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Detection of Antibiotic Resistant organisms in Water Sources in Okada Town, Edo State, Nigeria

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ABSTRACT

Water is an indispensable resource for the existence of all living things, human beings especially. This study investigated the physicochemical properties of drinking water and water used for domestic purposes in Okada town to ascertain their suitability for consumption and also the possible detection of antibiotic resistant organisms in such water sources. A total of six water samples were obtained for the study. Standard methods were employed in the estimation of the physicochemical properties of the samples, isolation and characterization of bacterial isolates from the samples. Antimicrobial susceptibility tests were performed on the isolates using the Kirby-Bauer disc diffusion method. The physicochemical properties of all the samples fell within the normal range when compared to the WHO maximum permissible levels. The isolates obtained from three samples were Gram positive cocci and Gram negative bacilli bacteria and had a probable identity of Staphylococcus sp, Coagulase negative Staphylococcus sp, Klebsiella sp, Escherichia coli. The Bacterial isolates were resistant to various antibiotics such as the cephalosporins, gentamicin, cloxacillin, augmentin, nitrofurantoin, ampicillin and ciprofloxacin. The detection of antibiotic resistant organisms is of public health importance and may be possible reason for prolonged therapy of water borne diseases. Accurate diagnosis and appropriate administration of drugs will help in the control of antibiotic resistant bacteria. Proper treatment of water especially before consumption is recommended.

Key Words: *Physicochemical analysis, Water, Microorganisms, Antibiotic resistance, Public health.*

1.0 INTRODUCTION

Water is essential in every aspect of life. From the lowest of life forms to the most complex, water is indispensable for survival. Metabolic reactions occurring in biological systems are aqueous based and as such it validates the fact that water constitutes about 65-75% of the total weight of an average human being. In addition, water is employed in a wide range of human activities including agriculture and manufacturing. Good drinking water therefore is not a luxury but one of the most essential requirements of life itself [Olajubu and Ogunika, 2014]. Water has to comply with certain physical, chemical and microbiological standards to ensure that water is palatable and safe for drinking before it can be described as potable [Taiwo et al., 2010]. These standards are defined globally by standards issued by WHO [WHO, 2004] and NSDWQ [NSDWQ, 2007]. Potable water is defined as water that is free from disease producing microorganisms and chemical substances deleterious to health [Olajubu and Ogunika, 2014]. There are two types of water pollution, namely; point source pollution which occurs as a result of release of harmful substance directly into the body of water, and non-point source pollution which occurs as a result of indirect introduction of pollution into water bodies/sources from the environment [Singh and Gupta, 2016]. According to the World Health Organization, contaminated water, inadequate sanitation, and poor hygiene cause over 80% of diseases in developing countries [Amangabara and Ejenma, 2012]. It has been reported that water borne diseases such as cholera, typhoid fever, bacillary and amoebic dysentery kill at least 3.4 million people every year [Agha, 2006]. During passage through the ground, water dissolves minerals in rocks, collects suspended particulate matter particularly those from organic sources as well as pathogenic microorganism from fecal matters

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[Onuh and Isaac, 2009]. Certain minerals such as the heavy metals are toxic although, some of the heavy metals such as zinc, manganese, nickel and copper acts as micro-nutrients at low concentrations. At high concentrations, they are detrimental to health. Other elements such as arsenic, bismuth, cadmium, mercury, lead, titanium have no apparent metabolic function and are very toxic to biological systems. In Nigeria, water occurs both in rural and urban areas. It has been estimated that nearly 1.5 million people lack safe drinking water. Raw sewage, garbage and oil spill serve as sources for contamination [Alfarra, 2010]. Microbial pathogens associated with water pollution include *Salmonella*, *Shigella*, *Camphylobacter*, *Vibrio*, *Yersinia*, *Giardia* and *Cryptosporidium* species [WHO, 1996].

