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Water Quality Assessment, Antibiotic Resistance and Plasmid Profiles of Bacteria Isolated from Asa River, Ilorin, Nigeria

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Abstract

Bodies of water are usually being polluted by wastes from domestic and industrial sources thereby making them unfit for use. Hence, this study aimed at assessing the water quality from Asa River, Ilorin, Nigeria in terms of bacteriological and physicochemical parameters. The bacteriological parameters assessed were heterotrophic bacterial count, total coliform, faecal coliform, identification of the isolates, antibiotic resistance patterns, and plasmid profile of the isolates. Whereas, the assessed physicochemical parameters were pH, total chloride, suspended solid, and total hardness. The heterotrophic bacterial count, total coliform, and faecal coliform counts ranged from 7.6 x 10^3 to 3.2 x 10^6 cfu/ml, 9 to 24000 MPN/ 100ml and 0 to 6.0 x 10^3 cfu/ml respectively. Bacteriological analysis showed that the water contained arrays of bacteria heavily contaminated with pathogens. The genera of bacteria isolated were *Escherichia, Pseudomonas, Brevundimonas, Enterococcus, Burkholderia, Enterobacter, Proteus, Pasteurella* and *Aeromonas*. The pH, total hardness, total chloride, and suspended solids of the water ranged from 6.2 to 7.9, 128 to 291 mg/l, 6.21 to 17.04 mg/l and 170 to 310 mg/l respectively. All the Gram-negative bacteria were resistant to cefuroxime, cefixime, augmentin, and ceftazidime. Whereas all the Gram-positive bacteria were resistant to cefuroxime, cloxacillin, augmentin, and vancomycin. The bacteria also showed multiple antibiotic resistance. The resistance of *Pasteurella multocida* to nitrofurantoin was plasmid mediated. After plasmid curing, *Brevundimonas diminuta* was susceptible to ciprofloxacin and intermediately susceptible to gentamicin, while *Pseudomonas fluorescens* was susceptible to ofloxacin and ceftazidime and intermediately susceptible to ciprofloxacin. It was concluded that the water from Asa River at the different sampling points was unsafe in term of bacteriological as well as some physicochemical qualities. It is recommended that this water should not be used in its present state without treatment. Discharge of industrial, agricultural and domestic wastes should be stopped; and open defecation at the bank of this river should be discouraged.

Keywords: River water, antibiotic resistance, contamination, appraisal

Introduction

Water is an important solvent used for drinking, washing, bathing, cooking, irrigation, aquaculture, recreation, religious bath, hydroelectricity generation, industrial purposes and as coolant [1]. It is necessary for the sustenance of plants, animals, and human life. Water bodies such as rivers being constantly polluted is a global problem and is critical in developing

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countries. Large quantity of pollutants entering the water bodies rendered the self-purification through the interaction of physicochemical and biological mechanisms as inefficient [2].

Microorganisms get into water bodies such as rivers through disposal of human and animal wastes, sewage and the release of untreated or improperly treated effluents from industries [3]. The management of water quality is therefore of special importance. Some common causative agents of water borne diseases are bacteria such as *E. coli*, *Salmonella* spp., *Shigella* spp., *Vibrio cholerae*, *Camylobacter* spp. and others [4].

Antibiotic resistance is the ability of a microorganism to withstand the effects of antibiotics. Microorganisms resistant to one particular antibiotic can develop resistance against others with a similar chemistry. Some factors which could lead to antibiotic resistance include misuse of antibiotics, improper diagnosis, non-compliance and self-medication by patients, drug counterfeiting and widespread use of antibiotics in livestock production [5].
Fresh water constitutes favorable living ecosystems for numerous organisms. Many bacteria found in freshwater could harbor antibiotic resistance genes. These genes are mainly inserted in plasmids, transposon or integron and are able to be transferred among water and soil bacterial community via horizontal gene transfer [6].

