Original Research Article

MULTI-DRUG RESISTANT BACTERIAL STATUS OF BOREHOLE WATER IN KOGI STATE UNIVERSITY, ANYIGBA

Abstract

Transmission of pathogens through water is of grave public health concern. Bacteria are of major concern because of the pathogenecity and etiologic agents of life threatening infections.

The multi-drug resistant (MDR) bacterial status of the borehole water samples from Kogi State University, Anyigba was studied. The samples were collected from various borehole water sources and analyzed for MDR bacteria. A total of seven isolates (*Echerichia coli*, *Bacillus* sp, *Klebsiella* sp, *Pseudomonas* sp, *Staphylococcus* sp, *Streptococcus* sp and *Salmonella* sp) were identified by standard microbiological methods. Phenotypic identification of antibiotic resistance profile using the disk diffusion method was carried out. *Pseudomonas* sp, *Streptococcus* sp, *Staphylococcus* sp, *Escherichia coli* and *Klebsiella* sp. were found to be 100% resistant to all the imported antibiotics while 55.6% and 66.7% resistance were recorded for *Salmonella* sp. and *Bacillus* sp respectively. Percentage resistance to all indigenous antibiotics recorded were 40% for *Pseudomonas* sp., 30% for *Salmonella* sp, 40% for *Escherichia coli*, 30% for *Klebsiella* sp, 20% for *Staphylococcus* sp, 20% for *Streptococcus* sp, and 10% for *Bacillus* sp. The results showed that all the isolates were multi drug resistant (MDR) and the presence of these organisms poses great risk to the university community as well as individuals that consume the water.

Key words: , antibiotics, bacteria, borehole, multi-drug resistant.

Introduction

Potable water is an essential ingredient for good health and the socio-economic development of man (Udom*et al*., 2002) but it's lacking in many societies. Clean water is priceless and a limited resource that man has begun to treasure only recently after decades of pollution and waste (Sinderberg, 2003). World population cannot be sustained without access to safe water (Brainstein, 2007). It is therefore important to conjunctly consider both water quality and quantity in water resources management (Xinghui*et al*., 2009). Borehole water become unsuitable for domestic use as a resource due to contamination that makes it unfit for many purposes (Agbaire andOyibi, 2009). Standards and guidelines in water quality stem from the need to protect human health. Borehole water serves as the major source of drinking water in the local population of Nigeria. Since only few can afford and rely on purified and treated bottled water for consumption. Chowdhury (2006), stressed the importance of groundwater as a source of potable water in Africa and constitutes about two thirds of the freshwater resources of the world. Ground water provides a reasonably constant supply for domestic use, livestock and irrigation. (Carlow *et al.*, 2011) stated this source can buffer the effects of rainfall variability across seasons. In many arid and semi-arid areas of Africa boreholes water is a means of coping with water deficiencies in areas where rainfall is scarce or highly seasonal and surface water is extremely limited (Agbaire andOyibi, 2009).

Underground water naturally is water from the atmosphere and earth surface that has percolated through the coarse particulate network of the soil, which serves as natural filter against microbial contaminants, into the water table and thus is expected to be of better quality than the surface water. Underground water is gradually becoming over exploited in Nigeria due to the inability of the government to provide adequate potable water supply to all communities in the country (Ayantobo *et al.*,2013). There are several rules guiding the digging of wells, but because of poverty some cannot afford the sinking of deeper wells called boreholes and thus employ the services of local well diggers who most times hand-dig wells irrationally and even sometimes closer to locations like soak away pit, latrine, and sewage septic tanks that usually encourages microbial contamination of such wells (Ayantobo *et al.*, 2013). Some of these hand-dug wells are not hygienically protected and are never treated from time to time (Ayantobo *et al.*, 2013). Most are made in a way that provides easy access to reptiles and insects that defecate or sometimes get drowned in the wells. The manner in which some even draw water from the wells encourages contaminations through the rope and containers being used. The world health organization (WHO) has defined portable water as water in which the physical, chemical and microbiological quality is within acceptable limit (WHO, 2004). However, the truth is, over one billion people worldwide have no access to portable water (WHO, 2000). This has resulted to increased cases and spread of waterborne diseases throughout the world (Obeta, 2013). WHO reported an estimation of over two million deaths as a result of waterborne diseases and over four billion diarrhea cases worldwide annually. In Africa, the WHO has estimated that a child has five episodes of diarrhea in a year with about 800, 000 deaths of children per year from diarrhea and dehydration (WHO, 2000). These have been attributed to the presence of bacteria pathogens in the drinking water which resulted into various waterborne diseases such as cholera, typhoid fever, bacillary dysenteries and many gastrointestinal diseases (Adeyinka,2014). Drinking of water contaminated with human and animal faeces exposes individuals to high risk of microbial infections, especially faeces from infected or carriers of waterborne disease-causing agents.

