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Bacteriological Quality and Prevalence of Multidrug Resistant Gram-negative Bacteria from Surface and Underground Domestic Water Sources in Selected Locations in Ibadan, Oyo State, Nigeria

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Authors' contributions

This research work was carried out in collaboration between the two authors. Author OSA designed the study, supervised the collection of samples, carried out the bench work, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the literature searches. Author OJF collected samples and carried out the bench work. The two authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To examine the bacteriological quality and prevalence of multidrug resistant (MDR) Gram-negative bacteria in surface and underground domestic water sources in Ibadan.
Study Design: Descriptive cross-sectional study.
Place and Duration of Study: Collection of water samples was in Ibadan, Oyo State, Nigeria; Analysis of water samples and characterisation of bacterial isolates was at the laboratory of the Department of Pharmaceutical Microbiology, University of Ibadan, between September 2015 and November 2015.
Materials and Methods: Twenty six water samples involving 2 collections each at 2 weeks intervals, from 13 different selected sources (8 hand-dug wells, 3 flowing rivers and 2 dams) were collected sequentially within Ibadan. Determination of total viable bacteria count was by pour-plate method, presumptive coliform count was by broth-dilution method, Bacterial identification was by

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standard methods and antibiotic susceptibility testing was by disc-diffusion method.
Results: The mean values of the total viable counts for the first water sample collection ranged from 3.1×10^2 to 6.2×10^4 cfu/mL and 2.4×10^2 to 6.1×10^4 cfu/mL for the second collection with the mean difference statistically significant for samples from sites AJR, DDR, ADM, EDM, WW-B and WW-G ($P > .05$). Most Probable Number of Coliforms ranged from 20 to >180/100 mL. A total of 7 bacteria genera including *E. coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp., *Serratia* spp., *Providencia* spp. and *Enterobacter* spp. were isolated. Among the isolates, 100% showed resistance to ampicillin, over 80% to amoxicillin, amoxicillin-clavulanic acid, cefuroxime and cefotaxime, 60% to ceftazidime, over 50% to ciprofloxacin and aztreonam, <40% to ofloxacin, nitrofurantoin and gentamicin while 100% were susceptible to the carbapenems. A total of 90.9% exhibited MDR phenotype.
Conclusion: In this study, the presence of high level of coliforms with MDR phenotype in surface and underground water used domestically in Ibadan signifies a public health hazard that requires urgent attention.

Keywords: Coliforms; bacteriological; multidrug resistance; gram-negative; viable count.

1. INTRODUCTION

Water is an important component of all living things and is the most abundant component on the earth [1-3]. However, one of the greatest challenges facing most developing countries such as those in Africa is the availability and access to safe drinking water [4,5]. Water is very essential for the survival of plants, animals, and humans [6]. Chemically, water is the combination of two hydrogen elements with single oxygen molecule and ideally this is expected to be pure without any other chemicals or particles being present for human use. However, in nature, water is found in combination with other elements usually introduced to it by several external factors [7]. In Nigeria, several efforts have been made by the government to provide potable water for the increasing population but still, over 52% of its population lack access to safe drinking water [8].

There are several sources of water. They include Surface water (examples of which are rivers, streams, dams, lagoons and oceans), underground water (examples of which are wells and boreholes) and atmospheric water (examples of which are rain, dew, snow, hail, ice, and atmospheric moisture). The rain water usually should be the purest among the three main sources when collected directly from the atmosphere without the falling droplets touching any object. However, apart from dissolved atmospheric gaseous contaminants, microorganisms usually carried in the atmosphere by air current are also major contaminants of rain water [9,10]. Surface water is the most exploited of the three main sources [11] and is also the most abused with high level of microbial contamination. Apart from the fact that surface water flow over the earth surface where soil microbial contaminants diffuse into the water, several human and animal activities also affect the microbial quality of surface water. In most places in Nigeria where surface water, particularly flowing river or stream are used for domestic purposes, people also use it for recreational purposes such as swimming, and out of ignorance, disposal of sewage and refuse especially during rainy season [12]. Some individuals often defecate into the water believing that animals in the water body will feed on it.

Underground water naturally is water from the atmosphere and earth surface that has percolated through the coarse particulate network of the soil, which serves as natural filter against microbial contaminants, into the water table [2] and thus is expected to be of better quality than the surface water. Underground water is gradually becoming over exploited in Nigeria due to the inability of the government to provide adequate potable water supply to all communities in the country [11]. There are several rules guiding the digging of wells, but because of poverty some cannot afford the sinking of deeper wells called boreholes and thus employ the services of local well diggers who most times hand-dig wells irrationally and even sometimes closer to locations like soak away pit, latrine, and sewage septic tanks that usually encourages microbial contamination of such wells [11]. Some of these hand-dug wells are not hygienically protected and are never treated from time to time [11]. Most are made in a way that provides easy access to reptiles and insects that defecate or sometimes get drowned in the wells. The manner in which some even draw water

from the wells encourages contaminations through the rope and containers being used.

