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Microbiological quality of water in a city with persistent and recurrent waterborne diseases under tropical sub-rural conditions: The case of Kikwit City, Democratic Republic of the Congo

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ABSTRACT

The availability of safe drinking water in sub-Saharan countries remains a major challenge because poor sanitation has been the cause of various outbreaks of waterborne disease due to the poor microbiological quality of water used for domestic purposes. The faecal indicator bacteria (FIB) used in the present study included *Escherichia coli* (*E. coli*) and *Enterococcus* (ENT). FIB and aerobic mesophilic bacteria (AMB) were quantified during July 2015 (dry season) and November 2015 (rainy season) in order to assess the quality of drinking water from wells (n = 3; P1–P3), and two rivers, the River Lukemi (RLK, n = 3) and River Luini (RLN, n = 2) in the city of Kikwit, which is located in the province of Kwilu in the Democratic Republic of the Congo. Kikwit is well known for its outbreaks of persistent and recurrent waterborne diseases including *Entamoeba*, *Shigella*, typhoid fever, cholera, and Ebola Viral Hemorrhagic Fever. Consequently, *E. coli*, ENT, and AMB were quantified in water samples according to the standard international methods for water quality determination using the membrane filtration method. The FIB characterization was performed for human-specific *Bacteroides* by PCR using specific primers. The results obtained revealed high FIB concentrations in river samples collected during both seasons. For example, *E. coli* respectively reached 4.3×10^4 and 9.2×10^4 CFU 100 mL^{-1} in the dry season and the wet season. ENT reached 5.3×10^3 CFU 100 mL^{-1} during the dry season and 9.8×10^3 CFU 100 mL^{-1} in the wet season. The pollution was significantly worse in the wet season compared to the dry season. Surprisingly, no faecal contamination was observed in well water samples collected in the dry season while *E. coli* and ENT were detected in all wells in the wet season with values of 6, 7, and 11 CFU mL^{-1} for *E. coli* in wells P1–P3, respectively and 3, 5, 9 CFU mL^{-1} for ENT in the same wells. Interestingly, the PCR assays for human-specific *Bacteroides* HF183/HF134 indicated that 97–100% captured in all analyses of isolated FIB were of human origin. The results indicate that contamination of *E. coli*, ENT, and AMB in the studied water resources increases during the wet season. This study improves understanding of the microbiological pollution of rivers and wells under tropical conditions and will guide future municipal/local government decisions on improving water quality in this region which is characterised by persistent and recurrent waterborne diseases. Although the epidemiology can be geographically localised, the effects of cross border transmission can be global. Therefore, the research results presented in this article form recommendations to municipalities/local authorities and the approach and procedures can be carried out in a similar environment.

1. Introduction

Waterborne diseases represent a major human health risk in many parts of the world, especially in developing countries. Most people in South and Southeast Asia and Sub-Saharan African

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countries still practice open defecation leading to the faecal contamination of water resources (UNICEF and WHO, 2015). In fact, Prüss-Ustün et al. (2014) reported that unsafe drinking water alone accounted for 50% of deaths in Sub-Saharan Africa out of a total of 50 million deaths in 2012. Furthermore, diarrhoeal diseases, mainly due to microbial water contamination, cause approximately 1.7 billion illnesses and 2.2 million deaths per year (Montgomery and Elimelech 2007; WHO, 2016). The majority of cases are located in Sub-Saharan Africa and south Asia where the economic situation and lack of effective infrastructure means a large part of the population. Furthermore, in some of these regions uses highly contaminated surface waters, shallow wells, boreholes, springs or stream waters for irrigation, and domestic or drinking purposes (Mwanamoki et al., 2015; Rochelle-Newall et al., 2015; Kapembo et al., 2016). Most of the rivers, lakes, and lagoons in some regions of Sub-Saharan Africa are receiving untreated hospital and industrial effluent, mining effluents, and urban storm runoff affected by anthropogenic pollutants due to intensive and uncontrolled urbanization (Nola et al., 2013; Atibu et al., 2013; Mwanamoki et al., 2014; Laffite et al., 2016; Kilunga et al., 2016). In addition, diseases can also be transmitted during recreational activities and domestic activities such as bathing in polluted rivers, lakes, or seawater (Noble et al., 2003; Pachepsky and Shelton, 2011; Thevenon et al., 2012; Devarajan et al., 2015a). The estimated population of the Democratic Republic of the Congo (DRC) is 65.7 million inhabitants but despite the potential of its rich fresh water network, more than 75% of the population have no access to in-home piped water (UNEP, 2011) and has to resort to potentially contaminated rivers, streams, wells, and springs as an alternative water resource. These water sources are often polluted with micropollutants and pathogenic organisms which have the potential to significantly impact human health (Mubedi et al., 2013; Tshibanda et al., 2014; Mwanamoki et al., 2015; Kapembo et al., 2016; Laffite et al., 2016). The primary contact with these polluted water sources is by recreational activities, bathing, and drinking.

