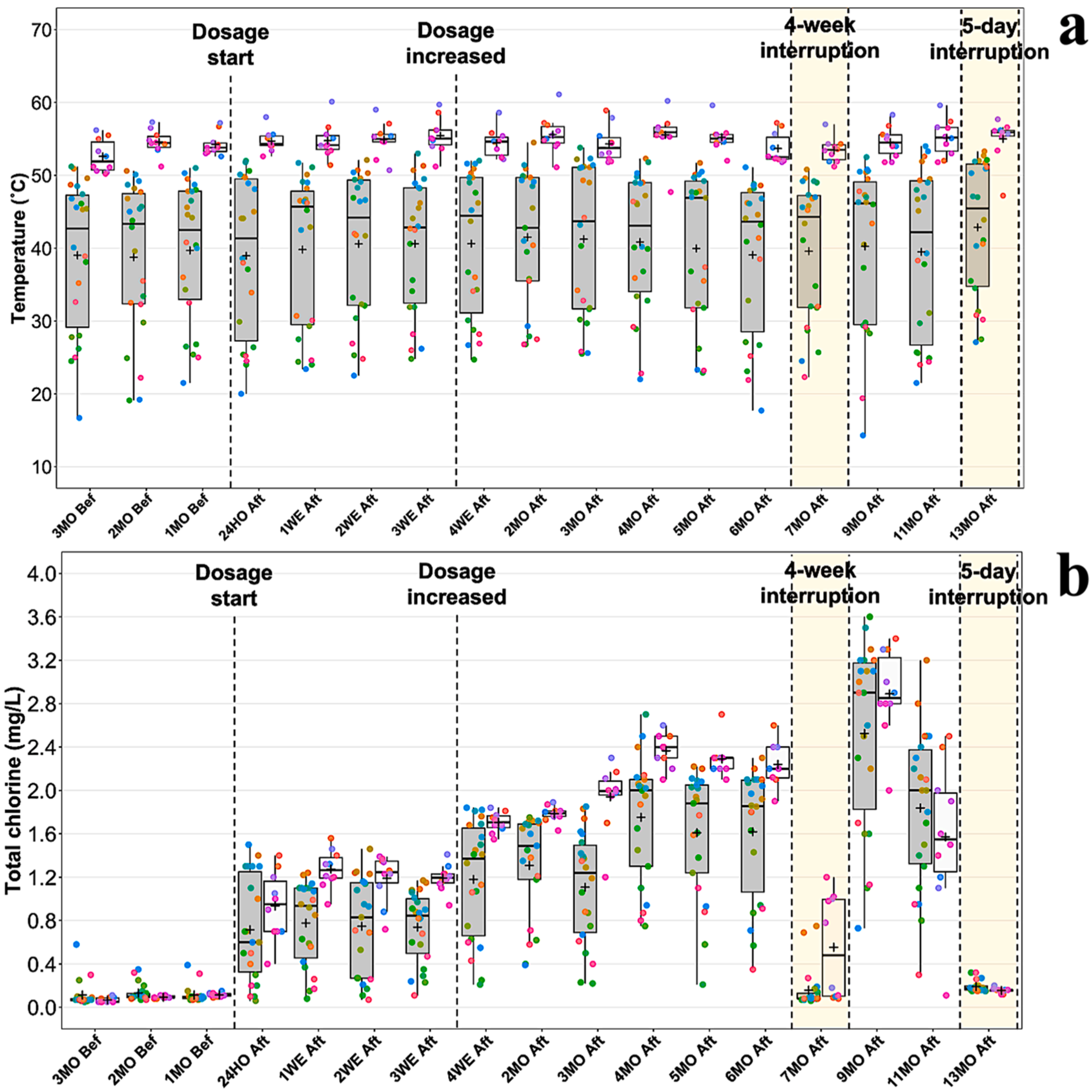

Fig. 1. Box plots of (a) Temperature and (b) Total chlorine over time at distal (grey boxes, $n = 22$) and system sites (white boxes, $n = 10$). Legend: Black cross – Mean, Horizontal black line – Median, Boxes – 25th and 75th percentiles, Colored dots – Raw data per sampling site per location, MO – Month, WE – Week, Bef – Before, Aft – After.

μg/L for lead and an aesthetic objective of 1000 μg/L for copper – also increased from 19 % to 29 % for lead and from 0 % to 9 % for copper.

The hospital's aging unlined cast iron risers, secondary copper piping, and leaded brass fixtures and fittings most likely contributed to these increases, corroborating previous findings on the impact of disinfectant shift on copper, lead, and iron release from disrupted and corroded oxide layers (Edwards et Dudi, 2004; Edwards et al., 2011). As expected, monochloramine had a greater impact on metal concentrations at distal sites compared to those located within the HWS, which showed less variability and minor increases. At some distal sites, concentrations oftentimes spiked largely over MAC or aesthetic standards, reflecting more extended stagnation periods (Zlatanović et al., 2017), larger surface-to-volume ratios (Ling et al., 2018), and the accumulation of particulates which can exacerbate the release of metals.

