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Bacteriology of Opa River, Ile-Ife, Nigeria and its public health implication on selected bordering areas

Onana Vera E.[1*], Afolabi Olusegun T.[2], Aladesanmi Omolara T.[1] and Adepoju Kayode A.[1]

[1]Institute of Ecology and Environmental Studies, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria.
[2]Department of Community Health, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria

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This study determined the bacteriological quality of water from the Opa River and assessed the impact of human behaviour on the quality of water along the river course. Twenty five water samples were collected over a period of five weeks at five selected points based on population clusters along the river. A hundred questionnaire items were administered to randomly selected residents to elicit information about the water body and predominant communal behaviours. Water samples were analyzed by standard microbial techniques and isolates were identified. The isolates obtained were tested for their resistance to antibiotics via standard methods. Descriptive statistical techniques were used to obtain the minimum and maximum values of Total Heterotrophic Bacterial (THB) count and Total Coliform Bacterial (TCB) count. The analysis of variance (ANOVA) test was used to compare the means. Heterotrophic bacteria load at the five sampling points ranged between 2 x 10^6 and 13 x10^6 cfu/ml while the most probable number of coliforms in 100 ml of samples was between 34 and 1600. The organisms isolated from the samples included those of the Genera *Citrobacter Enterobacter Escherichia, Proteus, Klebsiella, Salmonella, Serratia, Yersinia, Shigella, Pseudomonas* and *Vibrio*. *Escherichia coli* had the highest percentage frequency of occurrence (23.73%) and was isolated at all sampling points. In all areas, communal behaviour towards the river was generally poor. Some respondents reported symptoms associated with water borne illnesses which included vomiting (28.5%), passing out of watery stool (57.2%) and headache and dizziness (14.3%). The Opa River was found to be mostly contaminated by multiple antibiotic resistant organisms. Opa River could be a source of potential pathogens associated with water borne diseases and could consequently; serve as a pathway for human contamination, most especially of dwellers located along its banks that depend on Opa River water.

Key words: Pathogens, waterborne disease, contamination, communal behaviours, antibiotic resistance.

INTRODUCTION

There has been noticeable increase in the number of Drinking and recreational water health related publication

*Corresponding author. E-mail: veraonana@gmail.com

between 1980 and 2015. Research productivity on water related diseases from Asia and Africa have equally witnessed an upward increase in the last few years (Sweileh et al., 2016).

Waterborne diseases are rampant in sub Saharan Africa due to lack of access to clean water and poor sanitation. In Nigeria, contamination of drinking water with pathogens has also been reported in several towns (Olowe et al., 2016). Waterborne disease outbreaks occur either when public drinking water supplies are not adequately treated after contamination with surface water or when surface water contaminated with enteric pathogens is used for recreational purpose (Johnson et al., 2003).

In developing countries, the two main water problems man contends with are the quantity and quality of water (Adeniyi, 2004; Olajuyigbe, 2010a). Only about 58 per cent of Nigerians have access to safe water (UNICEF with WHO, 2012). Thus, most households have to resort to drinking water from wells and streams especially in the rural and suburban communities. These water sources are largely untreated and generally harbour pathogens causing waterborne diseases such as cholera, typhoid fever and hepatitis (Rahman et al., 2001; Adekunle, 2004; Fenwick, 2006).

Waterborne diseases are caused by pathogenic microorganisms which are directly transmitted when contaminated fresh water is consumed. Contaminated fresh water, used in the preparation of food, can be the source of water borne diseases. Many rivers, streams and wells worldwide are affected by faecal contamination leading to increased health risks to persons exposed to the water (Simmons, 1994; Obiri-Danso et al., 2009). Pathogenic bacteria that may be associated with faecal contamination include pathogenic strains of *Escherichia coli, Campylobacter species, Salmonella* species*, Shigella* species and *Vibrio cholerae*. In addition to these organisms causing human diseases, resistance to antibiotics has made treatment of the diseases they cause more difficult (Lamikanra and Okeke, 1997; Okeke et al., 2007).

According to the Nigerian Bureau of Statistics (NBS), cholera with symptoms of vomiting, watery stool, dehydration, fatigue, renal failure and occasional muscle cramps has been an extreme burden on Nigerians and often occurs rapidly and progresses to a large scale of outbreak (NBS, 2010).Newspapers in 2011 reported the death of one person and the hospitalization of 20 others following a cholera outbreak in Osogbo, the capital of Osun state. Also in 2012, a cholera outbreak at the Ede north and South Local government areas of Osun State left three people dead and 42 others hospitalized (Moses, 2012).

