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Evaluation of Physicochemical and Bacteriological Properties of Borehole Water in Selected Areas of Benin City, Edo State

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Abstract
Water is important in African towns and cities, and its quality is paramount to public health. This study therefore, assessed the physicochemical and bacteriological properties of borehole water samples from agricultural, animal farm, and residential areas in Benin City, Edo state. Physicochemical and bacteriological analyses of water samples were based on standard methods. Sheep blood agar was used for hemolytic bacterial isolation, and isolates were subsequently assessed for antibiotic resistance to common antibiotics using Kirby-Bauer disc diffusion method. Physicochemical parameters of borehole water samples assessed were within World Health Organization (WHO) standard with the exception of Total Suspended Solids (TSS). Bacterial analyses revealed counts ($1.01\pm0.10\times10^4$ to $4.80\pm0.10\times10^4$ cfu/mL) that were above WHO standard of 100 cfu/ mL as well as high coliform count (1.20 ± 0.23 - 3.00 ± 0.63 MPN/10 mL) above WHO standard of 0 MPN/ml. *Shigella* sp. (23.29%) and *Bacillus cereus* (21.92%) were the two isolates that occurred most in the study. Hemolytic bacterial pathogens recovered were *Bacillus cereus, Staphylococcus aureus, Bacillus subtilis* and *Micrococcus* sp. All four pathogens were resistant to the antibiotic ceftriaxone but *Staphylococcus aureus* had multiple antibiotic resistant index (MRI) of 0.8 before plasmid curing. MRI decreased in all pathogens with *S. aureus* having 0.4 while *Bacillus subtilis* and *Micrococcus luteus* had multiple drug resistant (MDR) of 0.1 after plasmid curing. This study has shown that borehole water could be sources of bacterial pathogens and therefore, should be properly treated before use. This would help to reduce the incidence of waterborne infections in communities.

Keywords: Borehole water, Physicochemical, Bacteria count, Coliform, Antimicrobial Resistance, Public health

Introduction
Water is one of the most important and precious natural resources. It is essential in the life of all living organisms from the simplest plants and microorganisms to the most complex living system known as human body (WHO, 2011). Groundwater represents the world's most extracted raw material (982 km^3 /annum), and supplies approximately 31.5% (2.2 billion people) of the global population with domestic drinking water (Andrade *et al.*, 2018). For example, 138.5 million Americans get their daily drinking water from a groundwater source (US EPA, 2015). Groundwater exists within earth's surface and becomes borehole water when pumped or conveyed to the surface via a network of pumps. It is an important source of drinking water in African towns and cities however, quality is been compromised because of anthropogenic sources of pollutions (Cronin *et al.*, 2007).

Access to safe drinking water has improved over the last decades in most parts of the world, but approximately 1.1 billion people still lack access to safe potable water, and over 2.6 billion worldwide lack access to adequate sanitation which causes water-borne diseases (Ademola *et al.*, 2011; Adebisi *et al.*, 2016). In Nigeria, majority of the rural populace do not have access to potable water (Oyedeji and Moninuola, 2011). Only few people can afford and rely on treated bottled water for consumption therefore, borehole water serves as major sources of drinking and domestic water used by the local populace (Adejuwon and Mbuk, 2011).

Groundwater pollution occurs when different anthropogenic pollutants released on the ground make their way down into groundwater. Intensive livestock and agricultural farming could contribute to groundwater contamination as these are sources of nitrate (Sahoo *et al.*, 2016). Again, residential areas with septic systems could be potential sources of nitrate, pathogenic bacteria, viruses and synthetic organics into groundwater (Cronin *et al.*, 2007). Pathogens such as *Salmonella* spp., *Shigella* sp., *Vibrio cholerea* and *Escherichia coli* that are shed into water bodies and perhaps groundwater through fecal contamination perpetuate many diseases such as typhoid fever, cholera and diarrhoea. Factors such as soil type, pore size, porosity, season, flooding and water table level play important roles in ground water pollution (Ademola *et al.*, 2011). Flooding is a major concern since it is widely accepted that climate change will increase the frequency and intensity of significant flood events into the future (Andrade *et al.*, 2018).

The chemical, physical and biological characteristics of groundwater are of major importance in determining whether or not the water is suitable for drinking and domestic use (Okonko *et al.*, 2009). This study determined the physicochemical and bacteriological properties of borehole water samples from agricultural, animal farming and residential areas in Benin City, Edo State.