3.3. Impact of monochloramine on microbial loads

3.3.1. Legionella

Before treatment, 61 % (40/66) of distal sites were culture-positive for L. *pneumophila*, with concentrations ranging 10 to 19,226 MPN/L (Fig. 2a). Nearly half of these (47 %) exceeded the 100 MPN/L alert threshold recommended in *Legionella* guidelines for HCFs, and roughly a quarter exceeded 1000 MPN/L, a value requiring immediate action to control growth (VHA, 2021; HSE, 2024). Elevated temperatures across the HWS likely inhibited culturability, as only four samples out of 30 samples were positive to L. *pneumophila* and all system sites were below 100 MPN/L. Nevertheless, L. *pneumophila* gene copies were detected at 98 % (52–21,000 gc/L) and 100 % (300–2170 gc/L) (Fig. 2b) of distal and system sites, respectively. At distal sites, *Legionella* spp. concentrations varied widely (10^2–10^6 gc/L), whereas system sites exhibited more consistent concentrations (10^3–10^4 gc/L) (Fig. 2c). On average, L. *pneumophila* accounted for 19 % (distal sites) and 25 % of all *Legionella* spp. (system sites) during baseline months. Serogroups 2–15 were overwhelmingly dominant, accounting for 98 % of the pre-treatment environmental samples. Despite serogroup 1 being responsible for the majority of nosocomial LD cases in Europe (Beauté et al., 2020) and the US (Kunz et al., 2024), hospitals have also reported cases caused by other serogroups and species. In fact, the two nosocomial LD cases originating from this hospital belonged to serogroup 10. Notably, the underperforming return line from Facility E frequently had the highest L. *pneumophila* concentrations (47–79 MPN/L) during baseline months, stressing the need to maintain hot water temperatures above 55 °C as a

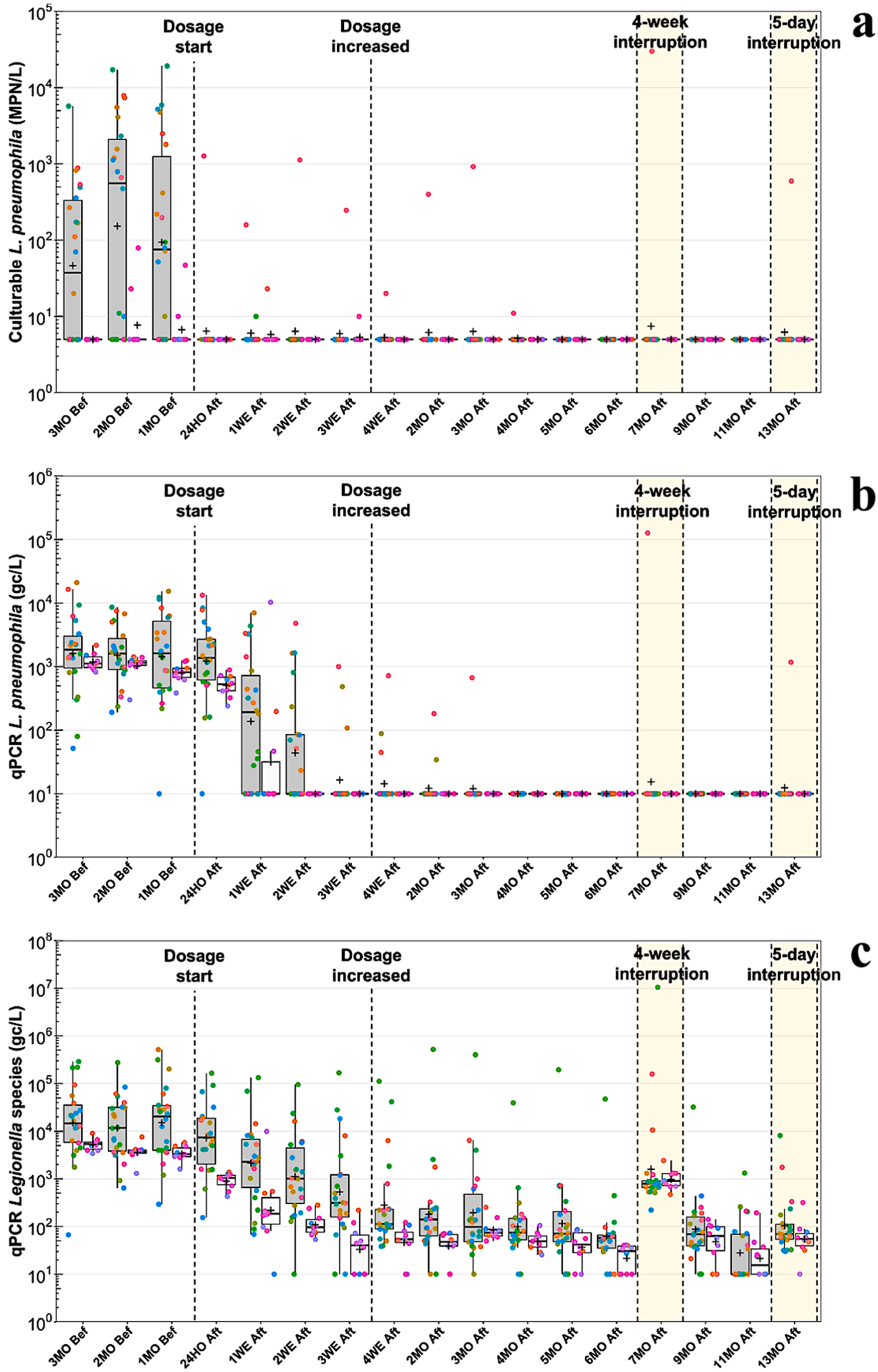

Fig. 2. Box plots of (a) Culturable Legionella pneumophila, (b) qPCR Legionella pneumophila, and (c) qPCR Legionella species over time at distal sites (grey boxes, $n = 22$) and system sites (white boxes, $n = 10$). Legend: Black cross – Mean, Horizontal black line – Median, Boxes – 25th and 75th percentiles, Colored dots – Raw data per sampling site per location, MO – Month, WE – Week, Bef – Before, Aft – After.

first-level control measure.

