Microbial Community Structure and Diversity in Drinking Water Supply, Distribution Systems as well as Household Point of Use Sites in Addis Ababa City, Ethiopia

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Abstract
Understanding ecology of microbiomes in drinking water distribution systems is the most important notion in delivering safe drinking water. Drinking water distribution systems harbor various microbiota despite efforts made in improving water infrastructures in the water industry, especially, in developing countries. Intermittent water supply, long time of water storage, low water pressure, and contaminated source water are among many of the factors responsible for poor drinking water quality affecting health of people. The aim of this study was to explore microbial diversity and structure in water samples collected from source water, treated water, reservoirs, and household points of use locations (taps). High-throughput Illumina sequencing technology was employed by targeting the V4 region of the 16S rRNA gene and the V1–V3 region of the 18S rRNA gene to analyze the microbial community structure. Proteobacteria followed by Firmicutes, Bacteroidetes, and Actinobacteria were the core dominating taxa. Gammaproteobacteria was also dominant among other proteobacterial classes across all sampling points. Opportunistic bacterial genera such as Pseudomonas, Legionella, Klebsiella, Escherichia, and Actinobacteria, as well as eukaryotic microbes like Cryptosporidium, Hartmannella, Acanthamoeba, Aspergillus, and Candida were also abundant taxa found along the distribution systems. The shift in microbial community structure from source to point of use locations was influenced by basic factors such as residual chlorine, intermittent water supply, and long-time storage at the household. The complex microbiota detected in different sampling sites in this study brings drinking water quality problem which further causes significant health problems to both human and animal health. Treatment ineffectiveness, disinfection inefficiency, poor maintenance actions, leakage of sewage, and other domestic wastes are few among many other factors responsible for degraded drinking water quality in this study putting health at high risk. Findings of this research provide important and baseline information to understand the microbial profiles of drinking water along source water and distribution systems. Moreover, knowing the microbial profile will help to design proper water quality assurance approaches.

Keywords Disinfection · Illumina sequencing · Microbiota · Opportunistic genera · Taxa · Water supply systems

Background
The primary goal of centralized drinking water treatment and distribution systems is to supply safe and risk-free drinking water to consumers; however, it is the most challenging system as distribution systems host complex microbial assemblages. Despite continuous effort has been exerted in the development of urban water infrastructure, waterborne disease is still common in the cities of developing countries due to intermittent treated water supply which leads to excessive storage, low pressure events, poor integrity of distribution system piping, and contaminated source waters [1–4]. Sustainable development goals (SDGs) are targeting these challenges and propose to achieve universal and equitable access to safe and affordable drinking water for all by 2030 [5]. Understanding the microbial community structure and profile should be given top priority for developing effective water quality monitoring strategies and for delivering safe drinking water as well as protection against health-risk waterborne pathogens [2, 3].
Although treatment alone using disinfectants is sometimes enough to produce microbiologically safe water, different steps of treatment systems should be employed to achieve the goal of producing safe drinking water from raw water sourced either from the surface or ground. The failure of source water protection from contaminants and inability of disinfection technologies to eliminate microorganisms lead to deterioration of water quality in most domestic and industrial potable water supplies. This may happen as a result of the entrance of pathogens and other pollutants directly into the distribution network from the treatment plant or due to re-growth and survival of microorganisms in the pipe walls which are later released into the flowing water through pipe breakages or joint leakages [3]. Lack of access to adequate and safe drinking water is the primary problem among most of the world population and this fact is true especially in sub-Saharan and South Asian regions. Bacteriologically good-quality drinking water at its source could easily be highly exposed to contaminants during distribution, delivery, storage, and handling at households and therefore cannot meet its standard quality for drinking purpose [6].

Ethiopia is in the forefront of the countries in sub-Saharan Africa with insufficient access to safe drinking water, which poor drinking water and inadequate water supply contribute to 50% of waterborne diseases [7, 8]. In Addis Ababa, the capital city, rapid increase in population growth, unregulated urban expansion [9], poor waste management practices [10], intermittent water supply services [11, 12], and insufficient operation and maintenance of treatment and distribution systems [13] are not only the driving forces for drinking water contamination but also limit the capacity of water supply utility and efficiency of sewage discharging capabilities. The old age of piping systems in Addis Ababa, degraded infrastructure, and cross-connected distribution systems have all an inevitable potential for contamination of drinking water through distribution systems and at the household.

The source water, treatment, and drinking water distribution systems in Addis Ababa City are assumed to harbor various complex microbial assemblages which seriously affect the water infrastructures and the quality of water delivered to consumers which inherently pose health risks. Although more attention is being directed to understand the microbial community profiles of Addis Ababa’s drinking water systems, studies conducted on the assessment of drinking water quality across various regions in Ethiopia and in Addis Ababa City were limited only in the detection of specific indicator organisms using culture-dependent techniques and hence very defective in exploring community structure and diversity of various microorganisms found across distribution systems, storage facilities, and household point of use sites [6, 14, 15]. Despite their convenience for routine monitoring, culture-based methods tend to underestimate the actual number of microorganism and the diversity and potential presence of both opportunistic and pathogenic microorganisms in water samples due to poor cultivability [16] or growth competition by non-pathogenic heterotrophic plate count (HPC) bacteria [17]. Next-generation sequencing platforms are useful for wide range identification and investigation of drinking water microbiota without the need to employ cultivation and have begun to be used in both resource-rich and constrained settings [18]. By employing next-generation sequencing techniques, it is possible to detect and identify the presence and diversity of pathogenic microorganisms that cannot be achieved with classical methods [19]. Therefore, this study was fundamentally important to fill this gap by studying the whole microbial profile across water supply systems of two water treatment plants in Addis Ababa City.