Contamination of water bodies has increasingly become an issue of serious environmental concern. In the case of underground waters like bore holes, this may arise from construction process of a borehole, drilling fluids, chemical casings and other materials which may find their way into the well thereby polluting the water (Angulo*etal*., 1997). An open well during the construction stage can also be a direct route for contaminants from the surface to the aquifer thereby providing an ideal opportunity for chemical casing and bacteriological pollution to occur (Brainstein, 2007). Even if no source of anthropogenic contamination may exist, there is potential for natural levels of metals and other chemicals to be harmful to human health.

The occurrence and spread of antibiotic-resistant bacteria (ARB) are pressing public health challenges worldwide, and aquatic ecosystems are recognized reservoir for ARB as well as antibiotic resistance genes (ARGs). The emergence of antibiotic-resistant bacteria limits the clinical use of antibiotics. So, there is increasing concern that existing antibiotics would not be potent any longer against these pathogens. It is in view of this that the borehole water sources from Kogi State University Ayingba were assessed to ascertain the multi-drug resistant bacteria status of the water.

MATERIALS AND METHODS

Sample collection: The borehole water samples were collected randomly from the university main borehole water sources in sterile containers and held in iceboxes until delivery to the laboratory.

Isolation and enumeration: Serial dilution was carried out for each of the samples before inoculation on Nutrient agar and MacConkey agar. One ml of the borehole water was diluted in 9ml to give tenfold serial dilution. With the aid of a sterile syringe, 1ml of the sample was aseptically transferred from the various dilution factors into the sterile nutrient agar petri dishes. Enumeration was done using the pour plate method.

Characterization and identification of bacteria: Identification and characterization of bacteria was done by isolation of bacteria in a pure form by streaking on nutrient agar to obtain a pure isolate and Gram staining was carried out as well as other biochemical tests contained in the standard diagnostic protocol (ISO 6885-1; 1999). The biochemical tests that were carried out include;

Catalase test: This test demonstrates the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide. The enzyme catalase mediates the breakdown of hydrogen peroxide into water and oxygen. The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide, and the rapid elaboration of oxygen bubbles. The lack of catalase is evident by lack of or weak bubble production.

The test was carried out by transferring a small amount of colony growth into a slide; a drop of 3% hydrogen peroxide was placed on the organism and observed for immediate bubbling.

Citrase test: This test detects the ability of an organism to use citrate as the sole source of carbon and energy. Use of citrate involves the enzyme citrase, which breaks down citrate to oxaloacetate and acetate. Oxaloacetate is further broken down to pyruvate and carbondioxide. Production of sodium bicarbonate as well as ammonia from the use of sodium citrate and ammonium salts results in alkaline pH. This results in a colour change. Bacterial colonies were picked and inoculated into a slope of Simmons citrate agar and incubated overnight at 37 degree Celsius. The organism that has the ability to use citrate, changes the color of the medium from green to blue.

Urease test: Urea is a di-amide of carbonic acid. It is hydrolyzed with the release of ammonia and carbon dioxide. Many organisms especially those that infect the urinary tract, have a urease enzyme which is able to split urea in the presence of water to release ammonia and carbon dioxide. The ammonia combines with carbon dioxide and water to form ammonium carbonate which turns the medium alkaline, turning the indicator phenol red from its original yellow color to bright pink.

