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ORIGINAL ARTICLE

Sanitary impact evaluation of drinking water in storage reservoirs in Moroccan rural area

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Abstract In Morocco, storage reservoirs are particular systems of water supply in rural areas. These reservoirs are fed with rainwater and/or directly from the river, which are very contaminated by several pathogenic bacteria. They are used without any treatment as a drinking water by the surrounding population. In this context, the aim of this study is to evaluate the impact of consuming contaminated water stored in reservoirs on health status for six rural communities located in Assif El Mal, Southern East of Marrakech. This was investigated using a classical methodology based on population survey and by molecular approach using PCR–DGGE technique to determine the intestinal bacterial diversity of consumers. The survey showed that, the residents of the studied area suffered from numerous health problems (diarrheal diseases, vomiting or hepatitis A) due to the lack of waste management infrastructures. The consumer's stool analysis by molecular approach revealed that numbers of *Escherichia coli*, *Aeromonas hydrophila* and *Clostridia*, were significantly higher in the diarrheal feces. In addition, PCR–DGGE study of the prevalence and distribution of bacteria causing human diseases, confirmed that, there is a relationship between water bacterial contaminations of storage reservoirs and microbial disease related health status. Therefore, water reservoir consumption is assumed to be the mean way of exposure for this population.

It's clear that this approach gives a very helpful tool to confirm without any doubt the relationship between water bacterial contamination and health status.

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1. Introduction

One third of the world's populations live in countries with some level of water stress. Due to the increase of human population and the resulting impact of human activity on the environment, water scarcity will increase in the future (Asano et al., 2007). Water resource contamination has harmful effects on the environment and human health (Emmanuel et al., 2009; Muhammad et al., 2011). Irregular water supply and insufficient treatment seem to be associated with self-reported diseases (Abu-Amr and Yassin, 2008). It is therefore important to understand the potential indicating that the natural and drinking water can contribute to the transmission of pathogenic microorganisms.

In 2006, Maged et al. (2006) reported a correlation between drinking water contaminated with bacteria and waterborne diseases such as diarrheal and hepatitis A. Moreover, an estimation game for 2 million children dies each year because of diarrheal disease (WHO, 2002). Almost all of them are living in developing countries and are less than 5 years of age. Children younger than 1 year account for more than 50 percent of these deaths, and the risk can be 2–3 times higher among children who are not exclusively breastfed (Arifeen et al., 2001; Bhandari et al., 2003). Many of these deaths are attributed to the use of unsafe drinking water.

Consequently, waterborne diseases are important public health issues, and many of them are derived from contact with contaminated water by human fecal material (Balarajan et al., 1991; Zahra and Jamil, 2001; Scott et al., 2002). Diarrhea can be classified as acute inflammatory disease of the intestinal tract. Its composition change has been related to different metabolic disorders and infections (Brigidia et al., 2001). Diarrhea induced by pathogens can cause dysbacteriosis which leads to changes in the intestinal microbiota and the destruction of protective microbial barrier (Chaofeng et al., 2011).

The bacteriological study by the isolation of bacteria on culture medium demonstrates that a very small proportion of these bacterial species (Nocker et al., 2007). For example, from 1012 bacteria in one gram of feces only 20–40% can be grown (Macfarlane et al., 2004; Suchodolski et al., 2004). Indeed, to identify one species by this technique, the biochemical or serological procedure used for that needs at least one week according to the standards of microbial analysis. To ensure a good public water quality, we must develop improved methods, more precise to identify human fecal pollution. Consequently, scientists have been searching for other rapid methods, that are very sensible to detect all bacterial diversity in environmental samples. Therefore, a molecular detection method is required, since such methods are highly specific and sensitive. The molecular approach is typically based on the detection and quantification of specific segments of the pathogen's genome (DNA or RNA).

These techniques allow researchers to speedily and exclusively detect microorganisms of public health concern. Additionally, recent methods have allowed immediate detection of numerous microorganisms in a simple test (Marcelino et al., 2006). They are the new techniques of multiplex PCR, real-time PCR, nucleic acid sequence-based amplification (NASBA), loop-mediated isothermal amplification (LAMP), oligonucleotide DNA microarray (Law et al., 2014), and magneto-DNA nanoparticle system (Chung et al., 2013).

The detection of bacteria in clinical microbiological research and diagnosis using molecular techniques has increased significantly (Tannock et al., 2004; Murray et al., 2005). Recently, the Polymerase Chain Reaction (PCR) technique has allowed fast and effective diagnosis of microbial infections due to its specificity and sensitivity (Sibleya et al., 2012). PCR–DGGE also has been performed for rapid changes tracking and diagnosing of bacterial diversity in healthy human neonates' intestinal tract (Favier et al., 2002) and in patients suffering from combined infections (Muyzer et al., 1993; Ariefdjohan et al., 2010). It is therefore apparent that this approach will contribute to the understanding of the genetic diversity of complex microbial communities.

Up to now, the nature and magnitude of endemic waterborne disease are not well characterized in Morocco. Epidemiologic studies can give an estimate of the waterborne risk along with other types of information. Endemic gastrointestinal illnesses are rarely seen by the medical authorities in Morocco, for the simple reason that the majority of gastrointestinal illnesses are not declared through the medical care system. In evaluating the drinking water risks, investigators must also study the factors and exposure risks.

Poor rural communities in Morocco, like those in other developing countries that do not have access to piped water, have mainly been reliant on other water resource harvesting systems as part of low cost strategies for improving water supply and sanitation. Typically, rainwater and surface water are collected and stored in traditional reservoirs and then conserved for drinking and cooking. It's the case of Assif El Mal valley (Marrakech region); our study site in which its water is very contaminated with many pathogenesis bacteria (Aziz et al., 2013). This bacterial pollution exposes the user population to many gastrointestinal illnesses.

Global studies have identified and analyzed the pathogenic bacteria in water that cause diarrhea, but there have been a few studies in Morocco, and none of them has evaluate the human impacts using a molecular technique.

The aim of this study is to investigate the human health impact due to contaminated drinking water stored in reservoirs, via an epidemiological study in the consuming population. This was done by (i) a survey questionnaire and (ii) by studying the 16S-rDNA diversity in children feces, using Polymerase Chain Reaction and Denaturing Gradient Gel Electrophoresis (PCR–DGGE) technique.

2. Methods

2.1. Study area

The basin of Assif El Mal is located on the north side of the High Atlas, one hundred kilometers southwest of Marrakech (Fig. 1). In the valley Assif El Mal, the population living in the plain suffers from drinking water shortage and lack of minimum hygiene conditions. The poor socioeconomic status of the local population does not enable them to dig wells. As a consequence, they are using an archaic method as the only source of water for any kind of use (consumption, watering of livestock, etc), water is stored in a kind of traditional cistern buried in the ground, called "*Matfya*" with no prior treatment. They are supplied by river and/or rain water through channels called "*Seguia*".