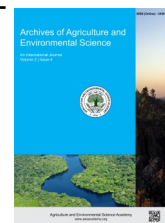This content is available online at AESA

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ORIGINAL RESEARCH ARTICLE

CrossMark

Bacteriological assessment of some borehole water samples in Mile 50, Abakaliki, Ebonyi State, Nigeria

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ABSTRACT

Water as excellent natural resource is meant to be of high quality to reduce the outbreak of water-borne diseases. Bacteriological load of some borehole water samples in Mile 50 Abakaliki were carried out to determine their potability. Fifteen borehole water samples were sampled during rainy and dry season from June to July and November to December 2018 respectively. The total bacterial count was determined by tenfold serial dilution method using peptone water. Eight bacterial species namely *Vibrio cholerae*, *Pseudomonas aeroginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella flexineri*, *Proteus vulgaris* and *Klebsiella pneumoniae* were isolated using standard analytical procedures. The bacterium that had the highest frequency of occurrence during both rainy and dry season's was *K. pneumoniae* with percentage frequency of 21.81% and 20.79% respectively, and *P. vulgaris* had the least value of 6.96% during rainy season. *E. coli* and *S. aureus* have the least value of 5.94% during dry season. Amoxicillin (30ug) was mostly resisted by the bacterial isolates why being was more susceptible to Ciprofloxacin (10ug) among the antibiotics used for susceptibility test. Two way analysis of variance (ANOVA) was used to determine the level of significance among the bacteriological analyses of both seasons. Therefore, there is need to create awareness about the present situation of the borehole waters and the necessity for further treatment of water by consumers, before it can be used for both drinking and domestic purposes to prevent disease outbreak in the area.

Citation of this article: Ibo, E.M., Umeh, O.R., Uba, B.O. and Egwuatu, P.I. (2020). Bacteriological assessment of some borehole water samples in Mile 50, Abakaliki, Ebonyi State, Nigeria. *Archives of Agriculture and Environmental Science,* 5(2): 179-189, https://dx.doi.org/10.26832/24566632.2020.0502015

INTRODUCTION

The quality of water is a vital concern for mankind since it is directly linked with human welfare. Since it is a dynamic system containing living as well as non-living organic, soluble as well as insoluble substances, its quality is likely to change day by day and from source to source. Only one percent of water is available on land for drinking, agriculture, domestic power generation, industrial consumption, transportation and waste disposal (Tahir *et al.,* 2008). The quality of water is more important compared to quantity in any water supply planning, especially for drinking purposes. Water quality standards are the foundation for the quality based control program and required for the treatment process (Nikoladze and Akastal, 1989). These standards support efforts to achieve and maintain protective water quality conditions (Omaka *et al.,* 2014; AlSuhaimi *et al.,* 2017). A primary concern of people living in developing countries is that of obtaining clean drinking water. Groundwater sources are getting contaminated due to human interference, such as waste dumping, effluent and sewage discharge without proper treatment. Municipal and industrial wastes, application of fertilizers, herbicides, pesticides, burning of coal, leaching from mining

activity further add to contamination of groundwater. These different sources of contamination may influence physical, chemical, and biological variables of groundwater (Olajuba and Ogunika, 2014). Open dumping of municipal solid wastes, is mainly the existing method of waste disposal used even in capital cities except perhaps among few and affluent institutions in Nigeria (Onwughara *et al.*, 2010). Groundwater contamination by leachates can transmit bacteria and disease. Typhoid fever is a common problem for the people of developing nations, many of them cannot afford to dig wells deep enough to reach fresh aquifers (Onwughara *et al.*, 2010; Reidl and Klose, 2002).

Faecal coliform bacteria are used as an indicator for the presence of any of these water-borne pathogens. Their presence in water however possibly detects the existence of disease causing organisms (Anhwange *et al.*, 2012). The paucity and unreliable water supply within Abakaliki has forced residents to increasingly depend on shallow wells and boreholes as the source of water for drinking and domestic use (Ibo *et al.*, 2020). These boreholes are dug shallow wells connected to constantly open overhead tank and pumped with sumo machine many of them are located close to household drainage systems and septic tanks and are therefore susceptible to contamination (Ibo *et al.*, 2020). A proper knowledge of the bacteriological properties of water meant for drinking and domestic purposes is crucial to avert a possible health hazard, therefore in this study; the bacteriological load of some borehole waters in mile 50 Abakaliki were evaluated.

MATERIALS AND METHODS

Study area

Abakaliki is the capital city and the largest town of the present day Ebonyi state in south-eastern Nigeria, located 64 kilometres Southeast of Enugu. The inhabitants are primarily members of the Igbo states with a population of about 79,284 (Hoiberg, 2010). It is predominated by traders and farmers, it is known for its local lead, zinc, salt and limestone mining (Cohen, 1998) (Figure 1).

Sanitary risk inspection of the sampled waters

The questions were adopted from (Umeh *et al.* 2020) and modified in accordance with objectives of this research. Vital information about the water such as depth, proximity of waters to farm lands, septic tanks, height of the slab/apron, interior concrete linings, were obtained by oral interview and visual analysis. The depth of the water was measured using calibrated long steel.

