Assessment of the Bacteriological Quality and Antibiotic Susceptibility Profile of Bacteria Isolated From Spring Water in South Eastern Nigeria

Okafor, C. N., Obire, O., Wemedo, S. A

Department of Microbiology, Rivers State Univesity, Nigeria

Abstract: **- The antibiotic sensitivity profile of bacteria isolated from IyiUba spring water in Udi local government area of Enugu State, Nigeria was investigated. Bacterial species were isolated on Nutrient Agar and a set of selective diagnostic media. The sensitivity of the isolates to 10 different antibiotics selected was ascertained on Muller Hinton Agar using the Kirby- Bauer disc diffusion method. The zone of inhibition of the isolates was measured in mm and the values were interpreted using the Clinical Laboratory Standard International (CLSI). Isolates were identified as *Staphylococcus* sp, *Bacillus* sp, *Pseudomonas* sp, *Escherichia coli*, *Klebsiella* sp, *Shigella* sp and *Vibrio* sp. The total heterotrophic bacterial count and total coli form count ranged from 4.45±0.07to 5.30±0.00 Log_{10}cfu/ml and 4.35±0.07 to 4.70±0.14Log_{10}cfu/ml respectively. Total Shigella count and Vibriod count ranged from 1.95±2.26[a] to 4.70±0.00Log_{10}cfu/ml and 1.95±2.76 to 4.90±0.00 Log_{10}cfu/ml respectively. The isolates were resistant to most of the antibiotics used in the study. The highest level of bacteria resistance were as follows; *Staphylococcus* sp., 100% to Cloxacillin and Cetriaxone, *Bacillus* sp., 66.67% to Cloxacillin and Erythromycin, *Pseudomonas* sp., 50% to Chloramphenicol, Cefuroxime and Streptomycin, *E. coli* 75% to Chloramphenicol, *Klebsiella* sp., 100% to Chloramphenicol and Streptomycin, *Shigella* sp., 100% to Amoxicillin, and *Vibrio* sp., 100% to Cefuroxime. The results showed that the spring water was contaminated and poses a threat to public health. The treatment of the spring water before consumption is therefore advocated. The isolates also exhibited multidrug resistance which poses a great threat to the management of public health.**

Keywords: **IyiUba spring water, bacteria, antibiotics, multidrug resistance, public health.**

I. INTRODUCTION

Water is an essential ingredient for the wellbeing and healthy life of humans and its public health significance cannot be over emphasized. Spring is a water of natural situation where water flows from an underground layer of water bearing permeable rock fractures to the earth's surface or emerges as spring. Spring water is used for variety of human needs primarily as a source of drinking water. Water is typically referred to as polluted when it is impaired by anthropogenic contaminants and no longer suitable for human use, like serving as drinking water (Ekubo and Abowei, 2011). This contamination caused by human activities adversely affects the health of people who consumes the water without treatment (Obire *et al*., 2008). For water to be considered potable for drinking, it is important to conduct thorough microbiological and physicochemical examinations. Potable water could be referred to as the water that is free from microorganisms that can cause diseases and chemical substances that are dangerous to health (Lamikanra, 1999). The contamination of spring water with fecal material, domestic and industrial waste may result in an increased risk of disease transmission to individuals who use those waters (Nguendo-Yongsi, 2011). Infectious diseases can be transmitted by water through fecal-oral route. Most important pathogenic pathogens transmitted by the water routes are *Salmonella typhi*, *E. coli*, *Camplobacter*, *Shigella*, *Cryptosporidium*, *Giardia*, and organisms causing diarrheas (Mahvi and Karyab, 2007. The World Health Organization (WHO) estimates that 3.4 million people, mostly children die every year from water-related diseases (Wilkes *et al*., 2009). There is significant increase in resistance of organisms to some specific antibiotics over a short period as a result of antibiotic abuse by humans or over use in animals (Abu and Wondikom, 2018). In Nigeria, majority of the rural areas do not have access to potable water, they depend on well, stream, and river water for domestic use. Therefore, the purpse of this study was to assess the water quality of IyiUgba spring by the use of bacterial counts, isolation and identification of pathogenic organisms and their level of resistance to antibiotics.

