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ASSESSMENT OF PHYSICOCHEMICAL QUALITY AND *VIBRIO* ABUNDANCE OF ABATTOIR EFFLUENTS DISCHARGED INTO IKPOBA RIVER, BENIN CITY, NIGERIA

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ABSTRACT
This study investigated the physicochemical quality and *Vibrio* abundance of abattoir effluent discharged into the Ikpoba River, Benin City, Nigeria with a view to determining its possible environmental and public health potentials. Physico-chemical (pH, temperature, turbidity, sulphate, nitrate, phosphate, BOD, TSS, and TDS), microbiological (*E. coli*, coliforms and *Vibrio*) and antibiogram analyses were conducted (using standard methods) on water samples and isolates from the abattoir effluent and its receiving Ikpoba River weekly for six weeks. The mean pH, temperature, sulphate and nitrate concentrations of the water samples fell within permissible limits while other physicochemical parameters such as turbidity, phosphate, BOD, TSS and TDS did not. Total heterotrophic bacterial counts ranged between $1.1\times10^3\pm0.28$ cfu/mL and $1.95\times10^6\pm48$ cfu/mL; mean *E. coli* counts varied from 0.0 to $4.9\times10^5\pm4.9$cfu/mL; and coliform counts ranged from 0.0 to $1.2\times10^6\pm28$ cfu/mL; while *Vibrio* density varied between 0.0 and $1.51\times10^6\pm70$ cfu/mL. Of the 50 selected presumptive *Vibrio* isolates, 9(18%) were confirmed as *Vibrio* spp.; 2 (22.2%) as *V. Vulnificus* and 3(33.3%) as *V. paraheamolyticus* using PCR techniques. The *Vibrio* isolates were resistant to oxacillin (96%), vancomycin (90%), amoxicillin (70%) and tetracycline (40%). The study demonstrated that the abattoir effluents compromised the physicochemical and microbiological qualities of the Ikpoba River and therefore portends serious environmental and public health risks.

KEYWORDS: Abattoir effluent, physicochemical quality, Vibrio, Public health, River water

INTRODUCTION
The disposal of abattoir effluent into natural bodies of water is a public health concern (Black *et al*., 1998), especially in developing countries like Nigeria, where abattoir effluents are discharged untreated. Availability of safe water is key to sustainable development, safe food production, quality health and poverty reduction (WHO, 2019). Rivers are a natural sources of domestic water for riverside communities in many developing countries. Therefore, water intended for human consumption must

not contain harmful chemicals (Balbus and Embrey, 2002). The World Health Organization (WHO, 2019) revealed that seventy-five percent (75%) of all diseases in developing countries arise from polluted waters. Pollution of a river first affects its chemical quality and then systematically destroys the community by disrupting the delicate food web (Joshi *et al*., 2009).

The physico-chemical components of water quality assessment give proper indication of the status, productivity and sustainability of a water body (Mustapha, 2008). Changes in physicochemical characteristics like temperature, turbidity and chemical elements of water such as pH, dissolved oxygen, nitrate, sulphate and phosphate provide valuable information on the water quality, source(s) of variations and their impacts on the functions and biodiversity of the reservoir (Mustapha, 2008). The physical and chemical properties of water immensely influence uses of a water body for the distribution and richness of biota (Unanam and Akpan, 2006). Each factor plays its own role but at the same time the final effect is the actual result of the interactions of all the factors. These factors serve as a basis for the richness or otherwise of biological productivity of any aquatic environment (Imevbore, 1970).

Abattoir waste, like many other wastes can be detrimental to humans and the environment if disposed indiscriminately (Chukwu and Chidiebere, 2011). In a bid to promote hygiene in slaughterhouses, abattoirs use large amount of water in processing operations; thus producing large volume of wastewater (Adonu *et al.*, 2017). This huge amount of wastewater produces obnoxious odour and could serve as nutrients that supports the growth of various pathogenic organisms (Coker *et al.*, 2001; Nafaranda *et al.*, 2006; Osibanjo and Adie, 2007).

Abattoir effluents have been reported to harbour a number of pathogens (including *Vibrio* spp) of public health significance; thus creating opportunity for outbreaks in the population (Hoffmann *et al*., 2010; Hassan *et al.,* 2012; CDC, 2018). Since 2010 several thousands of epidemic cholera outbreaks and non-*Vibrio cholerae* infections involving hundreds of deaths have been reported in Nigeria (Adagbada *et al*, 2012; Osunla and Okoh, 2017). Odjadjare and Igbinosa (2017) also reported that abattoir effluents are important reservoirs for multidrug-resistant *Vibrio* species that might contribute considerably to the recurrent episodes of cholera non-*Vibrio cholerae* infections in Nigeria.

Although epidemiologic surveillance constitutes an important component of the public health response, there is dearth of publicly available surveillance data on physicochemical quality and *Vibrio* abundance of abattoir effluents impacting the Ikpoba River, Benin City, Nigeria. Hence, the aim of this study was to assess the physicochemical quality and *Vibrio* abundance of abattoir effluent discharged into the Ikpoba River, Benin City, Nigeria with a view to determining its potential environmental and public health significance.

