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Bacterial diversity analysis of freshwater sources for human use in rural areas of the tropical Andean region of Colombia

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Abstract

Potable water supply and sanitization in rural areas in developing countries are still inadequate. The main risk associated with unsafe drinking water is the infection with pathogenic microorganisms. Objective: In this study, we investigate the bacterial diversity and the potentially pathogenic bacteria in water samples from different points of distribution in three rural villages from the Andean region of Colombia. Methods: Illumina libraries for water samples were prepared and sequenced using 300 bp paired-end MiSeq protocol, the bioinformatic analyses were performed with Mothur pipeline and the phyloseq package in Rstudio. Results: The microbial community composition showed statistically significant differences according to the village and the sample origin. Alpha, Beta, and Gammaproteobacteria were the dominant class detected in all water samples. The most relevant pathogenic genera detected in the surface were *Legionella*, *Mycobacterium*, *Yersinia, Burkholderia*, and *Rickettsia.* In the tap water samples, potential pathogens like *Streptococcus*, *Staphylococcus, Corynebacterium, Nocardia,* and *Escherichia/Shigella* were detected.

Keywords: drinking water, pathogenic bacteria, Colombian village, microbiota, metataxonomics

Análisis de la diversidad bacteriana en aguas superficiales usadas para consumo humano en zonas rurales de la región andina colombiana

Resumen

El suministro y potabilización del agua de consumo humano en las zonas rurales de los países en vías de desarrollo sigue siendo limitado. El principal riesgo asociado con el uso de agua no potable es la infección con microorganismos patógenos. Objetivo: En este estudio se investigó la diversidad bacteriana y la presencia de bacterias potencialmente patógenas en muestras de agua de diferentes puntos de distribución en tres asentamientos rurales de la región andina de Colombia. Métodos: Se prepararon y secuenciaron bibliotecas de amplicones (rDNA 16S) para muestras de agua utilizando la plataforma Illumina MiSeq con lecturas pareadas de 300 bases. Los análisis bioinformáticos se realizaron con el programa Mothur y el paquete estadístico Phyloseq en Rstudio. Resultados: La composición de la comunidad microbiana mostró diferencias estadísticamente significativas según el sitio y el origen de la muestra. Alfa, Beta y Gammaproteobacterias fueron las clase dominantes detectadas en todas las muestras de agua. Los géneros patógenos más relevantes detectados fueron *Legionella*, *Mycobacterium*, *Yersinia*, *Burkholderia* y *Rickettsia*. En las muestras de agua del grifo se detectaron patógenos potenciales como *Streptococcus*, *Staphylococcus*, *Corynebacterium*, *Nocardia* y *Escherichia /Shigella*.

Palabras clave: Metataxonómica, diversidad bacteriana, aguas superficiales, aguas de consumo humano no tratadas, 16S rDNA

Introduction

Access to safe drinking water and sanitization is still a public health problem in South America and the Caribbean. According to the Pan American Health Organization (PAHO), 28 million people lack access to an improved water source and 83 million people lack access to improved sanitation facilities[1]. Low coverage and quality services are more common and evident in low-income, vulnerable groups, and rural populations[2]. Pathogenic microorganisms are the most critical risk factor related to drinking water[3]. The main bacterial genera involved in waterborne diseases are enteric pathogens including *Vibrio*, *Salmonella*, *Shigella*, *Escherichia*, *Campylobacter*, among others[4].

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Most of the studies related to the drink water quality assessment have been performed using culture-dependent methods to identify indicator pathogens controlled in the drinking water regulation laws[5]. The advent of high throughput DNA sequencing techniques provides a powerful broad-spectrum and high-resolution tool for microbiota assessment in different types of environmental samples[6]. In this way, using metagenomic analysis tools, Kaestli *et al*. studied Australian water samples from drinking water distribution systems of three indigenous communities and successfully reported bacterial genera associated with fecal coliforms and other intestinal pathogens such as *Clostridium*, *Campylobacter*, *Corynebacterium*, *Escherichia coli*, *Mycobacterium*, *Legionella*, *Burkholderia*, and *Leptospira*[7]. In another report, 24 pathogenic and opportunistic bacterial species were detected in water samples using a metataxonomic approach in a rural area from Haiti in which *Klebsiella spp* was the most frequent pathogen[8]. In general, the microbial diversity of drinking water in Latin America has been poorly characterized. Using novel NGS/metataxonomic technologies it will be possible to observe both environmental microbiota as well common coliform pathogens and other bacterial pathogens not included in the actual water regulation laws[8].