The world health organization (WHO) has defined portable water as water in which the physical, chemical and microbiological quality is within acceptable limit [13]. However, the truth is, over one billion people worldwide have no access to portable water [14]. This has resulted to increased cases and spread of waterborne diseases throughout the world [15]. WHO reported an estimation of over two million deaths as a result of waterborne diseases and over four billion diarrhea cases worldwide annually. In Africa, the WHO has estimated that a child has five episodes of diarrhea in a year with about 800,000 deaths of children per year from diarrhea and dehydration [14]. These have been attributed to the presence of bacteria pathogens in the drinking water which resulted into various waterborne diseases such as cholera, typhoid fever, bacillary dysenteries and many gastro-intestinal diseases [16]. Drinking of water contaminated with human and animal feces exposes individuals to high risk of microbial infections, especially feces from infected or carriers of waterborne disease causing agents.

The City of Ibadan is the third largest metropolitan area by population and the largest metropolitan geographical area in Nigeria [17]. The city is inhabited by multi-ethnic groups from almost all the six geo-political zones of the country [17]. In most communities in Ibadan, rearing of domestic animals such as local chickens, cats, dogs, goats, sheep; herdsmen with cattle are very common. These animals like humans in the communities have equal access to the available unprotected surface water for drinking and bathing, hence this is a potential portal for water contamination with fecal materials. There have been several reports of indiscriminate refuse and waste disposal in the city [18] especially into water ways which have often lead to flooding. The two commonly engaged water sources for domestic use in Ibadan are the underground and surface water and several authors have reported that most of the water sources in the city are contaminated with coliforms like *Escherichia coli* which is an indicator of fecal contamination of water [2,11,19,20]. Coliforms themselves may not necessarily be harmful to human and animal health but their presence portends the most probable presence of other pathogenic bacteria like *Salmonella typhi*, *Shigella* spp., Enterotoxigenic *E. coli*, *Vibrio* spp. among others [21,22]. Several outbreaks of water-borne diseases have been reported in the city [11,23] and despite government effort in educating the communities on the implications of water pollution it appears the level of water pollution in the city is still on the high side.

In this era of high prevalence and spread of multidrug resistant bacteria isolates [24] which has made antibiotic treatment of most bacterial infections difficult, this study investigated bacteriological quality of randomly selected underground and surface water in Ibadan and further determined the antibiotic resistance profile and prevalence of multidrug resistance among the Gram-negative bacteria isolated.

2. MATERIALS AND METHODS

2.1 Study Area

The research was carried out in Ibadan mostly Ibadan north local government, Oyo State, Nigeria. The city is located between latitudes $7°00^1$ and $7°30^1$ and between longitudes $3°30^1$ and $4°00^1$ in southwestern Nigeria [11]. Ibadan is the largest indigenous city located within the coordinates; 7.3775°N, 3.9470°E, Nigeria, West Africa and has a land surface area of 828 km square with a population of approximately 2.6 million by the 2006 census [17].

2.2 Study Sample

Thirteen different water sources used for domestic purposes in Ibadan were studied. They include three different flowing rivers namely Orogun river (OOR), Dandaru river (DDR) and Ajibode river (AJR), two water dam namely Awba dam (ADM) and Eleyele dam (EDM), and eight different poorly protected hand-dug wells randomly selected in different communities within the city (labeled WW-A to WW-H).

2.3 Water Sample Collection

Water samples were collected from 13 random locations aseptically in wide-mouth 250 mL cleaned sterile bottles with screw cap closure. Water samples collected in the rivers and dams were made at a point half way between the edge and the center of the river or dam with the mouth of the bottle placed against the water current until it overflowed with water after which the bottle was covered aseptically. Water samples from

open wells were collected by submerging the sterile wide-mouth bottle completely into the well with the aid of a clean strong rope and withdrawing after the bottle was full and then aseptically capped. The bottles containing the water samples were then labeled and immediately transported to the laboratory of the Department of Pharmaceutical Microbiology, University of Ibadan for microbiological analysis. Water samples were collected twice from each location at an interval of two weeks from the first collection.

