MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Medicine and Medical Sciences (ISSN: 2354-323X) Vol. 10(12) pp. 292-297, December, 2022
Available online http://www.meritresearchjournals.org/mms/index.htm

DOI: 10.5281/zenodo.7477790

Original Research Article

Antibiogram of Bacteria Isolated from Borehole Water in Irrua and Environs, Edo Central District, Edo State-Nigeria

[*1]Osagie R.N., [2]Osagie O.J., [3]Osagie O.P., [4]Daibo O.O., [5]Oboh J.I.

[1]Department of Medical Microbiology and Parasitology, Faculty of Basic Clinical Sciences, College of Medical Sciences, Ambrose Alli University, Ekpoma, Edo-State

[2,3] Interns, AMR consortium of researchers, AAU-Ekpoma

[4]National Primary Healthcare Development Agency, Abuja-Nigeria

[5]Comprehensive Healthcare Centers, Irrua-Edo State

***Corresponding Author's E-mail: rachael.osagie@yahoo.com**

Abstract

Microbial load of borehole water sample from Irrua and surrounding settlements was determined using standard microbiological methods. Susceptibility of the bacteria isolated to commercial antibiotics was also assessed. Results showed that total viable count ranged from 0.12 x 10^2 to 2.8x10^2cfu/ml, while the total coliform count ranged from 4 to 16 MPN/100ml. Microbial count differed significantly among the areas of study. Predominant bacterial isolate include *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa and Lactobacillus species.* The antibiogram carried out using the disc diffusion technique showed that most of the isolated bacteria were highly resistant to the tested antibiotics but were susceptible to a few antibiotics tested. The zone of inhibition of the gram positive isolates ranges from 8mm to 13mm, while the zone of inhibition of gram negative isolates ranges from 7mm to 12mm. The antibiotics with the highest susceptibility on the Gram positive isolates (*B. cereus* and *Lactobacillus* sp) were Pefloxacin, Ciprofloxacin and Septrin. Ciprofloxacin had the best activity on the two Gram negative isolates. The organisms obtained in this study are of public healthimportance, hence water samples require proper treatment before domestic use to eliminate bacterial involved in waterborne disease outbreaks.

Keywords: Antibiogram, Bacteria, Borehole water

INTRODUCTION

Water is the medium of life's processes and man's daily physiological requirement of water is about 3.6 litres per day. About 70% of the human body weight is made up of water and many of the body function depend on it. Apart from the very essential use of water for drinking, it is required for wide range activities, such as food preparation, laundering, personal and environmental hygiene.

Many communities have been known to suffer from lack of safe drinking water while many rely on rivers, wells, rain water and boreholes. The coastal areas of Nigeria particularly the Niger Delta Basin, have suffered debilitating environmental degradation and pollution from human activities, manufacturing, and municipal discharges. Urbanization and municipal activities have contributed to the amount of wastes stream generated which results of the contamination in our environment (Onojake and Frank, 2013, Onojake *et al*., 2018). The availability of water in required quantity and quality is one of the process of the development of a community or nation, water is obtained from harvesting of rain, bodies of water (rivers, ponds) or from ground water (wells, spring and boreholes). The term ground water has been use to describe the subsurface water which is beneath water table in the soil and the geological formation that are fully saturated, which can be collected in high wells, tunnels, boreholes, or drainage galleries or, which flow naturally to the earth surface (Agbede and Adegoye, 2003). It is a vital source of water supply especially in places which experience long dry season that cause stream flow to stop and pond to dry. Ground water accounts for about 95% of global fresh water supplies. It is ideally suitable for drinking, requiring little or no treatment, and is widely distributed, dependable and usually inexpensive to develop (Kehinde, 1998).

Good drinking water supply to Nigerian's teeming populace is a perennial problem that has defied solution.As such, it has often attracted rhetorical commentaries with little or no practical solutions. Therefore, great concern must be given to the quality of drinking water as it is very critical for the overall socio-economic development of any society and, should engage the attention of individuals, groups, government and non-governmental organizations (Adetunde and Glover, 2010). The increasing pollution of surface water and industrial wastes coupled with the alarming cost of construction of water treatment plants and distribution network for human use has made ground water an attractive and important option in the social and economic development of many communities. Water obtained from borehole usually does not require elaborate treatment. Usually, the removal of hardness and chlorination are the common treatment required non-chlorinated borehole water when contaminated with microorganisms cause a number of infection of intestinal tract such as paratyphoid fever, cholera, dysentery, shigellosis and amoebiasis (Itah *et al.,* 1999). In safeguarding public water supplies, public health authorities and engineers rely on information obtained from the results of frequent bacteriological tests.

