Incidence of drug resistant bacteria and physicochemical properties of Ero Dam, Nigeria

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Abstract: An investigation on microbiological and physico-chemical properties of untreated and treated water from Ero-dam, Ikun-Ekiti was carried out in 2009. Samples of untreated and treated water collected from the dam and inflow rivers were analysed for total viable bacteria and coliforms using the standard methods. Antibiotic susceptibility testing was by NCCL technique. The pH ranged from 7.35 to 8.10 for both treated and untreated water sample while the temperature ranged from 24^0C to 28^0C. The total viable and coliform counts for all the water samples were generally high exceeding the internationally defined limits for drinking water. The isolates organisms were identified to be *E. coli*, *Klebsiella* spp S*taphylococcus* spp, *Salmonella* spp, *Shigella* spp, *Proteus* spp, *Bacillus* spp, *Pseudomonas aeruginosa*, *Streptococcus* sp, *Serratia marcescens* and *Falvobacterium* spp. Twenty one multiple antibiotic resistance patterns were demonstrated by the isolated bacteria. Recommendations for an improved rural water supply scheme are suggested.
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INTRODUCTION

Water that is safe to drink, pleasant to taste and usable for domestic purpose is termed potable water while contaminated water is one that contains microorganisms, chemicals, industrial or domestic waste or sewage so that it is unfit for its intended use (Pelczer 1999). Water that is absolutely pure is however not found in nature because natural water from all sources are associated with some kind of contaminants, their nature and amount varying with the sources of the water. There is therefore the need to subject raw source waters to treatment before consumption. Although contaminants in water are divided roughly into three categories: physical, chemical and biological, environmental risk assessment today reveals that the exposure to biological contaminants especially water-borne microbial pathogens needs to be given higher priority in treatment and regulatory programs for domestic water supplies (Crann 1986, 1988) .

Quality standards for treated drinking water vary from place to place. The objective of most treatment schemes is largely to reduce the possibility of the spread of water borne disease to the bearest minimum in addition to considerations for its wholesomeness and palatability in all respects (Edema et al. 2001). It is much undoubted that for any water treatment program, multiple barriers are essential to ensure the quality of drinking water; a single barrier cannot always be relied upon, as there might be technical or operational breakdowns. Expert recommendations suggest that using this approach, safe drinking water quality could be achieved even before the final treatment step, so that failure of any one process will not result in waterborne diseases. Sanitation, source protection, water treatment and protection of distribution and storage units are vital to making this a reality.

In many developing countries like Nigeria, unavailability of treated water for drinking and domestic usage has become a critical problem and it is a matter of great concern to communities depending on public water supply system. Furthermore, conformation with international and nationally defined microbiological standards is of

special interest because of the capacity of treated water from government municipalities to spread diseases within a large population (Okonko et al. 2008). Among issues identified in available literature as responsible for this are failures in the treatment and distribution processes. The call for increased surveillance and more studies that aim at identifying opportunities for improvement is thus a necessity.

With a view to identifying improvements in the current treatment system available at a mini-water treatment plant available for rural residents in South Western Nigeria, the study focused on the bacteriological and physico-chemical quality of treated and untreated water samples from Ero dam, Ekiti State, Nigeria.

MATERIAL AND METHODS

Samples were collected from different locations (raw, treatment point and consumer points) using clean and sterilized bottles. The raw samples were collected from Ayetoro-Ekiti river inflow, Igogo-Ekiti river inflow, Eda-Oniyo Ekiti river inflow, middle dam and dam outflow. Samples for the treatment point were collected from the reservoir while the consumer point samples were collected from the taps located along the street in Ikun –Ekiti. The samples were collected four times with a month interval and each batch of the samples were transported in ice to the laboratory and were processed for microbiological and physic-chemical analysis within 6 hours of collection.

