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Full Length Research Paper

Bacteriological, physicochemical and mineral studies on Awedele spring water and soil samples in Ado Ekiti, Nigeria

A. T. Odeyemi[1], A. C. Dada[2], O. R. Ogunbanjo[3] and M. A. Ojo[1]

[1]Department of Microbiology, University of Ado Ekiti, P. M. B 5363, Ado Ekiti, Nigeria.
[2]Institute of Ecology and Environmental Studies, Obafemi Awolowo University, Ile-Ife, Nigeria.
[3]Department of Environmental Management and Toxicology, University of Agriculture, Abeokuta, Nigeria.

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Enterococci **abound in faeces, survive long outside the enteric environment and possess unique ability to acquire and transfer antibiotic resistance. Recent studies have identified** ***Enterococci*** **as a relevant indicator of water quality. The current study focused on the prevalence of antimicrobial resistant forms of** ***Enterococcus*** **species isolated from Awedele spring water in Ekiti State Nigeria. The bacteriological and physicochemical quality of the water samples was also assessed. The mineral content of the adjacent soil samples was also determined. Antibiotic resistance among isolated** ***Enterococcus*** **species was highest to penicillin as all isolates demonstrated complete resistance to the antibiotics. Prevalence of gentamycin-resistant enterococci was lowest (48%). Mean total bacterial and enterococcus count ranged from 1.8 x 10^4 to 8.6 x 10^4 cfu/ml and 1.5 x 10^5 to 4.0 x 10^5 cfu/ml respectively. While calling for an improved data capturing system for drinking water surveillance in developing nations, the study highlights the need for continuous efforts aimed at instigating the required hygiene behavioral change among residents of rural settlements in the developing world.**

Key words: Antibiotic resistance, enterococcus species, spring water.

INTRODUCTION

Accessibility and availability of fresh clean water is key to sustainable development and an essential element in health (Adekunle et al., 2004). Unfortunately, almost half of the world population is without access to improved sanitation facilities and almost one billion people still lack access to improved drinking-water supplies (Gadgil and Derby, 2003). It is therefore no surprise that 2.2 million deaths per year which can either be prevented or minimized by improving access to drinking-water and sanitation are traceable to diarrhea diseases. The bulk of the burden is felt in developing nations where provision of public supply of drinking water is deficient owing partly to the lack of sufficient financial commitment towards existing infrastructure. People in rural settlements often resort to other alternatives as reliable source of water for drinking and to meet other domestic needs.

*Corresponding author: E-mail: adebowaletoba@yahoo.com, Tel: +2348032386094.

Unfortunately, given the prevalent appalling sanitary conditions in such locations, pollution of these alternative water sources by chemical and biological agents is not uncommon (Oluyege et al., 2009; Adesomoye et al., 2006). Although environmental risk assessment studies reveal 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), holistic surveillance programs take into consideration monitoring of the levels of both chemical and biological pollutants in water.

Generally, the presence of faecal pathogens in water is demonstrated by recoveries using assays or conventional laboratory media of indicator organisms (Kinzelman et. al., *2003).* Recent studies have shown that although *E. coli* and *enterococci* are equally acceptable or monitoring freshwater sources, enterococci may be the more relevant indicator of water quality (Kinzelman et. al., 2003). In particular*, E. faecalis, E. faecium, E. durans* and *E. hirae* are considered to be of faecal origin (God

Figure 1. Awedele Spring showing concrete protection.

Figure 2. Awedele spring and downstream flowpath under the influence of anthropogenic activities.

free et al., 1997). Because of their abundance in faeces and also due to their ability to survive long outside the enteric environment, the presence of enterococci is considered an indicator of fecal contamination and they are not considered 'generally recognized as safe (GRAS) (Domig et al., 2003; Mannu et al., 2003, Aparecida et al., 2007). Although Enterococci are not considered as primary pathogens, growing concerns abound that they rank among the leading causes of nosocomial infections (Arvanitidou et al., 2001). Added to this concern is their remarkable intrinsic resistance to several antimicrobials and their unique ability to exchange genetic material and to acquire additional resistance to many antimicrobials (Eaton and Gasson, 2001; Teixeira and Facklam, 2003). The past two decades have witnessed the rapid emergence of multiple drug resistant (MDR) enterococci (Mark et al., 1998). As the occurrence of antimicrobial resistance among enterococci is not only restricted to nosocomial settings, resistance strains dispersed through environmental sources may also act as potential reservoirs of antimicrobial resistance genes. The study investigated the bacteriological quality of Awedele spring water accessible to residents of a rural settlement in Western Nigeria. The study also set out to determine the antimicrobial resistance profiles among enterococcal isolates recovered from these water samples. Physico - chemical parameters were also determined and mineral analyses conducted for soil samples along the spring flow path.

