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Influence of dry season on *Salmonella* structural community and physicochemical characteristics of different streams at Uli, Anambra State, Nigeria.

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ABSTRACT

Contamination of water bodies had been a great threat to public health, especially in rural communities.This study was carried out to investigate the prevalence of *Salmonella* species, and physicochemical properties of stream water at Uli community. A total of 80 water samples were collected from five streams (Aluoha, Umuoma, Umuaku, AzuOwerri, and Umuegwungwu). The water samples were serially diluted using tenfold serial dilution, and were screened for *Salmonella*count, using standard plate count technique. The isolated organisms were identified via morphological appearances, biochemical test, and molecular characteristics. The physicochemical properties of the streams were determined using gravimetric and spectrophotometric methods. The pH (7.70 – 8.50) temperature (27.50 – 31.80°C) electrical conductivity (247.00 – 397.00µS/cm) turbidity (108.00 – 291.00 NUT) TSS (118.00 – 228.00 mg/L) TDS (114.00 – 221.00 mg/L) DO (2.27 – 4.41 mg/L) chlorine (19.14 – 54.45 mg/L) and nitrate (6.02 – 12.07 mg/L) were within WHO permissive limits except turbidity (108.00 – 291.00 NUT) phosphate (0.12 – 1.44 mg/L) and BOD (110.00 – 215 mg/L) in all the samples. Five *Salmonella* strains isolated from the studied water samples were SEFM366, SEYU39, STU288, SThydG and SPAOA13. Umuaku stream had the highest occurrence of the strains, while Umuegwungwu and Aluoha stream had the lowest. SEFM366 strain dominated, while the least strain was SThydG. This study has shown that streams at Uli community, particularly Umuaku are highly contaminated with *Salmonella*. Therefore, the streams require adequate treatment before optimizing them for domestic purposes. However, further microbiological research is necessary to evaluate the presence of other pathogens in different streams found in this community.
Keywords: Public health, stream, *Salmonella*, Uli, waterborne disease

INTRODUCTION

The quality of drinking water is a powerful environmental determinant of health of individuals in the society [1,2]. Drinking water safety is a foundation for the prevention and control of water borne disease outbreak and it can contribute to background rates of disease manifesting themselves on different time scales. Initiatives to manage the safety of water do not only support public health but often promote socio-economic development and wellbeing as well [3,4]. Research had shown that non-potable water is a reservoir of many pathogenic microorganisms that cause grave harm to human health, particularly enteric bacteria such as *Salmonella*, *Shigella* and *Escherichia* species [5,6]. These organisms are introduced into water bodies mainly by human activities such open defecation, indiscriminate refuse and sewage disposal [7,8]. *Salmonella* are found worldwide in both cold-blooded and warm blooded animals, and in the environment. They cause illness such as typhoid fever, paratyphoid fever and food poisoning [9]. *Salmonella* species contribute immensely to waterborne infection [10]. *Salmonella* cause salmonellosis which is introduced into human through fecal oral route, mainly by consumption of contaminated water. *Salmonella* species which causes typhoid epidemics according to [11] is characterized by a high rate of morbidity and mortality. The World Health Organization (WHO) estimates that 88 % of that burden is attributed to unsafe water supply, poor sanitation and poor hygiene. Most people infected with *Salmonella* develop diarrhea, fever and abdominal cramps 12 to 72 hours after infection [12]. Apart from microbial contamination of water, it had been also observed that some physicochemical parameters serve as indicators of water contamination, especially when they exceed standard limit speculated by WHO. Some of the parameters include turbidity, pH, electrical conductivity, biochemical oxygen demand, nitrate and phosphate [12].

