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The morphological and biochemical characteristics of bacterial isolates of selected borehole water Samples in Agbor, Delta State, Nigeria

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ABSTRACT

Potable water is one that is free from pathogens, low in compounds that are acutely toxic and have great long-term effects on human health.The morphological and biochemical characteristics of bacterial isolates of selected borehole water samples in Agbor, Delta State, Nigeria. Sixty water samples from fifteen boreholes were sampled in June and July, 2022 (rainy season); and January and February, 2023 (dry season). Antibiotic susceptibility test was done by adopting the Kirby-Bauer disc diffusion technique. All the isolated bacteria from the various borehole water samples showed varying levels of susceptibility to the five tested antibacterial agents (Levofloxacin (10ug), Ofloxacin (30ug), Ciprofloxacin (10ug), and Perfloxacin (10ug). Interestingly, ciprofloxacin recorded the highest zones of inhibition, while ofloxacin had the least zones of inhibition. From the data analysis, it was shown that there were significant differences in some of the parameters at 0.05 alpha level. This implied that seasonal variation had significant influence on the quality of water samples assessed. Therefore, there is need to create awareness about the quality of water from the boreholes and the necessity for treatment by the consumers, before it can be used for both drinking and domestic purposes, so as to prevent epidemics.

Keywords Morphological, biochemical, characteristics, bacterial, borehole, Agbor, Delta State and Nigeria.

INTRODUCTION

Potable water is that whichis odourless, colourless, practically tasteless and free from physical, chemical and biological contaminants [1]. Water is exploited by man for several commercial, agricultural,domestic and industrial usages; and the usage of water for any activity usually depends on the cleanness of the water. The quality of water is determined by its physical, chemical and microbiological characteristics [2]. The paucity of water supply in Nigeria has forced residents to depend on shallow dug boreholes as the sources of water for drinking and domestic purposes [2]. Groundwater sources are commonly vulnerable to pollution, which may degrade their quality. Generally, groundwater quality varies from place to place, and this sometimes depends on seasonal changes [3]; [4]. The quality of water also depends on thetypes of soils, rocks and surfaces through which it moves beneath the earth. Access to potable drinking water is a major public health issue in many parts of the world especially in most developing economies (Nigeria inclusive) where water hygiene and sanitation may still be poor.The people living in these countries usually get their primary source of water from boreholes, surface waters such as streams, ponds, rivers and the open skies when it rains.

Population growth coupled with increased industrialization, livestock farming and urbanization have led to frequent contamination of rivers [5]. Due to the inability of some governments to meet the ever-increasing water demand of their populace, most people often resort to groundwater sources such as boreholes as alternative water source. Borehole water therefore, is a primary source of water in most developing nations; and the chemical, physical and biological constituents of these sources of water is a critical public health issue that needs to be ascertained on periodic basis.Borehole water serves as one of the easily accessed and cheap commercial sources of drinking water for a greater number of the Nigerian populace; and they also infer that the conformation of these sources of water to lay down microbiological standards is of public health interest because of their capacity to spread diseases within a large population. The provision of potable water to both the rural and urban population is necessary to prevent public health hazards such as the emergence and spread of waterborne pathogens [6]; [7]. Agbor in Delta State depends mostly on ground water, its abstraction account for 20% of the totalwater usage. Currently, demands for groundwater usage have been increasing due to population growth and diminishing opportunities to economically develop potable water supplies [8]. The management of the resource is lagging behind the pace of development, and often, very little sanitary practice is exercised in its exploitation. The current groundwater resources development and supply status is unacceptably low and needs a major transformation [9]. The digging of more boreholes in Agbor brings the need to monitor the issue of water quality thatremains a major contender of its supposed existence in abundance essentially its quality is as equally important as its quantity. The quality of water is of vital concern for mankind since it is directly linked with human welfare. According to [10], the quality of public health depends to a greater extent the quality of groundwater. Physicochemical and bacteriological parameters of water indicate the safety of potable water [11] and their analysis is important for public Health and pollution studies [12].The increase in the prevalence of waterborne diseases across the world is alarming, and Nigeria isnot left out since some outbreaks of waterborne diseases have also been reported in this part of the world [13]; [14].Since borehole water is an important alternative source of potable water to most people in both therural and urban areas across the world, it is vital to evaluate their physicochemical and microbiological quality since these sources of water are usually at risk of pollution from human and other environmental activities. Thus, this study determined the physical, chemical andbacteriological quality of some selected borehole water sources in Agbor, Delta State owing to thefact that this source of water is commonly patronized in this region.