METHODOLOGY

Study Area

The study area for this investigation was an abattoir situated around the Ikpoba River at Ikpoba-Okha Local Government Area of Edo State, Nigeria (Coordinates: latitude 06^{o} 29′ N and longitude 05^{o} 22′ E). The Ikpoba River is located in Benin City, Edo State in Mid-Western Nigeria (latitude 06.5^{o} N, longitude 5.8^{o} E) (Okonofua and Oghayafedo, 2019). The River is a fourth order stream, with its headwater originating from North West of Benin City and flows north to south through the city (Benka-Coker and Ojior, 1995). The river flows through a dense rain forest where the allochthonous input of organic matter from the surrounding vegetation is derived through run-off from the surface of the soil. Ikpoba River empties into the Benin River system, the third largest in Nigeria (Ekhaise and Anyasi, 2005). The river serves as a source of water for domestic purpose including drinking, cooking and fishing. The water body receives a variety of wastes ranging from industrial, agricultural, domestic and other sources including abattoir effluents (Ekhaise and Anyasi, 2005).

Sampling

Water samples (1 litre) were collected weekly (for 6 weeks) in duplicates using pre-sterilized containers from four (4) different sites at the abattoir catchment including, abattoir effluent at the point of discharge into a canal linking the Ikpoba River (PS); the canal that carries abattoir effluent to meet the Ikpoba River (CP); 500 m upstream (US) and 500 m downstream (DS) from the point where the canal water makes contact with the Ikpoba River. Figure 1 shows the sampling sites as derived from Google map using a Global Positioning System (GPS). Samples were transported in cooler boxes containing ice packs to the Benson Idahosa University Microbiology Laboratory for analysis. All samples were analysed within 24 h of collection.

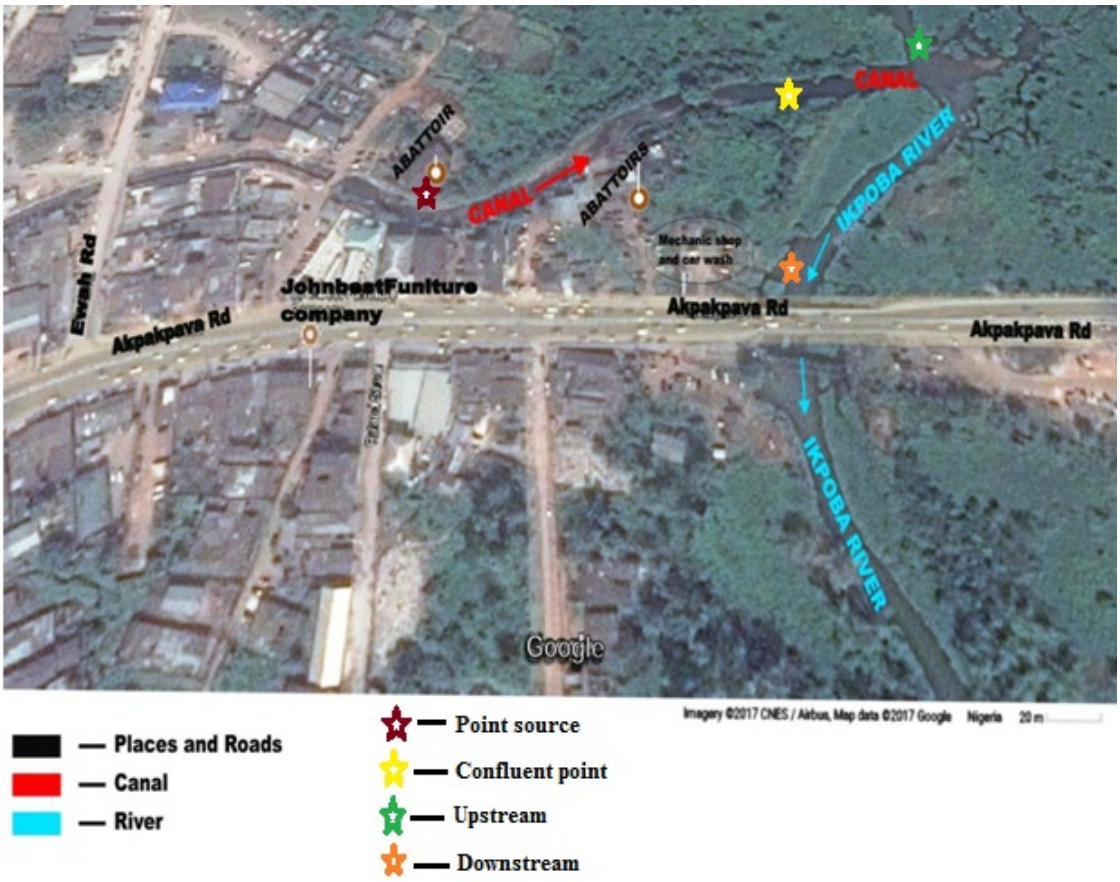

Fig. 1: Images of the abattoir catchment area
Source: www.googlewmaps.com

Physicochemical Analyses

Samples were subjected to physicochemical analyses using standard methods described by APHA (2005). Parameters studied included, pH, temperature, total suspended solids (TSS), total dissolved solids (TDS), phosphate, biological oxygen demand (BOD), nitrate and turbidity.

