Original Research Article

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

ABSTRACT

Introduction: Water is indispensable and it is life Water must be given the necessary attention at all times, water 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. **Methods**: Five of these 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. **Results**: Three genera of bacteria genus Klebsiella, Alcaligenes, and Salmonella were isolated from water samples. Total bacterial counts range from 0 to 32 × 10^2 CFU/ml. By using the MPN index, total bacterial counts in the water samples were from 0 to 39 for coliforms per 50 ml. **CONCLUSION:** The results of the study indicated that not all stream water is suitable for consumption and appropriate measures should be taken to clean and treat stream water regularly before drinking**.** The study aimed to prove whether stream water is suitable or not for human consumption in IYIUKWU in URUAGU AWGU L.G.A ENUGU state with a bacteriological analysis of that water in Nigeria.

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

INTRODUCTION

Water is indispensable and it is life Water must be given the necessary attention at all times, water 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 lyiukwu stream originates from the river Ogbuma flowing out of the cracks around Mgbidi Hill. 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: Iyiukwu and Eshea. lyiukwu 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 include a variety of viral, bacterial, algal, and protozoan agents. The estimated 4 billion cases and 2.5 million death from endemic diarrheal disease each year (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). The 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 the 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 and is more common among children less than five years of age and among adults 25-39 years old, also causes 120,000 deaths a year in the world. Cholera is particularly deadly in Africa, As it has become one of the most widespread epidemics since the 1970s of the last century. It affects 17 million people worldwide with more than 600,000 deaths, and 80% of these cases and deaths are in developing countries (Ademoroti, 2016).

Among 6 months to 12-year-old children admitted to the children & amp;#039;s hospital in Addis Ababa between 1984 & amp; amp; 1996 with typhoid, 25% developed intestinal perforations & amp;amp; 37% of those died (Chapman, 2013). Thus the bacteriological analysis of the Iyikwu stream is located in Uhuagu. Awgu Local Government Area, Enugu State determines the total count of the water sample, determines the coliform count (most probable count) in a water bacterial sample, and determines the type of bacteria present in the water (**<u>It appears to be an unclear paragraph; Please rephrase it clearly).</u>**

Prevention is the most effective way to limit morbidity and mortality associated with water-borne diseases, that include providing 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 the 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 the quality information needed in finalizing. The study aimed to prove whether stream water is suitable or not for human consumption in IYIUKWU in URUAGU 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 Lyiukwu 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

Preparation of MacConkey, Nutrient Plates, and Triple Sugar Indole Agar Slant

McConkey and Nutrient agar plates were prepared for bacterial culture and characteristics and also to distinguish between lactose-fermenting and non-lactose-fermenting organisms. TSI agar is used to determine carbohydrate fermentation and H2S production. They were prepared according to the manufacturer's instructions and sterilized by autoclaving at 121°C for 15 minutes at 15psi pressure. 20 ml of sterile agar was spread on sterilized Petri dishes and TSI were slanted McCartney bottles and then allowed to cool for 5-10 minutes to solidify. This agar was used for the total coliform count and total bacterial count..

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 I 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 two samples were inoculated into sterile petri dishes. Appropriate aliquots of sterile Nutrient agar were aseptically dispensed into the petri dish that containing the inoculants. The Petri dishes were gently swirled to ensure an 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 were 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 was counted.

Isolation Of Bacteria

Distinct bacterial colonies were randomly picked, using a flamed sterile inoculating loop, and subcultured onto fresh secondary plates (MacConkey) i.e. each colony was maintained as a pure culture. The method used for sub-culturing was the 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 by the manufacturer's instructions.

Biochemical Tests

Motility Test

The motility test was aimed at identifying motile bacteria. A drop of normal saline was placed on a sterile slide and a colony of test organisms was suspended and emulsified and then covered with a cover slip. The slide was examined microscopically using 10x and 40x objective Direction of different movements 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. The 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 test 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 the 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. ~~(and *Alcaligenes* spp and *Klebsiella* spp.???)~~ 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 pneumonia* 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 Lyiukwu 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 x 10'?? (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 sp., 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).