Physicochemical Analyses: The water sample temperatures were taken at the site of collection using a simple thermometer calibrated in ^{0}C [as described by Edema et al (2000)], electrical conductivity was measured with a CDM 83 conductivity meter (Radio Meter A/S Copenhagen, Denmark). Turbidity and pH were determined at site using Water Proof Scan 3+ Double Junction (Wagtech International, UK) and HI 98311-HI 98312 (Hanna) Water Proof EC/TDS and Temperature Meters (Wagtech International, UK). The water samples were then stored in the deep freezer until analyzed. Other physicochemical characteristics analysed were hardness determined by titrimetry; total dissolved solid and total suspended solid as determined by gravimetric method; acidity, alkalinity and sulphate were determined by titrimetry. Both nitrate and phosphate were determined colorimetrically by Spectronic-20 (Gallenkamp, UK) as described by the Official Methods of Analysis. Association of Official Analytical Chemists (Anonymous, 1990). Manganese was determined using atomic absorption spectrophotometer (Perkin-Elmer Model 403).

Microbiological analysis of water

Media used for the microbiological analysis of the water samples include Standard Plate Count Agar (SPCA), Eosine Methylene Blue (EMB), Salmonella-Shigella agar (SSA), Nutrient agar (NA), MacConkey agar and Potato Dextrose Agar (PDA). As described by Olutiola et al (1991), serial dilution method was used for total viable count and coliforms count. Appropriate dilutions (10^{-3} and 10^{-4}) of each sample were introduced with the aid of sterile pipette into prepared agar plates and incubated at 37^0C for 24 hours. With reference to Bergey's manual of determinative bacteriology (Buchanaaan and Gibbon, 1974), pure cultures of bacteria isolated were subjected to various morphological and biochemical characterization tests to determine their identity.

Antibiotics sensitivity testing

The antibiotics susceptibility of the isolates was determined by the disk diffusion method on Mueller-Hilton agar according to CLSI guidelines for antimicrobial susceptibility testing (Anonymous 2005). The bacterial isolates were tested against ABTEK disc antibiotics which comprised of Ampicillin (AMP), Cotrimoxazole (COT), Gentamicin (GEN), Nalidixic acid (NAL), Nitrofurantoin (NIT), Colistin (COL), Stretomycin (STR), Tetracycline (TET), Ciprofloxacin (CIP) , Penicilin (PEN) and Clindamycin (CLN). The inoculum was standardized by adjusting its density to equal the turbidity of a barium sulphate ($BaSO_4$) (0.5 McFarland turbidity standard), and incubated at 35^oC for 18 h. The diameter of the zone of clearance (including the diameter of the disk) was measured to the nearest whole millimeter and interpreted on the basis of CLSI guidelines for antimicrobial susceptibility testing (Anonymous 2005).

RESULTS

Physicochemical analysis

Table 1 shows results obtained for determination of selected physic-chemical parameters of analysed water samples. The pH ranged between 7.35 and 8.10. Other range of values obtained were 0.70 – 1.00 ms/cm (conductivity), 56-74 mg/L (total dissolved solids), 0.001-0.012NTU (turbidity), 0.00-0.001mg/L (sulphates and nitrates), 0.001-0.032mg/L (phosphates), 40-99.5mg/L (potassium), 0.00-4.89

(iron) and 0.00-0.40mg/L for zinc, copper and chromium levels. Arsenic and cobalt were not detected in all the water samples.

Microbiological analysis

Results obtained for the mean total viable counts (TVC)and coliform counts are presented in Table 2. The mean total viable counts for samples from Ayetoro-Ekiti river inflow (AER) was estimated to be 4.5 x 10^5 cfu/mL, while that of Igogo-Ekiti river inflow (IER), Eda-Oniyo-Ekiti river inflow (EER), middle dam (MD), dam outflow (DO), treatment point (TP) and consumers points (CP) were estimated to be 4.8 x 10^5, 3.5 x 10^5, 4.0 x 10^5, 5.3 x 10^5, 1.8 x 10^5 and 1.9 x 10^5 cfu/mL respectively (Table 2). Samples obtained from the dam outflow (DO) had the highest (TVC) of 5.3 x 10^5 cfu/mL while that of the treatment point (TP) had the least value of 1.8 x 10^5 cfu/mL. The results show a variation in TVC for all the samples collected except Eda-Oniyo-Ekiti rainflow which had a progressive increase in microbial loads throughout the period of investigation.

