Microbial Diversity of Source and Point-of-Use Water in Rural Haiti – A Pyrosequencing-Based Metagenomic Survey

Nabanita Mukherjee1, Debra Bartelli1, Cyril Patra1, Bhavin V. Chauhan1, Scot E. Dowd2, Pratik Banerjee1*

1 Division of Epidemiology, Biostatistics, and Environmental Health, School of Public Health, University of Memphis, Desoto Avenue, Memphis, Tennessee, United States of America, 2 Molecular Research LP (MR DNA), Shallowater, Texas, United States of America

* pbnerjee@memphis.edu

Abstract

Haiti endures the poorest water and sanitation infrastructure in the Western Hemisphere, where waterborne diseases cause significant morbidity and mortality. Most of these diseases are reported to be caused by waterborne pathogens. In this study, we examined the overall bacterial diversity of selected source and point-of-use water from rural areas in Central Plateau, Haiti using pyrosequencing of 16s rRNA genes. Taxonomic composition of water samples revealed an abundance of Firmicutes phyla, followed by Proteobacteria and Bacteroidetes. A total of 38 bacterial families and 60 genera were identified. The presence of several Klebsiella spp. (tentatively, K. pneumoniae, K. variicola and other Klebsiella spp.) was detected in most water samples. Several other human pathogens such as Aeromonas, Bacillus, Clostridium, and Yersinia constituted significantly higher proportion of bacterial communities in the point-of-use water samples compared to source water. Bacterial genera traditionally associated with biofilm formation, such as Chryseobacterium, Fusobacterium, Prevotella, Pseudomonas were found in the point-of-use waters obtained from water filters or domestic water storage containers. Although the pyrosequencing method utilized in this study did not reveal the viability status of these pathogens, the abundance of genetic footprints of the pathogens in water samples indicate the probable risk of bacterial transmission to humans. Therefore, the importance of appropriate handling, purification, and treatment of the source water needed to be clearly communicated to the communities in rural Haiti to ensure the water is safe for their daily use and intake.

Introduction

Access to safe water is a serious public health concern in Haiti, the nation with the poorest water and sanitation infrastructure in the Western Hemisphere [1]. The World Health Organization (WHO) estimates only 62% of Haitians have access to “improved water” sources [2]. The situation is even worse for those who live in rural areas; only 48% of Haiti’s rural population has access to such sources of water compared to 85% of the urban population [3].
Therefore, according to the World Bank’s most recent population data, an estimated 2.4 million people in rural Haiti lack access to improved water sources [4]. The WHO/UNICEF Joint Monitoring Programme (JMP) for Water Supply and Sanitation defines an improved water source as one that “by the nature of its construction or through active intervention, is protected from outside contamination, in particular from contamination with faecal matter” [5]. Categories of improved drinking water sources include piped water, public taps, boreholes or tubewells, protected dug wells, protected (capped) springs and rainwater. Taking into consideration the fact that improved drinking water is not necessarily safe water [6, 7] the proportion of the country’s rural population at an elevated risk of poor health due to exposure to contaminated drinking water is probably much higher than estimates indicate. In fact, the majority of those living in the countryside must rely on drinking untreated or unimproved water at least part of the time [1, 7].

Waterborne diseases, mostly infectious in nature, caused several thousand deaths in Haiti in recent years [1]. It is further estimated that the diseases spread by contaminated water are major causes of death of Haitian children under the age of five [8]. The cholera outbreak in 2010 which claimed more than eight thousand lives underscores the need to understand the overall risk of microbial contaminants transmitted through water [9]. In recent years, the Haitian government’s efforts to improve the situation included the creation of a regulatory body, the National Directorate for Potable Water and Sanitation (DINEPA) which deployed nearly 300 trained Communal Water and Sanitation Technicians (TEPACs) to serve rural communities [10]. Much of the work to improve both water quality and health in Haiti, however, continues to be driven by non-governmental organizations (NGOs) and international agencies [11]. Their approaches include installation of wells, capping springs and introduction of a wide variety of household or point-of-use (POU) water treatment systems. Studies examining the efficacy of these systems indicate the need for ongoing monitoring of both household and source water [6, 8, 11].

To date, most of the studies on the quality of water samples obtained from Haiti dealt with detection of specific organisms (such as *Vibrio cholerae*) using culture-dependent methods involving filtration followed by enrichment and plating or by polymerase chain reaction (PCR) based strategies [12–15]. However, the growth-dependent microbial assays may underestimate the potential presence of other non-target or non-cultivable microorganisms. The advent of the next generation sequencing (NGS) based metagenomics methods enable a broad spectrum identification of different microbes along with a high-resolution visualization of the overall microbiota of a given sample without the need of culturing the bacteria [16, 17]. Studies utilizing NGS techniques to understand the overall microbial composition, ecology, and diversity of different types of environmental media have gained remarkable attention from the scientific communities which is evident by a growing number of reports in recent years [18–21]. Moreover, recent studies indicate that metagenomic tools may identify new set of microbial parameters and targets for a wider, comprehensive, and more representative risk characterization [22, 23]. This eventually will address some key limitations of existing microbial risk assessment of water quality as the current microbial testing methods relying on detection of predetermined microbial indicators are often unable to accurately predict the occurrence of all microbial pathogen resulting in poor risk characterization [22]. Therefore, NGS-based metagenomics may be used effectively to gain in-depth knowledge about the comprehensive microbial quality and safety of the water available to the populations lacking access to improved water sources.

In the current study, we explored the overall bacterial ecology of selected source and POU water samples in a rural area of Haiti’s Central Plateau utilizing culture-independent pyrosequencing of the 16S rRNA genes. Using metagenomics approach, we comprehensively
assessed the bacterial diversity associated with the water sources and the POU water used as drinking water by household members living in the Central Plateau area.

