



The Incidence of *Vibrio Cholerae* as an Indicator of Pollution of Oyun River in Ilorin, Kwara State Nigeria

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ABSTRACT

Bacteria, which are the most common pathogens in water, gain entrance into water mostly through fecal contamination and this poses significant risk to human and animal health. The causative agent of cholera is globally autochthonous to the aquatic environment and it's not confined to only cholera endemic areas. This study is a survey of the microbiological quality of Oyun River and most importantly using the detection and incidence of *Vibrio cholerae* as an additional indicator of pollution of faecal origin. Molecular characterization and confirmation of bacterial isolates were done via Polymerase Chain Reaction (PCR) and DNA sequencing. The microbiological and molecular analysis revealed the presence of *E. coli*, *Streptococcus thermophilus* TH1435, *Vibrio Parahaemolyticus* wp_051483013, *Enterobacter cloacae* strain sugR_1, *Vibrio campbellii* HYO1 *Vibrio cholerae* and *Thermobaculum terrenum* in the Oyun River. Further sequence analysis revealed the presence of six new strains of enteric bacteria which were allocated the following accession numbers after deposition at the GenBank (MT275484; MT275485; MT275486; MT275487; MT275488; MT275489). The confirmation of isolates from the *Vibrio* genus and other enteric bacteria positions that the pollution is of faecal origin and thus, adequate monitoring to control activities along the riverside should be upheld by both the citizens and government authorities to avoid preventable outbreaks.

INTRODUCTION

Pathogenic or potentially pathogenic bacteria (organism) are normally absent from a healthy intestine unless infection occurs (Sharma, 2013). Bacteria, which are the most common pathogens in water, gain entrance into water mostly through fecal contamination and this poses significant risks to human and animal health (Figueras *et al.*, 2000).

Vibrio cholerae which is a "comma" shaped Gram-negative, facultatively anaerobic bacteria with a single, polar flagellum is the most important human pathogen belonging to the genus "Vibrio". Other species that are occasional human pathogens but with similar characteristics to *Vibrio cholerae* include *V. parahaemolyticus*, *V. vulnificus*, *V. mimicus*, *V. alginolyticus*, *V. fluvialis*, *V. furnissii*, *V. metschnikovii* and *V. hollisae* (Huq *et al.*, 2012).

The study revealed that the causative agent of cholera (*Vibrio cholera*) is globally autochthonous to the aquatic environment and it's not confined to only cholera endemic areas (Schuster *et al.*, 2011; Huq *et al.*, 2012). In the year 2000-2005, 18(46%) of the 39 African countries that reported cases of cholera had cases in all 6 years and most high endemicity were found in the Eastern, Southern, Central, and West African countries such as Benin, Burundi, Cameroon, the Democratic Republic of the Congo, Ghana, Guinea, Liberia, Malawi, Mozambique, Niger, Nigeria, South Africa, Swaziland, Togo, Uganda, United Republic of Tanzania, Zambia, and Zimbabwe (Gaffga *et al.*, 2017). Amongst these countries, four reported a high cholera density of about 200 cases/ 1,000,000 people: Mozambique (793/million), Liberia (594/million), Somalia (441/million), and the Democratic Republic of the Congo (242/million). In sub-Saharan Africa, 31(78%) of the 40 countries reported indigenous cases of cholera to WHO in 2005 (Gaffga *et al.*, 2017).

Nigeria is among the three major current cholera foci in the world (Piarroux and Faucher, 2010) and its outbreak in Nigeria and Lake Chad Basin began in 2009 with the first reports from a city in the far northeastern part of the country. Subsequent outbreaks were also reported from distant locales in Northern and Western Nigeria. In 2010, a severe outbreak spread throughout the country and was projected as the worst outbreak in Nigeria since 1991 because it was marked with the highest case fatality (Alagbada *et al.*, 2012). A comprehensive characterization of representative *V. cholerae* strains from sequential outbreaks in Nigeria by Marin *et al.*, 2014 reported that cholera outbreaks in Nigeria are driven by atypical El Tor strains. As at 2014 (30th October), there were about 4,536 cases of cholera which included 70 deaths in Maiduguri (Capital of Borno) with the highest number of cases been reported during week 41(5-11 October) (UNICEF, 2014) while in other locations, 1,040 cholera cases including 18