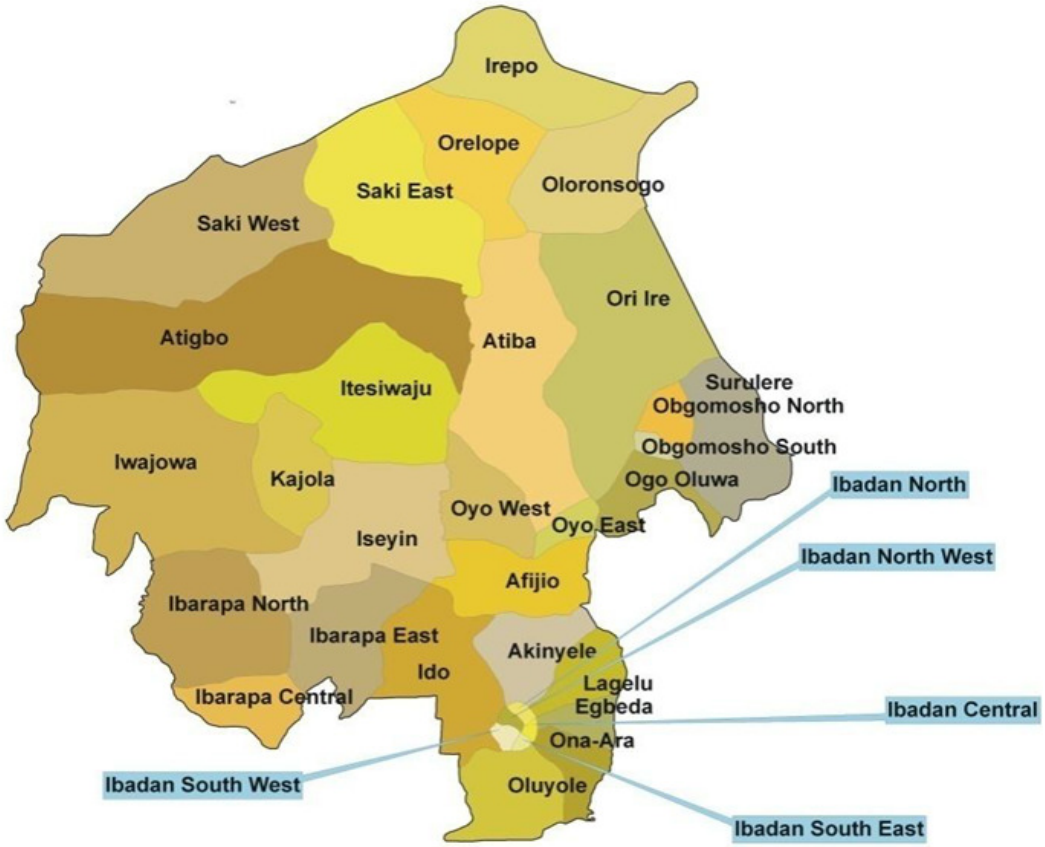

Fig. 1. The map of Oyo State
(Source: NPC, 2006) [17]

(A) **(B)** **(C)**

Fig. 2. A – Unhygienic river; B - Unhygienic hand-dug well; C – Well dug closer to a bathroom and toilet

2.4 Bacteriological Analysis of Water Samples

2.4.1 Bacteria total viable count

Using sterile distilled water, serial dilution of the water samples was done by adding 1 mL of the water sample into 9mL of sterile distilled water to give a 10^{-1} dilution. This was further diluted serially up to 10^{-4}. For each of the diluted water sample, 0.1mL of the 10^{-4} dilution was plated on Nutrient agar by surface spreading in triplicates. The plates were incubated in an inverted position at 37°C for 24 hours. The number of colonies after 24 hours of incubation was counted in the triplicate plates and each value used in the calculation of the total viable count as colony-forming-unit per mL (CFU/mL). This procedure was carried out on the two water samples collected at each location and data obtained entered into SPSS version 16 package for statistical analysis.

2.4.2 Presumptive coliform count

This determines the Most Probable Number (MPN) of coliform per 100 mL of the water sample. It involves a series of lactose broth tubes, one containing 50 mL of double strength MacConkey broth, five contained 10 mL double strength each, and another five tubes also containing 5 mL of single strength MacConkey broth each, all having a Durham tube suspended in an inverted position in each of the liquid medium for gas collection. The set of tubes, one containing 50 mL of double strength MacConkey broth and five tubes containing 10 mL each, were inoculated with 50 mL and 10 mL each, of the water sample respectively, while another five tubes containing 5 mL single strength MacConkey broth each, were inoculated with 1mL each, of the water sample. The tubes were incubated at 37°C for 24 hours. The presence of space (gas production) in the upper part of the Durham tube in the medium and the colour change of the medium from violet to yellow (acid production) simultaneously in anyone or all of the tubes is a presumptive evidence of the presence of coliforms in the water sample. The MPN of the coliforms in 100 mL of the water sample was estimated by comparing the number of positive tubes (gas and acid production) in each set of test with those recorded in the McCrady's probability table [25]. The magnitude of MPN is a qualification of the probability of the presence of pathogens [25]. This procedure was also carried out on the two water samples collected at each location and data obtained recorded.