In 1987, the directorate of food, roads and rural infrastructure (DFRRI) embarked on a nationwide rural water and sanitation project for the provision of 250 boreholes in each state of the federation. Also, the United Nations International Children Emergency Fund (UNICEF) assisted water sanitation project engaged in provision of potable water in rural communities through borehole projects in some selected states in Nigeria (Agbede and Adegoye, 2003). Although water can contain unwanted chemicals (from natural sources and agricultural activities), the greatest risk to human health is from faecal contamination of water supplies causing water - borne diseases. The consequent illnesses are mostly treated with antibiotics; unfortunately, there has been development of antimicrobial resistance by many strains of microorganisms which is now making it difficult to treat some infectious diseases. The wide consumption of antibiotics in humans and animals has favored the development of antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs), which are subsequently released through wastewater into the environment, including drinking-water sources (Sanganyado and Gwenzi, 2019; Banu *et al*., 2021). Additionally, antimicrobial resistance (AMR) may develop de novo in bacteria under the selective pressure of antimicrobial residues and other chemicals released into water bodies (Sakkas *et al*., 2019). Ingestion of these resistant bacteria in contaminated drinking water may not only result in infections that have a high risk of treatment failure, but also the possibility of horizontal gene transfer from environmental bacteria to human pathogens (Yuan *et al.,* 2020). Thus, water may be an important source for the spread of antimicrobial-resistant organisms and genes among humans and animals. Drug resistant strain have been reported among *Staphylococci spp, Gonococci spp, Pneumococci spp, Enterococci spp* (Riboldi *et al.,* 2009), and Gram negative bacteria including *Salmonella, Shigella, Klebsiella, Escherichia coli, Pseudomonas* as well as among *Mycobacterium tuberculosis* (Cheesbrough, 2004).

Aim

This research was conducted to ascertain the anti-microbial susceptibility pattern of organisms contaminating borehole water in Irrua and environs.

MATERIALS AND METHODS

Sample Location

Irrua is the Head Quarter of Esan Central Local Government situated in Edo Central senatorial district. The government has a total population of 137, 900 people (National population Commission of Nigeria web 2016) with ten political wards. Geographically, it is bounded in the East, by Esan North East Local Government, South by Igueben Local Government, North by Etsako West Local Government, and West by Esan West Local Government. It occupies a land mass of $253km^2$ and the Density of $545.1/km^2$ and the Geo Coordinate 6.44'20"N6.13086 E/6.219E/6.739:6.219 (NPC 2016). It's a rural Community with few civil servants, the occupation of the people are Farming, Trading, artisan and transportation (commercial taxi drivers and motorcycle riders).

Collection of samples

Three borehole water samples were collected from different sites at Irrua, Akho and Usugbenu (borehole water) in a sterile bottle. The bottles were immediately covered with the caps and labeled with the code number and taken to the laboratory for bacteriological analysis.

Microbiological Analysis

The test was conducted in the laboratory for the presence of heterotrophic bacteria and total coliform count. The coliform count of the water samples was determined using sterile MacConkey broth followed by incubation at 37°C for 24 hours. After incubation, the presence of coliform bacteria in the sample was detected by acid and gas production, which were revealed by the presence of air bubbles at the tip of the inverted Durham tube and by change of colour in the medium from purple and yellow. For the total heterotrophic count, 1ml of sample was inoculated to nutrient agar plate using pour plate method. These were incubated at 37°C for 24 hours and emerging colonies counted. The biochemical identification of the colonies was achieved with the aid of API 20E strips which were thereafter Gram stained and viewed under the microscope with the aid of the Bioevopeak binocular microscope (MSC-B102) at X40 and X100 magnification.

Cultural Characteristics

Characteristics growth of the bacteria on Nutrient, MacConkey, Eosin Methylene Blue Agar was observed and noted.

