Original Research Article

ASSESSMENT OF THE MICROBIOLOGICAL SAFTEY OF POTABLE WATER FROM RURAL SETTLEMENTS IN OWO LOCAL GOVERNMENT AREA OF ONDO STATE, NIGERIA

ABSTRACT

Access to quality drinking water is a major problem in rural settlements in Owo Local Government Area (L.G.A.) of Ondo State, Nigeria where surface and ground water sources (streams and wells) used for drinking are located near dump sites with faecal deposits. In spite of this, few data exists on the microbiological safety of water sources in these settlements. Therefore, this study investigated the microbiological safety of drinking water from 10 rural settlements in Owo (L.G.A), Ondo State Nigeria. Bacteriological analysis were carried out on water samples (wells and streams) that served as sources of potable water in these settlements to ascertain the quality of drinking water in these communities. Water samples were examined for total bacteria, total fecal coliform and total enterococci counts respectively. The detection of *Escherichia coli*, *Salmonella spp, Klebsiella aerogenes*, *Enterococci faecum*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were assessed by various biochemical tests. Fecal coliform contamination was detected in 35% of water samples (streams and wells) across the rural settlements while faecal enterococcal growth was also detected in 35% of the water samples from sample sources analyzed. The bacteriological loads of 65% of the sampled water from the different settlements were also found to be higher than the minimum value set for drinking water by W.H.O. Hence, the results from this study established the need for improved community access to potable water across these rural settlements by encouraging construction of toilet facilities and provision of proper waste disposal system by Local Government Authorities. Proper health education and strict monitoring of sanitary practices in these settlements by local health officials is also recommended across environmental biosaftey and containment of likely outbreaks in the nearest future.

Keywords: Drinking water, faecal coliforms, Owo L.G.A, Nigeria, Faecal enterococci, rural

INTRODUCTION

Access to safe drinking water is a basic human right as it is crucial to maintenance of community health status [1,2]. Nations maintain optimal health and rural development of their communities by a continual, steady supply of safe drinking water to their population [1-3]. However, drinking water is also the most important source of gastro-enteric diseases worldwide, mainly due to the fecal contamination of raw water or recontamination of drinking water at source and point of use [2-4]. About two thirds of drinking water consumed worldwide is derived from various surface water sources like lakes, rivers and open wells and it can easily be contaminated microbiologically by sewage or fecal discharges by animals or human [1-4]. As a result, water related diseases continue to be one of the major global health problems [2,3]. It is estimated globally that 80% of all illnesses are linked to use of unsafe and microbiologically poor water quality [5-7].

In developing countries however, about 1.8 million deaths per year are attributed to unsafe water, sanitation and hygiene, mainly through infectious diarrhea and gastro-enteric infections [1,3]. Gastro-enteric diseases remain a major killer in children as it is estimated that 17% of all child deaths under the age of 5 years in developing countries result from diarrheal diseases [7-8]. In developing countries such as Nigeria, most of the rural settlements are poor with lack of access to potable water supplies and hence they rely mainly on rivers, streams, wells and pond water sources for their daily needs [1,3,6-8]. Water from these sources is used directly by the inhabitants and the water sources are faecally contaminated and devoid of treatment before drinking [5-7]. Consequently, a significant proportion of residents in rural settlements of Nigeria are exposed to water-borne diseases and their complications [4,9]. Pathogenic contaminants in these water sources are derived from animal and anthropogenic sources

including humans in these settlements and this is mostly encouraged in areas with poor standards of hygiene and sanitation [1,5]. The sanitation crisis heightens when it is accompanied by poor health protection system associated with poor life standards of living common to many rural settlements in Nigeria [6].

The microbiological quality and safety of potable water in rural settlements of Owo L.G.A. of Ondo State Nigeria has been brought into question as most sources of potable water in known rural settlements are located around faecal and refuse dump sites, and there are no functional water storage facilities provided by local government authorities to these settlements for their health and safety. Hence, this study assessed the microbiological safety of drinking water in 10 rural settlements in Owo L.G.A. of Ondo State via bacteriological analysis of drinking water from surface and ground water sources in the study area and to highlight the associated possible public health risk factors.

MATERIALS AND METHODS

Study area description

Owo Local Government Area (L.G.A) is found in Ondo State, Nigeria with coordinates $7^{o}11N$ $5^{o}35E$/ $7.183^{o}N$ $5.583^{o}E$ [10]. It is located at 150 km north of Akure, Ondo State capital with an estimated population of 425,700 [10]. The 10 rural settlements under study focus for this research are: Alupe (A), Ago- Ebira (B), Ijebu (C), Ipele (D), Ipenme (E), Ode Oriya (F,) Utelu (G), Ohore (H), Ilale (I) and Isu Ada (J) settlements respectively.