In Ile-Ife, between 2008 and 2010, scores of water borne disease incidence were reported in various primary health centers across the city. Following the reported cases, a high prevalence of typhoid was indicated with over 1000 cases in the city as well as other water borne diseases like diarrhorea with about 400 cases and dysentery with about 200 cases. However, only few cases of cholera were recorded in Ile-Ife within those periods (Olajuyigbe, 2010b).

This research investigated the bacteriological quality of water from the Opa River and how its specific usage affected public health. The objectives included identifying potential pathogens, evaluating pathogenic load in the river and assessing the prevalence of water borne diseases in the neighbouring communities. All of this was done to understand how the activities of people impacted the bacteriological quality of the water and how the quality of the water in turn affected health in the locations of interest. This would be used to create a model for sensitization of local residents on harmful practices that affect surface water quality and invariably, health.

MATERIALS AND METHODS

Study site

The Opa River's source is in Esa-Oke in Osun-State and flows through many towns and villages before emptying into the Osun River at Asejire. Five different points (Figure 1) along the Opa River which flows through Ile-Ife were purposively chosen for sampling based on the presence of bordering communities along the river banks and the fact that the water from the river along those points was being actively used for domestic activities such as cooking, bathing and washing, small scale industrial activities, recreational activities and agricultural activities. The sampling points were Ajebamidele community, Alakowe area of Ile – Ife, the Abattoir located at Ede road popularly called Odo- Eran and the Saw mill locally called the Iso-Pako or Oke-Opa area.

Data source and sample size determination

Data used for this study were obtained from water samples collected from the five sampling locations along the Opa River as well as the responses of residents along the 500 m buffer zone created using Arc GIS 10.1. This was within the bordering communities along which the water samples were collected. Questionnaires were used to elicit information on communal behavior prevalent in the bordering communities in terms of water usage, water management, waste and sewage disposal methods and the prevalence of water borne diseases in the community.
The sample size was determined using the equation:

Necessary Sample Size = $(Z_{\alpha}^{2}*\sigma^{2})/d^{2}$ for an unknown population size (Smith, 2013), where Z-Score used was 1.645 based on the confidence level of 90%; standard deviation of 0.5 was used and a margin of error (Confidence Interval) of 8% was used.

Sample size = $((1.645)^{2} \times 0.5(1-0.5)) / (0.08)^{2}$
$(2.7060 \times 0.25) / 0.0064$
$0.6765/0.0064=105.7$