**Materials and methods**

**Study Area and Source of Water**

The study was conducted in a 22 square-mile rural area southwest of Hinche, the regional capital of Haiti’s Central Plateau. The majority of the estimated 20,000 residents of the area are subsistence farmers who speak predominantly Haitian Kreyol. The study area possesses no paved roads and lies 10–35 km from the nearest hospital. The primary sources of water for the community are springs, streams, and rivers. Within the study area, there were no capped springs or other functional improved water sources. Households in the study area utilize one of three types of water filtration systems introduced by a NGO from the United States that has been working in the area for several years. Systems in use include a two-bucket system that employs prechlorination followed by a string filter and granular activated carbon (GAC), and then post-chlorination; slow-sand filtration (biosand); and a 0.10 micron filtration system (Sawyer PointOne™). At the time of data collection, these systems had been in use from 9 years (two-bucket system) to six months (micron filter). The biosand filter was introduced approximately one year prior to the study. Within the study area, it was estimated that 600 households were using the two-bucket system, 20 households had a Sawyer PointOne™ filter and nine households had a biosand filter. A filtration system is absent in the majority of households in the region.

**Sample Collection and Processing**

A total of 116 source and POU water grab samples were collected in 100 mL Security-Snap™ Sterile Coliform Water Sample Bottles (Thermo Fisher, USA) from households and major water sources across eight villages in the study area in June 2015. Sources were the Hinquette and Tablon rivers and their tributaries. All water sources are located in public areas and their use does not require permission. Source locations were identified by household members who accompanied members of the research team and directed them to the location from which they collected household drinking water. Data collection did not involve any endangered or protected species.

Households with biosand and Sawyer PointOne™ filters were identified with the help of trained water technicians residing in the community. A snowball sampling technique was used to identify households with a two-bucket filtration system. Water samples were also taken from neighboring homes that lacked a filtration system. Each water sample location was geocoded using a Garmin eTrex 10 GPS Unit and recorded by the Universal Transverse Mercator (UTM) coordinate system. The geodata were then compiled and analyzed using ArcMap 10.4.1 (Esri Inc. USA) on OpenStreetMap basemap (openstreetmap.org) to produce the maps of water collection sites (S1 Fig). Water samples were stored at refrigeration temperature (4˚C) prior to processing by membrane filtration technique using pre-sterilized magnetic filter funnels fitted with sterile hydrophilic polyethersulfone (PES) 47 mm Supor™ 200 membrane disc filters with pore size 0.2 µm (Pall Corporation, Port Washington, NY, USA).

**DNA Extraction and Next Generation Sequencing**

Metagenomic DNA samples were directly extracted from PES membrane filters using PowerWater™ DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA), following the manufacturer’s protocol, and were quantified spectrophotometrically using a NanoDrop
spectrophotometer (Thermo Scientific, Wilmington, DE, USA). For pyrosequencing analyses, DNA from 95 samples was pooled into 18 groups based on the types of major sources (spring, stream or river), filter types, and location (S1 Table).

For metagenomics analyses, we performed bTEFAP® (MR DNA www.mrdnalab.com) described previously [21, 24, 25]. In this study, 16S universal Eubacterial primers 530F (GTGC CAGCMGCNGCGG) and 1100R (GGGTTNCGNTCGTTR) were used to evaluate the microbial ecology of each sample on the 454 GS FLX+ (Roche Diagnostics Corp, Branford, CT, USA) with methods via the bTEFAP® DNA analysis service utilizing the 454 FLX chemistry following manufacturer’s protocols as described in details previously [21, 24, 25].

Computational and Statistical Analyses

The sequence data with quality score 25 (Q25) were processed using a proprietary analysis pipeline (www.mrdnalab.com, MR DNA, Shallowater, TX, USA) and Operational Taxonomic Units (OTUs) clustering at 3% divergence (97% similarity) were defined as described previously [21, 24, 25]. The taxonomic classification of OTUs were done by using BLASTn against a curated database derived from GreenGenes/RDP/NCBI [26].

For data analyses, different statistical programs were used including "R", XLstat, NCSS 2007, and NCSS 2010. The overall bacterial diversity (alpha and beta diversity) was analyzed using Quantitative Insights Into Microbial Ecology (QIIME, www.qiime.org) as described previously [24, 25, 27–29]. The differences in bacterial genera between the source and POU water samples were compared using a controlled ANOVA procedure. Significance reported for any analysis is defined as p<0.05.

Accession Numbers

The metagenomic sequence datasets associated with this paper are archived at NCBI Sequence Read Archive (SRA) under the BioProject accession number of PRJNA322639. BioSample records are accessible with the following accession numbers: SAMN05172269, SAMN05172270, SAMN05172271, SAMN05172272, SAMN05172273, SAMN05172274, SAMN05172275, SAMN05172276, SAMN05172277, SAMN05172278, SAMN05172279, SAMN05172280, SAMN05172281, SAMN05172282, SAMN05172283, and SAMN05172284.

Results and Discussion

The concentrations of DNA extracted from water samples contained measurable amount of DNA (39–116 ng/μL), while negative controls with no cells had no detectable DNA (data not shown). A total of 116,660 sequences identified after stringent quality sequence curation within the bacterial kingdom. These sequences were utilized for final bacterial microbiota analyses of the eighteen sample groups which were based on the water types sampled (source versus POU samples collected from different villages), including the pooled samples from all water source (rivers or springs) and POU samples. The details of pooling samples and clustering into groups have been described in S1 Table. The average reads per sample was 6481. For alpha and beta diversity analysis, samples were rarefied to 6000 sequences and bootstrapped at 4500 sequences.