Aim of the study

The aim of this study was to determine the morphological and biochemical characteristics of bacterial isolates of some selected borehole water samples in Agbor, Delta State, Nigeria

MATERIALS AND METHODS

Study area

Agbor is the headquarters of Ika South Local Government Area of Delta State in the South Southern part of Nigeria. Agbor has a population in excess of 250,000 people and a land mass of 685km^2. The geographical coordinates of Agbor are 6.254^{o}C latitude, 6.194^{o}C longitude and 427ft elevation. The topography within two miles of Agbor contains only variations in the elevation with a maximum elevation of 367ft and an average elevation above sea level of 504ft. Agbor lies within the equatorial climate with two distinct seasons, the wet (April to September) and dry (October to March), high humidity of between 24^{o}C to 27^{o}C, which supports the rainforest vegetation. The discovery and exploration of oil in commercial quantity in Ekuku Agbor and environs have earned Agbor the status of oil producing city within the Niger Delta region of Nigeria. Agbor has the blessing of abundant natural water bodies. The people of Agbor are knownfor commerce and agricultural activities. Agbor hosts amenities such as University of Delta, Delta College of Nursing, 181 Army Amphibious battalion,National Psychiatric Hospital, General Hospital, Area Command of Nigeria Police Force, and the adorable Dein Royal Palace. The Deinof Agbor is the traditional ruler of Agbor Kingdom with over seventy communities [15]. A drive around the borehole locations was undertaken to enable proper capture of the accurate borehole points using the handheld Global Positioning System. The coordinates of the actual positions were acquired and inputted on the Google map. On-screen vectorization of features like towns, roads and boundaries were directly observed and recorded. The features from the Google map were zoomed to a very high resolution where all the features became very clear as represented in the samples map presented in this work. The mapping was carried out incollaboration with Geo-trust Project Services Ltd, a surveying firm in Agbor.