Isolation, Enumeration and Identification of Bacteria species

Serial dilution of samples was carried out, and aliquots of the diluted samples were inoculated onto appropriate agar plates (nutrient agar (total bacteria count), EMB Agar (*E. coli* counts) and Mackonkey Agar (coliform counts) following the spread plate method for enumeration of bacteria. Isolation of *Vibrio* spp. was carried out using alkaline peptone water (APW, Pronadisa, Madrid, Spain) as enrichment medium. Aliquots of the samples were inoculated into a test tube containing sterile APW and incubated aerobically at 35°C to 37°C for 6 to 8 hrs, before plating onto thiosulphate citrate bile-salt sugar (TCBS) agar for 18 to 24 hrs. Isolates suspected to be *E. coli* appeared as green colonies with metallic sheen on EMB agar; coliforms appeared as pink colonies on MacKonkey agar while *Vibrio* spp. appeared on TCBS agar as golden-

yellow and or greenish-blue colonies. Five to ten *Vibrio* colonies per plate were randomly picked from each sample and sub-cultured onto fresh TCBS agar plates. The pure isolates were subjected to preliminary identification using standard cultural and biochemical methods as described by Kaysner and DePaola (2004). The identity of presumptive *Vibrio* isolates were further confirmed using the PCR technique as described by Odjadjare and Igbinosa (2017).

Antibiotic Susceptibility Test

Susceptibility of presumptive *Vibrio* isolates to antimicrobial agents was performed using the disk diffusion method following guidelines established by the Clinical and Laboratory Standards Institute (CLSI, 2005) and using commercially available antimicrobial disks. A total of 10 antibiotic disks (Oxoid, United Kingdom) commonly used in human therapy were employed in the antibiogram test; they included amikacin (AK, 30µg), amoxicillin (A, 10µg), ceftazidime (CAZ, 30µg), ceftriaxone (CRO, 30µg), gentamicin (GM, 10µg), netilcilin (NET, 30µg), ofloxacin (OFX, 5µg), oxacillin (OX, 1µg), tetracycline (TE, 30µg), and vancomycin (V, 30µg).

Statistical Analysis

Calculation of means and standard deviation was done using Microsoft Excel Office 2010. Correlations (paired T-test) and analysis of variance (ANOVA) were performed using SPSS 23.0 version for windows programme (SPSS Inc.). Correlations and test of significance were considered statistically significant p values of <0.05 or <0.01.

RESULTS

Physicochemical Characteristics of Water Samples

Table 1 shows results of the physiochemical analysis of the water samples during this study. pH value was highest at week 5 (7.035±0.005) in US sample, and lowest at week 1 (5.61±0.01) in US samples. Temperature ranged from 22 °C to 30 °C with lowest record observed in week 3 (CP) and highest value on week 4 (PS). Other parameters varied as follows: BOD (1.03±0.01mg/L (US, week 4) to 35.37±0.265 mg/L (CP, week 1)); turbidity 0.03NTU (US, DS, week 4) to 5262.7±21.9NTU (PS, week 1)); nitrate (0.015±0.002 mg/L (US, week 2) to 21.7±0.04 mg/L (PS, week 2)); sulphate (0.047±0.001 mg/L (US, week 6) to 57.59±0.674 mg/L (PS, week 1)); phosphate (0.002±0.0 mg/L (DS, week 4) to 3.23±0.002 mg/L (PS, week 6); TSS (250±1 mg/L (US, week 4) to 10500±15 mg/L (PS, week 3)); and TDS (7.15±0.05 mg/L (US, week 2) to 103000±23 mg/L (PS, week 1)).

Table 1. Physicochemical properties of the test samples over the study period (Mean ± standard deviation)

