Evaluation of the Bacteriological Quality of the Drilling Water Analyzed at the National Health Laboratory during the First Half of 2019

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Abstract: *Nowadays, pollution problems are a growing danger for man and his environment. Groundwater, which is considered the most potable, is the most commonly used resource in developing countries. However, this water is very vulnerable to pollution and its protection is necessary at all levels. The objective of this study was to monitor the bacteriological quality of borehole water in certain localities of the country (Mali). The bacteriological analyses were carried out under a laminar flow hood, with deep seeding. The results of the study were assessed against the criteria defined by AMANORM. Of the 30 samples of drilling water analyzed, 13.33% were nonconforming and 86.67% conformed for Total Coliforms (TC), 23.33% of the samples were nonconforming and 76.67% conformed for Thermo-Tolerant Coliforms (TTC) parameters. For Mesophilic Aerobic Germs (MAG), 46.67% are non-compliant versus 53.33% compliance. For Fecal Streptococcus (FSS), 96.67% are compliant versus 3.33% non-compliant. During our study, the drilling water samples analyzed were globally compliant at 53.33%. The overall non-compliance rate is 46.67%. The good quality of drinking water ensures the good health of the population, which is a condition for the sustainable development of a nation.*

Keywords: Quality, bacteriological, Water, Drilling

1. Introduction

Water is a natural resource thanks to which life develops and sustains itself. Despite its abundance (covering 70% of the earth's surface), access to drinking water is a major public health problem [1]. According to the WHO, 1.8 billion people in the world used a source of water contaminated by fecal matter [2]. In 2010, 783 million people did not have access to safe drinking water [1]. The NGO Solidarity International reports that in 2018, one third of the world's population was drinking unsafe water and 2.6 million people would die each year from water-borne diseases. It estimates that by 2050, 40% of the world's population will face water shortages [3]. In Mali, as in most developing countries, water resource management is becoming increasingly problematic. In 2011, nearly half of the Malian population living in rural areas did not have access to safe drinking water [4]. The Minister in charge of Energy and Water has advanced in 2018, a rate of access to drinking water of 68% nationally [5]. The quality of the low drinking water coverage is threatened by open defecation. This is one of the main challenges of drinking water distribution in Mali [6]. When faecal matter contaminates water sources, especially groundwater, it leads to waterborne diseases such as cholera, typhoid fever, etc. and reinforces the problem of malnutrition. Diarrhea is one of the major waterborne diseases affecting children and the leading cause of child mortality [7]. Numerous studies have been undertaken to reduce the risks of water contamination in general and groundwater contamination in particular [5]. It is in this context that the advent of drilling, an expanding groundwater exploitation system in Mali to improve drinking water coverage, is taking place. However, borehole water is very vulnerable to pollution and still does not ensure good hygienic quality. Its protection is necessary at all levels. The only way to be sure of the quality of this water is to test it regularly [8]. This regular analysis is one of the missions of the National Health Laboratory (LNS). This is why during my internship at the LNS our study focused on "the evaluation of the bacteriological quality of the drilling water analyzed during the first semester". Indeed, the search for water quality indicator germs such as fecal coliforms and fecal streptococci must be permanent to prevent the consumption of water contaminated by pathogenic bacteria. The objective of this work was to monitor the bacteriological quality of borehole water in certain localities in Mali at the National Health Laboratory.

2. Equipment and Methods

2.1 Equipment

Nature of samples
These are drilling water samples

Taking samples
Drilling was done by buckling the end of the pipe, letting the water run for a period of time (two minutes). Sterile one liter vials were filled leaving an empty space at the edge. Some vials containing 10mg of sodium thiosulfate were used in case the water was treated with chlorine or derivatives. Sodium thiosulfate acts as a neutralizing agent for residual

chlorine that may be present in the water. It thus prevents the chlorine from acting between the time of sampling and the time of analysis and thus provides an accurate estimate of the number of microorganisms present in the water at the time of sampling. The samples were sent to the laboratory in a cooler at regular temperature. At the laboratory, the samples were recorded with a reference number. They were received and processed on the same day within a 12-hour time interval. After registration, a bench card was established for each sample containing the following information: assay number, reference number, sample name and parameters to be analyzed. After the analysis, the samples were stored in the refrigerator at 4 to 6°C until the end of the study.