In view of the prevalence of disposal of wastes into water body such as Asa River and indiscriminate use of antibiotics, it is necessary to assess the antibiotic resistance pattern and plasmid profile of the bacterial isolates gotten from this water. This research was done to assess the level of bacterial contamination of water from Asa River. The objectives of this study were to determine the physicochemical and bacteriological qualities of water from Asa River; conduct sanitary appraisal of the sampling points, identify the bacterial isolates; evaluate the patterns of antibiotic resistance of the bacterial isolates; and assess the plasmid profiles of the isolates.

Materials and Methods

Study Area

This present study was carried out on Asa River segments at Unity Road, New Yidi Road, Amilegbe and Oko-Erin in Ilorin, Kwara, Nigeria between 8°28’N 4°38’E to 8°31’N 4°40’E.

Sanitary Survey of the Water Sampling Points

A sanitary survey was done by scoring either yes or no for these parameters: absence of faeces, absence of dump, no discharge of industrial effluent into the river, no dumping of organic wastes at the site, and absence of vegetation at the site. The ratio of number of yes to the total number of parameters was multiplied by 100 to get the % sanitary score for each sampling point [7-8].

Collection of Water Samples

Water samples were collected from twelve different points along Asa River between January and March 2018. The water samples were collected using standard sampling methods [9]. The samples were quickly transported in ice chest to the laboratory for analysis [10-11].

Determination of Physicochemical Characteristics of the Water Samples

The physicochemical properties of the water samples assessed were pH, chloride content, total hardness, and suspended solids. They were determined according to standard methods [12-13].

Isolation and Enumeration of Viable Bacterial Counts

The heterotrophic bacterial counts were determined using pour plate technique with nutrient agar for isolation. Total coliform was determined using MacConkey broth as a medium and multiple tube fermentation method, while faecal coliform was determined using eosin methylene blue agar medium and spread plate technique [14].

Purification and Preservation of Isolates

The bacterial isolates were purified by subculturing on sterile nutrient agar and the pure cultures obtained were stored in the refrigerator for further use [15].

Characterization and Identification of Bacterial Isolates

The characterization of bacterial isolates was based on cultural characteristics and biochemical tests. The cultural tests done were Gram staining and colonial morphology, while the biochemical tests were oxidase, catalase, citrate, indole, methyl red, voges-Proskauer, nitrate reduction, urease, oxidation- fermentation, gelatin liquefaction, starch hydrolysis, phenylalanine decarboxylation, lysine decarboxylation, triple sugar iron agar and sugar utilizations. The sugars tested were lactose, glucose, xylose, mannitol, sucrose, maltose, sorbitol, trehalose, arabinose, rhamnose, raffinose, glycerol, cellobiose, fructose and adonitol [14-15]. Identification of Gram-negative bacteria were performed using Microbact^{TM} offline software for Gram-negative rods [16], while ABIS online was used for Gram-positive bacterial identification [17].

Determination of Antibiotic Resistance Patterns of the Isolates

The bacterial isolates were standardized using 0.5 McFarland standard [7]. The standardized culture was spread on the surface of sterile set plate of Mueller Hinton agar using sterile swab stick. Multiple antibiotic discs were placed on the agar surface and incubated at 37^{o}C for 24 hours. The Gram-negative antibiotic discs CM-12-NR100 and CM-12-PR 100 manufactured by Rapid labs, were used for the Gram-negative and Gram-positive bacteria respectively. In order to determine the sensitivity of the isolates to vancomycin, Oxoid single antibiotic disc was used. The diameter of the zones of inhibition of the isolates were then measured in mm [11, 18].

Multiple Antibiotic Resistance (MAR) Index of Bacterial Isolates

This was expressed as the ratio of number of antibiotics the bacterium showed resistance, to the total number of antibiotics tested [11]. The resistance was interpreted according to [18].