Sample collection

The borehole water samples for the bacteriological load were collected in both rainy and dry seasons from 15 boreholes at the following locations randomly. SDP, Ebonyi voice, Mile 50 layout, NEPA junction, Pastoral centre, Isiuke lane 1, Isiuke lane 2, Ibiam borehole, Ogodo borehole, Alugbara Eze, Amaike Aba road borehole, Obasi borehole, Eze and bros, Oroke market and Nkwoagu. All the sampling points were selected randomly within mile 50 in Abakaliki 30 Samples were aseptically collected from 15 different boreholes between June to July and November to December 2018. The borehole waters that were selected were those used for drinking and for other domestic purposes such as cleaning and farming. Samples for the bacteriological analysis were aseptically collected in one litersterile containers. Before then the water was left to rush for 3 minutes (This allows the nozzle of the tap to be flushed and any stagnant water in the service pipe to be discharged). A piece of cotton-wool soaked in ethanol and lighter was used to sterilize the faucet of the borehole and allow the tap to cool by running the water to waste for a few seconds before collection. Two samples were collected from each site at the interval of one week within both seasons (to ensure representative sampling). Collected samples were kept at 4°C in an ice box and transported to the laboratory for analysis within six hours (Cheesbrough, 2006).
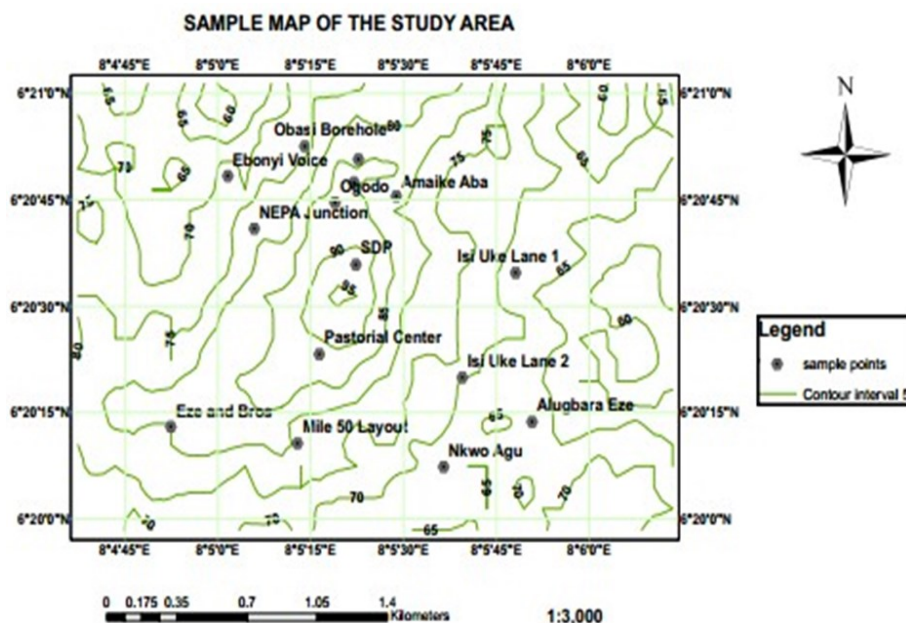

Figure 1. *Contour map of the study area.*

Examination of total bacterial count

The water samples that were collected from the boreholes were homogenized by stirring. The bacterial load of the water samples were determined by performing ten-fold serial dilution in test tubes containing sterile peptone water up to 10^{-4}. Nine millilitres (ml) of sterile peptone water were transferred aseptically into 4 sterile tubes labeled 10^{-1} to 10^{-4}, One ml of the borehole water sample was also aseptically transferred into the first tube (10^{-1}) with a sterile pipette and mixed using fresh pipette these were repeated until the 4th tube. The total viable count (Total plate count) were determined using the pour plate technique, cultured in duplicates. Nutrient agar was prepared according to the manufacturer's instruction and sterilized by autoclaving at 121°C for 15 minutes at 15 psi and was allowed to cool to 45°C before dispensing into sterile Petri-dishes containing 1ml of the samples from each of the dilution test tubes and allowed to solidify. A control was equally prepared without adding the sample. The plates were then inverted to prevent condensation droppings from the lid into the agar and incubated in the incubator at 37°C for 24 hours. The bacterial colonies ranging from 30 to 300 were counted and expressed in colony forming unit per ml (CFU/ml) (Agbabiaka, and Sule, 2010).

Colony forming unit / ml = Average number of colonies / aliquot volume × Dilution factor.

Determination of total and faecal coliform

This was done using the standard membrane filtration technique as described by Cheesbrough (2010). A sterile filtration apparatus was put in position and connected to a vacuum pump. The apparatus was rinsed by passing small amount of sterile water and the water sample to be analyzed through the funnel using the vacuum pump. The water samples were thoroughly mixed by inverting the container twenty-five times after which one hundred milliliters (100mls) of it was poured into the funnel containing the filter paper and slowly filtered through the membrane filter with the aid of the vacuum pump. Using sterile forceps, the membrane filters were removed from the filtration cup and transferred facing up on the surface of the Petri dishes containing the named medium for the culture of bacteria of interest. For total coliform, the plates were incubated at 37°C for 48 hour using MacConkey agar; for faecal coliform the plates were incubated at 44.5°C for 24 hour using Eosin methylene blue agar. After incubation, number of bacterial colonies were counted and expressed as colony forming units (CFU) per 100 ml.

Detection of *V. cholerae* and *V. parahaemolyticus*

Thiosulphate citrate bile salt sucrose (TCBS) agar was used as the growth medium. It was prepared according to manufacturer's instruction sterilized and cooled to 45°C before introducing into a Petri dish and allowed to solidify. The membrane filter paper was thereafter transferred to the surface of the medium using a sterile forceps. Incubation of the Petri dish was carried out at 37°C for 24 hours in an inverted position. The presences of yellow colonies were suspected to be *V. cholerae* and green colonies suspected to be *V. parahaemolyticus*.