Taxonomical composition revealed that the Firmicutes phyla was most common, followed by Proteobacteria and Bacteroidetes (Fig 1). The least bacterial diversity at the phylum level was found in water samples collected from Abrio and the Paradi spring which consisted mostly of Proteobacteria (86.3% and 91.8%, respectively). Prevalence of these phyla have also been reported previously from various types of water samples utilizing both culture-dependent and independent techniques [30–32]. Within these dominant phyla, the bacterial families with the
highest relative abundance across most samples were Aeromonadaceae, Bacillaceae, Bacillales, Bacteroidaceae, Bacteroidales, Clostridiaceae, Enterobacteriaceae, Moraxellaceae, Peptostreptococcaceae, and Porphyromonadaceae (Fig 2). Among these, Enterobacteriaceae, Bacillaceae and Clostridiaceae are known to include a variety of human pathogenic bacterial species transmissible through environments such as water [33–35].

The relative abundance and bacterial diversity at the genus level is presented in Fig 3. The most prevalent bacterial genus identified across all observed samples was Klebsiella. Other bacterial genera such as Acinetobacter, Aeromonas, Bacillus, Bacteroides, Clostridium, Dysgonomonas, Enterobacter, Escherichia, Parabacteroides, Parvimonas, Peptoclostridium, Shigella, and
**Yersinia** were commonly found in almost all samples. The genus *Citrobacter* was mainly found in spring water of Paradi (2.6%) but not in samples collected from other locations. The predominant bacterial genera in the water samples analyzed based on the relative abundance cut-off of 1.0% are shown in Figs 3 and S2. A high presence of *Exiguobacterium* was found in the POU water collected from Zabriko (11.7%) and Paradi (3.4%). Bacteria belonging to the genus *Kluyvera* were found mainly in spring water from the villages of Paradi (3.6%), Abrio (1.1%), Pedosant (1.0%), and Dominis (1.0%). *Porphyromonas* was only found in POU water from Paradi (8.6%), and the river water of Salmori (1.6%). *Proteiniphilum* was found in the source water of Salmori (5.9%) and POU water at Zabriko (1%). Both *Pseudomonas* and *Streptococcus* were found in POU as well as in the source water. *Pseudomonas* accounted for 9.1% relative abundance in the POU water samples from Salmori and 3.6% relative abundance in source water from Abrio. *Streptococcus* was found in the river water of Salmori (3.9%) and POU water samples collected from Dominis (1.1%). In addition, our results indicate that some bacterial genera were found exclusively in certain samples, for example, *Chryseobacterium* was only found in POU water from Paradi (8.1%), *Fusobacterium* and *Prevotella* only in POU water from Salmori.
samples in Layaye (3.5%, and 7.5%, respectively), *Macellibacteroides* in spring water from Dominis (1.1%), and *Shewanella* in spring water of Abrio (1.5%). Interestingly, some bacteria genus including *Candidatus symbiothrix* (8.8%), *Eubacterium* (2.8%), *Plesiomonas* (1.1%), and *Veillonella* (1.3%) were exclusively found in the river water in the Salmori village.

As indicated above, the most common bacterial genus in both source and POU water samples observed in our study was *Klebsiella* spp (belonging to the bacterial family Enterobacteriaceae). Bacteria of the genus *Klebsiella* are a frequent cause of nosocomial infections [36]. We identified the presence of several *Klebsiella* spp. (tentatively, *K. pneumoniae*, *K. variicola* and other *Klebsiella* spp.) in almost all the drinking water samples collected. Among them, *K. pneumoniae* is the most predominant estimated species, followed by *K. variicola* and other *Klebsiella* spp. Pathogenic *K. pneumoniae* has been associated with urinary tract infections as well as bacteremic liver abscess and can be transmitted easily through person-to-person contact and
coming in contact with a contaminated surface especially in a healthcare facility [36–38]. *K. pneumoniae* exhibits resistance to several classes of antibiotics, including aminoglycosides, chloramphenicol, fluoroquinolones, tetracycline, and trimethoprim/sulfamethoxazole [39]. One of the most potent antibiotic resistant strains among *Klebsiella* is carbapenem-resistant *K. pneumoniae* (CRKP). It has been reported that CRKP is resistant to most of the available antibiotics [40]. Hence the infections caused by CRKP are difficult to treat and thus have been associated with high rates of morbidity and mortality [41]. However, the high prevalence of *Klebsiella* spp. in these samples is not surprising as some of the *Klebsiella* species belong to human normal flora, especially found in skin, mouth and intestine [42]. Presence of entero-toxigenic *Klebsiella* species was reported in populations with gastrointestinal disorders in Haiti in reports published almost four decades ago [14].