In Colombia, 78% of the population has access to drinking water. However, the rural areas are at high risk since the 45,2% of the rural water purification plants show quality failures[10]. The Colombian Ministry of Health reports that about 1,300 children die each year from diarrheal diseases caused by drinking unsafe quality water[9]. In this regard, the government objectives include the universal and equitable access to safe drinking water. Nonetheless, the monitoring of indicator pathogens (*E. coli* and fecal coliforms) is mainly realized in urban areas[10]. A report of the Colombian Ministry of Health informed that *E. coli* and fecal coliforms were detected in 23,8% and 32,9% of water samples collected in rural and urban areas, respectively.

In the current study, we explored the bacterial diversity of the river intake water and tap water collected from three villages in the Andean region of Colombia, using 16S rDNA sequencing. These communities obtain water by collecting it from surface water of nearby rivers and storing it in improvised tanks, and, in most cases, the water is not treated with any chemical or physical methods before it gets to its final consumers. Bacterial pathogens were detected in the tap water, this could affect the health of the communities.

Materials and Methods

Study area and sampling

This study was conducted in the rural area from three municipalities of the Antioquia Department within the Andean region of Colombia: Village El Carmelo (El Peñol municipality), village Curiti (Liborina municipality) and village La Linda (Jardin) (Table 1). All the villages are located in the department of Antioquia (Fig.1).

- The municipality of Liborina is located in the middle west of the department of Antioquia (6 ° 40 '59' 'North, 75 ° 48' 0 '' West), the average temperature is 26 ° C, with an area of 217 km2, of which 99% corresponds to the rural area[11], a total population of 10,028 people, of which 2,296 are located in the urban area and 7,732 in the rural area[12]. The drinking water coverage is 100% in the urban area and 35% in the rural area[13]. The rural community of Curiti is located in the southwest of the municipality of Liborina. This rural community has a water supply system without purification treatment, and they take their water from two rivers.
- The municipality of El Peñol is located in the eastern subregion of the department of Antioquia (6 ° 13 '08' 'North, 75 ° 14' 31 '' West). El Peñol has a total area of 148 km2, an average annual temperature of 19 ° C[14]. The economy is based on agriculture with plantations of tomatoes, bananas, peas, beans, coffee, blackberries, cabbage, carrots, paprika, among others, as well as extensive livestock. The total population of El Peñol is 21,049 inhabitants, of which 11,022 and 10,027 make up its urban and rural population, respectively[12]. The potable water coverage is 100% in the urban areas, while in rural areas, it is 73%[15]. The village of El Carmelo is located to the south of the municipality, the inhabitants of the village do not have access to potable water.
- The municipality of Jardin is located in the southwest region of the department of Antioquia (5 ° 36 ' 0 " North, 75 ° 49 ' 1 " West), the average temperature is 19 ° Celsius. Its extension is 224 km^2. Its total population is 14,518 inhabitants,

Table 1. Description of the water samples collected in the different villages and distribution site

Sample code	Sample origin	Municipality-Village
CUIW	Intake water	Liborina-Curiti
CUWT	Storage tank	Liborina-Curiti
CUTW1	Tap water	Liborina-Curiti
CUTW2	Tap water	Liborina-Curiti
CUTW3	Tap water	Liborina-Curiti
CUDW	Downstream water	Liborina-Curiti
CAIW	Intake water	Penol-El Carmelo
CAWT	Storage tank	Penol-El Carmelo
CATW1	Tap water	Penol-El Carmelo
CATW2	Tap water	Penol-El Carmelo
CATW3	Tap water	Penol-El Carmelo
CADW	Downstream water	Penol-El Carmelo
LLIW	Intake water	Jardin-La Linda
LLWT	Storage tank	Jardin-La Linda
LLTW1	Tap water	Jardin-La Linda
LLTW2	Tap water	Jardin-La Linda
LLTW3	Tap water	Jardin-La Linda
LLDW	Downstream water	Jardin-La Linda

