Water quality and microbial diversity in cisterns from semiarid areas in Brazil

Fellipe Alves, Thorsten Köchling, Julio Luz, Sylvana Melo Santos and Savia Gavazza

ABSTRACT

Harvesting rainwater is a common practice worldwide, particularly in areas with no access to a public water supply or insufficient groundwater reserves. More than two million people living in semiarid regions of Brazil consume rainwater stored in cisterns, and little information is available regarding the water quality. Despite the initial good quality of the rainwater, its harvest and storage can introduce contaminants that must be eliminated before consumption. To evaluate the influence of handling, cistern age and precipitation on the quality of harvested rainwater, we monitored seven cisterns in the semiarid Brazilian Northeast over 4 years. Microbial and physicochemical parameters were monitored once a month, and denaturant gradient gel electrophoresis (DGGE) was performed at the end of the monitoring period. Coliform bacteria were detected in 100% of samples, while *Escherichia coli* were observed in 73.8%. The alkalinity and conductivity were the highest for the recently built cisterns due to the dissolution of construction materials. The DGGE of the 16S r DNA did not reveal the presence of *E. coli*. Instead, DGGE bands sequencing indicated that species primarily affiliated with *Alphaproteobacteria* were present in all cisterns, indicating the presence of microbial ecosystems capable of purifying and stabilizing the stored rainwater.

Key words | cisterns, first flush, microbial diversity, PCR-DGGE, rainwater quality, semiarid

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ABBREVIATIONS

CFU	colony-forming units
DGGE	denaturant gradient gel electrophoresis
HDPE	high density polyethylene
NTU	nephelometric turbidity units
PCR	polymerase chain reaction
TDS	total dissolved solids

INTRODUCTION

Water scarcity is a problem that affects approximately 1.2 billion people on every continent; over 500 million more people are approaching this status. Another 1.6 billion people, accounting for almost one-quarter of the world population, face economic water shortage: countries lack the necessary infrastructure to move water from rivers and aquifers (FAO 2007).

In Brazil, the geographical distribution of water is not uniform. The Brazilian semiarid region has suffered from a lack of water for many years (Simões *et al.* 2010); it is characterized by an average annual rainfall of below 800 mm, an aridity index less than or equal to 0.5 (between 1961 and 1990), and a drought risk above 60% (between 1970 and 1990) (Ministry of National Integration 2005). Because the rainfall only occurs during 3–4 months of the year, there are over a million homes without any source of water during the dry period, which begins 4 months after the end of the rainy season (Ministry of National Integration 2005).

Pesqueira and Caruaru, the towns studied in the present work, are both in northeastern Brazil; the conditions in

doi: 10.2166/wh.2014.139

these towns are precarious. The conventional public drinking water supply system covers 60% of the population, distributing water 9 and 5 days/month for the habitants living in the urban and rural areas, respectively (SNIS 2011).

Cisterns are commonly used to collect water for domestic use on farms and in areas with little rainfall (Abdulla & Al-Shareef 2009; Sturm *et al.* 2009). Because the technology is relatively inexpensive and simple, the construction of cisterns has been an immediate solution to the lack of water which is suitable for human consumption during periods of drought for many families (Palla *et al.* 2012). Although rainwater collection and storage systems provide an efficient solution, ensuring the quality of the stored water is critical (Lima *et al.* 2011; Lee *et al.* 2012).

Ecological studies of rainwater harvesting systems using culture-independent approaches frequently reveal common phylogenetic groups, such as the phyla *Proteobacteria* and *Bacteroidetes*, their respective subgroups, and members of both *Verrumicrobia* and *Actinobacteria*, regardless of the geographical location of the sampling sites (Eichler *et al.* 2006; Kim & Han 2011; Aizenberg-Gershtein *et al.* 2012). Most methods used to assess drinking water quality rely on techniques based on isolating and culturing a series of indicator organisms (total coliforms; *Escherichia coli* (*E. coli*)) or an indistinct mass of various microorganisms (heterotrophic plate count). This approach has critical shortfalls including a lack of proof that the total heterotrophic counts indicate the presence of pathogenic species (Allen *et al.* 2004), and the fact that some abundant species cannot be cultured.

Complementary methods that analyze the community structure and identify the members and key players in these ecosystems, such as polymerase chain reaction-denaturant gradient gel electrophoresis (PCR-DGGE) and PCR-16S rDNA cloning, provide a less biased and more complete picture. While the presence of enterobacteria should always be a reason for concern in drinking water supplies, the actual quality of this water must be evaluated while accounting for the presence of other bacteria that are often beneficial to the community and abundant in these samples. This culture-independent study of microbial communities in rainwater harvesting systems in Brazil identified the dominant species and compared the community structures within a set of cisterns from two different locations, accounting for ecological parameters, such as richness and diversity.

In Brazil, approximately 470,000 cisterns have been installed in the semiarid region, and the federal government intends to install 1.5 million cisterns through the 'Águapara-Todos' program ('Water for All', Ministry of National Integration 2011). The cisterns are usually installed in houses scattered throughout the rural areas; in these locations, constructing a conventional water network is difficult or not economically viable. For these households, harvested rainwater is the only source of water for drinking and food preparation. A standard system installed by the Water for All government program consists of a concrete cistern able to hold 16,000 l (for households with five people that are located in areas with 9 months of dry season per year; ASA 2013) and galvanized steel chutes connected to PVC pipes that move the collected water from the roof to the cisterns. Usually, an initial flush diversion is performed manually by disconnecting the PVC pipes and reconnecting them after an unspecified amount of time.