Materials and Methods
Sampling Location
This study was carried out in Ikpoba-Okha Local Government Area, Benin City, Edo State. Edo state is located in southwestern part of Nigeria at longitude 6.6°N and 5.9°E. It is about 40 miles from the Gulf of Guinea.

Borehole Water Sample Collection
A total of 45 Borehole water samples (5 samples each in replicate) were obtained from three (3) different locations; an agricultural area (as location A), animal farm area (as location B), and residential area (as location C) in Benin City, Edo state. Borehole nuzzles were sterilized with 70% acetone and allowed to run for two minutes before sample collection using sterile containers. Samples for Dissolved Oxygen (DO) and Biochemical Oxygen Demand (BOD) were collected in 250mL bottles with stoppers, 1mL each of Winkler's solutions A (MnSO4) and B (Akali-iodide-Azide) were added to the samples on site to fix the Oxygen. All samples were collected between the hours of 7.00 am and 9.00 am and carefully labeled and transported in ice-cooler to

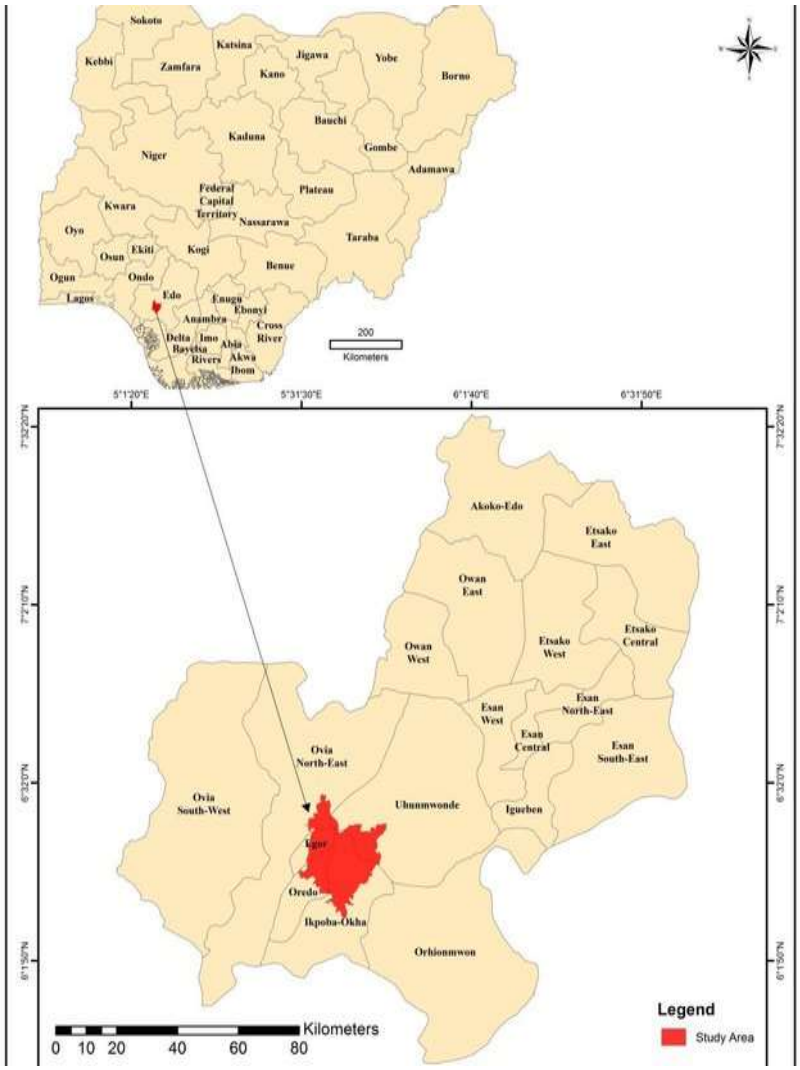

Figure 1: Map of study location (Ikpoba-Okha Local Government Area, Benin City, Edo State.

the laboratory for analysis within 4 h of collection (Tsegahun *et al.*, 2017).