74 **Study and sampling design**

75 A descriptive analytic study was used to examine the bacteriological quality of drinking
76 water from ground and surface water sources in the 10 settlements listed above. Water samples
77 from wells and streams that served as major potable water source in these settlements were
78 collected via simple random sampling methods.

79 **Sample collection**

80 A total of 20 water samples were collected from both ground water and surface water
81 sources across 10 rural settlements of Owo L.G.A. in December, 2016. Out of these, a total of 10
82 well water samples and 10 stream water samples were collected from different locations across
83 the rural settlements using a simple random sampling technique. Ethical approval was obtained
84 from the local health management authorities before samples were obtained. The samples were
85 collected aseptically into labeled sterile universal bottles (250ml) and stored in ice packs before
86 bacteriological analysis. All the samples collected were analyzed in the laboratory within 6hr of
87 sample collection.

88 **Sample preparation and Standardization of Inoculum**

89 [1, 11] was adopted for water sample preparation and Inoculum standardization in which
90 sterile distilled water was used as diluents and a 1ml of each stock was taken using a sterile
91 syringe into 9ml of sterile distilled water for serial dilution procedure in sterile test tubes under
92 aseptic conditions until four different dilutions were obtained. Thereafter, a 1 ml of each dilution
93 factor was used for inoculating already prepared Nutrient Agar (for total bacterial counts),
94 MacConkey Agar (for total faecal coliforms) and Bile Esculin Agar (for total faecal enterococci
95 counts), incubated for bacterial isolation at 37^{o}C for 24 hours [1,12-16]. After the incubation

time, the culture plates were observed for determination of colony forming units and thereafter, the fourth dilution factor was established as the standard for the isolation of the microbes due to easy numerical estimation of the colony forming units on the agar plate of the last dilution factor [1,17].

Biochemical characterization and identification of isolates

The methods described by [1, 17-19] were adopted by subjecting the various obtained sub cultured distinct colonies to wide arrays of biochemical tests for characterization and identification. Gram staining technique, Catalase test, Motility test, Sugar fermentation (glucose, sucrose, lactose, mannitol and triple salt iron) tests, Methyl Red/Voges Proskauer test, Oxidase test, Coagulase test and Catalase tests were carried out on the distinct isolates obtained after sub culturing [17,19]. The distinct biochemically characterized colonies were then further sub cultured on MacConkey Agar and Bile Esculin Agar respectively; incubated at 37^{o}C for 24 h [12, 17,19]. Thereafter which the identity of the bacteria isolates was determined after their growth on these selective media.

Preservation of Isolates

The identified pure isolates of *Staphylococcus aureus, Escherichia coli, Enterococcus faecium, Pseudomonas aeruginosa, Klebsiella aerogenes* and *Salmonella spp* were preserved on Nutrient Agar Slants and stored at 4^{o}C as described by [13, 17,19].

Data analysis

Analyzed sample treatments were replicated thrice; data means obtained were subjected to a 2-way analysis of variance and treatment means were separated using Duncan's New Multiple Range test at $P \leq 0.05$ level of significance [1,5].

RESULTS

The means of the total bacterial count, total faecal coliforms count and total faecal enterococci counts of the samples analyzed from different colony forming units after incubation were subjected to statistical analysis using Duncan's New Multiple Range test at $P \leq 0.05$ level of significance as represented in Tables 1, Tables 2 and Tables 3. Bacterial isolates from the sample sources analyzed were verified by various biochemical tests as represented in Table 4 while a total of 90 isolates of *Staphylococcus aureus* (31), *Escherichia coli* (16), *Enterococcus faecium* (10), *Pseudomonas aeruginosa* (12), *Klebsiella aerogenes* (8), and *Salmonella spp* (13) were screened out from the water samples collected (Table 5). Generally, it was observed that the total bacterial, faecal coliforms and enterococci loads of the samples from the surface water (stream) were higher than those of the ground water (well) across the rural settlements.

Furthermore, the total bacterial count (TBC) of 80% (8 out 0f 10) of surface water samples (streams) across the rural settlements were above the specified standard of 5 cfu/ml (colony forming unit per ml) [14-16]; while the TBC of 50% (5 of 10) of ground water samples (well) were higher than the WHO specified standard (Table 1). The faecal coliforms load of 40% (4 out of 10) of surface water samples (streams) from the rural settlements were above the specified WHO standard (≤ 3 cfu/ml) [14-16] while in ground water samples (wells) only 30% (3 out of 10) samples from the settlements were higher than the specified standard (Table 2).