MATERIALS AND METHODS

Study site

The spring (Figure 1) is situated in Awedele community, Basiri within the metropolis of Ado-Ekiti, the capital of Ekiti State, with an estimated population of 2.3 million people (Federal Republic of Nigeria 2006 Population Census).

Sampling

Using aseptic techniques, water samples were collected directly from the spring and from five different points along the flow path (Figure 2) of the spring using 250 ml sterile sized-bottles. Samples were transported to the laboratory on ice and analyzed within 4 h after collection. Water samples collected for mineral analysis were chemically preserved by the addition of 5ml concentrated HNO_3 per litre of the sample.

Enumeration of bacterial population

Determination of bacterial load in water samples were done in triplicates. The total numbers of culturable heterotrophic bacteria were determined by serial dilution and plating on general purpose media. Serial dilutions of water samples (1 ml fresh volume) were made with one-fourth strength Ringers solution. Plate counts of culturally viable bacteria were made on Tryptone Soya Agar (TSA; Oxoid, Basingstone, Hampshire, England) amended with 0.1 g/l cyclohexamide. The plates were inoculated with 1ml of water inoculum and cultured at 37°C for 24 h.

Isolation and characterization of *Enterococcus* species

Isolation of *Enterococcus* species was carried out on Bile Esculin agar. The bacterial isolates were identified as described by Barrow and Feltham (1993).Pure cultures of isolates were kept on nutrient agar slants at 12°C until used. The isolates were identified on the basis of cellular morphology following Gram stain, and results of biochemical testing, including catalase production, growth in 6.5% NaCl broth, haemolytic activity and motility (Devriese et al., 1992).

Antibiotic susceptibility test

The antibiotics susceptibility of the isolates was determined by the disk diffusion method on Mueller-Hilton agar according to CLSI (2005). The bacterial isolates were tested against seven ABTEK disc antibiotics which comprised Fusidic acid, Tetracycline, Penicillin, Erythromycin, Clindamycin, Trimethropim, Gentamycin and Sulfamethoxazole. The inoculum was standardized by adjusting its density to equal the turbidity of a barium sulphate

Table 1. Physico-chemical analysis of water samples from awedele spring water in Ado-Ekiti.

Parameters	Water samples					
Appearance	A	B	C	D	E	F
	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
Colour	Colourless	colourless	colourless	colourless	colourless	colourless
Conductivity	1.0 x 100	1.0 x 100	1.05 x 100	2.0 x 100	2.1 x100	2.1 x 100
Odour	Odourless	Odourless	odourless	odourless	odourless	odourless
Turbidity(NTU)	0.001	0.002	0.021	0.050	0.060	0.060
PH	4.8	5.0	5.0	5.6	6.7	6.8
Temperature (^{0}C)	29	29	29.5	29.5	30.5	30.5
Dissolved oxygen	1.5	1.5	1.5	2.0	1.5	2.0
Total dissolved solid (mg/l)	0.0036	0.0274	0.0158	0.0186	0.0116	0.0118
Total Suspended solid (mg/l)	0.0032	0.0202	0.0140	0.0020	0.0046	0.0042
Hardness (mg/l)	46	40	68	90	58	60
Alkalinity	20	30	150	100	110	90
Acidity	0.0002	0.0006	0.0008	0.0009	0.0012	0.0014
Phosphate(mg/l)	0.015	ND	0.005	0.020	0.019	0.001
Sulphate (mg/l)	0.060	0.048	0.033	0.100	0.095	0.090
Nitrate (mg/l)	0.290	0.088	0.120	0.050	0.034	0.075
Chloride (mg/l)	0.700	0.200	0.500	0.500	0.900	1.000

A, B, C, D, E and F – Point of collection of the water samples.