Motility Test

This was carried out on the semi - solid agar medium or nutrient gelatin contained in test tubes. The tubes were heat - sterilized and allowed to gel. Using flamed straight needle, each tube was stab inoculated to two third (2/3) of its depth and incubated at 37°c for 24 hours. Motility was positive when cloudiness existed around the stab (or point of inoculation) due to the migration of motile bacteria from the point of inoculation.

Antibiotic Susceptibility of Bacteria Isolates

The Modified Kirby-Bauer Susceptibility testing technique as described by Cheesebrough was used for the sensitivity test. Actively growing culture of the bacteria was streaked on nutrient agar and allowed to dry for 5 minutes before placing the multi-disc antibiotics on the cultured plates. Contact between the antibiotics discs and the culture was ensured by gently pressing the disc with sterile forceps. Within 30 minutes of applying the disc, the plates were incubated at 37°C for 24 hours. Zones of incubation were determined as mm diameter. The antibiotics discs used were ciprofloxacin (10µg), streptomycin (30µg); Ampliclox (30µg), Pefloxacin (10µg), Septrin (30µg), Tarivid (10µg), Gentamycin (10µg), Argumentin (30µg), Chloraphenicol (30µg), Sparfloxacinn (10µg) and Zinnacef (20µg).

Data Analysis

Data for microbial contaminants in borehole water samples were recorded and analysed for total coliforms, *E. coli*, pH, turbidity, susceptibility profile, mean and standard deviations were calculated from the results of the analysis of the three samples per sampling point. Water quality results were compared with the World Health Organization drinking water standards.

RESULTS

Results of the bacteriological quality of borehole water collected from Irrua, Akho and Usugbenu communities revealed a mean total viable bacterial count range of 0.12 x 10^2 to 2.80 x 10^2. The total viable count of water samples was higher in Irrua and the lowest was in Uugbenu. This is shown in Table 1.

Table 2 showed the total coliform count (MPN/100ml) of the borehole water sample and this ranged from 4-16/100ml of sample. The bacteria isolated from the borehole water sample include *Bacillus cereus, Escherichia coli, Lactobacilus species and Pseudomonas aeruginosa,* as shown in Table 3.

Table 4 shows the cultural, morphological and biochemical characteristics of the isolated bacteria.
Table 5 and 6 showed the antibiotics sensitivity pattern of the Gram positive and Gram negative bacteria isolates.

DISCUSSION

The range of the total viable count (0.12 x 10^2 to 2.80 x 10^2 Cfu/ml) and total coliform count (4 - 16 MPM/ 100ml) of borehole water samples studies as shown in table 1 and 2 respectively revealed a value higher than the recommended WHO standard for such water (WHO, 1997). However, similar high loads of microorganisms have been reported in various boreholes and well water used in other parts of Nigeriaas shown by the findings of Lobina and Mercy (2015) who reported atotal bacterial count in the borehole water sampled ranged from <1 to 44.1 cfu/100ml. Agbu *et al*. (1988), Itah *et al*. (1996) as well as Nnenna (2014) all reported similar coliform counts. The microbial loads also differ significantly among locations. This was probably due to the non-standardization of construction of borehole inIrrua town and neighboring communities; neither were there any standardized treatment in line with WHO and UNICEF (2000) Global Water Supply and Sanitation Assessment reportwhich stated that the formation of preferential flow from strong hydraulic gradients result from abstraction of groundwater which causes reduction in attenuation process but increases groundwater contaminants concentration. Also, UNICEF (1998) appraisal of borehole water samples from Owerri showed that for over

Table 1. Mean total viable count (cfu/ml) of bacteria in water examples

Sample area	Count cfu/ml
Irrua	2.80×10^2
Akho	2.20×10^2
Usugbenu	0.12×10^2

Table 2. Total coliform count (cell/100ml) of borehole water

Sample Area	Volume of sample in each bottle 50 ml		10ml		MPN of coliform/100ml
	No of Bottle	No of Positive	No of Bottle	No of Positive	
Irrua	1	0	5	3	4
Akho	1	1	5	4	16
Usugbenu	1	1	5	4	16

Table 3. Bacteria isolated from borehole water

Bacteria	Irrua	Akho	Usugbenu
Bacillus cereus	+	+	-
Escherichia coli	-	+	-
Lactobacillus sp	+	-	+
Pseudomonas aeruginosa	-	+	+