Physiochemical parameters	Week 1				Week 2				Week 3				Week 4				Week 5				Week 6				Standards
	PS	CP	US	DS	PS	CP	US	DS	PS	CP	US	DS	PS	CP	US	DS	PS	CP	US	DS	PS	CP	US	DS	
pH	6.395 ± 0.005[b]	6.49± 0.05	5.61± 0.01	5.665± 0.015	6.31 ± 0.01	6.395± 0.005	6.165± 0.015	6.265± 0.015	6.325± 0.005	6.625± 0.005	6.135± 0.005	6.355± 0.005	6.885± 0.005	6.8± 0.01	6.905± 0.045	6.91 ± 0.01	6.93 ± 0	6.955± 0.005	7.035± 0.005	7.03 ± 0	6.885± 0.005	6.905± 0.005	5.725 ± 0.005	5.905 ± 0.005	6-9[a]
Temperature (°C)	26.5 ±0.5	24.5± 0.5	24.5± 0.5	24± 0.1	27.25± 0.25	26.5± 1.5	25.75± 1.75	25.5 ± 1.5	23.25± 0.25	24± 0.2	22.25± 0.25	22.5 ± 0.5	30± 0.05	29.5 ± 0.15	25.5 ± 0.15	25± 0.2	25.5 ± 0.5	27± 0.11	25.5 ± 0.25	25.5 ± 0.35	27.75± 0.25	28± 0.17	25.75 ± 0.25	26.25 ± 0.25	≤25°C[a]
BOD_5 (mg/L)	35.28 ± 0.68	35.37 ± 0.265	1.68± 0.125	2.00 ± 0.1	29.45± 0.05	28.60± 0.2	1.49 ± 0.01	1.89 ± 0.015	29.30± 0	30.03± 0.075	1.80 ± 0	1.30 ± 0	29.66± 0.055	29.61± 0.03	1.03 ± 0.01	1.14 ± 0	31.43± 0.015	33.42± 0.015	1.41 ± 0.01	1.57 ± 0.12	28.61± 0.005	32.78± 0.025	1.63 ±0.015	1.16± 0.02	30 mg/L[d]
Turbidity(NTU)	5262.7± 21.9	4027.7± 37.1	25.0± 2.0	37.1 ± 3.0	4977.8± 0.4	4214.9 ± 0.5	84.6 ± 0.2	115.1± 1.3	4585.7± 0.5	3527.2± 0.3	175.1± 0.8	176.5± 1.3	41.351± 0.000	1.442± 0.037	0.037± 0.000	0.035± 0.002	13.979± 0.497	12.969± 0.004	0.135± 0.001	0.145± 0.001	11.370± 0.090	11.545 ± 0.125	0.180 ± 0.000	0.045 ± 0.01	5.0 NTU[b]
Nitrate (mg/L)	19.818± 0.335	15.573± 0.441	0.044 ± 0.032	0.046± 0.013	21.7 ± 0.042	14.828 ± 0.006	0.015± 0.002	0.026± 0.002	16.865± 0.045	13.138± 0.002	0.130± 0.005	0.171± 0.002	1.472± 0.002	13.639± 0.056	0.073±0.004	0.081±0.002	13.979± 0.497	12.969± 0.004	0.135±0.001	0.145±0.001	11.370± 0.090	11.545 ±0.125	0.180 ±0.000	0.045 ±0.01	10.0 mg/L[c]
Sulphate (mg/L)	57.587± 0.674	46.696± 0.624	3.672 ± 0.840	20.575± 0.215	49.033± 0.020	41.743 ± 0.003	3.573± 0.334	22.442± 0.797	49.392± 0.002	40.008± 0.021	17.133± 0.031	18.636± 0.150	14.742± 0.104	36.470± 0.240	9.590±0.140	10.223± 0.044	3.227± 0.002	1.594± 0.002	0.067± 0.001	0.075± 0.001	3.711± 0.004	1.260± 0.042	0.047 ± 0.001	0.056 ± 0.003	400.0 mg/L[c]
Phosphate (mg/L)	1.728 ± 0.086	1.578 ± 0.080	0.004 ± 0.000	0.007± 0.002	1.328± 0.000	1.479± 0.011	0.015± 0.001	0.026± 0.000	1.518± 0.059	1.421± 0.021	0.056± 0.000	0.056± 0.002	1.472±0.002	1.442±0.037	0.037±0.000	0.002±0.00	3.227±0.002	1.594±0.002	0.067±0.000	0.075±0.001	3.231±0.002	1.260± 0.042	0.047 ±0.000	0.056 ±0.003	0.005 mg/L[b]
Total Suspended Solids (mg/L)	4100± 0.1	4500± 0.1	310± 0.3	500 ± 0.5	9300± 0.4	6100± 0.1	300 ± 0.2	500 ± 0.05	10500± 15	3050 ± 0.15	500 ± 0.1	110 0± 0.11	1270 ±0.1	1340 ± 0.2	250 ±1	280 ± 0.28	2880 ± 0.4	3240 ± 0.01	1640 ± 0.4	3080± 0.31	1410± 0.3	1045± 0.45	1540 ±0.4	1675± 0.17	35mg/L[d]
Total Dissolved Solids (mg/L)	10300 ± 0.23	850± 0.02	800± 0.01	900 ± 0.1	1780± 0.34	1140± 0.14	7.15 ± 0.05	8.75 ± 0.15	1700 ± 0.2	3750 ± 0.15	26.25± 0.65	31.6 ± 0.4	1670 ±130	1850 ± 0.42	14.05± 0.125	16.3 ± 0.3	1150 ±0	1855 ± 0.15	16± 0.0	15.05± 0.35	1331±0.11	1415.5 ± 0.16	13.7 ±0.33	10± 041	450mg/L[c]

Legend: BOD_5 - Biological oxygen demand; [a]DWAF, 1996a; [b]DWAF, 1996b; [c]WHO, 2006; [d]DEA, 2014

Bacterial Counts

Total heterotrophic bacterial counts ranged between $1.1\times10^3\pm0.28$ cfu/mL (US, week 5) and $1.95\times10^6\pm48$ cfu/mL (CP, week 6) (Table 2) while the mean E. coli counts varied from 0.0 (US, weeks 2 – 6; DS weeks 2 - 4) to $4.9\times10^5\pm4.9$cfu/mL (CP, week 6) (Table 3). The highest coliform count ($1.2\times10^6\pm28$ cfu/mL) was observed at CP in week 6; whereas the lowest (0.0) was recorded at DS in weeks 2 and 3 (Table 4). *Vibrio* spp. were not isolated at UP and DS in weeks 2 and 3; however, the highest Vibrio count ($1.51\times10^6\pm70$ cfu/mL) was observed at PS in week 4 (Table 5).

Statistical Analysis

Vibrio count varied significantly ($P<0.05$) with sampling points. Although Vibrio counts in week 6 was significantly different ($P<0.05$) from those of other points (except counts for week 4), there was no significant difference in Vibrio counts for other treatments in relation to sampling weeks. Coliform and E. coli counts did not correlate significantly with Vibrio count. Similarly, total bacterial count (TBC) did not significantly correlate with Vibrio counts; whereas, TBC significantly ($P<0.01$) correlated with E. coli and coliform counts (see supplementary materials.

