Original Research Article

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

ABSTRACT

The majority of the population of the Iyiukwu community in the Awgu Municipal Area of Enugu State in Nigeria depends on the Iyiukwu stream for its water supply. Due to the recent dcrease in cases of waterborne diseases, a study was conducted to examine the bacteriological characteristics of the Iyiukwu 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 × 102 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 it is life Water must be given the necessary attention at all times ,water is not a luxury and it is one of the most essential amenities of life itself. The supply of safe drinking water to all has therefore engaged the attention of many individuals, groups, govermental organizations and private organizations (Olayemi, 2014).

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 shea stream took the route of the farm where it is been used to water crops during planting season.

Waterborne pathogens, including a variety of viral, bacterial, algal and protozoan agents, account for much of the estimated 4 billion cases and 2.5 million deaths 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).

Increase in human population has exerted an enormous pressure on the provision of safe drinking water, especially in developing countries (Umeh er 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 and Parashar *et al.* 2015). Recorded that more than 2 million persons, mostly children less than 5 years of age, die of diarrheal disease. Cholera, affects all age groups.It is more common among children less than 5 years of age and among adults 25 - 39 years old.Worldwide cholera causes 120,000 deaths a year.It is particularly deadly in Africa where epidemics have become wider spread and more frequent since the 1970s Typhoid fever as well occurs in all parts of the world where water supply and sanitation aresub-standard.It affects 17 million people worldwidewith more than 600,000 deaths.Almost 80% of these cases and deaths are in developing countries, (Ademoroti, 2016).Among 6 months to 12-year-old children admitted to the children's hospital in Addis Ababa between 1984 & 1996 with typhoid, 25% developed intestinal perforations & 37% of those died (Chapman, 2013).

Thus the bacteriological analysis of lyikwu stream located in Uhuagu. Awgu Local Government Area, Enugu State determines the total count of the water sample, determine the coliform count (most probable count) in a water bacterial sample and determine the type of bacteria present in the water.

Prevention is the most effective way to limit morbidity and mortality associated with water-borne diseases. Critical to prevention efforts are 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

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 with 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 PLATES

MacConkey agar plates needed for sub-culturing, and that will also be used in differentiating Lactose fermenting from Non-Lactose fermenting organisms were prepared by dispensing 20mls of the sterilized MacConkey agar on sterile petri dishes, and allowed to settle down, in other to solidify.. **PREPARATION OF NUTRIENT PLATES**

Nutrient agar plates needed for sub-culturing for cultural characteristics examination were also prepared. Sterilized nutrient agar that has been allowed to cool for 5-10minutes were dispensed on sterile petri dishes (20 mls), and allowed to settle and solidify.

PREPARATION OF TRIPLE SUGAR INDOLE AGAR SLANT

Triple Sugar Indole agar which is a medium used in determining carbohydrate fermentation and H_2S production, after autoclaving for 15 minutes at 121°C was dispensed into McCartney bottles. The bottles were slanted and allowed to solidify.

LABORATORY MATERIALS

The MacConkey agar, Triple Sugar Indole (TSI) agar and Nutrient agar which were needed for the total coliform count, total bacterial count and biochemical tests were all made available. They were prepared according to the manufacturer's instructions and sterilized by autoclaving at 121°C for 15 minutes at 15psi pressure.

STERILIZATION OF MATERIALS

Glass-wares such as test tubes, measuring cylinder, McCartney bottles needed for analysis were properly sterilized by autoclaving them at 121°C for 15mins at 15psi pressure.

BACTERIOLOGICAL QUALITY DETERMINATION

SERIAL DILUTION

1ml of sample (water sample) was aseptically transferred into a test tube of sterile distilled water. 10 fold serial dilutions was carried out; this was done by consecutively adding Iml of the previous dilution in a 9ml of the sterile distilled water until a 10^{-5} dilution was reached. This process was carried out for the 2 different water samples, andalso 5mls syringe was used for the 2 samples each.

The 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} for all two 2 samples were plated by using the pour plate method.

POUR PLATE METHOD

One milliliter from the 10-1, 102, 103, 10* and 105 from all 2 samples were inoculated into sterile petri dishes. Appropriate aliquots of sterile Nutrient agar were aseptically dispensed into the petri dish already containing the inoculants. The petri 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 was 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 to solidify, the plates were inverted and incubated at 37°C for 24hours.After incubation, the number of pink colonies were counted.

