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FULL LENGTH ARTICLE

Microbial assessment and prevalence of antibiotic resistance in polluted Oluwa River, Nigeria

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Abstract Antibiotics are emerging environmental contaminants, causing both short-term and long-term alterations of natural microbial communities due to their high biological activities. The antibiotic resistance pattern of bacteria from anthropogenic polluted Oluwa River, Nigeria was carried out. Microbial profiling and antibiotic sensitivity tests were carried out on water and sediment samples using 13 different antibiotics. Microorganisms isolated include those in the genera *Bacillus*, *Micrococcus*, *Pseudomonas*, *Streptococcus*, *Proteus* and *Staphylococcus*. The microbial count of isolates from water samples ranged between 94.10×10^2 Cfu/100 ml and 156.20×10^2 Cfu/100 ml while that of sediment samples ranged from 2.55×10^4 Cfu g^{-1} to 14.30×10^4 Cfu g^{-1}. From the water isolates, 100% resistance to antibiotics was found in *Micrococcus* spp. and *Pseudomonas* spp. while another *Micrococcus*, *Streptococcus*, *Staphylococcus* and *Bacillus* spp. showed between 40% and 90% resistances. From the sediment isolates, 100% resistance to antibiotics was found in a *Bacillus* spp. and *Pseudomonas* spp. while another *Bacillus*, *Micrococcus*, *Staphylococcus*, *Streptococcus* and *Proteus* spp. showed between 70% and 90% resistances. Multiple antibiotic resistance (MAR) was shown by all the isolates and *Bacillus*, *Micrococcus* and *Pseudomonas* spp. showed the highest resistances (100%) to all antibiotics. Thus, Oluwa River is not safe for public consumption.

Introduction

Antibiotics have over the decades been used for both human and animal disease treatment. They are however continuously found in the environment due to poor metabolism in the body. It is not yet clear and arguments among scientists increase daily about the involvement of man and his many anthropogenic activities in the spread of resistance elements in microorganisms. Several studies have reported lack of tangible relationship between anthropogenic activities and antibiotic resistance in bacteria and many believe that the elements that selects for resistance are naturally present within microbial genome (Davis and Anandan, 1970; Hughes and Datta, 1983; Barlow and Hall, 2002; Hall and Barlow, 2004; D'Costa et al., 2006, 2011; Wright, 2007, 2010; Baltz, 2008; Brown and Balkwill, 2009; Thaller et al., 2010; Toth et al., 2010; Bhullar et al., 2012; Cox and Wright, 2013). On the other

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hand, evidence abound that increased bacterial resistance to antibiotics and the transfer of resistance elements is a modern phenomenon having a strong link with anthropogenic activities (Knapp et al., 2010; Bhullar et al., 2012).

Besides the human health risks posed by the presence of antibiotic resistant bacteria in the environment, and the unwanted presence of antibiotics in water bodies, concern for the ecological fate and environmental threat of these drugs in the aquatic milieu is becoming a global phenomenon (Kümmerer, 2009). Bacterial resistance to antibiotics has been considered as a global public health menace. Different kinds of antibiotic resistant bacteria (ARB) are continuously detected in various environments ranging from aquatic to terrestrial ones. There is high possibility of resistance being spread by ARB from the environment to related human pathogenic microorganisms through numerous routes thereby suppressing the effectiveness of antibiotics (Threedeach et al., 2012).

A global strategy has been proposed by the World Health Organization to contain antibiotic resistance regarding its potential threat to both public and environment health (Pruden et al., 2006). Because of the high microbial biomass and abundant nutrients, as well as various antimicrobial agents, polluted water bodies represent a favorable habitat for both the survival of ARB and the transfer of antibiotic resistance, from where they spread resistant bacteria into subsequent aquatic and terrestrial environments (Bouki et al., 2013). Various ARB including multiple antibiotic resistant bacteria, have previously been encountered in a large number of water systems (Luczkiewicz et al., 2010).

Water pollution and reduction in quality is a major contributor to global freshwater scarcity, stressing the need for more integrated water management and monitoring (Dahunsi et al., 2014). Microbial and sediment pollution have been documented to be significant concern for rivers and streams and pathogens have also been known to impair or threaten more kilometers of water bodies than any other aquatic pollutant. In the same vein, bacterial pollution of water can result in unsafe drinking water, restrictions on recreation opportunities, and closures of shellfish beds (US EPA, 2010).

Sediment contamination has been reported to be the second leading cause of impairment to water bodies according to US EPA (2010), and this is because suspended sediment can directly impact aquatic organisms and can also increase water treatment costs in channel and reservoirs. Previous researchers have reported that several microbial contaminants are constantly adherent to sediment particles (Oliver et al., 2007), most of which are re-suspended from stream bottoms during the rising storm incidence. Besides, pathogens and sediment are usually both transported in water, either separately or adsorbed together.

