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The Culturable Microbial and Chemical Qualities of Some Waters Used for Drinking and Domestic Purposes in a Typical Rural Setting of Southwestern Nigeria

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Abstract: The culturable microbial and chemical qualities of some spring, well and sachet waters used for drinking and domestic purposes in a typical rural community in Nigeria were assessed using standard methods. Heteroptrophic bacterial count ranged appreciable in the order of 10^1 to 10^7 cfu mL^{-1}. Fungal densities were low and ranged between 0 and 18 cfu mL^{-1}, while coliform and Salmonella-Shigella counts varied between 3.8 and 803 cells/100 mL and 1.8 and 20.6 cfu mL^{-1}, respectively. Seventeen bacterial and nine fungal species were isolated from the water samples and all the bacteria isolates showed multiple antibiotic resistance to at least seven of the eight to eleven antibiotics used for specific isolates. The concentrations of elements in the water samples ranged in the following order: Mg (0-14.11 ppm); Mn (0-0.503 ppm); Zn (0-0.377 ppm); Ca (0->10.0 ppm); Cu (0-0.049 ppm); Ni (0-0.6 ppm); As (0-2.2 ppm) and Fe (0-8.7 ppm). The unacceptably high levels of As, Ni and Fe in most of the water samples, as well as the microbiological qualities suggest that the use of such waters for drinking and domestic purposes poses a serious threat to the health of the users and calls for the intervention of the appropriate control agencies.

Key words: Culturable microbial, chemical quality, well, spring, packaged waters

INTRODUCTION

Water is one of the earth's natural resources and one that is very essential to life as most indispensable to humans, animals and other living things. It has various uses such as drinking, cooking and agricultural i.e. farming and irrigation[1].

The type of water usually used in human activities is freshwater, which makes up about 3% of world water. Two third of that is frozen polar ice caps and glaciers. The remaining 1% of the total world water supply is freshwater available either as surface water or ground water. The contamination of natural water with fecal material, domestic and industrial sewage and agricultural and pasture runoff may result in an increased risk of disease transmission to humans who use such waters[2,3]. Portable water is that intended for human use and must be clean, clear and odourless and the standards must be strictly maintained to prevent outbreak of water borne diseases such as typhoid fever[4]. Traditionally, the presence of coliform bacteria in drinking water has been seen as an indicator of fecal contamination through cross connection, inadequate treatment, or an inability to maintain a disinfectant residual in the water distribution system[5].

The provision of portable water has for a long time been a major problem in developing urban and rural areas of Nigeria, a phenomenon associated with poverty and a common stance in most developing regions of the world[6]. This has led to overdependence on untreated alternative drinking and domestic water sources such as dug wells, rivers and packaged (such as bottled or sachet) waters, with their attendant health risks. Of these three alternatives, the packaged (sachet) water (popularly called pure water in Nigeria) has gained superior popularity in recent times. However, over the years concerns have been raised about the microbial quality of packaged water marketed worldwide[7,8]. Several studies have documented the detection of coliforms and heterotrophic bacteria in bottled water in counts which far exceeded national and international standards set for portable water for human consumption[8,9] and pathogens like *Escherichia coli*,

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Pseudomonas spp. and *Pseudomonas* spp. have been demonstrated to survive and multiply in bottled water with a potential to cause outbreaks in consumers[10].

Also, there have been increasing interests in the chemical composition of drinking water in recent years especially heavy metals and metalloids like arsenic[11]. Even in developed countries, there have been reports of unacceptable concentrations of some chemicals in some drinking water[12-14], resulting in controversy as regards the definition of the Maximum Allowable Concentration (MAC) values for certain elements in spring, drinking, thermal and surface waters and this has been and still is, the subject of chemical and biological research and political debate, in several countries[15,16] till date as a result of their health implications[17,18].

The high incidence (unpublished report) of gastrointestinal disorders amongst residents of Esa-Oke community which was suspected to be associated with waterborne infections led to the conceptualization of this study into the wholesomeness of the different water sources used for drinking and/or domestic purposes in this typical rural setting of Nigeria.

MATERIALS AND METHODS

Study area location and collection of water samples: Esa-Oke is a typical rural town in the rain forest region of southwestern Nigeria located within the coordinates 7°30' N and 5°00' E. The water samples used for this study were aseptically collected from this locality and comprises 10 dug wells, 5 packaged water and 3 spring water samples. The samples were collected in the months of January, February and March of 2004.

