Bacteriological Evaluation of Well Water Samples from Selected Community Markets in Ijebu-Remo Axis of Ogun State

Okunye, Olufemi Lionel[1]. Idowu Philip Adegboyega[2]. Kolade Titilayo Teniola[3]

[1]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Olabisi Onabanjo University, Ogun State.
[2]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Ibadan Nigeria
[3]Centre for Entrepreneurship Development, Yaba College of Technology, Yaba Lagos.

ABSTRACT

The quality of portable water and treatment of water borne diseases are critical public health issue. This study was carried out in Ijebu-Remo communities-axis of Ogun State to evaluate the bacteriological and physicochemical quality of in-situ community market well water for the presence of coliforms and other associated potential pathogens. A total of 24 wells from selected 6 market locations were examined. They were found to be laden with coliforms, *Pseudomonas* and *Klebsiella* in varying percentage. The potential hydrogen concentrations of the 24 wells examined were acidic with the exception of 2 wells with a pH 7. The total viable count varied between 7×10^6 to 47.5×10^6. Though the regressive analysis to determine the significance of the extrinsic and intrinsic values of the sampled water extends beyond the P-values≤0.5, the total viable count from the 24 well water sampled were grossly contaminated. Therefore, the water is unsafe for drinking to avoid water borne infection of unpredictable magnitudes.

Keywords: Bacteriological evaluation, Well water, Community markets. Ijebu-Remo.

INTRODUCTION

Water is a transparent, tasteless, odourless and colourless chemical substance and it is vital for all known forms of life, even though it provides no calories or organic nutrients. Water that contains large numbers of bacteria may be safe to drink. The important consideration, from microbiology standpoint, is the kinds of microorganisms that are present. Water from streams, lakes, community shallow or deep well water that contains multitudes of autotrophs and saprophytic heterotrophs is portable as long as pathogens for humans are lacking. The intestinal pathogens such as those that cause typhoid fever, cholera and bacillary dysentery are of prime concern. The fact that human faecal materials are carried away by water in sewage systems that often empty into rivers and lakes present a colossal sanitary problems; thus, constant testing of municipal or community water supplies or sources for the presence of faecal microorganisms is essential for the maintenance of water supply (Harold, 2002). The origin of water of water on the earth is not clear so far. However, the current presumption is that the primordial earth had no oceans, and perhaps very little atmosphere. It is believed that the volatile constituents bound in the earth's crust oozing as water to the earth originates from multiple volcanic compressions. This way, perhaps this remarkable combination of hydrogen and oxygen, called water,

came into being and eventually became an indispensable component of the earth's environment (Agrawal, 2012). A well is an excavation or structure created in the ground by digging, driving, or drilling toaccess groundwater in underground aquifers(Kirby, 1997). Water is not only essential to life but it is the predominant inorganic constituent of living matter, forming, in general, nearly three quarters of the weight of the living cell. It makes up to some 75 percent of the body weight of an adult human (Agrawal, 2012). Organisms which contain relatively small amounts of water are generally in dormant state or show very slow development, seeds and certain invertebrates that live in arid environments are examples. Of the three states in which water occurs in the ecosphere, gas, solid and liquid – only the last is an indispensable resource as far as human activity is concerned. Inland surface water account for barely 0.02 per cent (Kirby, 1997). Ground water contains a little amount of organic matter. This is the reason why microorganisms cannot multiply rapidly in such water. The growth of microorganisms in water mainly depends on the amount of available mineral nutrients and the dissolved oxygen present in it. It has been observed that as the amount of organic matter increases in water, the number of microorganisms also increases but up to certain limit.