These physical and chemical parameters greatly influence microbial proliferation and water pollution. Water pollution can be introduced by either direct sources which include: effluent outfall from factories and refineries, waste treatment plants etc., that emit fluids of varying quality into water bodiesor by indirect sources which include contaminants that enter water bodies from soil/ground water stems and from the atmosphere via water. Water pollution affects the entire biosphere-plants and organisms living in these bodies of water. In almost all cases, the effect is damaging not only to individual species, but also to the natural biological communities [13]. Water pollution has catastrophic implication on many factors ranging from human health, ecosystem, death of animal, destruction of aquatic life and economic losses. In Nigeria, mainly in Anambra State, diseases caused by *Salmonella* is one of the killers of children.*Salmonella* species is considered as one of the causative agents responsible for food poisoning endemics in Nigeria and neighboring countries [14]. Several researchers had worked on bacteriological and physicochemical characterization of water such as [9], but little information had been documented on the influence of dry season on *Salmonella* structural community and physicochemical characteristics of different streams at Uli, Anambra State. Hence, this work is aimed at evaluating the influence of dry season on *Salmonella* structural community and physicochemical characteristics of different

streams at Uli, Anambra State. The result obtained from this work would contribute immensely in combating waterborne infections within and outside the state.

MATERIALS AND METHODS

Study Area: The study was conducted at Aluoha, Umuaku, Umuoma, AzuOwerri, and Umuegwungwu streams, Uli, Ihiala Local Government Area, Anambra State. Uli is a village located between latitudes 5.47°N and 5.783°N and longitude 6.52°E and 6.87°E on the South eastern part of Nigeria. Uli extends westward to the confluence of the rivers of Atammiri and Eyinja, and across Ushamlake down to the lower Niger region. Uli has rainforest vegetation with two seasonal climatic conditions: rainy season and dry season, which is characterized by the harmattan between December and February. Uli is characterized by double maxima of rainfall with a light drop in either July or August known as dry spell or August break. The annual total rainfall is about 1,600 mm with a relative humidity of 80 % at dawn.

Sterilization of the Container: The containers used for sample collection were washed with detergent and water. After washing, it was thoroughly rinsed and sterilized with 70 % ethanol. The already sterilized containers were left to stand on a bench moppedwith 70 % ethanol for 60 seconds to remove tiny droplets in the container. Then, the container was aseptically closed.

Sample Collection: Water samples were collected following the method employed by Iheukwumere and Iheukwumere (2015). Water samples were collected by lowering the plastic container inside the water body, 30 cm deep, the water overflowed the container before withdrawal. The sampling points were approximately 100 m away from one another. The samplings were done in triplicate. After collection, the samples were covered and placed in a cooler containing ice block to maintain the temperature before laboratory analysis. The sampling procedure was repeated for the month of November, December, January, and February.

Sterilization of Glass Wares: The glass wares were sterilized using electric oven. The glass wares were washed with detergent and rinsed with clean water. These were dried and placed inverted inside of the oven and set the thermostat at 160°C for 1 h.

Preparation of Media for Isolation: The media used for this study include: *SalmonellaShigella* Agar (Biotech), Nutrient Agar (Biotech), Nutrient broth (Biotech),Tripple Sugar Iron Agar (Biotech), and Simmon Citrate Agar (Biotech). The media were prepared according to the manufacture's instruction and sterilized by autoclaving at 121°C, 15psi for 15 minutes.

Isolation and Purification of the Isolates: Tenfold serial dilution was done in which 9 mL of distilled water were dispensed into test tubes each and 1 mL of the stock water was dispensed into the first test tube and was vortexed after which 1 mL was taken from the tube using another pipette and was transferred into the second testtube. The process continued until the last tube after which 1 mL was discarded. Inoculation was carried out by aseptically inoculating 1.0 mL of the sample on SSA using pour plate method and the incubation was done inverted at 37°C for 24 h. After 24 h incubation, the grown colonies were sub-cultured by streaking a single colony aseptically on sterile poured plate, and this was incubated at 37°C for 24 h (Arora and Arora, 2008).