Microbial and chemical analyses: The microbial analyses carried out included estimation of the total culturable aerobic bacteria and fungi population, as well as the Coliform and Salmonella-Shigella counts. Total bacteria and fungi counts were done using standard spread plate technique in accordance with the descriptions of Seeley and vanDenmark[19] and FAO[20], while coliform and Salmonella-Shigella were estimated as described elsewhere[21,22]. Representative bacterial isolates were purified and identified based on cultural and biochemical chacateristic[23,24], while the fungal isolates were identified as described before[25]. The antibiotic susceptibility pattern of the bacteria isolates were analysed by the agar diffusion method[26] using specific multodiscs for gram positive (Abtek Biologicals, Liverpool, UK) and gram negative (Polytest laboratories, Enugu, Nigeria) bacteria isolates.

Fig. 1: Map of Southwester Nigeria showing study location (Esa-Oke) in red

The concentrations of Mg, Mn, Fe, As, Cu, Ca, Zn and Ni were determined using Alpha model-4 atomic absorption spectrophotometer (CHEMTECH ANALYTICAL LTD, PN) with the use of an air-acetylene flame and single element hollow cathode lamp.

RESULTS

The culturable aerobic bacterial densities of the well waters throughout the sampling periods ranged in the order of 10^2-10^7 cfu mL^{-1}, while the spring and packaged water counts ranged in the orders of 10^3-10^4 and 10^1-10^2 cfu mL^{-1}, respectively. Fungal densities were generally low ranging from 0-18 cfu mL^{-1} (Table 1). Whilst all the water analyzed contain bacteria in varying amounts, the percentage of the samples containing fungi ranged between 40 and 100% (Table 1).

Coliform densities were observed to range between 3.8 and 803 cells/100 mL (Fig. 2). Whilst all the wells and spring water samples contained coliform throughout the sampling period, only 80 and 40% of the packaged water samples in the months of January and March respectively contained coliform organisms and non of all the packaged waters samples contained *E. coli* (Table 2). Also, the Salmonella-Shigella counts ranged between 1.8 and 20.6 cfu mL^{-1} (Fig. 3).

A total of 17 bacteria isolates were identified, two to species level and fifteen to genus level (Table 3). The well samples were richer in bacterial diversity, followed by the spring and the packaged water, respectively. A similar trend was observed for the fungal diversity (Table 4), which were more in the well waters, followed by the spring and the packaged waters, respectively.

All the bacteria isolates showed multiple antibiotic resistances to at least seven of the eight to eleven antibiotics used for specific isolates. (Table 5)

The concentrations of Mg, Ca, As and some heavy metals (Mn, Zn, Cu, Ni and Fe) in the water samples were observed to vary appreciably in the different water

Table 1: Heterotrophic bacteria and fungi densities of the different water types sampled in the months of January, February and March 2004

Samples	Range of microbial counts (cfu mL^{-1})* Bacteria January	February	March	Fungi January	February	March
Well	7.7×10^2-1.2×10^7 (100)	2.1×10^3-2.7×10^7 (100)	1.6×10^3-2.0×10^7 (100)	0-18 (90)	0-8 (90)	0-7 (80)
Spring	6.5×10^3-2.0×10^4 (100)	1.6×10^4-2.9×10^4 (100)	4.8×10^3-2.6×10^4 (100)	2-9 (100)	1-3 (100)	0-5 (33)
Packaged	10-200 (100)	23-640 (100)	2-520 (100)	0-7 (20)	0-8 (80)	0-1 (40)

* Values in parenthesis represent % of samples containing bacteria/fungi.

Table 2: Number of water samples that contained coliforms (%)

Sample	Occurrence of coliforms (%) January	February	March
Well water	100 (30)	100 (30)	100 (70)
Packaged water	80 (0)	100 (0)	40 (0)
Spring water	100 (0)	100 (67)	100 (67)

Values in parenthesis represent % occurrence of *E. coli*

Table 3: List of bacteria species isolated from the water samples

Isolates	Samples Well	Spring	Packaged
Corynebacterium spp.	√		
Acinetobacter spp.		√	
Bacillus spp.			√
Aeromonas spp.	√		√
Klebsiella spp.	√		
Pseudomonas spp.	√		
Proteus spp.	√		
Micrococcus spp.	√		
Lactobacillus spp.	√		
Flavobacterium spp.	√		
Chromobacterium lutuem	√		
Serratia marscencen	√		
Staphylococcus spp.		√	
Salmonella spp.	√		
Shigella spp.	√	√	
Escherichia coli	√		
Citrobacter spp.		√	
Total	13	5	2

Table 4: List of Fungi species isolated from the water samples

Isolates	Samples Well	Spring	Packaged
Aspergillus niger	√	√	√
Aspergillus fumigatus	√	√	√
Aspergillus flavus	√		
Mucor spp.	√	√	√
Fusarium spp.	√		
Microsporium spp.	√	√	
Trichoderma spp.		√	
Cephalosporium spp.	√	√	
Alternaria spp.	√		√
Penicillium spp.	√		√
Total	9	6	5

types in the following order: Mg (0-14.11 ppm); Mn (0-0.503 ppm); Zn (0-0.377 ppm); Ca (0->10.0 ppm); Cu (0-0.049 ppm); Ni (0-0.6 ppm); As (0-2.2 ppm) and Fe (0-8.7 ppm) as shown in Table 6.

