Research Article

Bacteriological Quality and Antibiogram of Bacteria Isolated from Aquatic Environments in Three Local Government Areas of Ebonyi State, Nigeria

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ABSTRACT

The aim of this study was to determine the bacteriological quality and antibiogram of bacteria isolated from aquatic environments in Ebonyi State, Nigeria**.** A total of 736 water samples, comprising borehole (n=215), ponds (n=111), rivers (n=144), streams (n=117), and wells (n=149) respectively, were collected from three local governments areas (Abakaliki, Ebonyi, and Ikwo) of Ebonyi State over a period of eight (8) months. The collected water samples were analysed using standard microbiology techniques. Isolates were further characterized using API 20E identification system. Total bacterial count was carried out using pour plating technique while antibiotic susceptibility test was done using Kirby-Bauer disc diffusion test. A total of 308 bacterial isolates; *Aeromonas hydrophila* (13.9 %)*, Escherichia coli* (15.9 %), and *Vibrio cholerae* (11.8 %) were obtained in this study. Water samples from Elinwovu River in Abakaliki L.G.A had the highest bacteria load (5.06 x 10^4 cfu/ml) while borehole water from the same study location had the lowest (1.50 x 10^4 cfu/ml). There was a significant difference ($p < 0.05$) in the bacteria load levels in sampled water sources. Bacterial isolates were highly resistant (100 % - 81.6 %) to penicillin, sulphamethoxazole, kanamycin, azithromycin, streptomycin, cephalothin, and cefuroxime but highly susceptible (100 % - 83.1 %) gentamicin, meropenem, ciprofloxacin, imipenem, and chloramphenicol. The multiple antibiotic resistance index values of isolates ranged from 0.47 to 0.50. Our findings reveal the need for treatment of all water samples to make it potable for human consumption and domestic uses. Also, the presence of antibiotic-resistant bacteria in the water samples is of serious concern as this could cause serious public health problems, especially waterborne diseases if not properly tackled.

Keywords: *Aeromonas hydrophila, Escherichia coli, Vibrio cholerae*, aquatic environments, bacteriological quality, antibiogram.

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INTRODUCTION

Aeromonas hydrophila, *Escherichia coli*, and *Vibrio cholerae* are among a myriad of bacterial organisms commonly found in natural water bodies, and are potential invasive pathogens for those who suffer trauma while submerged in water[1]. Antibiotic resistance genes are emerging contaminants posing a potential worldwide human health risk. The spread and aggregation of antibiotic-resistant genes into multidrug-resistant pathogens is challenging life-saving antibiotic therapies. Antibiotic resistance genes in the environment have drawn great concern due to their health risk[2]. The emergence of antibiotic resistance causes increased mortalities and substantial costs; expenses have recently been estimated to be over 1.5 billion euros every year in Europe alone. The presence of antibiotic resistant bacteria and antibiotic resistance genes in the environment pose special challenges as environmental contaminants[3]. Antibiotic resistant bacteria and antibiotic resistance genes can persist and multiply, and antibiotic resistance genes can spread by horizontal gene transfer mediated by mobile genetic elements within and beyond the original point of occurrence, making possible the exchange of resistance traits between environmental bacteria and human pathogens[4]. Resistance genes are commonly encountered on, or associated with, mobile genetic elements such as plasmids, integrons, and transposons. This enables their transfer within and between bacterial cells and species, and their genetic context risk of transfer from a source environment, and onwards into clinically relevant bacteria[5]. It is reasonable to assume that such transfer of genes from the environment will occur in the future, and