Antibiotic Susceptibility Profile of Presumptive Vibrio Isolates

The antibiotic susceptibility pattern of presumptive Vibrio isolates is presented in Table 6. Vibrio isolates (50 strains) showed resistance to a broad range of antibiotics including, oxacillin (96%), vancomycin (90%), amoxicillin (70%) and tetracycline (40%). However, the isolates were sensitive to ceftazidime (56%), ceftriaxone (54%) and ofloxacin (42%). No Vibrio isolate was resistant to ofloxacin and neticilin while many were moderately sensitive to these two antibiotics, as well as gentamycin.

Table 2: Mean total heterotrophic bacterial counts of water samples during the six-week study

Sampled points	Total Bacterial Counts (cfu/mL) (mean ± SD)					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Point source (PS)	$1.98\times10^5\pm0.14$	$2.85\times10^5\pm0.7$	$1.97\times10^5\pm0.19$	$1.19\times10^5\pm0.2$	$1.79\times10^5\pm0.15$	$1.67\times10^6\pm0.64$
Confluent point (CP)	$1.16\times10^5\pm0.24$	$1.71\times10^6\pm0.14$	$3.5\times10^4\pm0.2$	$2.3\times10^5\pm0.5$	$2.88\times10^5\pm0.19$	$1.95\times10^6\pm0.48$
Upstream (US)	$4.05\times10^5\pm0.35$	$2.75\times10^5\pm0.4$	$2.6\times10^5\pm0.3$	$1.1\times10^3\pm0.28$	$2.68\times10^4\pm0.36$	$1.63\times10^4\pm0.49$
Downstream (DS)	$5.2\times10^4\pm0.14$	$4.9\times10^4\pm0.21$	$5.6\times10^4\pm0.35$	$2.7\times10^4\pm0.14$	$8.4\times10^4\pm0.2$	$7.1\times10^3\pm0.28$

SD – Standard deviation

Table 3: Mean *E. coli* counts of test samples during the six week study

Sampled points	*E. coli* Counts (cfu/ml) (mean ± SD)					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Point source (PS)	$2.0\times10^4\pm0.7$	$8.0\times10^3\pm0.11$	$6.0\times10^3\pm0.85$	$1.14\times10^4\pm0.22$	$2.9\times10^4\pm0.56$	$1.9\times10^5\pm0.28$
Confluent point (CP)	$5.5\times10^3\pm0.71$	$1.3\times10^4\pm0.14$	$1.4\times10^4\pm0.14$	$7.4\times10^3\pm0.78$	$2.8\times10^4\pm0.49$	$4.9\times10^5\pm0.49$
Upstream (US)	$1.8\times10^4\pm0.42$	0±0	0±0	0±0	0±0	0±0
Downstream (DS)	$3.1\times10^4\pm0.28$	0±0	0±0	0±0	$5.1\times10^4\pm0.53$	$1.13\times10^4\pm0.12$

SD – Standard deviation

Table 4: Mean total coliform count on MacConkey Agar

Sampled points	Total coliform count (cfu/ml) (mean ± SD)					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Point source (PS)	$3.25\times10^4\pm0.19$	$7.15\times10^5\pm0.12$	$5.15\times10^5\pm0.35$	$2.8\times10^5\pm0.85$	$6.0\times10^4\pm0.89$	$1.56\times10^4\pm0.28$
Confluent point (CP)	$3.5\times10^4\pm0.42$	$9.25\times10^5\pm0.7$	$8.1\times10^5\pm0.28$	$8.65\times0^5\pm0.35$	$2.7\times10^5\pm0.49$	$1.2\times10^6\pm0..28$
Upstream (US)	$2.9\times10^4\pm0.14$	$99.5\times10^2\pm0.71$	$3.7\times10^4\pm0.1$	$1.3\times10^3\pm0.1$	$7.65\times10^3\pm0.35$	$1.15\times10^4\pm0.31$
Downstream (DS)	$3.1\times10^4\pm0.14$	0±0	0±0	$7.5\times10^3\pm0.4$	$5.7\times10^4\pm0.42$	$1.39\times10^5\pm0.14$

SD - Standard deviation

Table 5: Mean *Vibrio* count of test water samples during the six-week study

Sampled points	Total *Vibrio* Counts (cfu/mL) (mean ± SD)					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Point source (PS)	$2.09\times10^4\pm0.12$	$1.84\times10^4\pm0.56$	$2.43\times10^5\pm0.63$	$1.51\times10^6\pm0.7$	$2.97\times10^5\pm0.19$	$1.14\times10^6\pm0.63$
Confluent point (CP)	$3.02\times10^4\pm0.18$	$1.46\times10^4\pm0.56$	$8.70\times10^4\pm0.68$	$9.4\times10^4\pm0.56$	$2.70\times10^5\pm0.37$	$2.4\times10^5\pm0.28$
Upstream (US)	$1.48\times10^4\pm0.24$	0±0	0±0	$0.8\times10^1\pm0.42$	$1.05\times10^1\pm0.10$	$3.0\times10^1\pm0.23$
Downstream (DS)	$3.05\times10^4\pm0.21$	0±0	0±0	$0.7\times10^1\pm0.28$	$3.05\times10^2\pm0.42$	$1.1\times10^1\pm0.28$

SD - Standard deviation

Table 6: Antibiotic susceptibility and resistance pattern of the isolated *Vibrio* spp