Pollution of water by petroleum and allied products is a universal environmental phenomenon in places where there is exploration or processing of petroleum deposits (Abdelgawad et al., 2008). Bitumen is a sticky, highly viscous liquid or semi-solid usually found in most crude petroleum and in some natural deposits and therefore referred to as a pitch. It is composed of several high boiling point compounds and molecules with relatively low carbon to hydrogen C:H ratio (Yoon et al., 2009). A large deposit of natural bitumen occurs in the bitumen belt of South-western Nigeria. The toxicity of a material has been shown to be the most common measure of its potential environmental impact and this is applicable to bitumen whose impacts on the Nigerian physical environment especially communities in Ondo State are enormous as it contains heavy metals.

Due to the importance of Oluwa River as the major water source for drinking and other domestic usages within these communities, its sanitary level is of great concern. Thus in continuation of the few chemical toxicity studies on the environmental impacts of natural bitumen deposits and other contaminants in Oluwa River Ondo State, South Western Nigeria, the aim of this preliminary study was to evaluate the microbial population and the antibiotic resistant pattern of heterotrophic bacteria in the water and sediment of this polluted river as this will assist in the determination of the pollution impact on the bacterial isolates and the evaluation of the public health implications from the ARB.

Materials and methods

Description of collection site

Ondo State constitutes an economically significant part of South-western Nigeria and has one of the largest fresh and coastal areas in the country. It is located in the coordinate of Latitude 6° 35′ 19 N, Longitude 4° 50′ 3 E and Altitude 61 m. This is where bitumen was first spotted in Nigeria in 1910 and two bitumen observatory wells were dug in the State in the 60 s during the early explorative activity of Nigerian natural bitumen. A large deposit of natural bitumen occurs in the so called bitumen belt of South-western Nigeria. The seepage of the bitumen material exists especially during the dry season when temperature is above 37 °C where it occurs as a free flowing liquid. Oluwa is a major river of industrial, agricultural and environmental significance which winds through many communities within the State. The river receives continuous seepage from bitumen exploration apart from domestic and agricultural deposits besides other activities carried out along its course and from its many tributaries which in turn contribute to its pollution (Fig. 1).

Sample collection

180 water samples in this study were collected from 20 different sites (19 polluted sites and 1 unpolluted site that was used as control for the study) along the river course during the dry season of 2011 ($n = 80$) and wet season of 2012 ($n = 100$). These sites were selected after due consultation with local authorities and a water assessment monitoring group, who identified these sites as having poor water quality due to usage for domestic activities by the inhabitants of Agbabu community. All samples were collected during low tide and sample collection during the dry season was carried out when there was no rainfall for more than 2 months or when there was rainfall, not more than 2 mm for at least 15 days prior to sampling. In contrast, sample collection during the rainy season was done when the sampling sites had received more than 100 mm rainfall few days prior to sampling. From each site, grab water samples were collected into 5 L sterile plastic bottles with screw caps from 30 cm below the water surface and transported on ice to the laboratory for analysis within 6 h of collection. Sediment samples were collected from four different locations (A, B, C, D) and another one control at

approximately two week intervals, only when it has not rained 4 days prior to sampling. The control samples were taken from points not affected by pollution. Fifteen 9.5 cm^3 replicate sediment samples were collected from a depth of approximately 200 m downstream.

Water quality evaluation

Water samples for analysis were collected into new high-density Polyethylene terephthalate (PET) screw-capped containers of 1.5 L capacity. The PET containers and stoppers were thoroughly washed with distilled water thrice and once with the water meant for sampling before collection according to the description of Khan et al. (2012) and Dahunsi et al. (2014). At each site, one bottle was filled with water without acid (for microbial analysis) while the other bottle was filled with the water from the same point and acidified with a few drops of 5% Nitric acid (HNO_3) to stop the activities of microorganisms and was later used for metal analysis. The water samples were transported to the Central Research Laboratory in Ladoke Akintola University of Technology, Nigeria and kept in a refrigerator at 4 °C.

Physical parameters including pH (HI 9024-C, Hanna Instruments, Smithfield, RI, USA), temperature (HI 98517, Hanna Instrument), salinity (HI 19311, Hanna Instrument), electrical conductivity (HI 2315, Hanna Instr.), and total dissolved solids (TDS) (VSI 22, VSI Electronics Private Limited, Punjab, India) were analyzed in- situ using the hand digital meters that are mentioned above. Dissolved oxygen of the samples was analyzed using the azide modification of Winkler's method (APHA, 2012). As described in APHA (2012) standard methods, chloride was determined by titration while the Ultraviolet spectrophotometer screening method was used in the determination of the major anions using a UV spectrophotometer (DR 2800, HACH, Washington, USA) (Khan et al., 2012; Dahunsi et al., 2014). In order to ensure reliability and reproducibility, blank, standard and pre-analyzed samples were analyzed after every 10 samples (Dahunsi et al., 2014). Metal analysis was done with the aid of an atomic absorption spectrophotometer (AAS) (Sens AA 3000, GBC, Australia) using the method in APHA (2012).