Physicochemical Analysis of Borehole Water Samples

The physicochemical properties of the borehole water samples were determined using standard methods for analysis of water (APHA, 1999; AOAC, 2000). Parameters determined were: Temperature, pH, Electrical Conductivity, Total Dissolved Solids, Total Suspended Solid, Turbidity, Dissolved Oxygen, Biological Oxygen Demand, Chemical Oxygen Demand, Phosphate, Sulphate, Ammonium nitrate and Colour.

Bacterial Isolation and Identification

The spread plate method was used for the determination of total heterotrophic bacteria count. Ten-fold serial dilution of each water sample was prepared aseptically in physiological saline 10^{-1} to 10^{-4} and 0.1mL aliquot of each dilution was placed on nutrient agar plates in triplicate. All incubation was conducted at 34°C for 24 – 48 h under aerobic conduction. After incubation the number of discrete colonies was counted in terms of Colony Forming Units (CFU). Pure culture of isolates was identified and characterized on the basis of their cultural, morphological and biochemical characteristics (Cheesbrough, 2006). Total fecal coliform counts were obtained using the Most Probable Number (MPN) method (APHA, 1999).

Determination of virulent factor

Hemolysin production: All isolates (*Bacillus cereus, Staphylococcus aureus, Micrococcus* sp., *Bacillus subtilis*, *Shigella* sp., *Salmonella* sp. and *Escherichia coli*) were cultured on sheep blood agar media. Plates were incubated at 37 °C for 24 h and then checked for zones of hemolysis around colonies.

Antibiotic sensitivity testing

Hemolysin positive isolates were subjected to antibiogram characterization. Pathogens were tested for resistance and sensitivity to different antibiotics using the Kirby-Bauer disc diffusion method. Bacteria grown actively between 18 and 24 h on Mueller-Hinton agar with inoculum corresponding to 10^6 cells (McFarland's standard) was streaked on Mueller-Hinton plates using a sterile inoculating loop. Then appropriate discs were used to impregnate the media using sterile forceps according to the methods delineated by Clinical Laboratory Standard Institute (CLSI 2013). Results were interpreted as susceptible (S), intermediate (I) or resistant (R) according to standardized CLSI chart (CLIS, 2013).

Plasmid Curing

Furthermore, isolates that were multi-resistant during antibiotic sensitivity experiment were subjected to plasmid curing by the method

described by Bauer *et al*. (1966). The acridine orange required for plasmid curing, was prepared directly before use by dissolving 7.5 mg acridine orange in 100 mL sterile distilled water. A hundred microliter (100µL) suspension of the standardized bacteria isolate was inoculated in nutrient broth containing the acridine orange solution and incubated for 48 h at 35°C. Similarly, control cultures were prepared without acridine orange in nutrient broth. After incubation, serial dilutions were prepared from each of the acridine orange-culture suspensions and were spread onto Mueller Hinton plates to obtain single colonies. The plates were then incubated for another 48 h. After 48 h, the antibiotic susceptibility patterns of these isolates were again determined in vitro by using the standardized agar disc- diffusion method (Bauer *et al*., 1966).

Statistical analysis

The data generated were analyzed by one –way ANOVA (analysis of variance) using Genstat 12th edition analytical package as well as non-parametric T-test. Differences in mean were compared by Duncan's multiple range tests.

Results

The physicochemical parameters of borehole water samples from agricultural, animal farm and residential areas are shown in Tables 1, 2 and 3. All studied physicochemical parameters were within WHO standard except TSS which was higher than the WHO recommended limit. Most tested parameters in the different water samples from the three areas were significantly different ($P < 0.05$) with exceptions of temperature and colour ($P > 0.05$).

Table 1: Physicochemical parameter of borehole water from agricultural area (A1 – A3)