that we can expect pathogens to pick up additional resistance determinants from the environmental resistome[6]. Aquatic ecosystems are considered important matrices for the release, mixing, persistence and spread of antibiotic resistant bacteria and antibiotic resistance genes associated with horizontally transferable genetic elements. Little is known about environmental contamination with antibiotic resistant bacteria in developing countries, like Nigeria. These are critical knowledge gaps since various risk factors favouring the development and spread of antibiotic resistance exist in these environments[7]. Naturally occurring antibiotic-resistant bacteria and antibiotic resistance genes in the aquatic environment are selected for and enriched for by antibiotics found in sewage and agricultural runoff, which result from the widespread and increased use of antibiotics. Historically, concerns about the microbial quality of drinking water have focused on the occurrence of pathogens in drinking water distribution systems[8]. Antibiotic-resistant bacteria are emerging biological contaminants of the environment, they originate mainly from hospital, industrial and community wastewater, animal farms, agricultural lands and wastewater treatment plants. In the aquatic environment, antibiotic resistance determinants may become part of the environmental gene pool, spread horizontally, and may be transferred to humans and animals colonizing bacteria through food and drinking water[9]. Once resistance is genetically encoded it can spread rapidly within a population of bacterial species, or even to another bacterial species through transduction, transformation, conjugation or transposition[9]. Therefore, the aim is to study the bacteriological quality and antibiogram of bacteria isolated from aquatic environments in Abakaliki, Ebonyi and Ikwo Local Government Areas of Ebonyi State.

MATERIALS AND METHODS

Sample Collection

Water samples were collected for a period of eight (8) months (February to September, 2018) covering both dry and rainy seasons. Water samples from five sampling sources namely: stream, river, well, borehole and pond from designated areas were aseptically collected for this study. A total of 736 water samples comprising boreholes (215), ponds (111), rivers (144), streams (117), and wells (149) were collected. For easy identification, collected water samples were labelled appropriately with codes. Water samples collected and period of collection is shown in Table 1. Water samples were collected using grab sampling procedure in accordance with standard methods for examination of water as recommended by Standard Method for microbiological analysis. The water samples for microbiological examination, were collected in non-reactive borosilicate glass bottles of 500 ml capacity each that had been cleansed and rinsed carefully, given a final rinse with distilled water and sterilized. Samples were taken from each source by holding the bottle near its base with hand and plunging it, neck downward, below the surface. Then turning the bottle until neck points slightly upward and mouth is directed toward the current. The sampling bottle were not filled to the brim as 20 to 30 mm space were left for effective shaking of the bottle[11]. Each sample was collected in triplicates. For the collection of borehole samples, the sampling protocols previously described[10] were strictly adhered to during sample collection. Each sample for analysis was collected in sterilized 500 ml glass bottles with screw caps after pumping water sample to waste for 5 to 7 minutes. Care was taken not to allow air bubbles into the bottles during collection. Triplicate samples were taken from each sampling point aseptically into sterilized 500 ml glass bottles, kept in an ice chest and transported immediately to microbiology laboratory of Ebonyi State University, Abakaliki for bacteriological analysis.

Determination of total bacteria count of the water samples

The total bacteria count was carried out by the use of pour plate technique. A ten-fold serial dilution was carried out by measuring 1 ml of each water sample into nine (9) ml of sterile distilled water in a test tube blocked with cotton wool and mixed properly to make a dilution of 10^{-1}. A mixture of 1 ml of the sample and sterile distilled water was introduced into another test tube containing 9 ml of sterile distilled water blocked with cotton wool to make serial dilutions of 10^{-2}. This procedure was repeated in turn until a dilution of 10^{-7} was obtained. From 10^{-2} to 10^{-7} diluents, 1 ml each of the diluents was pipetted separately into different sterile Petri dishes containing plate count agar (PCA) and the plates was incubated at 37 ^{o}C for 24 hours.

Table 1: Number of samples collected and sample sources

S/N	Sample source	Number of samples collected			Total
		Rainy Season	Dry Season		
		February – April	May– July	August – September	
1	Boreholes	52	70	93	215
2	Ponds	24	22	65	111
3	Rivers	38	40	66	144
4	Streams	15	48	54	117
5	Wells	21	50	78	149
	Total	150	230	356	736

Isolation of Bacteria

Discrete colonies obtained from the plate count agar plates was further sub-cultured onto freshly prepared plates of selective and differential media such as *Aeromonas* isolation agar base, MacConkey agar, thiosulphate citrate bile salts sucrose agar plates. The petri dishes were placed in an inverted position in the incubator for 24 hours at 37 ^{0}C to obtain pure cultures. Presumptive morphological identification of the colonies was done by observing their individual appearance on the selective media used. The colonies were stored in test tubes containing Peptone water for cultural/bacteriological identification, biochemical and sugar fermentation and was further characterizations using the API 20E (Biomerieux S.A., Marcy-l`Etoile/France) identification system.