ANTIBIOTICS	ANTIBIOGRAM Number(%) of strains(n=50)		
	Sensitive	Intermediate	Resistant
Vancomycin	0	5(10%)	45(90%)
Ceftriaxone	27(54%)	17(34%)	6(12) %
Tetracycline	9(18%)	21(42%)	20(40%)
Gentamycin	0	37(74%)	2(4%)
Amikacin	3(6%)	45(90%)	2(4%)
Amoxicillin	15(30%)	0	35(70%)
Oxacillin	0	2(4%)	48(96%)
Ofloxacin	21(42%)	29(58%)	0
Neticilin	3(6%)	47(94%)	0
Ceftazidime	28(56%)	17(34%)	5(10%)

Identification of Vibrio spp

Vibrio species were presumptively identified using cultural, morphological and biochemical characteristics. Out of the 50 presumptive *Vibrio* isolates identified using cultural/biochemical techniques, PCR analysis confirmed 9(18%) as *Vibrio* spp (Figure 2); whereas 2 (22.2%) and 3(33.3%) isolates were confirmed as *V. vulnificus* and *V. paraheamolyticus* respectively (Figures 3 and 4).

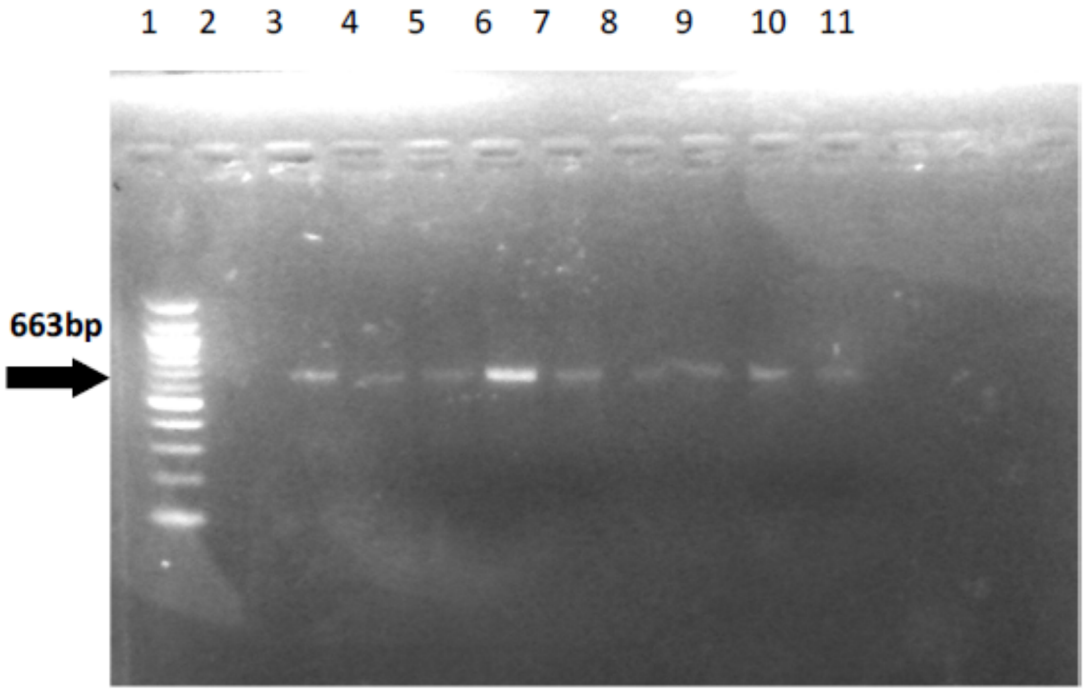

Fig. 2: PCR amplicons of *Vibrio* isolates from abattoir effluents
Lane 1- 1kb DNA Ladder, 2- negative control, Lane 3-11, PCR amplicons for *Vibrio* isolates