Parameter	A1	A2	A3	P-value	WHO limit
pH	4.9±0.05	5.21±0.02	5.24±0.03	0.00	6.5-8.5
E.C (µS/cm)	25.3±0.55	27.0±0.39	32.1±0.66	0.00	1000
Temp (°C)	27.3±0.07	26.6±0.10	27.3±0.09	0.00	20-30
TDS (mg/l)	40.6±0.10	43.2±0.90	43.4±0.93	0.06	500
TSS (mg/l)	19.9±0.24	22.6±0.77	19.0±0.42	0.00	< 10
Turb (NTU)	1.21±0.02	0.95±0.01	1.07±0.02	0.00	1-5
Alkali (mg/L)	4.77±0.02	5.68±0.05	6.71±0.09	0.00	30
NO_3 (mg/L)	1.62±0.02	2.95±0.02	1.90±0.01	0.00	40-50
BOD (mg/L)	3.87±0.04	3.01±0.02	2.68±0.09	0.00	<10
DO (mg/L)	4.38±0.02	3.21±0.03	3.37±0.04	0.00	5-15
COD (mg/L)	8.48±0.07	8.42±0.05	7.23±0.11	0.00	10
P (mg/L)	1.23±0.02	1.23±0.02	1.34±0.01	0.00	5
S (mg/L)	2.42±0.02	3.06±0.01	2.56±0.02	0.00	250
Ammonium. nitrate (mg/L)	0.87±0.01	0.94±0.01	0.63±0.21	0.21	10
Colour (TCU)	0.09±0.00	0.09±0.00	0.10±0.00	0.18	15.0

Table 2: Physicochemical parameter of borehole water from Animal Farm Area (B1 – B3)

Parameter	B1	B2	B3	P value	WHO limit
pH	4.66±0.06	5.08±0.08	5.20±0.02	0.00	6.5-8.5
E.C (µS/cm)	20.3±0.68	14.0±0.83	27.8±0.43	0.00	1000
Temp (°C)	26.3±0.25	27.3±0.92	26.5±0.41	0.07	20-30
TDS (mg/l)	51.5±0.31	54.5±0.34	40.2±0.81	0.00	500
TSS (mg/l)	18.7±1.27	18.0±0.94	19.6±1.63	0.07	< 10
Turb (NTU)	1.15±0.02	0.85±0.08	1.01±0.02	0.00	1-5
Alkali (mg/L)	4.38±0.04	5.12±0.07	5.42±0.02	0.00	30
NO_3 (mg/L)	1.91±0.04	1.13±0.02	1.84±0.03	0.00	40-50
BOD (mg/L)	3.26±0.05	3.21±0.03	3.10±0.02	0.02	<10
DO (mg/L)	4.08±0.02	4.12±0.01	3.42±0.01	0.00	5-15
COD (mg/L)	6.79±0.04	7.66±0.07	6.45±0.08	0.00	10
P (mg/L)	1.18±0.02	1.25±0.01	1.42±0.02	0.00	5
S (mg/L)	3.65±0.03	3.65±0.02	2.72±0.07	0.00	250
Ammonium nitrate (mg/L)	0.78±0.02	0.72±0.01	0.78±0.01	0.03	10
Colour (TCU)	0.09±0.01	0.09±0.00	0.10±0.00	0.26	15.0TCU

Table 3: Physicochemical parameter of borehole water from Residential Area (C1 – C3)

Parameter	B1	B2	B3	P value	WHO limit
pH	4.65±0.04	4.76±0.04	5.58±0.17	0.00	6.5-8.5
E.C (µS/cm)	28.1±0.51	13.4±0.25	24.1±0.30	0.00	1000
Temp (°C)	26.2±0.04	26.8±1.00	26.9±1.00	0.02	20-30
TDS (mg/l)	46.8±1.50	51.3±1.69	42.7±0.57	0.00	500
TSS (mg/l)	24.5±0.37	20.1±0.47	24.4±0.57	0.00	<10
Turb (NTU)	0.88±0.03	1.07±0.02	0.94±0.02	0.00	1-5
Alkali (mg/L)	5.82±0.10	6.32±0.06	6.77±0.04	0.00	30
NO_3 (mg/L)	1.92±0.02	1.96±0.01	1.99±0.01	0.01	40-50
BOD (mg/L)	3.13±0.05	2.99±0.05	3.33±0.07	0.01	<10
DO (mg/L)	3.69±0.05	3.61±0.03	4.22±0.03	0.00	5-15
COD (mg/L)	8.38±0.06	6.81±0.07	8.38±0.05	0.00	10
P (mg/L)	1.17±0.02	1.27± 0.02	1.55±0.02	0.00	5
S (mg/L)	2.18±0.02	3.18±0.01	2.35±0.02	0.00	250
Ammonium. nitrate (mg/L)	0.75±0.00	0.82±0.02	0.76±0.03	0.07	10
Colour (TCU)	0.09±0.00	0.25±0.16	0.90±0.00	0.37	15.0TCU