ISOLATION OF BACTERIA

Distinct bacterial colonies were randomly picked, using flamed sterile inoculating loop and sub-cultured onto fresh secondary plates (MacConkey) ie. 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 for differentiation between gram positive and gram negative bacterial strains. A drop of sterile distilled water was placed on a neat and clean glass slide, and a single isolated colony of 24 hours old culture was mixed in it. The smear was made by spreading the culture. This smear was air dried and fixed by rapidly passing the slide three times over the flame. It was then flooded with crystal violet for 1 minute and then washed off with distilled water. Then gram's iodine solution was added to the smear and the glass slide was left for one minute and rinsed with distilled water. This step was followed by the application of decolorizing agent (ethanol) for 30 seconds. Decolorizing agent was immediately washed with distilled water and the smear was counter stained with safaranine for one minute. The slide was washed with distilled water; air dried and was observed under the microscope.

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 was used to differentiate those 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 into the hydrogen peroxide solution. It was observed for immediate active bubbling for positive test.

Oxidase Test

This was carried out to identify bacterial species that will produce the cytochrome oxidase enzyme. Pseudomonas aeruginosa and Escherichia coli were used as positive and negative controls respectively. A piece of 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 few a seconds showed a positive test.

UREASE TEST

This test was aimed at identifying Klebsiella spp. that produce urease enzyme, which hydrolyze urea to give ammonia and carbondioxide. Proteus and Salmonella were used as control positively and negatively 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 18-24hours and examined, thereafter a pink color in the medium showed 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. was identified Simon's citrate agar medium was prepared in a slant bijou bottle, then using a sterile wire loop was used to inoculate the test organism onto the slant medium and incubated at35°C for 48hours after which it was examined for color formation. A bright blue color in the medium gave, positive citrate lest. Klebsiella pneumonia and Escherichia coll were employed as positive and negative controls respectively.

INDOLE TEST

This test was carried out for indole production by test organism which is important in identifying entero bacteria A sterile wire loop was used to inoculate a colony of test organism into 2ml of peptone water containing tryptophan. The tube was stored and incubated at 37°C for 24hours. Kovac's reagent was added to the medium. Observation of red coloration on the surface layer within 10minutes showed a positive result.

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 and the following results were drained from the analysis.

The total bacterial counts in the samples ranged from 0.00 to 39.0x 10' as shown in table

I;below. Range of coliform counts from 0.00 in sample B and C; it's 35 in sample A and E, and 3 in sample D, (table 2).

Three bacterial genera were identified from the water samples, namely, *Alcaligenes*

sp. Klebsiella spp. and Salmonella spp.

Klebsiella spp. Was isolated from sample A, both sample B and sample C had no growth of *Klebsiella and Alcaligenes spp*., Salmonella spp. was isolated from sample D, and *Alcaligenes spp*. was isolated from sample A and sample E, making it a total of he isolates gotten from the analysis, (table 3).

TABLE 1: TOTAL BACTERIAL COUNT OF IYIUKWU STREAM, UHUAGU, AWGU LOCAL GOVERNMENT AREA, ENUGU STATE.

Samples	**Total bacterial count(cfu/ml)x10^4**
A	$32x10^4$
B	0.00
C	0.00
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 IYIUKWU STREAM, UHUAGU, AWGU LOCAL GOVERNMENT AREA, ENUGU STATE.

Samples	**50mls test tube**	**10mls Test tube**	**5mls 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 IYIUKWU STREAM, UHUAGU, AWGU LOCAL GOVERNMENT AREA, ENUGU STATE.

Bacteria	Sample A	Sample B	Sample C	Sample D	Sample E
Klebisella spp	+	-	-	-	-
Alcaligenes Spp	+	-	-	-	+
Salmonella spp	-	-	-	+	-

Key:

+= Present

-= absent

TABLE 4: CULTURAL CHARACTERISTICS AND BIOCHEMICALCHARACTERIZATION OF ISOLATED MICROORGANISM FROM IYIUKWU 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, CONCLUSION AND RECOMMENDATION

DISCUSSION

The threat of water borne diseases in contaminated water and food continue to raise increasing concerns over the years. Water, even when obtained from well treated stream, may still present contaminants.

The result hence 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 other stream. The mean coliform count shows that sample B was relatively satisfactory and sample A had 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) were of the opinion that streams were most times contaminated as a result of closeness to the bush where refuse are dumped and faeces are passed and they are washed into the stream by rain water.

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 worthy to note that most homes in the village lack modern facilities; including toilet and the cost of construction is high. Hence, people have fast adapted to de situation by indiscriminate use of the bushes as a toilet and also washing of cloth and other house hold utensils and most times food items inside the stream.