2.4.3 Isolation and identification of coliforms and other gram-negative bacteria

Loop full of broth from the coliform-positive tubes of each water sample was streaked on Eosine Methylene Blue (EMB), MacConkey (MCA) and Pseudomonas cetrimide agar (PCA) plates. The plates were incubated at 37°C for 24 hours in an inverted position. Presence of *E. coli* was determined on EMB agar as green metallic sheen colonies characteristic of *E. coli*. Presence of other Gram-negative bacilli was also determined on EMB and MCA, and *Pseudomonas* spp. on PCA, based on their cultural and morphological characteristics. Each of the different colonies was separated into pure culture by continuous streaking on the different media. The pure cultures were further identified by Gram's reaction and standard biochemical tests such as indole, catalase and oxidase test. The isolates were also identified using Microbact™ GNB 12E Identification kit (Oxoid Ltd, Basingstoke, Hants, UK). This procedure was carried out only on the first water samples collected at each location.

2.5 Antibiotic Susceptibility Test

The bacterial isolates were subjected to antibiotic susceptibility testing against thirteen antibiotics selected from seven classes (penicillin, cephalosporin, aminoglycoside, fluoroquinolone, monobactam, carbapenem and nitrofuran) using the disc-diffusion method. Bacteria suspension was prepared from overnight nutrient broth culture of the isolates and diluted to 0.5 McFarland standard with sterile distilled water. With the aid of sterile cotton swab, the bacteria suspension was spread over the surface of Mueller Hinton agar to give a monolayer of bacteria cell on the agar surface. Standard antibiotic disc were then aseptically placed at equal distance on the inoculated agar plate. The antibiotics used are ampicillin (10 µg), amoxicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), cefuroxime (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), aztreonam (30 µg), imipenem (5 µg), ertapenem (5 µg) and nitrofurantoin (300 µg). The plates are then incubated at 37°C for 24 hours in an inverted position. The zones of inhibition were recorded and interpreted as sensitive or resistance according to the Clinical Laboratory Standards Institute guidelines [26]. Isolates exhibiting resistance to three or more classes of antibiotics were classified as MDR [27].

3. RESULTS

The results of the total viable count of the entire aerobic bacteria in cfu/mL and the most probable number of coliforms per 100 mL of the water samples are presented in Table 1. The mean values of the triplicate total viable counts for the first water sample collection ranged from 3.1×10^2 to 6.2×10^4 cfu/mL (standard error of mean (S.E.M) ranged from 8.8×10^1 to 3.3×10^2) with standard deviation (S.D) ranging between 1.5×10^1 and 1.5×10^3 while for the second collection ranged from 2.4×10^2 to 6.1×10^4 cfu/mL (S.E.M rage $6.7\times10^\circ$ to 1.2×10^3) with S.D ranging between 1.2×10^1 and 2.0×10^3. Some of the wells (WW-C, WW-E and WW-F) recorded higher number of aerobic bacteria compared to the rivers and dams. The differences between the mean values of the total viable count (cfu/mL) of the first and second water samples collected after analysis using Paired-Samples T test was statistically significant for samples from AJR, DDR, ADM, EDM, WW-B and WW-G (Table 1). All the water sources had higher (>180 MPN/100 mL) most probable number of coliforms per 100 mL except for two well water samples WW-B and WW-H having 20 and 40 MPN/100 mL respectively. Table 2 showed the different cultural and biochemical tests used in the identification of bacteria isolated from the water samples. The standard identification kit, Microbact™ GNB 12E (Oxoid Ltd, Basingstoke, UK) identified bacteria isolates to their specie level at over 90% probability for all the Enterobacteriaceae. The distribution of the various bacteria species in the different water samples are presented in Table 3. All the river and dam water samples and one of the wells (WW-C) were positive for *E. coli*. All the water sources had at least two or more bacteria except for one of the dams (ADM) which was contaminated with only *E. coli*.

Fig. 3 showed the overall resistance profile of the bacteria isolated from the water samples. All the isolates were resistant to ampicillin, over 80% were resistant to amoxicillin, amoxicillin-clavulanic acid, cefuroxime and cefotaxime while 60% were resistant to ceftazidime, 57.6% to ciprofloxacin, 54.5% to aztreonam, 39.4% to ofloxacin and 36.4% to nitrofurantoin. All (100%) the isolates were susceptible to imipenem and ertapenem.