Indole test: Indole is performed on bacteria species to determine the ability of the organism to convert tryptophan into indole. The pure bacteria culture was grown in peptone broth for 24 hours. After incubation, 5 drops of kovac's reagent was added to the culture broth. Positive result shows the presence of a red or red violet color in the surface alcohol layer of the broth, negative result appear yellow.

Methyl Red: It is used to identify and characterize enteric bacteria based on their pattern of glucose metabolism. An isolate was inoculated into a tube with a sterile transfer loop; the tube was incubated at 35 degrees Celsius for 2 days. After incubation, 2.5ml of the medium is transferred to another tube. Five drops of pH indicator methyl red is added to this tube, the tube is gently rolled between the palms of the hand to disperse the methyl red. A red color representsa positive test that is, they subsequently metabolize pyruvic acid to other acids. A yellow color represents a negative result.

Voges-Proskauer test: Alpha-nephthol and potassium hydroxide is added to the incubated bacteria (VP broth). A cherry red color indicates a positive result while a yellow-brown color indicates a negative result.

Multiple tube fermentation technique: Coliforms are detected in 3 stages-presumptive, confirmed and completed. In the presumptive test, dilutions from the water sample are added to tubes of lactose broth medium with a pH indicator and incubated for 24 to 48 hours. After the period of time, the development of gas and how much produced was examined. In the confirmed test, samples are streaked from the positive presumptive tubes at the highest dilutions onto plates of differential media, eosin methylene blue (EMB) agar that contains lactose. Since coliforms produce acid from lactose, and the eosin methylene blue dyes are absorbed under acid conditions, the coliforms form dark-centered colonies with or without metallic sheen; these colonies indicate a positive confirmed test. In the completed test, separate colonies are selected from the confirmed test and inoculated in lactose broth and on a nutrient agar slant for 24 hours at 35^{o}C. If gas and acid are produced in lactose broth, and the isolated microorganism is a gram negative non-endospore-forming rod, it indicates a positive completed test.

Anti bacteria susceptibility testing: The Kirby-Bauer method was adopted for the antibacterial susceptibility If the bacteria are using the Mueller-Hinton agar. The isolates in broth form were poured on the molten agar and allowed to solidify before application of the antibiotics on the plates. Sensitivity to the antibiotic by the bacteria was indicated with a clear ring or a zone of inhibition seen around the disc. A ruler was used to measure the diameter of the disk plus the surrounding clear area in millimeters (mm).

Results

TABLE 1: COLONIAL MORPHOLOGY OF ISOLATES

Samples		Shape	Colour	Elevation	Edges	Consistency	Probable organism
A	1	Round	Blue green	Convex	Entire	Dry	*Pseudomonas* sp
	2	Round	Milky	Flat	Entire	Moist	*Streptococcus* sp
	3	Round	Yellow	Raised	Entire	Moist	*Salmonella* sp
	4	Round	Milky	Convex	Entire	Moist	*Staphylococcus* sp
B	1	Circular	Yellow	Convex	Entire	Moist	*Escherichia coli*
	2	Round	Pink	Flat	Entire	Mucoid	*Klebsiella* sp
	3	Round	Yellow	Raised	Undulate	Mucoid	*Bacillus* sp

TABLE 2: GRAM REACTION AND BIOCHEMICAL TESTS OF ISOLATES

SAMPLE	GRAM REACTION	SHAPE	BIOCHEMICAL TESTS						PROBABLE MICROORGANISMS
			IND	MR	VP	CAT	CIT	URE	
A1	-	ROD	+ve	-ve	+ve	+ve	+ve	+ve	*Pseudomonas sp*
A2	+	COCCI	-ve	+ve	-ve	-ve	+ve	-ve	*Streptococcus sp*
A3	-	ROD	-ve	-ve	-ve	+ve	-ve	-ve	*Salmonella sp*
A4	+	COCCI	-ve	+ve	-ve	+ve	+ve	-ve	*Staphylococcus sp*
B1	-	ROD	+ve	+ve	-ve	+ve	-ve	+ve	*Escherichia coli*
B2	-	ROD	-ve	-ve	+ve	+ve	+ve	+ve	*Klebsiellasp*
B3	+	ROD	-ve	+ve	-ve	-ve	+ve	-ve	*Bacillus sp*

