BACTERIOLOGICAL EXAMINATION OF WELL WATER IN AGO-IWOYE AND ANTIBIOGRAM

Onajobi I. B.[1*], Banjo A. O.[1], Laba S. A.,[2] Samson O. J[1.], Nana C. O[1]., Adeleke, S. O[1]. & Abiona T. O.[1]

[1] Department of Microbiology, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.
[2] Department of Biological Sciences, Al-Hikmah University, Ilorin, Nigeria.
Email: onaobii@yahoo.com, **Phone:** 07065913072.

Abstract
This study aims to investigate the physicochemical parameters, bacteriological quality and antibiotic susceptibility profiles of bacterial isolates from well water used for domestic purposes by households in Ago-Iwoye southwestern Nigeria. The multiple tube fermentation method (MPN) was employed for the evaluation of total coliforms. The total bacterial plate count was done using the pour plate technique. Identification of isolates was done using standard methods. Antibiotics sensitivity and profile was determined using the disk diffusion method. The total coliform count within the eight (8) different wells ranged from $1.0x10^3$ to $2.3x10^3$ Cfu/ml while the total coliform count using the MPN of bacteria ranged from 3 to 93 bacteria per 100 ml. The total viable count ranged from $3.0x10^3$-$1.09x10^4$ Cfu/ml. The incidence of fecal coli form ranged from $5.0x10^2$ to $1.7x10^3$ Cfu/ml. A total of eight (8) genera of bacteria were isolated which includes *Escherichia coli, Klebsiella* sp*, Aerobacter aerogenes, Shigella* sp*, Serratia* sp*, Salmonella* sp*, Enterobacter* sp *and Staphylococcus aureus*, with the highest level of resistance exhibited by *Enterobacter* sp while *Escherichia coli* was highly sensitive to all antibiotics except augmentin. It was concluded that well water were of poor bacteriological quality indicative of health risk to the households living on it and that well water was not safe for consumption.
Keywords: Antibiotics, Bacteriological, Coliforms, Pathogens, Physicochemical, Well water.

INTRODUCTION

Water is a colourless, transparent, odourless liquid which forms the seas, lakes, rivers, and rain and is the basis of fluids of living organisms (Onajobi *et al.,* 2017a). It is composed of the chemical elements H_2 and O_2 and it exists in gaseous liquid and solid states. It is essential to life and is fundamentally important to all living things (Onajobi *et al.,* 2017b). Water constitutes about 90% by weight of the human body and nearly three quarters of the weight of the human cell (William *et al*., 2002). Also, water of good drinking quality is of basic importance to human physiology and indispensable to man's existence. Water is the source of energy and governs the evolution and functions of the universe on earth (Kataria *et al*., 2011). Water for human consumption and use are derived from different sources which can be either natural or artificial. Natural sources include rainfall, well, borehole, spring, river, stream, sea, ocean and lake while artificial water sources include distilled water, purified or treated water (Bello *et al*., 2013a).

In a city like Ago-Iwoye, where access to safe, clean water source or supply is poor, as water becomes scarce, people tend to use any locally available water source without any form of pretreatment. Well water is considered the main source of water supply and is used for various purposes like domestic, recreational, environmental, industrial and agricultural activities. Many households in Ago-Iwoye make use of well water as their source of drinking water due to the unavailability of portable water source. Well water is regarded as a reliable source of water supply due to the fact that it is often unpolluted as a result of restricted movement of pollutants in the soil profile and on a global scale; ground water represents the world's largest and most important source of fresh potable water (Gambo *et al*., 2015).
The objectives of this study are to collect well water samples from different wells in Ago-Iwoye and Determination of the physical, chemical parameter, MPN/100ml and isolates bacteria present in the well water samples. Biochemical identification and characterization, enumerate indicator organisms in the well water sources and to determine the sensitivity profiles of the pathogenic isolates to commonly used antibiotics.

MATERIALS AND METHODS

Sample Collection

Well water samples were collected randomly from eight different wells within the same geographical location, namely, itamerin, olopomerin, Ago-garage, chips, omu, ijesha road, sabo, onabamiro, in Ago-Iwoye town in Ijebu North Local Government Area of Ogun State, with the use of sterile sample bottles. The sterile bottles were tied with a strong string to a piece of metal of about 400g. The bottle caps were removed aseptically and the weighed bottle was lowered into the well, the opened bottle was allowed to sink below the water and was pulled up after observing there were no more bubbles from the bottle. The sterile bottle was gently raised out of the well without allowing the bottle to touch the sides of the wells. The caps were carefully replaced and sample bottles were labeled appropriately (Dawn *et al*., 2017).

