

Understanding the Microbiological Quality of Drinking Water at the Point of Consumption with Citizen Science

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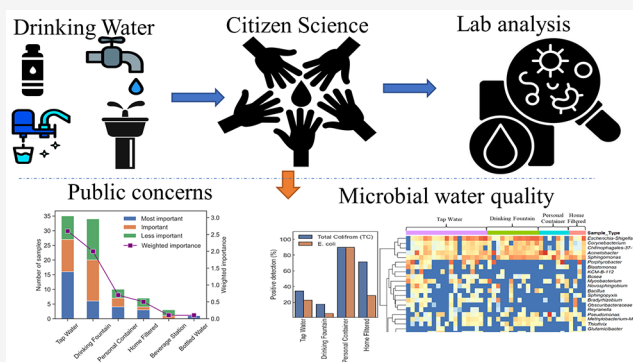
Article Recommendations



Supporting Information

ABSTRACT: Water quality monitoring at the point of consumption by consumers can improve the understanding of the microbial risk of drinking water. In this study, drinking water samples were collected by citizen science participants according to their normal drinking sources and behaviors. Among six different sample types, tap water received the most attention per sampling frequency (39%) and weighted importance score, followed by drinking fountain (38%) and personal container (21%). Enumeration of *Escherichia coli* and the total coliform as indicator organisms showed consistent and good quality in water samples from tap water and drinking fountains but significantly higher detection frequencies (90 and 90%, respectively) and median concentrations (14 and 252 CFU/100 mL, respectively) in water samples from personal containers. Microbial community analysis based on 16S rRNA gene amplicon sequencing showed that the microbial communities in water samples from personal containers were less diverse than microbial communities in the other water types, the latter of which showed high similarity in the microbial composition and α and β diversity. Several genera that include species of opportunistic pathogens were detected across samples from drinking water sources, while higher total relative abundances of these genera were detected in water samples from personal containers than others.

KEYWORDS: drinking water, microbiological water quality, citizen science, opportunistic pathogens



INTRODUCTION

Waterborne diseases due to contaminated drinking water remain a significant public health concern, causing tremendous disease burden and healthcare costs.^{1,2} Effective treatment processes and routine monitoring of the microbiological water quality are powerful tools to ensure drinking water safety at the treatment plants, while maintenance of an adequate disinfection residual in the distribution network has been required to prevent regrowth of pathogens.³ In spite of these treatment and preventive measures, microbial contamination can still occur during water distribution due to various internal or external events. The internal events can include the gradual depletion of chlorine residues over time (especially with long water ages), failure in the plumbing system due to equipment breakage, poor system design, and improper maintenance.^{4,5} External events can be natural disasters such as hurricane⁶ and flooding,⁷ which can overwhelm the treatment processes and preventive measures and introduce microbial contaminants into drinking water.

Previous studies on drinking water quality mainly focused on the water at the treatment plant or within the distribution system, which is essential to prevent large-scale contaminations and outbreaks but does not necessarily ensure the water quality at the point of consumption. The point of consumption typically

refers to tap water but should also include home filters and secondary containers, as individual water consumption behaviors may also affect the microbiological quality of drinking water. For example, home water treatment strategies (e.g., filtering and refrigerating) and cleaning and maintenance of personal water containers are factors that can alter drinking water quality after the water tap.^{8,9} Monitoring drinking water quality at the more diverse point of consumption can assess actual risks, yet the complexity of water distribution systems and the diversity of water consumption behaviors make this challenging. A few previous studies conducted a survey of water quality at the point of consumption, where the water sampling design and implementation were conducted by research scientists.^{9,10} In recent years, citizen science, which involves citizens in the scientific sample and data gathering,¹¹ has been adopted in many areas of scientific research, including the United Nations (UN) Sustainable Development Goals (SDG) reporting,¹² monitoring

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of river water quality,¹³ shoreline water quality,¹⁴ chlorine surveillance in fresh and drinking water sampled by water utilities,¹⁴ and lead detection in drinking water childcare centers.¹⁵ The citizen science approach presents unique opportunities for data collection that enables unique access to the diverse point of drinking water consumption.

In this study, drinking water samples were collected by citizen science participants from their normal drinking sources. A survey of the importance of the different types of drinking water was completed by the participants. Drinking water samples were analyzed through cultivation-based enumeration for two indicator organisms, including *Escherichia coli* and total coliform (TC), to indicate the microbiological water quality. The samples were also analyzed with cultivation-independent 16S rRNA gene amplicon sequencing to determine the overall microbial community structures. The microbiological water quality data and the microbial community data were analyzed with respect to the importance expressed by the participants and the drinking water sample types.