deaths were reported by 37 local government areas (LGAs) from six states (Bauchi, Kaduna, Kano, Plateau, Taraba, and Zamfara) and 23,324 cases had been reported, including 301 deaths (CFR 1.3%) from 109 LGAs in 18 states. Likewise, in 2013, 11 cases and 1 death was reported around the same period from five LGAs in three states (WHO, 2014).

Oyun river which is known to be natural dumpsite for industrial effluent is also been used for a wide range of activities such as irrigation, fishing and most importantly domestic use. There is a need to evaluate the level of bacteria (pathogenic) present in this river and most importantly the toxigenic *Vibrio cholerae* existing either in the culturable and non-culturable but viable state (VBNC) based on its environmental condition which is known to cause a deadly disease called Cholera.

MATERIALS AND METHODS

Study Design:

This study is a survey of the microbiological quality of Oyun River and most importantly the detection of *Vibrio sp* as an additional indicator of pollution from faecal origin.

Sampling Site:

Oyun river is located on 8° 34' 60' N and 4° 34' 0' E in Kwara state. The sampling sites along the river were selected at strategic segments, assessed to determine the level of activities and tagged A, B, and C for easy identification. These sampling points were underneath the bridge at Unilorin Dam (tagged "A"), Underneath the Oyun River (tagged "B") and underneath the bridge at Jimba Oja (tagged "C").

Population:

The population of people living in towns along the length of Oyun River is estimated to be over 500,000

Ethnicity:

The Yoruba's are predominant and co-exist with the Hausas, Igbo's, Fulani's, Bororo's, Nupe's and migrant workers from other States.

Occupation/Activities:

Activities around the three sampling sites include: farming, cattle rearing, irrigation, washing, bathing, fishing, block making factories, commercial water supply for various municipal uses, drinking, domestic use, dumpsite, bush burning, grass clearing, discharge of sewage effluents and recreational activities.

Climate:

Like other parts of Nigeria, the rainy season is usually between April and November, and the dry season between December and March with annual rainfall varying from 1000mm to 1500mm. Generally, the mean monthly temperature is mostly high throughout the year.

Sterilization of Materials:

All glassware used at all stages of analysis was thoroughly washed with detergent, rinsed properly with distilled water to remove all traces of residual washing compound and properly drained. They were then sterilized in a hot air oven at about 160°C for one hour, according to Fawole and Oso, 2007 procedures, while some autoclavable glass containers were sterilized at 120°C for 15 minutes. Metal instruments and workbench surfaces were properly disinfected by swabbing with cotton wool soaked in 70% ethanol.

Sample Collection:

The water samples were collected in labelled sampling bottles from the different selected segments along Oyun river tagged A, B, and C. The sampling was carried out twice a week for the period of 8 weeks with adherence to the WHO standard of water sampling collection (WHO, 2008). All samples were collected in the early hours of the day (7.00 am – 9.00am) and the capped bottles containing the water sample were protected with aluminum foil to guide against dust and handling which thus reduces the risk of contamination. To collect the river water, the stopper was removed carefully and the bottle filled to near full capacity with the open end against the flow of water. The samples were collected from a minimum of three feet from the bank and at about 50mm

below the water surface. The samples were then immediately taken to the laboratory for analysis. A largely appropriate amount of water sample was collected to increase the chances of a sufficient number of cells for a valid representation of the water. In addition, this also allows for proper sampling of *V. cholerae* which could be in VBNC state (Anwar *et al.*, 2012).

Media Preparation:

All culture media and reagents used in this work were prepared according to the manufacturers' specifications. These media include Nutrient agar (NA), Eosin Methylene Blue agar (EMB), MacConkey Sorbitol agar and Thiosulfate Citrate bile salts (TCBS) agar.