Characterization and Identification of the Isolates: The sub- cultured isolates were characterized and identified using their colonial and morphological descriptions, biochemical characteristics and the molecular characteristics (Ouma*et al*., 2016). The colonial description was carried out to determine the appearances of the isolates on agar media plates, their sizes, edges, consistencies, and optical properties of the isolates.

Gram Staining: This was carried out using the modified method of Willey *et al*. (2008). In this process, a thin smear of the culture was prepared on a clean grease free slide, air dried and heat fixed. The smear was floodedwith crystal violet solution for 60 seconds and rinsed with water. It was then covered with Gram's iodine for 60 seconds and rinsed with water. Alcohol (95 % w/w ethanol) was used to decolorize the slide content for 10 seconds and rinsed with water. The smear was then counter stained using safranin solution for 60 seconds, rinsed and air-dried. The stained smear with a drop of immersion oil was then observed under the light microscope using X100 objective lens.

Motility test: This was carried out using the method described by Willey *et al*. (2008). The medium used was semi-solid agar. It was prepared by mixing 17.00 g of bacteriological agar with 20.00 g of nutrient broth in 1 litre of distilled water. Heat was applied to dissolve the agar and 10.00 mL amounts were dispensed into test tubes and sterilized by autoclaving. The test tubes were allowed to stand in a vertical position. Inoculation was done by making a single stab down the center of the test tube to half the depth of the medium using a standard stabbing needle. The test tubes were incubated at 37°C for 24 h. Motile bacteria swarm and gave diffused spreading growth that was visible to the naked eye.

Biochemical Tests: The following biochemical tests were carried out:

Sugar fermentation test: This was carried out using method described by Willet *et al*. (2008). This test was done to ascertain the ability of the isolates to metabolize some sugars (glucose, xylose, ducitol, maltose, arabinose, inositol, mucate, and lactose) with the resultant production of acid and gas or either. One litre of 1 % (w/v) peptone water was added to 3 mL of 0.2 % (w/v) bromocresol purple and 9 mL was dispensed in the test tube that contained inverted Durham's tubes. The medium was then sterilized by autoclaving. The sugar solutions were prepared at 10 % (w/v) and sterilized. One milliliter of the sugars was dispensed aseptically into the test tubes. The medium was

then inoculated with the appropriate isolates, and the cultures were incubated at 37°C for 48 h, and were examined for the production of acid and gas. A change in color from purple to yellow indicated acid production, while gas production was assessed by the presence of bubbles in the inverted Durham's tubes.

Hydrogen Sulphide production: This was carried out using the method described by Willey *et al.* (2008). This was performed using triple sugar iron (TSI) agar. The TSI agar was prepared according to the manufacture's instruction. This was sterilized using autoclave and allowed to cool to 45°C. The isolate was aseptically inoculated by stabbing vertically on the medium and streaked on the top and inoculated at 37°C for 24 – 48 h. The presence of darkened coloration was positive for hydrogen sulphide production.

Methyl Red test: This was carried out as described in the manual of Microbiology by Willey et al. (2008). The glucose phosphate broth was prepared according to the manufacture's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37°C for 48 h. After incubating, five drops of 0.4 % solution of alcoholic methyl red solution was added and mixed thoroughly, and the result was read immediately. Positive tests gave bright color, while negative tests gave yellow color.

Citrate utilization test: This was carried out as described in the Manual of Microbiology by Willey *et al.* (2008). The Simmon's Citrate Agar was prepared according to the manufacture's direction and the isolates were inoculated by stabbing directly at the center of the medium in the test tubes and incubated at 37°C for 48 h. Positive test was indicated by the appearance of growth with blue color, while negative test showed no growth and the original green color was retained.

Catalase test: This was carried as described in the Manual of Microbiology by Willey *et al.* (2008). A smear of the isolates was made on a cleaned grease-free microscopic slide. Then a drop of 30 % hydrogen peroxide (H_2O_2) was added on the smear. Prompt effervescence indicated catalase production.