Microbial analysis

1.0 mL and 1 gram from each water and sediment sample were serially diluted, and then plated in duplicate on Nutrient agar, MacConkey agar, Mannitol salt agar and Salmonella-Shigella agar. All plates were incubated appropriately for 2–48 h. Colonies which developed on the plates were counted and recorded using colony forming unit per ml (Cfu/ml) of the

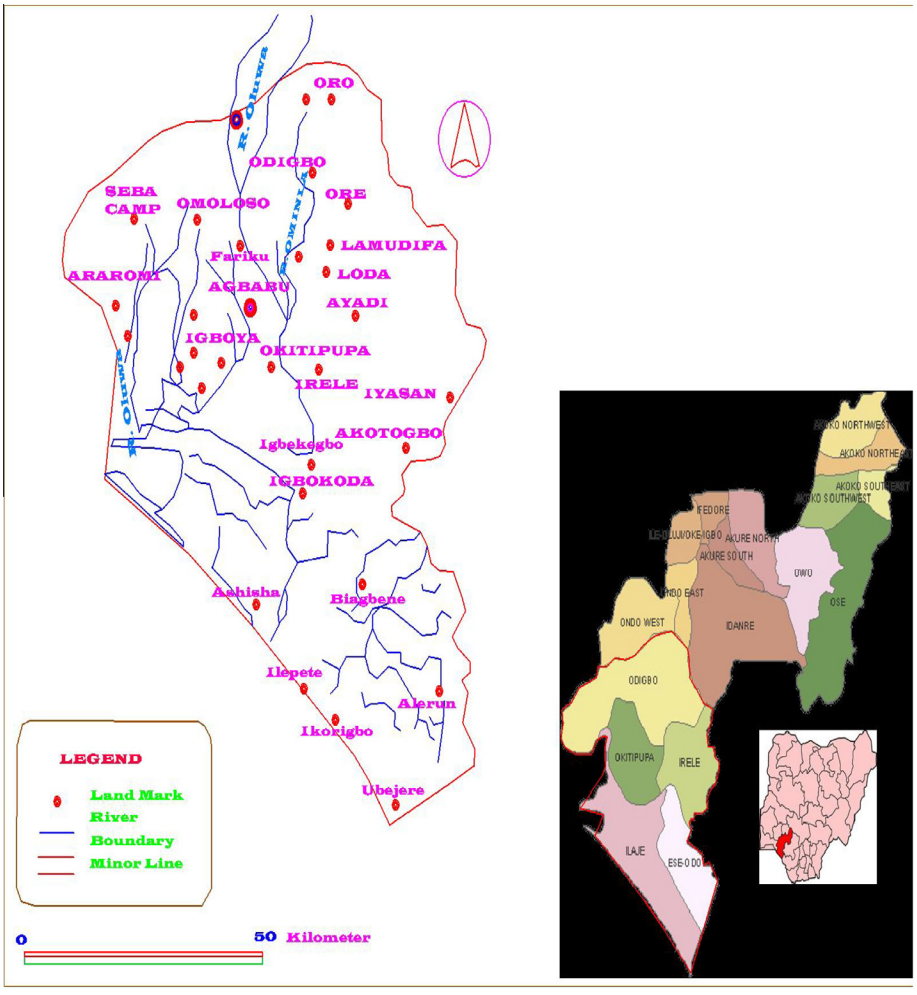

Figure 1 Map of study area.

Table 1 Physicochemical qualities of water and sediment of Oluwa River.