MATERIALS AND METHODS

Study Site, Participant Recruitment and Training, and Sampling Collection. The research was conducted in Honolulu, Hawaii, United States, in April 2018. Recruitment of citizen science participants followed a protocol approved by the Institutional Review Board (IRB) of the University of Hawaii at Manoa. Advertising flyers on campus were used to recruit 30 participants, and all participants attended an introductory workshop that focused on providing basic layman's information on drinking water sources, treatment, distribution network, potential contamination issues, quality parameters, and laboratory methods for drinking water monitoring and sample collection procedures.

For drinking water sample collection, participants were provided a sampling kit with all supplies needed and a written sampling procedure modified from the sampling guidance by the United States Environmental Protection Agency (EPA).¹⁶ Each participant collected three drinking water samples of their choosing, from their normal drinking water sources and following their individual drinking behaviors, resulting in a total of 90 samples. The drinking water distribution system in Honolulu uses groundwater directly as source water without further treatment except for adding a low level of free chlorine to provide disinfectant residual. Drinking water samples were collected in 500 mL of sterilized polyethylene wide-mouth bottles that contained 0.5 mL of 10% sodium thiosulfate to neutralize residual chlorine. The collected samples were stored in a cooler with ice packs and were returned to the laboratory for immediate analysis (i.e., within 12 h after sample collection).

Ranking of Importance. To understand the diverse drinking water sources at the point of consumption and their respective importance to the participants, each participant ranked the three samples collected in terms of importance (i.e., most important, important, less important). The importance ranking information was used to calculate a weighted importance score ($\bar{X}_w = \sum_{i=1}^3 \frac{X_i \times N_i}{N_{\text{tot}}}$) for each drinking water sample type collected, which takes into consideration both the individual importance rank and sampling frequency. $\bar{X}_w$ is the average important score for one type of drinking water, which was weighted by the sampling frequency of the group; X_i is the important scores assigned by the participants, which descend in importance/concern from 3 to 1; N_i is the sampling frequency

for one type of drinking water with the same importance score; N_{Tot} is the number of participants of sampling (i.e., 30). An example of the calculation of the weighted importance score is shown in Supporting Materials Table S1.

Cultivation-Based Assessment of Drinking Water Quality. The microbiological quality of the drinking water samples was assessed by enumerating *E. coli* and TC by membrane filtration, according to the EPA Method 1604.¹⁷ In brief, 100 mL of drinking water samples were filtered through a 47 mm, 0.45 μm pore size cellulose ester membrane filters. The membrane filters were then placed on MI agar plates and incubated at 35 °C for 24 h. After incubation, blue colonies indicate the breakdown of the chromogen indoxyl- β -D-glucuronide (IBDG) by the *E. coli* enzyme β -glucuronidase and were counted as *E. coli*, while fluorescent colonies under longwave ultraviolet light (366 nm) indicate the breakdown of 4-methylumbelliferyl- β -D-galactopyranoside (MUGal) by the TC enzyme β -galactosidase and were counted as TC.

DNA Extraction and 16S Amplicon Sequencing. Microbial communities in the drinking water samples were analyzed by cultivation-independent 16S rRNA gene amplicon sequencing through next-generation sequencing (NGS). Aliquots of the water samples (100 mL) were filtered, as described above, and used for total genomic DNA extraction, polymerase chain reaction (PCR) amplification of 16S rRNA gene fragments, NGS amplicon sequencing, and bioinformatic analysis by following the procedures described previously.¹⁸ Briefly, the filters were cut into small pieces before total genomic DNA was extracted by using a PowerSoil DNA Extraction Kit (Qiagen). The V4 hypervariable region of the 16S rRNA gene was first amplified with the F515/R806 bacterial/archaeal primers¹⁹ for 30 cycles. PCR products were then reamplified by PCR for 15 cycles by using F515/R806 primers linked with the sequencing adapters CS1 (5'-GCTGCGCGCAACGGC-GAAG-3') and CS2 (5'-TCCCGGCAGAGTTCCCAT-3'). Successful amplification was confirmed by the presence of DNA bands with the expected size after gel electrophoresis of the PCR amplicons. Library preparation of amplicons, multiplex indexing, and subsequent sequencing on an Illumina Miniseq platform was performed by the DNA service facility at the University of Illinois at Chicago to generate paired-end sequence reads with a length of 2×153 bp. The average sequencing depth per sample was 172,168 sequence reads ($\sigma = 16,658$).

Bioinformatic and Microbial Community Analysis. Bioinformatic and microbial community analyses were performed within QIIME2 (v. 2022.11).²⁰ Sequence quality control and detection of amplicon sequence variants (ASVs) were performed using the DADA2 method²¹ within QIIME2. Only ASVs with frequencies larger than 5 reads were included in subsequent analysis. The ASVs were aligned to the Silva SSU 132 99% database²² for taxonomy assignment. The α diversity (within-sample diversity) and β diversity (between-sample diversity) were calculated using the ASV data. For α diversity, ASV richness, Shannon index, and phylogenetic diversity (normalized to the sequencing depth) were calculated. For β diversity, nonmetric multidimensional scaling (NMDS) analysis was performed using the Bray–Curtis dissimilarity. Along with the NMDS analysis, ellipses of multivariate t-distribution at a 95% confidence level were generated by the *stat_ellipse* function in the R environment for sample grouping. Relative abundances of assigned taxa were visualized by heatmaps with the *heatmap* package²³ in the R environment,²⁴ while taxa clustering was

performed based on the Euclidean distance matrix of abundances in samples.