Determination of Physicochemical Parameters of Water Samples Along Oyun River:

Parameters such as Temperature (mercury bulb thermometer), pH (met rohm 632 pH meter), Total Solids (TS), Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Electrical conductivity, Turbidity and Total hardness (Fawole and Oso, 2007).

Bacteriological Analysis:

The total bacterial count was done using the standard plate count methods for the examination of water and wastewater). Total coliform counts (TCC) were carried out via the multiple tube test fermentation technique which is made up of three-stage tests; the presumptive (using MacConkey broth), the confirmed (using Brilliant Green Lactose Bile broth) and the completed (using Eosin Methylene Blue agar) test. The 3:3:3 true test was used and this quantitative coliform test was carried out according to the method of Fawole and Oso, 2007. Furthermore, the multiple tube techniques were also used to enumerate the thermotolerant coliforms but with an increase in temperature from 37°C to 44°C for 24 to 48 hours which is characteristic of their uniqueness as an indicator organism. The pure cultures obtained were then inoculated onto agar slants in McCartney bottles, incubated at 37°C and stored in the refrigerator at 4°C.

Characterization and Identification of Bacterial Isolates:

The identification of Bacterial isolates was based on colonial morphology: (colony colour, shape, size, optical characteristics, edges, elevation, and consistency) which were observed macroscopically on the plates after incubation. Cellular characteristics that involve Gram stain reaction, cell shape, and motility test and biochemical characteristics involving Catalase test, Methyl Red-Voges Proskeur test, Methyl red, Citrate test, Oxidase test, starch hydrolysis test and Triple sugar iron test (Fawole and Oso, 2007).

Molecular Characterization and Confirmation of Isolates:

This was done in three stages namely: DNA extraction/isolation, polymerase chain reaction (PCR) and sequencing of the amplified DNA.

DNA Extraction/ Isolation:

The extraction was carried with the use of QIAamp DNA mini extraction kit (250) (cat no. 51306). Buffer AE was placed into 70°C water bath followed by the addition of 180ul of ATL to the isolate. 20ul of proteinase K was then added and then incubated at 56°C for complete lysis. Shaking heat block was set at 500rpm lysis and completed in 2-3hours. The tube was briefly centrifuged to collect condensation and 200ul of Buffer AL was added and mixed by vortexing for 15 seconds. This tube was incubated at 70° C for 10minutes before being centrifuged again to collect its condensation. Afterward, 230ul of ethanol (96-100%) was added and vortexed for 30seconds. The sample was carefully applied to QIA amp spin column and centrifuged at 6000g for 1minute. The spin column was placed in a clear 2ml collection tube and the filtrate was discarded. This was followed by the addition of 500ul of buffer AW1 and then spun at 6000g for 1minute. This set up was also placed in a collection tube and filtrate discarded. 500ul of Buffer AW2 was added and the previous step repeated (Spinning at 6000g for 1minute and spin placed in a collection tube and filtrate

discarded). The tube was then centrifuged at full speed for 3 minutes and placed in a column labelled 1.5ml tube. 200ul of the preheated (70°C) Buffer AE was added, incubation was carried out at 70°C for 5minutes and then centrifuged at 6000g rpm for 1minute. The filtrate solution (-200ul) was then placed back into the spin column. The previous step was repeated (i.e. addition of 200ul of the preheated (70°C) Buffer AE, incubate the tube at (70°C) for 5minutes and then centrifuge at 6000g rpm for 1minute). The spin-column was finally discarded, and then Agarose and Nanodrop were run.

Polymerase Chain Reaction (PCR):

Polymerase Chain Reaction (PCR) was done using primers for amplification of the bacterial genomic DNA. A cocktail mixture of extracted DNA was made by adding 1µl of PCR buffer, 1 µl of 25 M MgCl₂, 0.5 µl of 5pMol forward primer, 0.5 µl of 5pMol reverse primer, 1 µl of DMSO, 0.8 µl of 2.5MmDNTPs, 0.1 µl of Taq 5, 2 µl of DNA and 3.1 µl of water. The mixture was placed in PCR thermal cycler. The PCR program consisted of an initiation step at 94°C for 5 minutes followed by denaturation step at 94°C for 30 seconds, primer annealing at 56°C for 30 seconds, elongation step at 72°C for 45 seconds, after the 36th cycle being the last one, a final elongation step at 72°C for 7 minutes was accomplished. This was held at 10°C after which the amplicon was then loaded on 1.5% agarose gel.