Parameters	Value	WHO	NIS
Water samples			
Temperature (°C)	24.97 ± 0.03	–	Ambient
pH	6.87 ± 0.05	6.5–8.5	6.5–9.5
Electrical conductivity (μs cm^{-1})	0.15 ± 0.01	1000	1000
Alkalinity ($CaCO_3$ mg l^{-1})	58.78 ± 0.48	–	–
Total hardness (mg $CaCO_3$ l^{-1})	4.01 ± 0.25	< 200	150
Total solids (mg l^{-1})	1320 ± 32.23	< 1500	–
Total dissolved solid (mg l^{-1})	429.65 ± 21.44	< 1000	500
Total suspended solid (mg l^{-1})	9.19 ± 0.26	500	500
Biological oxygen demand (mg O_2 l^{-1})	13.83 ± 0.23	–	–
Dissolves oxygen (mg O_2 l^{-1})	2.71 ± 0.22	6.0	–
Chemical oxygen demand (mg l^{-1})	165.38 ± 3.41	–	–
Chloride (mg l^{-1})	7.03 ± 0.15	–	250
Nitrate (mg l^{-1})	1.84 ± 0.79	50	50
Sulfate (mg l^{-1})	9.91 ± 0.41	500	100
Phosphate (mg l^{-1})	58.93 ± 3.23	–	–
Cadmium (mg l^{-1})	0.12 ± 0.05	0.003	0.003
Chromium (mg l^{-1})	0.58 ± 0.05	0.05	0.05
Lead (mg l^{-1})	0.19 ± 0.01	0.01	0.01
Zinc (mg l^{-1})	2.79 ± 0.17	–	3.000
Iron (mg l^{-1})	0.06 ± 0.01	–	0.300
Copper (mg l^{-1})	0.13 ± 0.07	2.000	1.000
Nickel (mg l^{-1})	ND	2.0	1.0
Fluoride (mg l^{-1})	0.22 ± 0.02	NA	NA
Manganese (mg l^{-1})	0.43 ± 0.02	NA	NA
Sediment samples			
Cadmium (mg l^{-1})	7.81 ± 0.83	0.003	0.003
Chromium (mg l^{-1})	22.26 ± 0.83	0.05	0.05
Lead (mg l^{-1})	13.01 ± 0.95	0.01	0.01
Zinc (mg l^{-1})	71.33 ± 1.70	–	3.000
Copper (mg l^{-1})	11.67 ± 0.24	2.000	1.000
Nickel (mg l^{-1})	15.08 ± 0.35	2.0	1.0
Manganese (mg l^{-1})	33.27 ± 2.57	NA	NA

WHO = World Health Organization (2011); NIS = Nigerian Industrial Standard (2007).

Table 2 Mean microbial count of microorganisms isolated from water and sediment.

Organism	Count (×10^2 Cfu/100 ml)
Water samples	
Bacillus spp.	156.20
Pseudomonas spp.	120.03
Streptococcus faecium	94.10
[$] *Micrococcus* spp. (a)	110.03
Micrococcus spp. (b)	112.03
Staphylococcus aureus	94.10

Organism	Control (×10^4 Cfu g^{-1})	Location A (×10^4 Cfu g^{-1})	Location B (×10^4 Cfu g^{-1})	Location C (×10^4 Cfu g^{-1})	Location D (×10^4 Cfu g^{-1})
Sediment samples					
Pseudomonas spp.	[*]6.39 ± 0.03	[*]14.30 ± 1.40	[*]11.80 ± 1.06	[*]13.07 ± 1.25	[*]6.39 ± 0.08
Proteus vulgaris	5.30 ± 0.02	[*]9.93 ± 1.15	[*]10.10 ± 1.05	[*]6.57 ± 0.07	[*]6.43 ± 0.09
Micrococcus spp.	[*]2.55 ± 0.10	[*]12.33 ± 1.33	[*]8.23 ± 1.02	[*]7.70 ± 0.20	[*]4.40 ± 0.01
Staphylococcus aureus	5.30 ± 0.13	[*]8.00 ± 1.01	10.97 ± 1.22	[*]6.47 ± 0.04	8.63 ± 0.02
Bacillus spp.	9.19 ± 1.03	[*]19.24 ± 2.10	16.11 ± 2.02	[*]16.11 ± 2.03	11.06 ± 1.04
Streptococcus faecium	[*]3.21 ± 0.01	[*]10.51 ± 1.02	[*]12.01 ± 1.03	[*]6.40 ± 0.02	[*]6.80 ± 0.01

[#] Significant difference between respective rows.
[*] Significant difference between respective columns.
[$] Water isolate from control.

sample using previous methods (Lateef et al., 2005; Guo et al., 2013; Hussain et al., 2013). Sub-culturing was carried out on distinct colonies until pure cultures were obtained and were transferred onto slant bottles containing freshly prepared agars. Individual colonies were purified and identified by morphological and biochemical techniques using the method of Jolts et al. (1994).

Determination of antibiotic sensitivity

In order to evaluate the bacterial resistance to 13 types of antibiotics used in this study, disk diffusion assay was employed. Bacterial cultures were grown at 35 °C for 18 ± 2 h in tryptic soy broth (TSB; Difco), diluted to 1×10^7 Cfu/ml in tempered 0.75% agar (45 °C; Difco), mixed gently, and poured onto Muller-Hinton agar (MHA; Difco). After solidification, antimicrobial susceptibility test disks (BBL™ Sensi-Disc™, BD Diagnostics, Sparks, MD, USA) were applied and plates were incubated at 35 °C for 24 h. The added antibiotic concentrations for ARB in polluted water have previously been defined as the maximum value of the minimum inhibitory concentrations (MICs) for *Enterobacter*, *Enterococcus* and *Staphylococcus* spp. resistant to that antibiotic (CLSI, 2011), since these three are the common species related to human health in polluted water. The antibiotics used are as follows: Erythromycin (Ery), 15 µg; Ciprofloxacin (Cip), 5 µg; Cotrimoxazole (Cot), 10 µg; Pefloxacin (Pef), 10 µg; Gentamicin (Gen), 10 µg; Ceftriazone (Cef), 5 µg; Chloramphenicol (Chl), 30 µg; Streptomycin (Str), 10 µg; Ofloxacin (Ofl), 30 µg; Amoxicillin (Amx), 25 µg; Augmentin (Aug), 30 µg; Nitrofurantoin (Nit), 25 µg and Tetracycline (Tet), 30 µg. All antibiotic disks used were supplied by Oxoid Ltd. (Basingstoke, Hampshire, England), antibiotics were dissolved in Milli-Q water prior to testing and were diluted with the appropriate medium immediately before the tests. A susceptible *Escherichia coli* strain was used to confirm the potencies of all the antibiotics prior to the test.

