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Analysis of the bacterial community composition in acidic well water used for drinking in Guinea-Bissau, West Africa

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ABSTRACT

Potable water is a resource out of reach for millions worldwide, and the available water may be chemically and microbiologically compromised. This is particularly acute in Africa, where water-networks may be non-existent or restricted to a small fraction of the urban population, as in the case of Guinea-Bissau, West Africa. This study was carried out seasonally in Bolama (11°N), where unprotected hand-dug wells with acidic water are the sole source of water for the population. We inspected the free-living bacterial community dynamics by automated rRNA intergenic spacer analyses, quantitative polymerase chain reaction and cloning approaches. The results revealed a clear seasonal shift in bacterial assemblage composition and microbial abundance within the same sampling site. Temperature, pH and turbidity, together with the infiltration and percolation of surface water, which takes place in the wet season, seemed to be the driving factors in the shaping and selection of the bacterial community and deterioration of water quality. Analysis of 16S rDNA sequences revealed several potential pathogenic bacteria and uncultured bacteria associated with water and sediments, corroborating the importance of a culture-independent approach in drinking water monitoring.

Introduction

The provision of safe drinking water should be a main concern in any society since water is a basic need to human development, health and well-being (WHO, 2009; Kormas et al., 2010). However, outbreaks of diseases transmitted *via* the use of contaminated drinking water remain a serious health concern worldwide, particularly in underdeveloped countries (Eichler et al., 2006). Each year, about 350 million people suffer from waterborne pathogens, causing 99.8% of the deaths in developing countries, 90% of which are related to children (WHO, 2006).

For many decades, microbiologists relied primarily on standard cultivation methods that targeted indicator organisms to assess microbial diversity of natural ecosystems, and more specifically to monitor the overall microbial quality of drinking water (Sartory, 2004). More than 99% of microorganisms are non-culturable by routine techniques (Brettar and Höfle, 2008), including many waterborne pathogens, (e.g. *Vibrio cholerae*), that can remain in a viable but non-culturable state (Vezzulli et al., 2013). Thus, drinking water monitoring following the international quality standards does not necessarily ensure good quality for the final consumer (Burtscher et al., 2009).

The application of molecular biological methods has unveiled the presence of a vast diversity of microorganisms previously undetected, allowing the fast, effective and simultaneous analysis of the microbial community in multiple samples from different and diverse ecosystems (e.g. Zhao et al., 2008; Tang et al., 2013), including the detection of scarce, waterborne pathogens (e.g. Aw and Rose, 2012; Johnson et al., 2012; Rashid et al., 2013). Unfortunately, such practices are still not routinely applied in the field of drinking water quality monitoring (Berry et al., 2006).

The WHO and UNICEF (2010) reported that 37% of the people without access to improved sources of drinking water live in sub-Saharan Africa. In Guinea-Bissau, water supply and sanitation cover

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less than half of the population (INEC, 2004) and no wastewater treatment is available. Cholera is epidemic in the country, and in the 2008 epidemic 14,228 cases of cholera were diagnosed and 225 people died (Luquero et al., 2011). The 2012 outbreak yielded 3280 cases and 22 deaths (Bordalo, unpublished data). Bolama is the capital island of the Bijagós Archipelago, off the coast of Guinea-Bissau (11°N) and the former colonial capital. The old water network is presently non-operational, and the population (6500 inhabitants) retrieves water from open/unprotected shallow hand-dug wells (normally not exceeding 15 m), by means of a bucket. The wells are recharged during the wet season and exposed to human and animal contamination. Traditionally, the population defecates on the wild, and the distance of the few existing latrines is often insufficient to avoid contamination of the well water with human pathogenic microorganisms. In addition, domestic animals wander freely. These conditions may foster waterborne and vector-borne diseases that can be particularly lethal to populations with poor nutritional status and scant access to any health system, as is the case of Guinea-Bissau. Therefore, microbial contamination in drinking water should be analyzed to provide records for control programs to be established before health and the subsequent economic problems emerge.

Although there are some studies concerning water quality in Africa (Abdelrahman and Eltahir, 2011; Chigor et al., 2012), few have been undertaken on shallow well water (Bordalo and Savva-Bordalo, 2007; Pritchard et al., 2007), and hardly any of these studies applied molecular methods. This research was undertaken to inspect the abundance and diversity of Bacteria present in the microbial community in the well water available to Bolama island population, and to relate the results to the prevailing water quality on a seasonal basis. The 16S rRNA gene approaches were applied as a supplementary tool to current international standard practices, and to detect potential microbiological risks.