Purification was achieved via the addition of 20ul absolute ethanol to the PCR product and then incubated at room temperature for 5 minutes. After incubation, it was spinned down at 10000 rpm for 15 minutes and the decantation of the supernatant was done. The Spinning protocol was repeated (10000 rpm for 15 minutes) followed by the addition of 40ul of 70% ethanol and the supernatant was decanted. After decantation, it was left to air-dry and about 10ul of ultrapure water was added and checked for amplicon on 1.5% agarose gel.

DNA Sequencing:

The PCR product was sequenced using Big-Dye Terminators v 3.1 Cycle Sequencing Kit following the instructions provided by the manufacturer. The sequencing reaction was purified and loaded on the 3130xl genetic analyzer from Applied Biosystems to give the sequences.

The sequenced nucleotides obtained were identified on the National Centre for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) Program. The matching sequence with the highest percentage identity was chosen using megablast (that is, a highly similar sequence). The deposition of sequence with disparity to available strains was submitted for evaluation and allocation of an accession number.

Analysis of Result:

The respective results were statistically analyzed to determine significant relationships. This was done via SPSS version 20 and Microsoft Excel package. This computed result was used to give a lucid representation of the study.

RESULTS

The result presented in Table 1 shows the analysis of the water samples collected along the river oyun for the physicochemical parameter. The temperature of the collected water samples across the sample locations were around 29°C and pH slightly above neutral. Total solids, total suspended solids,

electrical conductivity, turbidity, total hardness, and sulphate level were highest in location B and then location A respectively. The bacteriological analysis presented in Table 2 shows the highest bacterial count, total coliform count, and total vibrio count. Location C was noticed to have the highest count, followed by B and then A respectively. Figure 1 shows the respective molecular weight of the amplicons of the isolates from the respective growth media after extraction where four were blank (Lane 2, 4, 5, and 6). Sequence analysis and blast result of the amplicons confirms the presence of *E. coli*, *Streptococcus thermophilus*-TH1435, *Vibrio Parahaemolyticus* wp_0-51483013, *Enterobacter cloacae* strain sugR_1, *Vibrio campbellii* HYO1, *Vibrio cholerae*, and *Thermobaculum terrenum* while six of the analysed sequences was confirmed as new strains of enteric bacteria from genus Enterobacterales. The allocated accession number from the GenBank (NCBI) after evaluation are MT275484 (*Enterobacter cloacae* strain OMIIMMA01); MT275485 (*Erwinia iniecta* strain OMIIMMA05); MT275486 (*Enterobacter cloacae* subsp. *dissolvens* strain OMIIMMA06); MT275487 (*Enterobacter ludwigi* strain OMIIMMA08); MT275488 (*Shigella flexneri* strain OMIIMMA09) and; MT275489 (*Bacillus subtilis* strain OMIIMMA010) respectively.