($BaSO_4$) (0.5 McFarland turbidity standard), and incubated at 35^{o}C for 18 hours. 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 guideline (CLSI, 2005).

Physicochemical analyses

The water sample temperature was taken at the site of collection using a simple thermometer calibrated in ℃, 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 determined were hardness determined by titrimetry; total dissolved solid and total suspended solid were 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 AOAC (1990).

Soil analysis

Metal analyses were carried out using flame atomic absorption spectrophotometer (GBC Avanta version 1.31). The calibration curves were prepared separately for all the metals by running different concentrations of standard solutions. The instrument was set to zero by running the respective reagent blanks. Average values of three replicates were taken for each determination. The detection limits for Fe, Zn, Cu, Ni, Cr, Pb and Cd were 0.05, 0.008, 0.025, 0.04, 0.05, 0.06 and 0.009 (mg l) respectively. Manganese was determined using atomic absorption spectrophotometer (Perkin-Elmer Model 403).

RESULT

The physicochemical analysis of the spring water samples revealed that, all the samples are colorless, odorless and clear. The values obtained for temperature, total hardness, dissolved oxygen and pH are stated in Table 1. The turbidity of the water samples (absorbance reading taken at 540 nm wavelength) generally increased across gradient and ranged from 0.001 - 0.60 NTU. The values obtained for other parameters determined ranged from 4.8 to 6.8 (pH), 29 to 30.5℃ (Temperature), 1.5 to 2.0 (Dissolved oxygen), 0.0036 to 0.0274 Nmg/l (total solids), 0.002 to 0.0202 mg/L (Total suspended solids), 40 to 90 mg/L(Hardness), 20 to 110 (Alkalinity), 0.0002 to 0.0014 (Acidity), 0.00 to 0.19 mg/l (Phosphate), 0.033 to 0.100 mg/l (Sulphate), 0.034 to 0.290 mg/l (Nitrate) and 0.2 to 1.0 mg/l (chloride).

The result of mineral analysis of soil samples collected from the various sites is presented in Table 2. Results obtained indicated the presence of some essential micronutrients such as iron, manganese, zinc and copper whose concentrations all fall within tolerable limit. The soil samples contain an appreciable amount of iron and zinc but recorded the least values of 0.888 to 1.556 µg/g for copper in all the sampled sites. Some heavy metal pollutants (nickel, arsenic, chromium and lead) were also detectable in the soil samples. Cadmium contamination was not detectable in all the soil samples. Chromium was the highest contaminant recorded in the soil samples with a value ranging between 1.334 to 3.668 µg/g. Lead contamination at sites E and F which both recorded 2.222

Table 2. Metallic ion contents (µG/G) of soil samples from Awedele spring water in Ado-Ekiti.

Parameters	Soil samples				
	B	C	D	E	F
Fe	19.000	35.000	21.000	20.200	31.800
Mn	3.556	7.554	2.888	6.444	4.222
Zn	9.312	13.736	10.942	7.916	7.216
Cu	1.112	0.888	1.112	1.112	1.556
Ni	0.572	0.286	0.286	0.858	0.286
As	1.600	1.200	0.800	0.400	0.400
Cr	3.000	1.334	3.668	2.668	2.000
Cd	N.D	N.D	N.D	N.D	N.D
Pb	0.664	0.442	0.442	2.222	2.222

B, C, D, E and F- Point of soil collection along Awedele spring water at exact point where water samples for physicochemical analysis were collected. There is no soil sample for sample 'A' because the water sample for A was collected before the water touched ground. N.D - Not detected.

µg/g were significant while arsenic contamination at sites B and C with values of 1.600 µg/g and 1.200 µg/g are equally high. Total bacterial and *enterococcus* count of water samples from Awedele Spring water samples are presented in Table 3. The lowest total bacterial and *enterococcus*, 1.8 x 10^4 cfu/ml and 1.5 x 10^5 cfu/ml respectively was observed for samples taken directly from the outburst of the spring (before it comes in contact with the soil) while increasingly higher counts for total bacterial and *enterococcus* (8.6 x 10^4 and 4.0 x 10^5 cfu/ml) was observed for samples collected along the path of flow on the ground. A total of 50 isolates of *Enterococcus* species were encountered in the study. The antibiotic resistance pattern of these encountered isolates from Awedele Spring water in Ado-Ekiti is presented in Table 4. The isolates demonstrated generally high level of resistance to each antibiotic tested in the study. Resistance among isolated Enterococcus species was highest to penicillin as all isolates demonstrated complete resistance to the antibiotics. Bacterial resistance to gentamycin was least (48%).