However, 50% (5 of 10) stream water samples had a total faecal enterococci load higher than the specified standard (≤ 0 cfu/ml) [14-16] while 20% (2 of 10) ground water samples had faecal enterococci growth higher than the specified standard (Table 3).

T	TOTAL BACTERIAL COUNT OF WATER SAMPLES ACROSS RURAL SETTLEMENTS (Cfu/ml)									
	A	B	C	D	E	F	G	H	I	J
S	21.30± 1.00^{c}	12.10± 1.43^{b}	5.70± 1.30^{a}	3.60± 1.00^{a}	22.11± 1.48^{c}	18.10± 2.00^{c}	3.10± 1.33^{a}	8.20± 2.10^{b}	21.90± 1.22^{c}	11.90± 1.20^{b}
W	9.80± 1.30^{c}	9.80± 2.00^{c}	3.50± 1.00^{b}	2.00± 1.00^{a}	11.80± 1.21^{d}	1.90± 1.10^{a}	2.60± 1.00^{b}	2.10± 1.20^{b}	9.80± 1.50^{c}	6.50± 1.00^{b}

Table 1: Total Bacterial counts of Water samples from Streams and Wells across 10 rural settlements

Keys: T- sample types, W- well, S- stream, A- Alupe, B- Ago-Ebira, C- Ijebu, D- Ipele, E- Ipenme, F- Ode Oriya, G- Utelu, H- Ohore, I- Ilale and J- Isu- Ada values with the same letter as superscript have no significant difference at $p \leq 0.05$ level of significance.

Table 2: Total faecal coliforms counts from water streams and wells across 10 settlements

T	TOTAL FAECAL COLIFORM COUNT OF SAMPLES ACROSS RURAL SETTLEMENTS (Cfu/ml)									
	A	B	C	D	E	F	G	H	I	J
S	10.90± 2.00^{c}	1.50± 1.00^{a}	2.60± 1.00^{a}	1.60± 1.00^{a}	11.20± 1.28^{d}	1.10± 1.00^{a}	0.60± 0.28^{a}	2.80± 1.10^{b}	0.80± 0.20^{a}	6.10± 1.31^{b}
W	3.40± 1.21^{c}	0.60± 0.20^{b}	0.00± 0.00^{a}	1.00± 0.40^{b}	5.80± 1.51^{c}	0.90± 0.10^{b}	0.00± 0.00^{a}	1.30± 1.00^{b}	0.00± 0.00^{a}	3.10± 1.00^{c}

Keys: T- sample types, W- well, S- stream, A- Alupe, B- Ago-Ebira, C- Ijebu, D- Ipele, E- Ipenme, F- Ode Oriya, G- Utelu, H- Ohore, I- Ilale and J- Isu- Ada values with the same letter as superscript have no significant difference at $p \leq 0.05$ level of significance.

Table 3: Total faecal enterococci counts from water streams and wells across 10 settlements

T	TOTAL FAECAL ENTEROCOCCI COUNT OF SAMPLES ACROSS RURAL SETTLEMENTS (Cfu/ml)									
	A	B	C	D	E	F	G	H	I	J
S	9.50± 2.00^{d}	0.00± 0.00^{a}	1.60± 1.00^{b}	0.00± 0.00^{a}	0.00± 0.00^{a}	3.80± 1.00^{c}	0.00± 0.00^{a}	1.80± 1.10^{b}	0.00± 0.00^{a}	5.40± 1.71^{c}
W	1.40± 1.21^{b}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	0.00± 0.00^{a}	1.33± 1.00^{b}

Keys: T- sample types, W- well, S- stream, A- Alupe, B- Ago-Ebira, C- Ijebu, D- Ipele, E- Ipenme, F- Ode Oriya, G- Utelu, H- Ohore, I- Ilale and J- Isu- Ada values with the same letter as superscript have no significant difference at $p \leq 0.05$ level of significance.

177 Table 4: Biochemical characteristics of isolates from the water samples across the rural settlements

I.	Gram Stain	Sugar Fermentation					O/C	COT	MR/VP	Growth on Media			N. I.
		Lac.	Glu.	Suc.	Mann.	TSI				NA	Mac. A	BEA	
S.A.	+ve (cluster cocci)	-ve	+ve	+ve	+ve	-ve	+ve/ +ve	+ve	-ve/-ve	Cream/ raised	-ve	-ve	31
E.C.	-ve (bacilli rods)	+ve	+ve	+ve	-ve	A/G	+ve/ +ve	-ve	+ve/-ve	Cream/ raised	+ve (pink)	-ve	16
E.F.	+ve (cocci chains)	-ve	+ve	+ve	-ve	-ve	+ve/ -ve	-ve	-ve/-ve	Milky/ lobate	-ve	+ve (pink)	10
P.A.	-ve (bacilli rods)	-ve	+ve	+ve	-ve	K/NF	+ve/ +ve	-ve	-ve/-ve	Cream/ raised	+ve (pink)	-ve	12
K.A.	-ve (bacilli rods)	+ve	+ve	+ve	-ve	A/G	+ve/ +ve	-ve	+ve/+ve	Cream/ raised	+ve (pink)	-ve	8
S.S.	-ve (bacilli rods)	-ve	+ve	-ve	-ve	K/H_2S	-ve/ +ve	-ve	-ve/-ve	Cream/ rasied	+ve (pale)	-ve	13