Statistical Analysis. Pearson correlation was used for linear correlation analysis. Data subjected to analysis of statistical difference were pretested for normality with a quantile–quantile (Q–Q) plot using the `scipy.stats.proplot` package of Scipy.²⁵ Data showed an $R^2 > 0.6$ of a least-squares regression in the Q–Q plot, which was considered a normal distribution. Statistical differences between different drinking water types were tested by one-way analysis of variance (ANOVA) (or Kruskal–Wallis ANOVA when not normally distributed). When the significant difference between groups is proven, pairwise Student's *t*-test and Dunn test were used as post hoc tests for ANOVA and Kruskal–Wallis ANOVA, respectively, using the `scikit-posthocs` package.²⁶ A *P* value < 0.05 was considered to indicate statistical significance. Statistical analysis was performed with Jupyterlab²⁷ in Python environment. Visualization was conducted by using Seaborn²⁸ and Matplotlib²⁹ in Jupyter in Python environment or `ggplot2`³⁰ and the `heatmap` package²³ in the R.²⁴

RESULTS

Drinking Water Sampling Preference. 30 citizen science participants collected 90 drinking water samples from six categories of drinking water types and provided ranks of importance and/or concerns (Figure 1). Among the six different

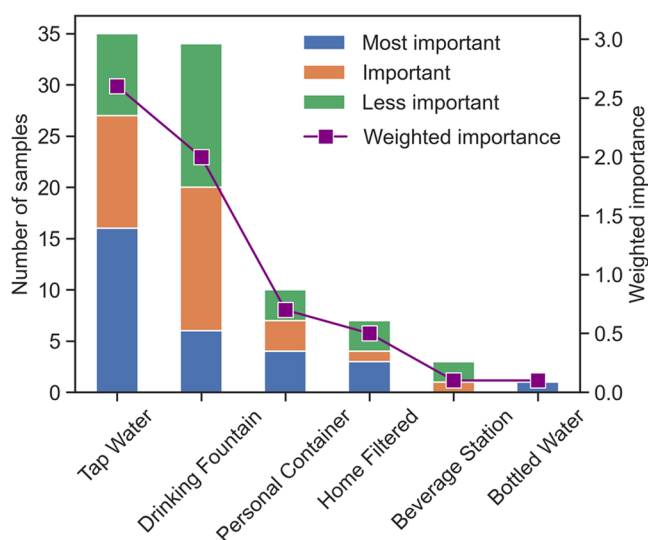


Figure 1. Number of samples collected from different drinking water types by citizen science participants (left axis, bar plot) and the weighted importance scores for different drinking water types (right axis, scatter plot).

sample types, tap water ($n = 35$, 39% of the samples) and drinking fountain ($n = 34$, 38%) received significantly higher sampling frequencies than personal container ($n = 19$, 21%) and home filtered ($n = 7$, 8%). Correspondingly, tap water and drinking fountain also received higher weighted importance scores of 2.6 and 2.0, respectively, than those for personal container (0.7) and home filtered (0.5). The number of samples collected from beverage station ($n = 3$) and bottled water ($n = 1$) were too small and thus were not further analyzed in statistical and sequencing analysis.

Water Quality Per Indicator Organisms. Cultivation-based enumeration of *E. coli* and TC in the water samples showed different detection frequencies and concentration levels

of the two indicator organisms in the different drinking water sample types (Figure 2A). When water samples from all six types were lumped together for analysis, *E. coli* was detected in 28% (25/90) and TC was detected in 33% (30/90) of all drinking water samples, and the detection frequency of *E. coli* showed a strong positive correlation with that of TC (Pearson's $r = 0.93$). Interestingly, detection frequencies of both *E. coli* and TC in different drinking water sample types exhibited opposite trends with that of the weighted importance indicated by the participants, as indicated by negative correlations (Pearson's $r = 0.78$ and 0.91 , respectively). The two sample types that received the highest weighted importance scores (i.e., tap water and drinking fountain) actually reported lower detection frequencies of *E. coli* (34 and 18%) and TC (23 and 6%) than some other types of drinking water sources, especially water from personal container (90% for both TC and *E. coli*) (Figure 2A).