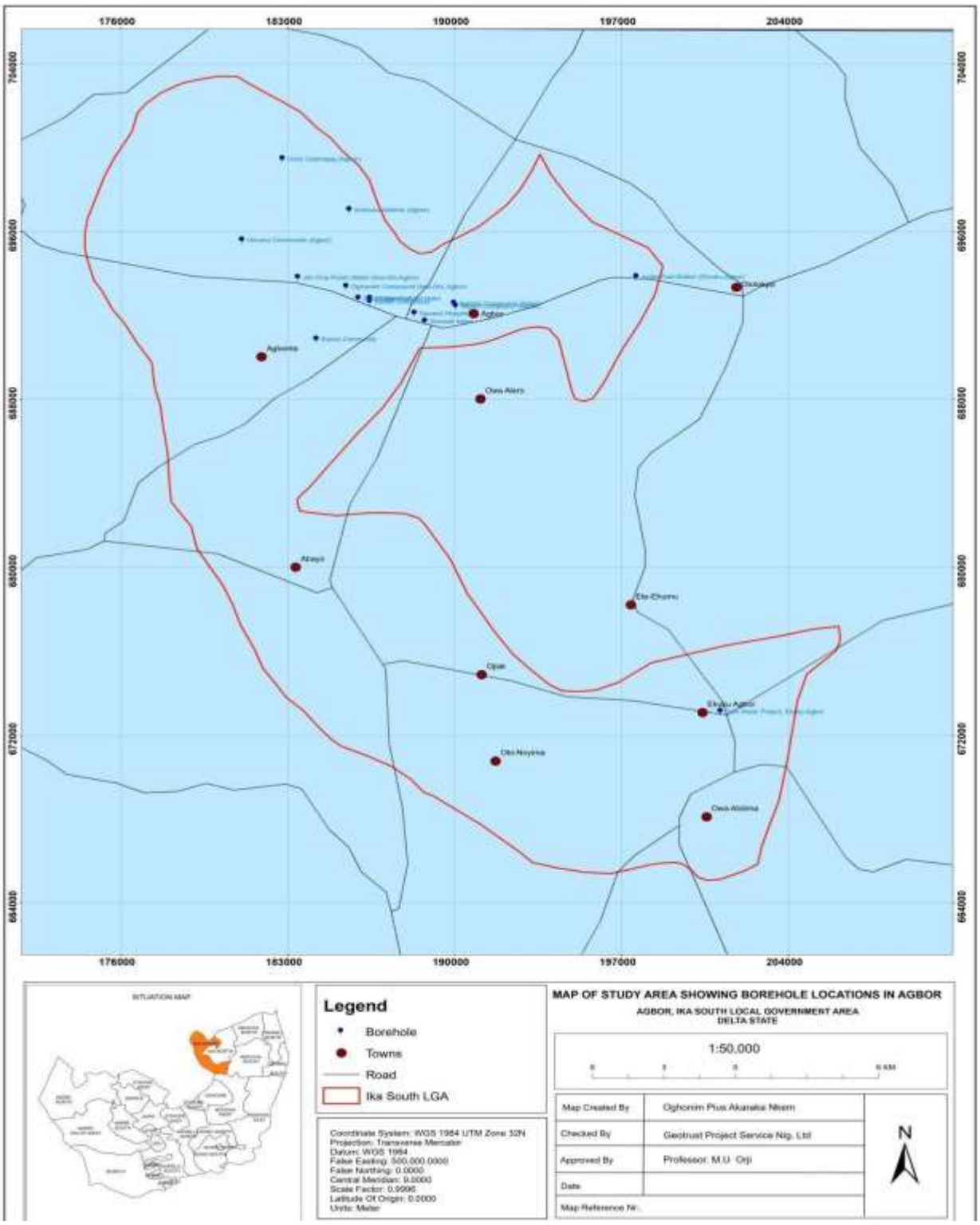

Fig. 1: Map of the study area, 2023. **Source: Geo-trust Project Services Ltd.**

Sample collection of bacteriological analysis

All the sampled points were selected randomly within Agbor, Delta State. Sixty (60) samples wereaseptically collected from 15 different boreholes between June and July, 2022 (rainy season); January and February, 2023 (dry season). The selected borehole waters were those used for drinking and for other domestic purposes. These water samples were collected in the morning period (7am – 9am). During the rainy season, fifteen samples were collected in June, analyzed and recorded, this was repeated in July and average of the values was taken, making it a total of thirty samples. This was also done in the dry season period to give a total of sixty samples for both seasons. Samples for physicochemical analysis were collected using one-litre plastic containers. The containers were first washed with sterile water and thereafter it was rinsed with water from the respective boreholes three times before collecting the water samples. Samples for the bacteriological analysis were aseptically collected in sterilized one-litre plastic container (which was sterilized by rinsing with 70% ethanol and then with sterile water and thereafter with the respective water sample three times before collection). The water was left to rush for 2 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 decontaminate the faucet of the borehole before collection. The collected samples were kept at 4°C in the cooler box packed with ice and transported to the laboratory for analysis within two hours as described by [16]. These boreholes were: Oghonim, Aziken, Agholor, Aliagwu, Dein, Golden Cocktail, Police,Ewuru, Jim Ovia, Omumu, Uvbe Ozanogo, Idumukwu, Central Hospital, Lucky Irabor, and Jodes.

Characterization and identification of the bacterial isolates

Cultural characteristics

The cultural characteristics of the respective isolates were examined and recorded.

Gram-staining and microscopic examination

This was done according to the procedure described by [16].

Each of the pure isolates was sub-cultured by streaking on fresh nutrient agar plate, incubated for 24 hours at 37^{0}C, and thereafter a smear was carefully prepared from each by adding a drop of sterile water on the microscopic slide and then the test organisms. The smear was prepared from each of the pure isolates, air-dried properly and heat-fixed by passingthe slide for three (3) times over the Bunsen flame. Fixed smears were covered with crystal violetstain for 60 seconds. The stain was rapidly washed off with sterile water. Each smear was coveredwith Lugol's iodine for 60 seconds, and the iodine washed off with sterile water. This was decolorized rapidly (two seconds) with acetone-alcohol and washed immediately with sterile water. Each smear was covered with counter-stain (safranin) for 60 seconds. The stain was then washed off with sterile water, the back of the slide wiped clean using a clean cotton cloth, and placed in the draining rack for the smear to air-dry. When completely air-dried, each of the smear was examined under a microscope at × 100 objectivelens to check the bacteria and recorded as Gram-positive (purple color) or Gram-negative (pink orred color).