Determination of Plasmid Profile of the Bacterial Isolates

Some of the Gram-negative bacterial isolates with MAR index up to 0.88 were selected for plasmid curing. The curing of plasmid was done by adding 0.1g of acridine orange into 100ml of distilled water. The mixture was sterilized by millipore filtration using millipore filter of diameter 0.45µm. This solution is equivalent to 1mg/ml of acridine orange. The sterile acridine orange (1ml) was added to 9ml of sterile nutrient broth to give a final concentration of 0.1mg/ml. The acridine orange-nutrient broth mixture was inoculated with 0.1ml of the standardized bacterial culture. The broth was agitated at 120 rpm for 48 hours. At the end of this period, the broth was sub-cultured on to sterile nutrient agar plate and incubated at 37^{o}C for 24 hours. The isolate retrieved was further used to determine if there will be any change in the antibiotic resistance after the curing process [11, 19-20].

Data Analysis

The data were means of 3 replicates ± standard deviation. The means were separated by one way analysis of variance using Duncan's multiple range test at $p<0.05$ [21].

Results

Physicochemical Characteristics of the Water Samples

The pH, total hardness, total chloride, and suspended solid contents of the water samples ranged from 6.2 to 7.9, 128 – 291mg/l, 6.21 to 17.04 mg/l and 170 to 310mg/l respectively (Table 1).

Bacteriological Counts of the Water Samples

The heterotrophic bacterial count, total coliform and faecal coliform counts of the water samples ranged from 7.6 x 10^3 to 3.2 x 10^6 cfu/ml, 9 to 24000 MPN/100ml and 0 to 6.0 x 10^3 cfu/ml respectively (Table 2).

Sanitary Appraisal of the Sampling Points

The sanitary appraisal scores of the sampling points ranged from 20 to 100% as most of the sampling points were littered with refuse, faeces, agricultural wastes as well as receiving effluents from industries (Table 3).

Characterization and Identification of Bacterial Isolates

The bacterial isolates gotten from the water samples were characterized and identified as *Proteus mirabilis, Enterococcus gallinarum, Aeromonas hydrophila, Pseudomonas syringae, Enterobacter agglomerans, Burkholderia pseudomallei, Pseudomonas fluorescens, Enterococcus* sp., *Escherichia coli, Pasteurella multocida, Enterococcus cloacae, Burkhloderia cepacia,* and *Brevundimonas diminuta*.

Table 1: Physicochemical characteristics of water samples from Asa River, Ilorin, Nigeria

Samples	pH	Parameters (mg/l)		
		Total hardness	Total chloride	Suspended solids
S1	$6.4^{a}\pm0.2$	$214^{f}\pm5$	$8.02^{bcd}\pm0.4$	$190^{bc}\pm5$
S2	$6.2^{a}\pm0.1$	$195^{e}\pm4$	$7.39^{abc}\pm0.2$	$180^{ab}\pm3$
S3	$7.4^{bc}\pm0.15$	$185^{d}\pm5$	$7.07^{abc}\pm0.3$	$200^{cd}\pm5$
S4	$7.2^{b}\pm0.0$	$128^{a}\pm0$	$8.17^{cde}\pm0.15$	$170^{a}\pm6$
S5	$7.4^{bc}\pm0.2$	$128^{a}\pm4$	$15.55^{g}\pm1.0$	$210^{d}\pm5$
S6	$7.6^{cd}\pm0.3$	$165^{c}\pm3$	$9.44^{ef}\pm0.2$	$300^{g}\pm10$
S7	$7.7^{cd}\pm0.2$	$213^{f}\pm3$	$10.26^{f}\pm0.7$	$240^{e}\pm5$
S8	$7.7^{cd}\pm0.1$	$224^{g}\pm4$	$9.37^{ef}\pm0.5$	$310^{g}\pm10$
S9	$7.9^{d}\pm0.3$	$211^{f}\pm11$	$9.09^{def}\pm0.4$	$180^{ab}\pm5$
S10	$6.2^{a}\pm0.1$	$291^{h}\pm10$	$17.04^{h}\pm2$	$300^{g}\pm10$
S11	$7.6^{cd}\pm0.2$	$146^{b}\pm6$	$6.82^{ab}\pm0.2$	$260^{f}\pm3$
S12	$7.5^{bc}\pm0.2$	$141^{b}\pm4$	$6.21^{a}\pm0.1$	$190^{bc}\pm5$