Study design and selection of sampling points

The study employed a cross-sectional study design. Five sampling

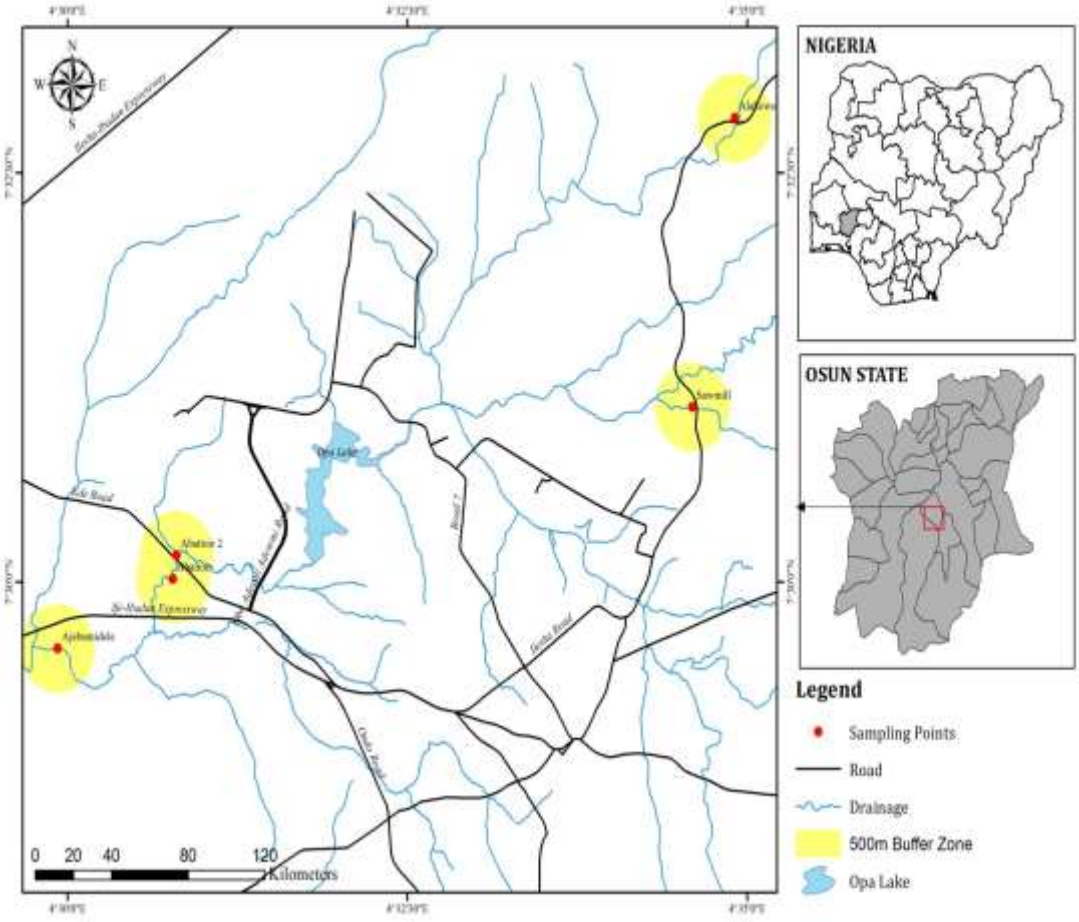

Figure 1. Map of study site showing sampling points and 500 m buffer zone.

points were selected purposively taking into consideration accessibility, availability of human settlements, and anthropogenic activities (domestic, small scale industrial and agricultural activities). The site locations and coordinates of the sampling points (Table 1) were determined using a portable global positioning system (GPS) set.

Sample collection

Twenty five water samples were collected from pre-selected sampling points (Table 1) over a period of five weeks from 25th August 2015 to 22nd September 2015. At each sampling point, sterile specimen bottles were used to collect water for bacteriological analysis. All surface water samples were collected directly by removing the cover cap and with the mouth facing upstream, plunging it downwards below the water surface. It was then slightly tilted upwards for the container to be completely filled with water. The water samples were placed in an insulated cold box and immediately transported to the microbiology laboratory for analysis.

A total of 100 questionnaires were distributed to residents to inquire about water use pattern and waste disposal practices in the study areas. Data from the questionnaires were analyzed using the SPSS statistical tool for descriptive and inferential analysis.

Enumeration of total heterotrophic bacteria

The number of viable bacteria in water sample was estimated based on standard techniques recommended in the Methods for the Examination of Water and Waste Water (APHA, 1998). A ten-fold serial dilution was done to thin out bacterial population in samples. 1 ml of serially diluted water samples was withdrawn and plated aseptically into properly labeled petri dishes. Then, about 20 ml of nutrient agar kept in molten form at 45°C in water bath to prevent heavy condensation was poured over the water sample and mixed properly by tilting and rotating the Petri dish on the work bench to ensure formation of a uniform layer as well as to prevent spilling. This was first done in a clock wise direction, then in an anti-clockwise direction, later to the left and to the right and finally, forwards and backwards to ensure that the microbes were well dispensed in the medium. The agar plates were allowed to set after which the plates were inverted and incubated at 35 ± 2°C for 24 h. After the incubation period, the plates were observed for growth and selected for count. The cultured plates in which the number of colonies fell within the statistically accepted range of number of colonies (that is 30 to 300 COLONIES per plate) and their respective duplicates were selected and counted. The average count per plate was multiplied by the reciprocal of the dilution factor at that dilution and expressed as the number of colony forming units (cfu) per milliliter of the original water sample. This represented the

Table 1. Site description and coordinates of sampling points.

Sampling location Code	Sampling point	Latitude (North)	Longitude (East)	Altitude (Meters)
A	Ajebamidele	7.493194	4.498778	233
B	Ede Road Abattoir	7.500030	4.512915	230
C	After Abattoir	7.502697	4.513340	231
D	Sawmill (Iso-Pako) Oke-Opa	7.51780	4.57722	267
E	Alakowe	7.54722	4.58180	282

viable cell count. Counting of colonies on plate was done manually.

Enumeration of total coliform bacteria

The most probable number (MPN) technique as described by American Public Health Association was employed for bacteria enumeration. The tubes in the first row held 10 ml of double-strength MacConkey broth as presumptive medium while the tubes in the second and third rows contained 10 ml of single-strength presumptive medium. With a sterile syringe, 10 ml of original water sample was aseptically inoculated into each of the five test tubes in the first row and 1 ml of water sample was also inoculated into each of the five tubes in the second row. For the third row of the last set of five test tubes, a 1:10 dilution was carried out on the water sample after which 1 ml of the 1:10 diluted sample was inoculated into each of the five test tubes in the third row. Durham tubes were inserted in all test tubes and corked for possible gas collection. The cultured tubes were carefully agitated to mix and dispense the inocula within the broth medium. They were incubated at 35°C for 72 h. Each tube was observed for microbial growth which was indicated by gas production and cultured media turbidity. The combined numbers of positive tubes in each set of arranged test tubes in order of least diluted to the most diluted was read out from the standard 5 tube MPN table to obtain the estimated number of coliform cells present in 100 ml of the original water sample. Also, positive broth cultures were sub-cultured on MacConkey agar, incubated for 24 h to isolate pure culture of organisms (APHA, 1998).