Bacteria with intrinsic resistance to antibiotics are found in nature and some organisms may acquire additional resistance genes from bacteria introduced into water or soil. Possible cause of resistance includes mutation or R-plasmid exchange between bacteria of the same or different species [Millan, 2018]. The common use of antibiotics in veterinary or agricultural practices for prophylaxis can contaminate the environment [Phares et al., 2020] including surface and underground water where they could be transferred to humans in drinking water and causing a rise in the levels of antibiotic resistance. Another common cause of antibiotic resistance in humans is the inappropriate use of antibiotics in treating and preventing human infections. When antibiotic resistant bacteria from humans are introduced into water for example through fecal contamination, emerging diseases would be very difficult to treat.

Globally, an estimated 2000 children under the age of five die every day from diarrheal diseases and of these about 1800 deaths are linked to water, sanitation and hygiene [UNICEF, 2013]. In Nigeria, the growing rate of industrialization has been reported by Aliyu and Amadu, 2017 to be gradually leading to contamination and deterioration of the environment especially the water sources which pose great public health hazards. This study aimed at the determination of physicochemical properties of water samples obtained from Okada town in Edo state Nigeria and the possible detection of antibiotic resistant bacteria in the water samples.

2.0 MATERIALS AND METHODS

2.1 Sample collection

Water samples were collected from six different water sources which included three different sources of borehole water, pond water, a brand of sachet and table water respectively. The samples were collected aseptically with the aid of sterile 500ml universal bottles. The samples were transferred to the laboratory for immediate analysis

2.2 Microbiological evaluation of samples

Standard isolation techniques were employed in the isolation of organisms using already prepared MacConkey agar plates [Olutilola et al., 1991]. Discrete colonies were picked out and sub cultured on Eosine methylene blue agar and Mannitol salt agar for appropriate characterization. Isolates were identified and characterized based on their cultural characteristics, colonial and microscopic appearance and biochemical reactions. Standard microbiological/biochemical methods were used in the identification of pure isolated bacteria [Brown, 2005]. The Biochemical tests included Gram staining reaction, Citrate, Catalase, Urease, Indole, Coagulase and Oxidase test.

2.3 Antimicrobial sensitivity test

The Kirby-Bauer susceptibility testing technique [Bauer et al., 1966] was carried out. Isolates were cultured on Nutrient agar overnight at 37^{o}C. The isolates were tested with 8 antibiotics which include; Ofloxacin(5µg), Erythromycin(10µg), Cloxacillin(5µg), Gentamicin(10µg), Augmentin(30µg), Ceftriaxone(30µg), Ceftazidine(30µg), Cefuroxime(30µg) for the gram positive isolates on Mueller Hinton agar plates. The gram negative isolates were also tested with 8 antibiotics similar to the ones used for the gram positive isolates except for Erythromycin, Cloxacillin and Ceftriaxone. Ampicillin (10µg), nitrofurantoin (300µg) and ciprofloxacin (10µg) were included in the susceptibility tests for the gram negative isolates. Incubation was performed at 37^{o}C for 24hours and results were also interpreted as sensitive and resistance based on the zones of inhibition.

2.4 Physicochemical analysis

The samples collected were analyzed for dissolved oxygen, pH, total metal determination, chloride, sulphate, phosphate, nitrate.

2.4.1 Dissolved oxygen

The Dissolved oxygen (DO) meter was calibrated prior to measurement with the appropriate calibration solution (5% HCl) in accordance with the manufacturer's instruction. The dissolved oxygen was measured for all the water samples.

2.4.2 pH

Samples were measured using a pH meter. The pH meter was calibrated with three standard solutions (pH 4.0, 7.0 and 10.0) before taking the measurements. The value of each sample was taken after submerging the ph probe in 100ml aliquot of each water sample and holding for a couple of minutes to achieve a stabilized reading. After the measurement of each sample, the probe was rinsed with de-ionized water to avoid cross contamination among different samples.