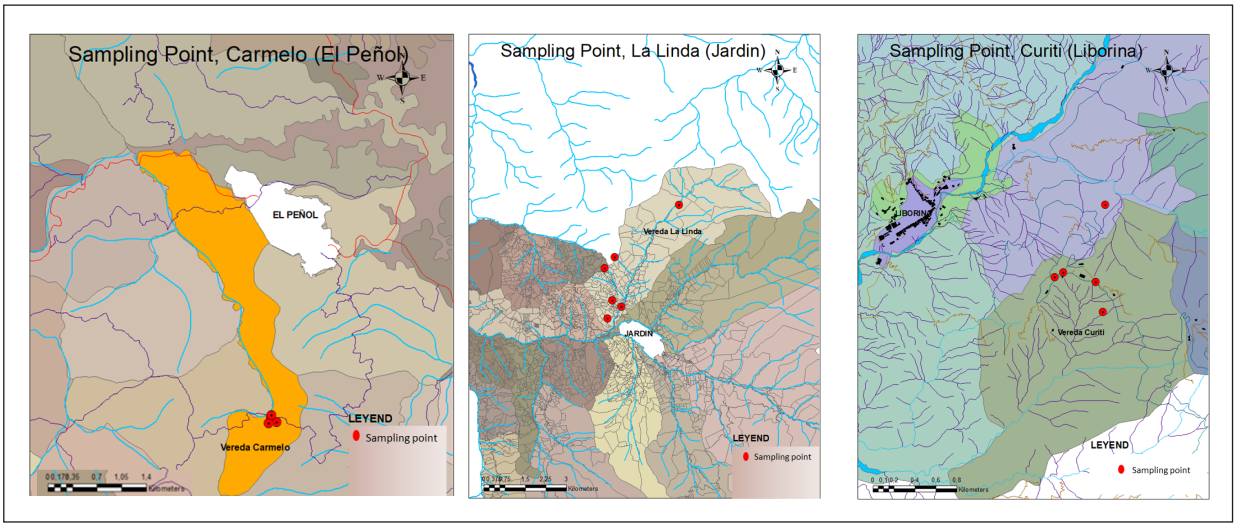

Figure 1. Maps representing the point of sampling in the municipality Jardin, El Peñol and Liborina

distributed in 7,659, in the urban area and 6,859 in the rural area[12]. Its mountainous topography characterizes Jardin. Its economy's base is a tourism and the cultivation of beans, sugar cane, cassava, potatoes, avocado, corn, and coffee. Its coverage in terms of drinking water is 99% in urban and rural areas, only in some rural communities there are water treatments by filtration and chlorination[16]. The rural community of La Linda is located 3 km from the municipality.

The water for human consumption in these communities is pumped from surface sources (rivers) into storage tanks without any treatment, except for Jardin in which there are improvised filters (without routine maintenance) for treating the water before the storage. In this study the selected sampling points were the water intake, the storage tank, tap water in the houses, and the town's downstream river. A total of 18 water samples were collected on January 2020. Ten liters of water were collected from each sampling site using clean, non-sterilized containers with a screw cap and transported on ice to the laboratory. The intake and downstream water samples were taken in the middle part and at a depth between 5 and 20 cm, the tank and tap water were taken through PVC pipes. The specifications of the Institute of Meteorology, Hydrology and Environmental Studies (IDEAM) and the British standard were followed for the sampling: Part 2: Guidance on sampling techniques and ISO 5667-3 Preservation and handling of simple water. (BRITISH STANDARD. Water quality —Sampling Part 2: Guidance on sampling techniques. BS EN 25667-2:1993 BS 6068-6.2:1991 ISO 5667-2: 1991 Incorporating Amendment No. 1). The water was filtered through membrane disc filters with a pore size of 0.22mm to ensures the biomass quantity for DNA recovery **(Membrane MCE 0.20um 47mm. Advantech MFS)**.

DNA extraction and Illumina sequencing

According to the manufacturer's instructions, genomic DNA was extracted directly from membrane filters within the first 24 h of sampling using DNAeasy PowerSoil Kit (Qiagen, Germany). DNA was quantified using the fluorescent probe Pico-Green (Invitrogen P11496) and DNA quality was assessed by gel electrophoresis (1% agarose).

Samples were normalized to a final concentration of 10 ng/µL. Illumina libraries were prepared and sequenced using 300 bp paired-end MiSeq protocol at Macrogen Inc. (Seoul, Republic of Korea) following the service provider recommendations. The V3-V4 hypervariable regions of bacterial and archaeal 16S rDNA gene were amplified with the primers Bakt_341F (5'- CCTACGGGNGGCWGCAG-3') and Bakt_805R (5'- GACTACHVGGGTATCTAATCC-3'). Forward and reverse primers contained the Illumina adapter, pad and linker sequences.