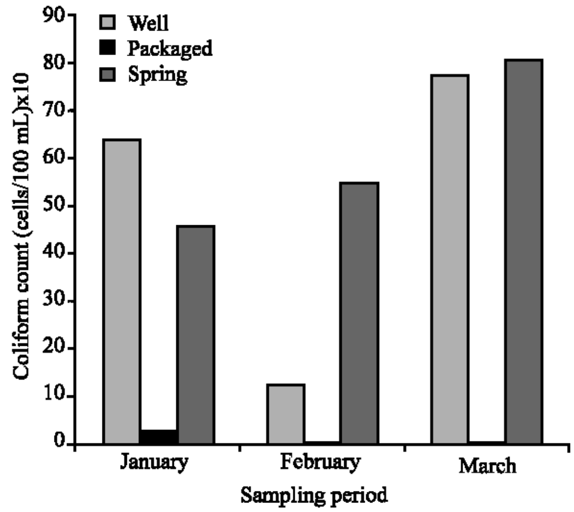

Fig. 2: Coliform densities of the different water types collected during the months of January, February and March 2004

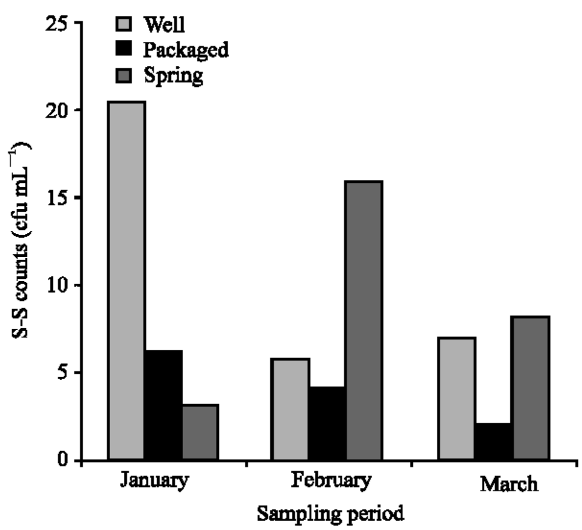

Fig. 3: Salmonella-Shigella densities of the different water types collected during the months of January, February and March 2004

DISCUSSION

The water microbial analysis revealed the presence of appreciable population of bacteria and fungi. The

Table 5: Antibiotic susceptibility pattern of the bacterial species isolated from the water samples. R = Resistant, S = Sensitive, Augmentine (30 μg) = AUG, Amoxycilin (25 μg) = AMX, Erythromycin (5 μg) = ERY, Tetracycline (10 μg) = TET, Cloxacillin (5 μg) = CXC, Gentamicin (10 μg) = GEN, Cotrimixazole (25 μg) = COT, Chloramphenicol (30 μg) = CHL, Nitrofurantoin (100 μg) = N, Cefuroxine (20 μg) = CF, Norfloxacin (10 μg) = NF, Nalidicic acid (30 μg) = NA, Ampicillin (25 μg) = AM, Ciproloxacin (5 μg) = CIP

Bacteria isolate	Test antibiotics													
	AUG	AMX	ERY	TET	CXC	GEN	COT	CHL	N	CF	NF	CIP	NA	AM
B1	R	R	R	R	R	R	R	S	nd	nd	nd	nd	nd	nd
B2	nd	nd	nd	S	nd	R	R	R	R	R	R	S	R	R
B3	R	R	R	R	R	S	R	R	nd	nd	nd	nd	nd	nd
B4	nd	nd	nd	S	nd	R	R	R	R	R	R	S	R	R
B5	nd	nd	nd	S	nd	R	R	R	R	R	R	R	R	R
B6	nd	nd	nd	S	nd	R	R	R	R	R	R	S	R	R
B7	nd	nd	nd	R	nd	R	R	R	R	R	R	S	R	R
B8	R	R	R	R	R	R	R	S	nd	nd	nd	nd	nd	nd
B9	R	R	R	R	R	R	R	S	nd	nd	nd	nd	nd	nd
B10	nd	nd	nd	R	nd	R	R	R	R	R	R	S	R	R
B11	nd	nd	nd	S	nd	R	R	S	R	R	R	S	R	R
B12	nd	nd	nd	S	nd	R	R	R	R	R	S	S	R	R
B13	R	R	R	R	R	R	R	S	nd	nd	nd	nd	nd	nd
B14	nd	nd	nd	R	R	R	R	R	R	R	R	S	R	R
B15	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B16	nd	nd	nd	S	R	R	R	R	R	R	R	S	R	R
B17	nd	nd	nd	R	R	R	R	R	R	R	R	S	R	R

