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PHYSICOCHEMICAL AND PHENOTYPIC VIRULENCE TRAITS OF C*LOSTRIDIUM* SPECIES ISOLATED FROM GROUND WATER SOURCES IN BENIN CITY.

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ABSTRACT

The study was carried out to isolate and identify *Clostridium* species obtained from water samples in different locations within Benin City. Physicochemical assessment of the water samples collected aseptically was carried. Also anaerobic **bacteria isolation and identification,** antimicrobial susceptibility tests and phenotypic virulence determinants were carried out using standard microbiological technique. The results revealed that the pH ranged from 6.06 - 7.59, temperature (25.3 - 29.4°C), electrical conductivity (13 – 159µS/cm), turbidity (0.21 – 1.83 NTU), total suspended solid (0.23 – 0.98 mg/ml), Alkalinity (0.12 – 0.50), Hardness (1.05 – 2.95 mg/ml), Phosphate (0.1 – 1.99 mg/L), Nitrate (0.03 – 1.05 mg/L), and Sulphate (0.12 – 1.0 mg/L) were within acceptable range delineated by World Health Organization for drinking water. The bacteriological analysis of water samples showed that the anaerobic bacteria count range from 4.50 cfu/100ml to 81.00 cfu/100ml. The identified anaerobic bacterial isolates were *Clostridium butyricum, Clostridium septicum,* and *Clostridium perfringens*. The highest-occurring anaerobic bacteria isolates from the water samples was *Clostridium butryricum* (50.00%) and *C. perfringens* (16.67%) was the least occurring. The phenotypic virulence properties of the anaerobic bacterial isolates showed that they had virulence determinants activity for Hemolysin proteins, DNAse and Gelatinase, but no Lipase enzyme activity. The antibacterial susceptibility testing revealed all the isolates were susceptible to Gentamicin (10 mcg), metronidazole (5 mcg), Erythromycin (15 mcg), Ciprofloxacin (5 mcg), Tetracycline (30 mcg) and Clindamycin (2 mcg) but on the other hand were resistant to Colistin (10 mcg) and Cefuroxime (30 mcg). However, the microbiological quality did not meet the SON standard for portable water, hence the need for quality assessment of drinking water sources and ensuring compliance with relevant standard to avoid risk to human health.

Keywords; Physicochemical, C*lostridium* spp, Virulence Properties, antibacterial susceptibility

INTRODUCTION

Water is unique compared to other commodities since it is crucial for attaining sustainable development in all of its facets - economic, social, and environmental (Soto *et al*., 2018). At various levels of treatment from the sources to the distribution network, water suppliers routinely collect (daily and periodically) and send water samples to water testing laboratories for chemical and microbiological analysis (Kumpel *et al*., 2016). Even with water flowing from a water sources, problems might occur due to leakages or network maintenance, exposing consumers and contaminating the water.

Benin City is located in Edo State, Southern Nigeria with a growing population. In Benin City, borehole digging is a constant practice in housing development projects because every home needs a water supply (Ezeanah, 2020). Nearly all homes, offices, and commercial buildings in Benin City have been constructed with boreholes. Due to Benin City's poor water table, it is less expensive to drill boreholes and hand-dig wells. The development of drilled bore holes and hand-dug wells can have a significant impact on the quantity and quality of groundwater reserves by introducing intense pressure due to massive water abstraction from this resource, which is extremely susceptible. The abundance of boreholes, particularly in Benin City, could pose a long-term threat to the ecosystem. Increased human activity in Benin City, especially the careless placement of septic tanks, soak-away pits, and pit latrines, as well as the disposal of garbage and other items that can leach into the groundwater, pose a serious threat to public health. The majority of the city's residents still use borehole and well water without appropriate or proper treatment.

The *Clostridium* genus encompasses a variety of Gram-positive, anaerobic, opportunistic pathogens which are ubiquitous, reaching from environmental to clinical settings. Although they form part of the normal flora in the intestinal tracts of humans and animals, they also cause severe gastrointestinal diseases and infections such as enteritis, botulism and gas gangrene (Graham *et al*., 2020). These pathogenic *Clostridium* species can be introduced into surface water systems through various sources, such as effluent from wastewater treatment plant and surface runoff from agricultural areas. *Clostridium* species (*C. perfringens, C. septicum* and *C. butyricum*) have been discovered and some of them are responsible for human diseases due to the formation of toxins (Uzal *et al*., 2018). Their spores are extremely resistant to harsh environmental conditions such as pH and temperature extremes and UV radiation and most importantly, disinfection treatment processes. The high levels of *Clostridium* species in water can be utilized as indicators of diffuse and point source fecal pollution or even to assess the inactivation of pathogenic protozoans and viruses in water treatment processes. The aim of this research is to isolate and identify *Clostridium* species, evaluate the antibiotic susceptibility pattern and pathogenicity of the bacterial isolates as an indicator of microbiological quality of water from borehole and well sources in Benin city.

MATERIALS AND METHODS

Study Area

This study was conducted in Benin City, the capital of Edo State in Nigeria, which is situated in the country's south-south geopolitical zone and has a total area of roughly 500 square kilometers. Benin City is bordered by the latitudes 6° 06' N and 6° 30' N and the longitudes 5° 30' E and 5° 45' E. The city is surrounded by a sedimentary deposit that consists of a top layer of reddish clay sand that covers extremely porous fresh water-bearing loose sands, as well as thin local clay and shale that are thought to have originated in braided streams. It is commonly thought to be highly porous, permeable and abundant in water production (Omorogieva *et al*., 2016).