II. MATERIALS AND METHODS

Description of study area

IyiUgba spring is located in Awhum, Enugu State of the eastern part of Nigeria. It is located in the village and it isn't well built but drains from the rock and flows in an earthen depression where water is fetched using bucket and there is a dump site at the entrance of the spring

Collection of spring water samples

The spring water samples were collected from IyiUgba in Udi local government area of Enugu State, Nigeria. The spring water samples were collected bimonthly for a period of six months, using 1litre capacity plastic bottles. The collected samples were stored in an ice packed cooler and immediately transported to the laboratory for further analysis.

Microbiological Analysis of the Spring Water Samples

Media for Cultivation and Enumeration and Isolation of Bacteria

Nutrient Agar was used for the cultivation and enumeration of total heterotrophic bacterial count, Thiosulphate citrate bile sucrose agar (TCBS) for vibriod count, Salmonella-Shigella agar (SSA) for Salmonella-Shigella count, and Mackonkey agar (MA) for total coliform count. The media were prepared according to the manufacturers instructions

Enumeration of the various bacterial populations in all the spring water samples was done as described by Odeyemi *et al*. (2010). The samples were diluted using the serial ten-fold dilution as described by Amadi *et al.* (2014). Using a one milliliter pipette, about 1ml of the spring water was aseptically introduced in to 9 ml of sterile normal saline in a test tube to give an initial dilution of 10^{-1} dilutions. Subsequent dilutions were done until a dilution of 10^{-6} was achieved. Aliquots(0.1ml) of appropriate dilutions were inoculated in duplicate onto sterile solidified Thiosulphate citrate bile sucrose agar (TCBS), Salmonella-Shigella agar (SSA), Mackonkey agar (MA) and Nutrient agar (NA), respectively. The innocula were evenly spread out with a sterile bent glass rod. The inoculated plates were later incubated at 37^{o}C for 24 hours and observed for growth. This was done for the different points of the different spring water. Counts of colonies was carried out only for plates that have colonies between 30-300 (Amadi *et al*., 2014) and representative colonies were sub-cultured onto sterile Nutrient agar plates to obtain pure cultures. Pure cultures were properly preserved in bijou bottles containing sterilized 10% glycerol and in agar slants. These were used for further identification tests and antimicrobial susceptibility tests.

Examination of Water Samples for Coliform bacteria

The multiple tube method (MPN) was used to ascertain the presence of coliform in the spring water. The MPN technique comprises of three (3) steps: Presumptive, confirmed and the completed tests (Cheesbrough, 2005).

Characterization and Identification of Bacterial Isolates from Spring Water Sample

Identification of the bacterial isolates was done based on the method described by Cheesebrough (2005). Method of characterization employed included colonial and morphology characteristics, and biochemical tests. The colonial characterization was done by observing the colour, elevation, shape, margin, opacity, size and texture of the colonies. Microscopy was carried out using gram reaction to group the bacteria into Gram positive or negative and to their shapes and sizes. The biochemical test carried out includes citrate utilization, oxidase, catalase, indole, motility, MRVP and sugars (sucrose, glucose, mannitol and lactose).

Antibiotic Sensitivity test of Bacterial Isolates

The antibiogram of the isolates from the water samples was ascertained on Mueller- Hinton agar using the Kirby- Bauer disc diffusion method (Bauer *et al.,* 1999). A total of 10 antibiotics corresponding to drugs used in the treatment of human and animal infections caused by gram negative and positive bacteria were employed in this study. Overnight cultures of the isolates were inoculated into peptone water and incubated at 37^{o}C for 3-4 hours. The density of the bacterial culture required for the assay was adjusted to 0.5 McFarland standards. The Muller-Hinton agar plates were uniformly inoculated by spotting 0.1 ml of the broth culture of each isolate and evenly spreading over the entire agar surface using a swab stick. The plates were thereafter allowed to dry for at least 10 mins. Using a sterile forceps, the antibiotic impregnated paper discs were aseptically placed on the surface of the Mueller-Hinton agar and incubated at 37 ^{o}C for 24 hours. A clear zone of inhibition around each antibiotic impregnated disc was measured in millimeter (mm). The degree of susceptibility of the test organism to each antibiotic was determined and interpreted as either sensitive (S), intermediate susceptible (I), or resistance (R) in accordance with Clinical Laboratory Standard Institute (2012).

Statistical Analysis

Data obtained during the study were subjected to statistical analysis of variance. One-way ANOVA was used to check for significance and Tukey test was used for mean separation. This was done using a computer-based program – SPSS version 22.