This study aimed to elucidate the microbial community present and their diversity and community structure across water distribution systems starting from source through treatment and along the distribution systems to storage and neighborhood tap for the two drinking water treatment plants serving Addis Ababa.

The specific objectives of this study are (i) studying the diversity of microorganisms available across the water supply systems and (ii) studying the dynamics and community structure of microorganisms across the water supply systems. Illumina sequencing was used with both 16S and 18S targets. The microbial diversity across different points of distribution lines and household points of use locations in the city was comprehensively investigated.

Materials and Methods

Study Site Description and Sample Locations

The study was conducted in Addis Ababa City, the capital of Ethiopia, which lies with GPS coordinates 9° 0’ 19.4436″ N and 38° 45’ 48.9996″ E at an elevation of 2355 m above sea level. The city largely relies on surface and ground water as the main sources for drinking, domestic, and industrial purposes. There are two surface water treatment plants, Legedadi and Gefersa, that provide water from Dire and Gefersa dams, respectively. The
treatment plants use conventional treatment that includes pre-chlorination, coagulation, flocculation, sedimentation, sand filtration, and post-chlorination, with a goal of maintaining an average chlorine residual of 0.8 mg/l in the distribution system.

For this study, a total of 38 samples were collected from several locations from source to tap along both the Legedadi \((n = 22)\) and Gefersa \((n = 16)\) systems (Table S1): Samples include source water entering into the treatment plant \((LS \text{ and } GS, n = 1 \text{ each})\); finished drinking water before entering into distribution lines \((LF \text{ and } GF, n = 1 \text{ each})\); reservoirs in the distribution system that stored treated water \((LR, n = 7 \text{ and } GR, n = 2)\); and household taps inside individual houses \((LT, n = 8 \text{ and } GT, n = 4)\) and storage tankers of individual houses \((LS, n = 5 \text{ and } GS, n = 8)\). Samples were collected in July 2015 based on established sampling procedures [20]. All glass sample bottles were sterilized by an autoclave \((121 \degree C, 15 \text{ min})\), then supplemented with sodium thiosulfate to quench residual chlorine in duplicate 2-l water samples per location. Prior to sample collection from each reservoir \((R \text{ samples})\) and household standpipe \((T \text{ and } S \text{ samples})\), the water was allowed to run for 10 min in order to ensure representative sampling and to avoid sampling stagnant water.

**Microbial Analysis of Water Samples**

**DNA Extraction from Water Samples**

Two liters of water per location was filtered through polycarbonate membrane filters \((0.22-\mu m \text{ pore size EMD MiliporeTM GTTP02500})\) and frozen immediately at \(-20 \degree C\). All filtered samples were shipped frozen using ice packs via overnight courier service to the Environmental Biotechnology Laboratory at the University of Michigan, USA, and arrived frozen. Each membrane filter was cut into four equally sized pieces using a sterile knife and placed into a single vial to facilitate DNA extraction. Total genomic DNA was extracted from each vial using a Power Soil DNA Isolation Kit \((\text{MoBio Laboratories, Carlsbad, CA})\) following the manufacturer’s protocol and instructions. The extracted DNA of each sample with total volume \(50 \mu l\) was ready for high-throughput sequencing process. The concentration and purity of the extracted DNA were determined using a NanoDrop ND-1000 spectrophotometer \((\text{NanoDrop Technologies, Wilmington, DE, USA})\) (Table S2) and stored at \(-20 \degree C\) until sequencing.
16S rRNA and 18S rRNA Gene PCR Amplification and Sequencing

Extracted DNA was submitted to the Core Genome Sequencing unit at the University of Michigan, USA. The hypervariable V4 region of the 16S rRNA gene was amplified from each sample using the dual-indexing sequencing strategy with primers 515F (5′-GTGCCACGAGCAGCAGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAAT-3′) (23, 24). PCR amplification was done in a total volume of 20 µl reaction mixtures containing 2 µl 10× PCR Buffer II AccuPrime HiFi, 11.85 µl sterile water, 0.15 µl polymerase (AccuPrime), 5 µl primer set (4 µM), and 1 µl sample DNA. PCR amplification conditions were 2 min at 95 °C for 1 cycle, at 20 s for 95 °C (30 cycles), 15 s at 55 °C (30 cycle), 5 min at 72 °C (30 cycles), and 10 min at 72 °C for 1 cycle. Library preparation, normalization, quality checking, cluster generation, and sequencing were conducted in the Core Genome Sequencing Lab of the UM according to the protocol of Illumina MiSeq platform.