Table 4. Morphological/Cultural Characteristics of Isolates

Cultural Characteristics	Gram Reactions	Biochemical Reactions	Sugar Fermentation	Identified Bacteria
Colonial appearance		MOT OXI IND CIT CAT MET URE COA	GLU. MAL, SUC. LAC. MAN	
Round pinkish colonies	Gram-ve rod	+ - + - - + - -	A/G A/G A A/G A/G	*Escherichia coli*
Round rapidly spreading creamy colonies	Gram +ve flat rod	+ - - - + + - -	A AAAA	*Bacillus sp*
Large creamy rod colonies producing diffusible pyocin pigment B- heamolytic	Gram –ve	+ + - + + - - -	A - - -	*Pseudomonas aeruginosa*
Usually long regular rods, non sporing, rarely motile	Grame +ve	+ - - - - - - -	- - - A -	*Lactobacillus sp.*

KEYS : MOT. = Motility OXL = Oxidase IND = Indole CIT. = Citirate CAT. = Catalase MET. = Methylred URE. = Urease COA. = Coagulase GLU. = Glucose MAL. = Maltose SUC. = Sucrose LAC. = Lactose MAN. = Mannitol Acid and Gas production A = Acid production + = positive - = negative

Table 5. Antibiotics sensitivity of Gram positive isolate (positive disc)

Antibiotics	Concentration	Zone of inhibition *Bacillus cereus*	(mm diameter) *Lactobacillus sp*
Pefloxacin	10µg	9	11
Gentamycin	10 µg	-	17
Ampiclox	30 µg	-	-
Zinnacef	20 µg	-	9
Amoxicillin	30 µg	-	11
Roceplin	25 µg	11	-
Ciprofloxacin	10 µg	12	10
Streptomycin	30 µg	-	-
Septrin	30 µg	11	13
Erythromycin	10 µg	8	-

Table 6. Antibiotics sensitivity of Gram negative isolate (Negative disc)

	Concentration	Zone of inhibition *Escherichia coli*	(mm diameter) *P.aeruginosa*
Septrin	30µg	-	7
Chloramphenicol	30µg	-	8
Ofloxacin	10µg	12	-
Ciprofloxacin	10µg	12	8
Amoxicillin	30µg	-	10
Augumentin	30µg	-	9
Gentamycin	10µg	6	11
Perfloxacin	30µg	9	-
Tarvid	10µg	-	-
Streptomycin	30µg	-	11

sixty water samples from boreholes within Owerri Zone, Imo State, Southern-east Nigeria shows that the PH of water samples is 6.0 to 6.5 on the average. Some of these boreholes were dug in close proximity to septic tank absorption fields which was also reported by Okhuebor and Izevbhuwa, (2020) from their findings on “Quality and Effect of Borehole Water Proliferation in Benin City, Nigeria and its Public Health Significance”. There is usually a high incidence of seepage of polluted water from the specific tank, resulting in contamination of the borehole water. On the other hand, tanks and reservoirs where the borehole water is stored prior to use could also be possible sources of contamination.

Although a number of the samples were chlorinated, some still harbored coliforms which may be attributed to a number of factors. It has been reported that coliforms can be found both in chlorinated and non-chlorinated water and that their total elimination from water would require knowledge of their population on such water determining the quantity of chlorine needed for their complete destruction on addition forming functional chlorinator (Cark *et al,*1992)

The result of the bacteria isolated from borehole is shown in table 3. The organisms isolated were bacillus cereus *Escherichia coli, Lactobabacillus species* and *Pseudomonas aeruginosa.* Table 4 showed the cultural, morphological and biochemical characteristics of isolated bacteria

The role of antibiotics in decreasing diseases, illness and/or death associated with bacterial infections in humans and animals cannot be overemphasized (Sawyer and McCarty1967). Theresults of antibiotic study revealed marked differences among bacterial isolates in their susceptibility and resistance patterns to antibiotics as observed in tables 5 and 6.