Although this government initiative has improved the quality of life for millions by providing water for those who previously walked long distances to collect water with buckets, the quality of the rainwater stored in the cisterns must be analyzed, particularly when these systems are not equipped with protective devices, such as pumps and first flush diversion systems. Consequently, this work was conducted to verify the quality of the water stored in cisterns in the Brazilian semiarid region, evaluating the effect of the use of a first flush diverter and the age of the cisterns. In addition, PCR-DGGE, a culture-independent technique, was used to analyze the structure and diversity of the bacterial communities in the monitored cisterns.

METHODS

Water quality monitoring

For this project, seven standard cisterns (as described in the Introduction) were selected for quality assessments based on the information collected about the structural characteristics and age of the tanks and roof area, as well as the socio-economic conditions of the communities. The monitoring

period was from January 2008 to December 2011. Characteristics of the monitored cisterns are given in Table 1.

The cisterns studied are located in the following communities in the state of Pernambuco, Brazil: Lajedo do Cedro (LC1, LC2, LC3 and LC4), in the municipality of Caruaru and Guaribas (GB1) and Canela de Ema (CE1 and CE2) within the municipality of Pesqueira (Figure 1). Cistern GB1 had a first flush diverter to automatically exclude the water from the first rainfall.

A first flush diverter discards the first millimeter of rainfall and is made of PVC pipe and fittings in a simple, quick and water-tight assembly (Figure 2). The device was patented by the Federal University of Pernambuco (patent number BR 20 2012 028275 0). When the rainfall begins, the system must be completely empty before collecting water from the first rain; this water washes contaminants off the roof and gutters. Based on the principle of communicating vessels, a set of pipes is filled to capacity, equaling 1 mm of rainfall, across the roof area. The subsequent precipitation contains lower levels of impurities and travels to the cistern. After each rainfall, the device must be manually emptied to continue functioning correctly. The government recommendations for operating and maintaining the cisterns include: (i) manually diverting the first rainfall, (ii) using only pumps to remove water from the cistern, (iii) keeping the cistern cover closed and (iv) storing only rainwater. During severe drought, however, households are often supplied with chlorinated, piped water.

The samples were collected by using the same procedure as was employed by the residents retrieving water from the cisterns. Buckets were used to draw water in six households, while only one family used a manual pump (GB1). One liter

Table 1 | Characteristics of the monitored cisterns

Variable	Cistern/location						
	Caruaru				Pesqueira		
	LC1	LC2	LC3	LC4	GB1	CE1	CE2
Cistern initial age[a] (years)	5	1	1	9	2	3	3
Number of people living in the house	3	1	4	8	3	4	5
First rainfall water discarded	M	M	M	M	A[b]	M	M
Water handling	Bucket	Bucket	Bucket	Bucket	Pump	Bucket	Bucket
Water source	Rain + piped water				Rainwater only		

M: Manual diversion of first rainfall water.
[a]Cistern age when the monitoring began in 2007.
[b]Automatic diversion of first rainfall water, installed in July 2009.

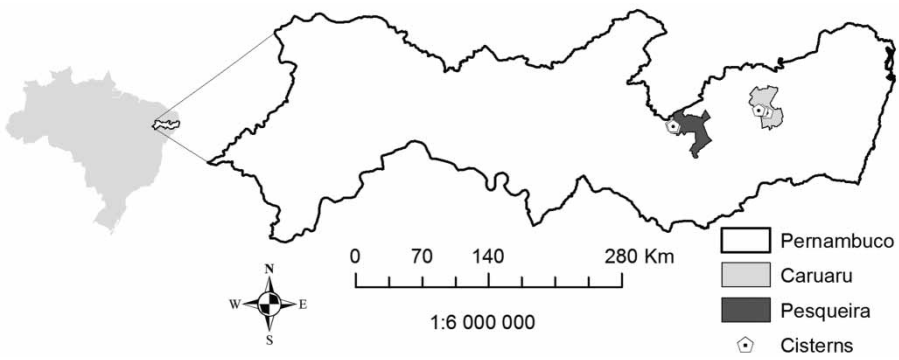

Figure 1 | Geographical locations of the monitored cisterns.

Figure 2 | Schematic illustration of the harvesting system. A: ceramic roof; B:– first flush diverter; C: cistern.

water samples were collected in sterile bottles and stored on ice while being transported to the laboratory for immediate analysis.

During the 4-year monitoring period, 37 samples were collected from the cisterns in Caruaru and 35 from the cisterns in Pesqueira. The parameters assessed (total alkalinity, conductivity, apparent color, chlorides, total hardness, dissolved oxygen, pH, salinity, total dissolved solids (TDS), turbidity, total heterotrophic bacteria, total coliforms and *E. coli*) were analyzed once a month according to the *Standard Methods for the Examination of Water and Wastewater* (APHA 2005).

Molecular ecology

Samples and collection

Rainwater samples from eight cisterns were collected for the biomolecular experiments. For each of the cisterns from the Pesqueira area (GB1 and CE2; Table 1), two samples were collected. The GB1-pump sample was taken from the pump used to draw water from the cistern and the GB1-rec sample was taken from the clay vessel used for storing the water before consumption. Sample CE2-cis was collected directly from the cistern and the CE2-filter sample was taken from the outlet of a clay reservoir with a microporous ceramic filter, which is used to remove the solid particles suspended in the water before consumption. This type of filter is very common in rural areas of Brazil. The other samples were taken from cisterns in the Caruaru region (LC1, LC3, LC4, LC5, LC6 and LC7).