Other bacterial species estimated in our study include pathogenic or potentially pathogenic *Aeromonas bestiarium, Aeromonas hydrophila, Bacillus anthracis, Bacillus cereus, Dysgonomonas gadei, Dysgonomonas capnocytophagoides, Enterococcus faecalis, Escherichia coli, Fusobacterium periodonticum, Kluwyera ascorbata, Parvimonas micra, Plesiomonas shigelloides, Porphyromonas spp., Prevotella nigrescens, Pseudomonas aeruginosa, Shewanella putrefaciens, Shigella spp., Streptococcus oralis, Veillonella parvula and Yersinia* spp. Additionally, some opportunistic pathogens such as *Acinetobacter baumannii, Acinetobacter junii, and Citrobacter* spp. were also identified in this study. The diversity of bacterial genera varied significantly between source and POU water (Fig 3). In POU water samples, bacterial genera such as *Aeromonas, Bacillus, Clostridium, Yersinia, Exiguobacterium, Pseudomonas, Porphyromonas, Chryseobacterium, Prevotella*, and *Fusobacterium* were found to constitute significantly higher potions of the community structure when compared to source waters (Figs 3 and S2). Most of these bacteria are known to transmit from environment (including water, air, soils, and foods) or via human to human pathways and cause various human diseases. For example, pathogenic *Aeromonas* species are ubiquitous in the natural environment and are often food- or water-borne [43, 44]. A prominent member of this genus found in this study, *Aeromonas hydrophila*, can cause a variety of diseases in humans from gastrointestinal disorders to more severe illnesses such as septicemia, meningitis, wound infections, etc. [45, 46]. *Bacillus* spp. (including *B. anthracis* and *B. cereus*), known to cause both gastrointestinal and non-gastrointestinal infections including severe disease such as anthrax [47, 48], were found extensively in both source and POU samples. Approximately 80% of the samples collected contained *Shigella* spp. and *Yersinia* spp, with high relative abundance in POU water samples. Both of these pathogens may pose a considerable public health threat in less developed countries by causing conditions such as bacillary dysentery, inflammatory autoimmune disorders, and even highly contagious diseases such as plague [49, 50]. Another pathogen *Kluwyera* spp. (*K. ascorbata*), found mostly in source water samples (spring or river) in our study, is known to cause urinary tract infection in humans [51, 52]. As revealed by our analysis, high frequency of OTUs related to pathogenic bacteria in POU water may indicate higher risk of human transmission, since these waters are used for drinking.

For alpha and beta diversity analysis, samples were rarefied to 6000 sequences and bootstrapped at 4500 sequences. A Rarefaction Curve (Fig 4) based on OTUs was constructed to estimate bacterial diversity. A 97% similarity of OTUs as revealed by the Rarefaction Curve modeling at the 3% divergence for each sample suggesting adequate depth of sequencing coverage [21, 24, 25]. Fig 4 reveals that the source water samples from Layaye (spring) were found to contain the highest level of species richness followed by river water samples from Salmori. This could be due, in part, to the location of the spring (adjacent to a river) with the possibility of overrun during heavy rains and cross-contamination caused by near-exclusive use of the spring by local residents. The POU water of Layaye (from two bucket and biosand filters)
showed the highest species richness among all the POU samples. The least species richness was observed in POU samples from Bwadem (biosand filter) and Salmori (two bucket system). Using weighted Principal Coordinates Analysis (PCoA) of the microbiome of each sample based upon UniFrac method, we observed no microbiome phylogenetic assemblage relationships among the sample groups. Filtered water samples (two bucket) from Paradi and Bwadem; and source waters from Pedosant (spring) and Zabriko (river) are found to be most distant from the central cluster of other samples (Fig 5). Some samples of different types (source vs POU) and places (geographical location) clustered together, for example, source water samples (spring) from Paradi and Abrio clustered together with POU sample from Bwadem. Similarly, spring water sample from Dominis clustered with filtered (two bucket) samples of Salmori. Therefore, the bacterial community compositions of these samples were independent of the type of place of collection indicating a species-level variability, which is not uncommon and has been attributed to a probable functional redundancy of different or same environmental habitats [53, 54].

Further, we evaluated the impact of water filtration on the composition of autochthonous microbiota of the source water. Filtration devices or certain components of filters (such as sand) may introduce certain species of bacteria associated with biofilm formation (“schmutzdecke”) which may eventually influence the microbial quality of the finished water as reported in previous studies [15, 55, 56]. In our study, we also found depletion or introduction of certain bacterial genera after-filtration in the POU water with reference to the autochthonous microbiota respective water sources (Table 1). It is evident that the bacterial depletion from source waters to the POU water varied across the villages and filters used, but we found no particular trend. For example, in Salmori village, the POU water showed a very high degree of bacterial genera depletion, where 15 out of 18 genera were found to be depleted. While in Zabriko
only 3 genera (out of 13 in source river water) were found to be depleted in the POU water. In this context, it is to be noted that DNA markers of bacteria detected in DNA-based methods (such as in metagenomics) can also originate from non-viable cells [57], and therefore, may not be directly associated with presence of viable bacterial cells and the filtration efficiencies of the filters. Interestingly, we detected new genera in the POU water samples (post-filtration) from some villages which were absent in the respective source waters (pre-filtration). All newly introduced genera (Table 1) in POU water samples in our study were reported to include species that are known to be efficient biofilm formers (as referenced here): Chryseobacterium [58], Exiguobacterium [59], Fusobacterium [60], Porphyromonas [61], Prevotella [62], Proteiniphilum [63], Pseudomonas [64], and Streptococcus [65].