Antimicrobial susceptibility studies

Antibiotic susceptibility of the isolates was determined using the Kirby-Bauer disc diffusion method according to the recommendations of the Clinical and Laboratory Standard Institute[11, 12]. The isolates were sub-cultured on nutrient agar, incubated at 37 °C for 18-24 hours. Then the colonies of each of the isolate were adjusted to 0.5 McFarland turbidity standard (equivalent to 1.5×10^8 cfu/ml) in sterile nutrient broth. The standardized broth culture was incubated for 10 minutes and using sterile a swab stick, the standardized broth culture of the isolates was inoculated onto Mueller-Hinton agar plates. The surface of the medium was streaked in four directions while the plates were rotated approximately 60° to ensure even distribution. The inoculated Mueller-Hinton agar plates were allowed to dry for a few minutes. The following standard antibiotic discs were used against the isolates; azithromycin (15 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (30 µg), cefpirome (30 µg), ceftriaxone(30 µg), cefotaxime (30 µg), cefuroxime (30 µg), erythromycin (10 µg), imipenem (10 µg), kanamycin (30 µg), cephalothin (30 µg), meropenem (10 µg), nalidixic acid (30 µg), norfloxacin (10 µg), ofloxacin (5 µg), oxytetracycline (30 µg), penicillin (10 µg), trimethoprim-sulfamethoxazole (25 µg), streptomycin (10 µg), tetracycline (30 µg). Sterilized forceps were used to place the antibiotic discs evenly on the inoculated Mueller-Hinton agar so that the disc should be about 15mm from the edge of the plate and not closer than 25 mm from disc to disc. After 30 minutes, the plates were inverted and incubated for 24 hours. A ruler was used to measure the diameter of each zone of inhibition in mm on the underside of the plate. The inhibitory zone diameter was interpreted as susceptible, intermediate or resistant according to the criteria of CLSI[11].

Multiple antibiotic resistance index (MARI)

The multiple antibiotic resistance index was calculated as the ratio of the number of antibiotics to which the bacterial isolates were resistant /the total number of antibiotics against which the isolates were tested[13].

Statistical Analysis

The raw data on the microbiological parameters obtained in the course of the study were presented as mean ± standard deviation in tables and bar charts while relevant data were interpreted using simple descriptive statistics such as minimum, maximum, and one-way analysis of variance (ANOVA) with the aid of IBM Statistical Package for Social Sciences (SPSS) version.

RESULTS

Table 2 shows the results of total bacterial count (cfu/ml) of water samples collected from different water sources in Abakaliki, Ebonyi and Ikwo local government area. Water samples collected from Elinwovu River in Abakaliki local government area had the highest bacteria load (5.06×10^4 cfu/ml) while borehole water samples from the same study location had the lowest bacteria load (1.50×10^4 cfu/ml). There was a significant difference ($p < 0.05$) in the bacteria load levels of water samples from all the study locations during the study period.

Table 2: Total bacterial count (cfu/ml) of water samples collected from different water sources

Abakaliki Local Government Area			
S/N	**Sample Sources**	**Mean No of Colonies**	**TBC (cfu/ml)**
1	Boreholes	36 ± 0.04	1.50×10^4
2	Ponds	46 ± 0.00	3.18×10^4
3	Elinwovu River	87 ± 0.06	5.06×10^4
4	Streams	49 ± 1.52	3.65×10^4
5	Wells	64 ± 0.12	4.32×10^4
Ebonyi Local Government Area			
1	Boreholes	41 ± 0.83	1.66×10^4
2	Ponds	62 ± 0.21	2.55×10^4
3	Ebonyi River	79 ± 1.65	4.55×10^4
4	Streams	57 ± 0.02	2.86×10^4
5	Wells	51 ± 0.51	2.32×10^4
Ikwo Local Government Area			
1	Boreholes	46 ± 0.66	2.16×10^4
2	Ponds	43 ± 0.73	2.11×10^4
3	Ebonyi River	63 ± 0.05	4.78×10^4
4	Streams	43 ± 0.62	2.41×10^4
5	Wells	53 ± 0.43	3.71×10^4