Table 6: Concentrations of elements (ppm) in the water samples: (a) represents Well water samples; (b) represents Spring water samples; and (c) represents Packaged water samples. Values in parenthesis (ppm) represents health-based recommended thresholds WHO[62]

Sample period	Elements concentrations of well water samples (ppm)							
	Mg	Mn (0.4)	Zn (3)	Ca	Cu (2)	Ni (0.02)	As (0.01)	Fe (0.3)
January	0.402-4.674	0-0.145	0.169-0.367	0.05-3.22	0-0.033	nil	0-2.6	0-4.0
February	0.460-11.497	0.051-0.503	0-0.290	0.12-5.11	0-0.017	0-0.3	0-0.7	0-7.9
March	0.498-14.11	0-0.073	0.196-0.274	0.13->10.0	0-0.049	nil	nil	0-3.2
	Elements concentrations of spring water samples (ppm)							
January	0.509-12.25	0-0.085	0.185-0.311	0-1.65	0-0.033	0-0.4	0.6-1.3	0-3.5
February	0.090-4.207	0-0.098	0-0.377	0.06-0.27	0-0.030	0.1-0.6	nil	0-2.4
March	0.231-10.83	nil	0.136-0.219	0.07-0.37	0-0.022	0-0.1	0-2.2	nil
	Elements concentrations of packed water samples (ppm)							
January	0.000-9.551	0-0.17	0-0.146	0-0.64	nil	nil	nil	0-8.0
February	4.580-7.04	Nil	0.12-0.274	0-0.38	nil	0-0.2	0-0.7	nil
March	5.650-10.01	0-0.16	0-0.301	0.07-0.32	0-0.02	0-0.3	0-1.6	0-8.7

presence of these organisms supports the phenomenon of ubiquity of microorganisms and their relatively high numbers is a cause for concern considering their health implication. The microbial population distribution observed in this study is similar to the range reported by many researchers[27,29] especially in relation to fresh water system[30]. The total bacterial count of well water was observed to be the highest followed by the spring and packaged waters in that order. The rather high microbial count observed in some cases in the month of February could be attributed to heavy rainfall resulting in the washing of debris into the unfortified wells from surrounding environment thus increasing the microbial load during this period.

The presence of such bacteria isolates as *E. coli* and *Klebsiella* spp. which are coliforms suggests that these waters have experienced some recent faecal contamination. The presence of *Bacillus* spp., a versatile organism in the packaged water could have been as a result of contamination from poor staff handling of the water samples. *Bacillus* spp. produces enterotoxin which could be deadly when ingested into the body. Also, the presence of *Pseudomonas* spp., a pathogenic organism renowned for its versatility and high incidence of multiple antibiotic resistances is a cause for concern[31].

The Multiple Tube Dilution method[32] used for coliform estimation revealed the presence of coliform bacteria in the different types of water samples. The coliform densities were higher in the well water samples than in the spring or packaged water samples. The packaged water samples had the lowest counts. This trend corroborates the findings of Brenner *et al.*[33]. In their report which focused on well water, tap water and vended water, they reported that the highest coliform counts were observed in the well water. The occurrence of coliform bacteria in these water can be viewed as an indicator of faecal contamination especially in cases where *E. coli* was observed. Similar observations were made by other authors[14,34,35].

It is given that, in the concept of an ideal faecal indicator, the sentinel organism does not grow outside the intestinal tract. However, some reports had shown that the regrowth of coliform bacteria occurs in finished drinking water and may not be related to faecal or pathogen contamination or to waterborne disease. This they argue is because factors controlling the regrowth of coliform bacteria is not well understood and hence there is no proper plan that utilities can adopt that can ensure total compliance with the elimination of total coliform[36].