2.4.3 Total Metal Determination

The concentrations in mg/L of three metals were determined in all the samples namely, Cu, Zn and Pb with the Atomic Absorption Spectrophotometer. The flame used for the analysis was air-acetylene mixture. A 100ml stock of multi-element solution was obtained from the laboratory of the Environmental Protection Agency, Accra. Standard solutions ranging from 0.2 to 5.0mg/l were prepared for calibration curves of the various metals. A blank solution was similarly prepared. The absorbance of the blank was taken before the analysis. A blank analysis was performed with distilled water treated with the sample treatment. Samples were digested for Zn, Pb and Cu using an aliquot of 5ml of concentrated nitric acid added to 50ml sample of water in a 100ml beaker. This mixture was heated on a hot plate to boil until the colour of the solution became pale and clear. The solution was heated on a hot plate to boil until its volume got to about 20ml. Another 5ml of concentrated nitric acid was added and the beaker was covered with a watch glass and heating continued for about 10 more minutes. A final 5ml acid was used to rinse the sides of the beaker. The solution was poured into a 50ml volumetric flask and topped with distilled water to the mark. Quantitative analysis was achieved by measuring the absorbance of a no of solutions of known concentration. A calibration curve and the equation of the line was used to determine the unknown concentrations based on its absorbance.

2.4.4 Choride

An aliquot of 50ml of sample was measured into a conical flask. The pH was adjusted to a range of 7-10 with H_2SO_4 for high pH samples and NaOH for low pH samples. Two drops of K_2CrO_4 indicator was added. Standard $AgNO_3$ solution of 0.01M was titrated against the resulting mixture above to a pink yellow end point. Blank titration with only the reagents and no water sample was also performed.

Chloride (mg chloride per litre)= X x N x 1000 x 35.5

In 1ml of sample, X= end point volume and N= Normality of AgNO3

2.4.5 Sulphate

A volume of 10ml of conditioning reagent was added to 25ml of the sample. This was followed by the addition of 0.3g of $BaCl_2$. The mixture was then diluted to 100ml with distilled water. Prepared samples were allowed to stand for 45 minutes. The concentrations were determined using the UV- visible spectrophotometer at 420nm. A blank without BaCl2 was prepared and analyzed at the same wavelength.

2.4.6 Phosphate

Standard solutions of 1, 2, 3, and 4µg/ml were prepared. To these preparations were added 2ml of combined reagent. The absorbance of the solutions after 10 minutes was taken at 655nm against a blank solution. A curve of absorbance against concentration was plotted. A blank analysis was performed with all the reagents without the samples for all the analysis.

2.4.7 Nitrate

Aliquots of 0.1, 0.2, 0.3 and 0.4ml of the stock solutions were measured into different 100ml volumetric flasks. To the different volume of stock solutions, 2ml of 0.1M NaOH was added followed by the addition of 1, 2, 3 and 4ml of colour developing reagent respectively. The mixtures were diluted to 100ml mark forming 0.25µg/ml, 0.50µg/ml, 0.75µg/ml and 1.00µg/ml respectively. The nitrate concentration was determined at wavelength 543nm absorbance. A blank analysis was performed with all the reagents without samples for all the analysis.

3.0 RESULTS

Out of the six water samples examined, a total number of six isolates were isolated from three water samples. The identified bacteria obtained from the water samples include *Staphylococcus sp, Coagulase negative Staphylococcus sp, Klebsiella sp,*

Escherichia coli. The pond water sample and the sachet water sample had two bacterial isolates respectively. One of the borehole water samples also had two bacterial isolates. Table 1 shows the frequency distribution of the isolates from the water samples.

Table 1 Frequency distribution of the isolates from the water samples

Isolates	*No of isolates*	*Frequency %*
Staphylococcus sp	*1*	*16.7%*
Coagulase negative Staphylococcus sp	*1*	*16.7%*
Klebsiella sp	*3*	*50%*
Escherichia coli	*1*	*16.7%*

The antimicrobial susceptibility test showed the presence of resistant isolates to antibiotics examined (Table 2 and 3).

