Brief Report

Linkage of In-Refrigerator Water Dispensing Systems to Elevated Exposure to Microbial Contamination at the Microbiome Scale

Abstract

In-refrigerator water dispensing systems are ubiquitous in residential homes with tap water as the inflow. Passage through these systems resulted in significant microbial growth in the water, with the abundance of potential opportunistic pathogens *Mycobacterium* and *Pseudomonas* increasing by 8,053- and 221-fold, respectively. Elevated exposure to microbial contaminants linked to in-refrigerator water dispensing systems may represent a significant public health concern.

Introduction

The supply of safe drinking water is critical to public health. Data on waterborne disease outbreaks suggest that drinking water consumption continues to be one of the most important pathways of exposure to waterborne pathogens.[1,2] Despite the intentional addition of disinfectant residual into treated drinking water for the suppression of microbial growth, microorganisms may propagate under certain circumstances during the delivery of treated water from a centralized treatment plant or well supplies to the point of use. This risk is particularly elevated in premise plumbing, the portion of water distribution system in buildings and homes characterized by long water age, extended stagnation, and dissipation of disinfectant residual.[3]

The in-refrigerator water dispensing system (IRWDS) is an extension of the premise plumbing that typically includes a carbon-block filter for additional treatment of tap water at the point of consumption. Given that carbon-block filters are known to remove disinfectant residual[4], passage of tap water through IRWDS may result in significant microbial growth in the water. Furthermore, IRWDS share the same characteristics that facilitate microbial growth in premise plumbing, i.e. long water age and stagnation. With the popularity of IRWDS, it is important to evaluate the public health risks of these systems. This report represents the first study to investigate the microbial exposure risks of IRWDS at the microbiome scale with 16S rRNA gene amplicon sequencing.

Methods

Water samples were collected in 10 single-family detached residential homes in Knoxville, TN, USA, served by public water supplies with free chlorine as the disinfectant residual, each having an IRWDS with a carbon-block filter and cold tap water as the inflow. A 1-liter sample from each IRWDS, referred to as refrigerated water hereafter, was collected for analysis early in the morning prior to any water usage. A 1-liter sample of cold tap water was collected simultaneously from the same residential home early in the morning prior to any water usage, serving as the control for the refrigerated water to evaluate the impact of the IRWDS on microbiological water quality. Free chlorine residual was measured in both tap water and refrigerated water samples immediately following collection using the standard DPD colorimetric method.[3] The level of overall microbial abundance in water samples was quantified as heterotrophic plate counts (HPC) in colony forming units (CFUs) as previously described.[5]

Microbiome-scale profiles of water samples were derived with 16S rRNA gene amplicon sequencing as previously described.[6] Briefly, microbial biomass was collected from each water sample via membrane filtration, followed by DNA extraction, PCR amplification of the V4 region of the 16S rRNA gene, high-throughput paired-end sequencing, and sequence analysis.[7,8] Raw sequences were deposited into the NCBI Sequence Read Archive database (SRA) with the accession numbers SRR22894290-SRR22894309.

The increase in abundance of a specific microbial taxon present in refrigerated water relative to that in tap water was quantified as "fold change" as previously described[9]:

$$\text{Fold Change} = \frac{F_{refrigerated\ water} \cdot MA_{refrigerated\ water}}{F_{tap\ water} \cdot MA_{tap\ water}} \qquad \text{Eq. 1}$$

where F is the fraction of microbial abundance in refrigerated water or tap water samples attributable to a specific taxon according to microbiome-scale profiles derived from 16S rRNA gene amplicon sequencing; MA represents the level of overall microbial abundance measured as HPC in refrigerated water or tap water samples.

Results

To determine the impact of IRWDS on microbiological drinking water quality, the level of overall microbial abundance, measured as HPC, was compared between refrigerated water samples and tap water controls. The HPC averaged 20±38 CFU/100mL in tap water (Fig 1A). In comparison, the average HPC for refrigerated water reached 93,060±167,387 CFU/100mL (Fig 1A). These results revealed significant increases in microbial contamination during the passage of tap water through IRWDS.