For eukaryotic organism detection, 18S rRNA gene fragments were amplified using universal primers (forward and reverse) Euk-A7F (5′-AAACCTGTTGATCCGTGCC AGT-3′) and Euk-570R (5′-GCTATGGAGCTGAATT AC-3′) which target the V1–V3 region of the 18S rRNA gene. PCR amplification was done in a total working volume of 770 µl containing 654.5 µl of sample library (containing AccuPrime Tag, PCR water, and 1× buffer II), 15.4 µl PhiX V3 as a control, and 100.1 µl of sample DNA. Library preparation, cluster generation, and sequencing were carried out using the Illumina MiSeq platform.

Bioinformatics and Statistical Analysis

The 16S rRNA reads generated from the Illumina MiSeq analysis were filtered, clustered, taxonomically assigned, and generally curated using MOTHUR (version 1.35.1) following the MiSeq SOP (https://www.mothur.org/wiki/MiSeq_SOP) [21]. Sequencing and PCR errors were reduced by using the make.contigs command. Furthermore, sequences that did not align to the SILVA database were removed through sequence screening, and precultering of rare sequences was merged into larger sequences [22]. The chimeras.vsearch command was used to detect and remove chimeric sequences through MOTHUR [23, 24]. Classification of high-quality sequences into OTUs at 3% distance (97% similarity) was carried out based on the Ribosomal Database Project (RDP) Training Set (version 9) [25]. Clustering of OTUs at 3% dissimilarity was done using the cluster.split command. Rarefaction analysis, Chao1, Shannon diversity, and evenness indices were computed using MOTHUR, version 1.35.1 [21]. A non-metric multidimensional (n-MDS) analysis based on the Bray–Curtis dissimilarity index was generated using PAST software (version 3) for clustering of sampling sites based on microbial composition similarity. The relative abundance bar graphs and box-whisker plot of phylum, class, and genus levels of the 16S rRNA sequence were plotted using IBM-SPSS (version 23) and XLSTAT tools. Heatmaps based on OTUs at the genus level were generated using ClustVis tool (web tool for visualizing clustering of multivariate data) [26]. The 18S rRNA gene sequences were submitted to MG_RAST web-based sequence analysis pipeline (http://metagenomics.anl.gov/) for qualitative analysis of the presence of public health-relevant parasitic and free-living eukaryotic organisms [27].

Results and Discussion

In this analysis, a total of 949,941 quality reads were processed and 22 phyla were retrieved from 729,385 curated sequences of the samples taken from 38 locations. Sequence clustering created 604 unique bacterial OTUs and 1230 eukaryotic OTUs. The rarefaction curves showed most of the sampling points failed to reach plateau, which suggested sampling frequency and sequencing depth should be improved to get higher coverage (Fig. S1).

The non-metric MDS ordination plot showed marked differences on the similarity of bacterial composition based on the distribution of OTUs (Fig. 2). Based on the analysis, samples having similar bacterial composition clustered together whereas those having different microbial assemblages are dispersed apart as shown in Fig. 2a and b. Shannon–Wiener (H’) and inverse Simpson (D−1) diversity indices calculated for samples from Legedadi water sources indicate bacterial diversity ranging between 0.6–1.7 and 1.5–14.3 respectively whereas Gefersa water sources had 0.7–1.7 and 0.7–40.3 of Shannon–Wiener (H’) and inverse Simpson (D−1) indices respectively. Untreated source water sample locations of Gefersa treatment plants (GS), reservoirs (LR2, LR4, and GR1), end of pipe/tap locations (LT3 and GT1), and storage tanker locations (LHS4 and GHST7), all from both treatment plants, had a lower Shannon–Wiener (H’) values less than one (Table 1).

Samples taken from the water (LF) leaving out Legedadi treatment plant had higher diversity and low richness compared to source water (LS), but water taken from the five reservoirs showed increasing trend of bacterial richness along their distance from the treatment plant. This might be due to loss and/or rapid decay of chlorine residuals along the reservoirs located in various degrees of proximity from the treatment plant where chlorination is applied. Evidently, LR5 located in the farthest end of the reservoirs had higher diversity and richness. Among the end of pipe water samples, increasing trend of diversity and richness was observed with respect to the reservoirs they are sourced from. For example,
LT1 and LT2, which are sourced from LR1, showed increasing bacterial diversity (1.5 to 1.7) and richness (104 to 376).

The water (GF) leaving out the Gefersa treatment plant showed slight decrease in richness compared with the source (GS) water. Water samples from the two reservoirs (GR1 and GR2) showed a slight increase in their diversity and richness across their distance from the treatment plant. Samples from the four taps (GT1–GT4) did not show consistent

Fig. 2 Non-metric multidimensional scaling (n-MDS) plot based on the Bray–Curtis similarity index to ordinate the similarity of each sample location of both Legedadi (a) and Gefersa (b) treatment plants on the bacterial taxa composition
The samples from storage tankers of both treatment plants showed variable degrees of diversity and richness. This could be explained by their difference in tanker material, age of tanker usage, stagnation of the water, and degree of cleaning frequency of the tanker. The increasing trend of bacterial diversity and richness in tap water samples of this study may be due to leakage of pipes and development of biofilms in the wall of pipes so that bacteria may get protection.