Biochemical tests

These biochemical tests were carried out as described by [16].

Catalase test

A colony of the test organism from the sub-cultured Petri-plate was smeared on a clean grease- free glass slide and two drops of 3% hydrogen peroxide (H_2O_2) was placed on it. The presence or absence of bubbles indicated a positive or negative result respectively.

Coagulase test

EDTA (Ethylene di-amine tetraacetic acid) bottle was used to dispense the blood to * avoidagglutination. The blood was allowed to settle. A drop of normal saline and a colony of the isolatewas smeared on a clean grease free glass slide. A drop of plasma was added and checked for agglutination within 10 seconds. EDTA is an anticoagulant. Coagulase is produced by *Staphylococcus aureus* and it converts fibrinogen to fibrin.

Citrate utilization test

The medium used was Simmon citrate agar. This agar medium was prepared according to manufacturer's instruction and autoclaved. Slants were made after which the test organisms were streaked onto the slant and incubated at 37^{0}C for 24hrs. A change in color from green to bright- blue colour indicated a positive result, but if the medium retained the green colour after incubationthen the bacteria is citrate negative.

Oxidase test

Sterile filter paper (they were wrapped in a foil and autoclaved) was placed in a sterile Petri-dish after which it was flooded with freshly prepared oxidase enzyme and excess was removed. Using a sterile wire loop, a colony of the test organism was removed and smeared on the filter paper. The development of a blue-purple colour within 10 seconds indicated positive result.

Urease test

Two hundred milliliters (200 ml) of water were sterilized and cooled before adding urea crystals (Christensen's modified urea broth) that was prepared according to manufacturer's instruction. Equal volume of urea agar base was prepared and sterilized in a 250 ml conical flask and cooled properly. The urea solution was then added to the cooled urea agar base (45^{0}C) and mixed gently to give amber color. Five mls were dispensed into test tubes for slant formation. The pure colonies of the test organisms were streaked atop the slant and incubated for 24 hours at 37^{0}C. Positive results were shown by pink colour, while otherwise proved negative.

Indole test

Peptone water was prepared according to manufacturer's instruction and 3mls were dispensed into test-tubes depending on the number of the test organisms, covered with cotton wool and foil then autoclaved. They were allowed to cool and a colony of the test organisms were inoculated. It was incubated at 37^{o}C for 24hrs and checked for turbidity, then 0.5ml of kovac's reagents were added into each of the test tubes and allowed to stand for 10 minutes. The presence of red-like ring on the surface indicated positive result. Note: Kovac's reagent was kept in a brown bottle to avoid oxidation which will give false result.

Motility test

Agar stab method was employed. The test organisms were sub-cultured in a sterile Petri-plate. Nutrient agar medium was prepared according to manufacturer's instruction and allowed to gel, a straight wire containing the colony of the test organisms were used in making a stab into the agar tube and incubated at 37^{0}C for 24hrs. The motile organisms moved away from lines of stab when the tubes were held to a bright light, indicating positive while non-motile ones grew along the stab made with the straight wire indicating negative result.

Voges-proskauer test

Glucose phosphate peptone broth (MR-VP broth) was prepared and one milliliter of cooled glucose phosphate peptone broth (MR-VP broth) was dispensed into sterile test tubes and pure culture of the test organisms were inoculated into the broth**,** incubated for 24 hours at 37^{o}C and turbidity checked. 0.6 ml of 5% alpha naphthol was added followed by 0.2 ml of 40% KOH. The tubes were shaked gently and allowed to stand undisturbed for 10 minutes. A positive test was represented by the development of a red color after 15 minutes or more. The test should not be read after standing for over one hour because negative Voges-Proskauer cultures may produce a copper like color, potentially resulting in a false positive interpretation. The test gives a negative result when yellow or copper colour is seen at the surface of the medium indicating that acetoin was absent.