*Corresponding Author: femfem111@yahoo.com 081 6555 8033

The number of bacteria and other microbes will always be higher in river passing by thickly populated cities than of the villages because persons living in cities, are continuously disposing sewage water and other waste products in rivers which contain a very high amount of mineral nutrients which is a medium for their growth. Moreover, the pH, temperature range and inorganic phosphate content as well as the situation of the lake and river also support the growth of microbes (Petrie, 1994). Water borne organisms can cause dysentery, typhoid fever, Salmonellosis and vibrio illness depending on the etiologic agent associated with each infection. And the socio economic impact is financial and manpower loss. On average a family spends about 10% of the monthly household income (Galibraith, 1987). The pH of water is a measure of the acid–base equilibrium and, in most natural waters, is controlled by the carbon dioxide–bicarbonate–carbonate equilibrium system. An increased carbon dioxide concentration will therefore lower pH, whereas a decrease will cause it to rise. Temperature will also affect the equilibria and the pH. In pure water, a decrease in pH of about 0.45 occurs as the temperature is raised by 25°C. In water with a buffering capacity imparted by bicarbonate, carbonate and hydroxyl ions, this temperature effect is subject to modification (APHA, 1989). The pH of most drinking-water lies within the range 6.5–8.5. Natural waters can be of lower pH, as a result of, for example, acid rain or higher pH in limestone areas. Humans use water in the home, in industry, in agriculture and for recreation. All life on earth depends on water. Many use water for many purposes which include drinking, irrigation, fisheries, industrial processes, transportation and waste disposal. Availability of basic infrastructures, financial constraints, illiteracy and bad governance are the major obstacles in the provision of water of good quality in developing countries. Ijebu-Remo, an axis of Ogun State in southwest Nigeria is less developed in terms of infrastures and basic amenities. Most of the towns and villages there are known with one or more than one community market that lack bore holes or treated tap water but with locally dug wells for water supply.   Treated pipe borne water is limited to urban areas for those that can afford it. This study evaluated the bacteriological and physico-chemical quality of some selected well water situated within some selected markets.

MATERIALS AND METHODS
Study Areas
pink rose ring colour were regarded to be due to faecal contamination of drinking water by

A total of twenty- four community dug well water sample of varied depths, four from each of the six selected markets; Ogunmakin, Ajebo, Ogere, Iperu, Sabo and Awolowo market in Sagamu   for this study   was randomly collected aseptically for bacteriological examination.
 Collection of water Sample
Water samples were collected with sterile 200 mL screwed capped glass bottles. The lid was aseptically removed and the bottles lowered into the well. The bottle was brought up to the surface and covered with a screw-cap with no air bubbles. All the sampled bottles were immediately labelled and transported in ice-pack to the laboratory for bacteriological analysis within 6 hours of collections.

Determination of pH of the Water Sample
The pH of each water sample collected was measured electrometrically with a glass electrode pH meter and was recorded for further analysis.

Bacteriological Analysis
The water sample was shaken thoroughly and a one-in-one thousand dilution of each sample was carried out with sterile distilled water. 1mL of the dilution (1:100) was inoculated into 20 mL of melted, cooled nutrient agar.   The nutrient agar was mixed thoroughly and poured into a sterile Petri-plate. This was allowed to solidify and then incubated at 37°C for 24 hours.   The number of discrete colonies were counted and expressed as colony forming unit per ml (cfu/mL).

Presumptive coliform test
A total of 11 tubes with Durham tube suspended, divided into 3 each were used for each of the six water samples. 50 mL of water samples was added to double strength MacConkey broth.   10 mL water sample was added to   10 mL of double strength MacConkey broth in 5 tubes and 1 mL of water sample was added to 5 mL of single strength broth each. The bottles and tubes were incubated at 37°C for 48 hours and were examined for acid and gas production.   Appearance of gas as indicated by Duham tubes displacement in the first 24 hours was recorded as positive for presumptive coliforms. The most probable number of coliforms per 100 mL of sample was estimated from probability table.

Differential Coliform Test
Subcultures were made from presumptive positive test into fresh tubes containing 5mL sterile peptone water.   The tubes were incubated at 44°C and examined after 48 hrs. 3 drops of Kovac's indole reagent was added to each tube. The development of

Escherichia coli.

Complete Confirmatory Test
A loopful of presumptive positive and negative cultures were streaked on MacConkey agar plate and incubated at $37^{o}C$ for 24 hours. The bacterial isolates obtained were inoculated on nutrient agar slopes for biochemical identification.

Biochemical Identification
Conventional microbiology tests; Gram stain, indole test, methyl red, Voges-Proskaeur, catalase test, oxidase test, citrate utilization and triple iron sugar test were carried out on the isolates.

Statistical analysis
Statistical analysis of the total viable bacteria count, the intrinsic and extrinsic of the examined well water were carried out by using analysis of variance interpretation model.

RESULTS
Different variations in the $_{p}H$ values relative to various depth obtained in this study as showed in Table 1 below where many of the $_{p}H$ values obtained are acidic with the exception of 4th well in Ajebo and Awolowo's markets which are both deep and neutral corroborates with the reports of WHO on testing wells to improve quality of water which states that the $_{p}H$ of water may varies due to the presence of nitrates, nitrites, lead, copper, metals and corrosion depending on the depth of such excavations which a function of texture of the land and environment.