### Table 1: Community diversity and richness estimators of the 16S rRNA amplicon sequences of samples from the two treatment plants along the distribution lines and their reservoirs. Note that for each sample name, the first letter denotes the source of water (G, Gefersa and L, Legedadi), the second letter denotes whether it is the untreated source water (S), finished treated water (F), water from reservoir (R), water from end of pipe/tap (T), and water from storage tanker (S). The descriptions of sample points of each location and their distance from the respective treatment plants are found at Table S1.

| Source     | Site type     | Sampling code | Shannon ($H'$) | Inv. Simpson ($D^{-1}$) | Chao I (lci, hci) | Distance from treatment plants |
|------------|---------------|---------------|----------------|--------------------------|------------------|-------------------------------|
| Legedadi   | Treatment plant | LS            | 1              | 2.4                      | 1476 (1420, 1551) | 0                             |
|            |               | LF            | 1.5            | 5.1                      | 109 (100, 135)    | 0                             |
| Reservoir  |               | LR1           | 1.2            | 10.4                     | 92 (73, 155)      | 15.6                          |
|            |               | LR2           | 0.6            | 1.5                      | 121 (115, 140)    | 21.3                          |
|            |               | LR3           | 1.6            | 5.4                      | 168 (158, 195)    | 22                            |
|            |               | LR4           | 0.6            | 5.7                      | 152 (138, 186.3)  | 23.7                          |
|            |               | LR5           | 1.3            | 7.7                      | 220 (206, 252)    | 27.5                          |
|            |               | LR6           | 1.4            | 7.3                      | 90 (81, 117)      | 23                            |
|            |               | LR7           | 1.5            | 6.8                      | 187 (179, 210)    | 23.5                          |
| Tap water  |               | LT1           | 1.5            | 4.8                      | 104 (95, 130)     | 16.5                          |
|            |               | LT2           | 1.7            | 14.2                     | 376 (360, 410)    | 18                            |
|            |               | LT3           | 0.6            | 2.2                      | 271 (261, 297)    | 22.6                          |
|            |               | LT4           | 1.2            | 4.2                      | 538 (521, 569)    | 23.2                          |
|            |               | LT5           | 1.3            | 8.6                      | 100 (92, 125)     | 24                            |
|            |               | LT6           | 1.4            | 5.4                      | 224 (208, 261)    | 24.9                          |
|            |               | LT7           | 1.7            | 6.3                      | 139 (119, 184)    | 24.5                          |
|            |               | LT8           | 1.6            | 5.6                      | 96(91, 111)       | 18.5                          |
| Storage tanker |             | LHS1         | 1.1            | 3.8                      | 91 (82, 117)      | 19.8                          |
|            |               | LHS2         | 1.5            | 8                       | 108 (87, 161)     | 20.5                          |
|            |               | LHS4         | 0.7            | 3.1                      | 118 (107, 152)    | 23                            |
|            |               | LHS5         | 1.3            | 4.2                      | 102 (80, 165)     | 23.7                          |
|            |               | LHS6         | 1.5            | 5.7                      | 205 (200, 243)    | 18.5                          |
| Gefersa    | Treatment plant | GS            | 0.9            | 7.7                      | 1584 (1540, 1644) | 0                             |
|            |               | GF           | 1              | 40.3                     | 1390 (1353, 1444) | 0                             |
| Reservoir  |               | GR1           | 0.9            | 5.7                      | 161 (145, 199)    | 8                             |
|            |               | GR2           | 1.5            | 17.8                     | 295 (274, 336)    | 9                             |
| Tap water  |               | GT1           | 0.7            | 4.3                      | 202 (185, 245)    | 8.5                           |
|            |               | GT3           | 1.4            | 4.3                      | 104 (95, 130)     | 12                            |
|            |               | GT4           | 1.2            | 4.1                      | 89 (73, 140)      | 14                            |
|            |               | GT5           | 1.4            | 3.8                      | 159 (144, 196)    | 19.5                          |
| Storage tanker |             | GHS1         | 1.5            | 4.4                      | 108 (9, 146)      | 7                             |
|            |               | GHS2         | 1.4            | 6.3                      | 74 (64, 104)      | 9.5                           |
|            |               | GHS3         | 1.1            | 5.3                      | 130 (121, 153)    | 11                            |
|            |               | GHS5         | 1.6            | 1.3                      | 101 (95, 122)     | 12                            |
|            |               | GHS6         | 1.1            | 18.2                     | 181 (16, 238)     | 14                            |
|            |               | GHS7         | 0.7            | 4.3                      | 63.67 (57, 87)    | 15.6                          |
|            |               | GHS8         | 1.3            | 5.1                      | 165.8 (152, 198)  | 20                            |
|            |               | GHS9         | 1.6            | 4.8                      | 100 (86, 141)     | 9.8                           |
against residual disinfectants [28]. Moreover, stagnation of water in the plumbing causes deterioration of water quality and potential pathogen proliferation which in turn results in major public health problems.

Inconsistent with this study, more diverse microbial taxa across metropolitan drinking water distribution systems at different geographic environments were detected and factors like source water, physicochemical parameters, and treatment process affect microbiota diversity across distribution systems [29].