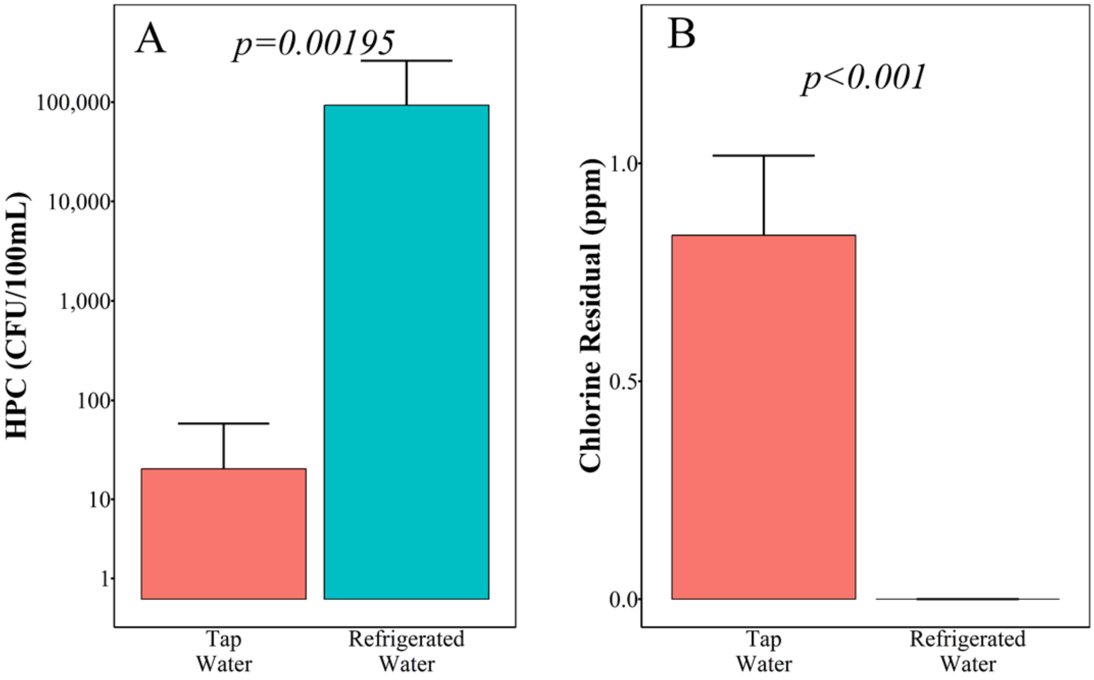

Fig 1. Impact of in-refrigerator water dispensing systems on microbiological water quality. (A) Comparison of HPC as a measure of overall microbial contamination between refrigerated water and tap water. (B) Comparison of chlorine residual as the disinfectant used for the suppression of microbial growth between refrigerated water and tap water. Data shown are means with error bars representing standard deviations. Statistical significance is indicated by the *p*-values according to the Wilcoxon signed rank test.

To further evaluate the microbial contaminants in refrigerated water, 16S rRNA amplicon sequencing was employed to evaluate the presence of *Mycobacterium* and *Pseudomonas*, two of the most common opportunistic pathogens in drinking water.[10] The fraction of microbial contaminants representing *Mycobacterium* was not significantly different between refrigerated water and tap water, averaging 6.68±5.45% and 9.19±11.82%, respectively (Fig 2A). In contrast, the fraction of microbial contaminants attributable to *Pseudomonas* was 0.04±0.07% in the refrigerated water, which was considerably lower than that of 10.97±12.11% in the tap water (Fig 2A).

