Physico-chemical and Bacteriological Quality of Boreholes and Evaluation of the Antibacterial Action of Disinfectants Used for the Microbiological Water Treatment

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors LUM and CMM designed the study and wrote the protocol. Authors HJMB and CMM wrote the first draft of the manuscript. Authors PTM and GBN managed the analyses of the study. Authors GNB and NM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Water pollution is currently at the top of the environmental agenda. In this study, the main objectives were (i) to assess the physicochemical and bacteriological quality of well and borehole water found in the vicinity of the University of Kinshasa and (ii) to assess the sensitivity of
1. INTRODUCTION

Water has always been inseparable from human activity. It is an essential resource for the continuation of life on planet earth and the palpable socio-economic development of a nation. However, in some countries of the South, the population does not have easy access to quality water resources. The water quality is one of the factors that hinder development in these countries [1]. M’nsari et al. [1] and Nouaytti et al. [2], reported that in the world, the pressure on water resources and particularly on groundwater resources is on the increase, mainly due to the growing demand due to the population explosion, and then, to agricultural and industrial development, urban expansion and so on.

In the Democratic Republic of the Congo (DRC), particularly in Kinshasa city, the access to water supplied by public distribution networks is limited, which justifies the use of well water (groundwater) in certain living environments for food, irrigation of agricultural land, animal husbandry and so on. The use of well water (groundwater from aquifers) in certain living environments for food, irrigation of agricultural land, animal husbandry, farming is also limited. Nevertheless, the food quality of aquifer water is a growing concern, as it is constantly facing the risk of converging natural and anthropogenic factors that lead to its degradation [1]. Thus, according to some authors, these waters represent a health hazard [3-5]. Chevalier [3] reported that aquifer waters are sometimes subjected to microbiological pollution and carry several diseases. Hence, the need to constantly monitor the physico-chemical or microbiological quality of these waters in order to prevent risks related to their use for food, agriculture, etc., and to ensure that they are safe for human consumption.

Furthermore, it should be recalled that *Escherichia coli* and enterococci are considered to be the preferred indicators of microbiological contamination of aquifer waters. However, some studies assert that, compared to *Escherichia coli*, enterococci are more resistant to harsh environmental conditions and persist longer in water; and such conditions are typical of groundwater, which is generally cooler and nutrient-poor [3,6,7]. The detection of enterococci in a groundwater sheet must raise a serious suspicion of faecal contamination and the presence of enteropathogenic microorganisms. Simmons et al. [8] reported some correlation between the presence of enterococci and faecal coliforms in untreated drinking water. Charrière et al. [9] demonstrated that the detection of enterococci was strongly associated with the presence of *E. coli* in groundwater distribution systems. Chevalier [3] added that there is an increased risk of developing gastroenteritis with a relatively small number of enterococci (3 to 10 bacteria /100 mL) and that groundwater in which enterococci have been identified should not be

**Escherichia coli and Enterococci** strains isolated from various selected water points to disinfectants used at the industrial scale (Chlorine and chlorine dioxide) or in the household (Aquatabs) for the microbiological treatment of water. Three sites having wells or boreholes were chosen for sample collection, namely: The University of Kinshasa and its vicinity (Kindele and Kemi districts). The Physico-chemical parameters were determined *in situ* and then in the laboratory; and concerned the following indicators: pH, conductivity and turbidity. Moreover, the bacteriological quality was assessed by detecting the presence of faecal pollution indicator bacteria (*E. coli* and *Enterococcus sp*). The susceptibility of *E. coli* and *Enterococcus sp* strains to antimicrobials generally used for the microbiological treatment of drinking water was assessed by the dilution method on agar medium. The physico-chemical analyses showed that: (i) acid pH values in the different water points under study ranging from 6.3-6.5, (ii) conductivity and turbidity values range between 28.5 and 146 NTU. The bacteriological analyses showed that the Kemi district was the only water point which exhibited contamination with fecal pollution indicator bacteria. The strains of *Enterococci* and *E. coli* isolated from that well were highly sensitive to chlorine dioxide and Aquatabs and less sensitive to chlorine while the strains of *E. coli* are highly sensitive to chlorine dioxide and Aquatabs compared to the strains of *Enterococci*. In conclusion, we can recommend to these populations, the use of these compounds for water treatment precisely Aquatabs for household.