Table 1: Intrinsic and extrinsic properties of the examined well water (depth of the well/$_{p}H$)

Location	Depth (M)	pH
Ogunmakin market	20.0	4.0
	11.7	3.2
	30.0	4.5
	35.0	5.2
Ajebo market	4.0	2.5
	42.0	5.7
	9.0	4.3
	60	7.0
Ogere market	30	4.3
	45	5.0
	12	3.0
	49	5.4
Iperu market	10	3.2
	27	4.1
	15	3.7
	32	4.3
Sabo market	30	4.2
	37	5.0
	53	5.9
	44	4.8
Awolowo market	14	3.2
	25	4.0
	33	4.5
	65	7.0

The bacterial isolates and coliforms loads as showed in Table 2 and 3 below agrees with the finding of WHO which states that coliforms and other bacterial community that could be presents in an excavated ground aquifer s(wells) are $_{p}H$, environment and depth dependent

Table 2: Distributions of bacterial isolates in the water well examined

Location	Code	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella*	*sp.*
Ogunmakin market	OM$_1$	28	-	24	
	OM$_2$	32	-	16	
	OM$_3$	22	-	18	
	OM$_4$	16	-	12	
Ajebo Market	AM$_1$	26	16	14	
	AM$_2$	24	14	8	
	AM$_3$	22	-	5	
	AM$_4$	28	-	15	
Ogere	OM$_1$	18	12	-	
	OM$_2$	22	10	-	
	OM$_3$	24	14	-	
	OM$_4$	36	-	-	
Iperu Market	IM$_1$	24	16	-	
	IM$_1$	28	14	8	
	IM$_1$	26	12	-	
	IM$_1$	26	-	9	
Sabo Market	SM$_1$	28	8	-	
	SM$_2$	24	14	-	
	SM$_3$	24	12	8	
	SM$_4$	30	16	10	
Awolowo Market	A'M$_1$	28	12	-	
	A'M$_2$	20	8	-	
	A'M$_3$	18	14	-	
	A'M$_4$	24	-	5	

Table 3: Total coliform count of the examined well water cfu/mL

Location	W$_1$	W$_2$	W$_3$	W$_4$
Ogunmakin market	32.5	12.5	25.0	7.0
Ajebo market	31.5	24.5	16.0	31.5
Ogere market	30.0	32.5	31.5	47.5
Iperu market	35.0	31.5	7.5	17.5
Sabo market	12.5	30.0	17.0	35.5
Awolowo market	25.0	7.0	32.5	25.0

Dilution factor 10^{-6} inoculum size 0.2mL

Cfu/ml = Viable count x Reciprocal of Dilution factor x Reciprocal of inoculums size

DISCUSSION AND CONCLUSION

The extrinsic and intrinsic factors of the selected well water sampled elicited variations in terms of depth and pH.   The deepest well (65M) within Awolowo market evaluated in the study had a pH 7.0 while the shallowest well (9M) at Ajebo market elicited pH 4.3 which is relatively acidic. This agrees with the reports of World Health Organization Guidelines *for Drinking-water Quality* that the optimum pH will vary in different supplies according to the composition of the water and the nature of the construction materials used in the distribution system, and the depth of the water but is often in the range 6.5–9.5. The effects of acids and alkalis depend on the strength of the acid or alkali and the concentration. Strong concentrated acids or alkalis are corrosive, whereas dilute and weak acids and alkalis are not corrosive. pH alone is not the primary determinant of adverse effects, and in water, acids and alkalis are normally extremely dilute. The pH of stomach fluid, which contains hydrochloric acid, is between 1.0 and 3.5, with a mean of approximately