Using controlled ANOVA we evaluated whether any specific bacterial genera were significantly different between the source water and filtered water samples. There were relatively few genera that were significantly different between the groups, the most notable being Klebsiella (Table 2). The significantly low abundance of Klebsiella in POU water as compared to
| Water Source                  | Depleted Genera                                                                 | Introduced Genera                                                                 |
|------------------------------|--------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| Layaye-Spring                | **Acinetobacter, Aeromonas, Bacillus, Bacteroides, Clostridium, Dysgonomonas, Enterobacter, Escherichia, Klebsiella, Parabacteroides, Peptoclostridium, Shigella, Yersinia.** | [Acinetobacter, Bacillus, Bacteroides, Clostridium, Enterobacter, Escherichia, Klebsiella, Peptoclostridium, Yersinia.](#) |
| Layaye-Point-of-Use (filtered, filter type 1) | **Acinetobacter, Bacillus, Bacteroides, Clostridium, Enterobacter, Escherichia, Klebsiella, Peptoclostridium, Yersinia.** | **Fusobacterium, Prevotella.** |
| Bwadem-River                 | **Acinetobacter, Aeromonas, Bacillus, Clostridium, Enterobacter, Escherichia, Klebsiella, Peptoclostridium, Shigella, Yersinia.** | **Acinetobacter, Aeromonas, Bacillus, Clostridium, Enterobacter, Escherichia, Klebsiella, Peptoclostridium, Yersinia.** |
| Bwadem-Point-of-Use (filtered, filter type 1) | **Acinetobacter, Aeromonas, Bacillus, Clostridium, Enterobacter, Escherichia, Klebsiella, Peptoclostridium, Yersinia.** | **Fusobacterium, Prevotella.** |
| Paradi-Spring                | **Acinetobacter, Aeromonas, Bacillus, Clostridium, Enterobacter, Escherichia, Klebsiella, Peptoclostridium, Shigella, Yersinia.** | **Enterobacter, Klebsiella, Peptoclostridium.** |
| Paradi-Point-of-Use (filtered) | **Acinetobacter, Aeromonas, Bacillus, Clostridium, Enterobacter, Escherichia, Klebsiella, Peptoclostridium, Shigella, Yersinia.** | **Chryseobacterium, Exiguobacterium, Porphyromonas.** |
| Zabriko-River                | **Acinetobacter, Aeromonas, Bacillus, Bacteroides, Clostridium, Dysgonomonas, Enterobacter, Escherichia, Klebsiella, Parabacteroides, Peptoclostridium, Shigella, Yersinia.** | **Bacillus, Citrobacter, Escherichia, Klyvera, Shigella.** |
| Zabriko-Point-of-Use (filtered) | **Acinetobacter, Aeromonas, Bacillus, Bacteroides, Clostridium, Dysgonomonas, Enterobacter, Escherichia, Klebsiella, Peptoclostridium, Shigella, Yersinia.** | **Chryseobacterium, Exiguobacterium, Porphyromonas.** |
| Dominis-Spring               | **Acinetobacter, Aeromonas, Bacillus, Bacteroides, Clostridium, Enterobacter, Klebsiella, Klyvera, Macellibacteroides, Parabacteroides, Peptoclostridium, Shigella, Yersinia.** | **Bacteroides, Dysgonomonas, Parabacteroides.** |
| Dominis-Point-of-Use (filtered) | **Acinetobacter, Aeromonas, Bacillus, Bacteroides, Clostridium, Enterobacter, Klebsiella, Peptoclostridium, Shigella, Yersinia.** | **Exiguobacterium, Proteiniphilum.** |
| Salmori-Spring               | **Acinetobacter, Aeromonas, Bacillus, Candidatus symbiothrix, Clostridium, Dysgonomonas, Enterobacter, Escherichia, Eubacterium, Klebsiella, Peptoclostridium, Plesiomonas, Porphyromonas, Proteiniphilum, Shigella, Streptococcus, Veillonella, Yersinia.** | **Candidatus symbiothrix, Bacteroides, Klyvera, Macellibacteroides, Parabacteroides, Shigella.** |
| Salmori-Point-of-Use (filtered) | **Acinetobacter, Aeromonas, Bacillus, Candidatus symbiothrix, Clostridium, Dysgonomonas, Enterobacter, Escherichia, Eubacterium, Klebsiella, Peptoclostridium, Plesiomonas, Porphyromonas, Proteiniphilum, Shigella, Streptococcus, Veillonella, Yersinia.** | **Candidatus symbiothrix, Bacteroides, Klyvera, Macellibacteroides, Parabacteroides, Shigella.** |

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corresponding source waters may indicate reduction of the overall coliform load of POU water which is primarily used for drinking or cooking purposes.

These findings were consistent with previously observed health complaints of area residents for which diarrheal disease, gastrointestinal disorders, unexplained fevers, and respiratory tract infections (especially in children) are commonplace (unpublished data).

Conclusions

In this study, the identification of bacterial communities in source and POU water samples was performed using pyrosequencing methods. The identification was determined by bacteria-specific nucleic acid markers regardless of viability status of the bacteria. These methodologies report non-viable or non-culturable cells along with culturable cells. Therefore, the results should be interpreted differently than those reported by growth-based and culture-dependent studies. A mere detection of the “presence” of certain bacteria in the water samples by sequencing-based methods (such as the present study) may not indicate the presence of live and active cells. Viability PCR (vPCR) or rRNA-based methods relying on detecting the synthesis of a species-specific rRNA precursor (pre-rRNA), RNAsec (metatranscriptomics), are required to confirm the presence of viable bacteria. In addition, functional information such as presence of virulence factors can be acquired by conducting real-time PCR-based assays. Nevertheless, the rich bacterial diversity and potential pathogenic organisms present in the water samples may represent a public health concern, and can serve as a great tool for risk assessment. The results of this study indicate the importance of ongoing water quality monitoring in rural Haiti supplemented with water sanitation and hygiene (WASH) education for the local population.

Supporting Information

S1 Fig. Map of study area.
(TIF)

S2 Fig. Relative abundance of bacterial diversity at the genus level in the source and point-of-use water samples. Bacterial genus abundance less than 1% were grouped as “Others”.
(TIF)

S1 Table. Sample Description.
(DOCX)
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Author Contributions
Conceptualization: PB DB.

Data curation: PB NM BC DB.

Formal analysis: PB NM SD CP BC.

Funding acquisition: PB DB.

Investigation: PB NM.

Methodology: PB NM SD BC.

Project administration: PB.

Resources: PB DB.

Supervision: PB DB.

Validation: NM SD.

Visualization: PB NM SD.

