

BACTERIOLOGICAL ANALYSIS OF IYUWKU STREAM WATER IN UHUAGU AWGU L.G.A ENUGU STATE, NIGERIA

ABSTRACT

The majority of the population of the Iyukwu community in the Awgu Municipal Area of Enugu State in Nigeria depends on the Iyukwu stream for its water supply. Due to the recent decrease in cases of waterborne diseases, a study was conducted to examine the bacteriological characteristics of the Iyukwu stream in order to protect public health from waterborne diseases. Five of this water samples taken from both the longitudinal profile and bottom level of the creek were tested for bacteriological properties using standard methods. Total bacterial counts were determined using the cast plate technique and total bacterial counts. From water samples three genera of bacteria genus *Klebsiella*, *Alcaligenes* and *Salmonella* were isolated. Total bacterial counts in water samples ranged from 0 to 32×10^2 CFU/ml. Total bacterial counts in the water samples analyzed ranged from MPN index 0 to 39 for coliforms per 50 ml. It was concluded that not all stream water is suitable for consumption and appropriate measures should be taken to clean and treat stream water on a regular basis before drinking.

Key words: *Salmonella*, *Stream*, *Cholera*, *waterborne diseases*

INTRODUCTION

“Water is indispensable and is one of the most essential amenities of life itself. The supply of safe drinking water to all has engaged the attention of many individuals, groups, governmental organizations, and private organizations” (Olayemi, 2014). The Iyukwu stream originates from the river Ogbuma flowing out of the cracks around Mgbidi. It started flowing out bit by bit at the spot where kids normally play around. With time, when the water was noticed, kids were stopped from playing at that spot and a way was made for the water to flow. After a short while, the water separated in two: Iyukwu and Eshea. Iyukwu stream took the route of the house where it is been used for domestic purposes and the shea stream took the route of the farm where it is been used to water crops during planting season. Waterborne pathogens a variety of viral, bacterial, algal, and protozoan agents. (Kosek *et al.* 2015) Water quality standards are designed to minimize known chemical and microbial risks. The term “safe” drinking water does not mean risk-free. It simply means that the risk is very low, below the ability of humans to quantify, or water treatment processes cannot further reduce water quality limits (Mustapha, 2018). “Increase in human population has exerted enormous pressure on the provision of safe drinking water, especially in developing countries” (Umeh *et al.*, 2016). “Unsafe water is a global public health threat, placing persons at risk for a host of diarrhea and other disease as well as chemical intoxication” (Hughes *et al.* 2015). “Unsanitary water particularly has devastating effects on young children in developing world” (Kosek *et al.* 2015; Parashar *et al.* 2015). “Recorded that more than 2 million persons, mostly children less than five years of age, die of diarrheal disease. Cholera affects all age groups is more common among children less than five years of age and among adults 25-39 years old. It affects 17 million people worldwide with more than 600,000 deaths, 80% of these cases and deaths are in developing countries” (Ademoroti, 2016). . Participants were children aged 6 months to 12 years who were admitted to the Addis Ababa Children's Hospital between 1984 and had typhoid fever in 1996, with 25% developing intestinal perforation. 37% died (Chapman, 2013). Therefore, the bacteriological analysis of the Iyukwu stream

Current is performed at Uhukwu in Awgu Municipality Region, Enugu State will determine the total number of water samples, determine the E. coli count (the most probable count) in the bacterial sample in the water, and determine the types of bacteria present in the water.

Prevention is the most effective way to limit morbidity and mortality associated with water-borne diseases, clean drinking water, temperature monitoring, proper wastewater treatment, monitoring of contamination of public waterways, and public education on proper sanitation (Baldursson et al., 2013). Thus the major obstacles experienced during the interval of making the project is the lack of some resources needed and lack of proper information about stream, which was due to the poor road network leading to the stream, also financial constraints, lack of equipment to carry out the tests and poor internet connection. But I was able to advance more in making sure the project produced quality information needed in finalizing. Aimed to prove stream water is suitable or not for human consumption in IYIUKWU in UhuAGU AWGU L.G.A ENUGU state with a bacteriological analysis of that water in Nigeria.