Statistical analysis

All statistical tests were performed using SPSS Version 19. The *t*-Test (Two sample assuming equal variances) at a *p*-value less than or equal to 0.05 was used to conduct the test of significance.

Results

The mean of the physical and chemical qualities of water and sediment samples of River Oluwa is shown in Table 1. On the other hand, the mean microbial count of organisms isolated from the water and sediment is shown in Table 2. From water samples, *Bacillus* species has the highest count of 156.20×10^2 Cfu/100 ml followed by *Pseudomonas* species and then *Micrococcus* species (*a* and *b*). *Streptococcus faecalis* and *Staphylococcus aureus* both recorded 94.10×10^2 Cfu/100 ml each. From the sediment on the other hand, bacterial count ranged between 2.55×10^4 and 19.24×10^4 Cfu g^{-1}.

Occurrence of bacterial isolates

As shown in Table 3, a total of 5 bacteria species (i.e. *Bacillus*, *Micrococcus*, *Pseudomonas*, *Staphylococcus* and *Streptococcus* species) were isolated from the water samples. *Bacillus* spp. had the highest occurrence (21.42%), *Micrococcus* spp. had 14.14% and *Pseudomonas* had 7.14% while *S. aureus* and *Streptococcus faecium* both had 3.57% each. A total of 6 bacterial spp. were isolated from the sediment. *Micrococcus* spp. and *Proteus vulgaris* had the highest occurrence (10.71%); followed by *Bacillus* spp. (6.57%). *Pseudomonas*, *S. aureus* and *S. faecium* had the lowest occurrence (3.57%).

Antimicrobial sensitivity

Results of the resistance of water and sediment bacterial isolates to the different antibiotic used are shown in Tables 4 and 5. *Bacillus* spp. was resistant to Ciprofloxacin, Chloramphenicol, Streptomycin and Amoxicillin while *S. faecium* shows resistant against Erythromycin, Ciprofloxacin, Cotrimoxazole, Pefloxacin, Ceftriazone, Chloramphenicol, Streptomycin and Amoxicillin. *Micrococcus* spp. (a) was resistant to only Ofloxacin, *Micrococcus* spp. (b) was resistant to all antibiotics while in the case of *S. aureus*, resistance was shown against Ciprofloxacin, Cotrimoxazole, Pefloxacin, Gentamicin, Ceftriazone, Chloramphenicol, Streptomycin and Amoxicillin. For the Gram negative bacterium; *Pseudomonas* spp. from the water samples, resistance was shown to all the antibiotics used. All the Gram positive isolates from sediment showed 100% resistance to Cotrimoxazole, Gentamicin, Ceftriazone, Chloramphenicol, Streptomycin, Pefloxacin and Amoxicillin while showing susceptibility to Erythromycin, Ciprofloxacin, Peflox-

Table 3 Occurrence of microbial isolates from water and sediment.

Water bacterial isolates		Sediment bacterial isolates	
Isolates	% Occurrence	Isolates	% Occurrence
Bacillus species	21.42 ± 1.21	*Pseudomonas* species	3.57 ± 0.22
[a]*Micrococcus* species	14.28 ± 1.20	*Proteus vulgaris*	10.71 ± 0.21
Pseudomonas species	7.14 ± 1.01	[b]*Micrococcus* species	10.70 ± 1.13
Staphylococcus aureus	3.57 ± 0.21	*Staphylococcus aureus*	3.57 ± 1.04
Streptococcus faecium	3.57 ± 1.10	*Bacillus* species	6.97 ± 1.01
		Streptococcus faecium	3.57 ± 0.22

[a] Water control isolate; $N = 180$ for water.
[b] Sediment control isolate; $N = 15$ for sediment.

Table 4 Resistance of bacterial isolates from water to individual antibiotic.