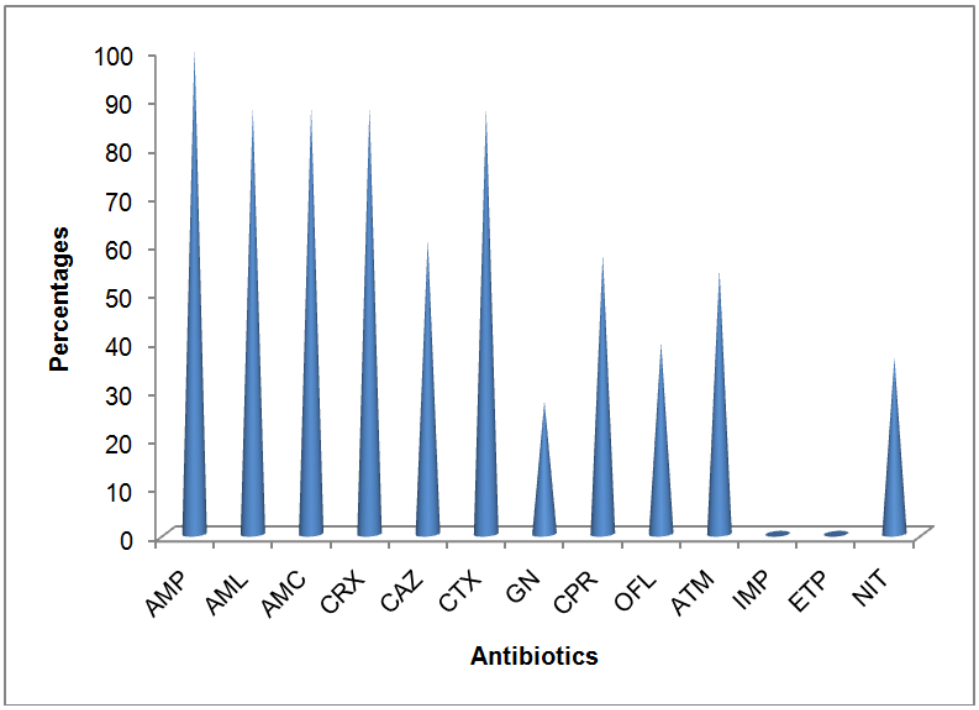

Fig. 3. Overall antibiotic resistance profile of the bacterial isolates

Keys: AMP – Ampicillin, AML – Amoxicillin, AMC – Amoxicillin-clavulanic acid, CRX – Cefuroxime, CAZ – Ceftazidime, CTX – Cefotaxime, GN – Gentamicin, CPR – Ciprofloxacin, OFL –Ofloxacin, ATM – Aztreonam, IMP – Imipenem, ETP – Etapenem, NIT – Nitrofurantoin

Table 1. Bacteria total viable count cfu/mL and most probable number of coliform per 100 mL of the water samples

Water sample	First collection			Second collection			*P* value for paired differences	MPN of coliform/100 mL	
	Mean	S.E.M	S.D	Mean	S.E.M	S.D		First collection	Second collection
AJR	3.1×10^{3}	6.7×10^{1}	1.2×10^{2}	4.1×10^{3}	5.8×10^{1}	1.0×10^{2}	.007*	180+	180+
DDR	4.0×10^{3}	8.8×10^{1}	1.5×10^{2}	4.1×10^{3}	8.8×10^{1}	1.5×10^{2}	.04*	180+	180+
OOR	4.6×10^{4}	5.8×10^{2}	1.0×10^{3}	5.2×10^{4}	1.2×10^{3}	2.0×10^{3}	.07	180+	180+
ADM	4.8×10^{4}	3.3×10^{2}	5.8×10^{2}	5.3×10^{4}	3.3×10^{2}	5.8×10^{2}	.003*	180+	180+
EDM	5.6×10^{4}	3.3×10^{2}	5.8×10^{2}	5.0×10^{4}	3.3×10^{2}	5.8×10^{2}	.02*	180+	180+
WW-A	5.2×10^{3}	8.8×10^{1}	1.5×10^{2}	4.9×10^{3}	3.3×10^{1}	5.8×10^{2}	.12	180+	180+
WW-B	3.1×10^{2}	8.8×10^{0}	1.5×10^{1}	2.4×10^{2}	6.7×10^{0}	1.2×10^{1}	.002*	20	20
WW-C	5.8×10^{3}	8.8×10^{1}	1.5×10^{2}	6.0×10^{3}	5.8×10^{1}	1.0×10^{2}	.07	180+	180+
WW-D	4.4×10^{3}	8.8×10^{1}	1.5×10^{2}	3.9×10^{3}	8.8×10^{1}	1.5×10^{2}	.12	180+	180+
WW-E	6.2×10^{4}	8.8×10^{2}	1.5×10^{3}	6.1×10^{4}	1.2×10^{3}	2.0×10^{3}	.53	180+	180+
WW-F	5.2×10^{3}	5.8×10^{1}	1.0×10^{2}	5.0×10^{3}	3.3×10^{1}	5.8×10^{1}	.12	180+	180+
WW-G	4.8×10^{4}	5.8×10^{2}	1.0×10^{3}	4.4×10^{4}	8.8×10^{2}	1.5×10^{3}	.02*	160	180+
WW-H	4.4×10^{4}	5.8×10^{2}	1.0×10^{3}	4.3×10^{4}	1.2×10^{3}	2.0×10^{3}	.42	40	20
Standard limit	1.0×10^{2}	NA	NA	1.0×10^{2}	NA	NA	NA	Nil	Nil