2.0, and there is a range of commonly encountered foods that are also of low pH. A direct relationship between human health and the pH of drinking water is impossible to ascertain, because pH is so closely associated with other aspects of water quality, and acids and alkalis are weak and usually very dilute. However, because pH can affect the degree of corrosion of metals as well as disinfection efficiency, any effect on health is likely to be indirect and due to increased ingestion of metals inadequate disinfection (HMSO,1978). Different variations in the $_pH$ values relative to various depth obtained in this study as showed in Table 1 above where many of the $_pH$ values obtained are acidic with the exception of 4th well in Ajebo and Awolowo's market which both deep and neutral corroborates with the reports of WHO on testing wells to improve quality of water which states that the $_pH$ of water may varies due to the presence of nitrates, nitrites, lead, copper, metals and corrosion depending on the depth of such excavations which a function of texture of the land and environment. The bacterial isolates and coliforms loads in this study agrees with the finding of WHO which stated that coliforms and other bacterial community that could be presents in an excavated ground aquifers(wells) are $_pH$ and depth dependent(WHO, 2007). The distributions of the bacterial isolates in the water sampled varied according to the environment surrounding the well. *Escherichia coli* was predominant in all the well sampled. At Ogunmakin market, the locally dug wells in an unkempt open places there were surrounded by various raw foodstuff seller's and there are no provisions for toilets and lavatory for marketers. *Escherichia coli* and *Klebsiella spp* were mostly predominant from the sample but no *Pseudomonas aeruginosa* were absent. The situation in Ajebo market which is a rural market is similar, though there are variation in the distribution of samples, *Pseudomonas aeruginosa* were recorded from two wells sampled. Ogere market is colonized by herders and artisans, the well water at this location was covered with a wooden plank, though there were litter of herbs around the well, there was no grown-up vegetation around this location.   There was no *Klebsiella sp.* in the samples but *Escherichia coli* and *Psuedomonas aeruginosa* were recorded. Iperu market and Sabo markets were predominantly known for herb sellers, farm produce seller, butchers, locally woven clothes and other fabric works. The well though covered with a metallic lid was located at the end of major gutter while Sabo well water was at the entrance of the major gate of the market. The distribution of the isolates in the two markets slightly varied. *Escherichia coli* were predominant than other

competing isolates. The Awolowo market was colonized by cereal sellers and locally manufactured provisions, *Klebsiella spp* was only recorded on the fourth well water sampled. *Escherichia coli* and *Pseudomonas aeruginosa* were recorded in varied numbers. With the exception of the pH of the water obtained from Ajebo and Awolowo marke 4th wells water respectively as shown in Table 1.0 that were neutral, the pH of the remaining water well examined were acidic. The pH values of water can be used to predict the parameter of microbial pathogens that can be obtained from such water. The values outside the recommended range can indirectly affect human health as secondary contaminants in water (McClanahan, 1974). The examination of bacterial isolates revealed that, *Escherichia coli,* an index of faecal contamination of water were predominant in all the 24 well water sample (Table 2.0). *Pseudomonas aeruginosa,* a nutritionally non-exacting bacteria were also recorded in 5 of the 6 wells   with the exception of Ogere market that has no pseudomonas growth and Ajebo market where wells number AM3and AM4 showed no growth. *Klebsiella spp* were also predominant in Ogunmakin and Ajebo market. This corroborates the findings of Lloyd and Helmer (1992) on surveillance of drinking water quality in rural areas. The results of the total coliform counts in the various samples of well water examined using inoculums size of 0.2mL from 10^{-6} dilution are shown in Table 3.0**.** The presence of coliforms in the water sampled varied from well to well . The values obtained in the Table 3.0 showed the significant contamination level of each well, which could be as a results of faecal oral contamination, hence an evidence of poor hygiene practises in and around the well location. Though the regressive analysis indicated no significant difference between the extrinsic and intrinsic values of the sampled water (P-values $\geq$ 0.05). The statistical significance could not be considered as more relevant than the clinical significance which is more important because of the pathogenic potential of the bacteria found in the water samples This is in agreement with the findings of Galbraith *et al*., (1987). The possible contamination ways could be due to poor hygiene, lack of treatment scheme for the water and every other dirty oriented marketing activities in and around those wells. It is imperative that the concern public health outfits should create awareness to the marketers and stimulate the sinking of water bore-holes in the various markets for use of stakeholders to prevent water borne infections and encourage healthy living.