III. RESULTS

The results of the total heterotrophic bacteria counts and total coliform count of the spring water are shown in Table 1. The mean value of total heterotrophic bacteria count obtained from January to June ranged from$4.45\pm0.07^{a}$to 5.30 ± 0.00^{d} Log_{10}cfu/ml. Mean value of total coliform count ranged between 4.35 ± 0.07^{a} to $4.70\pm0.14^{b}Log_{10}$cfu/ml. For total coliform count, the highest value was obtained in January, while the least was recorded in February.

Table 1: Total Heterotrophic Bacterial count and Total Coliform count from the Spring water

Months	THBC (Log10 CFU/ml)	TCC(Log10 CFU/ml)
January	4.80 ± 0.14^{abc}	4.70 ± 0.14^{b}
February	4.60 ± 0.14^{ab}	4.35 ± 0.07^{a}
March	4.45 ± 0.07^{a}	4.45 ± 0.07^{ab}
April	4.95 ± 0.07^{bcd}	4.55 ± 0.07^{ab}
May	5.10 ± 0.14^{cd}	4.65 ± 0.07^{ab}
June	5.30 ± 0.00^{d}	4.40 ± 0.00^{ab}

Means with similar alphabets across the columns indicates no significant difference at P ≥ 0.05

The fecal coliform count (MPN) obtained in the spring water ranged from 140 to 1600 MPN/100 ml. the highest was shown in January, March and June

Table 2Total Fecal Coliform Count (MPN) in the Spring Water

Month	**Total Fecal Coliform count (MPN/100ml)**
Jan	**1600**
Feb	**920**
Mar	**1600**
Apr	**140**
May	**350**
June	**1600**

The results of the total shigella counts and total vibrio count of the spring water are shown in Table 3. The mean value of total shigella count (TSC) obtained from January to June ranged from 1.95 ± 2.26^{a} to 4.70 ± 0.00^{a} Log_{10}cfu/ml. Mean value of total vibrio count ranged between $1.95\pm2.76^{a}$to $4.90\pm0.00^{a}Log_{10}$cfu/ml. The highest TSC was recorded in April, while the least was recorded in May. Therefore, the highest TVC was recorded in May while the least was recorded in January

Table 3 shows the total Shigella count and Total Vibrio count from the spring water

Months	TSC(Log10 CFU/ml)	TVC(Log10 CFU/ml)
January	2.45 ± 3.46^{a}	1.95 ± 2.76^{a}
February	4.35 ± 0.50^{a}	3.90 ± 0.14^{a}
March	4.15 ± 0.21^{a}	3.85 ± 0.07^{a}
April	4.70 ± 0.00^{a}	4.85 ± 0.07^{a}
May	1.95 ± 2.26^{a}	4.90 ± 0.00^{a}
June	4.35 ± 0.07^{a}	4.65 ± 0.07^{a}

Means with similar alphabets across the columns indicates no significant difference at P ≥ 0.0

The *Staphylococcus* species showed 100% susceptibility to Ciproflaxacin and Ofloxacin. They also showed 100% resistant to Cloxacillin and Cetriaxone while *Bacillus* species showed 100% susceptibility to Ciprofloxacin, some of the isolates were resistant to Cloxacillin and Erthromycin as shown in table 4.

Table 4 Antibiotic Susceptibility pattern of *Staphylococcus and Bacillus* species *5*

	***Staphylococcus* species**			***Bacillus* species**		
Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)	Susceptible (%)	Intermediate (%)	Resistant (%)
GN(10µg)	0	2 (50)	2(50)	3 (50)	1 (16.67)	2 (33.33)
CIP(5µg)	4(100)	0	0	6 (100)	0	0
CX(30µg)	0	1 (25)	3 (75)	1 (16.67)	2 (33.33)	3 (50)
LV(5µg)	1 (25)	0	3 (75)	5(83.33)	1 (16.67)	0
CL(10µg)	0	0	4 (100)	1(16.67)	1 (16.67)	4 (66.67)
AM(30µg)	0	1 (25)	3 (75)	0	3(50)	3 (50)
CT(30µg)	0	0	4 (100)	2(33.33)	3 (50)	1 (16.67)
E(10µg)	0	1 (25)	3 (75)	1(16.67)	1 (16.67)	4 (66.67)
CD(10µg)	1 (25)	1 (25)	2 (50)	3 (50)	2 (33.33)	1(16.67)
OF(10µg)	4 (100)	0	0	3 (50)	1 (16.67)	2(33.33)