Detection of *S. typhi* and *S.flexineri*

S. Shigella agar (SSA) was prepared according to manufacturer's instruction and cooled to 45°C before introducing into a Petri dish and allowed to solidify. One hundred millilitres (100mls) of the water sample were filtered through membrane filter paper. The membrane filter paper was thereafter transferred to the surface of the medium using a sterile forceps. Incubation of the Petri dish was carried out at 37°C for 24 hours in an inverted position. The presences of colourless colonies with black centers were suspected to be *Salmonella species* while colourless colonies without black centers were suspected to be *Shigella* species.

Detection of *P. aeruginosa* and *P. fluorescens*

Cetrimide agar (CA) was prepared according to manufacturer's instruction by weighing out the named gram into conical flask autoclaved and then allowed to cool to 45°C before dispensing into Petri dishes for solidification. The membrane filter paper was thereafter transferred to the surface of the medium using a sterile forceps. Incubation of the Petri dish was carried out at 37°C for 24 hours in an inverted position. The presences of greenish-yellow colonies were suspected to be *Pseudomonas* species.

Detection of *C. perfringens*

Reinforced Differential Clostridial Medium was prepared according to manufacturer's instruction with nystatin and allowed to cool to 45°C before dispensing into the Petri plate for solidification. Hundred (100mls) of the water sample were filtered through membrane filter paper. The membrane filter paper was thereafter transferred to the surface of the medium using a sterile forceps. Incubation of the Petri dish was carried out at 25°C for 72 hours in inverted position in an anaerobic jar after which the was no growth seen.

Characterization and identification of bacterial isolates

The morphological and biochemical characteristics of the isolates in a pure culture were determined using the general microbiological procedures (Cheesbrough, 2010).

Gram-staining and microscopic examination

This was done according to the procedure described by (Cheesbrough, 2010).

Biochemical tests

These biochemical tests were carried out according to (Cheesbrough, 2010). Catalase test, coagulase test, citrate utilization test, oxidase test, urease test, indole test, motility test, voges-proskauer test, methyl red test, sugar fermentation and hydrogen Sulphide Test.

Pathogenicity test (Haemolysis)

This was done according to the method described by (Murray *et al.*, 2003; Ryan *et al.*, 2004). Nutrient agar was sterilized by autoclaving at 121°C for 15 minutes. It was cooled to 45°C under room temperature and 5ml of whole blood (collected from Nnamdi Azikiwe University Medical Centre using Ethylene diamine tetra acetic acid (EDTA) bottle) was added to 100mls of nutrient agar aseptically and swirled properly. Twenty (20mls) of the prepared blood agar was dispensed into sterile Petri plates aseptically and a 24 hours old pure culture of each bacterial isolate was streaked on the blood agar, incubated upside down at 37°C for 24 hours. The pathogenicity of bacteria was confirmed by its ability to destroy the red blood cell component resulting to clear zones and greenish colour indicating beta and alpha haemolysis respectively.

Preparation of 0.5 McFarland standards

This is a barium sulphate standard against which the turbidity of the test isolates can be compared. One (1%) v/v solution of sulphuric acid was prepared by adding 1 ml of concentrated sulphuric acid into 99 ml of water and mixed well. One (1%) w/v solution of barium chloride was prepared by dissolving 0.5 g of dihydrate barium chloride ($BaCl_2.2H_2O$) in 50 ml of sterile water. Then the McFarland standard was prepared by adding 0.6 ml of 1 % w/v solution of barium chloride solution to 99.4 ml of 1% v/v sulphuric acid solution, and mix well to obtain a turbidity standard equivalent to 1.5×10^8 cells per ml. Three (3mls) of the turbid solution was transferred into a test tube and capped with cotton wool.

Preparation of standard inoculums of bacterial isolates

Using a sterile wire loop a 24-hour pure culture of the each bacterial isolate was collected and emulsify in 3ml of sterile physiological saline. A sheet of paper was used to match the turbidity of the bacterial suspension to that of McFarland turbidity standard. A sterile swab stick was used to inoculate the plate of Mueller Hinton agar with the bacterial isolate. Excess fluid were removed by pressing and rotating the swab against the side of the tube above the level of the suspension. The isolates were inoculated evenly over the surface of the medium. The inoculated Petri dish were covered and allowed for 5 minutes for the surface of the agar to dry. Using sterile forceps, the antimicrobial discs of interest were placed on the inoculated plate. (Disc was 15 mm from the edge of the plate and no closer than about 25 mm from disc to disc). Each disc was lightly pressed down to ensure its contact with the agar and incubated aerobically at 37°C for 24 hours.

Antibiotics susceptibility assay

Kirby-Bauer (1996) the Kirby-Bauer disk diffusion method was used to determine the antimicrobial susceptibility profiles of the bacterial isolates. Antibiotic multi-discs used consisted of Ciprofloxacin (10 µg), Amoxicillin (30 µg), Erythromycin (10 µg) Ofloxacin (5 µg), and Perfloxacin (30 µg). The medium used was Mueller Hinton (MH) agar. The medium was Prepared and steri-

lized as instructed by the manufacturer and poured into 90 mm diameter sterile Petri dishes to a depth of 4 mm (about 25 ml per plate). Pure cultures of organisms were enriched in normal saline to a turbidity of 0.5 McFarland standards. The MH agar was seeded with the pure cultures of organisms by swabbing using sterile swab stick. The antibiotic disks were applied using sterile forceps and sufficiently separated from each other in order to prevent overlapping of the zones of inhibition. The agar plates were left on the bench for 30minutes to allow for diffusion of the antibiotics and the plates were incubated inverted at 37°C for 24 hours. Results were recorded by measuring the zone of inhibition using meter rule and comparing with the NCCLS interpretive performance standard for antimicrobial disk susceptibility testing (NCCLS, 2002). The zones size of each antimicrobial, against each organism was interpreted as Susceptible, Intermediate, or Resistant.