**Keywords:** Water; borehole; well; treatment; disinfectants.
consumed. This explains our interest in evaluating the microbiological quality of our water samples based on two indicators of water pollution by faecal matter; namely: The simultaneous presence of *Enterococcus sp* and *E. coli*.

Moreover, the emergence of *E. coli* and enterococci strains resistant to antimicrobial agents and/or pathogens has been reported [10-13]. Diallo [11] asserted that some strains of *E. coli* are reported to be resistant to chlorine. This justifies our interest in evaluating the sensitivity of the bacterial strains mentioned above to disinfecting agents used at the industrial scale (chlorine, chlorine dioxide) or in a household (Aquatabs) for the microbiological treatment of water intended for human consumption. These analyses enabled us to assess the effectiveness of these antimicrobials for water treatment and to make recommendations to limit the health consequences of drinking water contaminated by strains of *E. coli* and enterococci resistant to these disinfectants (chlorine, chlorine dioxide and aquatabs) because they are potentially pathogenic.

2. MATERIALS AND METHODS

2.1 Water Sampling

2.1.1 Collection sites

Our various water samples were collected in some boreholes or wells found in the sites of the University of Kinshasa (UNIKIN) (borehole found behind the Polytechnic Faculty) or its surroundings, particularly in the Kindele and Kemi districts. The choice of the borehole found in the site of the UNIKIN, behind the polytechnic faculty, is justified by the fact that it constitutes a source of drinking water supply for the surrounding populations, notably students residing in Maluku village, and the inhabitants of the Kindele district in general. Also, the choice of wells in the Kindele and Kemi districts is justified by the fact that the rate of access to drinking water distributed by industrial caterers in these district are low and that in certain areas (notably the sites we investigated) the water from the aquifer is the only source of drinking water supply for household activities, animal watering, irrigation, etc. The Figure below shows our different study environments (Fig. 1).

![Fig. 1. Map indicating different study sites](image-url)
2.1.2 Method

Borehole and well water samples were collected in 250 mL sterile plastic vials and transported to the laboratory under isothermal conditions between 4°C and 6°C.

2.2 Determination of Physico-chemical Parameters

The analyses of the Physico-chemical quality concerned following parameters: pH, conductivity, turbidity and temperature. These parameters were measured using a multi-probe instrument (Hanna 98129). The idea was to immerse the electrode in water and then read the value for each specific parameter on the electronic display of the instrument after the values had been stabilized. The measurement of the various parameters mentioned above was carried out in situ and then in the laboratory.

2.3 Assessment of the Bacteriological Quality of Water Samples

The evaluation of the bacteriological quality concerned the identification of faecal pollution indicator bacteria, notably: E. coli and Enterococcus sp. It was carried out in the Environmental Monitoring and Molecular Biology Laboratory located at the Faculty of Sciences, Department of Biology, UNIKIN.

2.3.1 Preparation of culture media

2.3.1.1 Preparation of SBA medium (Slanetz Bartley Agar)

This medium was used for the isolation or culture of strains of Enterococcus sp. Prepare 20.75 g of the SBA medium powder which was diluted in 500 mL of distilled water; the solution was then boiled until completely dissolved.

2.3.1.2 Preparation of TBX (Tryptone Bile X-glucuronide medium)

For the preparation of the TBX medium that was used for the culture or isolation of E. coli, we first weighed 18.5 g of the power of this medium, which was diluted in 500 mL of distilled water. The solution was then boiled to accelerate dissolution and autoclaved at 121°C for 20 minutes.

2.3.2 Methodology

We performed successive decimal dilutions (10^0 and 10^-1) of the water samples collected upstream, which were then filtered from sterile filter membranes having a diameter of 47 mm and an average pore size of 0.45 μm; these membranes were then placed on the surface of the culture media (SBA: SlanetzBartley Agar or TBX: Tryptone Bile X-glucuronide medium) contained in petri dishes. These petri dishes were placed in an oven (WTB binder brand) at a temperature of 44°C for 24 hours (E. coli) or 48 hours (Enterococcus sp). After incubation, colonies of Enterococcus sp or E. coli were counted and the boxes kept in a refrigerator. Enterococcus sp strains were stained red while E. coli strains were stained blue.