The introduction of monochloramine rapidly suppressed (24 h) and nearly eliminated culturable L. *pneumophila* across all sites, regardless of residual concentrations (0.1–3.6 mg/L) (Fig. 2a). A single site (hand washing station) remained culture-positive (11–22,726 MPN/L) in the following months, despite receiving similar residuals (0.1–1.7 mg/L) than other tepid PoUs. Within 24 h of dosing, L. *pneumophila* became highly dominant (50–80 %) at all system sites, suggesting initial biofilm disruption releasing L. *pneumophila* into bulk water. Silva et al. (2024) found that L. pneumophila typically occupies the bottom layers of a Pseudomonas fluorescens biofilm. In contrast, this study suggests that in more diverse microbial communities, L. pneumophila likely resides in the top layers, making it more susceptible to sloughing under monochloramine exposure, thus explaining the long-term elimination of its reservoir. By the third week, qPCR L. *pneumophila* concentrations dropped below the limit of quantification in most samples, a decrease of up to 3-log. As the disinfectant quickly affected cell culturability, this 3-week period likely reflects a transition of L. *pneumophila* to a viable-but-non-culturable state before inactivation under prolonged monochloramine exposure. By the fourth month of treatment, L. *pneumophila* gene copies were undetectable. Given the pathogen's ability to regain culturability under favorable conditions, the progressive erosion of the qPCR signal underscores the complementary value of qPCR in monitoring long-term *Legionella* risks.

A gradual mean 2-log decrease in *Legionella* spp. gene copies occurred during the first month, further stabilizing at 10^2–10^4 gc/L, thus highlighting monochloramine's broad-spectrum effectiveness. The efficacy of such disinfectant to mitigate *Legionella* agrees with previous studies in legacy and complex HCFs, where similar target monochloramine concentrations (1–4 mg/L) (Marchesi et al., 2012; Casini et al., 2014; Duda et al., 2014, 2018; Coniglio et al., 2018; Marchesi et al., 2020; Lytle et al., 2021) or higher concentrations were used (6–10 mg/L) (Mancini et al., 2015). Notably, on the first day of treatment, L. *pneumophila* serogroup 1 were measured (42–843 gc/L) at six distal sites where only serogroups 2–15 were previously detected. Concentrations then quickly fell below detection limits, suggesting temporary