2.2 Methods

2.2.1. Preparation of culture media
Culture media were prepared according to the manufacturer's protocol.

2.2.2. Analysis of the bacteriological parameters of the water
For the bacteriological analyses we carried out under the laminar flow hood a deep inoculation of the water samples. The workstation was thoroughly cleaned and the boxes were numbered. The different germs were searched for: mesophilic aerobic germs, total coliforms, total coliforms, and fecal coliforms or thermo-tolerant fecal streptococci.

2.2.3. Research and enumeration of Mesophilic Aerobic Germs (MAG)
This analysis was carried out according to the method NF EN ISO 4833-1.Using a sterile pipette, 1mL was taken from the sample which was transferred to the Petri dish center for testing. The PCA medium in undercooling was poured, about 15mL in each Petri dish, then well homogenized by rotational movements and left to solidify for 10 to 15min. After complete solidification of the medium, we poured 5mL of the supercooled white agar onto the surface of the inoculated medium as a second layer and allowed to cool for 10 minutes. The cultures were incubated at 37°C for 24 hours. The whitish colonies were counted using a counter equipped with a magnifying glass. The result of the count was expressed in Colony Forming Units (CFU/mL).

2.2.4. Investigation and enumeration of Total Coliforms (TC)
This analysis was performed using method 08-050. Using a sterile pipette, 1mL was taken from the sample which was transferred to the Petri dish center for testing. The supercooled LAV medium was poured, about 15mL in each petri dish, then well homogenized by rotational movements and allowed to solidify for 10-15min. After complete solidification of the medium, 5mL of the supercooled VRBL agar was poured on the surface of the inoculated medium as a second layer and allowed to cool for 10 minutes. The cultures were incubated at 30°C for 24 hours. Pink or reddish colonies on HBVLR media were counted using a colony counter equipped with a magnifying glass.

2.2.5. Thermo-Tolerant Coliforms (TTC) detection and enumeration
This analysis was performed according to method 08-051. Using a sterile pipette, 1mL was taken from the sample which was transferred to the Petri dish center for testing. The supercooled HVLRB medium was poured, about 15mL in each petri dish, then well homogenized by rotational movements and allowed to solidify for 10-15min. After complete solidification of the medium, 5mL of the supercooled VRBL agar was poured on the surface of the inoculated medium as a second layer and allowed to cool for 10 minutes. The cultures were incubated at 44°C for 24 hours. Pink or reddish colonies on HVBV media were also counted by the same technique. The enumeration result was expressed in Colony Forming Units (CFU/mL).

2.2.6. Fecal streptococcal testing
We took 1mL of the sample to be analyzed which we put in a tube containing 10mL of Rothe broth. The mixture was well homogenized using a vortex. The cultures were incubated at 37°C for 24 hours. The positive sample had a haze at the bottom of the tube. The positive sample was subjected to the confirmatory test on Litsky broth. For this purpose 1mL of positive Rothe broth culture (presumptive medium) was transferred to a tube containing 10mL of Litsky broth (confirmatory medium) and incubated at 37°C for 24-48 hours. A negative control of Litsky broth was used under the incubation conditions.

2.2.7. Data processing
The results data were processed and analyzed using Epi-info version 7 and SAS software. The results were assessed against the criteria defined by the Malian standard MN-03-02/011:2011(AMANORM) and WHO 2016.

3. Results and Interpretation

3.1 Results

Table I: Concentration of microorganisms in different samples

Samples	MAG (CFU/mL)	TC (CFU/mL)	TTC (CFU/mL)	Fecal Strept
E_1	0	0	0	-
E_2	200	6	2	-
E_3	200	0	0	-
E_4	150	14	6	-
E_5	0	0	0	-
E_6	150	20	10	+
E_7	150	0	0	-
E_8	150	20	21	-
E_9	22	5	2	-
E_{10}	40	9	0	-
E_{11}	3	0	0	-
E_{12}	200	0	0	-
E_{13}	45	0	0	-
E_{14}	200	36	25	-
E_{15}	0	0	0	-
E_{16}	65	0	0	-
E_{17}	0	0	0	-
E_{18}	80	0	0	-
E_{19}	200	0	0	-
E_{20}	84	0	0	-
E_{21}	0	0	0	-