S1= Asa River along New Yidi road; S2= Asa River segment at Unity bridge (left side); S3= Asa River flowing across Amilegbe bridge (right side); S4= Fifty meters downstream from S3; S5= Asa River flowing across Amilegbe bridge (left side); S6= Fifty meters downstream from S5; S7= Asa River along Oko-Erin (left side); S8= Fifty meters from S7; S9= Asa River along Oko-Erin (right side); S10= Fifty meters from S9; S11= Asa River segment at Unity Road Bridge (right side); S12= Fifty meters downstream from S11.

Table 2: Bacteriological counts of water from Asa River water, Ilorin, Nigeria

Samples	Heterotrophic Bacterial Count (cfu/ml) x 10^4	Faecal Coliform (cfu/ml) x 10^2	Total Coliform Count (MPN/100ml)
S1	$120^{f}\pm7$	$20^{d}\pm0$	28^{a}
S2	$65^{d}\pm4$	$10^{c}\pm1$	24000^{e}
S3	$16^{b}\pm2$	$4^{b}\pm0$	93^{c}
S4	$80^{e}\pm4$	$8^{c}\pm0$	9^{a}
S5	$5.1^{a}\pm0.2$	$10^{c}\pm0$	43^{ab}
S6	$320^{g}\pm10$	$0^{a}\pm0$	2400^{e}
S7	$41^{c}\pm2$	$0^{a}\pm0$	2400^{e}
S8	$0.76^{a}\pm0.1$	$0^{a}\pm0$	93^{c}
S9	$2.8^{a}\pm0.2$	$10^{c}\pm1$	75^{bc}
S10	$48^{c}\pm3$	$40^{e}\pm2$	15^{a}
S11	$60^{d}\pm5$	$60^{f}\pm3$	150^{d}
S12	$120^{f}\pm8$	$0^{a}\pm0$	28^{a}

S1= Asa River along New Yidi road; S2= Asa River segment at Unity bridge (left side); S3= Asa River flowing across Amilegbe bridge (right side); S4= Fifty meters downstream from S3; S5= Asa River flowing across Amilegbe bridge (left side); S6= Fifty meters downstream from S5; S7= Asa River along Oko-Erin (left side); S8= Fifty meters from S7; S9= Asa River along Oko-Erin (right side); S10= Fifty meters from S9; S11= Asa River segment at Unity Road Bridge (right side); S12= Fifty meters downstream from S11.

Table 3: Sanitary survey and appraisal of Asa River, Ilorin, Nigeria

Sample Sites	Faeces	Refuse	Industrial Effluents	Organic Wastes	Vegetation	Sanitary Score (%)
S1	Yes	Yes	No	No	Yes	60
S2	Yes	Yes	No	Yes	Yes	80
S3	Yes	Yes	No	Yes	Yes	80
S4	Yes	No	No	Yes	Yes	60
S5	Yes	Yes	No	No	Yes	60
S6	Yes	Yes	No	No	Yes	60
S7	Yes	No	Yes	Yes	Yes	80
S8	Yes	Yes	Yes	Yes	Yes	100
S9	No	No	No	No	Yes	20
S10	Yes	No	No	No	Yes	40
S11	No	Yes	No	No	Yes	40
S12	Yes	Yes	No	No	Yes	60

S1= Asa River along New Yidi road; S2= Asa River segment at Unity bridge (left side); S3= Asa River flowing across Amilegbe bridge (right side); S4= Fifty metres downstream from S3; S5= Asa River flowing across Amilegbe bridge (left side); S6= Fifty meters downstream from S5; S7= Asa River along Oko-Erin (left side); S8= Fifty meters from S7; S9= Asa River along Oko-Erin (right side); S10= Fifty meters from S9; S11= Asa River segment at Unity Road Bridge (right side); S12= Fifty meters downstream from S11