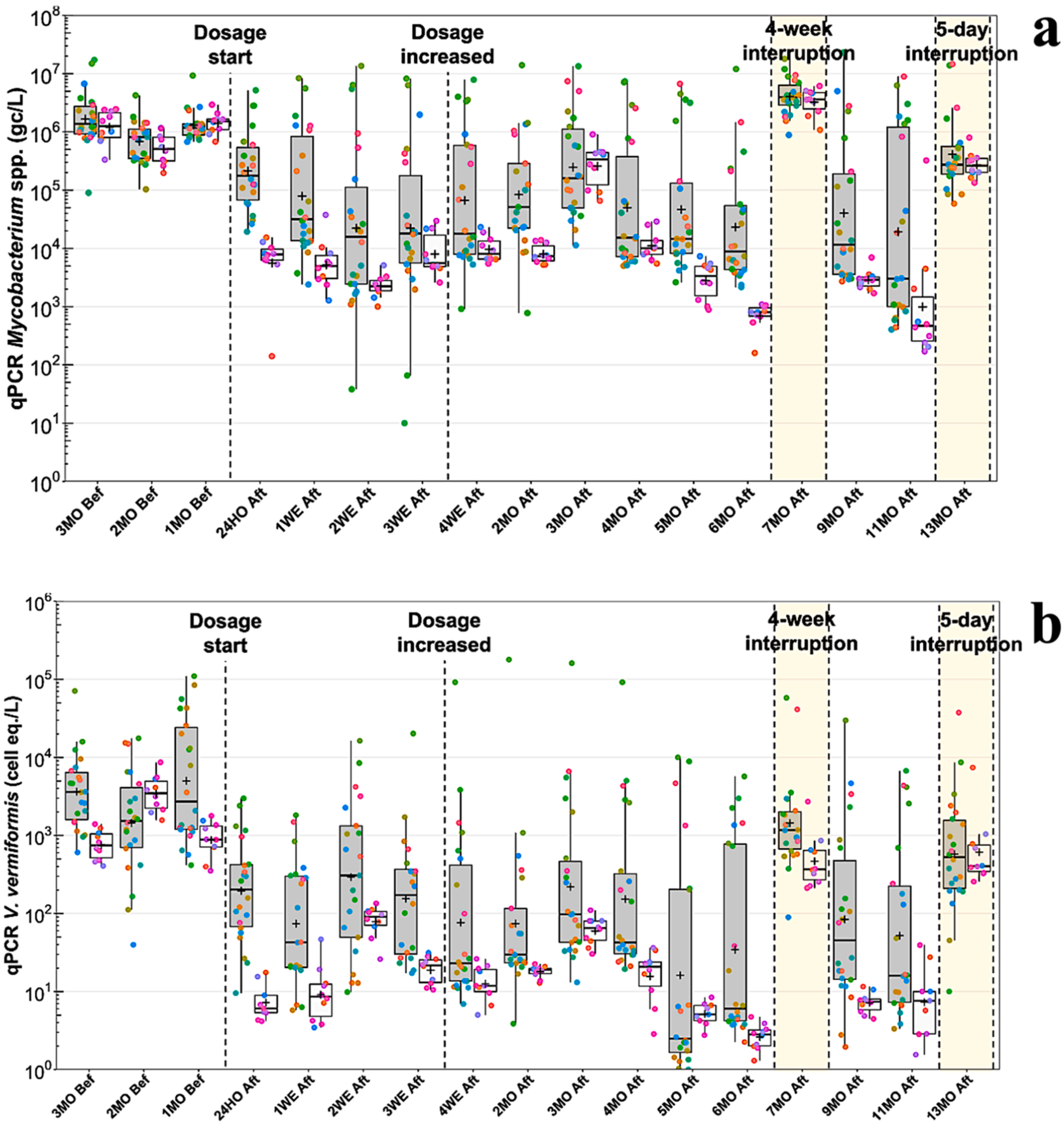

Fig. 3. Box plots of (a) qPCR Mycobacterium species and (b) qPCR Vermamoeba vermiformis over time at distal sites (grey boxes, $n = 22$) and system sites (white boxes, $n = 10$). Legend: Black cross – Mean, Horizontal black line – Median, Boxes – 25th and 75th percentiles, Colored dots – Raw data per sampling site per location, MO – Month, WE – Week, Bef – Before, Aft – After.

detachment of L. *pneumophila* serogroup 1 from adjacent biofilms triggered by monochloramine.

3.3.2. Nontuberculous mycobacteria

Prior to treatment, *Mycobacterium* spp. concentrations fluctuated between 10^5 and 10^7 gc/L, with comparable mean values at distal and system sites (Fig. 3a). After the onset of treatment, mean concentrations decreased by 1–2-log and 1–3-log at distal and system sites, respectively. However, system sites, which experienced more stable flow, temperatures, and residuals, showed less variability, underscoring the differential impacts of monochloramine between distal points and the central HWS. Notably, distal sites exhibited a wide range of NTMs concentrations post-treatment, spanning over five logs. This greater variability observed for NTMs compared to *Legionella* spp. under the same site-specific conditions, including wide ranges of operating temperatures (14–55 °C), water demand patterns, and residual concentrations (0.1–3.6 mg/L) can be attributed to the higher monochloramine disinfection CT (product of the disinfectant concentration and the contact time) required for NTMs. For example, a 3-log inactivation ($CT_{99.9\ \%}$) at ambient temperatures using monochloramine is achieved with <70 mg·min/L for different *Legionella* species, but requires 91–1710 mg·min/L for *Mycobacterium avium* depending on strain susceptibility (Taylor et al., 2000).

NTMs persistence despite monochloramine exposure is also due to the hydrophobic and complex lipid composition of their cell walls, which enhance resistance to disinfectants (Loret et al., 2019). More particularly, their ubiquitous detection in monochloraminated systems is corroborated by studies showing greater planktonic abundance compared to systems with free chlorine (Donohue et al., 2019), or biofilm-associated cells compared to systems without residuals (Waak et al., 2019). Nevertheless, the reductions in NTMs observed in this study align with previous findings (Lytle et al., 2021). However, the present study reveals a more progressive and continuous reduction in qPCR over the 11 months of treatment, corresponding to an increase in combined residuals as shown on Fig 1b Additionally, NTMs comprise a wide variety of species with varying disinfectant resistance, leading in distinct NTMs communities shaped by the type and disinfectant concentration (Yang et al., 2024), further supporting their survival.

3.3.3. Vermamoeba vermiformis

V. vermiformis ranged 10^2–10^5 cell equiv./L during baseline months (Fig. 3b). With monochloramine dosing, rapid 2-log mean reductions were measured at system sites within 24 h, with similar declines at most distal sites. This shows the disinfectant's effectiveness to reduce the amoeba population during the initial phase of treatment. Then, concentrations at all sites fluctuated slightly in the subsequent months, but showed a progressive long-term reduction, with mean levels remaining below the detection limit (<200 cell equiv./L).

L. *pneumophila* and certain NTMs are amoeba-resistant microorganisms that can evade phagocytosis, persisting intracellularly or replicating, especially under nutrient-limited conditions (Greub et Raoult, 2004). *V. vermiformis* is a well-documented host for L. *pneumophila* (Lau et Ashbolt, 2009) and a potential reservoir for NTMs (Delafont et al., 2014). Other pathogenic *Legionella* spp. co-occur with *Acanthamoeba* and *Naegleria fowleri* in engineered water systems, further supporting their reliance on these hosts for survival and replication (Logan-Jackson et Rose, 2021). Given the temperatures on Fig. 1a, it is likely that *V. vermiformis* was predominantly in its cyst form at system sites, as Cazals and colleagues (2022) observed a dominance of cysts above 55 °C under similar conditions. However, at distal sites, temperatures would likely support the presence of trophozoites, as the shift to the cystic form occurs above 40 °C, thereby allowing replication of L. *pneumophila* at PoUs.

Culturable and molecular concentrations of L. *pneumophila* did not correlate ($R < 0.08$, $p > 0.05$) to concentrations of *V. vermiformis* in pre- and post-monochloramine samples, whereas *Mycobacterium* spp. significantly ($p < 0.05$), but only weakly correlated to *V. vermiformis* ($R = 0.34$). This reflects differences in host specificity, preferential detachment from biofilms, survival strategies, and the targeted eradication of the L. *pneumophila* reservoir. In fact, L. *pneumophila* is known to infect various protozoa, including *Acanthamoeba* and *Naegleria* species (Lau et Ashbolt, 2009), which may explain its weaker correlation with *V. vermiformis*, except at the hand washing station where *V. vermiformis* was abundant. Conversely, NTMs may not rely as heavily on hosts for replication, allowing them to survive even when *V. vermiformis* populations were lower (during treatment), while still benefiting from intracellular protection.