Statistical analysis

Statistical analysis of data was done using a two way analysis of variance (ANOVA) to determine the level of significance among the bacteriological analyses of both seasons using SPSS 8.0 package.

RESULTS AND DISCUSSION

Groundwater has been considered as a safe source of drinking water. However, nowadays, the quality of drinking water is deteriorating (Yaduvams *et al.*, 2017). Therefore, the present study focuses on biological properties of the groundwater (borehole) samples collected from mile 50 Abakaliki and was compared with world health standard. During the sanitary risk assessment, it was discovered that most of the borehole were dug wells connected to (constantly open) overhead tank through a pipe and sumo machine for pumping the water, while some are drilled holes that are shallow and sited close to landfill dump site, septic tank, fuel station, poultry house, farm lands, metallurgical workshops, empty lands were people go for open defecation and dump refuse. These wells are often covered with dirty rusted iron sheathing with no proper concrete aprons raised above ground level to prevent contamination from run-off water. Most PVC piping used for pipe water distribution had breakages and leakages which might possibly introduce dirt and leachates from wastes into water. No proper drainage and sewer system existed within the area. Waste water from houses and masses of toxic waste from lead-acid batteries, used oil, junk spare parts, soldering among others from human activities were disposed of haphazardly straight onto the ground which often drained back into the water body.

Bacteriological characteristics of borehole water

The bacteriological parameters investigated in the borehole water during the rainy season were shown in Tables 1 and 2. Total bacterial count ranged between 41-67cfu/ml, total coliform count ranged from 6-23cfu/100ml, faecal coliform count ranged from 0-4cfu/100ml, *V. cholerae* count ranged from

0-7cfu/100ml and *P. aeruginosa* count ranged from 0-7cfu/100ml. The bacteriological parameter investigated in the borehole water samples during the dry season were shown in Tables 1 and 3. Total bacterial count ranged from 31-58cfu/ml, total coliform count ranged from 2-14cfu/100ml, faecal coliform count is from 1-3cfu/100ml, *V. cholerae* count ranged between 1-4cfu/100ml and *P. aeruginosa* count is from 2-4cfu/100ml.

The bacteria load for all the water samples analyzed in this study were generally high, and they exceeded the acceptable limit, high bacteria counts were recorded from all the boreholes across the seasons with borehole 11 having the highest total bacterial count of 67cfu/ml whereas borehole 7 recorded the lowest total bacterial count of 41cfu/ml during rainy season (Table 1). Borehole 11 had the highest total bacterial count of 58cfu/ml while borehole 12 had the lowest bacterial count of 32cfu/ml during the dry season. All the borehole water analyzed exceeded the WHO standards for drinking water quality for the bacterial count.

Table 1. Mean and logarithm of total bacterial counts from borehole water samples for rainy and dry season.

Borehole location	Mean (10^{-2}) (Rainy)	Log (10^{-2}) (Rainy)	Mean (10^{-2}) (Dry)	Log (10^{-2}) (Dry)
SDP	48	3.68	40	3.60
Ebonyi voice	47	3.67	42	3.62
Mile 50	44	3.64	37	3.56
NEPA	55	3.74	49	3.69
Pastoral	50	3.69	47	3.67
Isiuke lane 1	49	3.69	40	3.60
Isiuke lane 2	41	3.61	34	3.53
Ibiam	55	3.74	43	3.63
Ogodo	58	3.76	49	3.69
Alugbaraeze	49	3.69	42	3.62
Amaike Aba	67	3.82	58	3.76
Obasi	42	3.62	31	3.49
Eze& bros	44	3.64	37	3.56
Oroke market	55	3.74	43	3.63
Nkwoagu	55	3.74	48	3.68

Table 2. Occurrence of the bacterial isolates in the borehole water samples during the rainy season.

Borehole location	*S. aureus* (cfu/ml)	*E. coli* (cfu/100ml)	*V. cholerae* (cfu/100ml)	*P. aeruginosa* (cfu/100ml)	Total coliform (cfu/100ml)
SDP	5	2	3	0	6
Ebonyi	0	3	5	0	18
Mile 50	0	2	0	3	9
NEPA	4	3	4	5	9
Pastoral	6	0	3	0	9
Isiukelane1	2	0	0	3	15
Isiukelane2	3	0	0	0	17
Ibiam	0	2	0	0	13
Ogodo	7	4	7	7	23
Alugbaraeze	0	0	4	0	10
AmikeAba	6	3	6	6	17
Obasi	0	2	0	5	10
Eze& bros	4	2	3	0	11
Oroeke market	0	2	3	0	12
Nkwoagu	3	0	0	5	14

Table 3. Occurrence of the bacterial isolates in the borehole water samples during the dry season.