Gram positive isolates Organisms	Ery	Cip	Cot	Pef	Gen	Cef	Chl	Str	Ofl	Amx	**%
Micrococcus species (a)	−	−	−	−	−	−	−	−	+	−	90
Bacillus species	+	−	+	+	+	+	−	−	+	−	40
Streptococcus faecium	−	−	−	−	+	−	−	−	+	−	80
Micrococcus species (b)	−	−	−	−	−	−	−	−	−	−	100
Staphylococcus aureus	+	−	−	−	−	−	−	−	+	−	80
#Grand total (%)	60	100	80	80	60	80	100	100	20	100	
Gram negative isolates Organisms	Aug	Cef	Cot	Tet	Gen	Pef	Nit	Cip	Ofl	Amx	**%
Pseudomonas species	−	−	−	−	−	−	−	−	−	−	100
#Grand total (%)	100	100	100	100	100	100	100	100	100	100	

+, positive/susceptible; −, negative/resistant.
* Bacteria from control; others are from polluted water samples.
# Grand total, % resistance of isolates from water to each antibiotic.
** Cumulative % resistance of each bacterium to all tested antibiotics.

Table 5 Resistance of sediment bacterial isolates to individual antibiotic.

Gram positive Organisms	Ery	Cip	Cot	Pef	Gen	Cef	Chl	Str	Ofl	Amx	**%
Micrococcus species (a)	−	−	−	−	−	−	−	−	+	−	90
Staphylococcus aureus	+	−	−	−	−	−	−	−	+	−	80
Bacillus species (a)	−	−	−	+	−	−	−	−	+	−	80
Streptococcus species	−	+	−	−	−	−	−	−	+	−	80
Bacillus species (b)	−	−	−	−	−	−	−	−	−	−	100
#Grand total (%)	80	80	100	80	100	100	100	100	20	100	
Gram negative Organisms	Aug	Cef	Cot	Tet	Gen	Pef	Nit	Cip	Ofl	Amx	**%
Pseudomonas species	−	−	−	−	−	−	−	−	−	−	100
Proteus vulgaris	−	+	+	−	−	−	−	−	+	−	70
#Grand total (%)	100	50	50	100	100	100	100	100	50	100	

+, positive/susceptible; −, negative/resistant.
* Bacteria from control; others are from polluted sediment samples.
# Grand total, % resistance of isolates from sediment to each antibiotic.
** Cumulative % resistance of each bacterium to all tested antibiotics.

acin and Ofloxacin at different rates. Likewise, the Gram negative isolates from the sediment were 100% resistant to Augmentin, Tetracycline, Gentamicin, Pefloxacin, Nitrofurantoin, Ciprofloxacin and Amoxicillin and showed different rates of susceptibility to Ceftriazone, Cotrimoxazole and Ofloxacin. From the result obtained, all the isolates showed multiple resistance to all the antibiotics used. Four patterns of multiple drug resistance each were found from both water and sediment isolates with a number of the antibiotics ranging from 4 to 10 out of 13 used in the study (Table 6). Among all the isolates, *Bacillus*, *Micrococcus* and *Pseudomonas* spp. showed the highest (100%) MAR to all antibiotics.

Antibiotic resistant pattern of isolates from water and sediment samples

The antibiotic resistant pattern of isolates from water and sediment is shown in Table 6. For *S. aureus* among water isolates, the order follows Ciprofloxacin > Cotrimoxazole > Pefloxacin > Gentamicin > Ceftriazone > Chloramphenicol > Streptomycin > Amoxicillin; the order for *Bacillus* spp. is Ciprofloxacin > Chloramphenicol > Streptomycin > Amoxicillin; for *S. faecium*, it is Erythromycin > Cotrimoxazole > Pefloxacin > Gentamycin > Ceftriazone > Chloramphenicol > Streptomycin > Amoxicillin; for *Micrococcus* spp. (a), the order is Erythromycin > Ciprofloxacin > Cotrimoxazole > Pefloxacin > Gentamicin > Ceftriazone > Chloramphenicol > Streptomycin > Ofloxacin > Amoxicillin; for *Micrococcus* spp. (b), it is Erythromycin > Ciprofloxacin > Cotrimoxazole > Pefloxacin > Ceftriazone > Chloramphenicol > Streptomycin > Ofloxacin > Amoxicillin > Gentamicin and for *Pseudomonas* spp., the order is Amoxicillin > Augmentin > Gentamicin > Ceftriazone > Nitrofurantoin > Cotrimoxazole > Ofloxacin > Ciprofloxacin > Tetracycline > Pefloxacin. For sediment isolates, *Micrococcus* spp. had its resistant pattern to follow the order Erythromycin > Ciprofloxacin > Cotrimoxazole > Pefloxacin > Gentamicin > Ceftriazone > Chloramphenicol > Streptomycin > Amoxicillin; for *S. aureus*, it follows Ciprofloxacin > Cotrimoxazole > Pefloxacin > Gentamicin > Ceftriazone > Chloramphenicol > Streptomycin >

Table 6 Antibiotic resistance pattern of bacterial isolates from water and sediment.