As the concentrations of TC and *E. coli* in different types of drinking water did not all show normality (data not shown); thus, Kruskal–Wallis ANOVA was used to compare the difference between different drinking water types. Significant differences between different drinking water types were observed for concentrations of both TC ($P < 0.001$) and *E. coli* ($P < 0.001$). For TC, the median concentrations in the tap water samples and drinking fountain samples were < 1 CFU/100mL ($n = 35$) and < 1 CFU/100mL ($n = 34$), with average and geometric mean values summarized in Table S2. These two TC concentrations were also significantly lower than that in samples from personal container (median = 252 CFU/100mL, $n = 10$) ($P < 0.001$) (Figure 2B), while the TC concentrations in drinking fountain samples were also significantly lower ($P < 0.05$) than those in home-filtered water samples (median = 14 CFU/100mL, $n = 7$). For *E. coli*, the median concentrations in samples from tap water, drinking fountain, personal container, and home-filtered water were <1 CFU/100 mL ($n = 35$), <1 CFU/100 mL ($n = 34$), 14 CFU/100 mL ($n = 10$), and <1 CFU/100 mL ($n = 7$), respectively (Figure 2C). Similar to the results of TC, the highest median concentration of *E. coli* was also observed in water samples from personal containers, which were significantly higher than the other three drinking water types ($P < 0.006$).

Microbial Communities in the Drinking Water Samples. Microbial communities in the drinking water samples collected from tap water, drinking fountain, personal container, and home-filtered water ($n = 86$) were characterized by 16S rRNA gene amplicon sequencing. Because the low biomass in drinking water samples can often lead to low numbers of sequence reads, the sequencing data for all samples were first analyzed through rarefaction analysis to determine if the sequencing depths were sufficient based on the observed ASVs (Figure S1) and Shannon index (Figure S2). The observed ASVs and Shannon index both plateaued after a sequencing depth of 1500 clean reads (after quality trimming and denoising), which was subsequently used as the cutoff to exclude samples with an insufficient sequencing depth from further analysis. Out of the 86 samples sequenced, only 44 samples, including 19 tap water samples, 12 drinking fountain water samples, 7 personal container water samples, and 4 home-filtered water samples, met the cutoff value and were used in subsequent analyses.

Four microbial diversity indices, including the number of observed amplicon sequencing variants (ASVs), Shannon index, Faith's phylogenetic diversity, and Pielou's evenness of ASVs, in the different water types all obey normal distribution; thus,

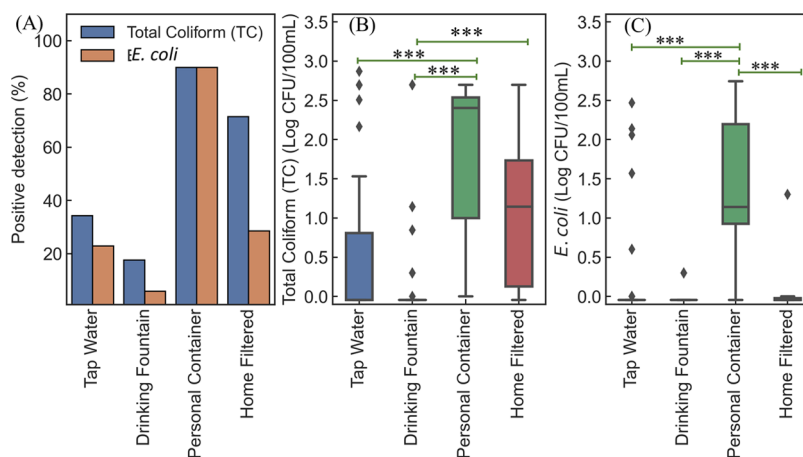


Figure 2. Detection frequencies of total coliform (TC) and *E. coli* (A); concentrations of TC (B) and concentrations of *E. coli* (C) in drinking water samples grouped by water types. ** indicates $P < 0.01$. 0.9 CFU/100 mL (log (-0.05)) was used for samples with no detection.