Borehole location	*S. aureus* (cfu/ml)	*E. coli* (cfu/100ml)	*V. cholerae* (cfu/100ml)	*P. aeruginosa* (cfu/100ml)	Total coliforms (cfu/100ml)
SDP	0	0	2	0	2
Ebonyi voice	0	0	0	0	5
Mile 50 layout	0	0	0	2	3
NEPA	0	0	2	0	4
Pastoral	0	0	0	0	0
Isiukelane1	0	0	0	0	2
Isiukelane2	0	0	0	0	10
Ibiam	0	0	0	0	2
Ogodo	3	3	4	0	14
Alugbaraeze	0	0	3	0	0
AmikeAba	2	2	0	4	7
Obasi	0	0	0	3	5
Eze& bros	1	0	0	0	5
Oroeke market	0	1	1	0	3
Nkwoagu	0	0	0	4	2

Table 4. Comparison of the average values of the bacteriological characteristics with the WHO standard.

Borehole location	Total bacterial count	Total coliform count	*E. coli* count	*V. cholerae* count	*S. aureus* count	*P. aeruginosa* count
SDP	44.00	4.00	2.00	2.50	2.50	0.00
Ebonyi	44.50	11.50	1.50	2.50	0.00	0.00
Mile 50	40.50	6.00	1.00	0.00	0.00	2.50
NEPA	52.00	6.50	1.50	3.00	2.00	2.50
Pastoral	48.50	4.50	0.00	1.50	3.00	0.00
Isiukelane1	44.50	8.50	0.00	0.00	1.00	1.50
Isiukelane2	37.50	13.50	0.00	0.00	1.50	0.00
Ibiam	49.00	7.50	1.00	0.00	0.00	0.00
Ogodo	53.50	18.50	3.50	5.50	5.00	3.50
Alugbaraeze	45.50	5.00	0.00	3.50	0.00	0.00
Amike Aba	62.50	12.00	2.50	3.00	4.00	5.00
Obasi	36.50	7.50	1.00	0.00	0.00	4.00
Eze& bros	40.50	8.00	2.00	1.50	2.50	0.00
Oroeke market	49.00	7.50	1.50	2.00	0.00	0.00
Nkwoagu	51.50	8.00	0.00	0.00	1.50	4.50
WHO (2006)	100	10	0	0	0	0

Table 5. Distribution of the bacterial isolates in the borehole water samples during the rainy season.

Borehole location	*S.aureus* (cfu/ml)	*E.coli* (cfu/100ml)	*V. cholerae* (cfu/100ml)	*P. aeruginosa* (cfu/100ml)	*S. typhi* (cfu/100ml)	*S. flexineri* (cfu/100ml)	*K.pneumoniae* (cfu/100ml)	*P. vulgaris* (cfu/100ml)
SDP	+	+	+	-	-	-	+	+
Ebonyi	-	+	+	-	+	-	+	+
Mile 50	-	+	-	+	-	+	-	+
NEPA	+	+	+	+	+	-	+	-
Pastoral	+	-	+	-	-	-	+	+
Isiukelane1	+	-	-	+	+	+	-	-
Isiukelane2	+	-	-	-	+	+	+	+
Ibiam	-	+	-	-	+	+	+	-
Ogodo	+	+	+	+	-	+	+	+
Alugbaraeze	-	-	+	-	+	+	+	-
AmikeAba	+	+	+	+	+	-	+	+
Obasi	-	+	-	+	-	+	-	-
Eze& bros	+	+	+	-	+	+	-	+
Oroeke market	-	+	+	-	+	+	+	-
Nkwoagu	+	-	-	+	-	+	+	+

Key: + = Organism isolated; -= Organism not isolated.

The average values of the bacteriological parameters investigated during rainy and dry seasons compared with the WHO standard were shown in Table 4. Total bacteria count ranged from 36.50-62.50cfu/ml and were within 30-300 WHO standard, 26.7% of the values of total coliform count were above the 10cfu/100ml WHO standard, 66.7% of the values of faecal coliform count were above the 0cfu/100ml WHO standard, *V. cholerae* count ranged between 1.50-5.50cfu/100ml above the 0cfu/100ml WHO approved limit, and *P. aeruginosa* count ranged from 1.50-5.00cfu/100ml above the WHO standard of 0cfu/100ml. The highest total coliform counts of 23cfu/100ml were recorded in sample 9 and lowest count of 6cfu/100ml in sample 1 during the rainy season (Table 2). While sample 9 had the highest total coliform count of 14cfu/100ml and sample 5 and 10 recorded the lowest value of 0cfu/100ml during the dry season (Table 3). Most of the coliform counts in the borehole water exceeded the WHO standard requirement of 10 total coliform counts per 100ml except sample 1, 3, 4, and 5 whose values were within the 10cfu/100ml for rainy season while sample 7 and 9 exceeded the WHO standard of 10cfu/100ml with the rest falling within during the dry season. This implies that the water samples with coliform count more than

10cfu/100ml were unfit for consumption. This finding is in agreement with the work carried out by Obiri-Danso *et al.* (2002) which stated that there were higher bacterial counts during rainy seasons than in dry seasons. It was observed that the number of bacterial count during the rainy season was higher than the count obtained in dry season. (Table 1) Several factors may be attributed to the differences in bacterial count. It could be due to the fact that water availability favours the movement and reproduction of the organisms. Surface runoff that flood through the streets may enter into the borehole water thereby depositing it contents into the water. Also cracked borehole pipes may be a source through which pollutants can get into the borehole water. Pipes used for water distribution were rusty thus allowing seepage of microbial contaminants into the borehole water. This result agrees with the findings of Nkwachukwu *et al.* (2013) that there are high counts of bacteria pathogens in most borehole water in some parts of Nigeria. The total number of bacteria in the borehole water for both rainy and dry season were analyzed using two-way analysis of variance at Alpha level of 0.05 which showed that there was extreme significant difference (*P = 0.0003*) between the water.