1. Material and methods

1.1. Description of study area

Bolama Island is located at 11°N and 15°W off the coast of Guinea-Bissau (Fig. 1). Four out of five wells (the only source of water for the population) receive fecal contamination and the water is acidic (pH 2.78–5.50) due to the dominance of sulfur rich sandy-clay soils with low organic matter content. The dry season is from November to May, and the wet season is from June to October. The environment has been researched and thoroughly described elsewhere (Bordalo and Savva-Bordalo, 2007).

1.2. Sample collection

Eleven major wells that supply most of the population with water for domestic use were visited in 2010 during the dry and wet seasons (Fig. 1). No water quality parameters were assayed during the wet season for sample #25 due to difficulties in accessing the sampling site (road obstructed by fallen trees). The island was divided into rural and urban (Bolama City) zones according to land use. The exact position of each well was obtained by means of GPS (Magellan 600, Magellan, Santa Clara, CA, USA). Water samples were collected using 500 mL plastic sterile flasks. All samples were kept in the dark, refrigerated in ice chests and processed in a field laboratory similar to the one described in Bordalo and Savva-Bordalo (2007). For microbial abundance and diversity analysis water samples were concentrated onto membrane filters, and immediately frozen at −20°C until further processing.

1.3. Analytical procedures

Temperature, conductivity, turbidity, dissolved oxygen, oxygen saturation, and pH were measured *in situ*, using a Hanna Instruments meter (HI9828, Hanna Instruments, Woonsocket, RI, USA). Samples for nitrate, nitrite, ammonium, aluminum, copper, iron, and chromium were assayed in a 12-V multiparameter Hanna photometer (HI83200, Hanna Instruments, Woonsocket, RI, USA) according to standard methods supplied by the manufacturer.

Samples for fecal coliforms (FC) and fecal enterococci (FE) enumerations were filtered onto sterile cellulose nitrate membranes (0.45 μm pore size, 47 mm diameter, GE Healthcare Life Sciences, Little Chalfont, UK), by means of a hand-pump, placed in mFC-agar (Difco, Franklin Lakes, NJ, USA) and Slanetz-Bartley agar (Oxoid, Waltham, MA, USA) plates, respectively, and incubated at 44.5°C for 24 hr (FC) or 48 hr (FE). Typical colonies were counted and results expressed as colony forming units (CFU)/100 mL.

1.4. DNA extraction

For DNA extraction, 100–400 mL water sample replicates were filtered onto sterile cellulose nitrate membranes (0.2 μm pore size, 47 mm diameter, GE Healthcare Life Sciences, Little Chalfont, UK) and kept at −20°C until nucleic acid extraction. Total community DNA was extracted using a modification of the cetyltrimethyl-ammonium bromide–polyvinylpyrrolidone–β mercaptoethanol extraction protocol described by Barrett et al. (2006). The DNA quality was checked on agarose gels and quantified on a Qubit fluorometer (Invitrogen, Carlsband, CA, USA) using the Quant-iT dsDNA assay.

1.5. Automated rRNA intergenic spacer analysis

Polymerase chain reaction (PCR) for automated rRNA intergenic spacer analysis (ARISA) was performed as previously described (Cardinale et al., 2004). Extracted DNA was amplified using an ITSF/ITSReub primer set (Appendix A Table S1), which amplifies the ITS1 region in the rRNA operon plus ca. 282 bases of the 16S and 23S rRNAs (Hewson and Fuhrman, 2004). ITSReub was labeled with the phosphoramidite dye 6-FAM (6-carboxyfluorescein). ARISA amplifications were performed in duplicate reaction mixtures containing 3× PCR buffer, 2.5 mmol/L $MgCl_2$, 1 μg of bovine serum albumin, 200 μmol/L of each dNTP (10 mmol/L, NZYTech, Lisbon, Portugal), 400 nmol/L of each primer (Stabvida, Lisbon, Portugal), 2.5 U of Taqmed polymerase (5 U/μL, Citomed, Lisbon, Portugal), and 2–6 ng of template DNA in a final volume of 25 μL. Thermocycling conditions followed those described by Cardinale et al. (2004). PCR combined products were examined on 1.5% agarose gel, purified using a GFX PCR DNA purification kit (GE Healthcare Life Sciences, Little Chalfont, UK), and quantified using the Quant-it dsDNA assay kit and the Qubit fluorometer (Invitrogen, Carlsband, CA, USA). A standardized amount of the purified PCR product was mixed with ROX-labeled genotyping internal size standard (ROX 1000, Applied Biosystems, Foster City, CA, USA). Sample fragments