*Keys: AJR – Ajibode river, DDR – Dandaru river, OOR – Orogun river, ADM – Awba Dam, EDM - Eleyele Dam, WW – Well water A – H, S.E.M – Standard Error of Mean, S.D – Standard deviation, NA – Not Applicable, * = statistically significant paired differences using Paired-Sample T Test at 95% confidence interval*

Table 2. Gram reaction, cultural and biochemical characteristic of the bacterial isolates

S/N	Gram stain	Shape	Cultural characteristics			Biochemical test				Suspected organism	Microbact™ GNB 12E ID Kit	
			Green metallic sheen on EMB agar	Growth on cetrimide agar	Lactose fermentation on macconkey agar	Motility	Indole	Catalase	Oxidase		Bacteria identified	Percentage probability
1	-	Rod	+	-	+	+	+	+	+	*E coli*	*Escherichia coli*	99.99
2	-	Rod	-	-	-	+	-			*Proteus* spp	*Proteus mirabilis*	99.81
3	-	Rod	-	-	-	+	+			*Proteus* spp	*Proteus vulgaris*	99.81
4	-	Rod	-	-	+		+			*Klebsiella* spp	*Klebsiella oxytoca*	95.67
5	-	Rod	-	+	+	+	-	+	+	*Pseudomonas* spp.	*ND*	ND
6	-	Rod	-	-	+					*Serratia* spp	*Serratia marcescens*	99.81
7	-	Rod	-	-	-	+	-			*Proteus* spp	*Providencia stuartii*	98.95
8	-	Rod	-	-	+	+	-			*Enterobacter* spp	*Enterobacter agglomerans*	99.95

Table 3. Distribution of the Gram-negative bacteria in surface and underground water sources

Water sources	*E. coli*	*P. mirabilis*	*P. vulgaris*	*K. oxytoca*	*Pseudomonas* spp.	*Serratia marcescens*	*P. stuartii*	*E. agglomerans*
AJR	+	-	+	-	-	-	-	-
DDR	+	+	-	-	-	-	-	-
OOR	+	-	+	-	-	-	-	-
ADM	+	-	-	-	-	-	-	-
EDM	+	-	-	+	-	-	-	-
WW-A	-	+	-	+	+	-	-	-
WW-B	-	-	+	+	-	-	-	-
WW-C	-	+	-	-	-	-	+	+
WW-D	-	+	-	-	-	-	+	-
WW-E	+	-	+	+	+	+	-	-
WW-F	-	+	-	-	+	-	+	-
WW-G	-	-	-	+	-	-	+	-
WW-H	-	+	-	-	+	-	+	+

Keys: AJR – Ajibode river, DDR – Dandaru river, OOR – Orogun river, ADM – Awba Dam, EDM - Eleyele Dam, WW – Well water A – H