2.4 Assessment of the Susceptibility of E. coli or Enterococcus sp Strains to Disinfectants Used for Microbiological Water Treatment

Three disinfectants were used namely: chlorine, chloramine dioxide and Aquatabs. Chlorine and chloramine dioxide are used for the microbiological treatment of water at the industrial scale; on the other hand, Aquatabs (sodium dichloroisocianurate) is used for the treatment of water at home. The agar dilution method was used to assess the sensitivity of E. coli and Enterococcus sp strains to the aforementioned disinfectants. This method consists of incorporating different concentrations of an antimicrobial to be tested into petri dishes containing an adequate culture medium, allowing the medium to cool and then inoculating the inoculum of the strains to be studied (in one or more parallel streaks, in a star pattern or by flooding). The individual plates are incubated in an oven at an appropriate temperature. After incubation, the Minimum Inhibitory Concentration (MIC) and/or Minimum Bactericidal Concentration (MBC) is determined by identifying the box containing the lowest concentration of the antimicrobial and on which no bacterial colony has been observed after 48 hours or 72 hours respectively [14].

2.4.1 Preparation of the bacterial suspension

A colony of Enterococcus sp or E. coli well isolated in a respective culture medium was diluted in saline water. The tubes containing the bacterial suspensions were shaken and let it for 30 to 60 minutes.

2.4.2 Preparation of different concentrations of chlorine dioxide and aquatabs and inoculation of inoculums

In fact, 120 mg of the chlorine or chloramine dioxide powders or aquatabs granules were diluted in 10
mL of distilled water to give a concentration of 12 mg/mL of each disinfectant. Dilutions ½ were then made to obtain the following concentrations: 6 mg/mL, 3 mg/mL, 1.5 mg/mL, 0.32 mg/mL, 0.16 mg/mL, 0.08 mg/mL, 0.04 mg/mL, 0.02 mg/mL, and 0.01 mg/mL. These were incorporated into the culture media (SBA or TBX) contained in petri dishes (15 mL/box). This allowed us to obtain petri dishes with the following final concentrations of the antimicrobials to be tested: 400 µg/mL, 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, 3.12 µg/mL and 1.56 µg/mL.

After solidification and cooling of the culture media contained in the petri dishes, 100 µL of the bacterial suspension was collected, inoculated and finally spread on the agar surface using a spreader. The various petri dishes containing the culture medium, the test extracts and the inoculum were finally incubated in an oven at 44°C. A petri dish containing no antimicrobial compounds was kept as a control.

3. RESULTS AND DISCUSSION

3.1 Determination of Physico Chemical Parameters

3.1.1 In situ determination

The findings of the physico-chemical quality of water analyses from the boreholes of the Polytechnic Faculty at UNIKIN or from the Kindele and Kemi districts are presented in the Table 1.

The Table 1 shows that the borehole or well water collected from the different sites has an acidic pH, ranging from 6.3 to 6.6 while the average is of 6.5 the International legal standards recommend a pH of between 6.5 and 8.5 for drinking water [15]. This indicates that the well or borehole water submitted for study is of good quality in terms of pH. According to the WHO, the conductivity values of drinking water must be between 400 and 1500 µS/cm [16]. Based on these standards, we estimate that the water from the boreholes surveyed meets the drinking water potability criteria concerning their conductivity: Kemi well (274.5 µS/cm), Kindele borehole (70.5 µS/cm) and UNIKIN borehole (40 µS/cm). In addition, the conductivity makes it possible to assess the number of dissolved salts (chlorides, sulphates, calcium, sodium, magnesium, etc.) in water [17]. Thus, by comparing the water conductivity values of the different water points surveyed, it is evident that the well water in the Kemi district has a high content of dissolved salts compared to that of the well in the Kindele district or the university site. Salghi, [17], Wanga et al. [18] and Sari [19] reported that water conductivity also varies according to the geological substratum crossed and about the physico-chemical load of the infiltration water or as a function of temperature.