Methyl red test

Glucose phosphate peptone broth (MR-VP broth) was prepared and 0.5 milliliters (0.5 ml) of cooled glucose phosphate peptone broth (MR-VP broth) was dispensed in sterile test tubes and pure colony of the test organisms were inoculated into the broth**,** incubated for 24 hours at 37^{o}C. A drop of methyl-red solution was added to the test tubes. A positive test was represented by the development of a bright-red color indicating acidity while yellow colour proved negative.

Sugar fermentation test

The sugars used were glucose, lactose, maltose and sucrose. One gram of the sugars was weighed into 4 different conical flasks (250mls). Seven hundred milliliters of peptone water were prepared according to manufacturer's instruction and bromothymol blue indicator was added until the colour changes to light blue. 175 mls each was dispensed into the various conical flasks harbouring the different sugars and mixed gently by swirling. Ten milliliters of the resulting solutions were dispensed into labelled test tubes with Durham tubes, capped and autoclaved at 115^{0}C for 15 minutes. The tubes were allowed to cool at room temperature and the isolates inoculated and incubated at 37^{0}C for 48 hours. Positive results were shown by acid and gas production. Gas production was indicated by the presence of gas at the top of the Durham tube. Acid production was indicated by change in color of the medium.

DNA extraction

This was done according to Maldi-Tof Mass Spectrometry (2011). 200 µl of each sample was added to a micro-centrifuge tube. 200 µl of BioFluid and Cell Buffer and 20 µl of Proteinase K was added to it and mixed thoroughly using a vortex for 10-15 seconds. The tubes were incubated the tube at 55^{o}C for 10 minutes on a heating block. One volume Genomic Binding Buffer (that is 420µl) was added to the digested sample and mixed thoroughly with a vortex mixer for 10-15 seconds. The mixture was then transferred to a Zymo-Spin™ IIC-XL Column in a Collection tube and centrifuged at 12,000 x g for a minute. The collection

tube was discarded with the flow through. 400 µl DNA Pre-Wash Buffer was added to the spin column in anew Collection Tube and centrifuged at 12,000 x g for a minute. The collection tube was emptiedand 700 µl g-DNA Wash Buffer was added to the spin column and centrifuged at ≥ 12,000 x g fora minute. The spin column was then transferred to a clean micro-centrifuge tube. 50 µl of DNA Elution Buffer was added directly on the matrix and incubated for 5 minutes at room temperature,then centrifuged at maximum speed for a minute to elute the DNA. The eluted DNA was stored ≤ -20ºC for future use.

Agarose gel electrophoresis

This was done according to Maldi-Tof Mass Spectrometry (2011).One (1) % agarose gel was prepared by dissolving 1g of Agarose in 100ml of 1X TAE Buffer. The mixture was heated to a clear solution using a microwave oven and allowed to cool to about50ºC. 8µl of gel stain (GR green) was added into the solution and mixed thoroughly. The agarosepreparation was carefully poured into a gel tray, with the gel comb in place and allowed to solidify.The tray was loaded into the gel tank and 1X TAE Buffer was poured into the tank, making surethat the gel was properly submerged. The gel comb was carefully removed. 3 µl of DNA wasmixed with 2 µl of loading dye and loaded into the holes. The tank was connected to the powerpack and set to run at 100 volts for 20 minutes.The bands were viewed using the gel documentation system.

Polymerase chain reaction

This was done according to Maldi-Tof Mass Spectrometry (2011). 12.5µl of One *Taq* Quick-Load 2X Master Mix with Standard Buffer (New England Bio labs Inc.) 0.5µl each of forward and reverse primers; 8.5µl of Nuclease free water and 3µl of DNA templatewas used to prepare 25µl reaction volume of the PCR cocktail. The reaction was gently mixed andtransferred to a pre-heated thermal cycler.

Amplification conditions for the PCR was as follows:

The mixture was left for three minutes at 94ºC to denature the DNA, followed by 35 cycles of denaturation at 94ºC for 20 seconds, primer annealing at 56ºC for 45 seconds and strand extension at 68ºC for 5 min on an Eppendorf nexus gradient Master cycler (Germany). PCR products were separated on a 2% agarose gel and DNA bands were visualized with syber gold.

Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual, while the sequencing kit used was that of Big Dye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA X were used for all genetic analysis.