MATERIALS and METHODS

Collection of Water Samples

Water samples were collected from Iyukwu Stream, Uhuagu in Awgu Local Government Area, Enugu State. The samples were collected in a sterile container. The sterile container was dipped to a depth of about 5-10cm from the surface of the water.

Preparation of Media

Bacteriological Quality Determination

Serial Dilution

1 ml of water sample was aseptically transferred into a test tube of sterile distilled water. 10-fold serial dilutions were carried out; this was done by consecutively adding 1 ml of the previous dilution in 9 ml of the sterile distilled water until a 10^{-5} dilution was reached. This process was carried out for the 2 different water samples, and also 5 ml syringe was used for the two samples each. The 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5} for all two samples were plated by using the pour plate method.

Pour Plate Method

One millilitre from the 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , and 10^{-5} from all samples were inoculated into sterile petri dishes. Appropriate aliquots of sterile Nutrient agar were aseptically dispensed into the petri dish containing the inoculants. The dishes were gently swirled to ensure even distribution of the sample. The plates were covered and allowed to solidify. After solidifying, the Petri dishes were inverted and incubated at 37°C for 24hrs, thereafter bacterial counts were obtained.

Coliform Test

Duplicate sets of plates using 1ml amount of each sample homogenate prepared. 15ml of MacConkey agar, melted and cooled to 45°C was added to each plate, mixed well, and allowed to set. Finally, another 5ml of MacConkey agar was used to overlay. After allowing them to solidify, the

plates were inverted and incubated at 37°C for 24 h. After incubation, the number of pink colonies counted.

Isolation Of Bacteria

Distinct bacterial colonies were randomly picked, using flamed sterile inoculating loop, and sub cultured onto fresh secondary plates (MacConkey) i.e. each colony was maintained as a pure culture. The method used for sub-culturing was streak-plate method. The sub-cultures were then inverted and incubated at room temperature for 24hrs. Distinct colonies developed in the secondary plates (i.e. the sub-cultured isolates), were transferred to agar slants for further studies.

Morphological and Biochemical Characterization

Cultural Characteristics

For the bacterial isolates, cultural characteristics were observed on Nutrient agar plates. The cultural characteristics include. Size, shape, surface, opacity, texture, elevation, and pigmentation were determined by visual observation.

Gram Staining

The Gram staining technique was used to differentiate between gram-positive and gram-negative bacterial strains

Biochemical Tests

Motility Test

Motility test was aimed at identifying motile bacteria. A drop of normal saline was placed on a sterile slide and colony of test organism was suspended and emulsified and then covered with a cover slip. The slide was examined microscopically using 10x and 40x objective. Direction of different movement gave a positive result.

Catalase Test

This test used to differentiate bacteria that produce enzyme catalase such as *Staphylococcus aureus* and *Escherichia coli* were used as positive and negative controls respectively. Hydrogen peroxide solution was filled into a sterile test tube. Then a sterile glass rod was used to collect several colonies of the test organisms and inoculate them into the hydrogen peroxide solution. The appearance of immediate bubbles was an indication of the positive result.

Oxidase Test

This was carried out to identify bacterial species that produce the cytochrome oxidase enzyme. *Pseudomonas aeruginosa* and *Escherichia coli* were used as positive and negative controls respectively. A filter paper was placed in a clean petri dish and 2-3 drops of fresh or nascent oxidase reagent was added. A smear of test organism was collected using a glass rod, smeared on the filter paper, and observed. Blue-purple color within seconds is an indicator of a positive test.

Urease Test

This test was aimed at identifying *Klebsiella* spp. that produces urease enzymes, by hydrolysing urea to give ammonia and carbon dioxide. *Proteus* and *Salmonella* were used as control positive and negative controls respectively. The test organism was heavily inoculated onto Christensen urea broth in a bijou bottle using a sterile wire loop and incubated at 35°C- 37°C for 18-24 h. and examined, thereafter a pink color in the medium showed a positive test.