Isolation and identification of *Vibrio cholerae*

Double strength alkaline peptone water was used as enrichment medium for *Vibrio Cholerae*. After preparation, 5 ml of the double strength alkaline peptone water was dispensed into MacCartney bottles and sterilized at 121°C for 15 min. The peptone water was standardized to the required pH of 8.6 and 5 ml of the water sample to be cultured was then dispensed into each MacCartney bottle containing the sterile double strength enrichment medium using sterile syringe and incubated at 35°C for 24 h. After incubation, sub culture was made on thiosulfate-citrate-bile salts-sucrose (TCBS) agar plates and plates were incubated at 35°C for 24 h. Suspicious yellow colonies were gram stained and tested for motility and oxidase production according to the methods of Theron et al. (2000).

Anti-microbial susceptibility test

Sensitivity of pathogenic bacteria to different classes of antibiotics was assessed by disc diffusion method using ABTEK DT NEG-1 sensitivity discs. Sterile Diagnostic Sensitivity Test (DST) Agar was prepared along with a 0.5 MacFarland equivalent standard of an 18-24 h old pure culture of the test organisms. The standard organism solution (in peptone water) was flooded through the surface of the agar plate and allowed to dry for 15 to 20 min under the laminar air flow to pre-diffuse before sterile Gram negative antibiotics disc containing: Augmentin (30 µg), Ofloxacin (5 µg), Gentamicin (10 µg), Nalidixic acid (30 µg), Nitrofurantoin (200 µg), Cotrimoxazole (25 µg), Amoxycillin (25 µg) and Tetracycline (25 µg) were placed gently on the surface of agar plates with sterile forceps to avoid deformation of the agar. The plates were incubated at 35°C for 24 h, after which the clear zone of inhibition was observed and measured using a millimeter rule. The values obtained were then compared with a recent inhibition standard table and strains were classified as sensitive, intermediate or resistant (Cowan and Steel, 1993).

RESULTS

Bacteriology of the Opa river and distribution of isolates

The bacteriological flora (Table 2) of water samples examined in a period of five weeks along the Opa River comprised a total of 14 distinct species of isolates that were well replicated in almost all of the five sampling points. The isolates belong to the same phylum (proteobacteria) and class (gammaproteobacteria), three different orders and families (Enterobacteriaceae, Pseudomonaceae, Vibrionaceae) as well as eleven distinct genera. They are: *Citrobacter koseri, Enterobacter aerogenes, Escherichia coli, Proteus mirabilis, Klebsiella aerogenes, Citrobacter freundii, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa, Proteus vulgari, Serratia marcescens, Yersinia enterocolitica, Shigella dysentariae* and *Vibrio cholera* and are presented in Table 2 alongside their distribution across various sampling points. Furthermore, water samples from all points were positive for potentially pathogenic organisms (coliforms and some other bacteria capable of causing water borne disease). However, Ajebamidele showed the highest frequency of occurrence for all pathogens except *Vibrio cholera*, *Enterobacter aerogenes* and *Proteus mirabilis* (Figure 2).

Table 2. Frequency/percentage frequency of occurrence of Isolates across sampling points.

Isolated Pathogens	Sampling points						
	Ajebamidele	Ede Road Abattoir	After Abattoir	Oke-Opa	Alakowe	Frequency of occurrence	% F (N=118)
Citrobacter koseri	+	+	+	-	+	6	5.08
Enterobacter aerogenes	+	+	+	-	-	6	5.08
Escherichia coli	+	+	+	+	+	**28**	**23.73**
Proteus mirabilis	+	+	+	-	+	4	3.39
Klebsiella aerogenes	+	+	+	+	+	7	5.93
Citrobacter Freundii	+	+	+	+	+	9	7.63
Klebsiella pneumonia	+	+	+	+	+	7	5.93
Salmonella typhi	+	+	+	+	+	**15**	**12.71**
Pseudomonas aeruginosa	+	+	+	+	+	7	7.63
Proteus vulgaris	+	+	-	-	-	3	2.54
Serratia marcescens	+	-	-	-	-	2	1.69
Yersinia enterocolitica	+	+	-	-	-	3	2.54
Shigella dysenteriae	+	+	+	+	+	**12**	**10.17**
Vibrio cholera	+	+	+	-	-	**9**	**7.63**

- Absent; +, Present.

Table 3. Descriptive statistics of bacteria load at Opa river.

