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Assessment of the Coliform Bacterial Load of Some Drinking Water Sources in Dutse Metropolis of Jigawa State Nigeria

Ibrahim Khalil Abubakar (Corresponding Author)
Department of Biological Sciences, Faculty of Science, Federal University Dutse, Jigawa State, Nigeria
Email: ai4khalil@gmail.com

Mustapha Mohammed Abubakar
Department of Biological Sciences, Faculty of Science, Federal University Dutse, Jigawa State, Nigeria

Muhammad M. Abubakar
Faculty of Science, Federal University Dutse, Jigawa State, Nigeria

Abstract

Drinking water samples from 5 sachet water companies, 3 boreholes and 2 taps, collected from different locations of Dutse Metropolis of Jigawa State, Nigeria were analysed for coliform bacterial counts using the Membrane Filtration Technique. All the samples contained some amounts of total coliforms, but mostly within permissible levels. Thirty three percent (33%) of the samples from borehole, 60% from sachet water and 100% from the taps contained faecal coliforms, which indicates contamination. Cultures of the faecal coliforms obtained were morphologically identified using the gram-staining procedure and some series of biochemical tests were carried out in order to identify the organisms. The identified organisms were *Escherichia coli* (*E. coli*), *Klebsiella sp.* and *Citrobacter sp*. Presence of coliforms above the regulatory set standards indicates contamination and un-safeness of the water for drinking. Presence of organisms such as *E. coli*, *Klebsiella sp.* and *Citrobacter sp.* necessitates improvement in monitoring and water hygiene practices to improve the quality of drinking water in the study area.
Keywords: Membrane filtration; Total coliforms; Faecal coliform; Contamination; *E. coli*; *Klebsiella sp.*; *Citrobacter sp.*

1. Introduction

Water, one of the most important and most abundant compounds on earth, is vital to the survival of any organism. It is an essential nutrient that is involved in all basic physiological functions of the body [1]. Safe drinking water, which is essential for the survival of humans and animals, is unavailable to about one billion of the world's people living in poverty [2]. The World Health Organization highlighted at least seventeen different genera of bacteria that may be found in drinking water sources which can cause disease in man [3] and animals [4]. Water resources, used for domestic, industrial and agricultural activities, have been the most exploited natural system since the world began [5]. Highly producing animals require the provision of a large amount of clean, fresh water [6]. Water is a nutrient of extreme importance for animals and is intimately involved in a wide array of bodily functions. It serves as the universal solvent in the extracellular and intracellular compartments, as 99% of all molecules in the body are carried in it [7].

Water related diseases continue to be one of the major health issues in Nigeria where the level of sanitation is very low, and lack of awareness of the implication of consuming contaminated water is high, which lead to high rate of reported cases of water borne diseases. Contamination of water has been a major source of waterborne diseases especially in the developing and underdeveloped countries. The rate of water borne diseases across Nigeria's 206 million people [8] is increasing yearly. In Dutse, the capital of Jigawa State, Nigeria, there have been reported cases of water related disease in recent years. The need to assess the quality of drinking water, therefore, becomes very imperative.

The main aim of the current research was to assess the coliform bacterial load of some drinking water sources in Dutse Metropolis of Jigawa State, Nigeria. The specific objectives included to:
  a. Ascertain the quality of drinking water by microbiological analysis.
  b. Determine the load of faecal and total coliforms in different water sources.
  c. Identify the coliforms present in the drinking water sources.
  d. Compare the assessed coliforms with set regulatory standards.

2. Material and Method
2.1. Sample Site Selection and Sample Collection

Drinking water samples from boreholes and taps were collected at three different locations (Yalwawa, Gida-Dubu and Garu) of Dutse Metropolis (latitude 11^0 45'N, $9^0$20'E at 444 meters above sea level) of Jigawa State,

Nigeria. Samples from popular sachet water companies were also collected for the analysis. A total of ten samples were collected for this work as follows: five from different sachet water companies, three from borehole pumps and two from taps. Water samples were collected using sterile bottles of at least 200ml capacity. In order to prevent contamination, the mouth of the taps and borehole pipes were flamed and the water allowed to run for some seconds before being allowed to fall into the sample bottles. The bottles were held at the base, to avoid contact with hands. All samples were labeled with the details of collection and were transported to the laboratory in an insulated cool bar for analysis.

2.2. Processing of Samples

All the materials for this analysis were kept clean and sterile. The media used was Membrane Lauryl Sulphate Broth (MLSB) and was prepared according to the manufacturer's specification.

2.3. Membrane Lauryl Sulphate Broth Media Preparation [9]

i. 1.9 gram of MLSB powder was dissolved in 25 ml distilled water in a beaker. The mixture was heated, but not boiled, to ensure the powder was fully dissolved.
ii. The medium was then poured into 50 ml plastic bottles and the bottle tops left slightly loose (not tightened).
iii. The bottles containing the media were sterilized upright in an autoclave at $121^{o}C$ for ten minutes and then removed, their tops tightened and allowed to cool to room temperature.
iv. After the media had cooled, about 2 ml were then poured onto each membrane pad sufficiently, ensuring the pad was fully saturated.