One liter rainwater samples were collected in sterile bottles, placed immediately on ice and filtered through 0.22 μm pore-size cellulose ester membranes (Fmaia, São Paulo, Brazil). The membranes were stored at −20 °C until DNA-extraction.

Total community DNA extraction

The filter membranes were subjected to the DNA extraction method of Urakawa *et al.* (2010), substituting the lysing matrix tubes with 1.5 ml microcentrifuge tubes containing 100 μl of 150–212 μm glass beads (G9018; Sigma-Aldrich, São Paulo, Brazil).

Polymerase chain reaction

A nested PCR protocol was used to amplify the bacterial 16S rDNA. During the first round, the 27F/1492R bacterial primer set was used to amplify the major portion of the gene (Lane 1991). These amplicons were used as a template for the second round of PCR; this round used the 968F-GC and 1392R primers (Ferris *et al.* 1996), to target the V6–V8 regions of the 16S rRNA gene.

All PCR reactions were carried out using a hot start in 2 mM $MgSO_4^{2-}$, 0.2 mMdNTPs, 0.2 μM of each primer, and 1 unit of *Taq* polymerase (Platinum Taq; Invitrogen Life Technologies, NY, USA). For the first round of PCR, the initial denaturation step (4 min at 94 °C) was followed by 32 amplification cycles of 30 s at 94 °C, 30 s of annealing at 55 °C and product extension for 2 min at 68 °C. The protocol was completed after an additional extension step of 10 min at 68 °C. The second round protocol consisted of 4 min at 94 °C; 32 cycles of 45 s at 94 °C, 45 s at 40 °C, and 1 min at 68 °C; and a final step at 68 °C for 5 min.

Denaturant gradient gel electrophoresis

DGGE was performed on a DCode Mutation Detection System (Bio-Rad, Hercules, CA, USA). The gels were stained with ethidium bromide (0.5 μg/ ml), and several bands were excised from the gel with a surgical blade. The bands were re-amplified with the 968F and 1392R primers before being bidirectionally sequenced by Macrogen Inc. (Seoul, South Korea).

Comparative analysis of the 16S rDNA sequences

The sequencing chromatograms were edited with Chromas (Technelysium, Brisbane, Australia); the forward and reverse sequences of each band were joined with Genedoc (Nicholas *et al.* 1997). The reconstructed sequences were checked for possible chimeras both manually and with Bellerophon (Huber *et al.* 2004). One sequence (band 5) was chimeric and was excluded from further analyses. BLAST (Altschul *et al.* 1990; Zhang *et al.* 2000) searches against the NCBI database (TL/16S_ribosomal_RNA_Bacteria_and_Archaea) and the RDP (Ribosomal Database Project) classifier tool (Wang *et al.* 2007) were used to determine the phylogenetic affiliation of the sequenced bands.

Statistical analysis of denaturant gradient gel electrophoresis data

The DGGE banding patterns were translated into a presence/absence matrix; a dissimilarity table was generated by calculating the Jaccard index. The samples were clustered using Ward's method and visualized in a dendrogram. The computations were carried out using the R Software Environment for Statistical Computing (R Development Core Team 2011) using the program's base libraries and the vegan package (Oksanen *et al.* 2012).

To analyze the biodiversity, the densitometry curves of the lanes of three replicate PCR-DGGE experiments were recorded with ImageJ (Abràmoff *et al.* 2004). The relative peak intensity (peak area) of each discrete band compared to the intensity of the whole fingerprint was used to estimate the number of individuals (p) belonging to an operational taxonomic unit (i) in a sample with S different species (richness, number of bands) via the Shannon-Wiener diversity index, using the $H' = -\sum pi\ln(pi)$ formula (Shannon 1948). Shannon's index of evenness was calculated according to $E = H'/\ln_S$, where $\ln_S$ represents H'_{max}. Simpson's index of biodiversity (Simpson 1949) was calculated in the same fashion using $D_1 = 1 - \sum pi^2$. The R/vegan software was used to compute the indices.

RESULTS AND DISCUSSION

General characteristics of the water from the monitored cisterns

The mean values (after 4 years of monitoring) of the physicochemical parameters are shown in Table 2.

Relatively high pH, alkalinity and conductivity levels were found in the most recently built cisterns (LC2 and LC3) due to the dissolution of the material (precast

Table 2 | Arithmetic mean and standard deviation of the main physicochemical parameters

