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Bacteriological Quality Analysis of Water in Storage Tanks in A University Community, South Southern - Nigeria

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Abstract
The use of unsafe water supplies and microbial contaminated water may pose serious health challenge to users. The aim of the study was to determine the level of bacterial contamination of the various water sources and the suitability for human use and consumption in University of Calabar Community, Nigeria. A total of 30 water samples were obtained from the University storage tanks and analysed for the presence of bacteria. The level of feacal coliform count, total coliform count and heterotrophic bacterial count was analysed using membrane filtration method and standard culture method on a differential and selective media. The samples were cultured on MacConkey and Nutrient agar. The isolates obtained from the above media were subcultured into slants of nutrient agar. Isolates were subjected to Gram staining and biochemical tests. The feacal coliform, *Escherichia coli* was isolated from all the water samples. Total coliform counts ranged from 1cfu/ml - 92cfu/ml while total heterotrophic bacterial count ranged from 1cfu/ml to 161cfu/ml. The bacteria species isolated were *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus* species*, Enterobacter aerogenes,* Coagulase negative *Staphylococcus, Proteus* species, *Lactobacillus* species and *Listeria* species. The results obtained from this study have shown high level of bacterial load which makes the water unsuitable for human consumption. Further treatment may be needed as the bacteria isolates from the water samples point to feacal contamination which may be due to inadequate treatment of water, contamination while in the storage tanks or passage through contaminated pipes supplying the community. The consumers may be at high risks of enteric bacterial infections.

Key words: Water, Bacteriological quality, storage tanks, Community

Introduction

Water is a transparent, tasteless, odourless and colourless mobile liquid except in large volumes where it appears blue. It freezes at 0°C (32°F) and boils at 100°C (212°F). Water is a basic necessity of life. In its absence higher animals survive only a few hours or days. It is essential to sustain life as it is used for several purposes including: washing, cooking, food processing, swimming among others. Therefore, portable water supply should be made available to consumers (WHO, 2004). The main sources of water are: rain, wells, streams, springs, ponds, oceans etc.

On a sanitary point of view for domestic water usage, water can be classified as polluted and unpolluted water (Twort *et al*., 2000). Portable water is colourless, odourless, tasteless and free from poisonous corroding and staining substances as well as diseases causing organisms (Diersing, 2009). The pollution of tap water could originate from contamination from water pipes and storage tanks, biological agents from human faeces especially bacterial pathogens. The coliform bacteria have always been the principal concern in setting health-based targets for microbiological quality of drinking water (Nwinyi *et al*., 2020). Common indicator bacteria are; coliforms example, *Escherichia coli, Streptococcus faecalis* and *Clostridium perfringens.* These bacteria are excreted in large

numbers by man and animals. Their presence in water indicates: the contamination of water by faecal matter, inability to kill or remove the organisms during purification or treatment (Ochei and Kolhatkar, 2007).

The World Health Organization (WHO) and National Agency for Food, Drug and Control (NAFDAC) Standard for portable water are: that in 100ml of water, coliform bacteria should be absent, and the total plate count for bacteria should be 100cfu/ml of water (United Nations, 2018). The quality of water should not be overlooked since contaminated water can cause health hazards when consumed.

Materials and methods

The study was an experimental study carried out in the University of Calabar community. University of Calabar is situated in the South South geopolitical region of Nigeria. The University has undergone impressive growth, with the creation of numerous Faculties and Post Graduate School. Her motto is "Knowledge for Service". A total of 30 samples of water were collected from the taps of the storage tanks in the following sites: Hostels, College of Medical Sciences, Main campus, Vice Chancellor Administrative Block, Faculty of Science and Staff Quarters. Simple random sampling technique was used for the water sample collection. The water samples were collected in a sterile 100ml screw cap container. The sample was collected after sterilizing the mouth of each tap using cotton wool soak in 70% (v/v) alcohol (American Public Health Association, 2012). After collection, the water sample was transported to the laboratory in an ice box for bacteriological analysis within 1 hour of sample collection (Ochei & Kolhatkar, 2007). Table 1 show the various study sites and the number of tanks sampled. The water sample was aseptically filtered under vacuum through cellulose acetate membrane filter with diameter of 47mm and pore size of 0.45m. After filtration, a sterile flat edge forceps was used in carrying the membrane with the particles retained on the surface of the membrane. The membrane was placed with its grid side upward on a nutrient agar Plates or MacConkey agar plates and allowed to stand for 1hour, incubated for 24-48hrs at 44^0C for feacal coliform colonies (Plate 1).