2.4. Membrane Filtration Technique [9]

Growth pads were dispensed into different sterile petri-dishes using a sterile pad dispenser, and saturated with MLSB. Using sterilized forceps, the membrane filter was placed onto the bronze membrane support, with the grid side up. The forceps were sterilized using flame. The first water sample was poured into the filter funnel up to the 100 ml graduation and hand pump was applied to pass the water through the membrane. The sterile forceps were used to take the membrane out of the filtration unit after the water was filtered. The membrane was then placed on top of the pad which has been saturated with the MLSB media. The filtration was done for a second time, and was placed onto another MLSB media-saturated pad. The petri-dish lids were then placed back and labeled with sample number, place, date and time. The petri-dishes were then placed into two different petri-dish racks. This procedure was repeated for all the remaining samples. The filled racks were then placed into the incubator (the incubator provides places for inserting the two racks). $44^{o}C$ was set on the first rack for faecal coliform incubation, and $37^{o}C$ on the second rack for total coliform. The period of incubation for all the samples was 17 hours. After this period, the incubations set at $44^{o}C$ and $37^{o}C$ were all noted and each petri-dish from an incubator rack was removed and temperatures setting were recorded. The petri dishes were then placed on a flat surface for counting of the coliforms. The lids were then removed and all the yellow colonies, irrespective of size were counted within few minutes using hand lens.

2.5. Identification of the Coliforms
2.5.1. Gram Staining

The inoculum was taken from the culture using a sterile inoculation (wire) loop and placed on a sterile slide (slide smear). Gentian Violet was poured on the smear, allowed to stay for 30 seconds, and then washed gently with distilled water. Lugol's iodine was applied and allowed for 60 seconds then rinsed off. Ethanol was then poured on the slide, which was held at an angle, for about 15 seconds until no more violet colour was visible in the draining runoff. Neutral Red was applied and then washed off after 2 minutes. The slide was shaken and carefully rinsed to remove the excess water and air-dried. The dried slide was placed under the microscope and observed under X100 magnification [10].

2.5.2. Biochemical Tests

The following biochemical tests were done in order to identify the organisms after the gram staining. All the reagents used were prepared according to the manufacturer's specifications. All biochemical tests outlined herewith were carried out as specified by MacFaddin [11].

2.5.3. Indole Test

The isolates were grown in 5ml of nutrient broth at $37^{o}C$ for 24 hours. After the incubation, 3 drops of Kovac's indole reagent were added and shaken gently.

2.5.4. Citrate Test

The isolate was inoculated into Simmons' citrate agar slant in a bijou bottle and incubated at $37^{o}C$ for 24 hours.

2.5.5. Triple Sugar Iron (TSI) Test

The isolates were inoculated into 6 TSI agar slants by stabbing through the center of the medium to the bottom of the tube and streaking the surface of the agar slant. They were incubated at $37^{o}C$ for 24 hours.

2.5.6. Urease Test

Urea tube was inoculated with 3 loops-full of slant culture and incubated at 37°C for 24 hours, and then observed for the reaction.

2.5.7. Lysine Decarboxylase (LDC) Test

The isolates were inoculated into a sterile tube of lysine decarboxylase broth and incubated at 37°C for 24 hours.

3. Results

3.1. Number of Coliforms Present in Borehole Tap and Sachet Water Samples

Table 1 shows the number of coliforms present in borehole, tap and sachet water samples, each compared with the limits set by NIS [12].

Table-1. Number of Coliforms Present in Borehole, Tap and Sachet Water Samples

Location	Total Coliform (cfu/ml)	Faecal Coliform (cfu/ml)
Borehole		
Yalwawa	4	0
Gida-Dubu	7	2
Garu	5	0
Tap		
Yalwawa	11	3
Garu	8	2
Sachet		
Sample 1	4	1
Sample 2	12	4
Sample 3	7	1
Sample 4	4	0
Sample 5	2	0
Maximum Permitted Levels	10	0

All samples from the three boreholes, two taps and five sachets contain some total coliforms, although mostly within the maximum limit, but Yalwawa tap and sachet sample 2 exceeded the limit. Faecal coliforms for borehole samples from Gida-Dubu, tap samples from both Yalwawa and Garu and sachet samples 1, 2 and 3 exceeded the limits.

3.2. Identification of Isolates

Table 2 shows the results of the biochemical tests performed on the inoculums of the faecal coliforms.