Table 1: The physicochemical parameter of the selected segment along Oyun River

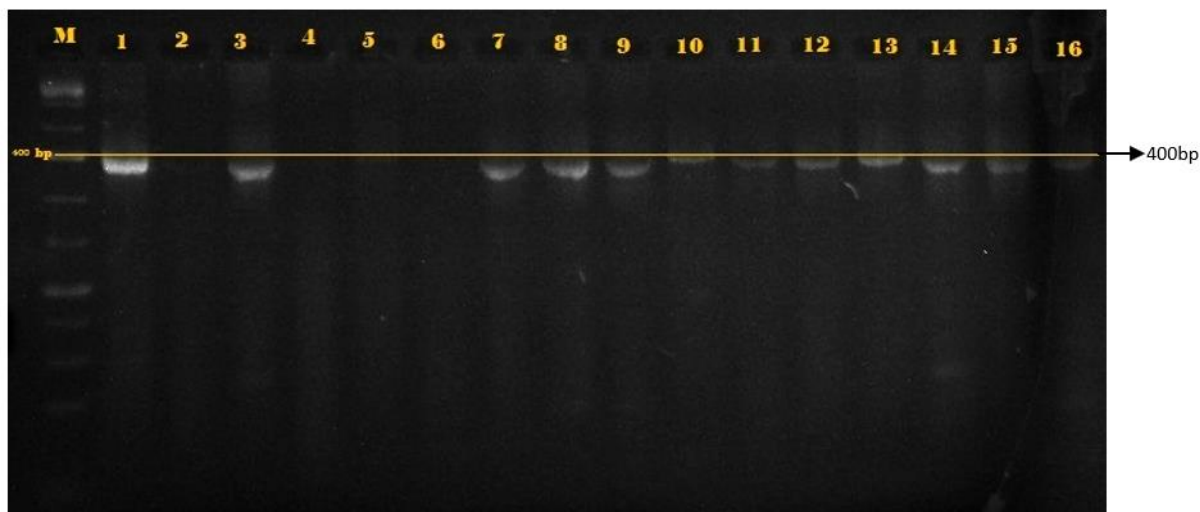
Physicochemical Parameters	Sampling Locations		
	A	B	C
Temperature (°C)	29.60±0.509	29.20±0.583	29.00±0.316
pH	7.76±0.050	7.60±0.089	7.58±0.086
Total Solid (mg/L)	48.55±0.016	50.15±0.016	36.75±0.016
Total Suspended Solid (mg/L)	20.38±0.007	24.05±0.013	16.55±0.009
Total Dissolved Solid (mg/L)	28.17±0.008	26.11±0.004	20.20±0.007
Electrical Conductivity (µs/cm)	132.01±0.006	164.22±0.010	130.65±0.014
Turbidity (NTU)	4.75±0.017	5.73±0.011	4.52±0.009
Total Hardness (mg/L)	42.06±0.024	43.30±0.053	41.54±0.017
Sulphate (mg/L)	12.38±0.009	14.56±0.012	12.32±0.007

NB: A= Unilorin dam; B= Oyun Bidge; C= Jimba Oja. ± = Standard Error

Table 2: The Bacteriological analysis of the collected water samples

Bacteriological Analysis	Sampling Locations		
	A	B	C
Total Bacterial Count 'TBC' (cfu/ml)	79.4±21.554	83±13.023	117.4±30.520
Total Coliform Count 'TCC'(MPN index/100ml)	26.4±3.17	50.0±17.38	105.2±29.52
Total Vibrio Count 'TVC' (cfu/ml)	20.4±5.83	38.8±2.42	93.6±8.00

NB: A= Unilorin dam; B= Oyun Bidge; C= Jimba Oja.
± = Standard Error

**Fig. 1:** PCR product analysis loaded on 2.0% agarose gel at 1kbplus Ladder Gene ruler from Thermo Scientific.

Marker =M

Isolate from TCBS= 1, 2, 5, 6, 7, 9, 11, 12, 15 and 16

Isolate from EMB= 8 and 13

Isolate from Sorbitol= 4, 10 and 14

DISCUSSION

The anthropogenic activities around the sampling site across Oyun River varied across the selected sites. Unilorin Dam which is situated in a tertiary academic area has a monitored level of activity and thus it can be deduced that pollution of faecal origin to this sampling site can mostly be as a result of cross-contamination from other polluted sites along Oyun river and maybe due to the period of this study which was the early part of the rainy season (usually characterized with a heavy concentration of materials as a result of runoff). Location B and C i.e. Oyun bridge and Jimba Oja are on the other hand located amongst populated area and thus pollution as a result of unmonitored anthropogenic activities is expected. This deduction correlates with a study by Kolawole *et al.*, 2011, which stated that different activities affect the water quality in different ways and magnitude.