Members of Phyla Firmicutes and Proteobacteria Dominate Reservoirs of the Two Treatment Plant Systems

A total of 22 phyla were retrieved from curated sequences of the water samples. The untreated raw water (LS) collected from the Legedadi treatment plant was dominated by members of the phylum Bacteroidetes followed by Actinobacteria accounting for 88% and 87% of relative abundance of the total sequences respectively. Raw water from Gefersa treatment plants was dominated by members of the phylum Proteobacteria accounting for 65% of the total sequences, followed by Actinobacteria which was 25% of the total sequences (Fig. 3). On the other hand, the LR2 reservoir receiving water from the Legedadi treatment plant was found to harbor members of the phyla Proteobacteria and Firmicutes, represented by 50% and 21% respectively. The remaining reservoirs which received water from this treatment plant also had higher dominance of Proteobacteria and Firmicutes with varying relative abundances as compared to other members of phyla. Similarly, finished water leaving the Gefersa treatment plant was found to harbor members of the phyla Proteobacteria and Bacteroidetes, represented by 57% and 24% respectively. From reservoir sample locations (GR1 and GR2), Proteobacteria were found in high dominance with relative abundance of 83% and 61% respectively followed by Firmicutes which accounted for 13% and 34% of the total sequences, respectively. In line with this present study, in a study done by Li et al., the most dominant bacterial taxa in raw water and subsequent sampling locations including finished water through drinking water distribution systems were Proteobacteria and Firmicutes followed by Actinobacteria and Bacteroidetes. These microorganisms are known inhabitants of drinking water ecosystems and network analysis showed that bacteria belonging to Proteobacteria tended to co-occur with each other [30]. Reservoirs of drinking water distribution systems are naturally very complex environments supporting the growth of diverse microbial assemblages which can adapt to different hydraulic conditions and nutrient availability [31] which supported the presence of dominant phyla Proteobacteria, Firmicutes, and Bacteroidetes in this present study. Moreover, the predominance of Actinobacteria and Bacteroidetes after disinfection of drinking water at various reservoir locations and in household storage tanker after disinfection was reported [32].

Persistently Highest Abundance of Proteobacteria Was Observed Irrespective of Distance from the Source

The bacterial taxonomical composition in this study revealed that in both treatment plants, Proteobacteria was the most predominant bacterial phylum in all the sampling sites clustered according to their sampling locations (Fig. 4). The presence of Proteobacteria in high dominance in locations far from treatment plants in this present study might be due to the presence of low amount
of residual disinfectant, which therefore favors re-growth, multiplication, and dominance of Proteobacteria and other microbial groups [33]. Moreover, the dominance of Proteobacteria among other phyla in drinking water distribution systems was due to its ability to adapt at low nutrient availability conditions and having the capacity of biofilm formation at different pipe material surfaces [30, 34, 35].

Phylum Proteobacteria in both near and far sampling locations of the Legedadi treatment plant had significantly higher relative abundance (P<0.01, Kruskal–Wallis test) of 86% and 84% respectively followed by Bacteroidetes (15% relative abundance) in near sample locations and Actinobacteria (17% relative abundance) in far sample locations. This clearly indicated a shift of bacterial community structure from near to far sample locations. Similarly, phylum Proteobacteria with a relative abundance of 81% and 87% respectively in both near and far sampling locations was the most dominant phylum followed by Firmicutes with a relative abundance of 11% (near) and 7% (far) respectively in the Gefersa treatment plant.

A study on metagenomic bacterial community from drinking water supply systems showed similar high dominance of Proteobacteria, followed by Bacteroidetes and Actinobacteria at various residential sample locations of the supply systems [1] and the dominance of these phyla in samples taken from raw water sources of a treatment plant, reaction tank, settling pond, and clear water tank in another study was reported [36].

**High Abundance of Class Gammaproteobacteria Was Found Across Sample Locations Along Distance from Sources of Treatment Plants**

The dominant class in both near and far sample locations of the Legedadi treatment plant was class γ-proteobacteria which accounted a relative abundance of 78% and 88% respectively (Fig. 5). The second dominant class was β-proteobacteria that accounted a relative abundance of 32% and 28% from the five classes respectively in near and far sample locations. Similarly, in water samples sourced from Gefersa, class γ-proteobacteria with a relative abundance of 74% and 75% followed by β-proteobacteria with a relative abundance of 21% and 66% in near and far sample locations respectively. Like the findings of this present study, there were researches done at different locations on microbial community structure in drinking water distribution systems and reported that δ-proteobacteria followed by β-proteobacteria were the most dominant bacterial groups with increased relative abundance starting from treatment plants down to distribution systems [16, 29, 37].

Along the sampling locations which were far from the Legedadi treatment plant, the abundance of α-, γ-, and
β-proteobacteria increased and similarly sampling locations far from Gefersa treatment plant, and the relative abundance of γ-proteobacteria and β-proteobacteria increased and this indicated that these bacterial groups may be able to reside and proliferate at different locations of the drinking water distribution systems without significantly affected by disinfectants [38].