Bioinformatics analysis

The Sequences were analyzed using Mothur's pipeline according to Miseq standard operating procedure (SOP) (17). Briefly, Paired-end (PE) reads were assembled using Mothur's tool "make.contigs" and then aligned to the SILVA 16S rDNA reference database. Sequences with ambiguous bases or homopolymers longer than 6 bases were removed. VSEARCH algorithm was used to filtering chimeric sequences. Nonbacterial lineages (chloroplasts, mitochondria, archaea and eukaryotes) were removed. Mothur's subroutine "dist.seqs" was used for clustering reads into operational taxonomic unit (OTU) at a nucleotide identity limit of 0.03. Data were normalized with the "totalgroup" method and rare OTUs with less than 3 sequences were removed. The phylogenetic classification was obtained with the Classifier Ribosomal Database Project (RDP) tool (80 bootstrap threshold) (18). The microbial diversity was calculated with the R package Phyloseq and Vegan. The Alpha diversity measurements including Chao1, observed species, Simpson, and Shannon index, were calculated for each sample. Beta diversity was calculated using non-metric multidimensional scaling (NMDS), the distance method was bray. The Core community was calculated with the R package microbiome according to the core microbiota analysis using amplicon data.

Statistical Analysis

Statistical Analysis was made through R v3.6.2 in RStudio and Phyloseq package. Statistical significance was considered when the P-value was less than 0.05 with the non-parametric tests Kruskal-Wallis, pairwise Wilcoxon and Analysis of variance using distance matrices (Adonis).

Accession number of sequences

Raw sequences were deposited in the SRA NCBI database under the bio project access number PRJNA662790.

Results

Alpha and Beta diversity analysis

An average of 33,904 sequences per sample was obtained after the quality filtering (low quality, chimeras, and nonbacterial origin), ranging from 32,813 to 34,607. Good's coverage estimator was between 88% and 99% for all the samples indicating an appropriate sampling effort for capturing most of the bacterial diversity. The rarefaction curves showed a good depth of sequencing (Fig. 2) The sequences were clustered into 35,236 Operational Taxonomic Units (OTUs) with 97% identity. The richness and diversity index were performed according to the village (Fig. 3) and sample origin (Fig. 4). These results showed a higher diversity in the intake water, but this observation was significant only for the observed index comparing the Carmelo (7333 OTUs observed) and Curiti (5856 OTUs observed) villages (p=0.045).

The non-metric multidimensional scaling (NMDS) analysis based on Bray-Curtis distance was applied for inferring the variations within the bacterial community structure grouping according to village or sample origin (Fig. 5). The stress value was 0.079. The obtained results showed a clustering of the bacterial community according to the village in the NMDS ordination space. Also, we detected a separation according to sample origin. This observation was supported by the Adonis test (p=0.001).

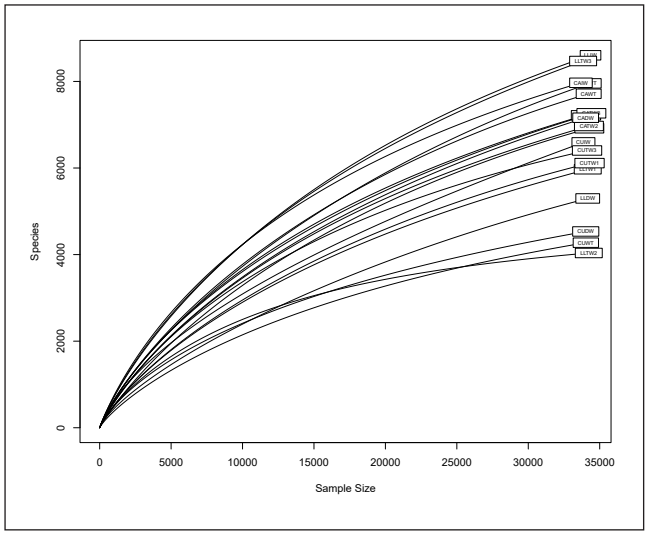

Figure 2. Rarefaction curves of the samples according to the village

Taxonomic composition in water samples

The Bacterial community structure was classified into 33 phyla dominated by Proteobacteria (42.5 to 67.32%) Bacteroidetes (1.88 to 23.68%), Actinobacteria (1.15 to 12.08%), and Acidobacteria (0.67 to 5.06%). A large proportion of sequences could not be effectively classified (3.84 to 25.17%) by the RDP classifier at 0.8 bootstrap threshold. We observed differences in the abundances of phyla according to the village and sample origin (Fig. 6). The sample collected in the intake water from Liborina village (CUIW) showed higher frequency in Actinobacteria and Acidobacteria phyla compared with the other samples. The core community was represented by 19 phyla detected in all villages included in the study. The frequency of Fusobacteria was higher in downstream water after the domestic activity from La Linda village (0.93%). Besides, this village showed a higher frequency of the candidate phylum WPS-2 (1.12%).

At the genus level, 619 genera were effectively classified in all samples. The dominant genera detected, belonging to Proteobacteria, were *Rhodoferax* and *Pseudomonas* (Fig. 7). *Flavobacterium* belonging to the Bacteroidetes phylum was the most frequent genus classified in the downstream water from La Linda village. Bacterial genera residing in the human intestinal tract were detected in fewer frequencies. However, we observed an enrichment of *Prevotella* and *Paludibacter* in the downstream water from La Linda Village.