Parameter	THB (Cfu/ml×10^6)	TCB (per 100 ml)
N	25	25
Min	2	34
Max	14	1600
Mean	6.68	969.36
Std. Error	0.76751	126.05611
Standard deviation	3.83753	630.28055
Skewness	0.584	-0.039
Kurtosis	-0.941	-1.979

THB, Total Heterotrophic Bacterial Count; TCB, Total Coliform Bacterial Count; Min, Minimum Value; Max, maximum value.

Table 4. ANOVA Statistics for Variation across Sampling Points of THB and TCB Counts in all sampling periods.

Count	N	Mean±SD	F	$P_{0.05}$
THB	25	6.68±3.83753	6.973	0.001**
TCB	25	969.36±630.28055	2.067	0.123

THB, total heterotrophic bacterial count; TCB, total coliform bacterial count.

Bacteriological load of the Opa River

Total heterotrophic bacterial (THB) count

The total heterotrophic bacteria (THB) count (Table 3) in Opa River ranged from 2×10^6 to 14×10^6 cfu/ml with a mean of 6.68×10^6 cfu/ml and a standard deviation of 3.84×10^6. The highest THB of 12×10^6 to 14×10^6cfu/ml count was recorded at Ajebamidele in all sampling periods. There was significant difference ($P<0.05$) in the total heterotrophic bacteria count across the various sampling points (Table 4).

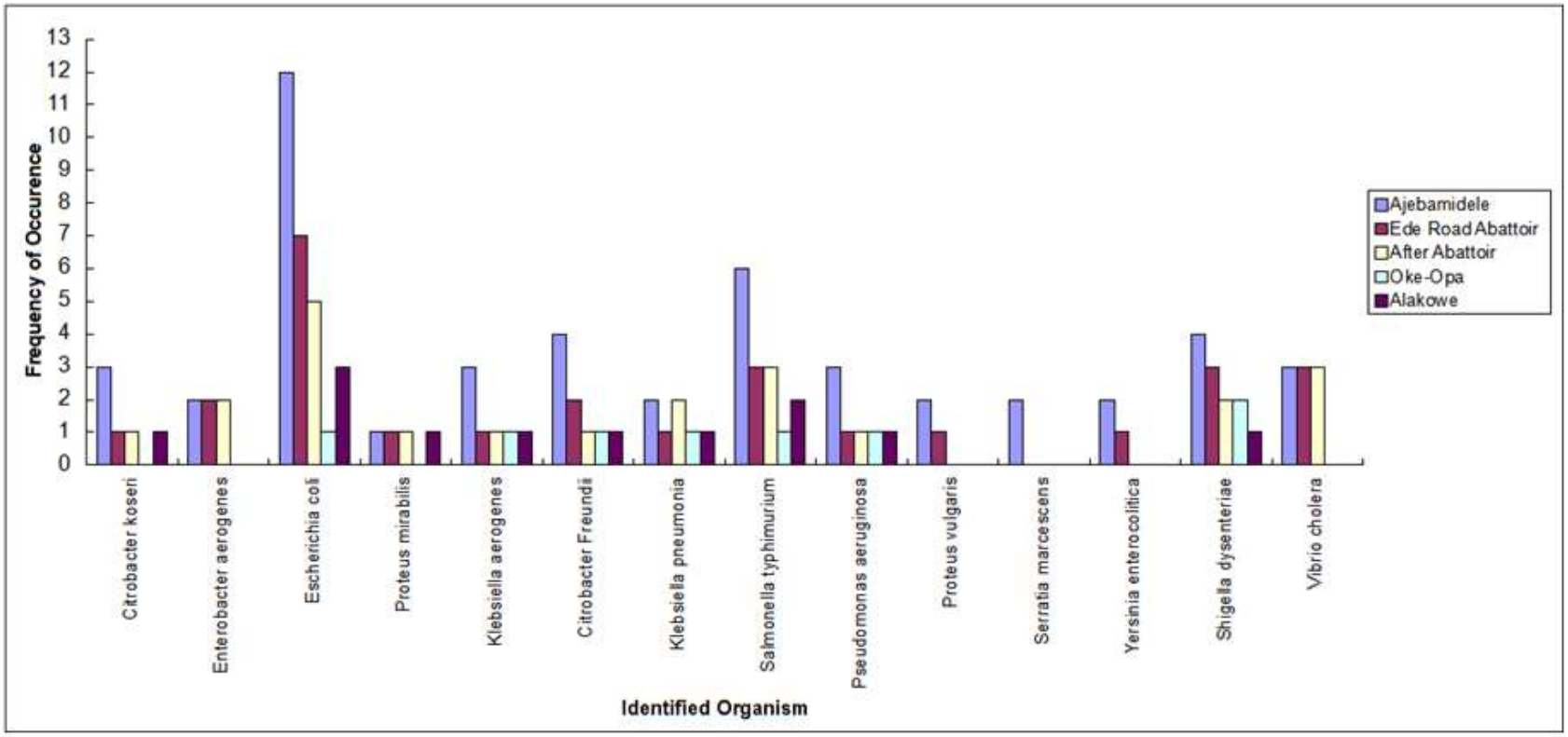

Figure 2. Frequency of Occurrence of Isolates along Sampling Points.