A mean total coliform count (TCC) of 3.9 x 10^5 cfu/mL was recorded for AER while IER, EER, D, DO, TP and CP were estimated to be 4.2 x 10^2, 3.9 x 10^2, 3.8 x 10^2, 4.7 x10^2, 8.0 x 10^1 and 1.6 x 10^2 cfu/mL respectively. (DO) had the highest number of coliforms (4.7 x 10^5 cfu/mL) while TP had the least value of 8.0 x 10^1 cfu/mL. There is a progressive decrease in coliform counts for DO and TP throughout the period of investigation. Also observed was the increase in coliform counts after the water had been treated (i.e. consumers points).

Sixty four (64) bacteria species were isolated from the samples collected (table 3). The isolated bacteria species were identified to be same with commonly encountered in aquatic environments. The isolates comprised *E. coli*, (40.6%), *Klebsiella* spp (15.6%), *Staphylococcus* spp (3.1%), *Salmonella* spp (6.25%), *Shigella* spp (4.69%), *Proteus* spp (4.69%), *Bacillus* spp (4.69%), *Pseudomanas aeruginosa* (4.69%), *Streptococcus* spp (3.1%), *Serratia marcescens* (1.56%) and *Flavobacterium* spp (3.1%).

Table 4 shows the antibiotic resistance pattern of bacteria isolates from Ero-dam. Fifty seven Gram negative and seven Gram positive bacteria were isolated. The gram-negative isolates were tested against gram-negative antibiotic discs while the other group were tested against gram positive discs. *E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus* spp exhibited resistance to all the antibiotics. In a similar vein, all encountered gram-negative isolates demonstrated high level resistance to ampicillin (100%). Ciprofloxacin was the most effective of all the antibiotics tested as only 9.3% demonstrated resistance to it. Multiple antibiotic resistance patterns of bacteria isolates from Ero-dam is shown on table 5. Bacteria isolates that exhibited resistance to two or more antibiotics were regarded as multiple antibiotic resistance strains. The results show that twenty one antibiotic resistance patterns were demonstrated by the isolated bacteria.

Table 1. Physiochemical properties of water samples from Ero dam.

Parameters	Sample collection points							Standard limits
	AER	IER	EER	MD	OD	TP	CP	
Temperature	24.00	24.70	24.60	24.00	24.00	26.00	28.00	-
P^H	7.90	7.40	7.60	7.60	8.10	7.35	7.35	6.5-8.5
Conductivity (ms/cm)	0.80	0.76	0.70	0.73	0.73	0.85	1.00	-
Total hardness (mg/L)	20.00	20.00	20.00	20.00	20.00	20.00	20.00	100
Total dissolved solids (mg/L)	60.00	57.00	56.00	57.00	56.00	62.5	74.00	None
Turbidity Abs. (540 nm) (NTU)	0.001	0.001	0.001	0.001	0.012	0.011	0.001	0
Sulphate (mg/L)	0.00	0.001	0.00	0.00	0.00	0.00	0.001	200
Nitrate (mg/L)	0.30	0.00	0.00	0.44	0.36	0.16	1.00	45
Chloride (mg/L)	28.20	26.40	21.30	18.40	28.20	28.36	42.54	200
Phosphate (mg/L)	0.020	0.002	0.00	0.004	0.00	0.032	0.02	_

K	(mg/L)	99.5	90.0	52.0	60.5	84.3	40.5	80.5	_
Fe	(mg/L)	0.10	0.20	0.20	0.10	N.D	N.D	48.9	0.30
Zn	(mg/L)	0.40	0.20	0.40	0.40	0.30	0.20	N.D.	5.00
Cu	(mg/L)	0.20	0.40	N.D.	0.2	N.D	N.D	N.D	1.00
Cr	(mg/L)	N.D	N.D	0.10	N.D	0.40	N.D	N.D	0.05
As	(mg/L)	N.D.	N.D	N.D	N.D	N.D	N.D	N.D	0.01
Co	(mg/L)	N.D.	N.D.	N.D	N.D	N.D	N.D	N.D	_