Based on temperature as a physical parameter for the presence of bacteria (Microbes) in a water environment, three groups of microbes are indicated: the Psychrotrophs, Mesophiles and the thermophiles. The result displayed in Table 1 revealed that the temperature range across the sites supports the growth and survival of most bacteria ($SEM \pm 0.469$). This result is in support of other studies on the river across Nigeria where they had a temperature that is within range of mesophilic bacteria (Kolawole *et al.*, 2011; Asa River, Ishaq *et al.*, 2016; River Benue).

The optimal pH for most bacteria has been stated to be within 6.5-7.5 (neutrophiles) because acidity or alkalinity generally inhibits microbial growth except for bacteria such as *Lactobacillus bulgaricus* (acidophile) and *Agrobacterium* (alkaliphiles). The result showed that the pH across the three locations was slightly above the previously stated standard pH range. This could be attributed to chemicals that might have been washed into the river from farming activity (fertilizer) or waste (domestic and/or industrial) along with the

sampling locations. The recorded pH across the sites favours the growth of most pathogenic human bacteria such as *E. coli* and that of alkaliphiles such as *V. cholerae* and *Alkaligenes faecalis* with optimum pH of 7.6 (WHO, 2016). This result correlates to a study on Asa River by Salaudeen, 2016 and on Benue River by Ishaq *et al.*, 2016. (Table 1)

It was noticed that the total suspended solids in this study are within the acceptable standard range of below 50mg/L for surface water regulation (EPA, 2016). This result correlates to the value of a study by Salaudeen, 2016. However, the high TSS value recorded in the water sample from Oyun Bridge could be due to the increased dilution factor from the increased precipitation and increased water level rise.

The result further revealed that the total dissolved solids which provide information on the quantitative measure of the dissolved ion as an indicator for general quality of water is low compared to other studies on River Benue (Ishaq, 2012), Asa River (Salaudeen, 2016) which had 50mg/L and above. This could be generally due to the lesser anthropogenic activity along the sampled area. The electrical conductivity limit provided by WHO is 500 μ s/cm (Anwar *et al.*, 2012) and this limit was not exceeded by any result from this study. Additionally, this study reveals a low salinity across the sampling area of Oyun River. This is of importance to aquatic vegetation because high salinity which can be measured by electrical conductivity decreases the osmotic pressure.

Water turbidity which can be caused by the presence of clay, silt, organic matter, phytoplankton, and other microscopic organisms were highest in the water sample from Oyun Bridge which is above the permissible standard level of 5NTU (Anwar *et al.*, 2012). This could be due to the fact that during the rainy season, the river receives a large volume of stormwater due to an increased flow rate which picks up material more quickly than in dry season. This result is in line with (Ishaq *et al.*, 2012;

Salaudeen, 2016), which also had high turbidity values that were above the standard. Excessive turbidity can cause problems with water purification and could be an indication of high microbial contamination.

The hardness of water is mainly due to the presence of calcium and magnesium ions and is an important indicator of the toxic effect of poisonous elements. From the presented result, the highest value recorded in Oyun Bridge for water hardness (Table 1). This could be due to the evaporation of water and the addition of calcium and magnesium salts by plants and living organism, it can also be attributed to the regular addition of large quantities of sewage and detergent into lakes from the nearby residential localities.

The sulphate values from the three sampling points range from $12.32 \pm 0.007 \text{ mg/L}$ (Jimba Oja) to $14.56 \pm 0.012 \text{ mg/L}$ (Oyun Bridge). The high values of sulphate may be due to the accumulation of the ion when washed into the river as a result of industrial and domestic activities (Table 1).

The total bacteria count, total coliform and the *Vibrio* count across the sampling period showed the least was recorded in Unilorin Dam while the highest was in Jimba Oja. This trend correlates with the anthropogenic study and physicochemical characteristics of the sites that were earlier presented by this study.