No of antibiotics	Resistance pattern	No of isolates	Organisms
Water			
4	Cip, Chl, Str, Amx	1	*Bacillus* species (a)
8	Cip, Cot, Pef, Gen, Cef, Chl, Str, Amx	6	*Staphylococcus aureus*
	Ery, Cot, Pef, Gen, Cef, Chl, Str, Amx	1	*Streptococcus faecium*
9	Ery, Cip, Cot, Pef, Gen, Cef, Chl, Str, Amx	2	*Micrococcus* species (a)
10	Ery, Cip, Cot, Pef, Cef, Chl, Str, Ofl, Amx, Gen	2	*Micrococcus* species (b)
	Amx, Aug, Gen, Cro, Nit, Cot, Ofl, Cip, Tet, Pef	2	*Pseudomonas* species
Sediment			
4	Cip, Chl, Str, Amx	1	*Bacillus* species (a)
8	Cip, Cot, Pef, Gen, Cef, Chl, Str, Amx	1	*Staphylococcus aureus*
	Aug, Nit, Gen, Ofl, Amx, Cip, Tet, Pef	3	*Proteus vulgaris*
	Ery, Cot, Pef, Gen, Cef, Chl, Str, Amx	1	*Streptococcus faecium*
9	Ery, Cip, Cot, Pef, Gen, Cef, Chl, Str, Amx	3	*Micrococcus* species (a)
10	Ery, Cip, Cot, Pef, Gen, Cef, Chl, Str, Ofl, Amx	1	*Bacillus* species (b)
	Amx, Aug, Gen, Chl, Nit, Ofl, Cip, Tet, Pef, Cot	2	*Pseudomonas* species

Ery = Erythromycin; Cip = Ciprofloxacin; Cot = Cotrimoxazole; Pef = Pefloxacin; Gen = Gentamicin; Cef = Ceftriazone; Chl = Chloramphenicol; Str = Streptomycin; Ofl = Ofloxacin; Amx = Amoxicillin; Aug = Augmentin; Nit = Nitrofurantoin; Tet = Tetracycline.

Amoxicillin; for *S. faecium*, the order observed was Erythromycin > Cotrimoxazole > Pefloxacin > Gentamicin > Ceftriazone > Chloramphenicol > Streptomycin > Amoxicillin; for *Bacillus* spp. (a), the order was Erythromycin > Ciprofloxacin > Cotrimoxazole > Gentamicin > Ceftriazone > Chloramphenicol > Streptomycin > Amoxicillin; for *Bacillus* spp. (b), it was Erythromycin > Ciprofloxacin > Cotrimoxazole > Pefloxacin > Gentamicin > Ceftriazone > Chloramphenicol > Streptomycin > Ofloxacin > Amoxicillin; for *Pseudomonas* spp., the order followed Amoxicillin > Augmentin > Gentamicin > Chloramphenicol > Nitrofurantoin > Ofloxacin > Ciprofloxacin > Tetracycline > Pefloxacin > Cotrimoxazole while for *P. vulgaris*, the pattern was Augmentin > Nitrofurantoin > Gentamicin > Amoxicillin > Ciprofloxacin > Tetracycline > Pefloxacin.

Analysis carried out revealed significant differences in the sediment bacterial populations in respect to the various organisms isolated from sediment samples. No significant difference however existed between all sediment bacterial counts from the four sampling sites in comparison with the control populations.

Discussion

The mean pH values of water samples from the river were found to be very weakly acidic (Table 1) and fall within the WHO and NIS permissible range of 6.5 to 9.5 which indicate that it is of good quality (Nigerian Industrial Standard, 2007; World Health Organization, 2011). Also, there is no observed statistical difference ($p < 0.05$) in the mean temperature values of the water samples thus falling within the acceptable temperature range (0–30 °C) for good surface water according to the submission of Chapman (1996). Therefore, the temperature of the water from Oluwa River could not be confirmed as an influencing factor for the observed bacterial population and the antibiotic resistance obtained in this research. It however could provide a favorable environment needed for the optimal proliferation of most of the bacteria isolated from the water in this study most of which are members of the Enterobacteriaceae growing optimally at mesophilic temperature range (20 °C and 32 °C).

The significant decrease ($p < 0.05$) in values for total suspended solids (TSS) and total dissolved solids (TDS) of the water falls within the permissible limits of 500 mg/L for TSS and 1000 mg/L for TDS which are the standard limits of both the WHO and NIS for good water quality. The high BOD and COD values in the water samples are indication that organic and inorganic pollutants are present in the river. The mean BOD values of all samples exceeded the permissible limit (3.0–6.0 mg/L) of the European Union (EU) for good quality water that will adequately support fishes and other aquatic life forms since the BOD value for unpolluted waters is usually $\leqslant$2 mg/L while values for polluted ones can be as high as $\geqslant$10 mg/L. The significantly high mean COD values also exceeded the permissible limit ($\leqslant$20 mg/L) for unpolluted surface water thus falling within the category of polluted waters (20–200 mg/L) according to Chapman (1996). The high BOD and COD values obtained are most likely as a result of bitumen influent and other pollutants in the river. A lower value was recorded for DO which implies that the river is depleted in oxygen and this could be attributed to the polluted nature of the water by bitumen and other pollutants. Values obtained for all metals in both the water and sediment sample are very high and above the WHO and NIS permissible limits for surface waters. This could easily be attributed to bitumen pollution as well since bitumen is known to contain heavy metals (Yoon et al., 2009).