Citrate Test

This test is based on the ability of an organism to use citrate as its source of carbon. ←Simon's citrate agar medium was prepared in a slant bijou bottle, and a sterile wire loop was used to inoculate the test organism onto the slant medium and incubated at 35°C for 48 hours after which it was examined for color formation. The positive citrate test appeared by forming a bright blue color in the medium. *Klebsiella pneumoniae* and *Escherichia coli* were employed as positive and negative controls respectively.

Indole Test

It is one of the most important tests to identify Enterobacteriaceae by their ability to produce indole. A sterile wire loop was used to inoculate a colony of test organisms into 2ml of peptone water containing tryptophan. The tube was stored and incubated at 37°C for 24 h, and then Kovac's reagent was added to the medium. The result was an indicator as positive by the formation of red color on the surface layer of the medium within 10 minutes.

Results

This study was carried out in order to evaluate the bacteriological profile of Iyukwu Stream, Uhuagu, Awgu Local Government Area, Enugu State. Samples of water from the stream were cultured using standard techniques. The results showed total bacterial counts in the samples ranged from 0.00 to 39.0×10^4 (Table 1). The range of coliform counts from 0.00 in samples B and C, 35 in samples A and E, and 3 in sample D (Table 2).

Three bacterial genera were identified from the water samples *Alcaligenes* spp., *Klebsiella* spp., and *Salmonella* spp. *Klebsiella* spp. was isolated from sample A, *Salmonella* spp. was isolated from sample D, and *Alcaligenes* spp. was isolated from sample A and sample E, but neither sample B nor C exhibited any growth of *Klebsiella* or *Alcaligenes* spp. (Table 3). **Cultural Characteristics And Biochemical characterization of Isolated Microorganism From Iyukwu Stream, Uhuagu Awgu Local Government Area, Enugu State showing Lactose fermenter (LF) and Non lactose fermenter (NLF) (Table 4) .**

Table 1: Total Bacterial Count of Iyukwu Stream, Uhuagu, Awgu Local Government Area, Enugu State.

Samples	Total bacterial count(CFU/ml) $\times 10^4$
A	32×10^4
B	0
C	0

D	0.03x10 ⁴
E	39x10 ⁴

Key: **A**= water sample from bowl 1, **B**= water sample from bowl 2, **C**= water sample from bowl 3, **D**= water sample from bowl 4, **E**= water sample from bowl 5

Table 2: Coliform Count of Water Samples From Iyukwu Stream, Uhuagu, Awgu Local Government Area, Enugu State.

Samples	50 ml Test Tube	10 ml Test Tube	50 ml Test Tube	MPN
A	1	4	4	35
B	-	-	-	NIL
C	-	-	-	NIL
D	-	1	2	3
E	1	4	4	35

Table 3: Prevalence of Bacteria In Water Samples From Lyiukwu Stream, Uhuagu, Awgu Local Government Area, Enugu State.

Type of Bacteria	Sample A	Sample B	Sample C	Sample D	Sample E
<i>Klebisella</i> spp	+	-	-	-	-
<i>Alcaligenes</i>	+	-	-	-	+
<i>Salmonella</i> spp	-	-	-	+	-

Key: += Present, -= absent

Table 4: Cultural Characteristics And Biochemical characterization of Isolated Microorganism From Iyukwu Stream, Uhuagu Awgu Local Government Area, Enugu State.

Isolates	Colonial morphology	size	gram staining	shape	indole	citrate	urease	motility	oxidase	TSI agar agent	Lactose Fermenters(LF)	Non-Lactose Fermenters(NLF)	Catalase
A	LF with Pinkish color And raised	2-3mm	Gram negative	Rod	-	+	+	-	-	NA	+	-	+
B	NLF is Flat	2-3mm	Gram negative	Rod	-	+	-	+	+	AG	-	+	+
C	NLF with Black dots	2-3mm	Gram negative	Rod	-	+	-	+	+	A	-	+	+
D	NLF which is flat	2-3mm	Gram negative	Rod	-	+	-	+	+	AG	-	+	+
P??													