Total coliform bacterial (TCB) count

The total coliform bacteria (TBC) count (Table 3) in Opa River across all points of sample collection ranged from 34 to 1600 per 100 ml of water sample analyzed with a mean of 969.36 and standard deviation of 630.28. The highest TCB count was recorded at Ajebamidele in all sampling periods although there was no significant difference (P>0.05) in total coliform bacteria count across the various sampling points (Table 4).

Water usage at the bordering areas

Water usage practice across the bordering areas was queried (Table 5). While 100% of respondents claimed not to engage in direct use by drinking, a majority of them (Ajebamidele, 70%; Ede Road Abattoir, 50%) attested to the fact that water from Opa River was used for domestic, agricultural, recreational, industrial and even spiritual purposes.

Waste and sewage management practices at bordering areas

Self-collection was the predominant form of waste collection in all the areas bordering Opa River that

Table 5. Water usage at the bordering areas.

Variable	Options (N=20)	Sampling Points										ANOVA	
		Ajebamidele		Abattoir		After Abattoir		Oke Opa		Alakowe		F	$P_{0.05}$
		F	%	F	%	F	%	F	%	F	%		
Is Opa water used?	Yes	14	70	10	50	6	30	7	35	5	25	7.755	0.001
	No	6	30	10	50	14	70	13	65	15	75		

Table 6. Waste and sewage practices at bordering areas.

Variable	Options	Sampling Points										ANOVA	
		Ajebamidele		Abattoir		After Abattoir		Oke Opa		Alakowe		F	$P_{0.05}$
		F	%	F	%	F	%	F	%	F	%		
Who collects the waste from your household?	Private waste collection	0	0	0	0	0	0	0	0	0	0	8.143	0.001
	Self-disposal	14	70	20	100	20	100	20	100	20	100		
	Government	6	30	0	0	0	0	0	0	0	0		
	Other means	0	0	0	0	0	0	0	0	0	0		
Alternative means of waste disposal	Water body	13	65	20	100	20	100	20	100	20	100	10.23	0.001
	Burning	7	35	0	0	0	0	0	0	0	0		
What system of sewage disposal is employed in your household?	Pit latrine	6	30	16	80	15	20	0	0	4	20	2.701	0.035
	Water closet system	2	10	4	20	5	80	20	100	13	65		
	None of the above	12	60	0	0	0	0	0	0	3	15		
If none, how is sewage disposed by you?	Water body	12	60	0	0	0	0	0	0	3	15		
	Buried	0	0	0	0	0	0	0	0	0	0		

were investigated (Table 6). In all bordering areas, 100% of respondents indicated that wastes were self disposed except for Ajebamidele. The major method of waste and sewage disposal was by dumping them into the Opa River as it was the most convenient form of disposal for respondents who live along the River bank. There was also significant difference ($P<0.05$) in waste collection methods, waste disposal methods and sewage disposal methods across all bordering areas.

Communal behaviour towards the Opa River

The communal behavior of occupants at the bordering areas towards the water body was generally poor (Table 7). It was observed that solid wastes, sewage and urine were indiscriminately thrown into the water body. In addition, agricultural wastes and waters from the local oil producing industry were dumped into the water body at Ajebamidele and Ede Road Abattoir while animal dung and other wastes were thrown into the Opa River. The river is also being used for spiritual baths and cleansing at Ajebamidele and Alakowe respectively.

Period prevalence of water borne disease at bordering areas and antibiotic susceptibility pattern of isolated bacteria to test antimicrobial agents

About 35% of the respondents at Ajebamidele experienced different symptoms related to water borne diseases. Five out of 7 of the respondents experienced the symptoms in a period of less than a week before the investigation was conducted while 2 suffered from such symptoms less than a month before the questionnaires were retrieved.

The antibiotic susceptibility pattern of bacteria isolated and identified at the Opa River was tested using eight antibiotics (Table 8) namely, Augmentin, Oflaxacin, Gentamicin, Nalidixic acid, Nitrofurantoin, Amoxicillin, Tetracycline and Cotrimazole (Table 8). Coliforms and other bacteria capable of causing water borne diseases

Table 7. Communal Behaviour towards Opa River at the bordering Areas.