Table 6. Distribution of the bacterial isolates in the borehole water samples during the dry season.

Borehole location	S. aureus (cfu/ml)	E. coli (cfu/100ml)	V. cholerae (cfu/100ml)	P. aeruginosa (cfu/100ml)	S. typhi (cfu/100ml)	S. flexineri (cfu/100ml)	K. pneumoniae (cfu/100ml)	P. vulgaris (cfu/100ml)
SDP	-	-	+	-	-	-	+	-
Ebonyi	-	-	-	-	+	-	-	+
Mile 50	-	-	-	+	-	+	-	-
NEPA	-	-	+	-	-	-	+	-
Pastoral	-	-	-	-	-	-	-	-
Isiukelane1	-	-	-	-	+	-	-	-
Isiukelane2	-	-	-	-	+	-	+	+
Ibiam	-	-	-	-	-	+	-	-
Ogodo	+	+	+	-	-	+	+	+
Alugbaraeze	-	-	+	-	-	-	-	-
AmikeAba	+	+	-	+	+	-	-	+
Obasi	-	-	-	+	-	+	-	-
Eze& bros	+	-	-	-	-	+	-	+
Oroeke market	-	+	+	-	-	-	+	-
Nkwoagu	-	-	-	+	-	+	-	-

Key: + = Organism isolated; -= Organism not isolated.

Table 7. Frequency and percentage occurrence of the bacterial isolates for both seasons.

Isolates	Total number of isolates for rainy season	Total number of isolates for dry season	Percentage frequency for rainy season	Percentage frequency for dry season
S. aureus	40	6	12.12	5.94
E. coli	25	6	7.57	5.94
V. cholerae	38	12	11.51	11.88
P. aeruginosa	34	13	10.30	12.87
S. typhi	36	14	10.90	13.86
K. pneumoniae	72	21	21.81	20.79
P. vulgaris	23	8	6.96	7.92
S. flexineri	62	21	18.78	20.79
Total	330	101	100	100

Occurrence and distribution of bacteria present in borehole water

The occurrence of the bacterial isolates in the borehole water samples during the rainy and dry season is shown in Tables 2 and 3. The distribution of the bacterial isolates in the borehole water samples during the rainy and dry season is shown in Tables 5 and 6. The organisms detected in the samples were marked as been positive (+) and those not detected in the samples were marked as negative (-). The Occurrence of the bacterial isolates in the borehole water samples during both rainy and dry season were analyzed using two-way analysis of variance at Alpha level of 0.05 which showed that there was extreme significant difference ($P< 0.001$) between the water. Proximity of the borehole water systems to waste water man-

agement systems may account for the high Total coliform counts observed in this study. This is similar to previous findings by Palamuleni and Akoth (2015), and Ugbaja and Otokunefor (2015), who isolated coliforms from potable borehole water systems located near waste water sewage systems. Also, long term usage of borehole may lead to the deterioration of the water quality, because the pipeline may become corroded with random cracks and in most cases clogged with sediment (Onemano and Otun, 2008). This will allow the passage of inorganic metals and bacteria. Uzoigwe and Agwa, (2012) reported that the presence of high total coliform in the water samples they analyzed provided an indication of waterborne problems and direct threat to human health and should be viewed as a serious concern.

Frequency and percentage occurrence of bacteria present in borehole water

Twenty-five and 6 colonies of *E. coli* were isolated during rainy and dry seasons from the borehole water samples with a percentage frequency of 7.57% and 5.94%, respectively. The presence of bacteria and total coliform in well water indicate contamination by human or animal wastes (Orebiyi *et al.*, 2010). Bacteriologically contaminated water could be the source of out breaks of water diseases such as cholera, dysentery, typhoid fever, diarrhea, and hepatitis (Okeke and Oyebande, 2009). Faecal coliform count obtained from the borehole water sample was of the range (1-4) cfu/100ml in both rainy and dry season more than the zero faecal coliform per100ml specification of W.H.O. It entails the growth of *E. coli* in most of the water sample analyzed. The faecal coliform count value showed that the waters are not acceptable for drinking and requires further treatment. The presence of *E. coli* in any water sample implies faecal contamination of such water (Ukpong and Okon, 2013). The presence of faecal coliforms in the analyzed water sample were attributed to the closeness of borehole water to pit latrines, septic tanks, animal rearing grounds and waste dumping sites hence not observing the 30m distance recommended for siting of boreholes. It is in line with the finding of (Uhuo *et al.* 2014; Ukpong and Okon, 2013) from the borehole water samples they examined. Since coliform is indicative of faecal contamination, the implication is that most of the water samples studied in mile 50 were of poor sanitary conditions, which is in agreement with the report of Banwart (2004) and Edema *et al.* (2001).