**Phylum Proteobacteria Was Highly Abundant in Running Tap and Storage Tanker Locations**

Phylum *Proteobacteria* was the most dominant phylum in received water samples from both Legedadi and Gefersa treatment plants. Significantly, the highest relative abundance (95%) of phylum *Proteobacteria* (*P* < 0.05, Kruskal–Wallis test) in running tap water followed by 87% in storage tankers was observed where there was a wide range of relative abundance in running tap water samples as compared to storage tankers received water from Legedadi (Fig. S2). Phylum *Firmicutes* was the second dominant phylum (44%) in Legedadi household tap water sample locations. Similarly, the relative abundance of *Proteobacteria* in tap water and storage tanker sample location of the Gefersa treatment plant was 98% and 92% respectively and *Firmicutes* (50%) followed by *Bacteroidetes* (42%) were the next abundant phyla in tap water samples whereas *Firmicutes* (12%) followed by *Actinobacteria* (5%) were the dominant phyla across storage tanker samples.

Several studies in consistency with this finding reported phyla *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, and *Firmicutes* as the most dominant phyla in samples taken from tap water of urban drinking water supply systems [39, 40], and hence this present study finding was not an eye-opening in its high-level detection.

The presence of bacterial taxa in wide range of their respective relative abundance at each sample location in this study was an indication of change in bacterial community from running tap and storage tanker locations throughout the distribution systems. Moreover, high dominance of bacterial taxa in tapes and storage tankers was probably due to resistance mechanisms against disinfectants and forming biofilms inside pipe surfaces and inside the wall of storage tankers as this fact was supported by scholars who did microbial community structure investigation in treated drinking water [41, 42]. The high dominance of bacterial taxa in running tap in this study compared to storage tanker sample locations might be changing water pressure through piping systems and general hydraulics which in turn causes detachment of some cells from biofilms and resuspension of sediment-associated bacteria. The presence of bacterial taxa to a lesser extent in storage tankers, on the other hand, in this study might be due to low water temperature and water stagnation which deters microbial metabolism and re-growth [43–45].

The presence of phylum *Actinobacteria* as the least proportion in treated tap water may be due to its vulnerability to disinfectant chlorine as this phylum is naturally dominant.
in raw and bulk water [46]. The dominance of *Proteobacteria* over taxa such as *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* in treated water samples collected from both running tap water and storage tanker in this study may be due to availability of nutrients under oxidative disinfection process which gives selective advantage for *Proteobacteria* having wide range of metabolic versatility and fast growth rate and also fundamental resilience to disinfection [32, 46]. The phyla *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* are the most common dominating bacterial groups in drinking water treatment systems [3, 37, 47].

Household Tap and Storage Tanker Locations Were Conducive Environments for the Dominance of Gammaproteobacteria

In running tap water and storage tanker samples sourced from Legedadi, class *γ-proteobacteria* was the most predominant among five identified classes between tap and storage tank samples with relative abundance of 92% and 88% respectively whereas in storage and tap samples sourced from Gefersa, class *γ-proteobacteria* with relative abundance of 71% and 97% respectively was observed.

The second abundant class was *β-proteobacteria* which accounted 28% and 46% in tap water and storage tanker locations of the Legedadi treatment plant and similarly 72% and 53% in tap and storage sample locations of Gefersa treatment plants respectively (Fig. 6).

Moreover, *δ-proteobacteria* was the other class detected in high relative abundance (74%) in the storage sample locations of the Gefersa treatment plant. Across all the tap water and storage tanker sample locations, there was a wide range in the relative abundance of all classes. On the other hand, *δ-proteobacteria* followed by *β*- and *γ-proteobacteria* were the most common and predominant bacterial groups usually found in residential water samples and chlorinated drinking water systems [1, 48]. There was significant variation in the relative abundance *γ-proteobacteria* among the four classes at each clustered sample location (*P* < 0.05, Kruskal–Wallis test).

The dominance of *γ-proteobacteria* shifted from the lowest to highest relative abundance across all clustered samples of tap water and storage tanker sample locations compared to other classes. Although *α-proteobacteria* has competitive advantage over other proteobacterial groups by its virtual nature of existing under low nutrient availability and its ability to degrade complex organic compounds for its nutrient need in disinfected water [46], the predominance of *γ-proteobacteria* in this study would be attributed to its survival under suppressed environments in addition to its ability of tolerance to different treatment processes and disinfectant agents [49–51]. Moreover, the relative abundance of *γ-proteobacteria* along the flow of...
the treatment process in another similar study showed that there was an increased pattern as compared to α- and β-proteobacteria classes which showed a decreasing relative abundance across the different steps of a treatment plant [30].

The presence of β-proteobacteria relatively with high percentage in potable water supplies may be the result of nutrient availability and due to formation of biofilms [52, 53] since this bacterial group is very sensitive to low nutrient availability and high residual disinfectant as compared to other proteobacterial classes. Although the effect of residual chlorine results in a dynamic shift of bacterial community structure [28–30, 32], the findings of this study showed γ-proteobacteria had comparatively selective advantage over other classes in stored and piped water samples after chlorination. From this, it can be inferred that there could be different mechanisms like high tolerance to disinfection stress and recovery during treatment.