In the present study, bacterial strains belonging to the genera *Bacillus* which are among bacteria better adapted to life in soil or water had the highest percentage of occurrence. Other Gram positive bacteria especially *Staphylococcus*, *Streptococcus*, *Micrococcus* etc. have been indicated extensively in previous studies (Lateef et al., 2005).

According to Harakeh et al. (2006), the emergence of antimicrobial resistant bacteria increases in environments where antimicrobials are indiscriminately used by the public. In Nigeria and other developing countries, acquired bacterial resistance to antimicrobial agents as obtained in this study is common and the complex socio-economic and behavioral

factors associated with this phenomenon include abuse of antibiotics among other factors. It has been reported that bacteria can obtain resistance by horizontal gene transfer of mobile genetic elements and that gross usage of antibiotic influences the selection of existing resistance mechanisms (Stokes and Gillings, 2011) and under the selective pressure of the antibiotics used in aquatic environment. Antibiotic resistance bacteria could persist and constitute an environmental reservoir as previously seen in the study by Bhullar et al. (2012) where antibiotic resistance was discovered in over 4 million year old culturable microbiome from Lechuguilla Cave, New Mexico. Similar antimicrobial resistance profiles were also reported in studies involving isolates from coastal environments, farmed fish and from tap water as observed by (Yano et al., 2014).

Koesak et al. (2012) have previously detected bacterial resistance against Ampicillin, Gentamycin, Erythromycin, Tetracycline and Ciprofloxacin at different times. In the present study, the percentage of resistance for antimicrobial agents ranges from 40% to 100%. The levels of antimicrobial agent resistance that have been reported range from 28.57% to 100%. Over 60% of the isolates tested showed resistant to over 40% of the antibiotics. This correlates with a study by Mydryk, 2002 in relation to the phenomenon of multiple drug resistances, multiple resistances may be coded on several genetic elements such as plasmids, mutational events or mobile genetic materials known as transposons. Also there can be horizontal gene transfer between microorganisms occurring spontaneously in nature, (Harakeh et al., 2006).

Results showed that all isolates in this study are resistant to more than 1 antibiotic (multiple drug resistances) which could be due to their long term exposure to bitumen and other pollutants in the river. Multiple bacterial resistances to drugs had earlier been reported in aquaculture environments (Hatha et al., 2005). Puah et al. (2013) had reported up to six different resistance patterns and resistance to at least one antibiotic was seen in 46 isolates (98%) while multidrug resistance (to two or more drugs) in 93% of tested isolates. Resistance to multiple antibiotics can lead to occurrence of newly emerging resistant bacteria which may be transmitted to consumers causing infections that are difficult to treat. The relatively high resistance of bacterial pathogens to antibiotics in this study agrees with the findings of Rakic-Martinez et al., 2011 who reported the prevalence of MAR bacteria in wastewater. The observed high frequency of bacterial resistance may not only result in the therapeutic failure in the river fauna population, but also endanger the health of the people who are at risk of infection with pathogens from these animals coupled with the possibility of plasmid transfer of resistance to human pathogenic bacteria (Schmidit et al., 2001). The prevalence of intrinsic multi-resistance to common antimicrobial agents has been documented (Wright, 2007; Baltz, 2008; Brown and Balkwill, 2009; Thaller et al., 2010; Toth et al., 2010; D'Costa et al., 2011; Bhullar et al., 2012; Cox and Wright, 2013). Thus proper programmes to monitor antimicrobial usage and resistance in bacterial from aquatic environments should be implemented in Nigeria as none is currently in place. The results obtained reveal the need for effluent/runoff treatment to avoid spread of AR bacteria in the aquatic environment and this had earlier been reported by Das et al. (2013).

In conclusion, the current research has described the microbial profile and antimicrobial resistance pattern observed among isolates from water and sediment of Oluwa River. Although intrinsic resistant is common among bacterial species in the environment, the possibility of horizontal gene transfer as a result of anthropogenic and other human activities (presence of bitumen and other pollutants in the present research) cannot be completely ruled out. As a result of this pattern of resistance to antibiotics by microorganisms and the health risk associated with it, it is important to avoid indiscriminate use of antibiotics in others to stop spread of drug resistance among microorganisms. Water quality management is a global issue and the protection of both surface and ground water from pollutants is fundamental to enhance the public health status of the populace. This study is only preliminary; extensive research into the possible long-term risks of water borne diseases via the ingestion of these drug resistant bacteria is advocated.

Conflict of interest

All authors hereby declare that there was no conflict of interest whatsoever throughout the period of this research. We all have agreed and approved this submission.

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