Key: GN=Gentamycin, CIP= Ciprofloxacin, CX=Cephalexin, LV=Levofloxacin, CL=Cloxacillin, AM=Ampicillin, CT=Cetriaxone, E=Erythromycin, CD=Clindamycin, OF=Ofloxacin

Table 5 showed the antibiotic susceptibility pattern of *Pseudomonas* species*and Escherichia coli*. The isolates were 100% susceptible to Gentamycin, Ciprofloxacin, Ofloxacin, Pefloxacin and Amoxicillin. Two of the *Pseudomonas sp* isolates were resistant to Chloramphenicol, Cefuroxime and Streptomycin while four isolates of *Escherichia coli* were 100% susceptible to Ciprofloxacin and Ofloxacin. Some of the isolates were resistant to Chloramphenicol (75%), Cefuroxime (25%), Streptomycin (25%) and Nitrofurantoin (50%)

Table 5 Antibiotic sensitivity pattern of *Pseudomnas* species *and Escherichia coli*

	***Pseudomnas*species**			***Escherichia coli***		
Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)	Susceptible (%)	Intermediate (%)	Resistant (%)
GN(10µg)	4 (100)	0	0	3 (75)	1(25)	0
CIP(10µg)	4 (100)	0	0	4 (100)	0	0
C(10µg)	0	2 (50)	2 (50)	0	1 (25)	3 (75)
OF(10µg)	4(100)	0	0	4 (100)	0	0
CF(10µg)	0	2 (50)	2 (50)	2(50)	1(25)	1 (25)
PF(10µg)	4 (100)	0	0	3 (75)	1 (25)	0
CT(30µg)	1(25)	3 (75)	0	1(25)	3 (75)	0
AX(30µg)	4 (100)	0	0	1 (25)	2 (50)	1(25)
ST(30µg)	2 (50)	0	2 (50)	2 (50)	2 (50)	0
N(100µg)	0	3 (75)	1 (25)	2 (50)	0	2 (50)

Key: GN=Gentamycin, CIP= Ciprofloxacin, C=Chloramphenicol, OF=Ofloxacin, CF=Cefuroxime, PF=Pefloxacin, CT=Cetriaxone, AX=Amoxicillin, ST=Streptomycin, N=Nitrofurantoin

Antibiotic pattern of *Klebsiella and Shigella* species in table 8 showed that *Klebsiella species* were 100% susceptible to Gentamycin, Ciprofloxacin, Ofloxacin, Pefloxacin, Cetriaxone and 100% resistant to Chloramphenicol and Streptomycin while *Shigella* species showed 100% susceptibility to Ciprofloxacin, Ofloxacin, Pefloxacin and Nitrofurantoin and resistant to Chloramphenicol (50%), Cefuroxime (50%), Cetriaxone (50%), Streptomycin (50%) and Amoxicillin (100%)

Table 6 Antibiotic sensitivity pattern of *Klebsiella and Shigella* species

	***Klebsiella* species**			***Shigella* species**		
Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)	Susceptible (%)	Intermediate (%)	Resistant (%)
GN(10µg)	2 (100)	0	0	1 (50)	1 (50)	0
CIP(10µg)	2 (100)	0	0	2 (100)	0	0
C(10µg)	0	0	2 (100)	0	1 (50)	1(50)
OF(10µg)	2 (100)	0	0	2(100)	0	0
CF(10µg)	0	2(100)	0	1(50)	0	1 (50)
PF(10µg)	2 (100)	0	0	2 (100)	0	0
CT(30µg)	2(100)	0	0	0	1(50)	1 (50)
AX(30µg)	0	1 (50)	1(50)	0	0	2(`100)
ST(30µg)	0	0	2 (100)	1(50)	0	1 (50)
N(100µg)	1 (50)	1(50)	0	2 (100)	0	0

Key: GN=Gentamycin, CIP= Ciprofloxacin, C=Chloramphenicol, OF=Ofloxacin, CF=Cefuroxime, PF=Pefloxacin, CT=Cetriaxone, AX=Amoxicillin, ST=Streptomycin, N=Nitrofurantoin

Table 7 showed the antibiotic sensitivity pattern of *Vibrio* species. The isolates were 100% susceptible to Ciprofloxacin, Pefloxacin and Cetriaxone. They also showed 100% resistant to Cefuroxime, then 33.33% to Gentamycin, Chloramphenicol, Amoxicillin and Streptomycin.