PHYSICOCHEMICAL PARAMETERS

The physicochemical properties of the sample were determined by measuring parameters such as pH, temperature, turbidity. The pH was determined using pH meter Wag WT 3020; colour and turbidity was determined using Wag WT 3020 turbidity meter (Sule, 2009).

TOTAL BACTERIAL COUNT

For each water sample, 1 ml of a (1:100) dilution was inoculated into 19 ml of molten nutrient agar, properly mixed and poured into a sterile Petri dish. The agar was allowed to set, and then incubated at 37°C for 24 hours. The formed colonies were counted and result expressed as Cfu/ml.

Total Coliform count

The multiple tube fermentation technique as described by Onajobi *et al.* (2017a) was used. In this method, varying amounts of water sample were added to double and single strength MacCon- key broth in sterile MacCartney bottles. The bottles were incubated aerobically at 37°C for 24 - 48 hours, after which they were examined for the production of acid and gas. Sterile distilled water was used as a negative control for each test batch. The presumptive coliform count was obtained by the most probable number (MPN) of coliform per 100 ml of water sample by making reference to the most probable number table (McCrady's probability table) after combination of various positive and negative results.

Feacal Coliform Count

The feacal coliform count was determined using Eosin Methylene Blue agar employing the pour plate technique. On Eosin Methylene Blue (EMB) agar, *E. coli* strains appeared as greenish metallic sheen colonies, and this was further confirmed by the ability of the organism to ferment lactose at the 44.5^{O}C while *Aerobacter aerogenes* appeared as large pinkish mucoid colonies (Burnett, 2013).

Bacteria isolation and biochemical characterization

Media used in this study includes nutrient agar, MacConkey agar, mannitol salt agar, eosin methylene blue (EMB) and *Salmonella Shigella*-agar. All media were prepared in accordance with manufacturer's specification. The bacteria isolates were characterized using morphological and biochemical characteristics, which includes Gram staining, motility, Indole, Methyl Red, Voges-Proskauer and Citrate (IMVIC), triple sugar iron, catalase, oxidase, coagulase, urease and sugar fermentation tests with strict adherence to standard methods. The purity of the bacterial isolates would be ascertained by plating on the different selective agar before carrying out biochemical tests.

Antimicrobial Susceptibility Testing

The Kirby-Bauer disk diffusion method was used to determine the antimicrobial susceptibility profiles of the bacterial isolates. The antimicrobial agents tested included chloramphenical (30µg), tetracycline (30µg), gentamycin (10µg), ciprofloxacin (5µg), nalidixic acid (30µg), ampicillin (10µg), nitrofurantoin (300µg), vancomycin (30µg), pefloxacin (5µg), ofloxacin (5µg), amoxicillin (10µg), streptomycin (10µg) (Oxioid, U.K), cotrimoxazole (25µg) and augmentin (30Jg). The medium used was Mueller Hinton (MH) agar (Onajobi *et al.* 2017a).

RESULTS

As shown in Table 1, the result indicates that the pH ranged from 6.0-7.0. The lowest was recorded from onabamiro, while the highest pH at the palace. All pH levels were within the standard of both WHO and

NAFDAC, except pH from onabamiro recorded at 6.0 which were lower than the standard for both WHO and NAFDAC. Water sample from olopomerin recorded the highest temperature of 28^0C. The turbidity of water sample was higher with a range of 3 at chips, onabamiro and sabo to 6 at ome. Turbidity of all well water samples were within the normal range of NAFDAC except in ome and well close to that of WHO this is indicating that the well water was relatively turbid, this may be due to parental rock activities.
The total viable count (TVC) in Table 2 indicates that well water (W1) from Ago-garage had the highest microbial load with the values of $1.09x10^4$ Cfu/ml and with well sample (W8) from sabo having the least microbial load of $3.0x10^3$ Cfu/ml which is higher than the recommended value as stipulated by WHO to be $1.0x10^2$ Cfu/ml. The results of the total bacteria count carried out on selective media shown in Table 2. Also recorded the highest value of $3.0x10^3$ Cfu/ml and the lowest value of $1.9x10^3$ Cfu/ml from well water sample W1 and W5 respectively. The bacteria count of *Salmonella* and *Shigella* species ranged from 0 Cfu/ml from sample W7 (onabamiro to $2.0x10^3$ Cfu/ml from sample W6 (ome).