Writing – original draft: NM DB PB.

Writing – review & editing: PB DB NM.

References
1. Patrick M, Berendes D, Murphy J, Bertrand F, Husain F, Handzel T. Access to safe water in rural artibo- nite, Haiti 16 months after the onset of the Cholera Epidemic. Am J Trop Med Hyg. 2013; 89(4):647–53. doi: 10.4269/ajtmh.13-0308 PMID: 24106191

2. WHO/UNICEF. World Health Organization/UNICEF Joint Monitoring Programme (JMP) for Water Supply and Sanitation Progress on Drinking Water and Sanitation: 2012 Update http://www.wssinfo.org/fileadmin/user_upload/resources/JMP-report-2012-en.pdf. Accessed 4/25/2016; 2012 [cited 2016 April 25]. Available from: http://www.wssinfo.org/definitions-methods/.

3. WHO. Progress on Sanitation and Drinking Water– 2015 update and MDG assessment: http://www.unicef.org/publications/files/Progress_on_Sanitation_and_Drinking_Water_2015_Update_.pdf. Accessed 4/25/2016; 2015 [cited 2016 April 25]. Available from: http://www.wssinfo.org/definitions-methods/.

4. World-Bank-Group. World Development Indicators (Haiti): http://data.worldbank.org/country/haiti. Accessed 4/25/2016; 2014 [cited 2016 April 25]. Available from: http://www.wssinfo.org/definitions-methods/.

5. WHO/UNICEF. World Health Organization/UNICEF Joint Monitoring Programme (JMP) for Water Supply and Sanitation Progress on Drinking Water and Sanitation: http://www.wssinfo.org/definitions-methods/ Accessed 4/25/2016; 2015 [cited 2016 April 25]. Available from: http://www.wssinfo.org/definitions-methods/.

6. Bain R, Cronk R, Wright J, Yang H, Slaymaker T, Bartram J. Fecal contamination of drinking-water in low- and middle-income countries: a systematic review and meta-analysis. PLoS Med. 2014; 11(5): e1001644. PubMed Central PMCID: PMC4011876. doi: 10.1371/journal.pmed.1001644 PMID: 24800926

7. Shaheed A, Orgill J, Montgomery MA, Jeuland MA, Brown J. Why "improved" water sources are not always safe. Bull World Health Organ. 2014; 92(4):283–9. PubMed Central PMCID: PMC3967570. doi: 10.2471/BLT.13.119594 PMID: 24700996
8. Wampler PJ, Sisson AJ. Spring flow, bacterial contamination, and water resources in rural Haiti. Environ Earth Sci. 2011; 62(8):1619–28.

9. Kupferschmidt K. Infectious diseases. Second bacterium theory stirs Haiti’s cholera controversy. Science. 2012; 336(6088):1493. doi: 10.1126/science.336.6088.1493 PMID: 22723386

10. Widmer JM, Weppelmann TA, Alam MT, Morrissey BD, Redden E, Rashid MH, et al. Water-related infrastructure in a region of post-earthquake Haiti: High levels of fecal contamination and need for ongoing monitoring. Am J Trop Med Hyg. 2014; 91(4):790–7. doi: 10.4269/ajtmh.14-0165 PMID: 25071005

11. Hill VR, Cohen N, Kahler AM, Jones JL, Bopp CA, Marano N, et al. Toxigenic Vibrio cholerae O1 in water and seafood, Haiti. Emerg Infect Dis. 2011; 17(11):2147–50. doi: 10.3201/eid1711.110748 PMID: 22099121

12. Kahler AM, Haley BJ, Chen A, Mull BJ, Tarr CL, Turnsek M, et al. Environmental surveillance for toxigenic Vibrio cholerae in surface waters of Haiti. Am J Trop Med Hyg. 2015; 92(1):118–25. doi: 10.4269/ajtmh.13-0601 PMID: 25385860

14. Klöpplein FA, Short HB, Engert RF, Jean L, Weaver GA. Contamination of the small intestine by enterotoxigenic coliform bacteria among the rural population of Haiti. Gastroenterology. 1976; 70(6):1035–41. PMID: 773737

16. Riesenfeld CS, Schloss PD, Handelsman J. Metagenomics: genomic analysis of microbial communities. Annu Rev Genet. 2004; 38(6):525–52. doi: 10.1146/annurev.genet.38.072902.091216 PMID: 15568985

19. Zhou J, He Z, Yang Y, Deng Y, Tringe SG, Alvarez-Cohen L. High-throughput metagenomic technologies for complex microbial community analysis: open and closed formats. MBio. 2015; 6(1). PubMed Central PMCID: PMCPMC3424309.

24. Dowd SE, Callaway TR, Wolcott RD, Sun Y, McKeohan T, Hagevoort RG, et al. Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). BMC Microbiol. 2008; 8:125–. doi: 10.1186/1471-2180-8-125 PMID: 18652685

25. Dowd SE, Sun Y, Wolcott RD, Domingo A, Carroll JA. Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) for microbiome studies: bacterial diversity in the ileum of newly weaned Salmonella-infected pigs. Foodborne Pathog Dis. 2008; 5(4):459–72. Epub 2008/08/21. doi: 10.1089/fpd.2008.0107 PMID: 18713063

26. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol. 2006; 72(7):5069–72. Epub 2006/07/06. PubMed Central PMCID: PMC1489311. doi: 10.1128/AEM.03006-05 PMID: 16820507
27. Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics. 2010; 26 (19):2460–1. Epub 2010/08/17. doi: 10.1093/bioinformatics/btq461 PMID: 20709691