KEY: A= first isolate from samples A, B= Second isolate from Sample A, C= isolate from Sample D, D= isolate from Sample E, += Positive, - = Negative, NAG= No acid and gas production, AG = Acid and gas production, A = acid production

DISCUSSION

The threat of **waterborne** diseases in contaminated water and food **continues** to raise increasing concerns over the years. Water even when obtained from **a well-treated** stream, may still present contaminants. The result obtained showed that the stream water examined was moderately contaminated with microorganisms. The presence of *Klebsiella* spp., *Alcaligene* spp., and *Salmonella* spp. in the various water samples indicated the possibility of **faecal** pollution in other **streams**. The mean coliform count shows that sample B was relatively satisfactory and sample A had **a** high coliform count. Contamination of this stream may have resulted from sewage from surface or sub-surface water which may have washed straight down the stream. The vessels used by the villagers for collecting the water from the stream may also have been a source of contamination. Caircross and Feacher (2018) **thought** that streams were most times contaminated as a result of closeness to the bush where refuse **is** dumped and **faces** are passed and washed into the stream by **rainwater**. **The high** coliform count obtained from the stream examined may be attributed to the incessant use of the bushes close to the stream for defecation without observing proper sanitation. It is that most homes in the village lack modern facilities including **toilets** and the cost of construction is high. Hence, people have **quickly** adapted to the situation by **indiscriminately using** the bushes as a toilet and also washing **clothes** and other **household** utensils and most times food items inside the stream. Okafor (2015) Stream sited away from bushes where faeces are passed **microbe**, while stream sited close to bush where faeces are passed has **high** microbial load. Has led to many cases of diseases, especially typhoid paratyphoid fever in villages. Perhaps, if the stream is sited away from the refuse Amp and bushes, a better quality of water will be obtained. Better protection the from pollution can be achieved by building modern facilities in homes to the bushes and by educating the villagers on the dangers of making of contaminated water so as to stop them from dumping refuse, defecating, and washing clothes and other materials in and around the stream.

CONCLUSION

Stream water is believed to be a semi-pure form of water because of the purification properties of the soil, however, source of contamination could be due to improper design, proximity to, refuse dump sites, and various human activities can serve as of contamination. Therefore, good sanitation of environment, proper cleaning and treatment of water control of human activities affect quality of drinking water. Water quality should be controlled in order to minimize acute problem of water-related diseases. Domestic treatment of stream water is also an important means of enhancing water quality, as is regular cleaning of water reservoirs with proper cleaning chemicals. Constant monitoring of water quality stands as a good means of detecting earlier the deviation of drinking water from the standard.

RECOMMENDATION

Measurable techniques should be taken in curbing the challenges of water supply in Uhuagu community, and some of these techniques :

- Improve sanitation facilities by providing toilets and latrines that flush into sewer or safe .
- Promote good hygiene habits through education. 35 percent cases of diarrhea can be reduce due to Proper hand washing with soap and water.
- Implement rainwater harvesting systems to collect and store rainwater for drinking or recharging underground aquifers. Build wells to extract ground water from underground aquifers.
- Provide home water treatment capability through the use of filters solar disinfection, or flocculants, to make drinking water safe.
- Promote low-cost solutions, such as chlorine tablets or plastic bottles that can be exposed to sunlight, to improve water quality
- . **Recommendation** Addressing water supply challenges in Woofug communities requires the use of measurable technology. These techniques include:
 - Improve sanitation by providing toilets and latrines with access to sewers or safe areas.
 - Promote good hygiene practices through education. 35 percent of diarrhea can be relieved by washing hands properly with soap and water.
 - Install rainwater harvesting systems to collect and store rainwater for drinking or replenishment of underground aquifers. Build a well to pump groundwater from an underground aquifer.
 - Provide domestic water treatment options through the use of filters, solar disinfection, or flocculants to make drinking water safe.
 - Promote low-cost solutions such as chlorine tablets and plastic bottles that can be exposed to sunlight to improve water quality.

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