Table 2 Antibiogram of the gram positive bacterial isolates

S/N	*Isolate*	*Caz 30ug*	*Crx 30ug*	*Gen 10ug*	*Ctr 30ug*	*Ery 10ug*	*Ofl 5ug*	*Aug 30ug*	*Cxc 5ug*
1	*Staph sp*	*18R*	*18R*	*20S*	*18R*	*20I*	*18S^{X}*	*18R*	*20R*
2	*CoN Staph sp*	*R*	*R*	*17S*	*15R*	*R*	*11R*	*R*	*R*

Key- Cxc- Cloxacillin, Gen- Gentamicin, Ctr- Ceftriaxone, Ery- Erythromycin, Caz- Ceftazidine, Ofl- Ofloxacin, Crx- Cefuroxime, Aug- Augmentin, x-zone of inhibition in mm, *Staph sp- Staphylococcus sp, CoNStaph sp-* Coagulase negative *Staphylococcus sp*

Table 3 Antibiogram of the gram negative isolates

S/N	*Isolates*	*Caz 30µg*	*Crx 30µg*	*Gen 10µg*	*Cpr 5µg*	*Nit 300µg*	*Ofl 5µg*	*Aug 30µg*	*Amp 10µg*
1	*Kleb sp*	*R*	*R*	*15R*	*15R*	*18S*	*17I*	*9R*	*7R*
2	*E. coli*	*8R*	*R*	*12^{X}R*	*18I*	*18S*	*20I*	*8R*	*R*
3	*Kleb sp*	*R*	*10R*	*5R*	*10R*	*12R*	*R*	*R*	*R*
4	*Kleb sp*	*R*	*25I*	*R*	*20I*	*R*	*20I*	*25S*	*R*

Key: Amp- Ampicillin, Gen- Gentamicin, Nit- Nitrofuantoin, Cpr- Ciprofloxacin, Caz- Ceftazidine, Ofl- Ofloxacin, Crx- Cefuroxime, Aug- Augmentin, x-zone of inhibition in mm, *Kleb sp- Klebsiella sp, E. coli- Escherichia coli*

The resistance phenotype of isolates recovered from the water samples showed the least number of antibiotics that isolates were resistant to was four (4) and the highest number of antibiotics was eight (8). Table 4 and 5shows the resistance phenotype of gram positive and gram negative isolates

Table 4 Resistance phenotype of gram positive isolates

Isolates	*Antibiotics*	*Number*
Staph sp	*Caz, Crx, Ctr, Aug, Cxc*	*5*
CoNStaph sp	*Caz, Crx, Ctr, Ery,Aug, Cxc, Ofl*	*7*

Table 5 Resistance phenotype of the gram negative isolates

Isolates	*Antibiotics*	*Number*
Kleb sp	*Caz, Crx , Gen, Cpr, Aug, Amp*	*6*
E. coli	*Caz, Crx, Gen, Aug, Amp*	*5*
Kleb sp	*Caz, Crx, Gen, Cpr, Nit, Ofl, Aug, Amp*	*8*
Kleb sp	*Caz, Gen, Nit, Amp*	*4*

Table 6 Physiochemical test results

Parameters	**Samples**					
	A	B	C	D	E	F
Ph	6.51	6.95	6.92	6.55	6.69	6.51
DO(mg/L)	6.24	6.24	6.24	6.24	6.24	6.24
Chloride(mg/L)	12.70	3.53	4.70	13.99	14.80	15.00
Sulphate(mg/L)	0.44	0.29	0.35	1.32	0.77	0.83
Nitrate(mg/L)	1.89	0.40	0.62	3.02	0.87	0.80
Phosphate (mg/L)	0.17	0.10	0.14	0.17	0.18	0.20
Pb(mg/L)	-	-	-	-	-	-
Zn(mg/L)	0.4	-	-	0.03	0.06	0.056
Cu(mg/L)	0.2	0.31	0.23	0.5	0.25	0.19