Table 4. Antibiotic resistance profile of each bacteria species isolated

Antibiotic	Bacterial isolates/percentage antibiotic resistance							
	E. coli **(n = 6)**	***K. oxytoca*** **(n = 5)**	***P. mirabilis*** **(n = 6)**	***P. vulgaris*** **(n = 4)**	***P. stuartii*** **(n = 5)**	***E. agglomerans*** **(n = 2)**	***Pseudomonas*** **spp. (n = 4)**	***Serratia marcescens*** **(n = 1)**
AMP	6(100%)	5(100%)	6(100%)	4(100%)	5(100%)	2(100%)	4(100%)	1(100%)
AML	5(83.3%)	4(80.0%)	6(100%)	4(100%)	4(80.0%)	1(50.0%)	4(100%)	1(100%)
AMC	5(83.3%)	5(100%)	5(83.3%)	4(100%)	5(100%)	0(0.0%)	4(100%)	1(100%)
CRX	6(100%)	4(80.0%)	5(83.3%)	4(100%)	5(100%)	0(0.0%)	4(100%)	1(100%)
CAZ	4(66.7%)	3(60.0%)	1(16.7%)	3(75.0%)	5(100%)	0(0.0%)	3(75.0%)	1(100%)
CTX	6(100%)	5(100%)	3(50.0%)	4(100%)	4(80.0%)	2(100%)	4(100%)	1(100%)
GN	3(50.0%)	1(20.0%)	0(0.0%)	2(50.0%)	2(40.0%)	0(0.0%)	1(25.0%)	0(0.0%)
CPR	6(100%)	5(100%)	0(0.0%)	2(50.0%)	4(80.0%)	0(0.0%)	2(50.0%)	0(0.0%)
OFL	4(66.7%)	3(60.0%)	0(0.0%)	1(25.0%)	3(60.0%)	0(0.0%)	2(50.0%)	0(0.0%)
ATM	4(66.7%)	4(80.0%)	3(50.0%)	1(25.0%)	3(60.0%)	2(100%)	1(25.0%)	0(0.0%)
IMP	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
ETP	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	ND	0(0.0%)
NIT	3(50.0%)	0(0.0%)	1(16.7%)	1(25.0%)	2(40.0%)	1(50.0%)	3(75.0%)	1(100%)

Keys: AMP – Ampicillin, AML – Amoxicillin, AMC – Amoxicillin-clavulanic acid, CRX – Cefuroxime, CAZ – Ceftazidime, CTX – Cefotaxime, GN – Gentamicin, CPR – Ciprofloxacin, OFL –Ofloxacin, ATM – Aztreonam, IMP – Imipenem, ETP – Etapenem, NIT – Nitrofurantoin, ND – Not done

Table 4 showed the antibiotic resistance profile of each of the different bacteria species isolated. As in the case of ampicillin, 100% resistance was also recorded against amoxicillin, amoxicillin-clavulanic acid, cefuroxime and cefotaxime by all the isolates of *P. vulgaris*, *Pseudomonas* spp. and *Serratia marcescens*. Also, 100% resistance was recorded against ciprofloxacin by the isolates of *E. coli* and *K. oxytoca*. The two *E. agglomerans* isolated showed 100% resistance to cefotaxime and aztreonam. The *P. stuartii* isolates showed 100% resistance to amoxicillin-clavulanic acid, cefuroxime and ceftazidime. As in the case of the carbapenems, 100% susceptibility was recorded for *P. mirabilis* against gentamicin, ciprofloxacin and ofloxacin; *E. agglomerans* against amoxicillin-clavulanic acid, cefuroxime, ceftazidime, gentamicin, ciprofloxacin and ofloxacin and *Serratia marcescens* against gentamicin, ciprofloxacin, ofloxacin and aztreonam.

The distribution of the MDR isolates among the different water sources is presented in Table 5. Overall, 90.9% of the entire Gram-negative bacteria isolated in this study were MDR. All the bacteria recovered from the rivers and dams are all MDR isolates while just 9.1%, all of which are from the different wells, did not exhibit MDR phenotype with respect to the panel of antibiotics used in this study.

4. DISCUSSION

Water is an important factor to life as no living things can do without it. However, it is important that water for domestic use be clean and potable for human and animal consumption so as to prevent outbreaks of waterborne diseases. In this study, all the water samples examined were found to have high total viable counts of aerobic bacteria and high numbers of coliforms per 100 mL above the WHO standard limit [28]. Similar report was made by Ayantobo et al. [11] on quality of different hand-dug well in Ibadan, Oyo State, Nigeria. This may likely be due to the level of hygiene around the water sources as reported by Ayantobo et al. [11]. Most unprotected streams, rivers and dams are accessible for humans (Fig. 2A) and animals and are usually heavily contaminated [29]. In this study, the communities where the river and dam water samples were collected rear some domestic animals such as cats, dogs, pigeon birds, goat and sheep that could visit the site to drink water and in the process defecate into the water. There are also reptiles found around the river and dam sites. Some people in the community often come into the river to swim, wash and sometimes pour garbages into flowing rivers or streams especially during raining season. All these reasons may be responsible for the high level of coliforms recorded in all the river and dam water samples in this study. However, high level of coliforms recorded in the well water sample correlated with the report of Ayantobo et al. [11] on unprotected hand-dug wells located closer to sewages or garbage dump site as experienced in this study. All the wells visited in this study are hand-dug wells some without visible protective measures and some poorly constructed in an unhygienic environment (Fig. 2B). The isolation of *E. coli* from all the water samples from the river, dam and one of the wells (WW-E) is an indication of recent fecal contamination. The well (WW-E) been closer to a toilet (Fig. 2C) suggest that the fecal substances find its way to contaminate the well as reported by Ayantobo et al. [11]. The statistically significant differences recorded between the first and second collections of the water samples from sites AJR, DDR, ADM, EDM, WW-B and WW-G suggest that the level of microbial contamination of these water sources changes with time depending on the source of contamination. For river or well located closer to a permanent garbage dump site, pit latrine or sewage septic tank, there is possibility of constant high level of microbial contamination with less variation with time, however, in the case of temporary location of contaminating source, the level of microbial contamination will only be higher in the presence of the contaminating source but lower in the absence. Temporary contaminating sources could be single time dumping of contaminated garbage or sewage closer to water sources.