DISCUSSION

The physicochemical parameters determined revealed values generally greater than values recommended by the World Health Organization (2008). Micronutrients in the analyzed soil samples were present below toxic level making them to be of minimum threat to biological existence. It must be mentioned that the iron and manganese content of the soils were very low, the copper and zinc content of the soils were just on the brink of the minimum value for tropical soils. Even when these values are slightly higher, the enrichment of Cu, Zn, Ca and Pb are mostly related to the geogenic inputs through weathering/erosion and run-off from the catchments area (Tijani et al., 2005). The micronutrient content of the sampled soils confirms that the humid forest zone soils are strongly weathered (van Wambeke 1991, Juo and Wilding 1996) and thus contain very low levels of mineral nutrients. Soil threshold for heavy metal toxicity is an important factor affecting soil environment capacity of heavy metal and determining heavy metal cumulative loading limit. For the soil-plant system, the heavy metal toxicity threshold is the highest permissible content in the soil (total or bioavailable concentration) that does not produce any phytotoxicity. All the soil samples showed the presence of four heavy metals namely nickel, arsenic, chromium and lead. The level of contamination of the metals was higher than the permissible limits. Chromium contamination was the most notorious resulting in several folds higher than the permissible concentration of 0.2 µg/g (IETEG, 2004). Chromium contamination may be from the parent material. Chromium is present as chromite in igneous rocks and to a lesser extent in sedimentary and metamorphic rocks, it had also been found to be associated with magnesium and nickel in some ultra basic rocks this may explain the high value of nickel concentration (IETEG, 2004). Increase in the lead concentration noticeable with increasing distance from the spring may be attributed to increase in vehicular traffics, disposal of electronic wastes and other anthropogenic activities as sampling progresses towards densely populated area. High levels of toxic elements in the soil samples lead to destabilization of ecological balance as they find their way into the food chain thus posing health hazard to public health and the environment at large (Nkono and Asubiojo, 1998; McLaughlin and Mineau, 1995; Sinha, 1997).

The mean total bacteria counts as obtained for all the well locations were remarkably high and all were in the 10^5 - 10^6 range. This raises cause for alarm especially when the water source is available as a drinking water choice for residents of the community. A total of 50 *enterococcus* isolates were encountered during the

Table 3. *Enterococci* count of water samples from awedele spring water in Ado-Ekiti.

Water sample	Total count (CFU/ml)	
	Enterococcal count	**Bacterial count**
A_1	1.5×10^4	0.5×10^5
A_2	2.0×10^4	2.5×10^5
A_3	2.0×10^4	3.0×10^5
A_4	1.5×10^4	0.5×10^5
Mean value	1.8×10^4	1.5×10^5
B_1	4.0×10^4	1.0×10^5
B_2	3.0×10^4	1.5×10^5
B_3	2.0×10^4	1.0×10^5
B_4	2.5×10^4	1.5×10^5
Mean value	2.9×10^4	1.3×10^5
C_1	10.0×10^4	3.0×10^5
C_2	6.0×10^4	2.5×10^5
C_3	10.0×10^4	5.0×10^5
C_4	8.0×10^4	5.5×10^5
Mean value	8.6×10^4	4.0×10^5
D_1	2.5×10^4	1.5×10^5
D_2	1.5×10^4	0.5×10^5
D_3	1.5×10^4	0.5×10^5
D_4	2.5×10^4	1.0×10^5
Mean value	2.0×10^4	0.9×10^5
E_1	4.5×10^4	2.5×10^5
E_2	1.5×10^4	0.5×10^5
E_3	4.5×10^4	1.5×10^5
E_4	2.5×10^4	1.0×10^5
Mean value	3.3×10^4	1.4×10^4
F1	6.0 × 104	1.5 × 105
F2	1.5 × 104	1.0 × 105
F3	1.5 × 104	0.5 × 105
F4	2.0 × 1.54	0.5 × 105
Mean value	2.8 × 104	0.9 × 105