Antibiotics Susceptibility Assay

The antibiotic susceptibility of the bacterial isolates was determined using Kirby-Bauer (1996) disc diffusion technique. The plating medium was Mueller-Hinton agar and commercial antibioticsused were Levofloxacin (10µg), Ofloxacin (30µg), Erythromycin (10µg), Ciprofloxacin (10µg), Pefloxacin (10µg).

Preparation of 0.5 McFarland Standard and Standardization of Inoculum as described by Chessbrough (2010).

This is a barium sulphate standard against which the turbidity of the test isolates can be compared.1% w/v solution of barium chloride solution was prepared by dissolving 0.5 gram of dihydrate barium chloride ($BaCl_2.2H_2O$) in 50 ml of sterile water. 1% v/v solution of sulphuric acid solution (H_2SO_4) was prepared by adding 1ml of concentrated sulphuric acid into 99 ml of sterile water, mixed by swirling.McFarland standard was prepared by adding 0.6ml of 1% w/v solution of barium chloride solutionto 99.4ml of 1% v/v solution of sulphuric acid solution to obtain a turbidity standard, which is equivalent to 1.5×10^8 cells per ml. A small portion (3ml) of the resultant turbid solution was transferred into sterile test-tube and plugged with cotton wool.Using a sterile wire loop, 3 well-isolated colonies of each of the test organisms was emulsified in3ml of normal saline, which was also plugged with cotton wool. With the aid of a sheet of paper, and in a good light, the turbidity of the suspension was matched with the turbidity of the standard, and each of the standardized inocula kept for use but not later than 15 minutes. If the suspension is not turbid enough, more bacteria can be added but if too turbid, it can be diluted with more normal saline which is the diluent. Muller-Hinton agar was prepared according to manufacturer's instruction (Appendix iii). It was allowed to cool to about 45ºC before dispensing into the labelled Petri-plates and allowed to gel. The standardized inocula were seeded onto the gelled medium using swab sticks. The commercially prepared antibiotic discs were placed atop the medium using sterile forceps and incubated at 37ºC for 24 hours. Zones of inhibition were measured using meter rule. Interpretationof the zone sizes was done by referring to the standard tables according to NCCLS guidelines andfor consideration whether the organism is susceptible, intermediate or resistant to that particular antibiotic as described by [17].

Data Analysis

Data analysis was done using a two-way analysis of variance (ANOVA) to determine the disparitybetween the physicochemical and bacteriological parameter during both seasons.

RESULTS

The 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 1. *Escherichia coli*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Proteus mirabilis* strain LYRY45, *Proteus mirabilis* strain AMJ230, and *Salmonella enterica* were identified

Table 1: Morphological and biochemical characteristics of the bacterial isolates in borehole water samples

Isolates	Colony morphology	Gram reactions	Microscopy	Motility	Catalase	Citrate	Oxidase	Coagulase
1	Green metallic sheen on EMB	-	Straight rods	Motile	+	+	-	-
2	Circular lemon green on CA	-	Rods	Motile	+	+	+	-
3	Circular lemon green on CA	-	Rods	Motile	+	+	+	-
4	Mucoid pink in MA	+	Rods	Non-motile	+	+	-	-
5	Colourless mucoid colonies	-	Rods	Motile	+	+	-	-
6	Colourless mucoid colonies	-	Rods	Motile	+	+	-	-
7	Translucent smooth black small round colonies on SSA	-	Rod	Motile	+	+	-	-

positive. = Negative EMB = Eosine methylene blue agar. CA = Cetrimide agar. SSA = Salmonella-Shigella agar. MA = MacConkeyagar.