In addition, the results of the physico-chemical analysis showed that the water from the Kemi well is more turbid (146 NTU), followed by that from the well in the Kindele district (36 NTU) and UNIKIN (28.5 NTU). These values are higher than those recommended by the WHO [16]. A high water turbidity predicts not only a high load of suspended matter (organic debris, clays, microscopic organisms, etc.), but also pathogens [18,20]. Suspended matter (especially organic compounds) traps microorganisms and promotes their proliferation [18]. It should also be noted that turbidity disrupts disinfection while ultraviolet treatment and chlorination is ineffective with increasing turbidity [20]. According to Sari [19], the measurement of turbidity is of great interest in the control of raw water treatment and the high turbidity of the water reveals the precipitation of iron, aluminum or manganese due to oxidation in the network.

| Origin and type of sample | pH   | Conductivity (µS) | Turbidity (ppm) | Temperature (°C) |
|--------------------------|------|------------------|----------------|-----------------|
| UNIKIN (Borehole)        | 6.5  | 40               | 28.5           | 29.8            |
| Kindele (Borehole)       | 6.3  | 70.5             | 36             | 30.1            |
| Kemi (Well)              | 6.6  | 274.5            | 146            | 29.5            |
| WHO Standards            | 6.5-8.5 | 400-1500      | <5             | -               |
As to Naima et al. [4], the chemistry of groundwater depends on several factors, such as the general geology (the nature of the terrain under which the groundwater is located), the degree of chemical weathering of various types of rock, the quality of recharge water and the various sources of recharge to aquifers. In addition, the findings showed that the temperature measured in the various water points under study ranged between 29.5 and 30.1°C. According to the literature, it is essential to know the temperature of a water because it plays a very important role in the solubility of salts (involved in electrical conductivity), and especially of gases, the determination of pH, the degradation of biomolecules and the growth of microorganisms in an aquatic environment [15,18-21]. However, Sari [19], added that high temperature promotes the growth of microorganisms and can enhance the taste, odor and color of water.

Moreover, a temperature below 10°C slows down the chemical reactions in different water treatments.

### 3.2 Determination of Physico-chemical Parameters in the Laboratory

Table 2 gives the physico-chemical of different samples collected and brought to the laboratory.

The Table 2 shows that the physico-chemical parameters measured in situ varied slightly during the transport of samples to the laboratory. The conditions under which the samples were transported and the influence of the laboratory temperature would be responsible for the variations in pH, conductivity and turbidity values measured in situ. In fact, the temperature influences most of the physico-chemical and biological parameters of water [2,18,20].

### 3.3 Evaluation of Microbiological Quality

The findings of the analysis of the microbiological quality of our samples are presented in Table 3.

The Table 3 shows that the water from the boreholes in the Kindele district and the water from UNIKIN is free of microorganisms that are indicators of faecal pollution (E. coli and Enterococcus sp). In addition, bacteriological analyses revealed the presence of E. coli and Enterococcus in the water sample collected from the well in the Kemi district. On the basis of these findings, we consider that the water from the boreholes in the Kindele district and UNIKIN is of good quality from a microbiological point of view. According to international guidelines for drinking water, bacteria indicative of faecal pollution should not be found in 100 ml of drinking water sample submitted for a bacteriological analysis [19,22]. The presence of E. coli and Enterococcus sp in a water sample predicts fecal contamination of water and implies the existence of enteropathogenic microorganisms [22]. In addition, we consider the well water in the Kemi district to be unfit for consumption, as it is contaminated with faecal pollution indicator microorganisms [5]. The presence of enteropathogenic germs in a well constitutes a significant health risk for the inhabitants who draw water from the well. It should be noted that generally groundwater is bacteriologically pure and is a better solution than surface water in terms of health. However, the microbiological quality of groundwater can be altered by several factors, including poor management of run-off water, infiltration of wastewater into the water table as a result of various interventions (drilling wells without precaution, opening gravel, lost wells), changes in agricultural practices (replacement of grassland by intensive crops) [19,23,24,21,25].

Sari [19] reported that during heavy rainfall, groundwater may become cloudy or even be polluted by a groundwater table in a nearby river. Furthermore, groundwater may also be polluted from the ground by pesticide application and wastewater discharges from animal or human origin. The physico-chemical or microbiological quality of a water sample in the aquifer provides information on the degree of protection of the groundwater from which the water to be analyzed comes or the self-purifying power of the soil.

### 3.4 Evaluation of the Sensitivity of E. coli and Enterococcus sp Strains to Compounds Used at the Industrial Scale (Chlorine, Chlorine Dioxide) or in the Household (Aquatabs) for Microbiological Water Treatment

The findings on the susceptibility assessment of E. coli and Enterococcus sp strains isolated from the Kemi well are presented in the Table 4.