A, B, C, D, E and F - Points of collection of water samples along Awedele spring water
1, 2, 3, 4 - Period of collection.

study. Facklam and Peterson (2004) reported that water may have a wide range of organisms which include indigenous species, saprophytic species as well as human pathogen contaminant, such as *Enterococcus feacalis, E.faccium, E.durans E. avium* and other species of *Enterococcus*. Enterococci are readily recovered outdoors from vegetation and surface water, probably because of contamination by animal excrement or untreated sewage (Jett et al, 1994). This is much undoubted as preliminary visits to the study site revealed that human faeces and other wastes from anthropogenic activities (Figure 2) occasionally littered bushy locations close to the spring. Eventually they could be washed by rain water as run-off into the spring and thus contaminate it. This compromises the system as waste materials from humans and animals could find its way unchecked into the spring and other available water sources. Oluyege et al. (2009) also reports a similar case in a rural location in the same state of unchecked pollutant contamination by human and animal waste materials. As typical concentrations of enterococci in human stool are up to 10^8 cfu/g (Rice et al., 1995), it becomes critical if these faecal wastes are rich in antibiotic resistant strains of the pathogen. This could be expectedly true in a developing nation setting where antibiotics can be purchased without prescription and are readily available on demand stores (Kapil, 2005; Oluyege et al., 2009; Okeke and Lamikanra, 1995) thus making self-medication and antibiotic overuse by laypersons prevalent. Antibiotic use provides selective pressure favoring resistant bacterial strains; a prevailing

Table 4. Antibiotics resistance pattern of the isolates from Awedele spring water in Ado-Ekiti.

Isolates	FUS	ERY	TRM	SMX	TET	PEN	CLN	GEN
1	-	-	-	-	I	-	-	-
2	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	+
6	-	-	-	-	-	-	-	+
7	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	+
11	-	-	-	-	-	-	-	+
12	-	-	-	-	-	-	-	+
13	-	-	-	-	-	-	-	-
14	-	-	+	-	-	-	-	I
15	-	-	-	-	-	-	-	-
16	+	I	-	-	-	-	-	+
17	+	-	-	-	-	-	-	+
18	+	I	-	-	I	-	-	+
19	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-
21	-	-	-	I	-	-	-	+
22	-	-	-	-	-	-	-	-
23	-	I	+	-	-	-	+	+
24	-	-	-	-	+	-	-	+
25	+	-	-	-	-	-	-	+
26	-	-	-	-	-	-	+	+
27	-	-	-	-	+	-	-	+
28	-	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-
31	-	-	-	-	I	-	-	-
32	-	-	-	-	-	-	-	-
33	I	-	-	-	+	-	+	+
34	-	-	-	-	-	-	+	+
35	-	-	-	-	-	-	+	+
36	-	-	-	-	-	-	+	+
37	-	-	-	-	-	-	-	-
38	-	-	-	-	-	-	+	+
39	-	-	-	-	-	-	+	+
40	-	-	-	-	-	-	-	-
41	-	-	-	-	-	-	+	+
42	-	-	-	-	-	-	-	-
43	-	-	-	-	-	-	+	+
44	-	-	-	-	-	-	I	I
45	-	-	-	-	-	-	-	-
46	-	-	-	-	-	-	-	-
47	-	-	-	-	-	-	+	+
48	-	-	-	-	-	-	-	-

Table 4. Contd.

49	-	-	-	-	-	-	+	+
50	-	-	-	-	-	-	+	+
%resistant isolates	90%	94%	96%	98%	88%	100%	72%	48%

KEY- FUS = Fusidic acid; TET = Tetracycline; PEN = Penicillin; ERY = Erythromycin; CLN = Clindamycin; TRM = Trimethropim; GEN = Gentamycin; SMX = Sulfamethoxazole; Resistance - (-) Sensitive - (+) Intermediate M - (I).

custom of inappropriate use and self-prescribing antibiotics raises the risk of resistance and ultimately the probability of disseminating resistant bacteria emerging in faeces.