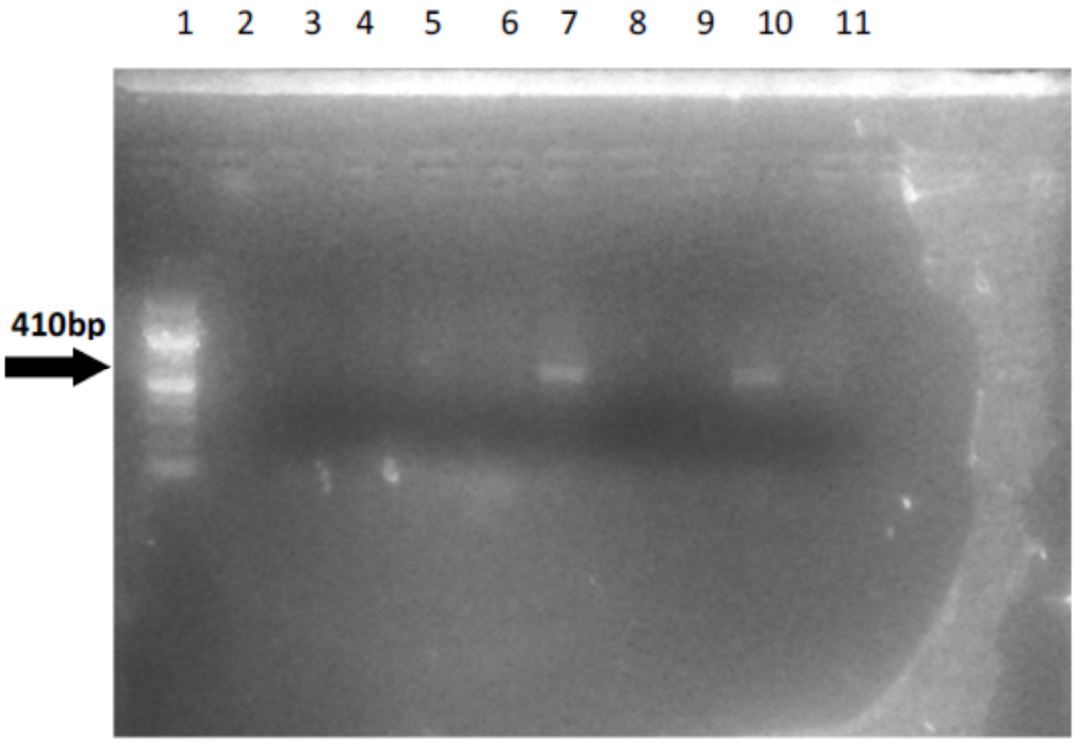

Fig. 3: PCR amplicons of *Vibrio vulnificus* isolates from abattoir effluents
Lane 1- 100bp DNA Ladder, 2- negative control, Lanes 7 and 10, PCR amplicons positive for the target isolates

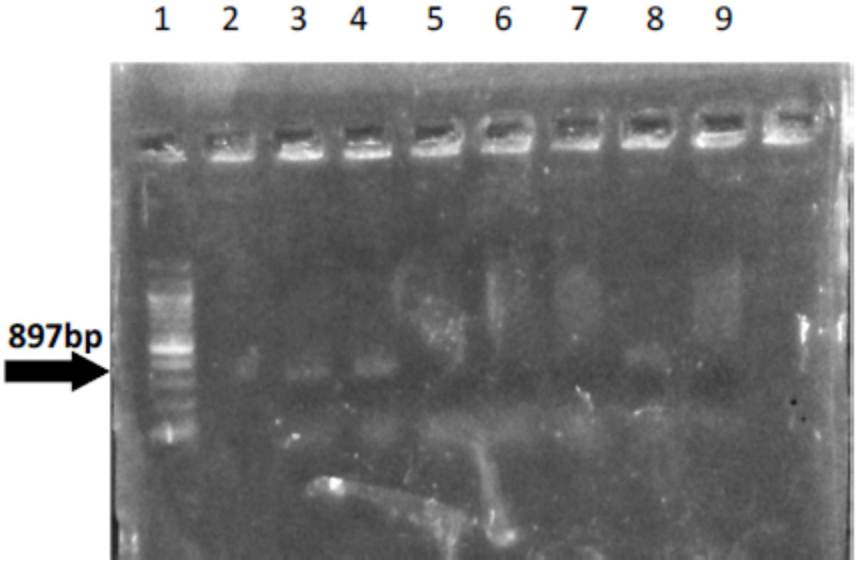

Fig. 4: PCR amplicons of *Vibrio* isolates from abattoir effluents
Lane 1- 1kb DNA Ladder; 2- negative control; Lanes 3, 4 and 8 were confirmed as strains of *Vibrio parahaemolyticus*

DISCUSSION

Ikpoba River is a readily available water resource used for several purposes by local inhabitants while also serving as receptacle for municipal and industrial wastes. Disposing untreated abattoir wastes into the Ikpoba river could derail the quality of the water resource with serious environmental and public health consequences (Hamaidi-Chergui *et al.,* 2013).

The mean pH, temperature, sulphate and nitrate concentrations of the water samples investigated were within the permissible limits (DWAF, 1996a,b; WHO, 2006). The phosphate level was generally higher than permissible limits (0.005mg/L) that would reduce eutrophication in aquatic ecosystems (DWAF 1996b); there are no recommended Nigerian permissible limits on phosphates for discharge of effluents into surface waters. Whereas turbidity and BOD_5 were higher than permissible levels at point source and confluent point for the duration of sampling, the reverse was observed for both parameters at the upstream and downstream sections of the river; indicating that the abattoir effluent was a considerable source of pollution to the receiving water body, in agreement with reports of Omole and Longe (2008). A similar trend was observed for TSS and TDS; further corroborating the impact of the abattoir effluent on the Ikpoba River in agreement with reports elsewhere (Igboanugo and Chiejine, 2012; Ologbosere *et al.,* 2016).

Total bacterial counts (TBC) similar to those observed in this study were reported at Aba and Ebutte rivers (Ezeronye and Ubalua, 2004; Omoigberale *et al*., 2013). Conversely, bacterial counts in this study were higher than those previously reported for Ikpoba, Foma, Ehor, Uke and Orogodo rivers respectively (Agbabiaka and Oyeyiola, 2012; Ihuma *et al*. 2016; Ologbosere, 2016; Esharegoma *et al*., 2018). High bacteria counts at point source and confluent point was indicative of the impact of abattoir effluent as a pollutant to the receiving watershed. However, the reduced bacterial loads at both upstream and downstream could be attributed to the dilution effect on the abattoir effluent before it flows into the Ikpoba River from the canal. This is in agreement with previous studies on the impacts of urban and biological activities on the quality of Ikpoba river (Odjugo and Konyeme, 2008; Ologbosere *et al.*, 2016).