Table 7 Antibiotic sensitivity pattern of *Vibrio* species

Antibiotics	Susceptible (%)	Intermediate (%)	Resistant (%)
GN(10µg)	1 (33.33)	1(33.33)	1(33.33)
CIP(10µg)	3 (100)	0	0
C(10µg)	1 (33.33)	1(33.33)	1(33.33)
OF(10µg)	3 (100)	0	0
CF(10µg)	0	0	3(100)
PF(10µg)	3 (100)	0	0
CT(30µg)	3 (100)	0	0
AX(30µg)	0	2 (66.67)	1 (33.33)
ST(30µg)	2(66.67)	0	1 (33.33)
N(100µg)	2 (66.67)	1(33.33)	0

Key: GN=Gentamycin, CIP= Ciprofloxacin, C=Chloramphenicol, OF=Ofloxacin, CF=Cefuroxime, PF=Pefloxacin, CT=Cetriaxone, AX=Amoxicillin, ST=Streptomycin, N=Nitrofurantoin

IV. DISCUSSION

Assessment of water for microbial population and identification of bacteria that constitute the drinking water ecosystem is necessary and important in public health. The total heterotrophic bacteria count from the spring water ranged from 4.40±0.14- 5.50±0.00 LOG_{10}cfu/ml. The highest total heterotrophic bacteria count was recorded in the month of June. The high population of heterotrophic bacteria count can be attributed to discharge and washing of organic matter into water bodies. The total heterotrophic bacteria counts in this study were above the permissible limit of 1.0×10^2cfu/ml which is a standard limit of heterotrophic count for drinking water (EPA, 2002). According to Bartram et al (2003), Significant changes in heterotrophic bacteria count serve as an alert for possible deterioration of water quality, triggering further investigation. The highest total coliform count was recorded in the month of January and February had the lowest record. High coliform indicates that human fecal matter had impacted the water, therefore high coliform count could be attributed to frequent human and animal contact with the springs. High heterotrophic and coliform bacteria count in spring water was also reported by Obire and Osigwe (2016), Balogun et al. (2013) with similar cause of contamination. The IyiUgba spring water did not comply with EPA standard of zero total coliform per 100 ml in drinking water (EPA, 2003). According to EPA standard, every water sample that has coliform must be analyzed for either fecal coliforms or *E. coli* to confirm contamination with human or animal waste and likely presence of pathogenic bacteria such as Gardia and Cryptosporidium (EPA, 2003). Total coliform, fecal coliform and *E. coil* are all indicators of drinking water quality (WHO, 2009). The high number of fecal coliforms recorded in the spring confirms the impact of human and animal activities going on in the spring. Another possible source of contamination could be run-off from waste dumps around the spring area. Occurrence of Shigella and Vibrio bacteriain the spring water sample indicated likely presence of pathogenic bacteria and must not be present in water, because they are of public health significance, having been associated with gastrointestinal infections such as diarrhea, dysentery and other form of infection (EPA, 2003). Other bacteria isolated in the spring water were *Staphylococcus sp*, *Bacillus sp*, *Pseudomonas sp*, *Escherichia coli* and *Klebsiella sp* and could alsopose public health significance. The antibiotic resistance levels of the isolated organisms were analyzed. This is important because the presence of antibiotic resistant bacteria increase the risk to human health. At least two of the *Staphylococcus sp* exhibited high level of resistant to all the antibiotics drug used except for Ciprofloxacin and Ofloxacin to which they were all susceptible. *Bacillus* species were susceptible to Ciprofloxacin and Levofloxacin. *Pseudomonas* species were resistant to Chloramphenicol (50%), Cefuroxime (50%), Streptomycin (50%) and Nitrofurantoin (25%). *E. coli* showed resistant to Chloramphenicol (75%), Cefuroxime (25%), Amoxicillin (25%), and Nitrofurantoin (50%). *Klebsiella sp* showed 100% resistant to Chloramphenicol and Streptomycin and 50% resistant to Amoxicillin. *Shigella sp* showed 50% resistant to Chloramphenicol, Cefuroxime, Cetriaxone, Streptomycin and 100% resistant to Amoxicillin. The *Vibrio sp* showed 100% resistant to Cefuroximeand one out of the three isolates were resistant to Gentamycin, Chloramphenicol, Amoxicillin and Streptomycin. The level of antibiotic resistance in this study was of health significance because of the danger in promoting multiple antibiotic resistant organisms. The consumption of water containing antibiotic resistant organisms from the spring may prolong the treatment of water related diseases.