3.4. Impact of monochloramine dosing interruption

In this study, both short (5-day) and prolonged (4-week) interruptions of monochloramine dosing did not lead in L. *pneumophila* increases. The absence of L. *pneumophila* rebounds at the majority of sites during interruption of dosage further supports the idea that monochloramine effectively suppressed the reservoirs, thus preventing any host-prey relationships. This contrasts with *Legionella* spp., which exhibited considerable increases during treatment interruptions, with gene copy concentrations increasing by more than one log at all sites during the longer dosage stop (Fig. 2c). These rebounds suggest that non-L. *pneumophila* species may have species-specific survival and resistance mechanisms during monochloramine treatment, while L. *pneumophila* remained overall effectively controlled. Notably, some distal PoU with limited water usage and ineffective control, such as the hand washing station showing persistent contamination to L. *pneumophila* and showerheads with elevated *Legionella* spp. concentrations (>10^4 gc/L), had the largest *Legionella* rebounds during interruptions. *Mycobacterium* spp. concentrations rebounded significantly (1–3-log), returning above or close to pre-treatment levels during the prolonged and shorter interruption periods, respectively (Fig. 3a). These apparent increases are attributed to detachment from biofilms caused by changes in water chemistry rather than rapid growth, given the slow proliferation rates of many NTMs species (>4 weeks) (Gupta et al., 2018). Additionally, one hypothesis for the striking efficacy of monochloramine in reducing L. *pneumophila* prevalence is its ability to induce amoebae to shift from their trophozoite active form to a cyst resistant form during selective pressures exerted by disinfectants, thereby preventing intracellular amplification of the pathogen (NASEM, 2019). In this study, large rebounds in *V. vermiformis* concentrations during discontinuation periods (Fig. 3b) suggest that encystment occurred during treatment, followed by detachment of encysted and active *V. vermiformis* when conditions became more favorable.

3.5. Influence of abiotic factors

3.5.1. Temperature

Temperature is a critical factor in controlling DWPIs in buildings, with temperatures below 50 °C previously being associated to increased culturable L. *pneumophila* positivity (Grimard-Conea et al., 2024) in large buildings and qPCR NTMs in residential buildings (Falkinham III, 2011). Prior to monochloramine treatment, the highest concentrations and detection rates of culturable L. *pneumophila* were measured within the temperature range of 30–50 °C, consistent with its optimal growth conditions (Hochstrasser et Hilbi, 2022), whereas lower occurrences were measured below 30 °C and above 55 °C (<8 %) (Fig. 4a). In contrast, high positivity was observed by qPCR for L. *pneumophila* (>92 %) (Fig. 4b) and *Legionella* spp. (>100 %) (Fig. 4c), regardless of the temperature. As monochloramine nearly eliminated L. *pneumophila*, no dependence to temperature could be observed post-treatment, unlike *Legionella* spp. for which mean concentrations of data points above 55 °C were 2-log lower than that between 20 and 40 °C. *Mycobacterium* spp. (Fig. 5a) and *V. vermiformis* (Fig. 5b) were ubiquitous in all samples during baseline months. Notably, the combination of elevated