Among the organisms isolated from water samples collected from Abakaliki local government area, *Escherichia coli* was the most frequently isolated (n = 118); followed by *Aeromonas hydrophila* (n = 103) while *Vibrio cholerae* had the least number of occurrence (n = 87) as shown in Table 3. *Aeromonas hydrophila* was the most frequently isolated (n = 110) organism in water samples collected from Ebonyi local government area, followed by *Escherichia coli* (n = 101), with *Vibrio cholerae* recording the least (n = 93) as shown in Table 3. *Escherichia coli* was the most frequently

isolated (n = 130) bacteria in water samples collected from Ikwo local government area, followed by *Aeromonas hydrophila* (n=88), while the least frequency was recorded for *Vibrio cholerae* (n = 77). The occurrence frequency of bacterial isolates in the water samples showed no significant difference ($p > 0.05$) during the study period.

Antibiotic susceptibility test results is shown in Tables 4 to 6. *Aeromonas hydrophila* isolates were highly resistant to penicillin (100 %), trimethoprim-sulfamethoxazole (100 %), cephalothin (97.1 %), streptomycin (96.1 %), kanamycin (95.1 %), azithromycin (90.3 %), cefpirome (88.3 %), cefuroxime (87.4 %), norfloxacin (81.5 %), and nalidixic acid (78.6 %) but susceptible to gentamicin (96.1 %), meropenem (94.2 %), ciprofloxacin (89.3 %) imipenem (88 %), chloramphenicol (84.5 %), tetracycline (73.8 %) and cefotaxime (61.2 %) (Table 4).

Escherichia coli isolates exhibited resistance to penicillin (98.3 %), trimethoprim-sulfamethoxazole (96.6 %), kanamycin (95.8 %), streptomycin (91.5 %), cefpirome (90.7 %), cephalothin (89 %), azithromycin (72.9 %), cefuroxime (72.9 %), norfloxacin (75.4 %), and nalidixic acid (73.3 %) but highly susceptible to meropenem (93.2 %), gentamicin (90.7 %), imipenem (86.4 %), chloramphenicol (83.1 %), ciprofloxacin (79.7 %), tetracycline (73.7 %), and cefotaxime (65.3 %) (Table 5).

Vibrio cholerae isolates were highly resistant to streptomycin (96.5 %), penicillin (95.4 %), cephalothin (95.4 %), trimethoprim-sulfamethoxazole (93.1 %), cefpirome (89.7 %), norfloxacin (89.7 %), kanamycin (88.5 %), azithromycin (81.6 %), nalidixic acid (75.9 %), cefuroxime (78.2 %), ceftriaxone (74.7 %), and oxytetracycline (73.6 %), but susceptible to gentamicin (100 %), chloramphenicol (97.7 %), meropenem (97.7 %), tetracycline (95.4 %), ciprofloxacin (94.3 %), imipenem (87.4 %), ofloxacin (82.8 %), and erythromycin (81.6 %) (Table 6).

Table 3: Frequency distribution of bacteria isolated from water samples collected from different water sources