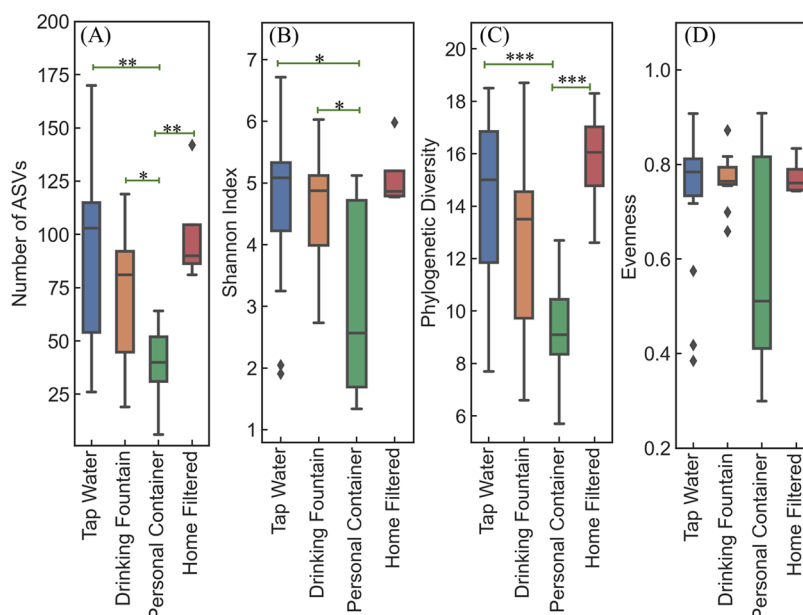


Figure 3. Comparison of α diversity indices, including the number of observed amplicon sequencing variants (ASVs) (A), Shannon index (B), Faith's phylogenetic diversity (C), and Pielou's evenness (D), in drinking water samples grouped by the four drinking water types, based on the saturated sequencing depth (i.e., 1500). ***, **, and * indicate a P value < 0.01 , 0.05 , and 0.1 , respectively.

ANOVA test and post hoc pairwise t -test were used to investigate the statistical differences. Overall, samples from the four water sources exhibited significantly different ASV richness ($P = 0.010$), Shannon index ($P = 0.019$), and Faith's phylogenetic diversity ($P = 0.004$), but not significantly different Pielou's evenness ($P = 0.070$) (Figure 3). Especially, the post hoc test indicated a significantly lower α diversity in water samples from personal container, with ASV richness lower than tap water and drinking fountain, Shannon index lower than tap water and drinking fountain, and Faith's phylogenetic diversity lower than tap water and home-filtered water (Figure 3). No significant difference in α diversity was observed between the tap water, drinking fountain water, and home-filtered water.

The tap water, drinking fountain water, and home-filtered water also exhibited high similarity in the microbial composition (β -diversity) at the ASV (Figure 4A), genus (Figure 4B), and family levels (Figure S3), as indicated by the absence of distinct clusters according to the three drinking water sample types. The significant overlap in the 95% confidence regions of the different

sample types indicated that the three different drinking water samples shared the majority of ASVs. Similar to the α diversity indices (Figure 3), personal container water samples exhibited a more varied microbial composition than the other three water types, as indicated by the generally broader 95% confidence regions and the sample points outside the overlap region of the other three water types, at all of the three levels tested.

Major Genera and Opportunistic Pathogens in Drinking Water Samples. In this study, we surveyed the microbiomes of the drinking water samples through 16S rRNA gene next-generation sequencing and detected 2,187 different ASVs, which can be assigned to 34 phyla and 415 genera. A total of 37 major genera, which were defined as those with relative abundances of no less than 5% in at least one sample, were detected across the 42 drinking water samples from the four drinking water sample types (Figure 5). Corroborating the observation based on α and β diversity, the tap water, drinking water fountain, and home-filtered water exhibited high similarity in the major genera. Several major genera were commonly

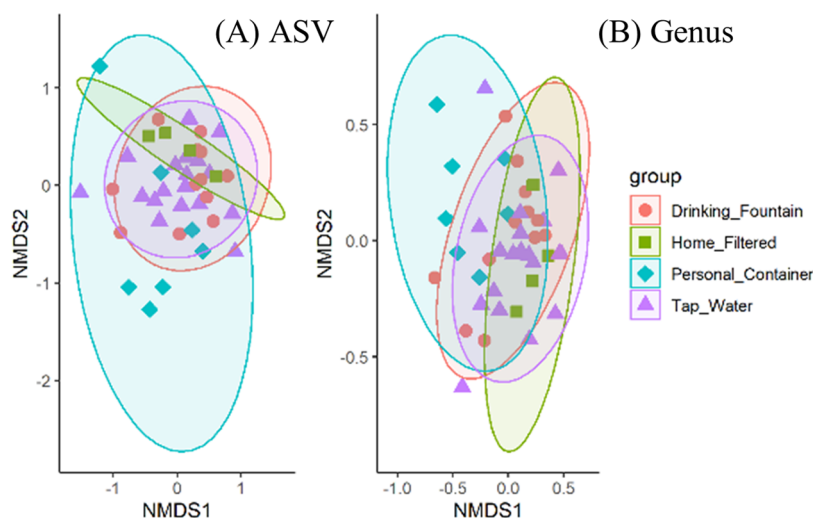


Figure 4. Nonmetric multidimensional scaling (NMDS) plots based on the Bray–Curtis dissimilarity distance to compare the microbial communities in the four drinking water sample types at different levels: amplicon sequencing variants (ASVs) (A) and taxonomic genera (B). The ellipses represent the 95% confidence regions for the different sample types.