Heterotrophic bacterial count in water samples from agricultural area, animal farm area and residential area were significantly different ($P < 0.05$). This was highest in animal farm area (1.2±1.05 to 4.80 ± 0.10 $\times 10^4$ CFU/mL) followed by agricultural area (1.4 ± 0.22 to 3.1±1.16$\times 10^4$ CFU/mL), but was least in residential area (1.01 ± 0.10 to 1.80 ± 0.89 $\times 10^4$ CFU/mL). Cultural, morphological and biochemical characterization of bacterial isolates showed four (4) Gram positive bacteria; *Bacillus cereus, Staphylococcus aureus, Micrococcus* sp. and *Bacillus subtilis* and three (3) Gram negative bacteria; *Shigella* sp., *Salmonella* sp. and *E. coli*. The percentage occurrence of bacterial isolates from borehole water samples in the different locations is shown in Table 4. *Shigella* sp. was the most occurring bacterial isolate (23.29%) while *Samonella* sp. was the least (7.2%).

Most Probable Number Index of water samples from agricultural, animal and residential areas is shown in Figure 2. Generally, coliform bacteria count within each area was significantly different ($P < 0.05$). Confirmatory test of water samples from agricultural, animal and residential areas showed that animal farm area had the highest coliform bacteria count (3.00±0.63 MPN/10.0mL).

Isolated and identified bacterial isolates were further tested for virulence based on hemolysin production (Table 5). *Staphylococcus aureus, Bacillus subtilis* and *Bacillus cereus* showed β-hemolysin while *Micrococcus* sp. showed α-hemolysin. *Shigella* sp., *Salmonella* sp. and *Escherichia coli* showed γ-hemolysin which means no zones of hemolysis.

Antibiotic susceptibility pattern of isolated pathogenic bacteria before plasmid curing is shown in Table 6. *Bacillus cereus, Staphylococcus aureus* and *Bacillus subtilis* were resistant to ciprofloxacin, pefloxacin, amoxicillin and ceftriaxone. *Staphylococcus aureus* and *Bacillus subtilis* were susceptible to chloramphenicol and zinnacef. All tested bacterial isolates were resistant to ceftriaxone. Multiple drug index showed that *Staphylococcus aureus* had the highest multiple drug resistance index of 0.8, followed by *Bacillus cereus* with that of 0.6 while both *Bacillus subtilis* and *Micrococcus luteus* had the least multiple drug resistance index of 0.5.

The antibiotic susceptibility pattern of pathogenic bacteria after plasmid curing is shown in Table 7. *Bacillus cereus, Staphylococcus aureus, Bacillus subtilis* and *Micrococcus luteus* were all susceptible to streptomycin, ampiclox, zinnacef, chloramphenicol and amoxicillin. However, *Bacillus cereus, Staphylococcus aureus* and *Bacillus subtilis* were still resistant to pefloxacin. Multiple drug resistance index of all pathogens reduced although *S. aureus* still had highest multiple drug resistance index of 0.4 while *Bacillus subtilis* and *Micrococcus luteus* had the least multiple drug resistance index of 0.1.

Discussion

Water quality is determined by its physicochemical and biological properties which are influenced by both human and natural factors. In this study, physicochemical and bacteriological quality of borehole water samples collected from agricultural, animal farming and residential areas were assessed. All the tested parameters were found to be within WHO standard with the exception of Total Suspended Solids (TSS) which was highest in water samples from Residential area. This could be as a result of silt, organic matter and sewage gaining access to the underground water (Ukpong and Okon, 2013; Palamuleni and Akoth, 2015). Other authors have also observed drinking water standards within WHO maximum permissible limit (Dhanaji *et al.* 2016; Atuanya *et al.* 2018) For example, the highest pH recorded in the study which was from residential area (5.58 ± 0.17) was within WHO pH limit of 6.5 - 8.5. Atuanya *et al.* (2018) similarly reported pH values of 5.6 – 6.6 in stored borehole water in Benin City. It has been reported that pH values of 6.5- 8.0 is good for water intended for consumption (Abdullahi *et al.* (2013). The lower pH values observed in agricultural and animal farm areas from the study sites may be attributed to anthropogenic activities such as use of fertilizers in agricultural lands of the study area. Again, water samples from agricultural area had the highest electrical conductivity and this could be attributed to agricultural run-off such as fertilizers, elevated temperature and low pH (Gambo *et al.*, 2015; Obuekwe and Osariemen, 2020).