This was confirmed by the morphological and biochemical characterization of the isolates from the well water samples as shown in Table 4 from the locations under study which were highly contaminated with one or more bacterial pathogens namely *Escherichia coli*, *Klebsiella* spp, *Serratia* spp, *Enterobacter* spp, *Aerobacter aerogenes*, *Shigella* spp, *Staphylococcus aureus* and *Salmonella* spp, eight genera in total shown in Table 4. As shown in Table 5 the percentage occurrence of the organisms isolated from the well sample showed *Klebsiella sp* (27%) to be the most common followed by *Escherichia coli (*18%), *Staphylococcus aureus* (14%), *Enterobacter* spp. (9%), *Serratia* spp. (9%), *Aerobacter aerogenes* (9%) *Shigella* spp. (9%) and *Salmonella* spp. (5%).

Antimicrobial resistance patterns among isolates recovered from each of the eight sampling sites are shown in Table 6. Escherichia coli was found to be sensitive to all other antibiotics used except Augmentin followed by Klebsiella species which was sensitive to four out of ten antibiotics then Serratia species and Staphylococcus species came lagging behind having been sensitive to just two antibiotics and Enterobacter taking the least position having just one ,while the highest level of resistance was exhibited by Enterobacter species having been resistant to six out of ten antibiotics assayed in this study followed by Staphylococcus aureus and serratia sp having four and three isolates resistant to antibiotics out of ten.

Table 1 Physicochemical properties of well water samples in Ago-Iwoye.

Samples	Colour	Odour	Temperature (^{0}C)	pH	Turbidity (NTU)
W1	Colourless	Odourless	26	6.8	4
W2	Colourless	Odourless	24	6.5	3
W3	Colourless	Odourless	28	6.7	5
W4	Colourless	Odourless	24	6.3	4
W5	Colourless	Odourless	21	7.0	5
W6	Colourless	Odourless	20	6.9	6
W7	Colourless	Odourless	22	6.0	3
W8	Colourless	Odourless	23	6.4	3
WHO	Colourless	Odourless	40.0	6.5-8.5	5-25
NAFDAC	Colourless	Odourless	Ambient	6.5-8.5	0-5

Keys: W-well water sample, W1-Ago- garage, W2- Chips, W3- Olopomerin, W4- Itamerin, W5- Palace, W6-Ome, W7-Onabamiro, W8-Sabo.

Table 2 Total bacteria count from well water samples in Ago-Iwoye.

Sample Codes	TVC cfu/ml	TCC MPN/100ml	TCC cfu/ml	FCC cfu/ml	MSA cfu/ml	MCA cfu/ml	SSA cfu/ml
W1	1.09×10^4	93	2.0×10^3	1.7×10^3	1.9×10^3	3.0×10^3	1.5×10^3
W2	8.3×10^3	75	1.5×10^3	1.0×10^3	1.5×10^3	2.7×10^3	1.0×10^3
W3	7.5×10^3	69	1.5×10^3	7.0×10^2	1.0×10^3	2.0×10^3	1.3×10^3
W4	7.2×10^3	44	2.1×10^3	1.0×10^3	1.5×10^3	2.5×10^3	1.8×10^3
W5	5.0×10^3	36	1.0×10^3	1.3×10^3	1.3×10^3	1.9×10^3	1.5×10^3
W6	9.5×10^3	93	2.3×10^3	1.6×10^3	2.1×10^3	2.8×10^3	2.0×10^3
W7	3.5×10^3	43	1.8×10^3	9.0×10^2	1.0×10^3	2.3×10^3	-
W8	3.0×10^3	3	1.0×10^3	5.0×10^2	-	2.1×10^3	3.0×10^2

Keys: TVC-Total viable count, TCC-Total coliform count, FCC-Fecal coliform count
MSA-Mannitol salt agar (selective *Staphylococcus aureus*), MCA-MacConkey agar, SSA-*Salmonella Shigella* agar (*Salmonella* sp.), W-well water sample, W1-Ago-garage, W2- Chips, W3-Olopomerin, W4-Itamerin, W5- Palace, W6-Ome, W7-Onabamiro, W8-Sabo.