Microbial pathogen analyses

Potentially pathogenic genera were identified in all the samples collected at different distribution points in the three villages, with differences in their frequencies (Fig. 8). *Legionella*, *Mycobacterium*, *Yersinia, Burkholderia* and *Rickettsia* were the dominant pathogenic genera reported in the surface water and water tank from La Linda village. These genera persisted in the tap water of two houses. Notably, the downstream water in this village was dominated by *Aeromonas* that was less frequent in the intake water. A higher frequency of some potentially pathogenic bacteria such as *Streptococcus*, *Staphylococcus, Corynebacterium* and *Treponema* was detected in the tap water from Carmelo village compared with the intake water and the water tank; this might suggest contamination in the pipeline. The water tank from Curiti village showed a higher frequency of *Leptospira, Escherichia/Shigella, Bordetella,* and *Serratia*. However, we observed a reduction in the frequencies of these bacterial genera in the houses' tap water with no apparent explanation.

Discussion

This study analyzed the bacterial microbiota from source, storage, tap water, and downstream water from three Andean rural communities in Colombia. The variations in the bacterial diversity within different rivers related to the geographical localization have been reported previously (figure 2)[19]. This observation confirmed our results since the beta-diversity Analysis showed a separation in the bacterial populations according to the village. Our results also demonstrate

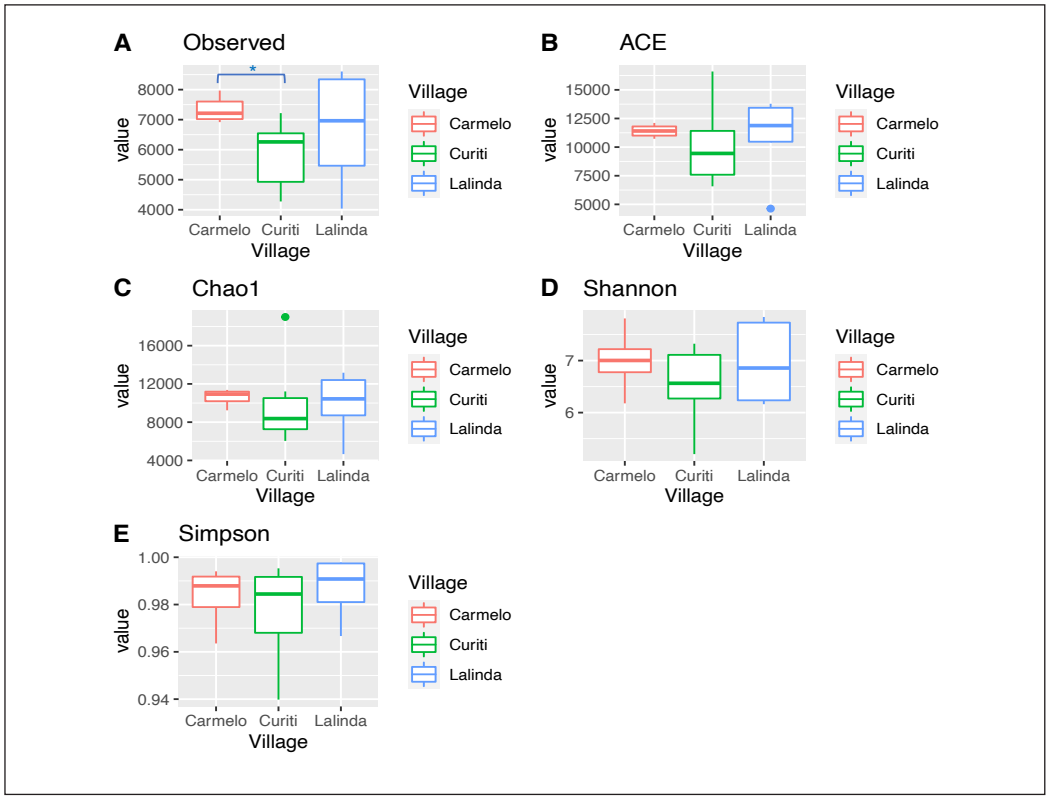

Figure 3. Alpha diversity indices in the water samples collected in three rural villages from Colombia. ACE (abundance-based coverage estimator). The OTUs observed index showed statistically significant differences between the villages Curiti and Carmelo, panel A ($p<0.05$).