Physicochemical Properties of the Stream Water

The physical parameters determined were turbidity, conductivity, pH, temperature, acidity, alkalinity, total solid, total dissolved solid, and total suspended solid. The chemical parameters determined were chloride, nitrate, phosphate, and sulphate in the water.

Turbidity, conductivity. temperature, and pH of the water sample: These were determined following the methods of Ouma*et al.* (2016). The turbidity of the water was determined using turbidiometer in Nephelometric Turbidity Unit (NTU). Conductivity of the water sample was determined using conductivity meter, whereas pH meter and Mercury in glass Thermometer were used to determine the pH and temperature of the samples, respectively.

Acidity of the water sample: This was done using the method of Ouma*et al.* (2016). Three drops of phenolphthalein indicator were added into the water sample and titrated with sodium hydroxide (NaOH). The titre value was determined and used for the determination of the acidity in mg/mL of $CaCO_3$.

$$\text{Acidity} = \frac{\text{Titre value} \times \text{Normality of NaOH used} \times 50000}{\text{Volume of water sample (mL)}}$$

Alkalinity of the water sample: This was carried out using the method of Ouma*et al.* (2016). Three drops of bromocresol green were added into the water sample and titrated with HCl. Thetitre value was determined and used for the determination of the alkalinity in mg/mL of $CaCO_3$.

$$\text{Alkalinity (mg/mL of } CaCO_3) = \frac{\text{Titre value} \times \text{Normality of Base used} \times 50000}{\text{Volume of water sample (mL)}}$$

Total solid in the water sample: This was carried out using the method of Ouma*et al.* (2016). The weight of crucible was taken and recorded, a known amount of water was measured into the crucible, and the weight was also measured and recorded. The crucible and its contents were heated in an oven at 105°C to dryness. The total solid was determined as follows:

$$\text{Total solid} = \frac{\text{Weight of crucible + dried sample – weight of empty crucible} \times 100/1}{\text{Volume of water}}$$

Total dissolved solid: This was carried out using TDS meter in mg/L.

Total suspended solid (mg/L) was determined as follows:

$$\text{TSS} = \text{TS} - \text{TDS}$$

Determination of biochemical oxygen demand (BOD) and dissolved oxygen (DO): This was determined using the method of Ouma*et al.* (2016). The dissolved oxygen was determined using DO meter. Two hundred and fifty milliliters (250 mL) of the water sample was

poured into 250 mL BOD bottle and incubated at 25° for 5 days. The difference in DO before incubation and after incubation was taken as BOD.

Determination of chloride level of the water sample: This was carried out using the method of Ouma*et al.* (2016). One milliliter of potassium chromate was added into the water sample and titrated with silver nitrate ($AgNO_3$). The titer value generated was used to calculate the amount of chlorine in the water sample.

Initial denaturation	Denaturation temp and time	Annealing temp and time	Extension	No. of circles
94°C	94°C	56 °C	72 °C	36
5 min	30 secs	30 secs		

Table 1: PCR Condition

Determination of nitrate level in the water sample: This was determined using the method of Ouma*et al.* (2016). A required volume of the water sample was measured into an evaporating dish and this was evaporated to dryness. Two milliliters of NaOH and phenol – disulphide acid was added and homogenized. The absorbance was taken at 410 nm. Potassium nitrate (KNO_3) was used as standard.

Determination of phosphate level in water: A total of 50 mL of the water sample was added into a 250 mL. Then 3 drops of phenolphthalein and 0.1 mL of NaCl was added and was boiled for 5 minutes. A few drops of 0.1NHCl was added until the pink color disappeared. One milliliter of the resulting solution was mixed with 2 mL of combined solution and the absorbance was measured at 420 nm using spectrophotometer .

Molecular Identification: Molecular characterization was done at National genetic research and biotechnology, Moore plantation, Ibadan (NAGRAB).