Variable	Options	Sampling Points									
		Ajebamidele		Abattoir		After Abattoir		Oke Opa		Alakowe	
		F	%	F	%	F	%	F	%	F	%
Children play in it?	Yes	20	100	20	100	20	100	20	100	20	100
	No	0	0	0	0	0	0	0	0	0	0
People urinate and excrete at the bank?	Yes	20	100	20	100	20	100	20	100	20	100
	No	0	0	0	0	0	0	0	0	0	0
Is domestic waste thrown into it?	Yes	20	100	20	100	20	100	20	100	20	100
	No	0	0	0	0	0	0	0	0	0	0
Is agricultural waste thrown into it?	Yes	20	100	20	100	20	100	20	100	20	100
	No	0	0	0	0	0	0	0	0	0	0
Do people bath/swim in it?	Yes	20	100	20	100	20	100	20	100	20	100
	No	0	0	0	0	0	0	0	0	0	0
Is it used for spiritual cleansing	Yes	20	100	0	0	0	0	0	0	20	100
	No	0	0	20	100	20	100	20	100	0	0

Table 8. Antibiotic susceptibility pattern of isolated bacteria to test antimicrobial agents.

Bacteria	MAR pattern	Number of Antibiotics Showing Bacteria Resistance	Percentage resistance of isolated bacteria
Salmonella typhi	AUG, NAL, NIT, AMX, TET, COT	6	75
Proteus vulgaris	AUG, NAL, NIT, AMX, COT	5	62.5
Shigella dysentariae	AUG, OFL, GEN, NAL, NIT, TET	6	75
Klebsiella pneumonia	AUG, NAL, NIT, AMX, TET, COT	6	75
Pseudomonas aeruginosa	AUG, AMX, TET, COT	4	50
Citrobacter freudii	AUG, NAL, NIT, AMX, TET, COT	6	75
Serratia marcescens	AUG, NAL, AMX, TET, COT	5	62.5
Yersinia enterocolitica	AUG, OFL, GEN, NAL, NIT, AMX, TET, COT	8	100
Escherichia coli	AUG, OFL, GEN,AMX, TET,	5	62.5
Klebsiella aerogenes	AUG, OFL, GEN, NAL, NIT, AMX, TET, COT	8	100
Vibrio cholerae	AUG, OFL, GEN, NAL, NIT, AMX, TET, COT	8	100

AUG-Augmentin; OFL, Ofloxacin; GEN, gentamicin; NAL, nalidixic acid; NIT, nitrofurantoin; AMX, amoxycillin; TET, tetracycline; COT, Cotrimazole; R, resistant; S, sensitive. Percentage (%) =f/N, where N = 8 (total number of antibiotics used) and f represents frequency of resistance per isolate.

(*Salmonella* Typhimurium 75%, *V. Cholerae* 100%, *K. aerogenes* 100%) were more resistant than sensitive to the antibiotics used.

Comparison of THB and TCB Counts to WHO and NIS Permissible Standards

THB and TCB counts of the twenty five water samples were compared to the World Health Organisation Permissible levels as well as the Nigerian Standard for drinking water quality permissible levels as presented in Tables 9 and 10.

DISCUSSION

All identified potential pathogens were either coliforms, which are indicators of feacal contamination, or other potentially pathogenic bacteria capable of causing water

Table 9. THB Count in Opa River in Comparison with the WHO and NIS Permissible Levels.

Sampling Periods	Ajebamidele	Ede Road Abattoir	After Abattoir	Oke-Opa	Alakowe	MPL (NIS 2007)	MPL (WHO 2007)
SP 1	12	5	7	2	4	10	100
SP 2	13	4	3	5	5	10	100
SP 3	10	9	3	6	6	10	100
SP 4	14	2	12	5	5	10	100
SP 5	13	4	2	7	7	10	100

Table 10. TCB Count in Opa River in Comparison with the WHO and NIS Permissible Levels.

Sampling Periods	Ajebamidele	Ede Road Abattoir	After Abattoir	Oke-Opa	Alakowe	MPL (NIS 2007)	MPL (WHO 2007)
SP 1	1600	1600	1600	34	540	0	1
SP 2	1600	540	540	540	350	0	1
SP 3	1600	540	540	1600	1600	0	1
SP 4	1600	350	180	350	350	0	1
SP 5	1600	1600	180	1600	1600	0	1

borne illnesses. This agrees with the work of Abdu et al. (2013) who also reported the contamination of Opa River by coliforms and other pathogens after microbiologically analyzing water from five locations along Opa River.

E. coli, as observed in this study showed the highest frequency of occurrence (23.73%) across the five sampling points. The high occurrence of *E. coli* in water samples at all points indicates recent and probably continuous fecal contamination of the water from River Opa by human activities and unhygienic behaviours of people living at the river banks which were identified by questionnaires specifically as excreting and urinating by the river bank due to lack of proper sewage disposal methods in the bordering areas. Ihejirika et al. (2011), in a similar study, attributed the isolation of *E.coli* in relatively high percentages from the Imo River to human activities. *S. typhi* showed the second highest frequency of occurrence (12.71%) across all sampling points. it is also corroborated by the findings of Arvanitidou et al. (2005) in Northern Greek waters that were used for recreational purposes.