AER – Ayetoro-Ekiti river inflow, IER – Inflow from Igogo-Ekiti river inflow, EERE – Eda Oniyo Ekiti River inflow,
MD – Middle dam, DO – Dam outflow , TP - Treatment point, CP – Consumer Point, N.D – Not detected

Table 2. Mean total viable bacterial and coliform counts of water samples from Ero dam (April-July, 2008)

Sources	Mean TVC	Mean TCC
Ayetoro river inflow	$4.5x10^5$	$3.9x10^2$
Igogo river inflow	$4.8x10^5$	4.2x102
Eda Oniyo river inflow	$3.5x10^5$	$3.9x10^1$
Mid-dam Ero	$4.0x10^5$	$3.8x10^2$
Ero dam outflow	$5.3x10^5$	$3.4x10^2$
Treated water (Ero)	$1.8x10^5$	$1.6x10^2$
Consumer point (Distribution/tap water)	$1.9x10^5$	$8.0x10^1$

Table 3. Distribution of bacterial isolates from untreated water samples from Ero dam

Bacterial isolates	Number of isolates	Frequency (%)
Escherichia coli	26	40.6
Klebsiella spp	10	15.6
Staphylococcus spp	2	3.1
Salmonella spp	4	6.25
Shigella spp	3	4.69
Proteus spp	8	12.5
Bacillus spp	3	4.69
Pseudomonas aeruginosa	3	4.69
Streptococcus spp	2	3.1
Serratia marceascens	1	1.56
Flavobacterium spp	2	3.1
Serratia marcescens	1	1.56
Flavobacterium spp	2	3.1

Table 4. Antibiotic resistance patterns of untreated water samples from Ero dam, Ikun-Ekiti

Bacterial isolates	N	AMP	COT	GEN	NAL	NIT	COL	STR	TET	CIP	PEN	CLN
Escherichia coli	26	100	84.6	53.8	50	57.7	34.6	53.8	96	50.4	-	-
Klebsiella spp	10	100	60	10	50	60	30	30	70	0	-	-
Staplococcus spp	2	-	-	100	-	-	-	-	100	50	100	100
Salmonella spp	4	100	75	50	50	25	25	0	25	0	-	-
Shigella spp	3	100	66.7	100	100	33.3	66.7	66.7	100	0	-	-
Proteus spp	8	100	75	37.5	62.5	87.5	62.5	37.5	100	0	-	-
Bacillus spp	3	-	-	33.3	-	-	-	-	87.5	0	33.3	0
Pseudomonas aeruginosa	3	100	100	100	66.7	100	66.7	100	100	33.3	-	-
Streptococcus spp	2	-	-	100	-	-	-	-	100	0	100	50
Serratia marcescens	1	100	0	100	100	0	100	0	100	0	-	-
Flavobacterium spp	2	100	100	100	100	50	50	100	100	0	-	-
Overall resistance	64	100	77.12	53.16	57.89	59.65	42.10	47.38	87.5	9.30	71.4	42.86

Ampicillin (AMP), Cotrimoxazole (COT), Gentamicin (GEN), Nalidixic acid (NAL), Nitrofurantoin (NIT) , Colistin (COL), Stretomycin (STR), Tetracycline (TET),Ciprofloxacin (CIP) , Penicilin (PEN), Clindamycin (CLN)

Table 5. Multiple antibiotic resistance patterns of some gram-negative bacterial isolates from Ero dam