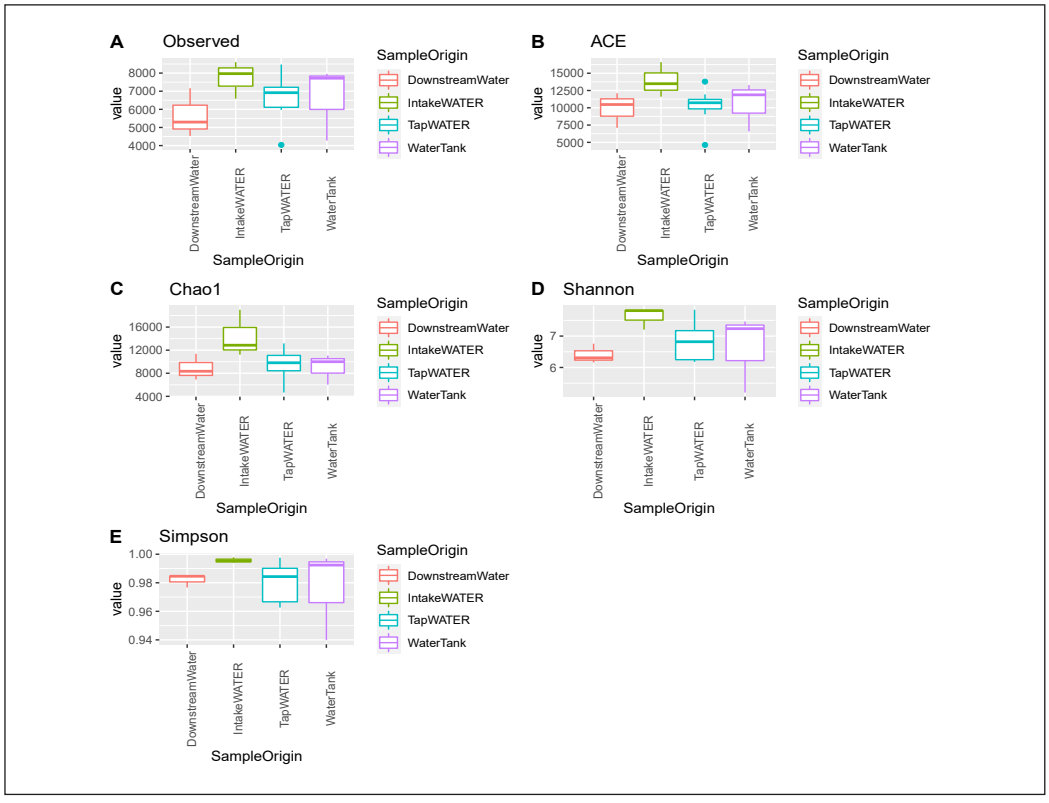

Figure 4. Alpha diversity in the water samples collected at four distribution points in the distribution network. ACE (abundance-based coverage estimator)

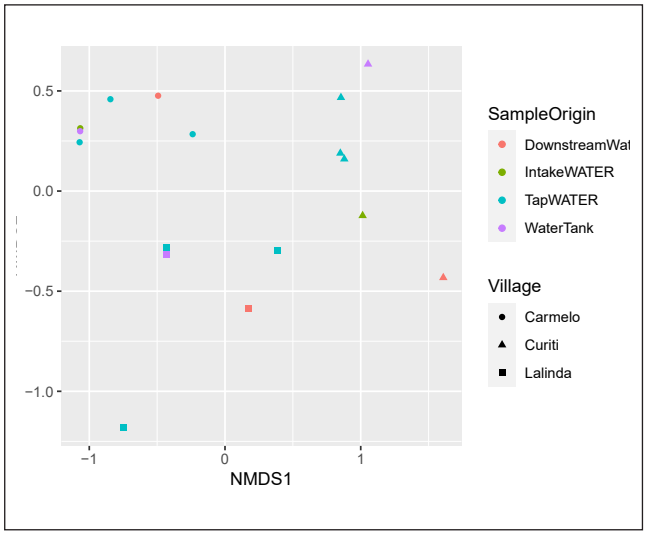

Figure 5. Non-metric multidimensional scaling (NMDS) plot showing differences in microbial composition in water samples according to the village and distribution point of collection.

the tremendous bacterial diversity present in the tropical Andean region's freshwater sources and the lack of knowledge of these bacterial communities due to the high frequency of unclassified bacteria detected in this study[20].