Key: IND= indole MR= Methyl red VP- Vogesproskaner, CAT- catalase, CIT= citrate and URE= ureas

TABLE 3: ANTIBIOTIC SUSCEPTIBILITY TEST ON SET 1(GRAM NEGATIVE)

Chemical class of antibiotics	Antibiotics	*Pseudomonas*	*Salmonella*	*E.coli*	*klebsiella*
Beta Lactam	PEF (10µg)	S	S	S	S
	AU(20µg)	R	R	I	I
	PN(20µg)	I	I	S	R
Aminoglycosides	CN(10µg)	R	I	R	R
	S(30µg)	R	I	R	S
fluoroquinolones	OFX(10µg)	S	S	S	S
	CPX(10µg)	S	S	S	S
	NA(30µg)	R	R	R	R
Cephalosporin	CEP(10µg)	S	R	R	I
Sulfonamides	SXT(30µg)	S	S	S	I
	Susceptible	50	40	50	40

Percentage (%)	Intermediate	10	30	10	30
	Resistant	40	30	40	30

Key: R=resistance, I= intermediate, S= susceptible, OFX= tarivid, CPX = ciproflox, AU= augmentin, CN=gentamycin,S = streptomycin, CEP= Ceporex , NA= Nalidixic acid, SXT= septrin, PN, ampicillin

TABLE 4: ANTIBIOTIC SUSCEPTIBILITY TEST ON SET 2(GRAM POSITIVE)

Chemical class of antibiotics	Antibiotics	*Staphylococcus*sp	*Streptococcus*sp	*Bacillus*sp
Fluoroquinolones	CPX(10µg)	S	S	S
	NB(10µg)	R	I	R
	LEV(20µg)	S	S	S
Aminoglycosides	CN(10µg)	S	S	I
	S(30µg)	S	S	S
Macrolide	E(30µg)	R	S	S
Beta Lactam	AML(20µg)	I	R	S
	APX(20µg)	I	R	I
Antitubercular	RD(20µg)	S	I	S
Chloramphenicol	CH(30µg)	S	I	S
Percentage (%)	Susceptible	60	50	70
	Intermediate	20	30	20
	Resistant	20	20	10

Key: R= resistant, S= susceptible, I= intermediate, CPX= Ciproflox, NB= Norfloxacin, CN= gentamycin, AMX= amoxyl S= streptomycin, RD= rifampicin, E= erythromycin, CH=chloramphenicol, APX= ampiclox, LEV=levofloxacin.

TABLE 5: ANTIBIOTIC SUSCEPTIBILTY TEST ON THE FOREIGN DISC

Chemical class of antibiotics	Antibiotics	*Pseudomonas* sp	Streptococcus sp	*Salmonella* sp	*Staphylococcus* sp	*E. coli*	*Klebsiella* sp	*Bacillus* sp
B-Lactam	Amp (10μg)	R	R	R	R	R	R	R
	OX(1μg)	R	R	R	R	R	R	R
	AUG(30 μg)	R	R	R	R	R	R	R
Macrolide	VAM(30 μg)	R	R	I	R	R	R	S
Cephalosporin	CAZ(30μg)	R	R	R	R	R	R	R
	CTX(30μg)	R	R	R	R	R	R	R
Tetracycline	TE(30μg	R	R	I	R	R	R	R
	DO(30μg	R	R	I	R	R	R	S
chloramphenicol	C(30μg	R	R	I	R	R	R	S
Percentage (%)	Susceptible	0	0	0	0	0	0	33.3
	Intermediate	0	0	44.4	0	0	0	0
	Resistant	100	100	55.6	100	100	100	66.7

Key: CTX= cefotaxime, CAZ= ceftazidime, AMP= ampicillin, OX= oxacillin, VAN= vancomycin, TE= tetracycline, DO= doxycycline, C= chloramphenicol. Aug= amoxicillin.