	Cistern/location							
	Caruaru				Pesqueira			Maximum acceptable value[a]
Parameter	LC1	LC2	LC3	LC4	GB1	CE1	CE2	
Total alkalinity (mg $CaCO_3$/L)	14.0 ± 19.8	13.1 ± 17.9	4.7 ± 9.3	3.9 ± 5.0	6.9 ± 9.3	6.2 ± 6.7	16.0 ± 15.0	ns
Electric conductivity (μS/cm)	118.8 ± 20.7	265.1 ± 102.0	147.0 ± 32.0	134.8 ± 43.9	75.6 ± 13.0	143.9 ± 47.6	92.8 ± 37.8	ns
Apparent color (μH)	8.3 ± 7.1	6.8 ± 5.2	7.1 ± 3.3	7.1 ± 4.0	2.3 ± 1.6	5.1 ± 3.4	5.9 ± 4.9	15
Chlorides (mg Cl^-/L)	7.0 ± 1.5	7.7 ± 1.4	9.3 ± 3.7	9.3 ± 6.2	5.9 ± 2.0	10.6 ± 4.7	5.9 ± 2.0	250
Total hardness (mg $CaCO_3$/L)	46.6 ± 12.9	46.5 ± 7.1	51.1 ± 12.4	47.9 ± 13.4	32.9 ± 5.4	55.0 ± 11.2	39.3 ± 8.8	500
Dissolved oxygen (mg O_2/L)	5.4 ± 1.1	5.8 ± 1.1	5.1 ± 0.9	5.3 ± 1.7	5.8 ± 1.3	6.1 ± 1.4	5.9 ± 1.5	ns
pH	7.6 ± 0.3	8.1 ± 0.4	8.0 ± 0.9	8.0 ± 0.8	7.8 ± 0.4	8.0 ± 0.5	8.0 ± 0.5	6.0–9.5
TDS (mg/L)	88.8 ± 42.0	119.1 ± 31.1	130.2 ± 86.3	118.3 ± 82.0	63.8 ± 24.7	81.8 ± 28.9	68.6 ± 23.4	1000
Turbidity (NTU)	0.8 ± 0.5	0.6 ± 0.3	0.6 ± 0.3	0.7 ± 0.7	0.4 ± 0.2	0.5 ± 0.3	0.6 ± 0.4	5

NTU: Nephelometric turbidity units.

[a]Maximum acceptable value for potable water (Brazilian legislation, Ordinance n° 2914/2011 of the Ministry of Health).

cement slabs) used to manufacture the cisterns. The mean alkalinity values were below 95.1 mg $CaCO_3$/l (LC2), and the pH ranged from 6.6 and 9.4 (LC4 and LC2 cisterns). According to potability standards established by Brazilian legislation (Ordinance n° 2914/2011 of the Ministry of Health), the pH value should range between 6.0 and 9.5, but a benchmark for the alkalinity and conductivity has not yet been established. In this study, pH values reached 9.4 near the maximum. The average conductivity was 154.6 μS/cm. In a study conducted on the island of Kefalonia (Greece), Sazakli *et al.* (2007) obtained an average conductivity value of 103.0 μS/cm in the rainwater stored in cisterns composed of reinforced concrete and built in the 1970s. The greater age of the cisterns may explain the lower values compared to those found in the cisterns, constructed more recently, in the Brazilian semiarid region.

The results for chlorides, total hardness and TDS were always below the maximum values, 250 mg Cl^-/l, 500 mg $CaCO_3$/l and 1,000 mg/l, respectively, established by Ordinance No. 2914/2011 of the Ministry of Health. In this project, we obtained average values of 7.6 mg Cl^-/l for the chlorides, 45.6 mg $CaCO_3$/l for the total hardness and 97.0 mg/l for TDS. In studies related to rainwater quality, other authors observed chloride concentrations of 1.9 mg Cl^-/l, a total hardness of 16.0 mg $CaCO_3$/l (Vialle *et al.* 2011) and a TDS value equal to 270.2 mg/l (Abdulla & Al-Shareef 2009). These results confirm that the material used to construct the cisterns interferes with the quality of the rainwater. Vialle *et al.* (2011) evaluated the quality of the rainwater stored in high density polyethylene (HDPE) tanks in a rural village in southwestern France, and Abdulla & Al-Shareef (2009) studied rainwater stored in 60 tanks distributed across Amman and Irbid (Jordan) that were made of various materials, such as brick, cement and concrete but not plastics. In the HDPE reservoirs studied by Vialle *et al.* (2011), the chloride concentrations and hardness values were lower than those in the present study, indicating a higher degree of particle solubilization, such as TDS, in tanks made of cement and concrete. The same material was used to construct the cisterns located in the Brazilian semiarid region.

The average apparent color of water from all cisterns was 6 mg Pt-Co/l (range 1–28 mg Pt-Co/l). Vialle *et al.* (2011) obtained an average of 18 mg Pt-Co/l for the observed color in the rainwater captured from roofs and stored in HDPE tanks. Brazilian law allows up to 15 mg Pt-Co/l in water intended for human consumption (Ministry of Health 2011). In this study, 8.1% of the samples exceeded this limit.

The age of the cisterns was correlated with the alkalinity, pH and conductivity. The younger the cistern, the higher the detected alkalinity levels (data not shown). During the first few years after a cistern is constructed, the variations in this parameter were large but tended to stabilize over time. This behavior was observed for the cisterns in Caruaru and in Pesqueira; the results were similar for pH and conductivity.

Heterotrophic bacteria and total coliforms were detected using the traditional microbiological tests at all collection points, throughout the entire monitoring period (Table 3). Brazilian law does not require the absence of these organisms to classify water as being suitable for human consumption when treatment is not performed in conventional water treatment plants. The tolerance for heterotrophic bacteria is 500 colony-forming units (CFU)/ml, and 46.6% of the samples exceeded this value. The average for the heterotrophic bacteria was 1,031 CFU/ml. Evans

Table 3 | Arithmetic means and standard deviations of the microbiological parameters

	Cistern/location						
	Caruaru				Pesqueira		
Parameter	LC1	LC2	LC3	LC4	GB1	CE1	CE2
Total heterotrophic bacteria ($10^2 \times$ CFU/100 ml)	14.0 ± 19.8	13.1 ± 17.9	4.7 ± 9.3	3.9 ± 5.0	6.9 ± 9.3	6.2 ± 6.7	16.0 ± 15.0
Total coliforms ($10^3 \times$ CFU/100 ml)	15.1 ± 19.8	17.1 ± 15.0	12.0 ± 14.2	12.2 ± 14.5	5.6 ± 7.3	8.9 ± 11.6	13.9 ± 17.0
E. coli ($10^2 \times$ CFU/100 ml)	2.0 ± 3.8	1.9 ± 4.6	6.4 ± 17.9	9.4 ± 15.8	1.1 ± 2.5	1.1 ± 2.5	3.0 ± 6.6

et al. (2006) presented values that ranged from 750 to 2,000 CFU/ml in a study conducted in the urban area of Newcastle (Australia), and Simmons *et al.* (2001) observed average values of 570 CFU/ml in the rainwater for domestic use in four rural districts of Auckland (New Zealand). The cisterns monitored in this study are located in rural areas, and this was a very important aspect when determining that most of the samples met the Brazilian Ministry of Health potability standards.