The results of the antibiotics sensitivity pattern of *Escherichia coli, Bacillus cereus* and *Lactobacillus species*showed high susceptibility to ciprofloxacin, ofloxacin and Pefloxacin. This can be attributed to the reduced or minimal use of these antibiotics in clinical practice and/or veterinary medicine within the sample location. This data is also in support with the findings of Talukdar *et al*. (2013) who reported a 91.75 % susceptibility profile of *E. coli* isolates from the various water sources to some prescribed antibiotics.

However, higher levels of resistance to Septrin, Chloramphenicol, Amoxicillin, Augumentin and Tarivid were observed in *P.aeruginosa* and *E. coli* isolates. This is in conformity with results to antibiotics resistance of *P.aeruginosa* isolated from water to vancomycin, ampicillin and gentamycin and as documented byRiboldi *et al*. (2009) and Onwa *et al*., (2019)who reported that bacteria pathogens isolated from underground water in Abakaliki were highly resistant to most of the antibiotic tested but were susceptible to Amikacin, Ofloxacin and Gentamycin while Osundiya *et al*., (2013) reported a 100% resistanceto ceftriaxone

A marked resistant pattern was also observed in*Bacillus cereus*isolated from all three sample locations to some of the tested antibiotics. This is at variance with susceptibility report for *Bacillus species* to erythromycin and septrin as reported by Umar *et al*, (2006). The variation in susceptibility and resistance of the isolates to the different antibiotics could be attributed to the difference in the concentration of antibiotics (Table 5 and 6), source of isolates and drug resistance transfer (Shewmake and Dillon, 1998). Emergence of multiple resistances to antibiotics by organisms has also been documented by some researchers (Cheesbrough, 2004).

CONCLUSION AND RECOMMENDATION

The high count of total coliform per 100 ml of water sample analyzed indicated the poor quality of borehole water within the sample communities. Rural dwellers are more than not left to take care of their water and other social needs which accounts for anon-conformity to WHO/UNICEF recommended standard. Therefore, proper treatment before domestic use to eliminate bacterial involved in waterborne disease outbreaks is essential.

The varying resistances to antibiotics by the isolates is of public health importance and attention should be focused on antibiotic agents which showed promising susceptibility on the action of most of the bacteria isolates as drugs of choice in the treatment of water borne infections.

Conflict of interest: None

Funding source: None (self- funded research)

Acknowledgement: Esan central local government area social mobilization committee

Weakness: Inability to access hard to reach community borehole in "last mile settlements"

Strength: Community support /approval of research by private borehole operators

Informed Consent Statement: Not applicable

REFERENCES

Adetunde LA, Glover RLK (2010). Bacteriological Quality of Borehole Water Used by Students' of University for Development Studies, Navrongo Campus in Upper-East Region of Ghana. *Current ResearchJournal of Biological Sciences 2*(6): 361-364.

Agbede, I.O., Adegoye, M.S. (2003): Assessment of the quality of borehole water in Benue State, Nigeria. *J. Agric. Sci. Technol. 13*: 38-52

Agbu AA, Alaribe HC, Singh, Adesiyun AA (1988) Bacteriological studies and chemical analysis of public Well water in Samaru and Zaria City in Northern Nigeria. *Nig. J. Microbiol. 8*: 88-98

Banu RA, Alvarez JM, Reid AJ, Enbiale W, Labi AK, Ansa EDO, Annan EA, Akrong MO, Borbor S, Adomako LAB (2021) Extended Spectrum Beta-Lactamase *Escherichia Coli* in River Waters Collected from Two Cities in Ghana. *Tropical Medicine. Infectious Diseases 6* : 2018–2020.

Bauer AW, Kirkby WMM, Sheris JC, Ture KM (1996) Antibiotics susceptibility testing by standardized single disc method. *Ame. J. Clin. Pathol. 45*: 493-496

Cheesbrough M (2004). Water related diseases testing of water supplies in: District Laboratory in tropical countries Cambridge University Press. Cambridge UK. Pp. 143-156.

Clark JA, Burger GA, Satationes LE (1982): Characterization of indicator bacteria in municipal raw water, drinking water and new main water supplies. *J. General Microbiol. 55*:1002 – 1013

He Y, Yuan Q, Mathieu J, Stadler L, Senehi N, Sun R, Alvarez PJJ (2020). Antibiotic Resistance Genes from Livestock Waste: Occurrence, Dissemination, and Treatment. *Clean Water 3*: 1–11.