The dominant phylum classified in all the studied samples was Proteobacteria (figure 5); this phylum was previously reported as dominant in several types of freshwater ecosystems and groundwater[21]. As in this study, *Alpha*, *Beta,* and *Gammaproteobacteria* were detected in the tap water produced from freshwater in Portugal and France[22, 23]. The persistence of *Alphaproteobacteria* in drinking water is related to their capability to survive in low levels of nutrients and the chlorine tolerance[23]. *Gammaproteobacteria* comprises known pathogenic species belonging to the families *Enterobacteriaceae*, *Pseudomonadaceae*, *Legionellaceae* and *Aeromonadaceae*. Members of *Burkholderiaceae* and *Comamonadaceae* belonging to *Betaproteobacteria* have been reported in drinking water, thanks to their capacity to their efficient stress response and biofilm formation[24].

Safe water supply improves the quality of life in the communities; however, in the villages included in this study, the water is carried to the houses from a river without any appropriate chemical or physical treatment. The impact of anthropogenic activities on water quality in these communities is related mainly to agriculture and domestic wastes. For this reason, microbiological contamination of the water sources is a relevant issue for these communities. Pathogens were detected in all phases of the improvised water distribution systems of these villages (figure 7). Our results are in concordance with the observations of[20], that reported dominance of *Pseudomonas, Mycobacterium* and *Aeromonas* in the surface water from several districts in Beijing.

Aeromonas presence in the intake water and downstream water in La Linda village in high frequency is a reason for public health concern because this bacterium is considered an emerging pathogen, especially in emerging economies[25]. Members of the genus have been isolated from aquatic habitats or inside various vertebrate hosts, including humans, fish, frogs, and crustaceans[26]. In humans, *Aeromonas* is responsible for gastrointestinal disturbances, septicemia, endocarditis, pneumonia, and conjunctivitis[27]. The high reports of *Aeromonas* in drinking water supplies are related to its capacity to colonize biofilms and resist the chlorine treatment[28]. *Aeromonas* has been isolated from surface water, ground water, chlorinated drinking water, and bottled mineral water in several countries such as Brazil[29]. Despite of the health significance of *Aeromonas* infections, no routine monitoring of water supplies searching this bacterium is performed.

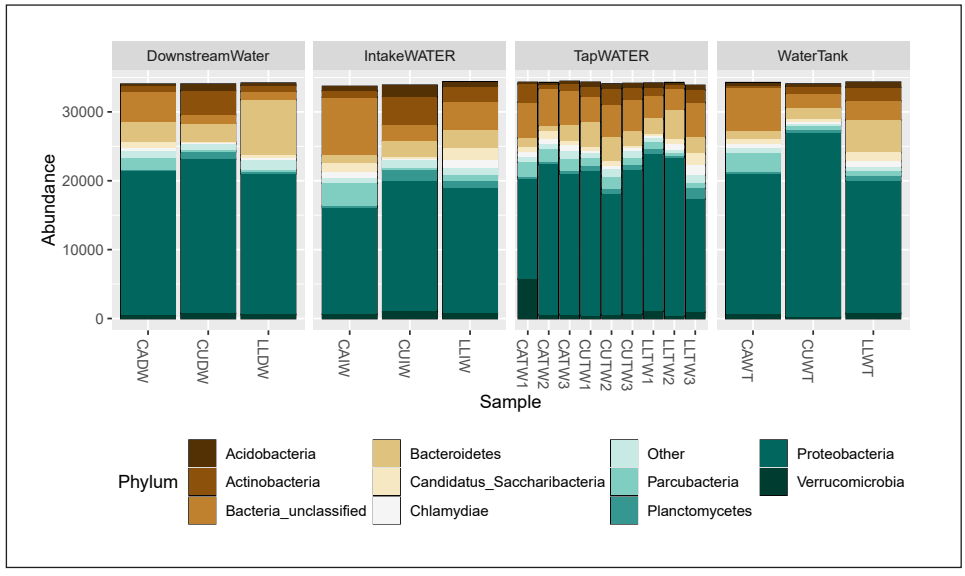

Figure 6. Top 10 of the bacterial community composition in the water samples at phylum level