V. cholerae was isolated from 8% of the water samples and its presence in water samples may have been due to animal contamination from birds, frogs, toads, crabs and fishes usually present in aquatic environments. The pathogen may have been introduced into the water from human feaces due to defecation along the river bank by dwellers of the bordering areas as reported by Ali et al. (2001). The presence of *Shigella spp*. (10.17%) in water samples might be due to the unsanitary condition of the areas bordering the Opa River most especially in Ajebamidele and Ede Road Abattoir and secondary fecal contamination from intermediary sources. Ihejirika et al. (2011) reported a 100% isolation of *Shigella spp* from water sources in Ahiazu Mbaise and the implication of such result was reported as a possible outbreak of shigellosis Emch et al. (2008).

The occurrence of *Citrobacter spp*, *Klebsiella* spp., *Enterobacter* sp. and other members of the family enterobacteriaceea in water samples from Opa River indicates further feacal contamination of the river as these pathogens are normally found in the gastrointestinal tracts of humans. While investigating the possible diarrheal disease potentials of water sources in Ahiazu Mbaise, Eastern Nigeria, Esomonu et al. (2012) identified similar organisms and reported their ability to cause infections in humans. All the pathogens isolated showed a pattern of multiple antibiotic resistance to the common antibiotics used in the susceptibility testing. *S. typhi* for instance recorded a 75% resistance to the following antibiotics: Augmentin, nalidixic acid, nitofuratoin, amoxicillin, tetracycline and cotrimazole. Isolated *V.cholerae* also showed a 100% resistance to all antibiotic used.

The pathogenic load recorded at each sampling point exceeded the World Health Organisation (WHO) and Nigeria Industrial Standard (NIS) permissible levels. Across all sampling points at all sampling periods, THB count showed a mean value of 6.68×10^6 cfu/ml of water sample analysed while TCB count, showed a mean value of 969.36 per 100 ml of water examined. These values extremely exceed the Nigerian standard for drinking

water quality (NIS 2007) and the WHO (2007) recommended limits for potable water. The Nigerian standard for drinking water recommends that the maximum permitted level (MPL) for human drinking water must not exceed 10 cfu/ml for heterotrophic bacteria count, 0 cfu/100 ml for total coliform count and 0 cfu/100 ml for *E. coli*, feacal *Streptococcus* and *C. Perfringes* spores, respectively (NIS, 2007). Also, freshwater quality criteria for domestic supply require that faecal bacteria levels should not exceed a geometric mean value of 100 cfu/100 ml while the drinking water criterion is 1 cfu/100 ml (WHO, 2001).

A high THB count is generally regarded as an indicator of poor biological quality of water (USEPA, 2003). The high levels of faecal bacteria recorded in this study as well as the presence of pathogens in the river clearly indicate that the Opa River receives faecal contaminants on a continual basis. This may be due to the fact that its tributaries are highly impacted by commercial, semi-industrial and domestic activities but the Ajebamidele and Ede Road tributaries may be considered as the most impacted due to direct sewage discharge from most homes in the Ajebamidele community into the river body and waste discharge from the dressing of carcasses at the slaughter house at the Ede Road Abattoir respectively.

All of these practices may predispose members of the bordering areas to water borne illnesses. However, members of the Ajebamidele community may be more vulnerable due to the fact that the highest pathogenic load values were recorded at Ajebamidele and most isolated coliforms and other pathogens capable of causing water borne diseases like *E.coli, Salmonella typhi* and *Shigella* spp. showed highest occurrence frequency at Ajebamidele than the other four bordering areas. This, coupled with the water usage system obtainable at Ajebamidele may make dwellers more vulnerable to water borne diseases than in the other four areas. Also, symptoms related to water borne illnesses were only observed at Ajebamidele. This is substantiated by the findings of Nwidu et al. (2008) which reported the frequent diagnosis of cholera, dysentery, typhoid fever and diarrhea in the Niger Delta region of Nigeria due to high levels of feacel contamination in River Amassoma.

Conclusion

Based on the findings from this study, it could be said that River Opa, which runs through many rural and semi-urban communities in Osun State, is contaminated with a wide array of antibiotic-resistant bacteria and potential pathogens that could predispose its users to water borne diseases. Therefore, water from the river may be unsuitable for domestic engagements without appropriate treatment measures as it could serve as a pathway for human contamination, most especially to dwellers located along its banks.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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