Abakaliki Local Government Area				
		No. of Isolates (%)		
S/N	**Sample Sources**	***Aeromonas hydrophila***	***Escherichia coli***	***Vibrio cholerae***
1	Boreholes	12 (11.65)	16 (13.56)	9 (10.34)
2	Ponds	20 (19.42)	22 (18.64)	17 (19.54)
3	Elinwovu river	30 (29.13)	35 (29.66)	27 (31.03)
4	Streams	18 (17.48)	20 (16.95)	14 (16.09)
5	Wells	23 (22.33)	25 (21.19)	20 (22.99)
	Total	**103 (13.9)**	**118 (16)**	**87 (11.8)**
Ebonyi Local Government Area				
No. of Isolates (%)				
S/N	**Sample Sources**	***Aeromonas hydrophila***	***Escherichia coli***	***Vibrio cholerae***
1	Boreholes	12 (10.91)	10 (9.90)	11 (11.82)
2	Ponds	23 (20.91)	18 (17.82)	16 (17.20)
3	Ebonyi River	34 (30.91)	32 (31.68)	26 (27.96)
4	Streams	15 (13.64)	16 (15.84)	18 (19.35)
5	Wells	26 (23.64)	25 (24.75)	22 (23.66)
	Total	**110 (14.9)**	**101 (13.7)**	**93 (12.6)**
Ikwo Local Government Area				
No. of Isolates (%)				
S/N	**Sample Sources**	***Aeromonas hydrophila***	***Escherichia coli***	***Vibrio cholerae***
1	Boreholes	7 (7.95)	10 (7.69)	8 (10.39)
2	Ponds	18 (20.45)	26 (20.00)	16 (20.78)
3	Ebonyi River	27 (30.68)	42 (32.31)	23 (29.87)
4	Streams	12 (13.64)	16 (13.85)	11 (14.29)
5	Wells	24 (27.27)	36 (27.69)	19 (24.68)
	Total	**88 (11.9)**	**130 (17.6)**	**77 (10.4)**

Table 4: Antibiotic susceptibility patterns of *Aeromonas hydrophila* isolated from water samples

S/N	Antibiotics	Disc Potency (µg)	Susceptible (%)	Resistant (%)
1	AZM	15	9.7	90.3
2	C	30	84.5	15.5
3	CIP	5	89.3	10.7
4	CN	30	96.1	3.9
5	CPO	30	11.7	88.3
6	CRO	30	34	66
7	CTX	30	61.2	38.8
8	CXM	30	12.6	87.4
9	E	10	43.7	56.3
10	IMP	10	88	12
11	K	30	4.9	95.1
12	KF	30	2.9	97.1
13	MEM	10	94.2	5.8
14	NA	30	21.4	78.6
15	NOR	10	18.5	81.5
16	OFX	5	38.8	61.2
17	OT	30	59.2	40.8
18	P	10	0	100
19	RL	25	0	100
20	S	10	3.9	96.1
21	TE	30	73.8	26.2

Key: AZM = azithromycin, C = chloramphenicol, CIP = ciprofloxacin, CN = gentamicin, CPO = cefpirome, CRO = ceftriaxone, CTX = cefotaxime, CXM = cefuroxime, E = erythromycin, IPM = imipenem, K = kanamycin, KF = cephalothin, MEM = meropenem, NA = nalidixic acid, NOR = norfloxacin, OFX = ofloxacin, OT = oxytetracycline, P = penicillin, RL = trimethoprim-sulfamethoxazole, S = streptomycin, and TE = tetracycline.

Table 5: Antibiotic susceptibility patterns of *Escherichia coli* isolated from water samples

S/N	Antibiotics	Disc Potency (µg)	Susceptible (%)	Resistant (%)
1	AZM	15	27.1	72.9
2	C	30	83.1	16.9
3	CIP	5	79.7	20.3
4	CN	30	90.7	9.3
5	CPO	30	9.3	90.7
6	CRO	30	55.1	44.9
7	CTX	30	65.3	34.7
8	CXM	30	27.1	72.9
9	E	10	40	60
10	IMP	10	86.4	13.6
11	K	30	4.2	95.8
12	KF	30	11	89
13	MEM	10	93.2	6.8
14	NA	30	26.3	73.7
15	NOR	10	24.6	75.4
16	OFX	5	55.1	44.9
17	OT	30	46.6	53.4
18	P	10	1.7	98.3
19	RL	25	3.4	96.6
20	S	10	8.5	91.5
21	TE	30	73.7	26.3

Key: AZM = azithromycin, C = chloramphenicol, CIP = ciprofloxacin, CN = gentamicin, CPO = cefpirome, CRO = ceftriaxone, CTX = cefotaxime, CXM = cefuroxime, E = erythromycin, IPM = imipenem, K = kanamycin, KF = cephalothin, MEM = meropenem, NA = nalidixic acid, NOR = norfloxacin, OFX = ofloxacin, OT = oxytetracycline, P = penicillin, RL = trimethoprim-sulfamethoxazole, S = streptomycin and TE = tetracycline.