REFERENCES

Adeniyi, J. (2003). Text of a Message delivered at the 31st Environment Day, Ibadan, Nigeria. *The Guardian Newspaper 5th June, 2003 p.37.*

Agrawal, A.K. and Pradeep Pariah (2012). Industrial Microbiology *ISBN(10): 81-88826-25-1 pg.608-615*

Anita, K.H. and Sidney, A.C (1992). Water borne Diseases in Encyclopedia of Microbiology *Academic Press Inc. p. 337-384.*

APHA (1989) Standard methods for the examination of water and wastewater. *17th ed. Washington, DC, American Public Health Association.*

APHA, (1992). Standard methods for the Examination of Water and Waste water. *Washington 17th Ed. Part 9000.*

ASTM (1976). Standard test for pH of water and waste water. In: *Annual book of ASTM standards. Part31. Philadelphia, PA, American Society for Testing and Materials, p. 178*

Chesebrough, M.(2002). Water related Diseases and Testing of Water supplies in District Laboratory Practice in Tropical Countries Part II. *Cambridge University Press P. 143-157.*

.Cowan S.T. (1981). Manual for the Identification of Medical Bacteria. *Cambridge University Press 2nd ed.*

Dadswill, J.V. (1990). Microbiological Aspects for Water Quality and Health *Journal of the Institutions of Water and Environment Management. Vol.4 (6) P.515-519.*

Duran, C.J. (1984). Safe Water for Every Family. *World Health No.6, Nov. 1984*
Galbraith, N.S. *et al* (1987). Water and Disease after Croydon: A Review of Water-borne and Water Associated Diseases in the U.K. *J IWEM, NO 1 Jan-Feb., 1987 P. 7-21.*

Harold, J. Benson (2002) Microbiology applications. *McGraw Hill,8th Edn. pg 40-47*
Hugo,W.B. and Russell, A.D.(1984). Pharmaceutical Microbiology. *Blackwell Scientific Publications. 3rd ed. P. 320*

HMSO (1978) The measurement of electrical conductivity and laboratory determination of the pH value of natural, treated and wastewaters. London, *Her Majesty's Stationery Office.*

Hutin, Y. Luby S. and Paquet, C. (2003). A Large Cholera Outbreak in Kano City: The importance of Hand Washing with Soap and The Danger of Street Vended Water. *J. Water & Health, Vol. 1, 2003.*

Illinois Dept. of Public Health (1999). Well Sampling for Coliform and Nitrate. *Environmental Health Fact Sheet.. www.idph.state.il.us/envhealth*

IPCS News(1993). Water Quality Guidelines. *The Newsletter of the International Program on Chemical Safety, Issue 4 :2*

Kirby, C. (1997). Shallow Aquifer Water Quality Risks. Water and Sanitation for All: Partnerships and Innovations. *23rd WEDC Conference, Durban, South African, 1997. P. 385-387.*

Lloyd, B. and Helmer, R.(1991). Surveillance of Drinking Water Quallity in Rural Areas. *L:longman Grp. UK., P, 1-4.*

Manitoba Health (2001). How do I Disinfect my well. *Well Water Fact Sheet March, 2001.*
Mendie, V.E. (2002). Cyclical growths of contaminants in drinking water packaged in polythene bag. *Nig. J. Pharmacy, 33, April-June, 2002, pg 27-32.*

McClanahan, M.A. and Mancy, K.H. (1974). Effect of pH on the quality of calcium carbonate film deposited from moderately hard and hard water. *Journal of the American Water Works Association*, 66(1):49–53.

Osho, A. and Fagade, O.E. (2000). Occurrence of Coliform and Faecal Streptococcus in Ago-iwoye Well Water. *Nig. J. Sci. Vol. 34, No 4 P. 323-329.*

Packam, R.F. (1990). Cryptosporidium and Water Supplies – The Badenoch Report *J. IWEM, 1990 4TH Dec. P. 78-580.*

Petrie, A.S. and Clapharm D.B (1994). Seasonal Variations in the Quality of Spring Waters Used as Water Supplies. *J. IWEM, 1994, 8TH June, P. 320-326.*

Strauss, B., King, W. and Hoey, J.R. (2001). A Prospective study of rural drinking water quality and acute gastrointestinal illness. *Biomed Central Public Health, 29th Aug. 2001. www.biomedcentral.com*

Water Resources (2002). Application of standards for bacteriological quality of public drinking water supplies and private wells: water quality standards. *Dept. of Environment, Gov. of Newfoundland & Labrador. . www.gov.nf.ca/env/Env/waterres*

WHO(1993). New drinking water guidelines. I*PCS news, issue 4.*

WHO (1996). Water and Sanitation Factsheets 112 Nov 1996 www.who.int/inf

WHO (2007). Guidelines for drinking water qualities who/sde/wsh/07.01/1