Antibiotic Resistance Patterns of the Bacterial Isolates

All the Gram-negative bacteria were resistant to cefuroxime, cefixime, augmentin and ceftazidime. Similarly, all the Gram-positive bacteria were resistant to cefuroxime, cloxacillin, augmentin and vancomycin. The MAR index of the Gram-negative and positive bacteria ranged from 0.5 to 1.0 and 0.56 to 0.89 respectively. Some of the bacterial isolates were susceptible to gentamicin, ofloxacin, nitrofurantoin, ciprofloxacin, ceftriaxone, erythromycin, and ceftazidime (Table 4).

Plasmid Profile of the Antibiotic Resistant Isolates

The MAR index of some of the Gram-negative bacteria did not change after plasmid curing. These included *P. mirabilis*, *P. syringae* and *B. pseudomallei*. However, after plasmid curing, the MAR index of *Brevundimonas diminuta*, *Pasteurella multocida* and *P. fluorescens* changed (Table 5).

Table 4: Antibiotic resistance patterns of the bacterial isolates

Gram-negative Bacteria									
	Zones of Inhibition (mm)								
	CRX	GEN	CXM	OFL	AUG	NIT	CPR	CAZ	MAR Index
P. mirabilis*	R	R	R	R	R	R	R	R	1.00
A. hydrophila	R	R	R	R	R	13(R)	R	R	1.00
P. syringae*	R	R	R	R	R	R	17(I)	R	0.88
***B. pseudomallei**n=1*	R	R	R	R	R	14(R)	R	R	1.00
P. fluorescens	R	R	R	R	R	R	R	R	1.00
E. agglomerans	R	R	R	R	R	24(S)	13(R)	R	0.88
E. coli	R	19(S)	R	26(S)	R	25(S)	25(S)	R	0.5
P. fluorescens	R	R	R	17(S)	R	27(S)	16(I)	R	0.63
B. diminuta*	R	11(R)	R	R	R	R	R	R	1.00
***B. pseudomallei**n=2*	R	R	R	R	R	R	R	R	1.00
E. agglomerans	R	R	R	11(R)	R	15(I)	11(R)	R	0.88
P. multocida*	R	R	R	R	R	R	R	R	1.00
P. fluorescens*	R	R	R	R	R	R	R	R	1.00
P. fluorescens	R	15(S)	R	17(S)	R	R	28(S)	R	0.63
B. cepacia	R	20(S)	R	15(I)	R	28(S)	24(S)	R	0.5
E. cloacae	R	R	R	25(S)	R	R	R	R	0.88
E. agglomerans	R	R	R	R	R	20(S)	R	R	0.88
***B. pseudomallei**n=3*	R	R	R	R	R	R	R	R	1.00

Gram positive bacteria	GEN	CRX	CTR	ERY	CXC	OFL	AUG	CAZ	VAN	
E. gallinarum	14(I)	R	20(I)	21(I)	R	14(I)	9(R)	R	R	0.56
***Enterococcus* sp.**	R	R	14(I)	R	R	16(S)	R	R	R	0.78
***Enterococcus* sp.**	18(S)	R	R	R	R	R	R	R	R	0.89
***Enterococcus* sp.**	14(I)	R	34(S)	R	R	34(S)	R	24(S)	R	0.56

R = Resistant, I = Intermediately susceptible, S= Susceptible, CAZ = Ceftazidime 30µg, CRX = Cefuroxime 30µg, GEN = Gentamicin 10µg, CXM = Cefixime 5µg, OFL = Ofloxacin 5µg, AUG = Amoxycillin/Clavulinate 30µg, CPR = Ciprofloxacin 5µg, NIT = Nitrofuratoin 300µg, ERY=Erythromycin 5µg, CXC= Cloxacillin 5µg, CTR=Ceftriaxone 10µg, VAN=Vancomycin 30 µg, * = selected for plasmid curing

Table 5: Antibiotic resistance patterns of Gram-negative bacteria after plasmid curing