Table 6: Antibiotic susceptibility patterns of *Vibrio cholerae* isolated from water samples

S/N	Antibiotics	Disc Potency (µg)	Susceptible (%)	Resistant (%)
1	AZM	15	18.4	81.6
2	C	30	97.7	2.3
3	CIP	5	94.3	5.7
4	CN	30	100	0
5	CPO	30	10.3	89.7
6	CRO	30	25.3	74.7
7	CTX	30	58.6	41.4
8	CXM	30	21.8	78.2
9	E	10	81.6	18.4
10	IMP	10	87.4	12.6
11	K	30	11.5	88.5
12	KF	30	4.6	95.4
13	MEM	10	97.7	2.3
14	NA	30	24.1	75.9
15	NOR	10	10.3	89.7
16	OFX	5	82.8	17.2
17	OT	30	26.4	73.6
18	P	10	4.6	95.4
19	RL	25	6.9	93.1
20	S	10	3.5	96.5
21	TE	30	95.4	4.6

Key: AZM = azithromycin, C = chloramphenicol, CIP = ciprofloxacin, CN = gentamicin, CPO = cefpirome, CRO = ceftriaxone, CTX = cefotaxime, CXM = cefuroxime, E = erythromycin, IPM = imipenem, K = kanamycin, KF = cephalothin, MEM = meropenem, NA = nalidixic acid, NOR = norfloxacin, OFX = ofloxacin, OT = oxytetracycline, P = penicillin, RL = trimethoprim-sulfamethoxazole, S = streptomycin and TE = tetracycline.

Multiple antibiotic resistance index (MARI) of bacterial isolates is shown in Table 7. The MARI values of bacterial isolates ranged from 0.47 to 0.50.

Table 7: Multiple Antibiotic Resistance Index (MARI) of bacteria isolated from water samples

S/N	Bacterial Isolates	MARI
1	*Aeromonas hydrophila*	0.50
2	*Escherichia coli*	0.48
3	*Vibrio cholerae*	0.47

DISCUSSION

Water must meet certain microbiological standards before it can be described as potable. The occurrence and spread of antibiotic-resistant bacteria are pressing public health problems worldwide, and aquatic ecosystems are a recognized reservoir for antibiotic-resistant bacteria[14]. This study was designed to determine the bacteriological quality and antibiogram of bacteria isolated from aquatic environments in Ebonyi State, Nigeria**.** In this study, a total of 736 water samples from boreholes, ponds, rivers, streams, and wells were collected from three local government areas (Abakaliki, Ebonyi, and Ikwo) of Ebonyi State over a period of eight (8) months. A total of 308 bacterial isolates, namely *Aeromonas hydrophila* (13.9 %), *Escherichia coli* (15.9 %), and *Vibrio cholerae* (11.8 %) were obtained from the 736 water samples observed in this study. The total bacteria counts for the various water sources used in our study were higher than WHO standard of zero (0) per 100 ml. The highest bacterial load was observed in water samples collected from rivers and wells while the least bacterial load was observed in borehole water samples. The total coliform counts for river water samples in our study is consistent with the study of Sadiya *et al.*[15] who reported that total coliform counts were highest in river water samples with a value of 1.03 x 10^2 cfu/ml. Iroha *et al.*[16] also reported high bacterial load with a value of 2.4 × 10^4 cfu/ml in river water samples. In a study conducted in Ilorin metropolis, Agbabiaka and Sule[17] reported a total bacterial count ranging from zero (0) to 2.30 x 10^2 cfu/ml in river water samples. In another study on selected boreholes used by people in Umuahia North