Bacterial properties of borehole water in selected areas of Edo State revealed that bacterial counts were above WHO standard of 100 CFU/mL as well as coliform count (0 CFU/mL). Total heterotrophic bacterial

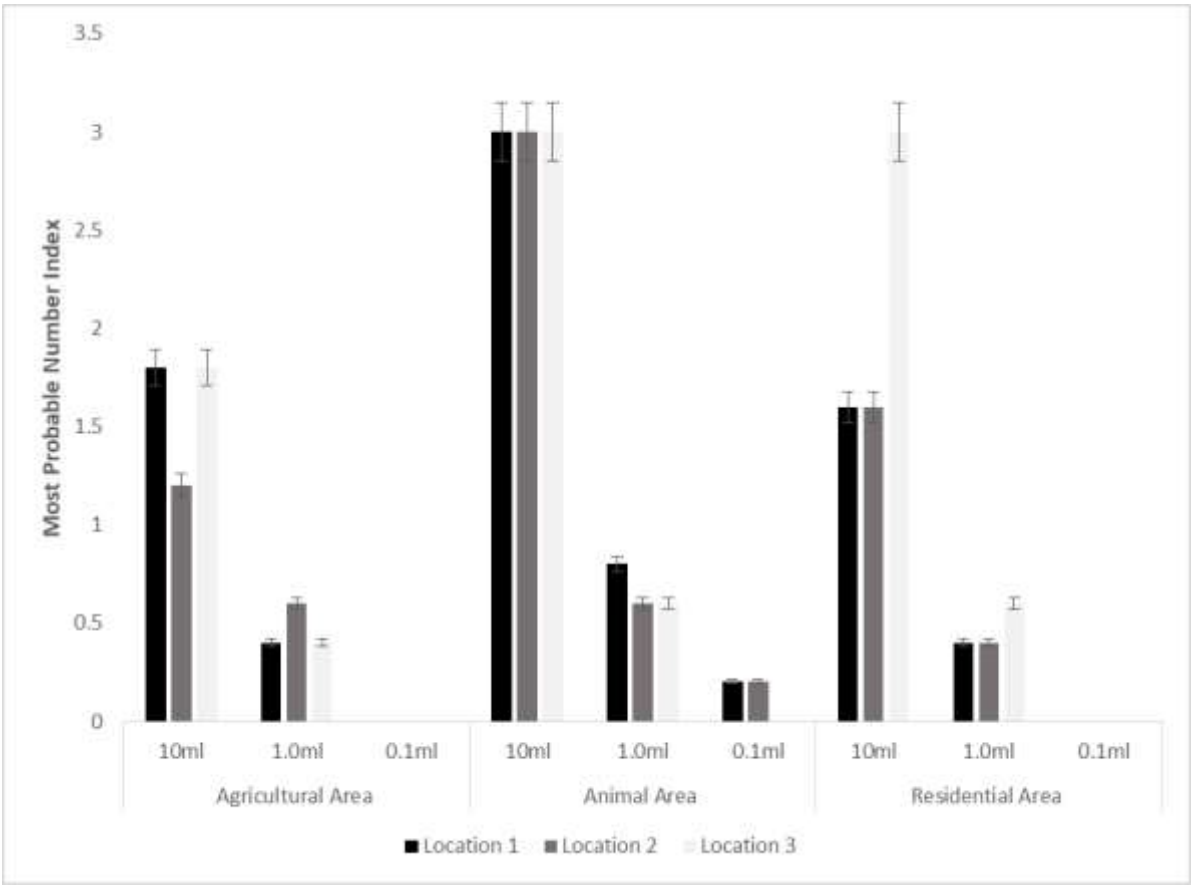

Figure 2: Confirmatory test of water samples from Agricultural, Animal and Residential areas

Table 4: Percentage occurrence of bacterial isolates from borehole water samples in the different locations

	Location				
Bacteria isolate	Agricultural Area	Animal Farm Area	Residential Area	Total	Occurrence (%)
Bacillus cereus	5	4	6	15	21.9
Staphylococcus aureus	5	2	3	10	14.5
Micrococcus sp	2	3	4	9	13.0
Bacillus subtilis	0	3	3	6	8.7
Shigella sp.	6	4	5	15	23.3
Samonella sp.	0	4	1	5	7.2
Escherichia coli	2	5	2	9	13.0
Total	20(27.4%)	25(34.2%)	24(32.9%)	69(100%)	100