With respect to the coliform bacteria, *E. coli* was detected at all collection points; 73.8% of the samples tested positive for *E. coli*, and the average for all cisterns was 287.5 CFU/100 ml. Vialle *et al.* (2011) determined 148 CFU/100 ml was the average *E. coli* content in the rainwater captured from roofs and stored in HDPE tanks. Vialle and colleagues' study was carried out in a village in France where the average temperature ranged between 7.9 and 18.3 °C; the difference in climate may influence the levels of *E. coli* because the average temperatures in the study region range from 23 to 27 °C, favoring the development of *Enterobacteriaceae*.

To evaluate the influence of the first flush device, the *E. coli* counts throughout the monitoring period are shown in Figure 3. Total coliforms and *E. coli* levels (5,600 CFU/100 ml and 104 CFU/100 ml) were among the lowest in the water from the cistern where the device was installed (GB1). This cistern also showed less variability in all parameters (smaller standard deviation) (Tables 2 and 3). In the case of total coliforms in GB1 the average value was 2.5 times lower than the average for the other points. Similar results were observed for the maximum values of total coliforms; GB1 also had results 2.5 times lower than the average maximum values for the other collection points, reinforcing the importance of performing the first flush, as previously reported (Souza *et al.* 2011; Lee *et al.* 2012), to clean the roof and the pipework.

Additionally, the rainwater stored in the rural cisterns of Pernambuco that were monitored in this study met the potability standards established by the Brazilian legislation for color and turbidity during at least 91.9% of the monitoring period; for *E. coli*, these standards were met 26.2% of the time. The lowest contamination levels were observed at the end of the rainy season, usually in July or August. At the end of the dry season, the contamination levels increased, especially when the first rainfall occurred, carrying impurities into the reservoirs.

Rainfall was the main external factor that influenced the quality of water stored in the cisterns because it reduces the concentration of some contaminants via dilution when the cisterns are filled. The increased monthly rainfall during the rainy season decreased total alkalinity, chloride concentrations, electric conductivity, total hardness, pH and TDS concentration in all cisterns throughout the 4 years of monitoring. The trends in the electrical conductivity results confirm this observation (data not shown).When comparing the average conductivity in January, April and July with the average of the following month (February, May and August) we observed 22.9, 20.6 and 8.4% decreases, respectively. This behavior can be attributed to the increased amount of

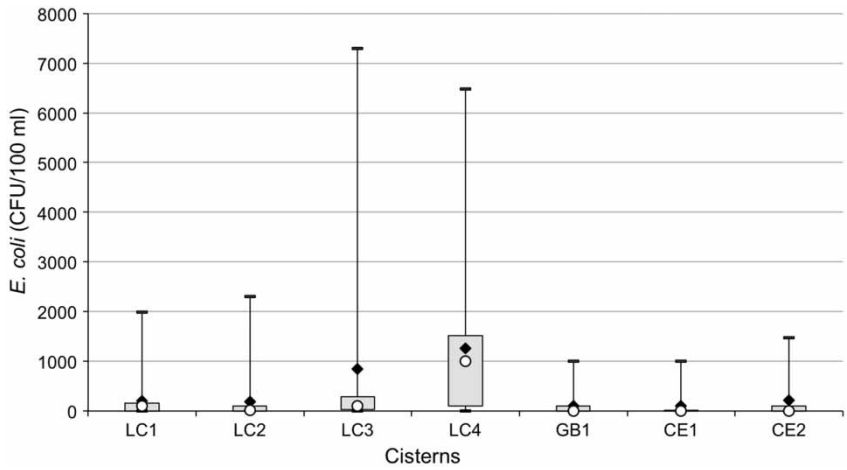

Figure 3 | Box plot diagram of *E. coli* in all monitored cisterns: – minimum; 25th and 75th percentile; ○ median; ◆ 50th percentile and – maximum.

precipitation during these months. Additionally, in small households (GB1, three people) with low cistern water consumption, the influence of dilution on the conductivity values was less noticeable.

In general, the coliforms counts, apparent color and turbidity increased due to the increased precipitation. In the first 4 months of 2010 at collection point LC2 (Caruaru), the monthly rainfall (57, 90, 120 and 118 mm; INPE 2010) must have carried impurities from the roof and gutters into the cisterns, increasing the total coliforms (in April the concentration was 91.4 times higher than in January, data not shown). In June, the intense rainfall (329 mm), compared with May (11 mm), must have reduced the level of coliforms because the contact surfaces were already cleaned by the earlier precipitation.