Isolates	Zones of Inhibition (mm)								
	CRX	GEN	CXM	OFL	AUG	NIT	CPR	CAZ	MAR Index
P. mirabilis	R	R	R	R	R	R	R	R	1.00
P. syringae	R	R	R	R	R	R	20(I)	R	0.88
***B. pseudomallei* n=1**	R	R	R	R	R	14(R)	R	R	1.00
B. diminuta	R	14(I)	R	R	R	R	25(S)	R	0.75
***B. pseudomallei* n=2**	R	R	R	R	R	8(R)	R	R	1.00
P. multocida	R	R	R	R	R	15(I)	R	R	0.88
P. fluorescens	R	R	R	25(S)	R	R	17(I)	23(S)	0.63
***B. pseudomallei* n=3**	R	R	R	R	R	R	R	R	1.00

R = Resistant, I = Intermediately susceptible, S= Susceptible, CAZ = Ceftazidime 30µg, CRX = Cefuroxime 30µg, GEN = Gentamicin 10µg, CXM = Cefixime 5µg, OFL = Ofloxacin 5µg, AUG = Amoxycillin/Clavulinate 30µg, CPR = Ciprofloxacin 5µg, NIT = Nitrofuratoin 300µg, n= number of the same species isolated

Discussion

The pH of 75% of the water samples were between 6.5 to 8.5 as allowed by guidelines [9]. In a study conducted on the unity segment of this sample, a pH range of 6.32 to 6.43 was obtained [10]. However, in this present study a pH of 6.2 was obtained at this same segment of Asa River water sample. Another study obtained alkaline pH in the range of 7.34 to 7.61 and 7.45 to 7.93 in year 1 and 2 of the bacteriological assessments of River Jataganga in Indian Himalayas [22]. The pH of water in this study was slightly acidic to slightly alkaline. Another study obtained water samples with pH slightly alkaline (7.42 – 8.09) [23].

Majority of the water samples (67%) had total hardness beyond the limit of 150mg/l permitted by guidelines [24]. These samples can be regarded as being hard and not suitable for laundry. Another study obtained total hardness ranging between 207 – 285 mg/l in the water quality assessment of Rawanduz River and Gali Ali Beg Stream in Iraq [25]. Furthermore, a different study obtained total hardness ranging between 132 – 344 mg/l [13].

The total chloride contents of all the water samples were below the limit of 200 – 300mg/l and 250mg/l as allowed by guidelines [9] and [24] respectively. Total chloride ranged from 6.21 - 17.04 mg/l in this study. Whereas total chloride ranged from 48.2 – 73.8 mg/l in a study of bacteriological and physicochemical qualities of Halabja drinking water in Iraq [12].

The suspended solid contents of all the water samples were above the limit of 25mg/l allowed in potable water. This trend of results of suspended solids was not unexpected since the water was highly contaminated. A range of 766.6 – 1498 mg/l of total suspended solids was obtained by Kolawole *et al*. [10] at Unity, Ilorin, Nigeria segment of the same Asa River. However, in this study a range of 180 – 260 mg/l was obtained at the same segment.

Furthermore, Oyeleke *et al*. [26] obtained suspended solids ranging from 1.50 – 30.17mg/l in the study of quality of water from tin mining pond.

The heterotrophic bacterial count was high in most of the sampling sites since the water samples were untreated. The highest bacterial count of 3.2×10^6 cfu/ml gotten in this study was above the range of 6.42×10^4 to 7.12×10^4 cfu/ml and 1.9×10^4 to 8.2×10^4 cfu/ml obtained by other studies [27-28]. This was lower than the highest value of 3.71×10^6 cfu/ml obtained by Ekhais and Omoigberale [29].