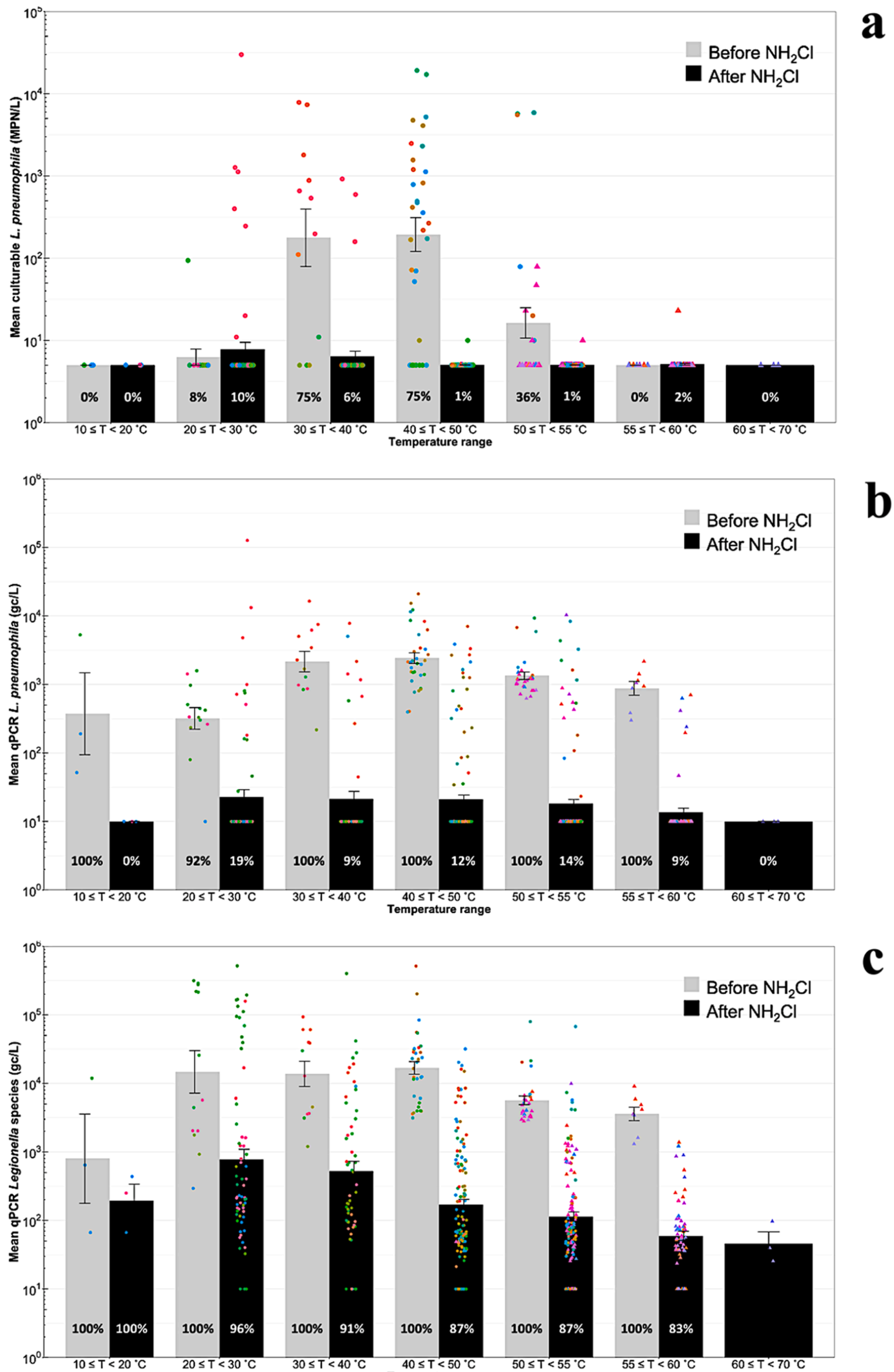

Fig. 4. Bar plots of mean (a) Culturable Legionella pneumophila, (b) qPCR Legionella pneumophila, and (c) qPCR Legionella species per temperature range. Legend: Bar plot – Mean values, Bracket – Error bars, Circle points – Distal sites, Triangle-shaped points – System sites.

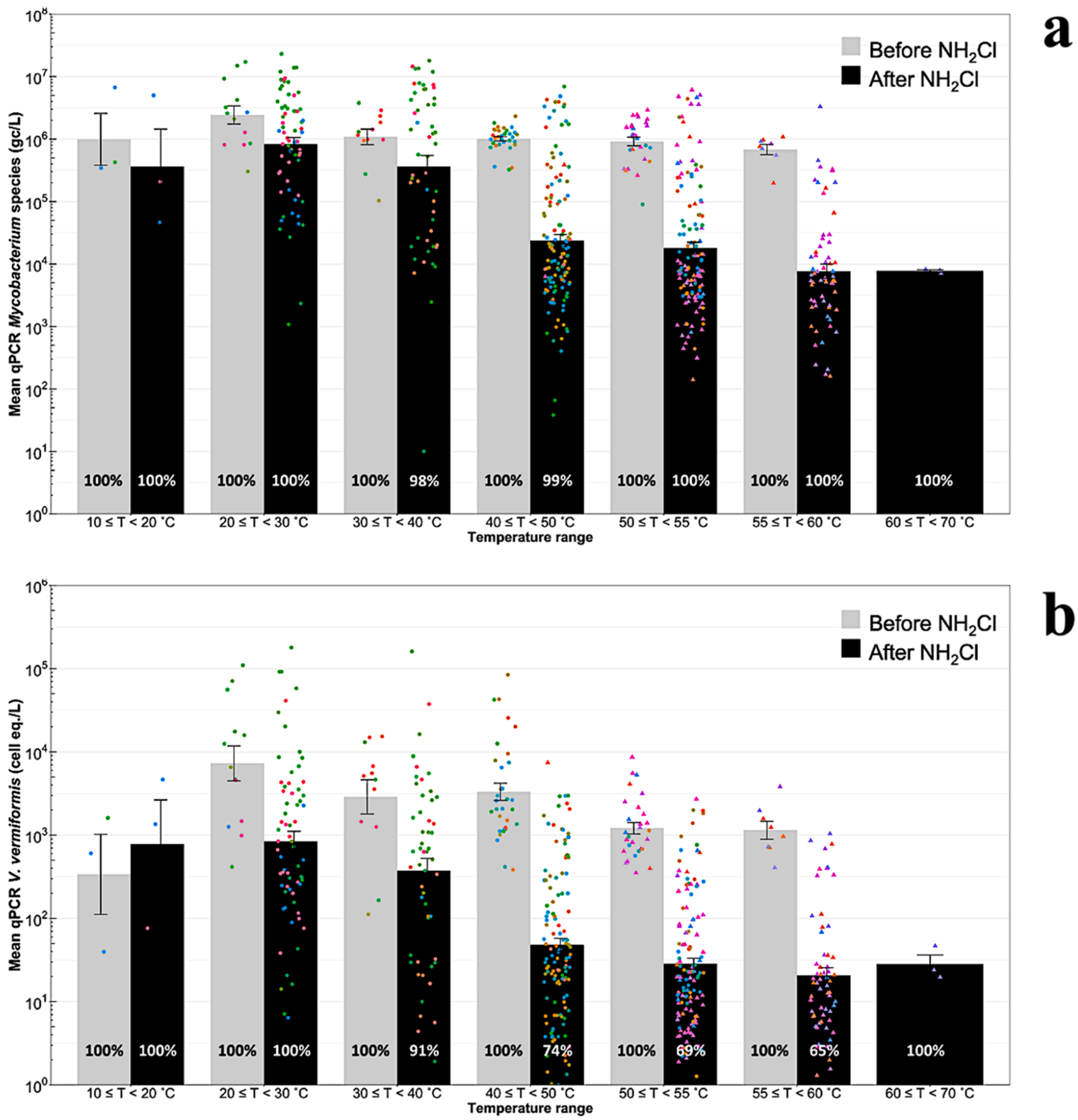

Fig. 5. Bar plots of mean (a) qPCR Mycobacterium species, (b) qPCR Vermamoeba vermiformis per temperature range. Legend: Bar plot – Mean values, Bracket – Error bars, Circle points – Distal sites, Triangle-shaped points – System sites.