E. coli occurrence was observed in the well water and spring water samples while it was totally absent in all the packaged water samples during the sampling months. In the report of Eckner[37], *E. coli* occurrence in the private wells was higher than those observed in the surface water and none was detected in the treated water or the water under production, thus supporting our observation. Arnade[38] reported that groundwater samples collected from sixty residential wells in Palm Bay, Florida contained very high counts of coliform bacteria. Similar observations have been reported elsewhere[3,39]. Later, Gallegos *et al.*[40] discussed the potential effects of groundwater degradation on public health and the need for guidelines to protect groundwater quality. Although we did not attempt to detect *E. coli* O157 strain, it is important to note that *E. coli* O157:H7 has the potential to multiply and then survive for over 300 days in artificially inoculated bottled water[41].

The absence of *E. coli* in the packaged water samples could be attributed to the fact that the majority of coliforms found in drinking water are injured[42,43]. Injury has been described as sub lethal physiological damage resulting from the exposure of microorganisms in drinking water to chemical, biological or physical factors that cause the organisms to lose the ability to grow on selective media which are satisfactory for growing normal cells[44-47]. The increased sensitivity of stressed coliforms to selective media can result in the failure to detect them or underestimation of their concentration in drinking water[43]. Ultimately, this can result in the use of unsafe water by the public with its attendant health risks[48]. It could also be that the packaged water is not as exposed to faecal contamination as the well and spring water sources, or that the process or conditions for the production of the packaged water are efficient.

The range of Salmonella-Shigella counts observed in this study are similar to previously reported observation[49] and exceeded the NAFDAC[50] accepted standard value of 1 cfu/100 mL. These pathogens presence in the well waters may be associated with uncontrolled opening of the wells as earlier highlighted, which invariably introduces contaminants through soiled materials from the water retrieval devices. Mayer[51] reported that, the risk factors in the portability of these well water samples are more than what can be over looked. Also the proximity of wells to sewage disposing facilities which allows infiltration of waste materials into surrounding water tables could also be a suitable source of contamination. The alarming number of the organisms found in the Spring water samples may be associated with the drainage runoff particularly during storms that has consummated water with human and animal faecal remains[51]. Such pathogens can then gain entrance into a body of water which ultimately serves as source of drinking water[52]. The presence of the Salmonella-Shigella bacteria in packaged water can also be associated with the source and processing of the water.

The multiple antibiotic resistant patterns of the bacteria isolates are a cause for serious concern for its attendant health impact. Biyela *et al.*[53] reported high incidence of multiple antibiotic resistant genes in aquatic systems and suggested that the widespread and indiscriminate use of antibiotics has led to the development of antibiotic resistance in pathogenic, as well as commensal microorganisms, such that resistance genes may be horizontally or vertically transferred between bacterial communities in the environment and the recipient bacterial communities may then act as a reservoir of these resistance genes.

The fungal communities were noted to be lower in all cases compared to bacterial communities, in consonance with our previous documentation on microbial diversities[30] and these species are traditionally common inhabitants of most tropical environments where they play their different roles to ensuring the stability of the ecosystem.

The main threats to human health from heavy metals are associated with exposure to such elements as arsenic. This metal along with others such as mercury and lead have been extensively studied and their effects on human health regularly reviewed by international bodies such as the WHO[54]. The health implications of excess consumption of these non-essential metals have been noted to result in neurological, bone and cardiovascular diseases, renal dysfunction and various cancers, even at relatively low levels[55-57].

While the concentrations of Mn, Zn, Ca and Cu were higher in the well waters, that of Ni, As, Cu and Fe were found to be higher in the spring water samples and the packaged (sachet) water contained unusually high amount of Mg. These observations are similar to that reported by Dixit *et al.*[58] who found high levels of manganese, copper, selenium and cadmium in final water supply of four treatment plants and 80 tube wells at Delhi. Also, Zhang[59] reported high levels of As beyond permitable limit in some counties in China. In a study of the chemical status of bottle water in Alabama[12], the concentrations of most water quality constituents analyzed, in most cases, were higher in the spring water brands compared to the purified or distilled brands of bottled water. The concentrations of Zn and Cu in all the water samples tested were below the recommended health based threshhold, while most of the water samples contained arcenic, nickel and iron concentrations that were orders of magnitude higher than the maximum allowable limit and this could portend serious environmental and health hazard to the consumers of such waters.

This study, therefore, suggests that there is the need for public awareness of the inherent dangers that would be associated with the consumption of these waters in their untreated forms, more so with all the high coliform densities that was observed in almost all the water samples. Also, the microbiological wholesomeness of waters meant for drinking, recreation and industrial uses must be made to comply with established standards of appropriate national and international legislative agencies, especially in the light of recent report[60] which suggest that 99.8% of deaths in developing countries are water, sanitation and hygiene-related deaths and that 90% are deaths of children. Besides, these sources of untreated drinking water could be veritable reservoirs of several other opportunistic pathogens of humans[61] and chemical poisoning.

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