Table 3 Morphological characteristics and biochemical tests of isolated bacteria

I/codes	Form	Margin	ELV	OPA/ SUR	Gram Rxn	CAT	OXI	CIT	IN	MOT	Probable identification
Na2	Smooth	Entire	convex	Trans lucent	-	+	-	-	+	+	*Escherichia coli*
Na4	Smooth	Entire	convex	Moist	-	+	-	+	-	+	*Enterobacter* spp
Na1	Circular	Entire	Flat	Rough	-	+	-	+	-	+	*Serratia* spp
Na5	Smooth	Entire	raised	Trans lucent	+	+	-	+	-	-	*Staphylococcus aureus*
Na7	Smooth	Entire	convex	Trans lucent	-	+	-	-	+	+	*Escherichia coli*
Em1	Smooth	Entire	convex	Trans lucent	-	+	-	-	+	+	*Escherichia coli*
Em2	Mucoid	Entire	raised	opaque	-	+	-	+	-	-	*Klebbsiella* spp
Em8	Mucoid	Entire	raised	opaque	-	+	-	+	-	-	*Klebbsiella* spp
Em4	Mucoid	Entire	raised	opaque	-	+	-	+	-	-	*Klebbsiella* spp
Em6	Mucoid	Entire	Flat	Moist	-	+	-	-	-	+	*Aerobacter aerogenes*
Em7	Circular	Entire	Flat	Rough	-	+	-	+	-	+	*Serratia* spp
Mc1	Mucoid	Entire	raised	opaque	-	+	-	+	-	-	*Klebbsiella* spp
Mc4	Smooth	Entire	convex	Moist	-	+	-	+	-	+	*Enterobacter* spp
Mc3	Mucoid	Entire	Flat	Moist	-	+	-	+	-	+	*Aerobacter aerogenes*
Mc6	Mucoid	Entire	raised	opaque	-	+	-	+	-	-	*Klebbsiella* spp
Mc8	Mucoid	Entire	raised	opaque	-	+	-	+	-	-	*Klebbsiella* spp
Ms3	Smooth	Entire	raised	Trans lucent	+	+	-	+	-	-	*Staphylococcus aureus*
Ms5	Smooth	Entire	raised	Trans lucent	+	+	-	+	-	-	*Staphylococcus aureus*
Ms7	Smooth	Entire	raised	Trans lucent	+	+	-	+	-	-	*Staphylococcus aureus*
Sa2	Circular	Entire	Flat	opaque	-	+	-	+	-	+	*Salmonella* spp
Sa6	Circular	Entire	convex	Rough	-	+	+	+	-	-	*Shigella* spp
Sa5	Circular	Entire	convex	Rough	-	+	+	+	-	-	*Shigella* spp

Keys: ELV-elevation, IN-indole, rxn-reactiion, OXI-oxidase, CIT- simmons citrate, SUR-surface, MOT-motility, OPA-opacity, Na- nutrient agar, Ms-mannitol salt agar, CAT-catalase, Em-eosin methylene blue agar, Mc-macconkey agar, I/codes-isolate codes, Sa-Salmonella-shigella agar.

Table 4 Distribution of bacterial pathogen isolated from well water samples in Ago-iwoye.

Sample codes	Bacterial isolates
W1	*Serratia* spp *Escherichia coli*, *Klebsiella* spp
W2	*Escherichia coli, Salmonell*a spp *Klebbsiella* spp
W3	*Staphylococcus aureus*, *Aerobacter aerogenes*, *Klebbsiella* spp
W4	*Enterobacter* spp *Klebbsiella* spp
W5	*Staphylococcus aureus*, *Shigella* spp
W6	*Shigella* spp *Klebbsiella* spp *Aerobacter aerogenes.*
W7	*Serratia* spp *Escherichia coli, Staphylococcus aureus.*
W8	*Klebbsiella* spp *Enterobacter* spp *Escherichia coli.*

Keys: W-well water sample, W1-Ago- garage, W2- Chips, W3- Olopomerin, W4- Itamerin, W5- Palace, W6-Ome, W7-Onabamiro, W8-Sabo.

Table 4.5 Frequency and percentage occurrence of bacteria isolated from well water samples in Ago-iwoye.

Bacterial isolates	**Number of occurrence**	**Percentage of occurrence**
Klebbsiella sp.	6	27%
Escherichia coli	4	18%
Staphylococcus aureus	3	14%
Enterobacter sp.	2	9%
Serratia sp.	2	9%
Aerobacter aerogenes	2	9%
Shigella sp.	2	9%
Salmonella sp.	1	5%
TOTAL	**22**	**100%**

Table 4.6 Antibiotics sensitivity pattern of isolated bacterial from well water in Ago-iwoye.

Antibiotics	Concentration (µg)	Measured	Zone	Of	Inhibition	(mm)	
		Escherichia Coli	*Klebbsiella* sp	*Salmonella* Sp	*Serratia* sp.	*Enterobacter* sp.	*Staphylococcus aureus*
Septrin	30	15	13	13	9	0	0
Chloramphenicol	30	16	13	14	12	0	7
Sparfloxacin	10	18	18	17	16	0	13
Ciprofloxacin	10	17	11	17	0	10	0
Amoxicillin	30	14	16	18	0	0	15
Augmentin	30	11	13	10	0	0	5
Gentamicin	10	18	10	13	13	10	0
Perfloxacin	30	15	18	14	17	18	12

Tarivids	10	19	17	17	14	13	17
Streptomycin	30	14	10	13	10	5	9

Data showed mean results of three observations, Key < 7- Resistance, 8-13-Moderate, >14- Sensitive.