temperatures (>50 °C) and monochloramine treatment resulted in the largest reductions in mean concentrations of these two organisms (>3-log), though this only significantly reduced positivity for *V. vermiformis*. These decreases align with the strong and significant negative correlations between temperature and qPCR concentrations of *Mycobacterium* spp. ($R=-0.56, p < 0.001$) and *V. vermiformis* ($R=-0.61$, $p < 0.001$) in monochloraminated samples, underscoring the added benefits of maintaining elevated temperatures alongside disinfection for their effective mitigation.

3.5.2. Disinfectant concentration

Mean concentrations of the targeted microorganisms for different total chlorine range during monochloramine dosing and interruption periods are presented in Table 1. Overall, both culturable and qPCR mean concentrations of L. *pneumophila* decreased as monochloramine levels increased, with qPCR signals becoming undetectable when total chlorine was maintained at 2 mg/L or more. Compared to baseline months, *Legionella* spp. showed the most significant average decrease (2-log) when total chlorine levels were maintained at 2–3 mg/L, suggesting that further increasing the dosage did not result in a more considerable reduction. Likewise, results from Table 1 indicates that total chlorine

Table 1
Mean concentrations of targeted microorganisms for different total chlorine range. LoD: Limit of detection.

Targeted microorganism	Before	Stop	0 ≤ Cl < 0.5 mg/L	0.5 ≤ Cl < 1 mg/L	1 ≤ Cl < 2 mg/L	2 ≤ Cl < 3 mg/L	3 ≤ Cl < 4 mg/L
L. *pneumophila* (MPN/L)	1.1E+03	5.4E+02	7.7E+01	2.3E+01	< LoD	< LoD	< LoD
L. *pneumophila* (gc/L)	2.6E+03	2.3E+03	6.8E+02	3.5E+02	3.4E+02	< LoD	< LoD
Legionella spp. (gc/L)	3.5E+04	1.9E+02	2.3E+04	2.0E+04	1.6E+03	6.0E+01	4.3E+01
Mycobacterium spp. (gc/L)	1.7E+06	2.9E+06	2.1E+06	1.9E+06	4.8E+05	4.3E+04	5.1E+03
V. vermiformis (cell eq./L)	7.7E+03	3.2E+03	1.8E+03	8.6E+03	5.1E+02	4.2E+01	2.3E+01

above 2 mg/L was effective to erode the qPCR signal of *V. vermiformis*, but the data suggest that higher residuals (>3 mg/L) would be required to achieve the most substantial reductions in *Mycobacterium* spp. concentrations. Due to chlorine demand during distribution throughout the hospital's HWS and dilution with cold water at tepid PoUs, concentrations of >2 mg/L are inconsistently reached at distal sites (**Table S2**).

3.5.3. Water use

The installation of flushing monitors at a subset of distal sites enabled the assessment of sites with very low water demand, defined as <30 s of continuous use over the course of a week. These devices only allowed the identification of extreme low usage, as 30 s of use per week remains very low. In this case, showerheads located in patient rooms within day cancer units, as well as the hand washing station at the entry of the neonatology ward, where hand sanitizer use is more prevalent, were rarely utilized. A modest increase of 0.2 mg/L in mean total chlorine (**Fig. S6**a) was measured in sites more regularly used. While no significant differences in L. *pneumophila* gene copies were observed between the different use regimes (**Fig. S6**b), a clear trend appeared with sites experiencing low water use showing significantly ($p < 0.05$) higher qPCR concentrations of *Legionella* spp. (**Fig. S6**c), *Mycobacterium* spp. (**Fig. S5**d), and *V. vermiformis* (**Fig. S6**e). These observations are consistent with results from another hospital, where significantly higher concentrations of qPCR *Legionella* spp. and *V. vermiformis* were also measured in extremely low demand regime (<2 h per month) (Nisar et al., 2023). Similarly, automatic flushing taps near dead legs in a large hospital only reduced the prevalence of L. *pneumophila* when operated for one minute every two hours, compared to less frequent flushing (one minute every six hours) (Totaro et al., 2018). Indeed, resume of flow with flushing can cause detachment and even biofilm sloughing after longer periods of distal stagnation (Bédard et al., 2018; Grimard-Conea et al., 2022).

4. Implications for future guidance and conclusions

The recurring costs and inconveniences of PoU filters, along with labor- and logistics-intensive remedial actions like heat shocks and hyperchlorination underscore the need for effective, long-term solutions to control DWPIs in HCFs. In this study, despite prior optimization of thermal regimes across the hospital's HWS, *in situ* monochloramine application was needed to achieve prolonged mitigation control of *Legionella*, NTMs, and *V. vermiformis*. Notably, monochloramine strikingly eliminated L. *pneumophila* reservoirs, reducing rapidly culturable (<24 h) and progressively qPCR concentrations (<4 weeks). Abundances of NTMs and *Legionella* spp., which comprise other pathogenic species, were further reduced and concentrations stabilized over the course of six months and four weeks, respectively. In this intervention study, total chlorine concentrations above 2 mg/L showed the greatest benefits on lowering abundances of *Legionella* spp., NTMs, and *V. vermiformis* at distal sites, which was only consistently reached when monochloramine was injected at 2.5–3.5 mg/L at the heater outlet. Additionally, a more aggressive dosage approach starting with the onset of treatment would limit the periods of persistence of *Legionella* spp. and NTMs. Overall, results emphasize the importance of multi-pathogen monitoring and risk assessments tailored to patient vulnerability throughout the duration of the intervention.