TABLE 6: MPN RESULT OF BOREHOLE WATER

Sample	Number of tubes giving a positive result			MPN (per 100ml)	95% confidence limit	
	10ml	1ml	0.1ml		Low	high
A	5	3	0	79	25	190
B	3	2	1	140	37	340

DISCUSSION

This study was conducted to assess the multi-drug resistant bacteria in the borehole water of Kogi State University Anyigba. A total of seven isolates were enumerated and characterized from the borehole water sampled. Bacteria isolated were *Escherichia coli*, Klebsiella sp, *Staphylococcus aureus*, *Salmonella sp*, *Bacillus sp*, *Streptococcus sp* and *Pseudomonas sp*. These organisms pose several health risks to consumers in general and immune compromised individuals in particular (Iroha*et al*., 2018). The presence of *Klebsiella* sp in the borehole water is unacceptable from the public health point of view and it agrees with the findings of Ogu *et al*., 2017.

Microorganisms isolated were identified with gram staining, three isolates (*Streptococcus* sp, *Staphylococcus* sp, and *Bacillus* sp) were gram positive while four isolates (*Pseudomonas* sp, *Salmonella* sp, *Escherichia coli*, *Klebsiella* sp) were gram negative.

Further identification with biochemical tests were carried out on the isolates. *Pseudomonas* sp was positive for catalase, citrate, urease, vogesproskauer, indole test but negative for methyl red. *Streptococcus* sp was positive for methyl red and citrate test but negative for indole, vogesproskauer, catalase and urease test. *Salmonella* sp was positive for catalase and urease test but negative for indole, methyl red, vogesproskauer and citrate test. *Staphylococcus* sp was positive for methyl red, catalase, citrate but negative for urease, voges-proskauer and indole test. *Escherichia coli* was negative for voges-proskauer and citrate but positive for indole, catalase, methyl red, and urease. *Klebsiella* sp was negative for indole and methyl red but positive to catalase, citrate, urease, voges-proskauer. *Bacillus* sp was positive to methyl red and citrate but negative to catalase, urease, voges-proskauer and indole.

Antibiotic susceptibility test was carried out for each isolate on an indigenous and imported disk. The percentage of resistance of the gram negative isolates on the indigenous disk ranges between 30-40%, intermediate ranges between 10-30% and the susceptibility ranges between 40-50%. The percentage of resistance of the gram positive isolates on the indigenous disc ranges between 10-20%, intermediate between 20-30% while susceptibility ranges from 50-70%. Most of the isolates had 100% resistance on the foreign disc.

The multiple antibiotic resistances of *E. coli* established in this study agree with other findings (Park *et al*., 2018). Strains of *E. coli* and *Salmonella* spp. accounted for several outbreaks worldwide, partly due to resistance to chloramphenicol, ampicillin, and trimethoprim (Uma *et al*., 2009).

The frequency of resistance to penicillin in the current study was high among the isolates as compared with resistance to chloramphenicol and ampicillin observed in the isolates obtained from the various water sources. *E. coli* resistance against ampicillin was observed by Çelebi *et. al*. (2007) and Olowe *et al*., (2008). Lastly, periodic monitoring of antibiotic sensitivity of the water sources is of importance to detect any changing patterns that may arise in future in order to keep pace with such changing patterns for better curative measures or policies formulation and implementation.

For the MPN (most probable number) method, the maximum acceptable concentration for drinking water is none detectable per 100ml and the samples exceeded this, which means that the borehole water is unsafe and this work agrees with the work of Okafor, 1985 on borehole water in Nsukka.

Conclusion

It can be deduced that most bacteria found in the borehole water of Kogi State University Ayingba are multi drug resistant because they showed resistance that cut across different classes of antibiotics. On this note chemical treatment of water, boiling of our drinking water, regular washing of overheard tanks and the use of water filters if practice would help in reducing the menace.

REFERENCES

Adeyinka S.Y, Wasiu J, Akintayo C.O 2014.Review on prevalence of waterborne diseases in Nigeria. *J. Adv. Med. Life Sci.*;1(2):1-3

Agbaire, P. O., and Oyibi, P. I. (2009).Seasonal variation of some Physico-chemical properties of borehole water in Abraka, Nigeria.*African Journal of Pure and Applied Chemistry, 3*(6), 116-118.