**Pseudomonas Was the Most Dominant Genus Across the Distribution Systems**

The relative abundances of all the identified genera from both Legedadi and Gefersa treatment plants are depicted in Fig. 7 and Fig. S3. The relative abundance of *Pseudomonas* in source water (LS) of the Legedadi treatment plant was 71% and showed a slight increase (77%) in finished water (LF). The trend fluctuated across the subsequent reservoirs from 55% in LR1 to 87% in LR5, with highest level (95%) in LR4. Drinking water is commonly inhabited by *Pseudomonas* species as this genus is characterized by high prevalence, wide distribution in drinking water, and its antibiotic resistance capacity [54]. Across tap water samples, *Pseudomonas* was the most dominant in LT5 (98%) followed by LT6 (96%), and water sample from the storage tanker, LS5, was found to contain 95% relative abundance (Fig. 6). Likewise, in Gefersa treatment plants, *Pseudomonas* was also the dominant genus which accounted for 98% in the tap water sample (LT5) located far from the treatment plant compared to low relative abundance (8%) of source water (GS) and
86% relative abundance from finished water sample (GF). Next to finished water sample, reservoir GR1 was found to contain 96% relative abundance of Pseudomonas and storage samples; GS5 and GS7 respectively contained 84% and 77% relative abundance of Pseudomonas. In drinking water distribution systems and pipes, Pseudomonas and Acinetobacter commonly reside coated with thin films (biofilm) as long as organic matter is available there, and these genera cause illness in young and immunocompromised people [55]. Moreover, distribution systems characterized by intermittent water supply; low water pressure; intrusions of pathogens, metals, and other chemicals through breakage and infrastructure inefficiency; and presence of organic matter which reduce residual chlorine as well as long time storage of water in the household storage tanker are all important mechanisms of water contamination by opportunistic pathogens like Pseudomonas [4]. In agreement with this finding, high relative abundance of Pseudomonas in chlorinated drinking water distribution systems was also reported [56].

The genus Pseudomonas was repeatedly described as the leading biofilm-forming bacterial groups in treated drinking waters [57] which hence might be the basic factor for its presence at high relative abundance in drinking water distribution systems of this study. Moreover, this genus has the ability to attach to pipe surfaces and get protection from the action of disinfection by forming extracellular polymeric substances (EPS) helping for cell-to-cell communication in addition to attachment to pipe surfaces [56, 58].

Acinetobacter was the second most abundant genus in Legedadi treatment plant samples, LS, with a relative abundance of 25%, tap water (LT1), 87% and storage tanker sample (LS2) with a relative abundance of 93% and similarly a relative abundance of 92% was observed in source water (GS) of Gefersa treatment plant. Furthermore, tap water sample (GT4) and storage tanker samples (GS2, GS3, GS6, and GS8) had a relative abundance of 93%, 99%, 61%, 63%, and 55% of Acinetobacter respectively. Acinetobacter is the most common causative agent of hospital-acquired infections particularly respiratory infections in susceptible individuals [59]. Similarly, it was reported that Acinetobacter was the most abundant in centralized drinking water treatment plants after disinfection by chlorine and several of its strains were found to survive without being affected significantly [60].

Moreover, Escherichia with 9%, 38%, 6%, and 19% was observed in finished water (LF) and three reservoirs (LR1, LR2, and LR3) respectively. In agreement with this study, several opportunistic pathogenic genera were identified from chlorinated drinking tap water with Escherichia as the predominant genus [61]. Legionella with 3% relative abundance was found in source water (LS), and very small percentage from finished water (LF), few of the reservoir (LR5), tap (LT2), and storage tanker (LS3). Interestingly, Legionella was found in the finished water (GF) only and not detected from the subsequent samples in Gefersa. Although in very small percentage, detection of Legionella in distribution systems of this study might be associated with presence of host protozoa, invasion of Legionella from biofilms inside the pipe surface and low impact of chlorination [62]. Furthermore, physical–chemical conditions associated with stagnant water-like accumulated sediment, tepid temperatures, excessive water age, and absence of residual disinfectant supported Legionella growth in premise plumbing systems.

The presence of these bacterial genera which are known to contain pathogenic species in drinking water treatment systems, storage tanks, public faucets, and individual tapes was also reported [57, 63–65]. The diversity and dynamics of microbial communities in centralized water treatment plants showed the dominant of potential pathogenic bacterial groups in different sampling locations [36]. The presence of these opportunistic pathogens in this study may be due to rapid recovery after chlorination, development of resistance to disinfection, disinfection inefficiency, leakage after treatment, and low disinfectant residual across subsequent sample locations of drinking water distribution systems [66]. Moreover, the ability to survive under high temperature (up to 40 °C) and the ability to adapt at low nutrient conditions of soil, fresh water, and drinking water systems [67] were the other factors which strengthen the presence of opportunistic pathogenic groups (Pseudomonas, Acinetobacter, and Legionella) in considerable abundance.