28. Eren AM, Zozaya M, Taylor CM, Dowd SE, Martin DH, Ferris MJ. Exploring the diversity of Gardnerella vaginalis in the genitourinary tract microbiota of monogamous couples through subtle nucleotide variation. PLoS One. 2011; 6(10):e26732. Epub 2011/11/03. PubMed Central PMCID: PMC3201972. doi: 10.1371/journal.pone.0026732 PMID: 22046340

29. Swanson KS, Dowd SE, Suchodolski JS, Middelbos IS, Vester BM, Barry KA, et al. Phylogenetic and gene-centric metagenomics of the canine intestinal microbiome reveals similarities with humans and mice. ISME J. 2011; 5(4):639–49. Epub 2010/10/22. PubMed Central PMCID: PMC3105739. doi: 10.1038/ismej.2010.162 PMID: 20962874

30. Rudi K, Berg F, Gaustad E, Tannes T, Vatn M. Ratios between Alpha-, Beta- and Gamma-proteobacteria in tap water determined by the ProteoQuant assay. Lett Appl Microbiol. 2010; 50(1):1–6. Epub 2009/10/31. doi: 10.1111/j.1472-765X.2009.02743.x PMID: 19874479

31. Balleste E, Blanch AR. Persistence of Bacteroides species populations in a river as measured by molecular and culture techniques. Appl Environ Microbiol. 2010; 76(22):7608–16. Epub 2010/09/21. PubMed Central PMCID: PMC2976185. doi: 10.1128/AEM.00883-10 PMID: 20851970

32. Wu H, Zhang J, Mi Z, Xie S, Chen C, Zhang X. Biofilm bacterial communities in urban drinking water distribution systems transporting waters with different purification strategies. Appl Microbiol Biotechnol. 2015; 99(4):1947–55. Epub 2014/09/26. doi: 10.1007/s00253-014-6095-7 PMID: 25253043

33. Mathers AJ, Peirano G, Pitout JD. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. Clin Microbiol Rev. 2015; 28(3):565–91. PubMed Central PMCID: PMC4405625. doi: 10.1128/CMR.00116-14 PMID: 25926236

34. Nicholson WL. Roles of Bacillus endospores in the environment. Cell Mol Life Sci. 2002; 59(3):410–6. PMID: 11964119

35. Fang MX, Zhang WW, Zhang YZ, Tan HQ, Zhang XQ, Wu M, et al. Brassinicinhibitor mesophilus gen. nov., sp. nov., a strictly anaerobic bacterium isolated from food industry wastewater. Int J Syst Evol Microbiol. 2012; 62(Pt 12):2018–23. doi: 10.1099/ijs.0.034660-0 PMID: 22307504

36. Rahimian J, Wilson T, Oram V, Holzman RS. Pyogenic liver abscess: recent trends in etiology and mortality. Clin Infect Dis. 2004; 39(11):1654–9. Epub 2004/12/04. doi: 10.1086/425616 PMID: 15578367

37.天河 WH, Kao CY, Yang DC, Tseng CC, Wu AB, Teng CH, et al. Clinical and microbiological characteristics of Klebsiella pneumoniae from community-acquired recurrent urinary tract infections. Eur J Clin Microbiol Infect Dis. 2014; 33(9):1533–9. Epub 2014/04/24. doi: 10.1007/s10096-014-2100-4 PMID: 24756209

38. Wang JH, Liu YC, Lee SS, Yen MY, Chen YS, Wang JH, et al. Primary liver abscess due to Klebsiella pneumoniae in Taiwan. Clin Infect Dis. 1998; 26(6):1434–8. Epub 1998/06/24. PMID: 9636876

39. Nathisuwan S, Burgess DS, Lewis JS 2nd. Extended-spectrum beta-lactamases: epidemiology, detection, and treatment. Pharmacotherapy. 2001; 21(8):920–8. Epub 2001/11/23. PMID: 11718498

40. Limbago BM, Rasheed JK, Anderson KF, Zhu W, Kitchel B, Watz N, et al. IMP-producing carbapenem-resistant Klebsiella pneumoniae in the United States. J Clin Microbiol. 2011; 49(12):4239–45. Epub 2011/10/15. PubMed Central PMCID: PMC3233008. doi: 10.1128/JCM.05297-11 PMID: 21998425

41. Heyland DK, Cook DJ, Griffith L, Keenan SP, Brun-Buisson C. The attributable morbidity and mortality of ventilator-associated pneumonia in the critically ill patient. The Canadian Critical Trials Group. Am J Respir Crit Care Med. 1999; 159(4 Pt 1):1249–56. Epub 1999/04/08.

42. Podschan R, Pietsch S, Holler C, Ullmann U. Incidence of Klebsiella species in surface waters and their expression of virulence factors. Appl Environ Microbiol. 2001; 67(7):3325–7. Epub 2001/06/27. PubMed Central PMCID: PMC930022. doi: 10.1128/AEM.67.7.3325-3327.2001 PMID: 11425763

43. Kivanc M, Yilmaz M, Demir F. The occurrence of Aeromonas in drinking water, tap water and the porsuk river. Braz J Microbiol. 2011; 42(1):126–31. PubMed Central PMCID: PMC3768929. doi: 10.1590/S1517-83822011000100016 PMID: 24031613

44. Palumbo SA, Bencivengo MM, Del Corral F, Williams AC, Buchanan RL. Characterization of the Aeromonas hydrophila group isolated from retail foods of animal origin. J Clin Microbiol. 1989; 27(5):854–9. PubMed Central PMCID: PMC267443. PMID: 2745695

45. Turska-Szewczuk A, Kozińska A, Russa R, Holst O. The structure of the O-specific polysaccharide from the lipopolysaccharide of Aeromonas bestiarum strain 207. Carbohydr Res. 2010; 345(5):680–4. doi: 10.1016/j.carres.2009.12.030 PMID: 20080230