The city of Kikwit is the largest and capital city of the new province of Kwilu in the Democratic Republic of the Congo (DRC). This city is notorious for its recurrent outbreaks of disease including waterborne diseases and the 1995 Ebola epidemic of Viral Hemorrhagic Fever. The latter is one of the most well studied epidemics to have occurred in the Democratic Republic of the Congo to date (Hall et al., 2008). However, no research or programs have been carried out to assess the quality of water used in this city according to seasonal variations. Consequently, there is no known data quantifying the faecal indicator bacteria (FIB) in streams, rivers, wells, and other water sources in the city of Kikwit.

FIB are commonly used to assess the hygienic safety of drinking, recreational, and shellfish waters because the presence of FIB indicates the possible presence of pathogens of faecal origin while their absence suggests that pathogens are absent (Anderson et al., 2005; Baghel et al., 2005). However, in tropical conditions, other pathogens such as viruses and protozoa need to be considered (WHO, 2004). In addition, different bacterial groups are commonly used to monitor water quality; firstly, total coliforms (TC) of which faecal coliforms (FC) are a subset and *E.coli* is the most important species; secondly, enterococci (ENT); and thirdly, aerobic mesophilic bacteria (AMB, heterotrophic plant count (HPC)). *E. coli* and ENT are generally considered to have the greatest sanitary significance while TC and AMB can also originate from non-faecal sources (*e.g.* Noble et al., 2004; Evanson and Ambrose, 2006).

A previous study by Kapembo et al. (2016) of shallow wells in the municipality of Bumbu, a commune in the city of Kinshasa (capital and largest city in the DRC) found that the septic tank systems influenced the human contamination of shallow wells during both dry and wet seasons. The contamination of drinking water in wells and streams used for domestic purposes varies considerably from nation to nation depending on the source, aspects of hygiene, water-supply and sanitation systems as well as regulations and infrastructure (Nola et al., 2013; Rochelle-Newall et al., 2015; Kapembo et al., 2016). Therefore, further studies in developing countries to assess the water quality in terms of human health are strongly recommended (in rural areas in different regions).

As a consequence, the main aim of the present study is to assess the seasonal variation of the quality of river and well waters used by populations in the city of Kikwit in the province of Kwilu, DRC. The assessment is based on (i) the quantification of FIB including *E. coli*, ENT, and AMB, and (ii) characterisation of the FIB isolated using the molecular approach in order to identify the possible cause(s) of water contamination. Water samples were analysed in both the dry and wet seasons to identify any changes in water quality with the season. Molecular analysis was also carried out to identify potential human sources of faecal pollution in wells and rivers.

2. Materials and methods

2.1. Study site description

This research was conducted in the city of Kikwit in the province of Kwilu, a rural part of the Southwest of Democratic Republic of the Congo located 500 km west of the capital Kinshasa (Fig. 1). The city of Kikwit has an estimated population of 1,000.000 inhabitants (Mutungu, 2014) in a total area of 92 Km^2. Here *Entamoeba, Shigella*, cholera, and other diarrhoeal diseases are great threats to public health affecting nearly 30% of the population each year. Despite its potential rich water resources, the city of Kikwit has chronic shortages of safe drinking water, poor sanitation, and suffers from a lack of basic infrastructure (electricity, urban transport, *etc.*). The subject and the study area were selected based on the critical condition of recurrent unhealthy waterborne disease epidemics in the city of Kikwit. Most of the people use untreated water from streams, rivers, and wells (considered to be main sources in this study) to obtain water for domestic purposes.

2.2. Water sampling procedure

In order to establish the seasonal variations of bacteriological parameters present in the water, water samples were collected during the dry season in July 2015 and during the wet season in November 2015. They were taken from three main wells used as sources of drinking water in Kikwit and two important rivers, the River Lukemi and the River Luini (tributaries of the Kwilu River). These two rivers drain the city of Kikwit and are the main rivers used to obtain water for domestic purposes (Fig. 2). The well water samples were taken directly from outlet pipes and labelled P1–P3 (Fig. 3A–C). River water samples were collected manually at 10–50 cm water depth and about 1 m from the shore and labelled as (i) RLK1, RLK2, and RLK3 for the River Lukemi and (ii) RLN1 and RLN2 for the River Luini. The sampling points in rivers were selected according to frequent human access for domestic purposes (Fig. 4), and the GPS data for geographical coordinates locating the various sampling sites are presented in Table 1 and mapped in Fig. 2. Water Samples were collected in triplicate from each sampling site using sterile plastic bottles without disturbing the bottom sediments. The heaviest rainfall in Kikwit occurs during October and November when average rainfall is approximately 230–250 mm whereas on average July is the driest month in Kikwit with a maximum rainfall of 5 mm. Once taken, the samples were stored in an icebox at about 4 °C and transported to the laboratory for analysis within 48 h.

2.3. Water physicochemical parameters

Physicochemical parameters of water including temperature (T), pH, dissolved oxygen (O_2) and electrical conductivity (EC)