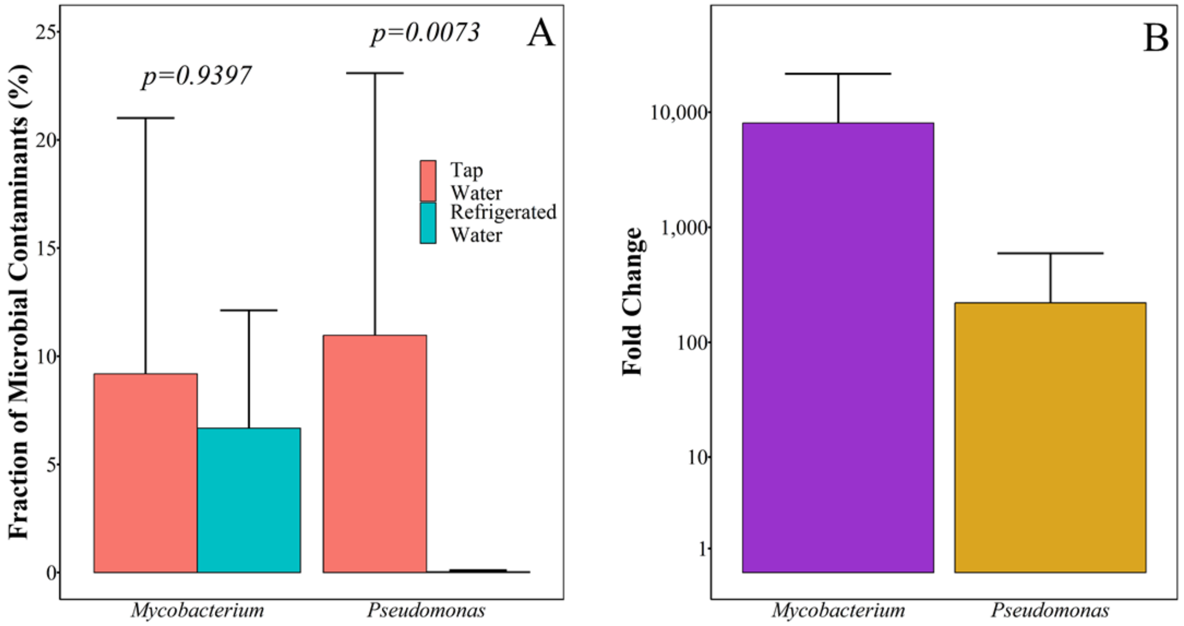

Fig 2. Impact of in-refrigerator water dispensing systems on water contamination by *Mycobacterium* and *Pseudomonas*. (A) Fractions of microbial contaminants attributable to *Mycobacterium* and *Pseudomonas* in refrigerated water and tap water controls as determined by 16S rRNA gene amplicon sequencing; statistical significance is indicated by *p*-values according to the Wilcoxon signed rank test. (B) Increases in the abundance of *Mycobacterium* and *Pseudomonas* due to the use of in-refrigerator water dispensing systems represented by fold change as calculated according to Eq. 1. Fold changes greater than 1.0 indicate the abundance of a specific taxon was higher in the refrigerated water than the tap water. Error bars represent standard deviations.

However, taking into consideration the drastic increases in overall microbial contamination measured as HPC in refrigerated water (Fig 1A), the abundance of *Mycobacterium* and *Pseudomonas* experienced average increases of 8,053-fold and 221-fold, respectively, following passage through the IRWDS (Fig 2B). The increased abundance of both opportunistic pathogens raises public health concerns with the use of IRWDS.

To identify potential causes of increased microbial contamination in refrigerated water, the level of chlorine residual was measured in tap water and refrigerated water samples. It was revealed that chlorine residual was not detectable in refrigerated water while averaging

0.84±0.18 ppm in tap water (Fig 1B). This loss of chlorine residual in refrigerated water corresponded to a significant increase in the level of microbial contamination in refrigerated water (Fig 1A). These results suggest the likely cause of increased microbial contamination in refrigerated water was the loss of chlorine residual in IRWDS.

Discussion

Results from this study show that the use of IRWDS led to sharp increases in microbial contamination of refrigerated drinking water (Fig 1A), which coincided with the loss of chlorine residual intended for preventing waterborne microbial growth (Fig 1B). Since carbon-based filtration materials, such as the carbon-block filters used in IRWDS, are known to remove chlorine residual[4], the loss of chlorine residual is thus expected following the passage of chlorine-containing tap water through IRWDS. These carbon-block filters are intended for pollutant removal at the point of use. However, the unintended consequence of disinfectant loss and subsequent increases in microbial contamination warrant further assessment of the public health risks associated with IRWDS.

Conclusions

IRWDS in residential homes resulted in elevated microbial contamination of drinking water, representing a potential public health concern. The deterioration in drinking water microbiological quality is likely due to unintended removal of disinfectant residual by carbon-block filters included in IRWDS, which warrants further assessments to mitigate potential risks of exposure to waterborne pathogens.

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References

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