Key- A- Borehole water, B- Table water, C- Sachet water, D- Pond water, E- Borehole water F- Borehole water, pH- Hydrogen ion concentration, DO- Dissolved oxygen, Pb- Lead, Zn- Zinc, Cu- Copper

4.0 DISCUSSION

Water samples were analyzed for their physiochemical properties and the possible detection of antibiotic resistant isolates. Although a limited number of isolates were obtained from the water samples, the results showed high level of resistance among the isolates. The presence of antibiotic resistant bacteria in drinking water is of public health importance as a result of promoting antibiotic resistant bacteria in humans. Antibiotic resistant bacteria have imposed a great challenge to treatment clinicians, clinical microbiologists and infection control and prevention practioners. This study shows that one of the possible ways antibiotic resistant bacteria can be promoted is by drinking untreated water. Results from this study correlates with results from the study by Nwachukwu and Emeruem, 2007 that reported a high percentage of bacteria isolates resistant to Ampicillin and tetracycline and a low percentage resistant to Ofloxacin isolated from sachet water produced and sold in Eastern Nigeria. Also, Nwachukwu and Otokunefor, 2003 reported sixty three Gram negative bacteria isolated from rural untreated drinking stream water supply with high resistance to Ampicillin. Results from another study by Oluyege et al., 2009 showed at least 10 percent of bacteria isolated from surface and underground water sources from south western Nigeria were resistant to four or more antibiotics thus exhibiting multiple antibiotic resistance patterns. The results of this study revealed that the *E. coli, Klebsiella spp. and Staphylococcus aureus* isolated in this study were resistant to more than four antibiotics. The number of antibiotics to which they were resistant ranged from 4 to 8 (Table 4 and 5). Isolation from water sources in a rural community like Okada town of antibiotic resistant pathogenic bacteria is thus a primary concern.

The results of this study show clearly that the sachet, pond and borehole water samples which serve as drinking water and also used for domestic purposes to rural communities could also serve as reservoirs for pathogens resistant to quite a number of antibiotics. Unfortunately, affordable treatment facilities in most rural settings are unavailable which rule out the possibilities of

microbial reduction before such waters are consumed and used for other domestic purposes. Among the resistant enteric pathogens encountered in this study were *E. coli* and *Klebsiella spp*. *Staphylococcus spp* was also isolated in this study. This finding is in agreement with the work of Efuntoye and Apanpa, 2005 that highlighted, *E. coli*, *Klebsiella spp*., *Staphylococcus spp*, *Bacillus, Proteus, Pseudomonas* as among the pathogens isolated from water samples from hand-dug wells. A regular monitoring of the water quality for improvement not only prevents disease and hazards, but also checks further pollution of water sources. The conservation of water sources is very necessary to provide safe water.

The intestinal tract of the human open population is considered to be an important reservoir of antibiotic resistant bacteria [Olofsson, 2006]. As far as possible, water sources must be protected from contamination by human and animal waste, which can contain a number of microorganisms. It becomes more challenging if these faecal wastes are rich in antibiotic resistant strains of pathogens especially in a developing nation setting like Nigeria. Antibiotics can be purchased without doctor's prescription and are readily available in open market like, hospitals, pharmacies, patent medicine [Okeke and Lamikanra, 1995, Hoge et al., 1998]. This is useful in making self-medication and antibiotic overuse by laypersons prevalent.

The pH values obtained in this study (6.51-6.95) fell within the WHO and Nigerian standard for drinking water maximum permissible levels of 6.5-8.5 [Okonkwo et al., 2006]. WHO standard specifies maximum permissible value under the Dissolved Oxygen (DO) test for water samples as >30mg/l [Akpoveta et al., 2011]. Dissolved oxygen value was 6.24 for all samples tested in this study and this was similar to the results reported by Akpoveta et al., 2011 (5.4- 6.2mg/L) and Okonkwo et al., 2008 (5.52-5.68mg/L) which correlates with WHO permissible value.