Local Government Area, Abia State, Nwachukwu *et al.*[18] reported total bacterial counts ranging from 0.32 x 10^2 to 1.38 x 10^2 cfu/ml. Aromolaran *et al.*[19] also reported that water samples collected from different drinking water sources in Ondo, south western Nigeria had total bacteria counts ranging from 1.31 x 10^5 to 4.20 x 10^5 cfu/ml. Oyedeji *et al.*[20] also confirmed the presence of high number (1.20 x 10^4 – 7.0 x 10^4 cfu/ml) of coliforms in drinking water sources (streams) in Osun State, Nigeria. The reason for the variations in the rates of bacteria count might be attributed to differences in sample locations and varying levels of development going on at each locality as well as the enumeration techniques employed. These variations might also be as a result of the location of study and/or time of water analysis. The high bacteria load of water samples in this study is of immense public health importance because of their implications in a number of waterborne diseases. Bello *et al.*[21] isolated *E. coli* in borehole and well water sources in Ogun State. This is in tandem with our study as we also isolated *E. coli* in borehole and well water samples. Odeyemi *et al.*[8], in a related study of water samples from Arinta waterfall Ipole-Iloro, Ekiti State, also reported the presence of *E. coli* and *Aeromonas* species with percentage occurrence frequencies of 31.1 % and 4.4 % respectively. In support of our study, Bello *et al.*[21] reported that *Aeromonas hydrophila* was the most common species isolated in their study with an overall prevalence of 91 %, followed by *Aeromonas sobria* with a 9 % prevalence in water sources in North Eastern Nigeria. In a similar study, Nwachukwu *et al.*[18] reported the isolation of *Escherichia coli* (13.04 %) and *Vibrio* species (2.17 %) in drinking water samples from Abia State. Alessandro *et al.*[22], in their study, recovered 231 *Aeromonas* strains, most of them belonging to *Aeromonas hydrophila* and *Aeromonas media* species. The result of this study agrees with the findings of Hayes[23] who reported that *Aeromonas hydrophila* was the most well-known of the species belonging to the genus *Aeromonas* that inhabits aquatic environments. It has been reported[14,15] that *Aeromonas* species are found worldwide in aquatic environments, including ground water, surface waters, estuarine and marine waters, drinking water and wastewater as revealed and authenticated by this study. Sadiya *et al*.[15] reported the isolation of *Aeromonas* species and *Escherichia coli* from rivers, boreholes, and well water sources in Abuja. Our study is consistent with the work of Ehiowemwenguan *et al.*[26] in Eyaen community Area of Edo State, were pathogens such as *E. coli* and *Vibrio cholera* were isolated. The presence of these bacteria in water sources can be attributed to indiscriminate human and animal defecation and general poor sanitation[27]. According to Jay[28] *Escherichia coli* presence in drinking water sources is an indication of enteric pathogens. *Vibrio cholerae* infection is a major cause of diarrhea and vomiting in Nigeria. In our study, bacterial isolates (*Aeromonas hydrophila*, *Escherichia coli*, and *Vibrio cholerae*) were generally highly resistant (100 % - 81.6 %) to penicillin, trimethoprim-sulfamethoxazole, kanamycin, azithromycin, streptomycin, cephalothin, and cefuroxime but highly susceptible (100 % - 83.1 %) to gentamicin, meropenem, ciprofloxacin, imipenem, and chloramphenicol. Our study is in agreement with that of Mohammad *et al.*[29] in Abu Dahbi of United Arab Emirates who reported that *Aeromonas* species were resistant to the penicillins, trimethoprim-sulfamethoxazole, and erythromycin but sensitive to tetracycline, chloramphenicol, aminoglycosides, cephalosporins, and quinolones. Liezl and Hafizah[30] reported that *Aeromonas* species in their study were susceptible to aminoglycosides, fluoroquinolones, 2nd and 3rd generation cephalosporins, and carbapenems but were resistant to tetracycline, amoxicillin and augmentin. The responses of *Escherichia coli* to antibiotics in this study is in tandem with the study of Mulamattathil *et al.*[31] who reported that all *E. coli* environmental isolates were highly susceptible to streptomycin and ciprofloxacin but resistant to erythromycin and trimethoprim-sulfamethoxazole. The result of this study is in accordance with the study conducted by Moore *et al.*[32] (2010) who reported that *E. coli* isolates were resistant to erythromycin and tetracycline. Suma *et al.*[33] in their study on surface and drinking water in Mafikeng, South Africa reported that *Escherichia coli* isolates were susceptible to ciprofloxacin. In another study, Prabhat *et al.*[34] reported that out of a total of 233 *Escherichia coli* isolates from household water supply in Bangladesh, 57 % were resistant to ampicillin, followed by 45 % to tetracycline, 37 % to nalidixic acid, 36 % to trimethoprim-sulfamethoxazole, 17 % to ciprofloxacin, 9 % to ceftriaxone, 8 % to chloramphenicol, and 1 % to gentamicin. The *Vibrio cholerae* isolates in this study showed resistance to streptomycin, penicillin, cephalothin, trimethoprim-sulphamethoxazole, cefpirome, norfloxacin, kanamycin, azithromycin, nalidixic acid, cefuroxime, ceftriaxone, and oxytetracycline, but susceptible to gentamicin, chloramphenicol, meropenem, tetracycline, ciprofloxacin, imipenem, ofloxacin, and erythromycin. Ehara *et al.*[35] who carried out studies in Kenya reported that majority of *Vibrio cholerae* isolates were resistant to tetracycline. In disagreement with our findings, Shukla *et al.*[36] reported that 100 % *Vibrio cholerae* were resistant to ciprofloxacin. The result of our study is in not in agreement with the report of Idika[37], who studied *Vibrio cholerae* during an outbreak of cholera in Lagos in 1997 and reported that the isolates were resistant to tetracycline. Ash *et al*.[38] also reported high level of resistance in Gram-negative bacteria isolates from rivers in the United States and also revealed that the isolates exhibited multiple resistance to a number of commonly used antibiotics such as ampicillin, tetracycline, and penicillin. In a recent study of *Vibrio cholera* O_1 isolated during a cholera outbreak in Edo State, Ukaji *et al*.[39] reported that all isolates were susceptible (100 %) to ciprofloxacin, pefloxacin and sparfloxacin while some strains susceptible to gentamicin, streptomycin and augumentin. This is also in agreement with our study. In this study, MARI values of bacterial isolates ranged from 0.47 to 0.50. MARI value ranging from 0.12 to 0.59 were also reported by Liezl and Hafizah[30]. The high antibiotic resistance frequencies observed among bacterial isolates in this study might be due to the selective pressure exerted by the use of antibiotics in the management of bacterial