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179 Keys: I.- Isolates, S.A.- *Staphylococcus aureus*, E.C.- *Escherichia coli*, E.F.- *Enterococcus faecium*, P.A.- *Pseudomonas aeruginosa,*
180 K.A.- *Klebsiella aerogenes,* S.S.- *Salmonella spp,* Lac.- Lactose, Glu.- Glucose, Suc.- Sucrose, Mann.- Mannitol, TSI- Triple Salt
181 Iron, O/C- Oxidase/ Catalase test, COT- Coagulase test, MR/VP- Methyl red/ Voges Proskauer, NA- Nutrient Agar, Mac. A.-
182 MacConkey Agar, BEA- Bile Esculin Agar, N.I.- Number of isolates, -ve- negative, +ve- positive, A/G- Acid/ Gas, K/NF- Alkaline
183 slant/ No fermentation, K/H_2S- Alkaline slant/ Hydrogen Sulphide produced.

Table 5: Distribution of identified isolates across the 10 rural settlements

I.	RURAL SETTLEMENTS									
	A	B	C	D	E	F	G	H	I	J
S.A.	6	1	1	2	1	5	5	4	3	3
E.C.	4	1	1	1	1	3	2	1	1	1
E.F.	3	1	1	2	-	-	-	1	1	1
P.A.	3	1	1	2	-	-	1	2	2	-
K.A.	2	1	1	1	-	-	1	1	1	-
S.S.	4	1	1	1	-	1	1	1	2	1

Keys: I.- Isolates, S.A.- *Staphylococcus aureus*, E.C.- *Escherichia coli*, E.F.- *Enterococcus faecium*, P.A.- *Pseudomonas aeruginosa,* K.A.- *Klebsiella aerogenes,* S.S.- *Salmonella spp,* A- Alupe, B- Ago-Ebira, C- Ijebu, D- Ipele, E- Ipenme, F- Ode Oriya, G- Utelu, H- Ohore, I- Ilale and J- Isu- Ada.

DISCUSSION

It was observed from this study that, the drinking water samples across all the 10 settlements are faecally contaminated by either faecal coliforms or faecal enterococci or even both. This is mainly due to sanitary practices across these settlements as sources of potable water serves other purposes such as bathing, waste disposal, faecal dumps and so-on asides drinking, this agreeing with other recent finding done by [5,11-13]. The total bacteria counts of all the samples were generally higher than the specified WHO standards as reflected in the results [14-16] but more importantly, the total bacteria counts of samples from surface water (streams) were generally higher than the total bacteria counts of ground water sample (wells) and this was also noticed in the case of total faecal coliforms counts and total faecal enterococci counts respectively as also indicated in the findings of [9,11-13].

Subsequently, the oral interviews conducted by authors with the inhabitants of these settlements revealed that the settlements lacked access to potable water or water storage facilities and are unwilling to see any potential harm in using stream water for their drinking and other domestic purposes; although bioethical concerns exist in their cultural belief that flowing water sources (streams) cannot be contaminated; the authors however, didn't press further to investigate this belief as it was beyond the scope of this research aim, similar bioethical concerns were also encountered in the reports of [1, 5, 12-14]. Local health demography of these rural settlements obtained from local health authorities suggests frequent relapse of gastro-intestinal infections and this research study accurately justifies why it is so, this agrees also with the findings of [1,9,11] .

Since the standard of living in these settlements are generally low with high poverty rates, it was obvious that adequate health care facilities and basic social amenities were not in

place, hence, the use of water bodies as vehicles for waste disposal had become a norm and such is the case of many rural settlements across developing African countries [6-7, 14-16, 18].

CONCLUSION

Urgent and adequate government aid and intervention is highly recommended for these settlements as the results of this research have proved potential danger of outbreak of gastro-enteric infection across these rural settlements. Moreso, improved access to potable water across these rural settlements, construction of toilet facilities and provision of proper waste disposal facilities by Local Government Authorities is strongly recommended. Proper health education and strict monitoring of sanitary practices in these settlements by local health officials is also recommended for environmental biosaftey and containment of likely outbreaks of infection in the nearest future.

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