V. CONCLUSION

The IyiUgba spring water is the major source of drinking water in the community. Findings from the study showed that the water quality is impacted mainly by human activities and animals. High microbial load and the presence of pathogenic organisms with their level of resistance are of serious concern. It is advisable to enlighten the community about the implication and encourage treatment of spring water before drinking. However human activities that increase the chances of contamination should be discouraged.

REFERENCES

[1]. Abu, G.O. and Wondikom, A.C. (2018). Isolation, characterization and antibiotic resistance profile studies of bacteria from an excavated pond in Port Harcourt Metropolis, Nigeria. *J. Appl. Sci. Environ. Manage*. 22(8): 1177-1184.

[2]. Amadi, E.N, Kiin-Kabari, D.B, Kpormon, L.B, Robinson, V.K.K. (2014). Microbial flora and nutritional composition of adult Palm - Wine Beetle (Rhychophorusphoenicus). International journal of Current Microbiology and Applied Science; 3(11) 189-192.

[3]. Batram, J., Cotruvo, J., Exner, M., Fricker, C. and Glasmacher, A. (2003). Heterotrophic counts and drinking-water safety: Significance of HPCs for water quality and human health. London, UK: IWA publishing. http://www.who.int/water_sanitation_health/dwg/hpc/en/index.html.

[4]. Bauer, A.W., Kirby, W.M.M., Strerris, J.C. and Turk, M. (1999). Antibiotic susceptibility testing by a standard single disk method. *American Journal of Clinical Pathology*. 45: 493-496.

[5]. Chesbrough, M. (2005). District Laboratory Practice in Tropical Countries, Part.Cambridge University Press U.K 55- 80.

[6]. Clinical and Laboratory Standards Institute. (2012). Reference method for broth dilution antifungal susceptibility testing of yeasts 4th Informational Supplement. CLSI document M27-S4, Wayne: Clinical and Laboratory Standards Institute.

[7]. Ekubo, A.T. and Abowei, J.F.N. (2011). Aspects of Aquatic Pollution in Nigeria. *Research Journal of Environmental and Earth Sciences.* 3(6): 673-693.

[8]. EPA. (2002). US Environment Protection Agency Safe Drinking water Act Amendment http://www.epa.gov/safewater/mcl.Html.

[9]. EPA. (2003). US Environment Protection Agency Safe Drinking water Act. EPA 816-F-03-016.

[10]. Lamikanra, A. (1999). Essential microbiology for students and practitioner of pharmacy, medicine and microbiology. 2nd ed. Amkra books, Lagos. P. 406.

[11]. Nguendo-Yongsi, H.B. (2011). Microbiological evaluation of drinking water in a Sub-Saharan Urban Community (Yaounde). *American Journal of Biochemistry and Molecular Biology*. 1(1): 68-81.

[12]. Mahvi, A.H. and Karyab, H. (2007). Risk assessment for microbial pollution in drinking water in small community and relation to diarrhea disease. Am. Eurasian J. Agric. Environ. Sci. 2: 404-406.

[13]. Malik, Q.A. and Khan, M.S. (2016). Effect on human health due to drinking water contaminated with heavy metals. *J. Pollut. Eff. Cont*. 5:1. Doi:10.4172/2375-4397.1000179.

[14]. Obire, O., Aguda, M and Ramesh, R.P. (2008). Impact of Human Activities on Drinking Water Quality. Journal of basic and Applied Biologigy, 2 (3&4): 52-58.

[15]. Obire, O and Osigwe, I.S. (2016). Bacterial Quality of Spring Water in Ihitte/Uboma LGA of Imo State, Nigeria. *Current Studies in Comparative Education, Science and Technology*, 3(2):149-155.

[16]. Odeyemi, A.T., Dada, A.C., Ogunbanjo, O.R. and Ojo, M.A. (2010). Bacteriologic, physicochemical and mineral studies 'on Awedele spring water and soil samples in Ado Ekiti, Nigeria. African Journal of Environmental Science and Technology 4(6), 319-327.

[17]. World Health Organization (WHO). (2009). Water safety plan manual: Step by step risk management for drinking water suppliers.

[18]. Wilkes, G., Edge, T., Gannon, V., Jokinen. C., Lyautey, E., Medeiros, D., Neumann, N., Ruecker, N., Topp, E. and Lapen, D.R. (2009). Seasonal relationships among indicator bacteria, pathogenic bacteria, Cryptosporidium oocysts, Giardia cysts, and hydrological indices for surface waters within an agricultural landscape. *Water Res*. 43:2209–2223