Bacterial isolates	No of isolates tested	No of antibiotics	MAR Frequency (%)	Resistotypes
Escherichia coli	26	4	11.5	AMP/GEN/COL/TET
		5	19.5	AMP/COT/NIT/COT/TET
		6	23.1	AMP/COT/NAL/STR/TET/CIP
		7	23.1	AMP/COT/GEN/NAL/COL/STR/TET
		8	7.6	AMP/COT/GEN/NAL/COL/STR/TET/CIP
Klebsiella spp	10	3	10.0	AMP/COT/STR
		4	30.0	AMP/COT/NIT/TET
		5	20.0	AMP/NA//NIT/COL/TET
		6	10.0	AMP/COT/NAL/NIT/COT/TET
		7	10.0	AMP/COT/GEN/NAL/NIT/STR/TET
Salmonella spp	4	3	25.0	AMP/COT//TET
		4	50.0	AMP/COT/NAL/NIT
		5	25.0	AMP/COT/GEN/NAL/TET
Shigella spp	3	5	33.3	AMP/GEN/NAL/COT/TET
		6	33.3	AMP/COT/GEN/NAL/STR/TET
		7	33.3	AMP/COT/GEN/NAL/NIT/STR/TET
Proteus spp	8	4	12.5	AMP/NAL/COT/TET
		5	37.5	AMP/COT/NAL/NIT/TET
		6	12.5	AMP/COT/GEN/NIT/COL/TET

DISCUSSION

The pH of all the water samples ranged between 7.35 and 8.10 (table 1). As supported by Ademoroti (1996), these values recorded for both treated and untreated water samples obtained from Ero river water supply could be considered as being within acceptable range for natural waters. According to Medera *et al.*, (1982), the P^H of most natural waters range from 6.5-8.5 while deviation from the neutral 7.0 is as a result of the $C0_2$/bicarbonate/carbonate equilibrium. The temperature range of 24-28^{0}C of the water sample is believed to have been influenced by the intensity of the sunlight as temperature rose on relatively hot days. This was also reported by Banwo (2006). All the water samples had dissolved solids below the prescribed maximum level required for potable water. The recommended maximum level of dissolved solids in drinking water is given as 500 mg/L. The value (20.00 mg/L) obtained for total hardness in the water samples was consistent and the same at the different sampling points. This value is below the permissible limit of 100 mg/L. The sulphate, nitrate, chloride and phosphate contents of the water sample are within permissible limit. The iron content of water sampled from the three river inflows ranged between 0.01 mg/L and 0.02 mg/L which was less than the prescribed standard limit of 0.30mg/l. Iron concentration and lead content in the water sampled at the consumer point far exceeds the standard limit for these metals in drinking water. This could be traceable to the dissolution of metallic ions into distributed water as it flows in the old dilapidated iron pipes used in the distribution system. Also, chromium detected in water sampled at Eda Oniyo Ekiti River inflow (EER) and at the dam outflow (DO) at respective values of 0.10 mg/L and 0.40 mg/L were above the maximum permissible level. Zinc concentration in the water samples were within tolerable limit.

Although variation existed in the observed mean total viable counts and coliforms count (Table 1), the values were generally high exceeding the acceptable limits for water. The present results obtained for total viable counts and coliforms count are similar to the results obtained by Okonko et al. (2008) in the study on water samples of Abeokuta and Ojota Lagos State, Banwo (2006) in the study on Nutrient load and pollution study of some selected stations along Ogunpa River in Ibadan. The results are also similar to those obtained by Edema et al. (2001), Fapetu (2000) and Otunola and Giwa (1994). According to a study by Baxter-Potter and Gilliland (1988) on straight river watersheds, when precipitation and stream flows are high, the influence of continuous sources of pollution such as finding individual sewage treatment plants, industrial and institutional sources and waste water treatment facilitates overshadows weather driven sources such as feed between run-off and urban storm water which leads to generation of faecal coliforms concentration. The persistence increase in microbial loads of Eda-Oniyo Ekiti river inflow (EER) which is the largest source of water supply to the dam may be as a result of illegal dumping of domestic wastes, livestock management, faecal deposit and waste dumps also affect bacteria concentration in run-off.

The coliforms count for the water samples ranged from 8.0 x 10^1 to 4.7 x 10^2 cfu/mL for both treated and untreated water samples. These values are high, it could be inferred that both untreated and treated water samples were contaminated. As the dam is available for various domestic use, it could be suggested that the high microbial loads may be due to human and animal contact with these water sources. Observed activities of fishermen, farmers and feeding water birds in the study location support this submission. The direct washing of legs, hands, clothes and utensils like cutlasses could also lead to contamination. Also the presence of nearby bushes as presented by a similar study (Banwo 2006) might increase the possibility of hide-outs for smaller mammals who frequently visit these water bodies to drink water and to pass waste products. The decrease in microbial loads for treated water samples (i.e. treatment point) could be attributed to filtration and disinfection process which the water had been subjected to at the treatment plant. However, these appear not effective enough to bring the microbial loads to levels below the acceptable WHO standard for drinking water. Ideally, the total coliforms count in drinking water should be zero (Anonymous 2006). Inadequacies in the supplies of chlorine, alum and other treatment materials owing to associated cost and logistic failures in the study location could be responsible for failures in the treatment process.