DGGE analysis

The bacterial 16S rRNA gene amplification and DGGE generated discrete banding patterns for all surveyed samples (Figure 4). The PCR-amplification of the DNA templates with primers specific for *Archaea* compared to a positive control confirmed the absence of archaeal species in the cistern samples (data not shown). The richness values measured using the number of bands per sample ranged from 10.3 ± 0.6 to 15.7 ± 0.6, and the studied cisterns appeared to harbor a diverse bacterial community with Shannon index values ranging from 2.12 ± 0.04 to 2.62 ± 0.02 (Table 4). The evenness was equally distributed between the samples, ranging from 0.88 ± 0.03 to 0.95 ± 0.01. These values did not reveal a location-dependent trend among the cisterns, and they fit into the wide range encountered in planktonic communities in various habitats, as described in other freshwater ecosystems (Yu *et al.* 2010; summarized in Troussellier *et al.* 2002). The diversity and richness values based on the DGGE gel analysis may depend on the primers chosen for the PCR step. While this technique enables the highly resolved discrimination of microbial communities by generating band patterns, comparing the

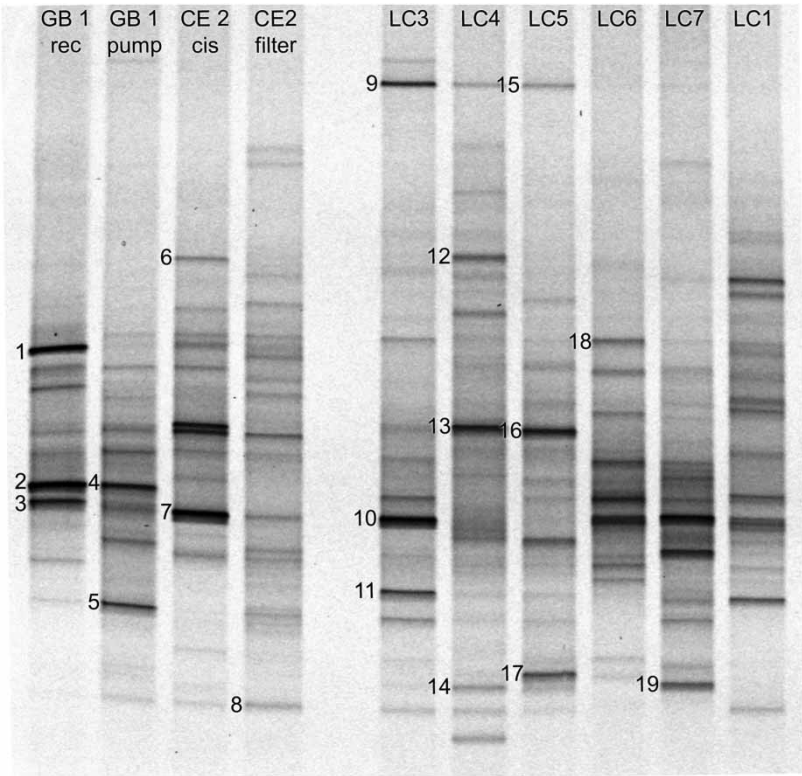

Figure 4 | DGGE analysis of bacterial 16S rDNA amplified from the total community DNA extracts of cistern water samples from Pesqueira (GB1, CE2) and Caruaru (LC1, LC3–LC7). The numbered bands were excised and sequenced.

Table 4 | Ribotype richness and diversity/evenness indices of the DGGE band patterns derived from cistern water samples[a]

Sample	Richness (number of bands)	Shannon-Wiener index of diversity	Simpson's index of diversity	Shannon's index of evenness
GB1-rec	10.3 ± 0.6	2.12 ± 0.04	0.87 ± 0.01	0.91 ± 0.00
GB1-pump	12.7 ± 0.6	2.22 ± 0.03	0.88 ± 0.00	0.87 ± 0.01
CE2-cis	12.0 ± 1.0	2.17 ± 0.16	0.86 ± 0.03	0.87 ± 0.04
CE2-filter	14.7 ± 0.6	2.56 ± 0.04	0.92 ± 0.00	0.95 ± 0.00
LC3	11.7 ± 0.6	2.16 ± 0.08	0.86 ± 0.01	0.88 ± 0.02
LC4	14.0 ± 2.0	2.31 ± 0.17	0.87 ± 0.03	0.88 ± 0.03
LC5	14.7 ± 1.2	2.41 ± 0.01	0.89 ± 0.00	0.90 ± 0.02
LC6	11.3 ± 0.6	2.13 ± 0.02	0.86 ± 0.00	0.88 ± 0.01
LC7	13.0 ± 1.0	2.36 ± 0.11	0.89 ± 0.01	0.92 ± 0.02
LC1	15.7 ± 0.6	2.62 ± 0.02	0.92 ± 0.00	0.95 ± 0.01

[a]Values are means ± standard deviations of three replicate PCR-DGGE analyses.

diversity values between studies is problematic without precise methodological standardization.

Thirteen out of the 18 sequenced and identified bands (72%) belonged to the phylum *Proteobacteria*, mainly to the class *Alphaproteobacteria*; this class is the most abundant phylogenetic group detected in this study (Table 5). The other sequences were affiliated with *Nitrospira* (2 bands), *Bacteroidetes* (1), *Cyanobacteria* (1) and *Firmicutes* (1). Members of the *Proteobacteria*, particularly the alpha- and beta-classes, are often present in high proportions in various freshwater habitats, including rainwater harvesting systems (Eichler *et al.* 2006; Evans *et al.* 2009; Kim & Han 2011; Poitelon *et al.* 2009; Kwon *et al.* 2011; Aizenberg-Gershtein *et al.* 2012). The eight rainwater cisterns in this study may harbor an ecosystem typical for this type of environment. While the nitrite-oxidizing *Nitrospira* species (bands 17 and 19) are also often found in freshwater environments, the detection of a species affiliated to the anaerobic genus *Clostridium* (band 12), which has a 98% sequence similarity to *C. celatum*, most likely indicates the presence of fecal contamination in cistern LC4. This finding agrees with the results of the *E. coli* counts where samples from LC4 showed the highest average values, a direct indication of fecal contamination.