Angulo, F. J., S. Tippen, D. J. Sharp, B. J. Payne, C. Collier, J. E. Hill, … D. L. Swerdlow. (1997).A community waterborne outbreak of Salmonellosis and the effectiveness of a boil water order. *Am. J. PublicHealth, 87*(4), 580-584.

Ayantobo. O.O, Oluwasanya G.O, Idowu.O.A, Eruola.A.O 2013 Water quality evaluation ofhand-dug wells in Ibadan, Oyo State,Nigeria. *Global Journal of Science FrontierResearchAgriculture and Veterinary*.;13(10):21-27.

Brainstein, J. (2007). *Trading the Rain, Should the World's fresh water resources be an international tradedcommodity?*

Carlow C. Roger, Alan M. McDonald, Alan L. Nicol, & Nick S. Robins. (2011). *Groundwater Security andDrought in Africa. Linking Availability, Access and Demand*

Çelebi.A, Duran .N, Öztürk. F, Açık. L, Aslan. G, and Aslantaş. O, (2007 "Identification of clinic uropathogen *Escherichia coli* isolates by antibiotic susceptibility, plasmid and whole cell protein profiles," *Advanced Molecular Biology*, vol. 1, pp. 31–40, 2007.

Chowdhury, S., Mazumder, M.A.J., Al-Attas, O., Husain, T. (2016). Heavy metals in drinking water: Occurrences, implications, and future needs in developing countries. Sci. Total Environ. 569–570, 476–488.

Iroha, C., Iroha, I., Nwakeze, E., Ajah, M. and Ejike, C. (2018). Evaluation of the metal content and bacteriological parameters of selected borehole water sources in Abakaliki, Nigeria. International Journal of Waste Resources. 8: 338

Obeta MC, Ocheje FJ. Assessment ofgroundwater quality in Ankpa Urban, KogiState, Nigeria.Environmental ResearchJournal. 2013;7(3):37-47.

Ogu, G.I., Madar, I.H., Olueh, A.A. and Tayubi, I. A. (2017). Antibiotic susceptibility profile of bacteria isolated from drinkingwater sources in Amai Kingdom, Delta State, Nigeria. Annual Research and Review in Biology. 14(1): 1-9.

Okafor, N. (1985). Aquatic and waste Microbiology (lsf ed.). Fourth Dimension Publishing Coy.P. Enugu. Pp 52.

Olowe .O.A, Okanlawon. B. M, Olowe. R. A, and Olayemi .A.B, "Antimicrobial resistant pattern of *Escherichia coli* from human clinical samples in Osogbo, south western Nigeria," *African Journal of Microbiology Research*, vol. 2, no. 1, pp. 8–11, 2008

Park. J, Kim. J. S, and Kim. S. (2018) "A waterborne outbreak of multiple diarrhoeagenic *Escherichia coli* infections associated with drinking water at a school camp," *International Journal of Infectious Diseases*, vol. 66, pp. 45–50,

Sinderberg, M. S. (2003). Higher Education Chemistry.In *The Molecular Nature of Matter and Change*.McGraw Hill.

Udom, G. J. Ushie, F. A., and Esu, E. O. (2002).A geochemical survey of groundwater in Khana and Gokanalocal government area of Rivers State, Nigeria.*J. Applied Sci. Environ. Manage, 6*, 53-59.

Uma. B, Prabhakar. K, Rajendran.S, Kavitha .K, and Y. L. Sarayu, 2009 "Antibiotic sensitivity and plasmid profiles of *Escherichia coli* isolated from pediatric diarrhea," *Journal of Global Infectious Diseases*, vol. 1, no. 2, p. 107,

WHO Guidelines for drinking-water quality,health criteria and other supportinginformation, 2nd Edition, World HealthOrganization, Geneva. 2004;2. WHO. Water Supply and Sanitation Council, Global water supply and sanitation assessment report 2000. New York, USA; 2000

WHO.Water Supply and SanitationCouncil, Global water supply andsanitation assessment report 2000.NewYork, USA; 2000.

Xinghui, X. Zhifeng Y., &Yuxiang W. (2009).Incorporating Eco-environmental Water Requirements in Integrated Evaluation of Water Quality and Quantity- A case study for the Yellow River.*Water ResourManage, 23,* 1067-1079.