Table-2. Results of the Biochemical Tests Performed on Inoculums of the Faecal Coliforms

Location	Indole	Citrate	Urease	TSI	LDC	Identified Organism
Borehole						
Gida-Dubu						
1st Inoculum	+	-	-	-		*Escherichia coli*
2nd Inoculum	-	+	+	-		*Klebsiella sp.*
Tap						
Yalwawa						
1st Inoculum	+	-	-	-		*Escherichia coli*
2nd Inoculum	-	-	-	+	-	*Citrobacter sp.*
3rdInnoculum	+	-	-	-		*Escherichia coli*
Garu						
1st Inoculum	+	-	-	-		*Escherichia coli*
2nd Inoculum	+	-	-	-		*Escherichia coli*
Sachet						
Sample 1						
Inoculum	+	-	-	-		*Escherichia coli*
Sample 2						
1st Inoculum	-	+	+	-		*Klebsiella sp.*
2nd Inoculum	+	-	-	-		*Escherichia coli*
3rd Inoculum	-	-	-	+	+	*Escherichia coli*
4th Inoculum	+	-	-	-		*Escherichia coli*
Sample 3						
Inoculum	-	-	-	+	-	*Citrobacter sp.*

4. Discussion

Most of the water samples from the boreholes, taps and sachets in the study area contained some amounts of coliforms. The identified organisms were *Escherichia coli* (*E. coli*), *Klebsiella sp.* and *Citrobacter sp*. Presence of *E. coli* in drinking water indicates that the water has been contaminated with human or animal faeces and can lead to gastroenteritis with symptoms like diarrhea [13]. *K. pneumonia*e is one of the human pathogens that present serious risk of disease whenever present in drinking water. It has been identified as important common pathogen for nosocomial pneumonia, septicaemia, and urinary tract infection, wound infections, intensive care unit (ICU) infections, and neonatal septicaemias [14]. The most important cause of *Klebsiella* spp. contamination of drinking water is the leakage of animal faeces into drinking water sources [15]. *Citrobacter freundii* is responsible for a number of significant opportunistic infections. It causes a variety of nosocomial infections of the respiratory tract, urinary tract, blood and several other normally sterile sites in patients [16].

Total coliform counts for borehole samples in all three locations were within the limits set by the World Health Organization. Borehole samples from Gida-Dubu had 2 faecal coliforms while those from Garu and Yalwawa had none. The organisms identified in samples that contained faecal coliforms were *E. coli* and *Klebsiella sp*. These organisms have also been identified in water from boreholes, both within and outside Nigeria [14, 17-19]. All tap water samples had faecal coliform counts above the set regulatory standards. This is similar to the findings of [20] for tap water in Enugu State, Nigeria and Abubakar and Abubakar [21] for wells and tap water in Bauchi Metropolis, Nigeria. In the current study, the organisms identified in the tap water samples were *Citrobacter sp*. and *E. coli*. These organisms have been reported by Shanker and Pindi [22] in various drinking water sources, by Staradumskyte and Paulauskas [23] in tap water and by Mbah and Muhammed [24] in sachet water and tap water samples. In the current study, 60% of the sachet water samples had faecal coliforms. None of the sachet water producers complied with the labeling requirement of the WHO [25] for drinking water of indicating manufacturing date, expiring date and batch number on the sachet. Oyedeji*, et al.* [26], reported that all brands of sachet water had total coliforms and 20% of the samples had faecal coliforms. The organisms identified in the sachet water samples of the current study were *E. coli*, *Klebsiella sp.* and *Citrobacter sp*. These organisms were also identified by Oluyege*, et al.* [27] in Ado-Ekiti, Nigeria.

Contamination of sachet water can occur: i) when packaging materials are not sterilized, ii) due to poor hygiene, iii) from carrier handlers or iv) from the environment in which the packaged water was produced. Environments such as kitchen, boy's quarters and motor garage used by some sachet water producers as their factories can contribute to contamination of the packaged water. Many Nigerians, who have resorted to drinking sachets water, since they are under the assumption that such products are "pure" and safe, may be affected. Potentially pathogenic microorganisms of faecal origin such as *E. coli* may find favourable condition and proliferate in drinking water distribution systems. The detection of these indicator organisms especially *E. coli* in water signifies a recent pollution because this organism cannot survive for long outside its natural habitat which is intestinal tract of animals [28].

5. Conclusion

The current work revealed that most of the drinking water in the study area did not meet the set standards as the coliform bacterial loads were above the limits set by the Nigerian Industrial Standard. None of the sachet water producers complied with the labeling requirement of the World Health Organization for drinking water as they did not indicate manufacturing date, expiring date and batch number on the sachets. All tap water samples had faecal coliforms, which could be as a result of pipe corrosions, damages or illegal connection. One of the boreholes, out of the three examined, had coliform counts above the standards. Samples from the remaining two boreholes meet the required standard limits and were considered fit for human consumption. In order to safeguard the health of the public, safe and good drinking water must be ensured for all. This can be achieved by i). regular monitoring of the water quality for improvement, prevention of disease and hazards by ensuring that the water resource do not get further polluted, ii. National Agency for Food and Drug Administration and Control (NAFDAC) intensifying efforts in monitoring the activities of producers and vendors of packaged drinking water, iii) NAFDAC ensuring that all sachet water contains both manufacturing and expiry dates as well as batch number for easy recall, iv) Periodic replacement of the old pipelines as well as corroded borehole pipes with new ones and v) Giving public lectures in order to enlighten the public on health hazards associated with contaminated drinking water.

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