In large and complex HCFs, temperature and disinfectant act as combined selective pressures. In this study, the combination of monochloramine exposure and temperature had mixed results. Before onset of treatment, elevated temperatures (>55 °C) were effective only at controlling culturable L. *pneumophila* occurrence, with no temperature dependency observed for the other targeted microorganisms. During monochloramine exposure, the largest reductions in *Legionella* spp., NTMs, and *V. vermiformis* were primarily observed at system points, exposed to higher residuals, thus underscoring the importance of maintaining consistent and adequate thermal control throughout the HWS. Nevertheless, as exposure risk is primarily driven by concentrations, the significant reductions in their abundances despite their ubiquitous occurrence indicates that long-term risks associated with these organisms were still drastically reduced. The push to reduce energy consumption by lowering heater temperatures must be balanced against the need to maintain water safety, as failing to control the growth of less sensitive organisms can result in considerable public health risks in HCFs.

Rebounds in *Legionella* spp., *Mycobacterium* spp., and *V. vermiformis* concentrations during dosage interruptions demonstrate their persistence within biofilms, which serve as critical reservoirs allowing survival under adverse conditions and release into bulk water when treatment is disrupted. Even a brief 5-day stoppage contributed to elevate microbial concentrations to nearly pre-treatment levels, highlighting the need to rapidly respond to operational warnings from the monochloramine generator. Therefore, continuous monochloramine application and quick response protocols to dosage alerts should be initiated and clearly defined in water safety plans (WSPs) to prevent pathogen resurgence. Possible rebounds of clinically-relevant species of *Mycobacterium* and *Legionella* other than L. *pneumophila* need further investigation to shed light on the benefits of multi-pathogen monitoring strategies and control measures. *V. vermiformis* was unlikely the primary host for L. *pneumophila* in this study, as no correlation was observed between their abundances. Nonetheless, understanding and managing the disruption of host-pathogen interactions remains crucial, especially in exploring the biotic factors contributing to the persistence and resurgence of these DWPIs.

In this study, monitoring both system and distal sites was essential for accurately assessing risk mitigation. Focusing solely on system sites would overlook the greater challenge of controlling DWPIs at distal points where exposure occurs. In general, results evidenced a higher DWPIs prevalence at distal sites, indicating that system sites, where water quality is typically monitored, were not predictive of distal sections. This reinforces the need for risk-based strategies in WSPs that extend beyond control points (system sites) to include comprehensive management of sentinel points (distal sites). Indeed, system sites alone do not provide a full picture of pathogen risks, particularly in buildings with diverse water use patterns and complex systems. Regular monitoring of DWPIs at sentinel sites is critical for early detection and timely intervention. Notably, after the onset of treatment, a greater variability in pathogen levels and plumbing metals was observed at distal sites, reflecting site-specific factors such as differences in water use patterns, disinfectant residuals, and plumbing configurations and materials. Oppositely, system sites, which were exposed to more consistent temperatures and residuals, showed less variability. This variability calls for tailored control measures, including increased flushing, temperature control, and PoU filters in high-risk areas.

Furthermore, tepid and low-use sites exhibited higher microbial concentrations, showing the necessity for targeted control measures at these higher-risk PoUs. Discarding first draws of water, through regular flushing of taps is essential to minimize DWPIs exposure. This preventive measure can be carried out by maintenance staff during inpatient room turnover, or through the installation of auto-flush devices in critical areas or manual scheduled flushing protocols with the use of sensor-based monitoring systems implemented at strategic PoUs. Small amount of flushing (<2 min) is typically required to reduce significantly microbial levels in first draws (Bédard et al., 2018; Grimard-Conea et al., 2022), although the frequency at which taps should be flushed remains site-specific (Grimard-Conea et Prévost, 2023). Tepid PoUs present additional challenges, as their operating temperatures consistently promote DWPIs growth. In the context of *in situ* monochloramine application, these sites are also compromised by diluted disinfectant concentrations resulting from the mixing of hot and cold water. While evidence of nitrification was not observed in this study based on variations of surrogate parameters (nitrogen species, dissolved oxygen, pH), tepid water temperatures may render these sites more vulnerable to growth of nitrifying bacteria. Therefore, regular flushing of tepid PoUs can prevent exposure to exacerbated pathogen growth, whereas in sections of the hospital housing very susceptible patients, installation of PoUs filters could provide a more robust layer of protection.

Finally, some transient release of metals (copper, lead, iron, manganese) was observed at both distal and system sites, as expected when shifting from free chlorine to monochloramine. However, with monochloramine injected in the HWS, the apparent increases in regulated metals are less concerning since hot water is not used for human consumption.

CRediT authorship contribution statement

Marianne Grimard-Conea: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Xavier Marchand-Senécal:** Writing – review & editing. **Sébastien P. Faucher:** Writing – review & editing. **Michèle Prévost:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2025.124107.

Data availability

Data will be made available on request.

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