Discussion

In developing countries, bacteriological contamination of drinking water has been one of the most serious problems leading to water borne infectious diseases (WHO, 2003; Haq, 2010; Acharjee *et al*., 2012; 2013). Present study was carried out to determine the microbial quality (actual microbial load), potability on the basis of the presence of indicator bacteria which indicates the chance of fecal contamination as well as the health associated risks. Moreover, next to the detection of indicator bacteria from the processed drinking water, the load of coli form and other pathogenic isolates present was determined to deliver a complete bacteriological data of well water samples used by households in Ago-iwoye for various domestic purposes; (drinking, bathing, washing and cooking). Also the antibiotic profile of pathogenic isolates was determined. The results of this study correlated with the report of Onajobi *et al.,* (2017a) who showed that the water samples from selected water sources was highly contaminated with coli form bacteria.

The high total coliform counts ($1.0x10^3$-$2.3x10^3$) observed in this present study was consistent with the result of Gambo *et al*., (2015). A study carried out in Cameroon (Bambui student residential area) as reported by Niba and Nchang, 2013 showed the microbial load of wells ranging from $0.2\text{-}7.3x10^4$ Cfu/ml exceeding the recommended limits(<500 Cfu/ml) corroborating this present study that well water are highly contaminated with values showing high presence and concentration of bacteria . This was confirmed by the characterization of the isolates from the well water samples from the locations under study which were highly contaminated with one or more bacterial pathogens namely *Escherichia coli*, *Klebbsiella* sp, *Serratia* sp, *Enterobacter* sp, *Aerobacter aerogenes*, *Shigella* sp, *Staphylococcus aureus* and *Salmonella sp*, eight genera in total. The most predominant was the enteric coli form *Klebsiella* spp (27%) followed by *Escherichia coli (*18%), *Staphylococcus aureus* (14%), *Enterobacter* spp (9%), *Serratia* spp (9%), *Aerobacter aerogenes* (9%) *Shigella* spp (9%), and *Salmonella* spp (5%).

These are pathogenic organisms of which most were mainly of fecal origin. Any water source used for drinking or cleaning purpose should not contain any organism of fecal origin (Onajobi *et al.,* 2017b). The reason for the gross contamination of well waters by pathogens as observed in this study may be due to shallowness of the wells which allows easy entrance of particles from the surroundings. It may also be due to poor sanitary condition around the areas where such wells are located or drawing water from the wells with contaminated containers, a practice that is common among the users since individuals bring along their own water containers (Onajobi *et al.,* 2017a). The high morbidity that is recorded from enteric diseases such as diarrhea, dysentery and typhoid fever in the country may be due to wide spread consumption of contaminated well water.

The high resistivity of the *Enterobacter* species to commonly used antibiotics was confirmed in this study, as reported by earlier studies carried out by Bello *et al.,* (2013c) and Onajobi *et al.,* (2017b). In this study, the findings of enteropathogens which were sensitive to commonly used antibiotics was a fortunate occurrence, but the existence of other multiple drug resistant bacteria with the susceptible ones increases the chances of transfer of antibiotic resistance to the sensitive ones. Thus, maintaining a pool of resistant bacteria with a pool of resistant genes for pathogens. High sensitivity of *Escherichia coli* to Antimicrobial agents tested was observed in this study.

Conclusion and Recommendation

This study has shown that there is a high incidence of contamination of well waters by pathogenic microorganisms, mostly well water samples from Ago-Garage (W1). This showed a high level of pathogenic bacteria contamination. This was followed by well water samples from Ome (W6). Well water samples W3, W4 and W7, used as source of drinking water was found to be non-portable. Samples W3, W4 and W7 recorded coliform count results that exceeded the standard limit set by WHO and NAFDAC to be 0/10 bacteria per 100ml. *Salmonella* / *Shigella* spp and *Staphylococcus aureus* were not detected in well water samples W7 and W8 respectively. The detection of feacal coliforms indicates feacal pollution of

the water supplies which was shown by the presence of fecal indicators such as *E. coli* and *Enterobacter.* Obtained results implicates that the majority of well water samples studied in Ago-Iwoye were of poor sanitary conditions. We strongly recommend that communities using well water as their source of water should be educated of the possible risks, when well water is used for human consumption. However, prudent use of antibacterial drugs, using the appropriate drug, at recommended dosage and duration, is one important means of reducing the selective pressure that helps resistant microorganisms to emerge.

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