Table 1: Location of water source and number of tanks sampled

Source of water sample	No. of tanks sampled
Hall 2	2
Hall 4	3
Hall 5	4
Hall 8	4
Hall 9	3
College of Med. Sci.	2
VC Admin. Block	2
Main Campus	4
Faculty of Science	2
Staff Quarters	4
Total	30

Culture

The Standard culture method for water samples using undiluted water sample was performed with 1ml of the water sample. This was inoculated on MacConkey agar medium. The plate was rotated clockwise and anticlockwise several times for the sample to mix properly and kept on the bench for 1 hour for the sample to settle. Plates incubation was at 44^0C for 24-48 hours. The Standard culture method was also used for the inoculation of the diluted water sample. This was carried out with the water samples diluted 1:10 and 1:100 respectively. One ml each of 1:10, 1:100 diluted samples and the undiluted water was inoculated on nutrient agar medium, incubated at 37^0C for 24 hours for total coliform colonies and at 22^0C for 72 hours for heterotrophic colonies. The enumeration of

colonies was done using the colony counter. Isolates were sub cultured on nutrient agar slants for further identification. Isolates were identified using Gram's staining method, coagulase, catalase, oxidase, urease and indole test methods (Ochei and Kolhatkar, 2007).

Data analysis

Data obtained from the study was analyzed using Epi Info 2010 (CDC, Atlanta, Georgia, USA) Statistical Software. Descriptive statistics was carried out. Frequency was calculated for categorical variables. Interaction between specific categorical variables was tested for significance using Chi square test. A p value of $\leqslant 0.05$ was considered statistically significant.

Results

The study was designed to provide information on microbial contamination of water in storage tanks in University of Calabar Community. A total of 30 water samples were obtained and analyzed. All the samples showed presence of microbes with a total number of 8 genera of bacteria obtained from filtered, undiluted and diluted water samples. Table 2 shows the feacal coliforms count using membrane filtration technique and standard culture method, total coliforms colony count and total heterotrophic bacterial colonies count from the samples. Hall 5 water tanks had the highest bacterial load (1.09cfu/100ml) followed by main campus water tanks (0.96cfu/100ml) while Hall 4 water tanks had the lowest bacterial load (0.45cfu/100ml). Hall 5 water tank had the highest feacal coliforms count by standard culture method on MacConkey agar (86cfu/ml) followed by Main campus tanks (83cfu/ml) while VC's administration Block tanks had the lowest bacterial load (28cfu/ml) (Table 2). Hall 4 had the highest total coliform count in standard culture method on nutrient agar 92cfu/ml while Hall 9 had the lowest total coliform count (60cfu/ml) (Table 2). The highest total heterotrophic bacterial count on nutrient agar was observed in main campus tanks (161cfu/ml) while staff quarter had the lowest bacterial load (86cfu/ml) (Table 2). Table 3 shows the feacal coliform count by membrane filtration technique on MacConkey agar. *Escherichia coli* was the most encountered feacal coliform bacteria (0.48cfu/ml) in Hall 5 and Faculty of Science respectively but least encountered (14cfu/ml) in College of Medical Science. In the standard culture method, *Escherichia coli* was the most encountered (37cfu/ml) feacal coliform bacteria in Hall 5 while VC Admin block had the lowest bacterial load.

Table 5 shows the total coliform count by standard culture method. *Escherichia coli* was the most encountered (30cfu/ml) in Faculty of Science water tank while *Klebsiella pneumonia* had the lowest bacterial load (3cfu/ml) in main campus. Table 6 shows that *Staphylococcus aureus* was the most encountered total heterotrophic bacterial isolates in main camp with the highest bacterial load while *Listeria* species was the lowest bacterial isolate (0cfu/ml) in hall 4, College of Medical Science and Faculty of Science with lowest bacterial load.