Morphological and biochemical characteristics of bacterial isolates continued

Isolates	Indole	Urease	Methyl red	Voges Proskauer	H_2S	Sucrose	Glucose	Maltose	Lactose	Identity
1	+	-	+	-	-	A	A/G	A/G	A/G	*Escherichia coli*
2	-	+	-	-	+	-	-	-	-	*Pseudomonas fluorescens*
3	-	-	-	-	-	-	-	-	-	*Pseudomonas aeruginosa*
4	-	+	-	+	-	A/G	A/G	A/G	A/G	*Klebsiella aerogenes*
5	-	+	+	-	+	-	A/G	-	-	*Proteus mirabilis* strain LYRY45
6	-	+	+	-	+	-	A/G	-	-	*Proteus mirabilis* strain AMJ230
7	-	-	+	-	+	-	A	A	-	*Salmonella enterica*

Key A= Acid, A/G = Acid and Gas

- = No acid and gas

The antibiotics susceptibility of the bacterial isolates in borehole water samples

The antibiotics susceptibility of the bacterial isolates to different concentrations of antibiotics weredenoted using S, I and R meaning susceptible, intermediate and resistant respectively when compared with [17] standard as shown in Table 2. The Antibiotics susceptibility of the isolates in the borehole water during both rainy and dry seasonwere analyzed using two-way analysis of variance at Alpha level of 0.05 which showed that therewas no significant difference ($p = 0.0863$).

Table 2: Antibiotics susceptibility of the isolates

Antibiotics (μg)	*Escherichiacoli*	*Pseudomonas fluorescens*	*Pseudomonas aeruginosa*	*Klebsiella aerogenes*	*Proteus mirabilis* strain LYRY45	*Proteus mirabilis* strain AMJ230	*Salmonella enterica*
Levofloxacin (10)	25	22	21	23	19	20	24
Ofloxacin (30)	19	21	20	24	22	18	23
Ciprofloxacin (10)	28	30	24	25	20	22	26
Perfloxacin (10)	23	21	19	21	25	28	24

Source: [17] performance standards for antimicrobial susceptibility testing;
Key<16: Resistance, 16-20: Intermediate, >20: Sensitive

DISCUSSION

Groundwater exploitation is generally considered as the only realistic option for meeting dispersed rural and urban water demand. Due to inability of governments to meet the ever-increasing water demand, residents' resort to shallow wells etc as alternative water resources for domestic purposes. The effect of uncontrolled disposal systems and other bad sanitary practices in Agbor can render groundwater unsafe for human, agricultural and recreational use, hence, posing a threat to human life and is therefore against the principle of sustainable development.The result of the bacteriological characteristics showed that Gram negative bacteria were dominantin the studied borehole water samples. The bacterial identification revealed the presence of seven isolates; *Escherichia coli*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Proteus mirabilis* strain LYRY45, *Proteus mirabilis* stain AMJ230, and *Salmonella enterica*. The isolates were detected in higher number in the rainy seasonthan the dry season. This may be attributed to increased contamination from surface water run-offs, steady rainfall, and bad sanitary practices in rainy season. The coliforms isolated were an indication of the contamination of the water with fecal materials. The fecal material maybe as a result of the proximity of boreholes to septic tanks, agricultural land, compost sites, and poor sanitary level. 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 antibacterial agents (Levofloxacin (10ug), Ofloxacin (30ug), Ciprofloxacin (10ug), and Perfloxacin (10ug) (Table 2). According to [17] standard, the isolates showed varying degrees of sensitivity to the antibacterial agents. Interestingly, ciprofloxacin recorded the highest zones of inhibition, while ofloxacin had the least zones of inhibition. These findings were similar to that of [18] who stated that 87.5%, 75% and 62.5% of the bacterial isolates from water samples were sensitive to Ciprofloxacin (10ug), Perfloxacin (10ug) and Ofloxacin (10ug) respectively. The variation in susceptibility of the isolates to different antibiotics could be attributed to the difference in location of the sample sources and level of drug resistance transfer among the bacteria within the communities where the boreholes are located.The results of the antibiotic susceptibility test did not vary significantly ($p < 0.0863$) at 0.05 alphalevel of significance against the isolates using two-way analysis of variance (ANOVA).

CONCLUSION

The water samples from the boreholes examined in Agbor, Delta State, Nigeria were of poor quality with regards to bacteriological parameters. The detection of total bacteria, total coliforms, faecal coliforms, and other pathogenic bacteria in significant numbers indicated that the water samples are not potable for human consumption and this may be attributed to poor sanitary practices by residents.