Thirty-eight and 12 colonies of *V. cholarae* were isolated from the borehole water sample during rainy and dry season with a percentage frequency of 11.51% and 11.88%, respectively. High number of *V. cholerae* more than the WHO standard of 0 was recorded in most of the borehole water samples in both rainy and dry seasons. With sample 9 having the highest value of 7cfu/100ml and 4cfu/100ml for rainy and dry season respectively. (Table 2 and 3) the presence of *V. cholera*e in the borehole water samples analyzed has a negative health implication as the organism causes cholera. This study showed that more *V. cholerae* was recovered from the water samples during the rainy season than in the dry season (Table 2 and 3). The finding of these work agreed with the report of Onuorah *et al.* (2018) who reported high Number of *V. cholerae* from hand pump borehole water in Onueke. Ten (66.7%) of the boreholes studied during the dry season met the WHO standard of 0cfu/100ml for *V. cholerae* while 40% complied with the standard during the rainy season.

Table 8. Morphological and biochemical characteristics of the bacterial isolates in borehole water samples.

Isolates	Colony morphology	Gram reactions	Microscopy	Motility	Catalase	Citrate	Oxidase	Coagulase
1	Golden yellow on NA	+	Cocci in clusters	Non motile	+	+	-	+
2	Green metallic sheen on EMB	-	Straight rods	Motile	+	+	-	-
3	Lemon green on CA	+	Rods	Motile	+	+	+	-
4	Translucent smooth black small round colonies on SSA	-	Rods	Motile	+	+	-	-
5	Mucoid pink in MA	+	Rods	Non motile	+	+	-	-
6	Yellow colony on TCBS	-	Rods in comma shape	Motile	-	+	+	-
7	Colourless colony on MA	-	Short rods	Motile	-	-	-	-
8	Round colourless colonies	-	Rods	Non motile	+	-	-	-

Key: + = positive; - = Negative.

Table 9. Morphological and biochemical characteristics of bacterial isolates continues.

Isolates	Indole	Urease	Methyl red	Voges Proskauer	H_2S	Sucrose	Glucose	Maltose	Lactose	Identity
1	-	+	+	+	-	A	A	A	A	*S. aureus*
2	+	-	+	-	-	A	A/G	A/G	A/G	*E. coli*
3	-	-	-	-	-	-	-	-	-	*P. aeruginosa*
4	-	-	+	-	+	-	A	A	-	*S. typhi*
5	-	+	-	+	-	A/G	A/G	A/G	A/G	*K. pneumoniae*
6	+	-	+	-	-	A/G	A/G	A/G	-	*V. cholerae*
7	+	+			+	A/G	A/G	A/G	-	*P. vulgaris*
8	-	-	+	-	+	-	A	A	-	*S. flexineri*

Key: A= Acid, A/G = Acid and Gas; -= No acid and gas.

Thirty-four and 13 colonies of *P. aeruginosa* were isolated from the borehole water sample during rainy and dry season with a percentage frequency of 10.30% and 12.87%, respectively. Forty and six colonies of *S. aureus* were isolated from the borehole water sample during rainy and dry season with a percentage frequency of 12.12% and 5.94%, respectively. Thirty-six and 14 colonies of *S. typhi* were isolated during rainy and dry seasons from the borehole water samples with a percentage frequency of 10.90% and 13.86%, respectively. Seventy-two and 21 colonies of *K. pneumoniae* were isolated during rainy and dry seasons from the borehole water samples with a percentage frequency of 21.81% and 20.79%, respectively. *P. aeruginosa* is an opportunistic nosocomial pathogen of immune compromised persons that is found in the soil, water, plants, the skin on moist parts of a healthy human body and most man-made environments. It is associated with urinary tract infections, wound infections, blood infections, dermatitis, osteomyelitis and community acquired pneumonias (Balcht and Smith, 1994). Its occurrence in drinking water is probably related more to its ability to colonize biofilms in plumbing fixtures (i.e. faucets, showerheads, and so on.) than its presence in the distribution system or treated drinking (Mena and Gerba, 2009). The presence of such bacteria as *P. aeruginosa* and *E. coli* is of significant value in determining the extent of water pollution. Pathogens such as *Klebsiella* and *S. aureus* were also isolated. This result agreed with the finding of Anyanwu and Okoli (2012) who reported these organisms from the evaluation of groundwater in Nuskka. *K. pneumoniae* was the predominant bacterium isolated from the water samples during both the dry and rainy seasons while *E. coli* was less frequently isolated during both seasons (Table 5). This result indicated that the environmental conditions were most favourable to *K. pneumoniae* than the other bacterial isolates.

C. perfringens were however not detected in any of the samples analyzed. This result conformed to the works of (Nkamare *et al.*, 2012; Abdullahi *et al.*, 2013; Olajuba and Ogunika, 2014). However, since *C. perfringens* were not detected in any of the samples, the result indicated that the water samples were free from pollution of remote periods.

Twenty-three and eight colonies of *P. vulgaris* were isolated during rainy and dry seasons from the borehole water samples with a percentage frequency of 6.96% and 7.92%, respectively. *P. vulgaris* inhabits the intestinal tracts of humans and animals and can be found in soil, water and faecal matter. It is an opportunistic pathogen that can infect the lung or wounds and frequently causes urinary tract infections, severe abscesses and nosocomial infections O' Hara *et al.* (2000). *P. vulgaris* was isolated more from rainy season than dry season; similar findings were reported by Ukpong and Okon (2013) from private and public borehole water in Uruan Local Government Area of Akwa Ibom State. This could be attributed to rain water which must have introduced bacteria in it to the boreholes and the environmental conditions which must have been more favourable to the organisms. Sixty-two and 21 colonies of *S. flexineri* were isolated during rainy and dry seasons from the

borehole water samples with a percentage frequency of 18.78% and 20.79%, respectively.