In contrast, several of the sequenced bands were phylogenetically related to the alphaproteobacterial genera; these genera include members with specific physiological traits that may enhance the quality of the water meant for human consumption. Bands 2 and 4 (GB1-rec and GB1 pump, respectively, Figure 4) could be assigned to the genus *Sphingosinicella*. Interestingly, the members of this genus can degrade peptidic xenobiotic compounds (Geueke *et al.* 2007) and microcystin; the latter is a bacteriotoxin produced by certain cyanobacteria (Maruyama *et al.* 2006). Toxin-producing cyanobacteria are commonly found in stagnant water. Although not detected by DGGE, the presence of cyanobacteria and their potentially harmful toxins should be considered in the studied cisterns due to the long intervals between rainfall episodes. Band 1 could be attributed to the genus *Comamonas*, which includes species exhibiting antifungal activity (El-Banna 2007), while the *Sphingobium* species (band 3 and 7) can degrade polyaromatic hydrocarbons and certain herbicides; this trait may prove beneficial for water from rural, agricultural areas, such as our study area (Prakash & Lal 2006; Sipilä *et al.* 2010). Furthermore, band 16 was related to *Rhodobacter*, which comprises members that degrade the pesticide *p*-nitrophenol. However, this reaction is light-dependent and will therefore not occur in the closed water harvesting systems (Blasco & Castillo 1992; Roldan *et al.* 1997). These examples indicate that the rainwater harvesting systems surveyed in this work might purify the cistern water, thus improving the quality of the drinking water.

The ecosystems are adapted to the nutrient-poor water in the storage cisterns, out-competing the potentially harmful microorganisms and degrading toxic compounds, such as heavy metals and pesticides; this phenomenon has been proposed previously (Evans *et al.* 2009) and is corroborated by our results. Additionally, the microorganisms in the studied cisterns were affiliated with bacterial groups capable of degrading the cyanobacterial toxins and polyaromatic hydrocarbons that might contaminate the rainwater storage reservoirs in rural areas similar to the one studied in this survey. The presence of these microorganisms may add water purifying properties to these systems.

Clay pots have been used for water storage for thousands of years. In this study, the sequencing results for a clay receptacle (GB1-rec) show that these devices may purify the contained water using specific microbial populations that may develop inside these containers; the three

Table 5 | Comparative sequence analysis of bacterial 16S rDNA DGGE bands from cistern water samples. Band numbers correspond to the positions indicated by the numbers in Figure 3

Band	Closest match (cultured strain)	NCBI access	%	Phylogenetic group
1	*Comamonas testosteroni* KS 0043	NR_029161	99	Betaproteobacteria
2	*Sphingosinicella soli* KSL-125	NR_043532	97	Alphaproteobacteria
3	*Novosphingobium tardaugens* ARI-1	NR_028630	96	Alphaproteobacteria
4	*Sphingosinicella soli* KSL-125	NR_043532	97	Alphaproteobacteria
5	Chimeric sequence	NA	NA	NA
6	*Runella limosa* EMB111	NR_043771	96	Bacteroidetes
7	*Sphingobium herbicidovorans* MBIC3166	NR_040807	96	Alphaproteobacteria
8	*Stella vacuolata* DSM 5901	NR_025583	93	Alphaproteobacteria
9	*Sulfurovum lithotrophicum* 42BKT	NR_024802	95	Epsilonproteobacteria
10	*Nisaea nitritireducens* DR41_18	NR_043924	92	Alphaproteobacteria
11	*Planktothricoides raciborskii* NIES-207	NR_040858	90	Cyanobacteria
12	*Clostridium celatum* DSM 1785	NR_026167	98	Firmicutes
13	*Rhodobacter sphaeroides* 2.4.1	NR_029215	96	Alphaproteobacteria
14	*Nisaea nitritireducens* DR41_18	NR_043924	93	Alphaproteobacteria
15	*Sulfurovum lithotrophicum* 42BKT	NR_024802	95	Epsilonproteobacteria
16	*Rhodobacter blasticus* ATCC 33485	NR_043735	97	Alphaproteobacteria
17	*Nitrospira moscoviensis* NSP M-1	NR_029287	97	Nitrospira
18	*Herminiimonas fonticola* S-94	NR_043090	97	Betaproteobacteria
19	*Nitrospira moscoviensis* NSP M-1	NR_029287	96	Nitrospira

[a]NA: data not available.

dominant bands belonged to microbial groups that are beneficial for water purification.

In addition, seven out of the nine bands related to the *Alphaproteobacteria*, including all of the aforementioned bands related to the phylogenetic groups involved in toxin degradation, were dominant bands in the gel, exhibiting the highest intensities within the corresponding samples. This phenomenon was observed in both sets of cisterns (Pesqueira and Caruaru). None of the non-proteobacterial sequences derived from the DGGE bands matched any of the dominant bands in the gel. These observations indicated that members of the *Alphaproteobacteria* probably dominate the microbial communities in several of the studied rainwater harvesting systems, acting as key players in their respective communities.