Table 5. Hemolytic bacterial isolates from Borehole water samples

Bacterial isolate	Hemolysin production
Bacillus cereus	β
Staphylococcus aureus	β
Micrococcus sp.	α
Bacillus subtilis	β
Shigella sp.	γ
Salmonella sp.	γ
Escherichia coli	γ

KEY:
α-hemolysis (greenish zones)
β-hemolysis (clear zone)
γ-hemolysis (no hemolysis)

Table 6: Antibiotic susceptibility of isolated bacteria before plasmid curing

Bacterial Isolate	CPX	S	SXT	E	PEF	CH	APX	Z	AM	R	MDR
Bacillus cereus	R	S	R	S	R	S	S	R	R	R	0.6
Staphylococcus aureus	R	R	R	R	R	S	R	S	R	R	0.8
Bacillus subtilis	R	R	S	S	R	S	S	S	R	R	0.5
Micrococcus letus	S	S	R	R	S	R	R	S	S	R	0.5

PEF: Pefloxacin (10 µg), CH = Chloramphenicol (30µg), APX= Ampiclox (30 µg), Z= Zinnacef (20 µg), CPX= Ciprofloxacin (10 µg), S= Streptomycin (30 µg), SXT= Septrin (30 µg), E= Erythromycin (10 µg), AM = Amoxicillin (30 µg), R = Ceftriaxone (25 µg),
MDR = Multiple Drug Resistance.
MDR index ≥ 0.2 (public health significance)
R= resistance
S = susceptibility

Table 7: Antibiotic susceptibility of isolated bacteria after plasmid curing.

Gram Positive	CPX	S	SXT	E	PEF	CH	APX	Z	AM	R	MDR
Bacillus cereus	S	S	R	S	R	S	S	S	S	S	0.2
Staphylococcus aureus	R	S	S	R	R	S	S	S	S	R	0.4
Bacillus subtilis	S	S	S	S	R	S	S	S	S	S	0.1
Micrococcus letus	S	S	S	S	S	R	S	S	S	S	0.1

PEF: Pefloxacin (10 µg), CH = Chloramphenicol (30µg), APX= Ampiclox (30 µg), Z= Zinnacef (20 µg), CPX= Ciprofloxacin (10 µg), S= Streptomycin (30 µg), SXT= Septrin (30 µg), E= Erythromycin (10 µg), AM = Amoxicillin (30 µg), R = Ceftriaxone (25 µg),
MDR = Multiple Drug Resistance.
MDR index ≥ 0.2 (public health significance)
R= resistance
S = susceptibility

count (34.2%) and coliform count (3.00 ± 0.63 MPN/10 ml) were highest in animal farm area. This may be as a result of animal wastes discharge filtering through to the groundwater (Abdullahi *et al.*, 2013). The presence of coliforms in the water samples could be an indication of fecal contamination of the borehole water samples (Uhuo *et al.*, 2014). Ukpong and Okon, (2013) similarly reported high coliform count in borehole water samples that did not meet the WHO standard in Akwa Ibom state, Nigeria. However, several authors have reported the absence of fecal coliforms in borehole water samples (Mustafa *et al.*, 2013; Onuorah *et al.* 2018; Onuorah *et al.*, 2019). Identified bacteria species (*Bacillus cereus, Staphylococcus aureus, Micrococcus sp., Bacillus subtilis, Shigella sp. Salmonella sp* and *E. coli*) isolated from the borehole water samples were similar to the study of Olajubu and Ogunika (2014) who isolated similar bacteria from borehole water samples in Akungba – Akoko, Ondo State, Nigeria. *Salmonella* species were also isolated from water samples used for domestic purposes in Okada town, Edo state (Josiah *et al.*, 2014). *Micrococcus* species isolated from the borehole water samples in this study is in agreement with the study of Young *et al.* (2010) who reported their presence in drinking water samples from rural health centers. *Micrococcus* sp was thought to have been transmitted through contact with borehole water storage system and pipelines.