These isolates with high frequency of occurrence are important human pathogens associated with a variety of infectious diseases such as gastroenteritis, typhoid fever, dysentery, cholera, urinary tract infection, etc. (Orji *et al.*, 2006; Uzoigwe and Agwa, 2012). The high number of these pathogens in the water samples from study areas needs public health attention. The high prevalence pathogens in this study are in agreement with the findings of Obi and Okocha, (2007) in selected borehole waters in World Bank Housing Estate, Umuahia and of Amajor *et al.* (2012) on enumeration and identification of pathogenic pollution indicators in different water sources used in processing root and tuber crops in Umudike, Umuahia, Abia State, Nigeria.

Morphological and biochemical characteristics of bacterial isolates

The morphological and biochemical characteristics of bacteria isolated from the borehole water samples during rainy and dry seasons were shown in Table 8. *S. aureus*, *P. aeruginosa*, *V. cholerae*, *E. coli*, *K. pneumoniae*, *S. typhi*, *P. vulgaris* and *S. flexineri* were identified.

Pathogenicity of bacterial isolates present in borehole water

The pathogenicity of the bacteria isolates detected in the samples were represented using positive (+) or negative (-) signs indicating their class of pathogenicity.

Antibiotics susceptibility of the bacterial isolates in borehole water

The antibiotics susceptibility of the bacterial isolates to different concentrations of antibiotics were denoted using S, I and R meaning susceptible, intermediate and resistant, respectively when compared with NCCLS (2002) standard as shown in Table 10.

Bacteria isolates were screened for their antimicrobial susceptibility pattern. All the isolated bacteria from the various borehole water samples showed varying levels of susceptibility to the five tested antimicrobial agents (Perfloxacin (10ug), Amoxicillin (30ug), Erythromycin (10ug), Ciprofloxacin (10ug) and Ofloxacin (10ug). Notably, higher levels of resistance to Amoxicillin (30ug), were observed by 62.5% of the bacterial isolates. This finding was similar to that of (Onuorah *et al.*, 2018). While 87.5%, 75% and 62.5% of the bacterial isolates where sensitive to Ciprofloxacin (10ug), Perfloxacin (10ug) and Ofloxacin (10ug) respectively. However, as observed in this study, the sensitivity of all isolates to ciprofloxacin was also reported by (Mulamattathil *et al.*, 2014). Results from their study, revealed that all environmental isolates which included *P. aeruginosa*, *E. coli* and *P. vulgaris* were completely sensitive to ciprofloxacin. They also recorded the resistance of isolates to erythromycin and amoxicillin. In another study by Onuoha (2015), *E. coli* from well water sources exhibited sensitivity to Ciprofloxacin and Perfloxacin. The variation in susceptibility and resistance of the

isolates to different antibiotics could be attributed to the difference in location of the sample sources and drug resistance transfer among the microorganisms within the communities where the boreholes are located. The findings from this study, suggests that the isolates may have acquired resistant genes to the tested antibiotics, probably due to exposure to sub-lethal doses in the environment or possession of intrinsic genes by the isolates. The Antibiotics susceptibility of the isolates in the borehole water during both rainy and dry season were analyzed using two-way analysis of variance at Alpha level of 0.05 which showed that there was extreme significant difference ($P = 0.0258$) between the water.

Table 10. Pathogenicity of the isolates.

Isolates	Alpha	Beta	Gamma
S. aureus	-	+	-
E. coli	-	+	-
V. cholera	-	+	-
P. aeruginosa	-	+	-
S. typhi	-	+	-
K. pneumoniae	-	-	+
P. vulgaris	-	-	+

Key: +: pathogenic (Beta); -: Non-pathogenic (gamma)

Table 11. Antibiotics susceptibility of the isolates.

Antibiotics (ᵤg)	*S. aureus* (cfu/ml)	*E. coli* (cfu/100ml)	*V. cholerae* (cfu/100ml)	*P. aeruginosa* (cfu/100ml)	*S. typhi* (cfu/100ml)	*S. flexineri* (cfu/100ml)	*K. pneumoniae* (cfu/100ml)	*P. vulgaris* (cfu/100ml)
Perfloxacin (10)	S	S	S	I	S	S	S	I
Amoxicillin (30)	R	R	R	R	R	R	R	R
Erythromycin (10)	S	-	-	-	-	-	-	-
Ciprofloxacin (10)	S	S	S	S	S	S	S	I
Ofloxacin (10)	S	S	S	R	I	I	S	S

Source: NCCLS (2002) performance standards for antimicrobial susceptibility testing; Key: <16: Resistance; 16-20: Intermediate; >20: Sensitive.

Conclusion

The assessment of the bacteriological properties in borehole waters located at mile 50 Abakaliki shows that most of the waters were contaminated with coliforms and faecal coliforms capable of causing health hazards to the consumers of the borehole waters. The results showed that the waters were more polluted in rainy season than in dry season. The bacterium that had the highest frequency of occurrence during both rainy and dry season's was *K. pneumoniae* with percentage frequency of 21.81% and 20.79% respectively, and *P. vulgaris* had the least value of 6.96% during rainy season. *E. coli and S. aureus* have the least value of 5.94% during dry season. Amoxicillin (30ug) was mostly resisted by the bacterial isolates among the antibiotics used for susceptibility test. Therefore, the boreholes require further treatment to meet the standards for potable drinking water. However, continuous monitoring should be conducted to determine the extent of pollution by bacteriological pollution indicators from time to time.

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