The analysis of the Table 4 shows that strains of E. coli and Enterococcus sp isolated from the Kemi well are highly sensitive to chlorine
Table 2. Determination of physico-chemical parameters of boreholes and well waters found in the vicinity of UNIKIN

| Origin and type of sample | pH  | Conductivity (µS) | Turbidity (ppm) | Temperature (°C) |
|---------------------------|-----|------------------|-----------------|-----------------|
| UNIKIN (Borehole)         | 6.2 | 38.5             | 20.5            | 26.1            |
| Kindele (Borehole)        | 6   | 66               | 34.5            | 25.9            |
| Kemi (Well)               | 6.1 | 280              | 148.5           | 28.9            |
| WHO Standards             | 6.5-8.5 | 400-1500         | <5              | -               |

Table 3. Bacteriological analysis of water samples from boreholes and well locate in the vicinity of UNIKIN

| Origin and type of sample | CFU/100 mL | Isolated bacterial strains | E. coli | Enterococcus sp |
|---------------------------|------------|-----------------------------|---------|---------------|
|                           |            | Dilution                    |         |
|                           |            | 10^0                        | 10^-1   | 10^-2         | 10^-3         |
| UNIKIN (Borehole)         | 0          | 0                            | 0       | 0             |
| Kindele (Borehole)        | 0          | 0                            | 0       | 0             |
| Kemi (Well)               | 10. 10^-2  | 30. 10^-2                   | 10. 10^-2 | 30. 10^-2     |

Table 4. Susceptibility of Enterococcus sp and E. coli strains isolated from the Kemi well to compounds used industrially (chlorine, chlorine dioxide) or in the household (Aquatabs) for microbiological water treatment

| Sampling site | Isolated bacterial strain | Chlorine | Chlorine Dioxide | Aquatabs (dichloro-isocyanurate) |
|---------------|---------------------------|---------|-----------------|----------------------------------|
|               |                           | MIC (µg/mL) | MIC (µg/mL) | MIC (µg/mL) |
| Kemi (well)   | Enterococcus sp           | 400     | 200             | 200                              |
|               | Escherichia coli          | 400     | 100             | 100                              |

dioxide and Aquatabs compared to chlorine. The lower susceptibility of the E. coli and Enterococcus sp strains isolated from the Kemi well to chlorine would predict the acquisition of chlorine resistance genes by these strains. Avery et al. [26], Sommer et al. [27], Diallo [11] and Mateo [28] also reported that some strains of E. coli survived after treatment with chlorine. While Gauthier [29], asserted that chlorine dioxide was more effective on some bacterial strains (Bacillus subtilis, B. mesentericus, and B. megatherium) compared to chlorine. Also, since chlorine is used at very high concentrations during microbiological water treatment, we consider the concentrations of chlorine used during our bacteriological screening to be low. This could explain the fact that chlorine was not effective on the strains tested like other products subjected to bacteriological screening [30]. The Table 4 also shows that E. coli strains are very sensitive to chlorine dioxide and Aquatabs compared to Enterococcus sp strains that were less sensitive to these products. This is consistent with the work of WHO [7] and Chevalier [3], which reported that enterococci are resistant to disinfectants compared to strains of E. coli and can survive harsh environmental conditions.

4. CONCLUSION

This study focused on the physico-chemical and microbiological characteristics of water from boreholes and wells in the vicinity of UNIKIN.

The findings of the physicochemical parameters revealed that the pH and conductivity values of the water from the boreholes or wells under study met the recommended standards for drinking water. Although these waters showed turbidity values exceeding those recommended by the WHO for drinking water.

In addition, bacteriological analyses showed that only 1/3 of the wells surveyed were contaminated...
with strains of bacteria indicative of faecal pollution. The strains of Enterococcus sp and E. coli isolated from the Kemi well were highly sensitive to chlorine dioxide and Aquatabs and less sensitive to chlorine while the strains of E. coli were highly sensitive to chlorine dioxide and Aquatabs compared to those of Enterococcus sp. Though, these products are used to purify water, it should be noted that they are not fully protecting i.e. after treatment the water is not totally free of pathogens.

However, further long-term studies are needed to confirm the existence of strains of E. coli or Enterococcus sp that are resistant to chlorine, chlorine dioxide or Aquatabs and/or to characterize them genetically. We believe that proper waste management (solid or liquid) and regular monitoring of surveyed wells or boreholes could prevent microbiological contamination.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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