According to Okafor (2015), stream sited away from bushes where faeces are passed contain less microbe while stream sited close to bush where faeces are passed has high microbial load, this has led to many cases of water borne 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 of the scam from pollution can be achieved by building modern facilities in homes so as to sop people using the bushes and by educating the villagers on the dangers of making se 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 toilet, refuse dump sites, and various human activities which can serve as source of contamination. Therefore, good sanitation of environment, proper cleaning and treatment of water source and control of human activities that 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 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 Uhuagu community; and some of these techniques includes:

- Improve sanitation facilities by providing toilets and latrines that flush into sewer or safe enclosure.
- Promote good hygiene habits through education. 35percent 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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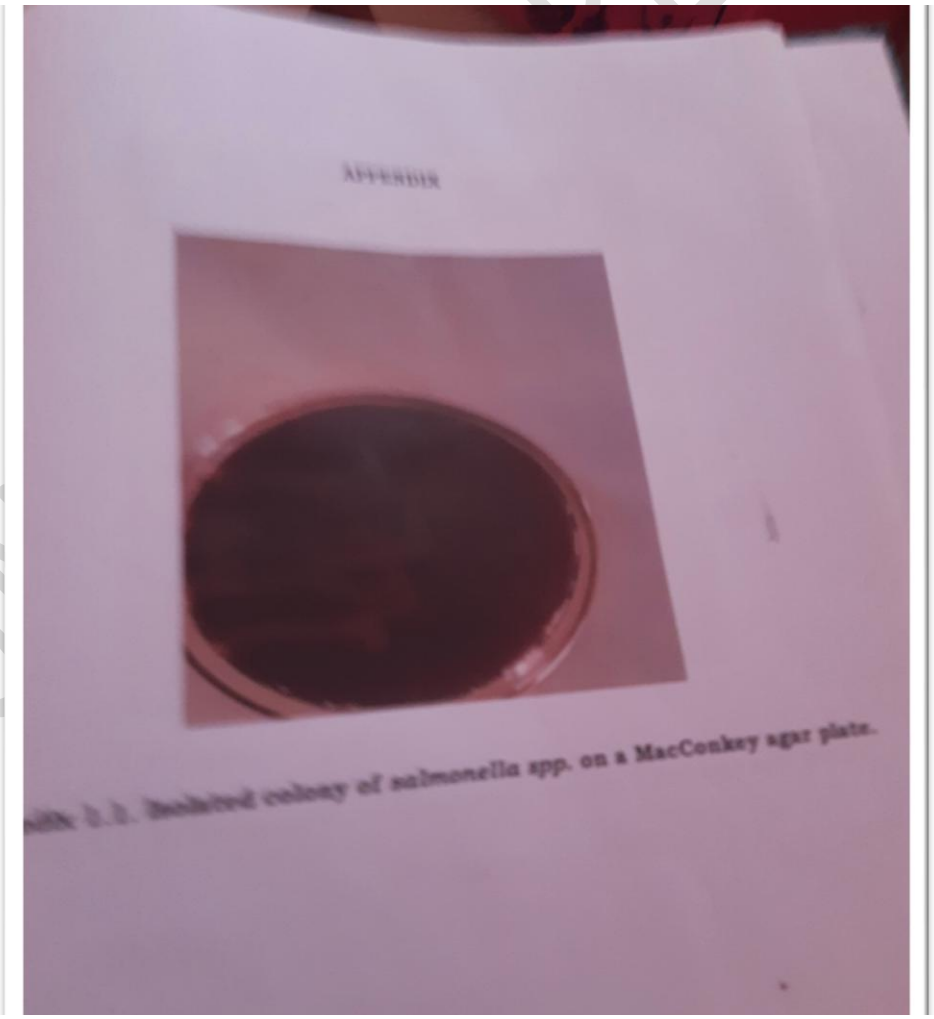

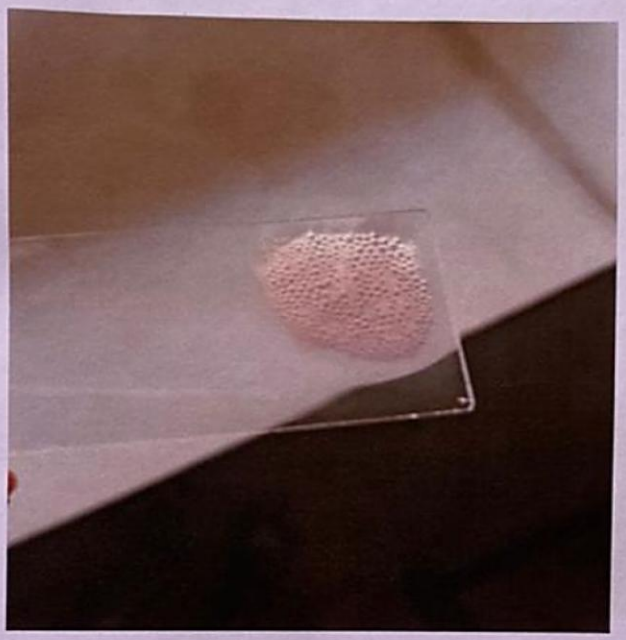

Appendix 1.3. A catalase positive test.+

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