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

Samples	Total bacterial count(CFU/ml)x10^4
A	$32x10^4$
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 Sampkes From Lyiukwu 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
Klebisella spp	+	-	-	-	-
Alcaligenes	+	-	-	-	+
Salmonella spp	-	-	-	+	-

Key: += Present ,-= absent

Table 4: Cultural Characteristics And Biochemical characterization of Isolated Microorganism From Lyiukwu 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 worth noting that most homes in the village lack modern facilities including toilets and the cost of construction is high. Hence, people have quickly adapted to de 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) explained the stream sited away from bushes where faeces are passed contains fewer microbe, while stream sited close to the bush where faeces are passed has a high microbial load. This has led to many cases of waterborne diseases, especially typhoid ~~ed~~ 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 scams from pollution can be achieved by building modern facilities in homes to stop people from using the bushes and by educating the villagers on the dangers of making ~~se~~ (use) 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, the source of contamination could be due to improper design, proximity to toilets, refuse dump sites, and various human activities that can serve as sources of contamination. Therefore, good sanitation of the environment, proper cleaning and treatment of water sources, and control of human activities affect the quality of drinking water. Water quality should be controlled in order to minimize the acute problem of water-related diseases. Domestic treatment of stream water is also an essential means of improving water quality and regular cleaning of water reservoirs with appropriate cleaning reagents.

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 the Uhuagu community, and some of these techniques include:

- Improve sanitation facilities by providing toilets and latrines that flush into the sewer or safe enclosures.
- 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
- . R**ecommendation** Addressing water supply challenges in Woofug communities requires the use of measurable technology. These techniques include: o Improve sanitation by providing toilets and latrines with access to sewers or safe areas. o Promote good hygiene practices through education. 35 percent of diarrhea can be relieved by washing hands properly with soap and water. o 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. o Provide domestic water treatment options through the use of filters, solar disinfection, or flocculants to make drinking water safe. o 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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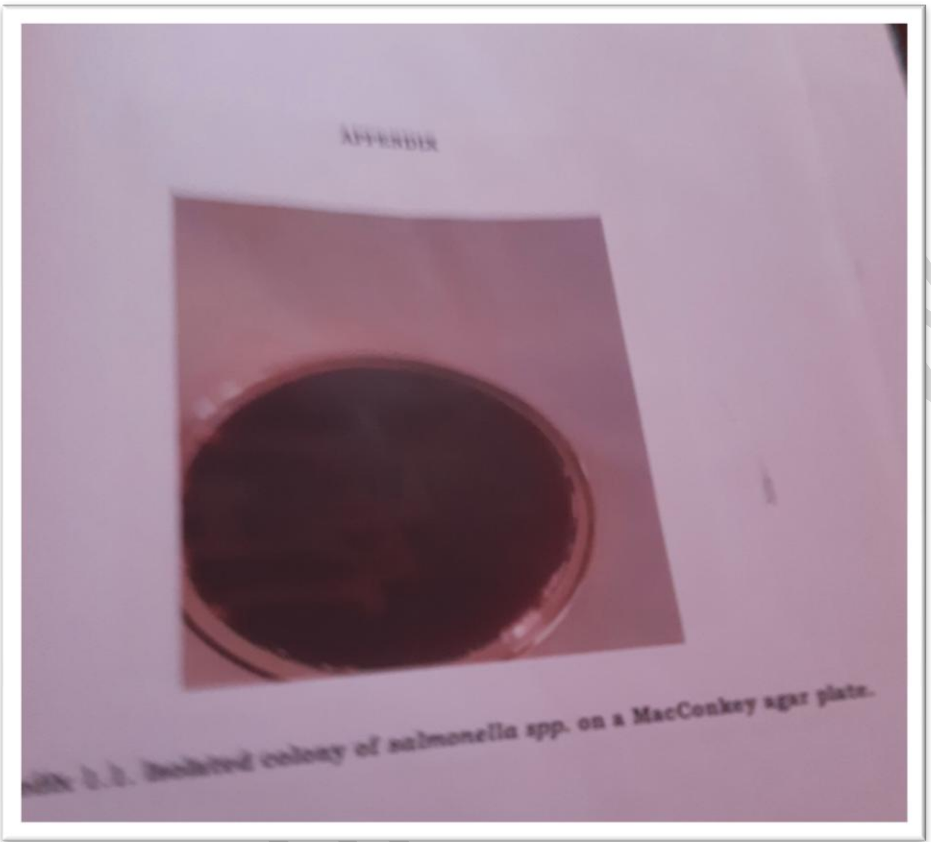
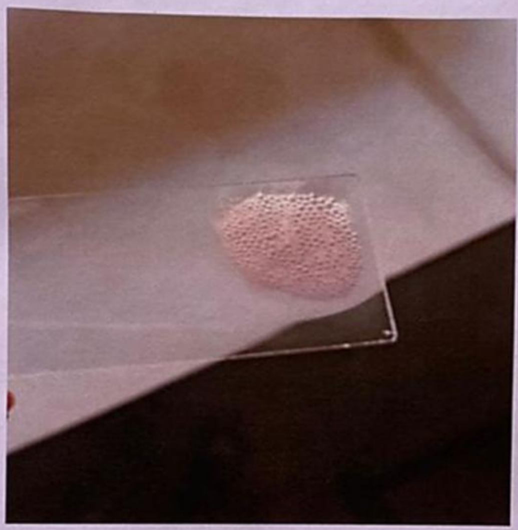
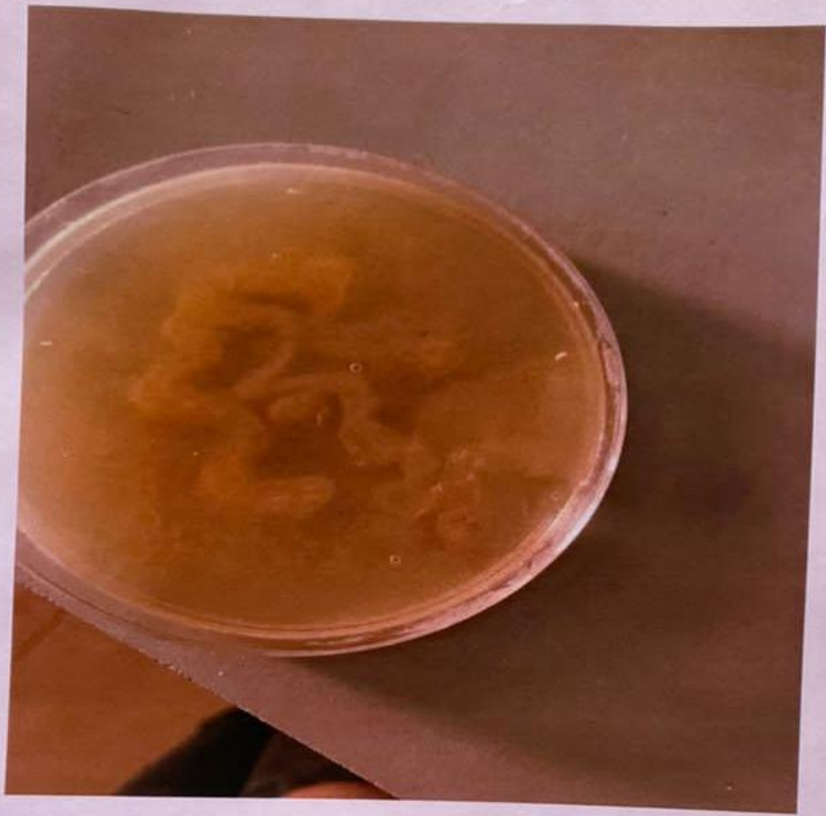




Appendix 1.3. A catalase positive test.+



Appendix 1.2. Isolated colony of *Alcaligenes spp.* On a Nutrient agar plate.