E_{22}	200	0	0	-
E_{23}	200	0	0	-
E_{24}	200	0	0	-
E_{25}	200	0	0	-
E_{26}	0	0	0	-
E_{27}	0	0	0	-
E_{28}	10	0	0	-
E29	47	0	0	-
E_{30}	0	0	0	-
Malian standard	≤100 CFU	≤10 CFU	0 PDUS	Absence

Table II: Analyses of variance for germ contamination of samples according to different parameters

Visit	DDL	MAG	TC	TTC
		Medium square	Medium square	Medium square
Samples	29	2.76***	0.69***	0.46***

***, significant at $P < 0.05$ and $P < 0.01$, respectively.

3.2 Interpretation

The test of variance on the analysis of the microbiological parameters of the different samples shows that there is a significant difference between the different samples and between the different parameters measured.

From Table I, we can see that the number of Total Coliforms varies from 0 to 36

The maximum value is observed at the level of E14. All these values are below the standard (10UFC/100mL) except for four samples (E_4, E_6, E_8, and E_{14}. As for Thermo-Tolerant Coliforms, they range from 0 to 25 CFU. The maximum value is observed in sample 14 (E_{14}). 7 samples out of 30 do not comply with the standards (0 CFU/100mL). In general, the presence of coliforms in water causes diarrheal problems in humans. The count of Mesophilic Aerobic Germs shows us that 16 samples out of 30 comply with the standards (100 CFU/1mL). The number of AMGs varies between 0 and 200 CFU. Also this table shows the presence of Fecal Streptococci on a single sample (E_6).

The overall compliance rate of the samples is 53.33% or 16/30 and 46.67% or 14/30 of non-compliance.

For Total Coliforms:
Out of 30 samples, 26 samples are in conformity with the standards, i.e. 86.67% and 4 samples are non-compliant, i.e. 13.33%. We can say that the 4 samples were contaminated by various sources (stored waste, latrines and septic tanks) likely to cause pollution in the surroundings of the drill holes.

For Thermo-Tolerant Coliforms:
23 samples out of 30 comply with the TTC criterion, i.e. 76.67% and 7 samples do not comply with the TTC criterion, i.e. 23.33%. We conclude that the non-compliance of the 7 samples may result in the presence of fecal waste and septic tanks in the vicinity of these drill holes.

For Mesophilic Aerobic Germs:
Out of 30 samples, 16 samples comply with the AMG standards, i.e. 53.33% against 14 non-compliant samples, i.e. 46.67%. The presence of MAG can be explained by the fact that this parameter includes all the bacteria in the environment. It is therefore a natural presence of bacteria in groundwater.

For faecal Streptococci:
29 samples, i.e. 96.67% are in conformity with the standards against 3.33% nonconforming. This only non-compliant sample was contaminated either by animals, latrines and septic tanks (fecal pollution of human origin) in the vicinity of these boreholes.
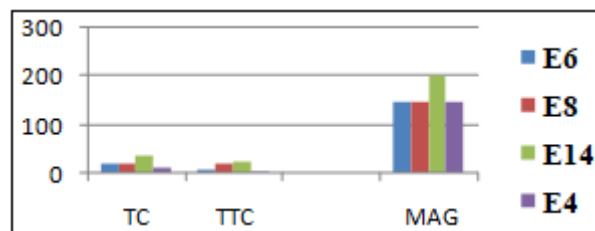
Figure 1: Non-compliance rate of samples

4. Conclusion

Borehole water is an important source of water supply in Mali. It is used for various purposes, especially for food. Contamination of these waters by bacteria of fecal origin constitutes a major risk of gastroenteritis for consumers. The causes are mainly the lack of sanitation and hygiene in families. Our study revealed a non-compliance rate of 46.67% of the bacteriological quality of the analyzed drilling water.

Of the 30 samples, 14 contained MAG, 4 TC, 7 TTC and 1 faecal Streptococcus. The poor bacteriological quality of these waters is linked to the presence of germs indicative of fecal contamination. This presence indicates the existence of pathogenic bacteria in the environment. However, the absence of coliforrms is not synonymous with the absence of other bacteria. Bacteria are present naturally and in large numbers in our environment: air, water, soil... They are not systematically pathogenic. They have the property to develop very quickly, if the environment is not too polluted.

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