Because most of the dominant bands in the DGGE were affiliated with *Alphaproteobacteria*, enterobacteria might not have been present in high proportions, nor did they constitute a metabolically important fraction of the population in the cistern water, even though they were detected using the standard culture-dependent plate count tests (total coliforms and *E. coli*). The temperature and the oligotrophic characteristics of the stored rainwater could impede their growth, enabling them to be out-competed by better adapted species, such as members of the *Alpha-* and *Betaproteobacteria* (Evans *et al.* 2009).

A cluster analysis of the DGGE banding patterns formed two principal groups that, at first, seem to reflect the geographical distance between the two locales of the sample sets because (with one exception) the set of samples from the Pesqueira site and those from Caruaru each cluster together (Figure 5).

Considering that all of the samples from cluster 1 are from cisterns exclusively fed by rainwater, and the samples from cluster 2 were fed by rain- and piped water, the clustering by type of water input is more cohesive. To confirm this observation, a study involving more cisterns with each type of water input would be necessary. The inclusion of an automatic first flush water diverter in cistern GB1 did not generate a distinct band pattern for the corresponding samples and might not have significantly impacted the

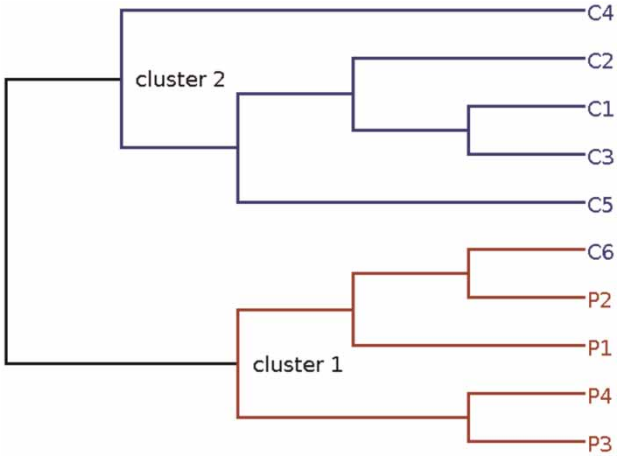

Figure 5 | Dendrogram visualizing the cluster analysis of the DGGE samples from Figure 4. C1–C5 indicate samples from Caruaru and P1–P4 samples from Pesqueira.

predominant bacteria in the planktonic community or the microorganisms deposited with the dust and animal feces on the roof of the catchment.

The seemingly random distribution of the Jaccard indices of dissimilarity as well as the richness and diversity indices between the individual cisterns suggested that each water cistern contained its own specific bacterial community. In contrast, the sharing of several bands, the overall dominance of alphaproteobacterial species and the clustering into two groups, which was most likely caused by the different water composition of water input, suggests a common structure and grouping of the bacterial ecosystems into two sets of rainwater harvesting systems that share ecological similarities.

CONCLUSIONS

This study contributes to knowledge about the quality of the rainwater stored in cisterns in physicochemical and microbiological terms, and the suitability of this water for human consumption.

Each of the monitored cisterns complied with the legally established drinking water standards at least once during the experiment; however, 100% of the samples contained coliforms, 73.8% contained *E. coli* and the heterotrophic plate counts exceeded the permissible maximum in 46.6% of the samples. Therefore, harvesting rainwater to supply potable water in semiarid Brazil has potential but still requires further optimization to entirely eliminate the pathogens. The physicochemical characteristics of the cistern water samples were satisfactory with the exception of color; this parameter exceeded the limits established by Brazilian law in 8.1% of the samples.

Older cisterns exhibited decreased total alkalinity, pH and conductivity, while larger amounts of precipitation were correlated with the decline in values for most parameters, except for the color and bacteriological parameters (heterotrophic bacteria, total coliforms and *E. coli*). The present ecosystems might possess the ability to purify and control the cistern water, as well as limit the persistence or population of harmful bacterial species. This assumption is supported by the detection of several bacterial groups, including species capable of degrading toxins, such as pesticides and polyaromatic hydrocarbons. The dominant members of the bacterial communities were *Alphaproteobacteria*; this group contains mostly harmless or useful species; although enterobacterial species were detected (total coliforms and *E. coli*), they were most likely not a dominant member of these ecosystems.

PCR-DGGE of the 16S rRNA gene is a relatively fast and economical method that might identify the functionally important members in microbial communities present in drinking water. This technique complements the conventional plate count techniques because the sequence analysis of the DGGE bands contributes important data regarding the composition of the microbial community. The taxonomic data partially resolve the problem inherent to the heterotrophic plate count, which is the lack of qualitative information. Furthermore, a cluster analysis of the DGGE banding patterns can be used to ascertain the compositional similarities between the microbial communities from different cisterns and help to find the possible causes of these similarities.

In summary, the monitored cisterns were an acceptable source of drinking water; however, to protect the consumer from contamination by potentially harmful microorganisms, quality-enhancing systems, such as small pore size filters that are used to eliminate particulate and bacterial components from the water should be employed at the point of use. These results were obtained by studying a semiarid

tropical region in Brazil. However, these data are significant on a broader scale because the contamination of harvested rainwater from roof run-off is a ubiquitous problem that affects human health; fortunately, these issues can be monitored and amended in other parts of the world using the measures applied in this paper.

ACKNOWLEDGEMENTS

The authors would like to thank the Brazilian agencies of FINEP and CNPq for their financial support and for providing scholarships for the first four authors. The authors are grateful to the families who use and maintain the cisterns for contributing to this research.

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First received 27 June 2013; accepted in revised form 3 November 2013. Available online 6 January 2014