46. Janda JM, Abbott SL. The genus Aeromonas: taxonomy, pathogenicity, and infection. Clin Microbiol Rev. 2010; 23(1):35–73. PubMed Central PMCID: PMC2806660. doi: 10.1128/CMR.00039-09 PMID: 20065325
47. Berger T, Kassirer M, Aran AA. Injectional anthrax—new presentation of an old disease. Euro Surveill. 2014; 19(32).
48. Kotiranta A, Lounatmaa K, Haapasalo M. Epidemiology and pathogenesis of Bacillus cereus infections. Microbes Infect. 2000; 2(2):189–98. PMID: 10742691
49. Anderson M, Sansonetti PJ, Marreyen BS. Shigella Diversity and Changing Landscape: Insights for the Twenty-First Century. Front Cell Infect Microbiol. 2016; 6:45. PubMed Central PMCID: PMC4835486. doi: 10.3389/fcimb.2016.00045 PMID: 27148494
50. Revell PA, Miller VL. Yersinia virulence: more than a plasmid. FEMS Microbiol Lett. 2001; 205(2):159–64. PMID: 11750796
51. Isozaki A, Shirai K, Mimura S, Takahashi M, Furushima W, Kawano Y. A case of urinary tract infection caused by Kluyvera ascorbata in an infant: case report and review of the literature. J Infect Chemother. 2010; 16(6):436–8. doi: 10.1007/s10156-010-0067-3 PMID: 20454915
52. Torre D, Crespi E, Bernasconi M, Rapazzini P. Urinary tract infection caused by Kluyvera ascorbata in an immunocompromised patient: case report and review. Scand J Infect Dis. 2005; 37(5):375–8. doi: 10.1080/00365540410021033 PMID: 16051578
53. Dinsdale EA, Edwards RA, Hall D, Angly F, Breitbart M, Brulc JM, et al. Functional metagenomic profiling of nine biomes. Nature. 2008; 452(7187):629–32. doi: 10.1038/nature06810 PMID: 18337718
54. Hamady M, Knight R. Microbial community profiling for human microbiome projects: Tools, techniques, and challenges. Genome Res. 2009; 19(7):1141–52. PubMed Central PMCID: PMC3776646. doi: 10.1101/gr.085464.108 PMID: 19383763
55. Hwang HG, Kim MS, Shin SM, Hwang CW. Risk assessment of the schmutzecke of biosand filters: identification of an opportunistic pathogen in schmutzecke developed by an unsafe water source. Int J Environ Res Public Health. 2014; 11(2):2033–48. PubMed Central PMCID: PMC3945583. doi: 10.3390/ijerph110202033 PMID: 24534769
56. Bai Y, Liu R, Liang J, Qu J. Integrated metagenomic and physiochemical analyses to evaluate the potential role of microbes in the sand filter of a drinking water treatment system. PLoS One. 2013; 8(4):e61011. PubMed Central PMCID: PMC3623876. doi: 10.1371/journal.pone.0061011 PMID: 23593378
57. Cangelosi GA, Meschke JS. Dead or alive: molecular assessment of microbial viability. Appl Environ Microbiol. 2014; 80(19):5884–91. PubMed Central PMCID: PMC4178667. doi: 10.1128/AEM.01763-14 PMID: 25038100
58. Lo HH, Chang SM. Identification, characterization, and biofilm formation of clinical Chryseobacterium gleum isolates. Diagn Microbiol Infect Dis. 2014; 79(3):298–302. doi: 10.1016/j.diagmicrobio.2014.01.027 PMID: 24796989
59. Bhotmange DU, Singhal RS. Identification of chondroitin-like molecules from biofilm isolates Exiguobacterium indicum A11 and Lysinibacillus sp. C13. J Appl Microbiol. 2015; 119(4):1046–56. doi: 10.1111/jam.12914 PMID: 26218551
60. Zilm PS, Rogers AH. Co-adhesion and biofilm formation by Fusobacterium nucleatum in response to growth pH. Anaerobe. 2007; 13(3–4):146–52. doi: 10.1016/j.anaerobe.2007.04.005 PMID: 17540586
61. Bao K, Belibasakis GN, Thurnheer T, Aduse-Opoku J, Curtis MA, Bostanci N. Role of Porphyromonas gingivalis gingipains in multi-species biofilm formation. BMC Microbiol. 2014; 14:258. PubMed Central PMCID: PMC4186565. doi: 10.1186/s12866-014-0258-7 PMID: 25270662
62. Passariello C, Gigola P. Adhesion and biofilm formation by periodontopathogenic bacteria on different commercial brackets. Eur J Paediatr Dent. 2013; 14(3):199–203. PMID: 24295004
63. Fernandez N, Sierra-Alvarez R, Amils R, Field JA, Sanz JL. Compared microbiology of granular sludge under autotrophic, mixotrophic and heterotrophic denitrification conditions. Water Sci Technol. 2009; 59 (6):1227–36. doi: 10.2166/wst.2009.092 PMID: 19342820
64. Zhang D, Li W, Zhang S, Liu M, Zhao X, Zhang X. Bacterial community and function of biological activated carbon filter in drinking water treatment. Biomed Environ Sci. 2011; 24(2):122–31. doi: 10.3967/0895-3988.2011.02.006 PMID: 21565683
65. Hwang G, Klein M, Koo H. Analysis of the mechanical stability and surface detachment of mature Streptococcus mutans biofilms by applying a range of external shear forces. Biofouling. 2014; 30(9):1079–91. doi: 10.1080/08957344.2014.969249 PMID: 25355611