**Sporadic Distribution of Eukaryote Communities Was Observed Across Sampling Locations**

Diverse eukaryotes were detected from all sampling locations starting from source water up to running tap and storage tanker samples across the distribution networks. The most common genera found across different sample locations of the distribution system were Hartmannella, Cryptosporidium, Cryptococcus, Acanthamoeba, Aspergillus, and Candida (Fig. 8). Hartmannella was found in 28% of all the samples sourced form the Legedadi treatment plant and 44% of samples sourced from Gefersa treatment plant. Furthermore, Cryptosporidium was also found in 22% of the samples in Legedadi and 38% of samples in Gefersa. Moreover, the genus Acanthamoeba was found in 56% of samples from Legedadi and 57% of samples sourced from the Gefersa treatment plant. Acanthamoeba, the most prominent parasitic protozoon transmitted through contaminated water, and Cryptosporidium, a causative agent of diarrheal disease in children in developing countries, are the main problems in urban drinking water supply systems [68].

The opportunistic fungal pathogens including Aspergillus were found in all sampling locations except source water (LLS) and reservoir (LR2) of the Legedadi treatment...
plant and also finished water (GF) and first reservoir (GR1) receiving water from the Gefersa treatment plant. Candida accounted 67% and 50% in Legedadi and Gefersa sample locations respectively whereas Cryptococcus was observed in 50% and 38% of the sampling locations of Legedadi and Gefersa as well. Although many species of fungi under the genera Penicillium and Aspergillus produce mycotoxins in food and beverages, interestingly there are species of Aspergillus which produce aflatoxin in cold drinking water storage tanks [69]. The human pathogen Candida species was reported in drinking water and surface water [70] and similarly, sequencing results from fungi in drinking water and biofilms showed a higher abundance of Cryptococcus [71]. A phytoplankton microalgae genus Cryptomonas was also detected in 50% and 44% of the sampling locations which received water from Legedadi and Gefersa treatment plants respectively. The unusual growth of genus Cryptomonas, a microalgae phytoplankton, plays for the presence of fishy odor in drinking water and hence contributes to low-quality water [72].

Hartmannella and Acanthamoeba serve as a host for many pathogenic waterborne organisms such as Legionella, Acinetobacter, Clostridium, and Mycobacterium [36, 73] and these host organisms are believed to be found in surface water, waste water, and drinking water systems in tropical regions where water temperature is 30 °C [74]. Although this study has not established the chlorine sensitivity test, the presence of Hartmannella in samples analyzed in this study showed its resistance to disinfection. A similar assessment of drinking water treatment plants showed very high residual chlorine concentration (0.8–3 mg/l) in produced water was insufficient for the removal of cysts of Acanthamoeba and other free-living amoebas [75].

Targeting 16S rRNA genes of specified regions (V4) with short read sequencing platforms cannot achieve the taxonomic resolution at species and strain levels which is afforded by sequencing the entire genome. Likewise, identification of microbial community using the 16S rRNA gene at the genus level may mask important levels of inter-genus population differences and heterogeneity, which remain inaccessible to short read 16S rRNA gene–based analysis. Furthermore, as a limitation of this study, under sampling in this study clearly implied further frequent sampling needs to be conducted to get sufficient sequence reads and to get bacterial populations in the study area.

**Conclusions**

To our knowledge, this study is the first in investigating the microbial community structure and dynamics of drinking water distribution systems in Addis Ababa City using Illumina MiSeq sequencing techniques. The microbial community structure, diversity, and dynamics of water samples collected from source water entering to treatment plants, finished water inside treatment plants, reservoirs, and from various household points of use pipes across the distribution system were investigated. Highly diverse microbial communities were detected across all sampling locations. Moreover, the community structure shifted throughout the distribution systems. The phyla Proteobacteria followed by Firmicutes, Bacteroidetes, and Actinobacteria were the highest predominant bacterial groups found.
in all the sampling locations. Unlike several researches which reported the predominance of Alphaproteobacteria in treated drinking water systems, Gammaproteobacteria was the predominant class found followed by Alphaproteobacteria and Betaproteobacteria. Potentially opportunistic pathogens like Pseudomonas, Legionella, Escherichia, Klebsiella, Proteus, and Acinetobacter were the dominant bacterial genera found in almost all the sampling locations. Free-living protozoan organisms like Hartmannella and Acanthamoeba which are hosts for opportunistic pathogens like Legionella and pathogenic ones like Cryptosporidium were also found in the samples analyzed.

Abbreviations GC: Gregorian calendar; HPC: Heterotrophic plate count; MASL: Meter above sea level; MG-RAST: Metagenomic rapid annotations using subsystems technology; OTUs: Operational taxonomic units; SDGs: Sustainable development goals; SOP: Standard operating procedures

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Author Contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Bable Atanfu Kassa, and primary data analysis was done by Bable Atanfu Kassa and Akeye Feleke Desta, Bayable Atanfu Kassa and Akeye Feleke Desta did data interpretation, and Bable Atanfu Kassa rewrote it. The first draft of the manuscript was written by Bable Atanfu Kassa with significant input from Akeye Feleke Desta and Fassil Asssefa. Fassil Asssefa did proof reading of the manuscript. All authors commented on previous versions of the manuscript and are responsible for the content of this paper.

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Data Availability Data provided in the paper and supplemental information are available from the corresponding author upon reasonable request.

Declarations

Ethics Approval and Consent to Participate Not applicable.

Consent for Publication Not applicable.

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