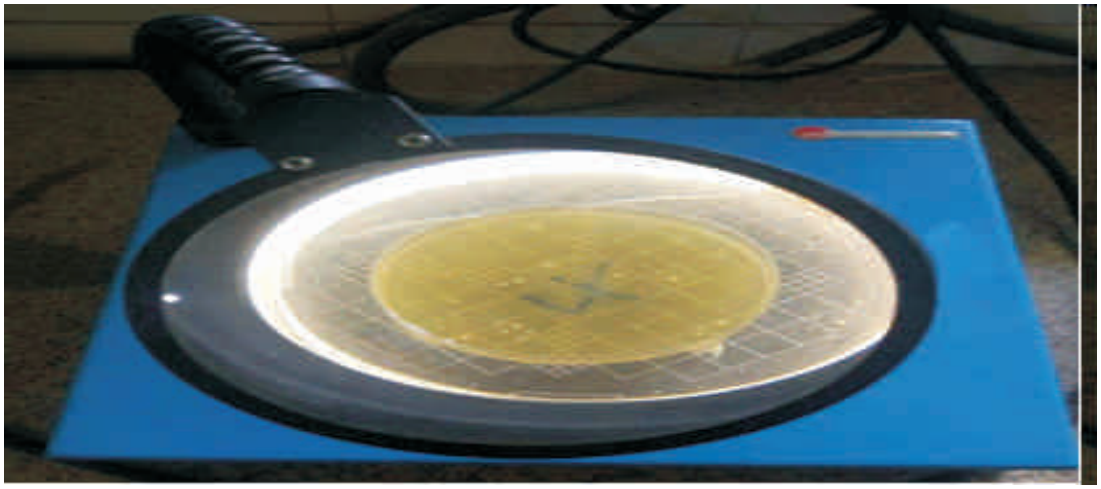

Plate 1: Colony count on nutrient agar Plate using colony counter

Table 2 Microbiological characteristics of University of Calabar Water Sources

Source of water	No. examined	Feacal coliform count		Total coliform count on nutrient agar (cfu/ml)	Total heterotrophic count on nutrient agar (cfu/ml)
		Membrane filtration technique on MacConkey agar (cfu/100ml)	Standard culture method on MacConkey agar (cfu/ml)		
Hall 2	2	0.51	36	78	118
Hall 4	3	0.45	48	92	108
Hall 5	4	1.09	86	69	115
Hall 8	4	0.88	70	75	127
Hall 9	3	0.67	32	60	101
College of Medical Sciences	2	0.60	58	85	97
VC Admin. Block	2	0.51	28	63	94
Main Campus	4	0.96	83	76	161
Faculty of Science	2	0.77	48	87	131
Staff Quarter	4	0.85	66	79	86
WHO standard		0cfu/100ml	0cfu/ml	0cfu/ml	100cfu/ml
EPA standard		0cfu/100ml	0cfu/ml	1-5cfu/ml	100cfu/ml

Key:
WHO - World Health Organization
EPA - Environmental Protection Agency

Table 3 Feacal coliform colony counts obtained by membrane filtration technique

Source of water	*Escherichia coli* colony count (cfu/ml)	*Streptococcus faecalis* colony Count (cfu/ml)	*Klebsiella Pneumoniae* colony Count (cfu/ml)
Hall 2	18	24	9
Hall 4	21	21	3
Hall 5	48	23	38
Hall 8	39	22	27
Hall 9	31	23	13
College of Medical Sciences	14	31	15
VC Admin. Block	28	5	18
Main Campus	43	28	25
Faculty of Science	48	10	19
Staff Quarter	37	26	22

Table 4 Feacal coliform count in standard culture method

Source of water	*E. coli* colony count (cfu/ml)	*S. feacalis* colony Count (cfu/ml)	*K. pneumonia* colony count (cfu/ml)
Hall 2	13	05	18
Hall 4	25	14	9
Hall 5	37	19	30
Hall 8	26	24	18
Hall 9	11	18	13
College of Medical Sciences	21	15	22
VC Admin. Block	9	3	16
Main Campus	32	23	28
Faculty of Science	16	4	28
Staff Quarter	29	13	24

Table 5 Total coliform bacteria colonies obtained using standard culture method from nutrient agar in 1ml of water sample

Source of water	*E. coli* (cfu/ml)	*P. aeroginosa* (cfu/ml)	*K. pneumonia* (cfu/ml)	*S. feacalis* (cfu/ml)	*Enterobacter* (cfu/ml)
Hall 2	28	16	14	10	10
Hall 4	26	14	19	17	16
Hall 5	18	12	8	13	18
Hall 8	23	18	13	9	12
Hall 9	18	13	9	14	6
College of Medical Sciences	26	16	8	22	13
VC Admin. Block	20	14	8	4	17
Main Campus	21	21	3	20	11
Faculty of Science	30	8	19	11	19
Staff Quarter	24	19	13	9	14