DNA Amplification using Eppendorf Thermocycler

Reaction mixture and volume pipetted: 10 x PCR buffer (2.5 mL), 25 Mm MgCl2 (1.00 mL), 5 pMol forward primer (1.00 mL), 5 pMol reverse primer (1.00 mL), DMSO (1.00 mL), 2.5 Mm dNTPs (2.00 mL), Taq polymerase 5 µ/ µl (0.1 mL), 10 ng/ µl DNA (3.00 mL) and water (13.4 mL).Total reaction volume = 25 µL

PCR product purification

Twenty(20 µl) of absolute ethanol was added to the PCR product and incubated at room temperature for 15 minutes. Spinning was done at 10000 rpm for 15 minutes. The supernatant was decanted and spun at 1000 rpm for 15 minutes. Forty (40 µL) of 70 % ethanol was added and the supernatant was decanted and air dried, then 10 µl of ultrapure water was added and the amplicon was checked on 1.5 % agarose.

Statistical Analysis

The data generated from this study were presented as mean ± standard deviation. The test for significance was carried out using LSD of SPSS software with one way – ANOVA at 95 %.

RESULTS

The result of total mean viable counts of *Salmonella* species in the water samples is presented in Table 2. From the result, Umuaku stream had the highest total mean viable counts of *Salmonella* species in November (16.00 ± 0.14), followed by Umuoma and Aluoha (12.00 ± 0.21) and Umuegwungwu(7.00 ± 0.14), whileAzuOwerri had the lowest counts of *Salmonella* species (1.00 ± 0.00) in February. The total mean viable counts of *Salmonella* species decreased drastically as dry season increases.

Table 2: Total mean viable counts of *Salmonella* species in the stream water samples

Stream	NOV(CFU/mL)	DEC	JAN	FEB
Umuaku	16.00 ± 0.14	11.00 ± 0.48	7.00 ± 0.37	6.00 ± 0.71
Umuoma	12.00 ± 0.21	9.00 ± 0.81	4.00 ± 0.00	4.00 ± 0.00
Aluoha	12.00 ± 0.05	7.00 ± 0.17	3.00 ± 0.00	3.00 ± 0.00
Umuegwungwu	7.00 ± 0.14	4.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.00
AzuOwerri	4.00 ± 0.37	3.00 ± 0.00	3.00 ± 0.00	1.00 ± 0.00

The result of morphological and biochemical identification of the isolates is presented in Table 3. From the result, the five isolates exhibited similar morphology on SSA. The colonies appeared colorless with dark center, rod shaped, raised with entire edge. The result obtained from biochemical reaction showed that all the isolates were motile, gram negative, catalase positive, methyl red positive, oxidase negative, citrate and H_2S positive except R isolate. All the isolates utilized mucate, glucose, D - arabinose and ducitol, while inocitol, xylitol, and sorbitol were poorly utilized by the isolates.

Table 3: Morphological and biochemical identification of the isolates

Parameter	Isolate M	N	P	Q	R
Appearances on SSA	Colorless and dark central	Colorless and dark central	Colorless and dark central	Colorless and dark central	Colorless and dark central
Elevation	Raised	Raised	Raised	Raised	Raised
Edge	Entire	Entire	Entire	Entire	Entire
Shape	Rod	Rod	Rod	Rod	Rod
Gram reaction	–	–	–	–	–
Catalase	+	+	+	+	+
Oxidase	–	–	–	–	–
Motility	+	+	+	+	+
Citrate	+	+	+	+	–
H_2S	+	+	+	+	–
MR	+	+	+	+	+
Mucate	+	+	+	+	+
Glucose	+	+	+	+	+
Inositol	–	–	+	+	–
Ducitol	+	+	+	+	+
Xylitol	+	+/–	+	+	+/ –
D–arabinose	+	+	+	+	+
Sorbitol	+	+/–	+	+/–	+

Key:
SSA= *Salmonella Shigella* Agar, H_2S: Hydrogen sulphide, MR: Methyl red

The result of nucleic acid quality is presented in Table 4. From the result, isolate Q had the highest concentration (166.4 ng/µL), followed by isolate M (122.70 ng/µL), isolate N (99.10 ng/µL), isolate R(77.70 ng/µL) while isolate P had the lowest concentration (48.8 ng/µL). Similarly, isolate Q had the highest absorbance at 260/280 nm (1.89) while isolate P had the lowest absorbance (1.81).