The values for chloride (3.53-15.00 mg/L) were lower than those reported by Okonkwo et al., 2008 which ranged from 9.4-17.4mg/L. The values are far much below the maximum permissible limit of 250mg/l set by WHO and the Nigerian standard for drinking water. Excessive chloride concentration in water increases rate of corrosion of metals in the distribution system depending on the alkalinity of the water. This can lead to increased concentration of metals in the water supply [Afolabi et al., 2012]. Chloride ion is one of the major ions in water generally associated with sodium and calcium. High concentration of chloride in water may lead to objectionable salty taste [Ehi-Eromosele and Okiei, 2012]. Sulphates are formed due to the decomposition of various sulphur containing substances present in water supplies. The sulphate ions ($SO4^{-2}$) occur naturally in most water supplies. Sulphate levels ranged from 0.29-1.32mg/L having levels slightly higher than a range of 0.12-0.37mg/L reported by Akpoveta et al., 2011. However the results fell within the WHO maximum permissible level of 250mg/L and therefore are incapable of causing bad odour. The phosphate level of the water samples ranged from 0.10-0.20mg/L which is within the limits set by WHO. This observation is also in agreement with the findings of other reports in similar studies [Aremu et al., 2011, Ezeribe et al., 2012].

Nitrates indicate the presence of fully oxidized organic matter. The high levels of nitrates in water samples contain high level of oxidized organic matter which appears in the form of soluble anions such as nitrates. Excess levels of nitrates can cause methemoglobinemia. Although nitrates levels that affect infants do not pose a direct threat to older children and adults, they do indicate the poor quality of water samples [Robert, 2006]. Nitrate (0.4-3.02 mg/L) was slightly higher than values of 0.15-0.55mg/L reported by Akpoveta et al., 2011. Copper values (0.19-0.5 mg/L) for the water samples in this study were higher than the values of 0.033-0.07mg/L reported by Akpoveta et al., 2011. Copper was detected in all the water samples and as toxicity is associated with continuous low level exposure, this can eventually lead to serious health effects. Contamination of drinking water with high level of copper may lead to public health hazards [Ehi-Eromosele and Okiei, 2012]. The occurrence of copper in water may be from brass taps or chemical leaching from coatings taken up from contact with surface during treatment or disinfections [Kazaure et al., 2015]. Both the nitrate and copper values fall within the WHO maximum permissible value of 10mg/l and 1mg/l respectively. Zinc was only present in four samples, ranging from 0.03-0.4 mg/L which were slightly lower than results 0.56-0.98mg/L reported by "Reference [Rim-Rukeh et al., 2008]" and had its values falling within the WHO maximum permissible value of 5mg/L. Lead was absent in all the samples, this contrasts with values of 0.012-0.016mg/L and 0.008mg/L reported by "Reference [Okonkwo et al., 2008, Akpoveta et al., 2011]. The absence of lead and low level of the parameters investigated in this study from the water samples could be due to the absence of industries in Okada and lack of proximity to possible sources of contaminants. The physicochemical properties of all the samples fell within the normal range when compared to the WHO maximum permissible levels.

5.0 CONCLUSION

The detection of antibiotic resistance among the isolates may have an important therapeutic implication that calls for caution in the indiscriminate use of antibiotics in humans. Antibiotic use provides selective pressure favoring resistant bacterial strains. Regular monitoring of antibiotic sensitivity of isolates from the water sources and also physicochemical characteristics of such 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. The presence of bacteria that are resistant to antibiotic poses a serious health hazard especially since such organism can serve as reservoir for antibiotic resistant genes that could be transferred to potentially pathogenic bacteria in the ecosystem.

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CONFLICT OF INTEREST

No conflict of interest is declared.