Key
E –Escherichia
P – Pseudomonas
S- Streptococcus

Table 6 Total heterotrophic bacteria colonies obtained using standard culture method from nutrient agar medium in diluted water sample

Source of water	*S. aureus* (cfu/ml)	*Lactobacills* (cfu/ml)	*Streptococcus* species (cfu/ml)	*P. aeruginosa* (cfu/ml)	*L. monocytoges* (cfu/ml)	*Enterobacter* (cfu/ml)	*E. Coli* (cfu/ml)	*K. pneumo-niae* (cfu/ml)
Hall 2	27	5	18	22	1	9	20	16
Hall 4	31	1	16	24	0	13	14	9
Hall 5	21	9	19	10	5	17	28	6
Hall 8	42	0	23	15	9	8	11	19
Hall 9	13	4	29	18	2	2	23	10
College of Medical Sciences	29	0	22	11	0	0	19	16
VC Admin. Block	33	1	13	8	7	0	21	11
Main Campus	55	11	26	20	2	7	22	18
Faculty of Science	31	8	21	17	0	16	29	9
Staff Quarter	28	0	12	14	4	0	16	12

Discussion

Coliform bacteria have been used over the years as indicators of the bacteriological quality of ground water (Odonko and Ampofo, 2013; Muazu *et al*., 2012). The presence of coliform is an index of bacteriological quality of water most especially the isolation of faecal coliform such as *Escherichia coli* which is an indication of faecal contamination. The presence of *E. coli* in water indicates recent infection or contamination or pollution because the organism cannot survive for a long period outside their natural habitat which is the intestinal tract of humans and animals. The World Health Organization, in 1997 recommended that water both treated and untreated water should have no *E. coli* in it. The growth and multiplication of bacteria especially faecal indicators are affected by so many environmental factors such as temperature which ranges from 25^0C – 45^0C, and humidity. The spread of diseases through faecal contamination of water sources particularly in developing country like Nigeria is a common phenomenon (Odonko and Ampofo, 2013; WHO, 2004).

Water that is potable for consumption should be free from disease-causing organism or large number of non-pathogenic organisms. Feacal coliform count measure moderate level of bacteria in 100ml of water sample in membrane filtration technique while the standard culture method measures a high level of bacteria load which exceed the limit of 0cfu/ml which is the standard for feacal coliform count for drinking water. The presence of these indicator organisms indicates that the water is not suitable for human health and may provide an indication for water borne diseases (WHO, 2012).

Total coliform count for the sample was high which exceed the World Health Organization and Environmental Protection Agency Level of 0cfu/ml and 1-5cfu/ml colony. It shows that the water source is contaminated which could be due to improper maintenance of the water source. Total heterotrophic bacteria count in some water sources in these works was generally high because some of the colony count exceed the WHO and EPA level of heterotrophic count for drinking water. The heterotrophic count is used to indicate the effectiveness of water treatment processes (Environmental Protection Agency, 2012).

This elevated level of total heterotrophic count in some water sources may have been as a result of stagnation in the piped distribution system. Total heterotrophic count does not indicate the presence of pathogenic bacteria but bacteria regarded as "opportunistic pathogen" may cause infection in immune compromised individuals.

In all the 30 water samples examined bacteriologically, the bacterial load exceeded the recommended zero level of coliform/ml. The high coliform count observed in this study was an indicator of presence of pathogenic organisms in the water samples.

Conclusion

The result obtained from this study has shown high level of bacterial load which makes the water unsuitable for human consumption. Further treatment needs to be done because the growth in the water samples points to feacal contamination which may be due to inadequate treatment of water, contamination while in the storage tanks and passage through contaminated pipes of the borehole. The consumers may be at high risks of enteric bacterial infections.

Recommendations

A good and proper sanitation of water tanks should be maintained by washing them quarterly every year to avoid accumulation of debris which favours the growth and multiplication of these organisms. A regular microbiological analysis of the treated water should be carried out to ensure that the treatment method is working effectively.

Conflict of interest declaration

None declared.

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