Table 4: Quality of Nucleic Acid used for the study

Sample ID	**Concentration of nucleic acid (ng/µL)**	**Quality at 260/280 nm**
M	122.70	1.82
N	99.10	1.85
P	48.8	1.81
Q	166.4	1.89
R	77.70	1.82

The result of molecular characterization of the isolates is presented in Table 5. From the result, all the isolates were identified and assigned accession numbers. Isolate M was identified as *Salmonella enterica subsp. enterica* Serovar Enterica strain DM 366, isolate N was identified as *Salmonella enterica subsp. enterica* Serovar Enteritidis strain YU39 (SE YU39), isolate P was identified as *Salmonella enterica subsp. enterica* Serovar Typhimurium strain U288 (STU288), isolate Q was identified as *Salmonella enterica subsp. enterica* Serovar Typhimurium strain hydG (ThydG) and isolate R was identified as *Salmonella enterica subsp. enterica* Serovar paratyphi A strain AOA13 (SPAOA13).

Table 5: Molecular characterization of the isolates

Isolate	Description	Max score	Total score	Query cover	E – value	Identity	Accession number
M	*Salmonella enterica subsp. enterica* Serovar Enterica strain DM 366	600	600	100 %	0.00	96 %	NG034789.1
N	*Salmonella enterica subsp. enterica* Serovar Enteritidis strain YU39 (SE YU39)	2844	2844	100 %	0.00	100 %	CP011428.I
P	*Salmonella enterica subsp. enterica* Serovar Typhimurium strain U288 (STU288)	2193	4386	100 %	0.00	98 %	CP003836.1
Q	*Salmonella enterica subsp. enterica* Serovar Typhimurium strain hydG (ThydG)	2183	2421	100 %	0.00	100 %	M64988.1
R	*Salmonella enterica subsp. enterica* Serovar paratyphi A strain AOA13 (SPAOA13)	300	300	58 %	4e-79	85 %	LC056024.1

The result of prevalence and percentage of the isolates in the water samples during dry season is presented in Table 6. From the result, SEFM366 from Umuaku stream had the highest prevalence and percentage occurrence of 9 (5.11 %), followed by SEYU39 from the same Umuaku stream (4(2.27 %)) while STD was only found at Umuaku stream with a low prevalence and percentage occurrence of 1 (0.57 %).

Table 6: Prevalence and percentage of the isolates in the stream water samples during dry season

Isolate	Aluoha	Umuoma	AzuOwerri	Umuaku	Umuegwungwu
SEFM366	6(3.41)	7(3.98)	4(2.27)	9(5.11)	4(2.27)
SEYU39	3(1.70)	2(1.14)	0(0.00)	4(2.27)	1(0.57)
STU288	2(1.14)	2(1.14)	0(0.00)	1(0.57)	1(0.57)
SThydD	0(0.00)	0(0.00)	0(0.00)	1(0.57)	0(0.00)
SPAOA13	1(0.57)	1(0.57)	0(0.00)	1(0.57)	1(0.57)