The faecal and total coliform counts were high in most of the sampling points. The faecal coliform counts ranging from 0 to 8.0×10^3 cfu/ml obtained in this study, was lower than the range of 1×10^2 to 8.0×10^4 cfu/ml as obtained by Edokpayi *et al*. [30]. The highest total coliform counts of 24,000 MPN/100ml obtained in this study was quite higher than the highest total coliform of 6200 cfu/ml, 1426 MPN/100ml and 350MPN/100ml obtained by other researches [10, 28-29]. Faecal coliform was isolated in 66.7% of the water samples and were a clear indication of water contamination with faeces. In a similar study, any total coliform and *E. coli* (faecal coliform) was not obtained in the quality assessment of Halabja drinking water [13].

Sanitary survey conducted revealed that the water was grossly contaminated by refuse, agricultural wastes, domestic and industrial wastes at most of the sampling sites. At some sampling points, there were direct human contacts with the water especially at Oko-Erin and New-Yidi Road, Ilorin, Nigeria. The water was being used for washing of rugs, plastic chairs and tables along New-Yidi segment and for irrigation along Oko-Erin segment. The use of contaminated water for irrigation could affect the consumers health. In another study, it was observed that there was no evidence of inadequate sewerage system along Haladja drinking water supply in Iraq [13]. However, there was an evidence of faecal contamination in this study.

Eighteen Gram-negative bacteria belonging to 8 genera were isolated. These genera were *Proteus, Aeromonas, Burkholderia, Pseudomonas, Enterobacter, Escherichia, Pasteurella*, and *Brevundimonas*. The dominant among these isolates were *Pseudomonas* (5 isolates) followed by *Enterobacter* and *Burkholderia* with 4 isolates each. Other genera had one isolate each. In another study *E. coli, S. typhi, V. cholerae, P. vulgaris, Y. enterocolitica, S. dysenteriae, S. enteritis* and *B. subtilis* were isolated from water from mining ponds [26]. Furthermore, Aishvarya *et al*. [22] isolated enterobacteriaceae such as *Citrobacter, Escherichia, Hafnia, Klebsiella, Salmonella* and *Serratia* as well as micrococcaceae such as *Micrococcus* and *Staphylococcus* in their study. In this study Gram-negative bacteria dominated the isolates.

All the four Gram-positive bacteria isolated in this study were enterococci. The enterococci could be of faecal origin since open defaecation occur at some of the sampling sites especially at Unity and Amilegbe segments. Enterococcal counts in the range of 1.0×10^2 to 5.7×10^3 cfu/ml was obtained by Edokpayi *et al*. [30] in their study of Nzhelele River in South Africa.

In this study, all the isolates exhibited MAR index greater than 0.2. The MAR index of *P. mirabilis, P. syringae*, and *B. pseudomallei* remained unchanged after plasmid curing. Their antibiotic resistance was non-plasmid mediated. In contrast, the antibiotic resistance of *P. multocida* to nitrofurantoin can be said to be plasmid mediated since it was intermediately susceptible to this antibiotic after the plasmid curing process with diameter of zone of inhibition of 15mm. *B. diminuta* after plasmid curing became intermediately susceptible to gentamicin and susceptible to ciprofloxacin. Furthermore, *P. fluorescens* n=1 became susceptible to

ofloxacin and ceftazidime but intermediately susceptible to ciprofloxacin after curing. Similar to this study, it was also reported that *P. fluorescens* was susceptible to ofloxacin, augmentin, nitrofurantoin and ciprofloxacin after plasmid curing [20]. In a study of Dasmeh *et al*. [31], it was demonstrated that acridine orange could be used to cure plasmid and was also used in this study. In addition, it was found that the resistance of some bacterial isolates to nitrofurantoin, ceftazidime, gentamicin, ciprofloxacin and ofloxacin decreased after curing with acridine orange and were plasmid mediated [32].

Conclusion

It is concluded from this study that water from Asa River, Ilorin, Nigeria should not be used in its raw state since it has failed most of the bacteriological and physicochemical parameters. Some of the bacteria isolated showed multiple antibiotic resistance patterns that were plasmid mediated. The discharge of effluents containing antibiotics into Asa River and dumping of refuse should be discouraged.

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