Shigella sp. (23.29%) and *Bacillus cereus* (21.92%) were the two isolates that occurred most in this study. This is similar to the report of Felföldi *et al.* (2010) who reported high prevalence of *Bacillus cereus* (22.16%) and *Shigella* sp (21.80%) in drinking water distribution system. Again, the work of Zhang *et al.* (2006) showed *Bacillus* species as the most prevalent bacterial pathogen in studied drinking water samples. *Salmonella* sp. was the least occurred bacterial isolate (7.2%) in the water samples in this study. This is similar to the report of Ekelozie *et al.* (2018) who showed that although *Salmonella* species was most prevalent (35.0%) in Reservoir water samples, they were least prevalent (7.45) in Borehole-water samples. It has been reported that the presence of *Salmonella* sp., in borehole water samples may be due to the sanitary condition around the areas where the boreholes are located (Taiwo *et al.* 2020).

Bacterial isolates virulence or pathogenicity was based on hemolysin production. *Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus* demonstrated clear β-hemolysis in the form of clearance zone along the streak on blood agar plate within 24 h of incubation at room temperature. Erova *et al.* (2007) reported that hemolytic proteins are commonly isolated from pathogenic bacteria, and β-hemolysin is one of the important bacterial virulence factors. Production of hemolysins by *Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus* agrees with the report of Bagyalakshmi *et al.* (2009) where the occurrence of hemolysin, lipase, protease, gelatinase and caseinase was established as virulence factors in *Micrococcus* sp. *Bacillus subtilis* and *Shigella* sp. isolated from Bhavani River, Tamil Nadu, India.

Bacillus cereus, *Staphylococcus aureus*, *Bacillus subtilis* and *Micrococcus* sp. isolated from the borehole water samples exhibited virulence because of their hemolysin production and may be capable of inducing waterborne infections. *Staphylococcus aureus* (β-haemolysis) is arguably the most prevalent pathogen of humans, and may cause up to one third of all bacterial diseases ranging from boils to toxic shock (Onuorah *et al.*, 2018). Again. Bacillus species are associated with gastrointestinal tract infections (Onuorah *et al.*, 2019). The pathogenic isolates from this study may pose severe health complications to humans such as bacteraemia, septic shock, and septic arthritis especially if they harbour virulence gene determinants (Onuorah *et al.*, 2018).

Staphylococcus aureus was resistant to ciprofloxacin, streptomycin, septrin, erythromycin, pefloxacin, ampiclox, amoxicillin and ceftriaxone. It also had the highest multiple antibiotic resistance index (0.8) followed by *Bacillus cereus* (0.6) before plasmid curing. This is similar to the studies of Wegener (2012) who reported multidrug resistance in *Staphylococcus aureus*, *P. aeruginosa* and *Bacillus cereus* to ciprofloxacin, pefloxacin, ampiclox, zinnacef and erythromycin. *Bacillus subtilis* and *Micrococcus* luteus were also resistant to more than two (2) tested antibiotics and this is of public health significance. This result is in agreement with the studies of Mwajuma (2010) who reported that *Bacillus subtilis*, *Escherichia coli*, K. pneumoniae and *Micrococcus* luteus were resistant to ceftriaxone, ampiclox, erythromycin and zinnacef. Similarly, Mbim *et al.* (2016), Jaran, (2015) and Tagoe *et al.* (2011) reported the existence of multidrug resistance in *E. coli, S. aureus* and *B. subtilis*. Generally, the development of drug resistant pathogens can be linked to indiscriminate use of antibiotics and resultant selective pressure on bacteria (Mohammed *et al.*, 2014).
Upon plasmid curing of *Bacillus cereus*, *Staphylococcus aureus*, *Bacillus subtilis* and *Micrococcus* luteus, they became sensitive revealing that their resistance could be plasmid borne.

Conclusion

Borehole water samples from agricultural, animal farm and residential areas in Ikpoba-Okha, Benin City, Edo state showed that most physicochemical parameters were within WHO standard except TSS. This high TSS could be attributed to organic matter or sewage gaining access to the underground water. Bacteriological analysis showed high heterotrophic bacterial count, coliforms, and some virulent bacterial isolates that are likely to cause waterborne infections. Again, these isolates exhibited multi-drug resistance that could be plasmid or non-plasmid encoded. Therefore, it is imperative to treat these borehole water samples before use especially as potable water. Continuous monitoring and proper sanitary measures should be strictly observed around the vicinity of these water sources.

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