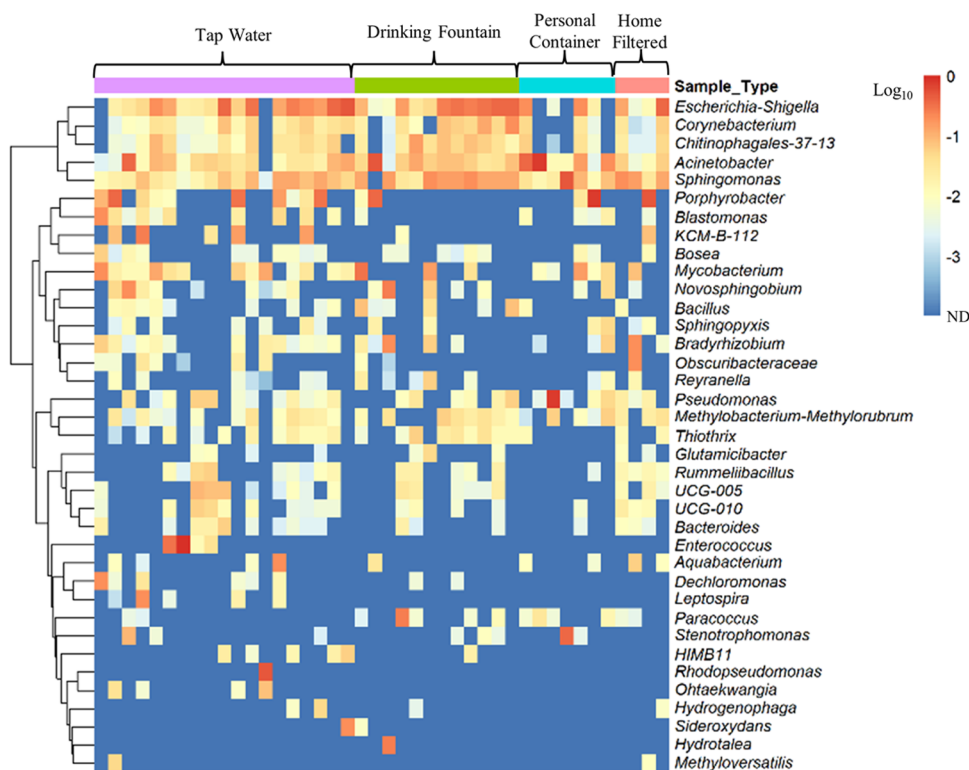


Figure 5. Major genera ($\geq 5\%$ in at least one sample) in the six types of drinking water. The scale is log 10 transformed relative abundance (unitless). Clustering of rows is based on the Euclidean distance matrix.

detected in all sample types with a high detection frequency. Those major genera frequently shared by the drinking water samples included *Escherichia-Shigella*, *Acinetobacter*, *Corynebacterium*, *Sphingomonas*, *Porphyrobacter*, and *Pseudomonas*, as the most abundant ones. Also, corresponding with the higher richness (based on observed ASVs and Shannon index), more major genera were also detected in the tap water and drinking fountain water than that in the personal container water samples where lower richness was observed. Clustering of some genera (based on the Euclidean distance of relative abundances across samples) was observed in the drinking water samples, for

example, *E. coli*, *Corynebacterium*, *Chitinophagaceae* 37–13, *Acinetobacter*, and *Sphingomonas*, suggesting the shared source of these microorganisms.

Among all genera detected in the drinking water samples, some fecal bacterial indicators and bacterial genera containing opportunistic pathogenic species were detected either as major genera ($\geq 5\%$), including *Escherichia*, *Mycobacterium*, *Pseudomonas*, *Acinetobacter*, *Bacteroides* and *Enterococcus* (Figure 5), or as nonmajor genera ($< 5\%$), including *Clostridium sensu stricto*, *Streptococcus*, *Vibrio*, and *Legionella* (relative abundance of nonmajor genera were not shown). *Acinetobacter*, *Escherichia*,

Pseudomonas, and *Mycobacterium* were detected in high relative abundance (Figure 5) and also a high detection frequency across all of the sample types (Figure 6). *Escherichia* was more

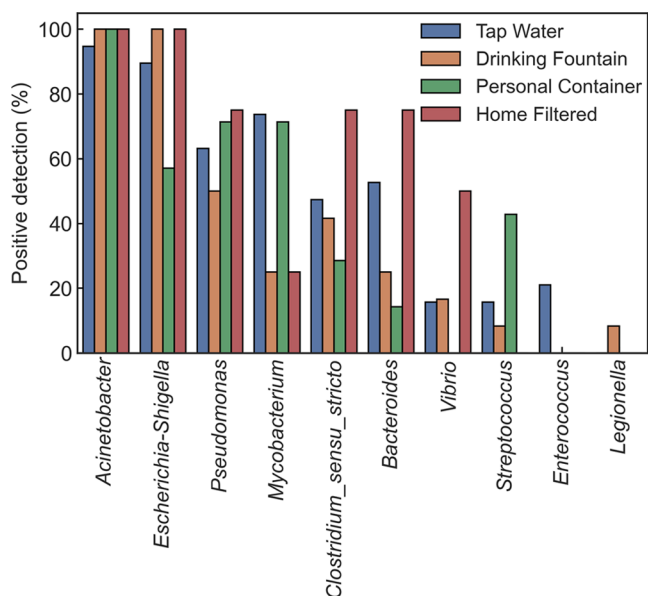


Figure 6. Detection frequency of bacterial genera containing opportunistic pathogens in drinking water samples of the four water types.