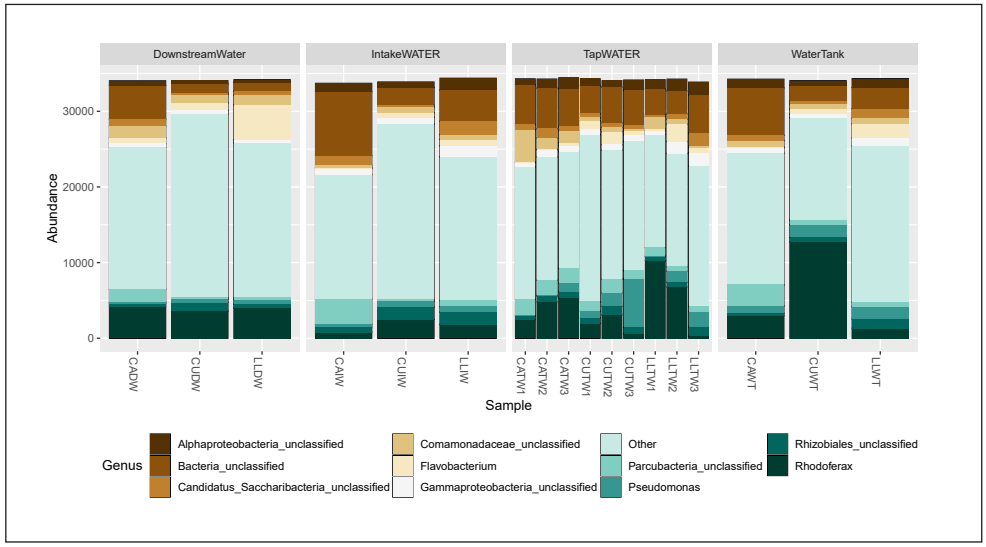

Figure 7. Top 10 of the bacterial community composition in the water samples at genus level

Storage tanks are essential components of the water distribution systems and, if not properly maintained and sanitized, it can increase the risk to human health since the long stagnation times of drinking water could foster the risk of infection with pathogenic bacteria[30]. Previous studies have reported the presence of *Pseudomonas*, *Legionella*, and coliforms in storage tanks[30]. These observations are consistent with our results in which *Escherichia/Shigella* and *Yersinia* were detected in higher proportion in water storage tanks. *Leptospira* also was detected in a storage tank from Curiti village; this bacterium has been isolated from drinking water mainly in areas where sanitization is insufficient[31].

In this study, the tap water showed a high frequency of *Legionella,* this genus comprises 59 species, most of them are of aquatic origin[32]. Potable water is the most important source of pathogenic species of *Legionella* related with lung infections[33]. This bacterium was detected in drinking water from Germany at temperatures above 50ºC and have been associated with biofilm formed in water piping. Legionnaire's disease outbreaks have been reported around the world. In these cases, the primary infection route is the inhalation of contaminated aerosols from drinking water, whirlpool spas, and cooling towers[34].

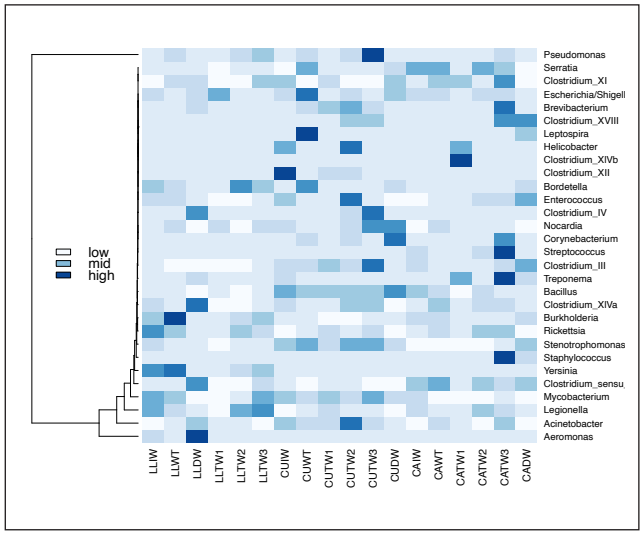

Figure 8. Heatmap showing the bacterial genus potentially pathogens detected in the water samples

Thirty potentially pathogenic bacterial genera were detected in the tap water of the three studied villages, some of them at significant abundance. This observation might help us understand the hazardous situation in which these people lives, and that this lack of safe drinking water could explain, at least in part, the burden of the enteric diseases of these populations. Despite in this study we do not perform viability test, this result could be an indicator of contamination because the DNA of dead cells is degraded in the environment and is less probable to detected through the sequencing platforms. To assess the real risk viability test should be performed. Pathogens such as *Escherichia/Shigella*, *Yersinia* and *Leptospira* pose a great threat to human health, especially to the children in these villages, and are consumed on a regular basis in the drinking water by these communities. The pathogens reported in the tap water from La Linda village could indicate that the improvised water filtration systems and without routine maintenance offer no improvement in the water quality. This situation causes the final consumer to be exposed to the intake of water contaminated with pathogenic microorganisms such as *E. Coli*, total and fecal coliforms, *Aeromonas*, and others, which generate adverse effects on human health.

Ethical disclosures

Protection of human and animal subjects. This research do not use animal nor human material.

Confidentiality of data. Not data that enable identity of participants was revealed

Conflicts of interest. The authors declare no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work.

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