Also observed in this study is the increase in microbial loads at the consumers point (i.e. the taps located around streets in the town). This may be attributed to the observed activities the tap. At some points, the direct washing of human clothing and washing of other house hold utensils around the tap. The presence of animals like pigs, goats etc. and the intense agricultural related activities going on around the consumers point could lead to contamination. The observation could also be due to leakages in the

pipelines during transmission as leakages in pipe water transmission pipelines have been reported to allow contaminants into the water transmission line. Another probable reason might be that the disinfectants used may be bacteriostatic and not effective enough to wipe out the microbes completely, this may results to reactivation of the static microbes after some days after disinfection.

As was reported in a previous studies (Banwo 2006, Okonko et al. 2008), the isolated bacteria species were identified to be the same with those commonly encountered in aquatic environments. The high number of bacteria present in this water and the fact that they mostly belong to the family Enterobaceriaceae present enough evidence that water from these sources are unfit for human consumption. The implication is that they could constitute serious risk to public health. The most common manifestation of water borne illness is gastrointestinal disturbances (nausea, vomiting and diarrhoea) and this sis usually of short duration. However, in susceptible or immunocompromised individual, the effects may be more chronic (e.g. kidney damage) or even fatal pathogenic bacteria such as *Salmonella* and *Shigella* can be responsible for severe gastrointestinal illness. *E. coli* has also been recognized to contribute to the incidence of diarrhoea (Sofola and Lawal 1983). Also numerous outbreaks of disease have been reportedly linked to contaminated drinking water generally (Bamiro 2007, Banwo 2006). In most cases, the drinking water was not treated or was improperly treated prior to consumption.

The situation is further complicated if these implicated pathogens are antibiotic resistant strains. Drug resistance could be transferred between members of the family Enterobacteriaceae with exchange of antibiotic resistance plasmids readily occurring at inter and interspecies level in raw sewage system (Richard et al. 1981). This observation, in conjunction with the selection pressure imposed by antibiotic usage, has increased the incidence of pathogenic strains that have acquired antibiotic resistance (Routman et al. 1985). In the present study, antibiotic-resistant bacteria were prevalent in Ero river water supply. This is not surprising since the intrinsic resistance of many organisms to antibiotics in aquatic environments is well documented (Roland et al. 2002). When these pathogens contaminate water, the population or individuals drinking contaminated water from these sources may have resistant strains easily becoming part of their mciroflora. As a result of selection pressure, such organisms may establish themselves within the individuals and become predominant microflora. Infections caused by such organisms are very difficult to treat (Ajayi and Akonai 2003).

In as much as water is essential for life, it could at the same time prove to be a potential reservoir of microorganisms that could pose significant threat to the consuming citizenry. Thus it is necessary that all the sources of contamination of water used for drinking and other domestic purposes in rural settings should be subjected to adequate treatment when faecal pollution is suspected. The water sources should be prevented from household activities and other uses. It is necessary to ensure that washing sites of domestic chores are kept at a distance of at least 15m especially in areas where channelled drainages is absent. Animal and humans should also be prevented from entering directly into the dam. Pro-poor informative programs that focus on the immediate and long-term effects of indiscriminate waste disposal and contamination from other anthropogenic activities on the quality of drinking water sources should be the embarked upon. As much as an efficient treatment system is desired at the dam, much effort should be channelled towards protecting the distribution system. Huge investments should again be allotted to the quality of pipelines used in distribution networks. Failure to embark on the suggested strategies may lead to increased risk of exposure to water borne pathogens which in turn could portend serious implication on public health outcomes in such settings.

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