frequently detected in tap water and drinking fountain water than that in personal container or home-filtered water. *Enterococcus*, *Vibrio*, and *Legionella* were only detected in tap water and (or) drinking fountain water but not in personal container or home-filtered water samples.

DISCUSSION

Public Concerns on Different Drinking Water Types. In this study, the drinking water samples were collected by citizen science participants to examine the water quality at the point of consumption. In total, drinking water from six different types, including tap water, drinking fountain, personal container, home-filtered, beverage station, and bottled water, were collected, covering a wide range of drinking water consumption. The different sampling frequency and weighted importance scores reflect the different levels of importance and/or concern that the participants reported on the six drinking water sample types (Figure 1). The easily accessible public drinking water supplies, including tap water and drinking water fountains, received the most attention in this study as indicated by their higher sampling frequencies and weighted importance scores than the other categories of drinking water sources. Home filtered, personal containers, beverage station, and bottled water received less attention, which may be due to additional costs or efforts involved in sampling or lower consumption frequencies by the participants.

Microbiological Quality of Different Drinking Water Sample Types. The microbiological quality of the drinking water samples was characterized using two distinct approaches: cultivation-based enumeration of traditional indicator organisms and cultivation-independent microbial community analysis based on 16S rRNA gene amplicon sequencing. Although time-consuming and labor-intensive, the cultivation-based enumeration methods are highly sensitive because of their enrichment

through selective growth and capability in differentiating viable bacterial cells from dead cells and environmental DNA. *E. coli* and TC are the two commonly used indicator organisms to detect the presence of fecal contamination in water because of their simple and cost-efficient detection compared to actual pathogens.

The two indicator organisms, *E. coli* and TC, were detected in some of the samples by the cultivation-based enumeration method, which may indicate potential fecal contamination or growth of these indicator organisms in drinking water during transportation in the distribution system and storage prior to consumption. It is most interesting to observe that much higher concentrations of both *E. coli* and TC were detected in water samples from personal containers than those collected from tap water and drinking fountain water. Beyond the quality of the original water sources (which were typically tap water or water fountain), water from personal water containers can also be affected by individual hygiene practices and maintenance habits (e.g., routine container cleaning). The results suggest potential contamination and regrowth of these indicator organisms within personal containers during filling and storage. A previous study also reported a higher detection frequency of *E. coli* in household drinking water storage tanks than in tap water.³¹ Contaminated hands play an important role in microbial contamination of drinking water during collection, transport, and storage, as the levels of fecal contamination on hands were positively correlated with fecal contamination in stored drinking water within households.³² Variations in regular cleaning and maintenance of personal water containers may also lead to the development of biofilms on the interior wall of personal water containers, which could contribute to the observed higher *E. coli* and TC counts.

TC is the most common drinking water quality parameter that frequently experiences exceedance, representing about 37% of all violations in the United States in the years 1982–2015.³³ In this study, we also observed a higher detection frequency and higher concentration levels of TC in water samples from home filters than that in tap water and drinking fountains. The results are in agreement with previous studies that showed that the installation of a point-to-use filter could increase the bacterial concentration in the drinking water.^{34,35} Water filter usage can trap and accumulate microorganisms onto the filter, and biofilms developed in an aged filter can result in increased microbial concentrations in the effluent.³⁴ Because of the varying biological stability of drinking water from the water treatment facility to the point of consumption, it can be affected by many factors, including nutrient conditions, residual disinfectants, and environmental conditions.³⁶

Microbial Community in Drinking Water. Based on 16S rRNA gene amplicon sequencing, all drinking water sample types in this study showed similar microbial community diversities (Figures 3 and 4). This finding is in alignment with a previous study where the high similarity in the microbial community in tap water and drinking fountain water was observed through metagenomic sequencing.³⁷ At the genus level, several genera were found abundant across the four drinking water sample types in this study, including *Escherichia-Shigella*, *Acinetobacter*, *Sphingomonas*, *Porphyrobacter*, *Pseudomonas*, *Mycobacterium*, and *Corynebacterium*. A previous study on drinking water using 16S rRNA gene amplicon sequencing also frequently reported the relative abundance of these genera, for example, *Sphingomonas*, *Enterobacter*, *Pseudomonas*, and *Mycobacterium* were found among the dominant genera in tap water.^{38,39}