In this study, 87.9% of the Gram-negative bacteria isolated were identified to belong to the members of the coliform bacteria while the remaining 12.1% was identified as *Pseudomonas* spp. Although these bacteria can be found in the soil and are not usually pathogenic, they could be opportunistic pathogens to some individual within the community especially children below 5 years of age and those that are immuno-compromised [30]. Some of these bacteria have been linked with certain outbreak of infections such as urinary tract infections, diarrhea, dysentery and others [30,31]. The results of the antibiotic susceptibility test revealed high level (>70%) resistance of the isolates to ampicillin, amoxicillin, amoxicillin-clavulanic acid, cefuroxime and cefotaxime with 90.9% of

Table 5. Distribution of the multidrug resistant bacteria in the water sources

Water sources	Number of isolates (n)	Isolates with MDR phenotype	
		Number (n)	Percentage (%)
AJR	2	2	100
DDR	2	2	100
OOR	2	2	100
ADM	1	1	100
EDM	2	2	100
WW-A	3	2	66.7
WW-B	2	2	100
WW-C	3	3	100
WW-D	2	2	100
WW-E	5	5	100
WW-F	3	3	100
WW-G	2	1	50
WW-H	4	3	75
Total (N)	33	30	90.9

Keys: AJR – Ajibode river, DDR – Dandaru river, OOR – Orogun river, ADM – Awba Dam, EDM - Eleyele Dam, WW – Well water A – H

the isolates exhibiting MDR phenotype. Percentage resistance of the isolates to gentamicin, ciprofloxacin, ofloxacin, aztreonam and nitrofurantoin falls below 60% with the carbapenem having 100% activity against all the isolates. This result correlated with results of some previous authors who had carried out similar study. Ayandiran et al. [32] worked on bacterial isolates from Obere river in Orile-Igbon, Oyo state and reported 100% resistance of the isolated bacteria to amoxicillin, amoxicillin-clavulanic acid, 90% to ceftriaxone, 35% to ciprofloxacin and 90% of the isolates exhibiting MDR phenotype. In another study by Odeyemi et al. [33] on isolated bacteria from Arinta Waterfall in Ipole-Iloro Ekiti state, they reported 100% resistance of the isolated Gram-negative bacteria to amoxicillin-clavulanic acid and ampicillin with 99.6% of the isolates exhibiting MDR phenotype. Atobatele and Awoseni [34] in their study on bacteria isolated from a lentic freshwater body in Iwo, Osun state, Nigeria reported 87% and 76% resistance of the isolated bacteria to amoxicillin and amoxicillin-clavulanic acid respectively with 88.5% of the isolates exhibiting MDR phenotype. All these various reports in and around Oyo state further supported the findings in this study of high level spread of MDR bacteria in domestic water sources in Ibadan and thus necessitate that awareness on adequate water treatment before use for domestic purposes be made to various communities through relevant channels.

5. CONCLUSION

This study reported high level of bacterial contamination, including coliforms and *Pseudomonas* spp., in surface and underground domestic water sources from selected locations in Ibadan city and thus certifies these water sources as unfit for domestic use. The high most probable number of coliform per 100 mL of the water samples recorded in this study was basically as a result of the sites and the unprotected condition of the hand-dug wells, as well as the rivers and dams. This thus, further elucidated the low level of awareness of the people in this community on the health hazards of using unwholesome water for domestic purposes and the danger of unhygienic digging of wells closer to sanitary facilities, waste dump sites and even burial ground. The level of dissemination of MDR bacteria in the different water sources calls for a serious and prompt attention to avoid an outbreak of waterborne infections due to these MDR bacteria strains. The ability of a non-pathogenic MDR isolate to transfer its MDR genetic trait to a pathogenic bacteria present in domestic water source is indeed a serious public health risk. Hence, effort must be made to create awareness and educate communities using underground and surface water for domestic purposes on the need for adequate protection from contamination and constant treatment of water before use.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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