Based on the findings of the study, antimicrobial resistance demonstrated by encountered enterococcal isolates from Awedele springs were remarkably high for all the tested antibiotics. The comparison of the percentage of resistant strains with previously published work is often complicated because previous researchers have used different numbers and kinds of antibiotics in their studies. However, our observations corroborate the report of Niemi et al., (1983) that demonstrated low incidence of antibiotic resistance to gentamycin. A similar study by Rice et al (1995) collaborates this phenomenon by affirming that high-level gentamycin are rarely detected among enterococci isolated from the aquatic environment. On the extreme end was resistance to penicillin (100%). Mark et al (1998) also assert that penicillin is among the few antibiotics that could show inhibitory, but not bactericidal, activity against the organism. Tansuphasiri et al. (2006) demonstrated significantly high antibiotic resistance of enterococci species to tetracycline. High prevalence of resistance to erythromycin and tetracycline as presented by this study was also demonstrated by previous studies (Ferria da Silva et al., 2006; Mondino et al., 2003; Vicela et al., 2006). The prevalence of these erythromycin and tetracycline-resistant enterococci could partly be traced to its abuse. Uncontrolled community use of antibiotics for empirical treatment of infectious diseases has been implicated as a cause of high prevalence of erythromycin-resistant enterococci (Arvanitidou et al, 2001; Phillips et al., 1990). Resistance to clindamycin among the enterococcal isolates was also high (72%). Supporting this observation, Mark et al. (1998) and Bhakid (2005) assert that like vancomycin use, the usage of clindamycin is equally or more often associated with infection with multiple drug resistant (MDR) enterococci. Enterococci have a remarkable capacity of expressing resistance to several groups of antimicrobials thus posing a daunting challenge to the world of clinical practice as the number of therapeutic options for medical interventions are significantly reduced (Mathur et al., 2003). The ubiquitous nature of the organism and its resistance to adverse environmental conditions is partly responsible for its ability to colonize different habitats and also its ability to spread easily through the food chain (Aparecida et al., 2007). As presented by this study, the prevalence of multiple resistances among environmental isolates of enterococci from sources that serve as drinking water to rural communities is thus a serious concern given the propensity to serve as reservoirs facilitating the spread of resistance traits to other non-resistant bacterial population. The lack of affordable treatment facilities in these rural settings rule out the possibilities of before-use microbial reduction of such available water sources thus exposing the public to significantly increased potential health hazards. This is especially true in typical developing nations where the cost, capital and commitment to effectively run such treatment systems are lacking. While the call is made for strengthened government efforts to increase access to safe water through the provision of associated infrastructure, it is noteworthy to mention that the provision of adequate water sources for drinking must be properly balanced with continuous efforts to instigate the required hygiene behavioral change among residents of rural settlements. 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. Failure to embark on such strategy 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.

The call for more surveillance studies especially on available water bodies in vulnerable populations of developing nations is therefore eminent. Beyond the species level, further studies that identify genetic cross-interactions and dynamics of pathogens in aquatic environments are still needed. Such genetic analysis should also aim to identify possible epidemiological linkages among antibiotic-resistant enterococci isolated from drinking water sources and clinical infections. Although several studies disjointed studies litter the world of academic literature, these are often non replicable and in most cases they are conducted using subjective methodologies or based on available resources due to inadequate funding. These and other intrinsic factors in the developing world affect objective-oriented research outcomes that aim to improve the health status and livelihoods of these populations. It is thus important for the international community through capacity building and institutional support to help strengthen efforts aimed at capturing in a sustainable manner the outcome of a range

of monitoring activities currently undertaken at all levels in the developing world. This will improve linkages between datasets on access to sanitation and drinking-water and how it relates to the burden of water-borne diseases. Undoubtedly, policy-makers will be better informed on the appropriateness and effectiveness of whatever policy or intervention that is applied and thus will be able to make timely decisions on water and sanitation infrastructure and service investment especially in most vulnerable locations.

Conclusion

The current study presented an analysis of the bacteriological and physicochemical studies conducted on Awedele spring water samples in Ado-Ekiti, Nigeria. Mineral studies on soil samples from the flow path of the spring were also conducted. The assessed water and soil samples were generally of poor quality and considered unfit for drinking. The study also suggests a possible link between behavioral patterns of rural residents and the prevalence of antibiotic resistant *enterococci* in the water samples collected from the spring. It is also suggested that interventions aimed at providing drinking water for rural residents in the developing world should be balanced with efforts that that promote the required hygiene behavioral change. It is hoped that these programs will ultimately allow for achievement of the desired public health outcomes in such settings.

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