The most important microbial risks in drinking water are associated with human pathogens, and several genera detected in the drinking water samples can contain opportunistic pathogens (Figure 6). Genera including *Escherichia-Shigella*, *Acinetobacter*, *Pseudomonas*, and *Mycobacterium* were among the most frequently detected, which were frequently reported in drinking water samples.^{40–42} The *Escherichia-Shigella* genera include several highly pathogenic *E. coli*⁴³ as well as *Shigella* spp.⁴⁴ Some *Acinetobacter* spp. are considered emerging pathogens due to their ability to persist in the environment and to resist various antimicrobial and sterilization agents.⁴⁵ *Mycobacterium* spp. and *Pseudomonas aeruginosa* are opportunistic pathogens commonly detected in water networks.^{46,47} This study also detected *Clostridium sensu stricto* (mainly *stricto* 1) that contains *Clostridium perfringens*, which is an important cause of foodborne illness. *Bacteroides* species are among the most abundant species in the feces of warm-blooded animals,⁴⁸ while human-associated *Bacteroides* spp. have been adopted as indicators of human fecal contamination markers in water environments.⁴⁸ Comparison of the total relative abundances of the bacterial genera containing opportunistic pathogens showed higher abundances in water samples from personal containers (median = 56%, *n* = 7) than those samples from tap water (median = 28%, *n* = 19), drinking water fountain (median = 28%, *n* = 19), and home-filtered water (median = 18.3%, *n* = 4).

Implications of This Study. This study investigated the microbiological quality of drinking water from different drinking water types at the point of consumption. This endpoint monitoring may provide a more accurate assessment of the microbial risks of the final drinking water quality than at treatment facilities. Numerous factors, including waning of disinfectant residuals and bacterial regrowth, interaction with biofilms in water pipes,⁴⁹ and potential contamination during pipe distribution, in-house transport, and storage,⁵⁰ may all contribute to a distinct endpoint microbiological quality at the point of consumption than at centralized monitoring points (such as treatment facilities). This study demonstrated the feasibility of using the citizen science approach to collect samples from diverse locations and sources in order to understand the microbiological risks of drinking water at the point of consumption. Another distinction of this study is that the drinking water samples were collected by citizen science participants in a manner consistent with their typical water consumption behaviors instead of conforming to the current regulatory ion sampling strategy. The drinking water regulatory sampling requires considerable flushing before sampling, which is designed to monitor water quality in the distribution network but is less likely to capture the microbiological risks at the point of consumption as such flushing is often not practiced before water consumption.

Among the different drinking water sample types, tap water received the most attention from the participants, followed by drinking fountain and personal container, as indicated by the sampling frequency and weighted importance score. The numerous samples collected from water taps and drinking fountains from diverse locations within the distribution network showed consistent good water quality based on culture-based enumeration of *E. coli* and TC. This is also supported by microbial community analysis, which showed high similarity in the microbial composition and α and β diversity among the different drinking water types. Interestingly, the highest detection frequencies and concentrations of *E. coli* and TC were actually observed in water samples from personal

containers than from other drinking water types. While water samples from personal containers showed the lowest diversity, suggesting selective enrichment of certain bacterial populations. This indicates that personal hygiene and secondary storage maintenance can lead to significant water quality deterioration after dispensing from the distribution network, which, however, may often be overlooked by consumers.

CONCLUSIONS

This study investigated the microbiological quality of drinking water at the point of consumption collected by citizen science participants. A survey of concern revealed that tap water received the most attention, followed by water from drinking fountains and personal containers among the different drinking water sample types. Cultivation-based enumeration of indicators *E. coli* and TC showed consistent and good water quality from tap water and drinking fountains but higher concentrations and detection frequencies of indicator organisms in water samples from personal containers despite the less sampling attention. Microbial community analysis based on 16S rRNA gene amplicon sequencing revealed a similar microbial diversity in samples from tap water, drinking fountain water, and home-filtered water, while significantly lower microbial diversity was observed in water samples from personal containers. Several genera including *Escherichia-Shigella*, *Acinetobacter*, *Pseudomonas*, and *Mycobacterium* that encompass opportunistic pathogens were frequently detected by sequencing across the drinking water samples.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsestwater.3c00184>.

Sampling procedure; calculation of the weighted importance score with examples; rarefaction analysis of sequencing data clarifying the required sequencing depth for analysis in this study; and similar microbial communities in the different drinking water sample types at the taxonomic family level (PDF)

Drinking water samples collected by the citizen science participants (XLSX)

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Author Contributions

CRedit: **Xingnan Li** data curation (lead), formal analysis (lead); **Prakit Saingam** data curation (equal).

Notes

The authors declare no competing financial interest.

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