infections in animals or humans in the area studied. The high prevalence of multiple antibiotic-resistant organisms in the drinking water distribution system could potentially pose serious public health threat to humans consuming this water. The presence of multidrug-resistant bacterial pathogens in the drinking water sources is an important health concern due to the risk of developing waterborne diseases and the health risks associated with immunocompromised patients living in the study area.

CONCLUSION

The total bacterial count in this study did not meet international standards as they were higher than the WHO standard of zero (0) per 100 ml, signifying a potential problem for human and animal health. The risk of infection is further enhanced by the presence of opportunistic pathogens (*Aeromonas hydrophila, Escherichia coli* and *Vibrio cholerae*) in water samples studied. Bacterial isolates obtained in this study were multidrug-resistant as they exhibited resistance to penicillin, sulphamethoxazole, kanamycin, azithromycin, streptomycin, cephalothin, and cefuroxime. Gentamicin, meropenem, ciprofloxacin, imipenem, and chloramphenicol were the most effective antibiotics against the bacterial isolates from the various water sources. Undesirable properties of water quality caused by the presence of multidrug-resistant bacteria can pose a negative impact on human health. The high prevalence of multiple antibiotic-resistant organisms in the drinking water distribution system could potentially pose a threat to humans consuming this water. Dissemination of antibiotic resistant bacteria into the aquatic environment may impact human health as they may cause serious enteric diseases and other waterborne diseases which might be difficult to treat among people, especially immunocompromised individuals who consume such contaminated water.

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