Although coliform and *E. coli* counts did not significantly correlate with *Vibrio* counts, they (total coliform and *E. coli*) were reported as reliable indicator of presence of microbial pathogens of faecal origin in the environment (Kei *et al.*, 2004; Gerardi and Zimmerman, 2005). Coliform population observed in this study exceeded the WHO recommended standard of less than 1 colony forming unit per 100 millilitres (WHO, 1998). The high coliform and *E. coli* populations recorded in this study was also consistent with the observation of Ewa *et al*. (2011) which reported that faecal coliforms are present in water contaminated by human and animal wastes. Furthermore, *E. coli* population in this study were higher than those reported by Edokpayi *et al*. (2013) in Nzhelele River, South Africa, and Maitera and Sudi (2011) who

investigated the coliform levels of some portions of River Gongola. However, the observation of this study is consistent with the report of Ezeronye and Ubalua (2004) on coliform quality of Aba River and river water sources of Venda communities, South Africa; Danube River, Budapest, and Manasbal Lake of Kashmir respectively (Obi *et al*., 2002; Bayoumi-Hamuda and Patko, 2012; Shafi *et al.*, 2013). The observation is generally worrisome because communities that use the Ikpoba river as domestic water resource are exposed to health risks from potentially pathogenic microorganisms such as *Cryptosporidium, Giardia*, *Shigella* and *Norovirus* (Taiwo *et al*., 2014).

The isolation of *Vibrio* spp. at all sampling points in this study suggests that the sampled locations were reservoir of pathogens of potential epidemic importance. Both point source and confluent points maintained high *Vibrio* populations throughout the sampling period; this is indicative of abattoir effluent as a source of potential pathogens to the receiving watershed (Adelegan, 2002). This observation is further corroborated by the significantly ($P<0.05$) higher *Vibrio* densities recorded in PS and CP compared to US and DS. Isolation of *Vibrio* spp. associated with abattoir effluents flowing into the Ikpoba river was previously reported by Odjadjare and Igbinosa (2017). Incidences of *Vibrio* spp. in Ikpoba river were also recently reported in the literature (Akatah *et al*., 2018; Akpe *et al.,* 2018). Although *Vibrio* counts in this study varied significantly ($P<0.05$) with sampling weeks, reports elsewhere suggests a high *Vibrio* population density in Woji river regardless of seasonal variations (Ojesanmi and Ibe, 2012). *Vibrio* counts similar to those observed in this study was reported by Ogbonna (2014) in Ogbu and Trans-Amadi abattoir effluents and their collecting river body (Otamiri River). There was no correlation between *V*ibrio count and coliforms count, and *Vibrio* count versus *E. coli* count; suggesting that coliform and *E. coli* might not always be reliable indicators of the presence of *V*ibrio spp. in water environments contrary to the report of Ashbolt *et al*. (2001).

Vibrio spp. isolated from the investigated samples showed varying levels of sensitivity and resistance to the antibiotics tested. Strains showed resistance to oxacillin (96%), vancomycin (90%), amoxicillin (70%) and tetracyclines (40%) in agreement with reports from Nigeria and other African countries (Materu *et al*., 1997; Opajobi *et al*., 2004; Odjadjare and Igbinosa, 2017). Resistance of some *Vibrio* spp. to antibiotics used in this study has been previously reported (Chikwendu *et al*., 2014); confirming reports of drug resistance among pathogenic bacteria in aquatic environments (Schmidt *et al*., 2000; Hatha *et al*., 2005). Atieno *et al.* (2014) also reported isolation of drug resistant *Vibrio* spp. from abattoir waste in Kenya. Antibiotic resistance is on the increase among bacterial pathogens, posing health risks due to changes in course of treatment (WHO, 1999; Levy and Marshall, 2005). Furthermore, antibiotic resistant bacteria are increasingly occurring in aquatic environments due to increased

antibiotics usage in agricultural industries whose wastes find their way into water bodies; and rivers are the major collecting body (Al-Ghazali *et al*., 1988; Pathak *et al*., 1998). Sensitivity of isolates of this study to ceftriaxone is consistent with the observation of Chiang and Chuang (2003) and Odjadjare and Igbinosa (2017); while sensitivity to ceftazidime (56%) is contrary to the report of Odjadjare and Igbinosa (2017) who documented resistance of *Vibrio* spp. from abattoir effluent to this antibiotic. However, in agreement with observation of this study Opajobi *et al.* (2004) reported strains of *Vibrio* spp. that were sensitive to ofloxacin.

Confirmation of *Vibrio parahaemolyticus* and *V. vulnificus* by PCR gives cause for concern as these species of *Vibrio* have gained global recognition, including in Africa, as causative agents of gastroenteritis (Su and Liu, 2007; Letchumanan *et al*., 2014; Wang *et al*., 2016). *V. vulfinicus* is spread through consumption of partially, undercooked, or contaminated marine products, causing deadly food poisoning which manifests through clinical features distinct from other *Vibrio* spp. (e.g. wound infection and septicemia) (Wang *et al*., 2015). In Nigeria, cases of gastroenteritis were traced to consumption of *V. parahaemolyticus*-contaminated seafoods (Adeleye *et al*., 2010). Adebayo-Tayo *et al*. (2010) also reported high prevalence of *Vibrio* spp. (including *V. parahaemolyticus* and *V. vulfinicus*) in water samples from Oron creek, Uyo. And incidence of *V. parahaemolyticus* and *V. vulfinicus* in seafoods sold in Nigerian cities were reported by Nsofor *et al*. (2010). These seafoods acquire *Vibrio* spp. from their aquatic environments, and spread to the population through the food chain. The study demonstrated that the abattoir effluent investigated was of poor physicochemical and microbial quality and therefore negatively impacted the Ikpoba River in lieu of its environmental and public health significance.

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