The result of average physicochemical characteristics of water samples from the five streams during dry season is presented in Table 7. From the result, Umuoma stream recorded the highest pH value of 7.90 ± 0.04 while AzuOwerri recorded the least pH value of 7.56 ± 0.05. However, all the sampled streams showed little variation in pH. Similarly, all the sampled streams had little variation in temperature, though the highest temperature was recorded at Umuoma stream (31.00 ± 0.20°C), while Umuegwungwu stream recorded the lowest temperature (30.18 ± 0.03°C). Highest electrical conductivity was recorded at Umuoma stream (371.25 ± 2.39 (µS/CM)), followed by Umuaku stream (369.75 ± 2.17 (µS/CM), while AzuOwerri

stream recorded the lowest electrical conductivity (331.50 ± 1.57 (µS/CM)).Umuaku stream recorded the highest turbidity (176.25 ± 1.78 NTU), while AzuOwerri stream had the lowest turbidity (143.00 ± 1.60 mg/L). Umuaku stream recorded the highest total suspended solid and total dissolved solid of 148.00 ± 1.52 mg/L and 201.00 ± 2.12 mg/L, respectively. The highest dissolved oxygen was recorded at AzuOwerri stream (2.75 ± 0.02 mg/L) whileUmuegwungwu stream recorded the lowest dissolved oxygen (2.47 ± 0.04 mg/L). Biochemical oxygen demand at Umuoma stream was the highest (199.50 ± 1.22 mg/L), while AzuOwerri stream had the lowest value of BOD (167.00 ± 1.33 mg/L). Umuoma stream recorded the highest nitrate and phosphate value of 7.79 ± 0.30 mg/L and 0.42 ± 0.02 mg/L, respectively while Aluoha stream had the highest value of chloride (45.66 ± 0.35 mg/L).

Table 6: Average physicochemical characteristics of water samples from the five streams during dry season

Parameter	Aluoha	Umuoma	AzuOwerri	Umuaku	Umuegwungwu	WST
pH	7.70± 0.06	7.90 ± 0.04	|7.56 ± 0.05	7.81 ± 0.05	7.60 ± 0.05	6.50-8.5
Temperature °C	30.20 ± 0.10	31.00 ± 0.20	30.90 ± 0.03	30.50	30.18 ± 0.03	30 ±2
Conductivity(µS/CM)	361.25 ± 2.50	371.25 ± 2.39	331.50 ± 1.57	369.75 ± 2.17	345.75 ± 1.96	<400
Turbidity(NTU)	156.00 ± 2.10	171.25 ± 2.39	143.00 ± 1.60	176.25 ± 1.78	151.25 ± 2.39	<5
Total suspended solid(mg/L)	141.50 ± 2.00	145.00 ± 1.59	130.00 ± 1.72	148.00 ± 1.52	136.00 ± 1.56	<500
Total dissolved solid(mg/L)	180.50 ± 2.20	189.50 ± 2.23	160.25 ± 1.86	201.00 ± 2.12	170.75 ± 2.38	<500
Dissolved oxygen(mg/L)	2.52 ± 0.02	2.70 ± 0.031	2.75 ± 0.02	2.62 ± 0.05	2.47 ± 0.04	5
Biochemical oxygen demand(mg/L)	187.50 ± 1.80	199.50 ± 1.22	167.00 ± 1.33	197.50 ± 1.84	178.75 ± 1.73	150
Chloride(mg/L)	45.66 ± 0.35	39.68 ± 0.20	41.90 ± 0.34	44.06 ± 0.36	39.14 ± 0.34	<250
Nitrate(mg/L)	7.42 ± 0.55	7.79 ± 0.30	6.26 ± 0.27	7.51 ± 0.27	7.34 ± 0.3445	
Phosphate(mg/L)	0.31 ± 0.04	0.42 ± 0.02	0.20 ± 0.01	0.38 ± 0.02	0.27 ± 0.02	0.1