REFERENCES

1. Engwa, A.G., Tagbo, N.R., Iyala C.P.J. and Unaegbu, M. (2015). Physicochemical and Microbial Analysis of Potable Water Sources in Enugu Metropolis. *Journal of Public Health and Epidemiology*, **7**(3):65-70.
2. Agwu, A., Avoaja, A.G. and Kalu, A.U. (2013). The Assessment of Drinking Water Sources in Aba Metropolis, Abia State, Nigeria. *Environmental Research*, **3**(4):72-6.
3. Trivedi, P., Bajpai, A. and Thareja, S. (2010). Comparative Study of Seasonal Variations in Physicochemical Characteristics in Drinking Water Quality of Kanpur, India with Reference to 200 MLD Filtration Plant and Groundwater. *Natural Science journal*, **8**(4):1-7.
4. Vaishali, P. and Punita, P. (2013). Assessment of Seasonal Variations in Water Quality of River Mini, at Sindhrot, Vadodara. *International Journal of Environmental Sciences*, **3** (14):24-36.
5. Edokpayi, J.N., Odiyo, O., Msagati, T.A.M. and Potgieter, N. (2015). Temporal Variations in Physicochemical and Microbiological Characteristics of Muudi River, South Africa. *International Journal of Environmental Research and Public Health*, **12**(4):28-40.
6. Josiah, J.S., Nwangwu, C.O.S., Omage, K., Akpanyung, O.E. and Amaka, D.D. (2014). Physicochemical and Microbiological Properties of Water Samples used for Domestic purposes in Okada town, Edo State, Nigeria. *International Journal of*
7. Olajubu, F.A. and Ogunika, F. (2014). Assessment of the Physicochemical and Microbiological Properties of Borehole Water Samples from Akungba-Akoko, Ondo State, Nigeria. *International Journal of Pharmaceutical Science and Research*, **5**(7):367-74.
8. Akpoveta, O.V., Okoh, B.E. and Osakwe, S. A. (2011). Quality Assessment of Borehole Water used in the Vicinities of Benin, Edo State and Agbor, Delta State of Nigeria. *Current Research in Chemistry*, **3**: 62-69.
9. Nwankwoala, H. O. (2011). An integrated approach to sustainable groundwater development and management in Nigeria. *Journal of Geology and Mining Research*, **3** (5), 123-130.
10. Rajana, A. (2010): Physicochemical Analysis of some Groundwater Samples of Kotputli Town Jaipur, Rajasthan India.*International Journal of Environmental and Pharmaceutical Research*, **1**(2): 111-113.
11. MacDonald, A. M. and Calow, R.C. (2009) Developing groundwater for secure *Water supplies in Africa.* Desalination, **248**, 46-56.
12. Kot, M. (2013). Do invading organisms do the wave? *Canada Applied Math. Quarterly*,**10**, 139- 170.

13. Obi, C.N. and George, P. (2011). The Microbiological and Physicochemical Analysis of BoreholeWaters used by Off- campus Students of Michael Okpara University of Agriculture, Umudike (MOUAU), Abia State, Nigeria. *Research Journal of Biological Sciences*, **6**(11):2-7.
14. Olobaniyi, S.B., Ogban, F.E., Ejechi, B.O. and Ugbe, F.C. (2004). Assessment of Groundwater Across Delta State: Effects of man, geology and hydrocarbon exploitation. *Final report*. Delta State University. 100p.
15. Cheesbrough, M. (2010). District Laboratory Practise in Tropical Countries. Part 2. 2nd edn.
16. NCCLS (2002). National Committee for Clincal Laboratory Standards. Performance standards forantimicrobial susceptibility testing. United States of America.
17. Onuorah, S., Nwoke, J. and Odibo, F. (2018) Bacteriological Assessment of the Public Hand-PumpBorehole Water in Onueke, Ezza South Local Government Area, Ebonyi State, Nigeria *International Journal of Photochemistry and Photobiology* **2**(2): 39-48.
18. Palamuleni, L. and Akoth, M. (2015) Physicochemical and Microbial Analysis of Selected Borehole Water in Mahikeng, South Africa *International* Journal *of Environmental Research Public Health* **12** (8): 8619–8630.

Oghonim, Pius Akaraka Nkem (2023). The morphological and biochemical characteristics of bacterial isolates of selected borehole water Samples in Agbor, Delta State, Nigeria. NEWPORT INTERNATIONAL JOURNAL OF BIOLOGICAL AND APPLIED SCIENCES (NIJBAS) 3(2):242-253