Itah AT, Etukudo SM, Akpan EJ (1996). Bacteriological and chemical analysis of some rural water supplies in Calabar, Nigeria, *West Afr. J. Biol. Appl. Chem. 41*: 1-10.

Kehinde OM (1998). The impact of industrial growth on ground water quality and availability: case study of Ikeja industrial area In: *Communiqué and Proceedings of Workshop jointly organized by the Federal Government Protection Agency and the Federal Ministry of Budget and Planning,* Abuja, FEPA, Lagos Pp. 25-35.

Lobina Palamuleni and Mercy Akoth (2015). Physico-Chemical and Microbial Analysis of Selected Borehole Water in Mahikeng, South Africa. *Int. J. Environ. Res. Public Health12*(8): 8619-8630.

Nnenna DP (2014). Implications of Borehole Water as a Substitute for Urban Water Supply: The Case of Egbeada Federal Housing Estate Owerri, Imo State. *Int. J. Innov. Edu. Res. 2*: 10-14.

Okhuebor SO, Izevbuwa OE (2020). The Quality and Effect of Borehole Water Proliferation in Benin City, Nigeria and its Public Health Significance. *Advanced Microbiology Research 4*: 13-21

Onojake MC, Frank O (2013). "Assessment of heavy metals in a soil contaminated by oil spill: a case study in Nigeria". *Chemical Ecology.,29*(3): 246-254.

Onojake MC, Sikoki FD, Omokheyeke O, Akpiri RU (2018). "Surface water characteristics and trace metals level of the Bonny/New Calabar River Estuary, Niger Delta, Nigeria".*Applied Water Science*, DOI 10.1007/s/3201015-0306-y.

Onwa NC, Uzomaka IC, Maduako AL, Elom EE, Ikeanumba MO, Nwode VF (2019). Antibiotic Susceptibility of Bacterial Species Isolated From Underground Waters in Abakaliki Metropolis of Ebonyi State, Nig. *Int. J. Pharma. Sci. Invention 8*(2): 55-65

Osundiya OO, Oladele RO, Oduyebo OO (2013). Multiple antibiotic resistance (MAR) indices of *Pseudomonas* and *Klebsiella* species isolates in Lagos University Teaching Hospital *Afr. J. Clin. Experimental Microbiol.14*(3): 164-168.

Riboldi GP, Frazzon J, D'azevedo PA, Feazzon APG (2005). Antimicrobial resistance profile of *Enterococus* specie isolated from food in Southern Brazil. *Brazilian J. Microbiol. 40*: 125-128

Sakkas H, Bozidis P, Ilia A, Mpekoulis G, Papadopoulou C (2019). Antimicrobial Resistance in Bacterial Pathogens and Detection of Carbapenemases in *Klebsiella Pneumoniae* Isolates from Hospital Wastewater. *Antibiotics, 8*: 85-89

Sanganyado E, Gwenzi W (2019). Antibiotic Resistance in Drinking Water Systems: Occurrence, Removal, and Human Health Risks. *Sci. Total Environ*. *669*: 785–797. [CrossRef] [PubMed]

Sawyer CN, McCarty PL (1967). Chemistry of sanitary engineers, 2nd edition. McGraw Hill, New York. P. 57

Shewmake, R.A., Dillon, B. (1998). Food Poisoning: Causes, Remedies and prevention. The Practical Peer - Review *J. Prim. Health Care Physician103* (6) 10-14

Talukdar PK, Rahman M, Rahman M, Nabi A, Islam Z (2013). Antimicrobial resistance, virulence factors and genetic diversity of Escherichia coli isolates from household water supply in Dhaka, Bangladesh. PLoS 1(8): 10-15

Umar AF, Tahir F, Yerima MB (2006) Antimicrobial sensitivities of bacillus cereus isolates in food solid in Bauchi metropolis to some selected antibiotics. *Nig. J. Microbiol. 20* (3): 1460 -1464.

UNICEF (1998) Appraisal of Borehole Water Samples from Owerri. Unpublished UNICEF document

W.H.O (1997) Guidelines for drinking water quality, Drinking water quality control in small communities supplies WHO General, Switzerland *3*: 121- 130

WHO & UNICEF (2000): Global Water Supply and Sanitation Assessment 2000 Report.WHO, Geneva, Switzerland.