Key: NTU= Nephelometric turbidity unity, WST= WHO standard

DISCUSSION

The World Health Organization standard for *Salmonella* species in potable water is zero [7]. The present study revealed the presence of *Salmonella* species in all the sampled streams. It was observed that the water samples from Umuaku stream recorded the highest *Salmonella*count, whereas water samples from AzuOwerri stream recorded the least *Salmonella* count.The high count of *Salmonella* species recorded is mostly attributed to the organic deposits predominantly from human and animal sewage and refuse such as unsanitary habit of sewage disposal, unhygienic attitude of disposing polluted materials as reported by [8]. It was also observed that the total mean viable count of *Salmonella* species decreased drastically as dry season increases. High *Salmonella* counts were recorded during November while low *Salmonella* counts were recorded during February in all the sampled stream (Table 2). The reduction in *Salmonella* count could be attributed to unfavorable environmental condition for bacterial proliferation, which supports the observation made by [11]. The morphological features and Gram reaction of the isolates showed that they are Gram negative bacteria (Table 3). Also, their biochemical characteristics provided a detailed

information such as production of catalase, utilization citrates as source of carbon, mixed fermentation of sugars, sugar salts, and sugar alcohols. The isolates identified as *Salmonella* species agreed with the isolation made by [7], who studied the prevalence of enteric bacteria isolated from water and fish. The nucleic acids extracted from the isolates were within the purity range (1.80 – 1.89), which contributes immensely in ideal molecular characterization of the isolates as shown in Table 4. The molecular characterization of the isolates as shown in Table 5 revealed the presence of five *Salmonella* serovars which corroborate with the findings of many researchers [6]. The high prevalence and percentage occurrence recorded at Umuaku stream could be attributed to high pollution of the stream which enhanced microbial proliferation (Table 6) as also observed by [9]. Several studies had shown that optimal physicochemical parameters of water bodies promote the survival and growth of *Salmonella* species [5]. The slightly basic nature of the sampled streams is within the WHO standard for drinking water(Table 7). Research had shown that pH is one of the parameters that defines water quality, because the solubility of substances that contaminates water is influenced by pH. Similar pH values were obtained by [7] who analyzed physicochemical and bacteriological quality of groundwater (pH=6.22 – 7.85). The temperature of the streams was within the WHO standard, though there was a slight elevation recorded at Umuoma stream. The temperature is favorable for checking excessive turbidity recorded in all the sampled streams as reported by [5]. The observed temperature is capable of encouraging proliferation of pathogenic bacteria such as *Salmonella* species, which reduces water potability [4]. The low dissolved oxygen recorded in all the sampled streams could be responsible for low nitrates observed due to their conversion to nitrites [4]. The sampled streams had moderate electrical conductivity and total dissolved solid, which could be attributed todissolved ions such as $Ca^{2+,}$ Cl^{-}, $Mg^{2+,}PO4^{2-}$, $NO3^{-,}$ and HCO3 [6]. Though some of these ions are needed for strong bone and teeth, yet the streams are unwholesome for human consumption, due to the alkaline content which causes skin and eye irritation as reported by [2]. Nitrates and phosphates found in the sampled streams could have emanated from fertilizer applied on nearby soil, which correspond to the analysis done by [7]. The biochemical oxygen demand (BOD) recorded in all the sampled streams exceeded the WHO standard, which could be attributed to high organic matter in the streams. The microbial decomposition of the organic matter eventually reduced the dissolved oxygen, thereby elevating the BOD level [8]. This discovery shows that the sampled streams, particularly Umuaku and Umuoma have poor quality. Research had revealed that poor quality water is unwholesome for drinking and domestic purposes when there is an inadequate treatment. However, such streams can only be used for irrigation during cultivation of crops as reported by [4].

CONCLUSION

Water is important for daily function of life and knowing the quality of water may help to reduce the incidence of various water – borne diseases and infections transmitted through rivers and streams. This study has shown the presence of *Salmonella* species in five studied streams during dry season, which shows that the water is microbiologically unsterile, and the physicochemical parameters analyzed also revealed the presence of some chemical substances which are capable of jeopardizing human health. Therefore, there is need for adequate control of indiscriminate refuse and sewage disposal in the streams, and adequate treatment is necessary before the streams can be optimized for domestic purposes. However, further research is needed to ascertain the prevalence of *Salmonella* species during rainy season.

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