Earlier researches proposed that the extent of contamination by the easily decomposable organic matters is reflected in total bacteria count or the total number of heterotrophic count while the extent of the size of the contamination by faecal substance is reflected in the number of faecal coliforms. The trend of high bacteria count, total coliform count and *Vibrio* count that is noticed in Oyun river which exceeds the stated guidelines by Nigerian Standard of Drinking water quality (anwar *et al.*, 2012) (TCC of 10cfu/mL maximum and thermotolerant coliform of $< 0 \text{ cfu/mL}$) is in correlation with other research by Okonko *et al.*, 2008; Kolawole *et al.*, 2011.

The increase in bacteria load noticeably in Oyun Bridge and Jimba Oja can be attributed to the activities around these areas. During the sampling period, it was noticed that sewage dumping, rearing of cattle's (which cross the shallow areas to drink while defecating along), domestic washing (dirty and likely contaminated clothing's and materials), farming and bush clearing were common around the river and this correlate with a statement in a study by Kolawole *et al.*, 2011 which deduced that refuse dumping, swimming and fishing activity among others is capable of increasing the bacterial load of the river. The abundance of these bacteria load which was significant in the three location along Oyun river could also be due to the wet/ rainy season (which was at its early stage) during which the sampling was carried out because water level generally increases as well as runoffs from farms, market environments and dumpsites which are often offloaded into the river. This deduction correlates to a study in Walkerson, Canada by Connor, 2002 which discovered that an outbreak of *E. coli* related disease was traced to the runoff from contaminated cattle faeces into the borehole drinking water supply in the town.

Faecal contamination which was proved by the presence of faecal coliform bacteria and most importantly by the isolation of *Vibrio cholerae* from the samples is a confirmatory of pollution from human or animal origin and this is line with the study by Okonko *et al.*, 2008 which analysed the microbiological and physicochemical parameter of water samples in Abeokuta.

The significant level of *Vibrio* which is above the permissible standard of zero cfu/ml (WHO, 2008) noticeable in the examined water samples also correlates to a study in Zaria by Bulu *et al.*, 2015 on the isolation and characterization of *V. cholerae* from water sources. Isolation of *E. coli* on MacConkey Sorbitol agar which is specific for strain 0157:H7 commonly found in the human gut further confirms the origin of the pollution in Oyun River. *Vibrio cholerae* and

E. coli are generally found in abundance in (infected) human and animal faeces but could also be found in sewage, effluents, natural water and soils that are subjected to recent faecal contamination (Anwar *et al.*, 2012).

Polymerase chain reaction analysis as presented on plate one revealed that, of the sixteen isolates that were prepared for the protocol, four had no DNA while twelve of the remaining isolate had detectable DNA. Most of the band with DNA that was analysed on the agarose gel had a weight of 400- 430bp which correlates with other similar research that involved PCR confirmation of *Vibrio* spp. such as Cindy, 2011 but this is contrary to the study by Bulus *et al.*, 2015 which had a bp of 208. This difference is however expected due to the different segments of the DNA that could have been detected and analysed. This further emphasizes the role of DNA sequencing which provides detail information about the isolated genetic material. DNA sequence result after a blast on the NCBI search engine confirmed the selected isolates which are mostly from faecal origin.

In Nigeria and in other developing countries, surface waters are an important source of drinking water, agricultural irrigation, recreational and domestic purposes and in support to the result from this study which clearly showed heavy pollution of Oyun River, outbreaks linked to sewage discharge from treatment facilities, animal faeces and runoffs have been reported by Chalmers *et al.*, 2000; Taiwo *et al.*, 2012.

In conclusion, the confirmation of isolates from the *Vibrio* genus and other enteric bacteria such as *Enterobacter cloacae* strain OMIIMMA01, *Erwinia iniecta* strain OMIIMMA05, *Enterobacter cloacae* subsp. *dissolvens* strain OMIIMMA06, *Enterobacter ludwigi* strain OMIIMMA08, *Shigella flexneri* strain OMIIMMA09 and *Bacillus subtilis* strain OMIIMMA010 as deposited at the GenBank posits that the pollution is of faecal origin. Adequate sensitization and monitoring of activities along the riverside

should be upheld by both the citizens and government authorities to avoid preventable outbreaks.

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