Collection of Water Samples

Water samples were randomly collected from different districts within Benin City. The groundwater samples comprise of seven boreholes and two well water. Samples were collected into 250 ml sterile sampling bottles observing aseptic procedures and immediately transported to the laboratory for analysis.

Physico-chemical tests (Water Quality Test)

The evaluation of water quality involves the assessment of various physico-chemical parameters that can provide information about its suitability for different purposes such as drinking, recreational activities or industrial use. Several equipment and processes are used to measure and analyze these parameters. Different physicochemical parameters amenable to water quality assessment, namely, pH, temperature, salinity, dissolved salts measured as electrical conductivity, total suspended solid, essential elements and their corresponding compounds (nitrates, phosphates, sulphate), dissolved oxygen, biological oxygen demand and carbon-oxygen demand (NSDWQ, 2007; WHO/UNICEF, 2021).

Culture, isolation and identification of *Clostridium* species in borehole and well water

Water samples were analyzed immediately after collection, for the presence of heterotrophic and total coliforms using membrane filtration method (USEPA, 2009). Aliquots of 100ml from each sample were filtered using 0.45 µm paper filters. The filters were placed on Nutrient agar and Eosin methylene blue agar plates and were incubated aerobically at 37 °C for 24 hrs. Colonies isolated were then sub-cultured on Vogel-Johnson agar, Salmonella Shigella Agar and Pseudomonas Centrimide Agar for bacteria identification. The isolates were subjected to both preliminary Gram staining and confirmatory biochemical identification tests such as; indole, oxidase, citrate utilization and Triple Sugar Iron tests (Forbes and Weissfeld, 1998; Prescott, 2001).

Antibiogram:

Antimicrobial susceptibility studies were carried out by the modified Kirby-Bauer disk diffusion method, according to the guidelines of the Clinical Laboratory Standard Institute (CLSI, 2017) The *Clostridium* spp isolated were adjusted to 0.5 McFarland turbidity standards and applied onto Mueller-Hinton (MH) agar plates using sterile swab sticks. Single antibiotics disks (Antibiotics disks used were: AG - Amoxicillin (30mcg), CIP - Ciprofloxacin (5mcg), TE - Tetracycline (30mcg), GEN - Gentamycin (10mcg), CB - Cefuroxime (30mcg), E - Erythromycin (15mcg), CD - Clindamycin (2mcg), M - Metronidazole (5mcg), CS - Colistin (10mcg) were aseptically placed on the swabbed MH agar plates at a distance of 20mm apart using sterile forceps. All susceptibility test plates were incubated at 37°C for 18–24h. The zone of inhibition was measured, recorded, and interpreted as susceptible (S) and resistant (R) using standard antibiotic breakpoints as stated by the CLSI (2017). Also multiple antibiotics resistance index (MARI) was determined.

Pathogenicity Testing

Testing for pathogenicity was used to determine a microorganism's capacity to infect or cause disease in a host organism. It assists with comprehending the virulence mechanisms and possible dangers connected to particular diseases. Hemolysin, DNAse, Gelatinase and Lipase test were carried out as described by Bergey *et al.* (2009) and Tille and Forbes (2014).

Isolated *Clostridium* Spp were inoculated onto 5% sheep blood agar and incubated overnight at 35°C. Hemolysin production was detected by the presence of a zone of complete clearance of erythrocytes around the colony as observed against transmitted light (Roberts, 1957; Tille and Forbes 2014).

Bacterial isolates were inoculated into prepared DNase agar and incubated at 37°C for 24hrs. Positive result is visible if the bacterium breaks down the DNA, causing the medium to lose its viscosity or to develop a clear halo around the bacterial colony (colourless around the organism). Negative result is visible if the medium remains green (Tille and Forbes 2014).

Gelatinase production/protease activity: Gelatinase activity was demonstrated using gelatin agar. The gelatin agar plate was inoculated with *E. coli* at 37°C for 24 hours. After incubation, the plates were flooded with mercuric chloride solution. Development of the zone of opacity surrounding the colonies was considered positive for gelatinase production (Sharma *et al*., 2007).

Spirit blue agar (SBA) was prepared and autoclaved. The medium was poured into sterile petri dishes after cooling. Isolates were inoculated into labelled plates by streaking with sterile wire loop and plates were incubated at 37°C for 24hrs. Positive result is observed when the bacterium breaks down the lipids, causing the medium to turn opaque or develop a chalky-white appearance (clear zone) around the organism. Negative result shows no clear zone around the organism (Pascoal *et al*., 2018).

RESULTS

The results of physiochemical properties of water samples from different sampling points in Benin City was evaluated as presented on Table 1. The pH ranged from 6.06 - 7.59, while temperature (25.3 - 29.4°C), electrical conductivity (13 – 159 µS/cm), turbidity (0.21 – 1.83 NTU), total suspended solid (0.23 – 0.98 mg/ml), Alkalinity (0.12 – 0.50), Hardness (1.05 – 2.95 mg/ml), Phosphate (0.1 – 1.99 mg/L), Nitrate (0.03 – 1.05 mg/L), and Sulphate (0.12 – 1.0 mg/L) were within acceptable range delineated by WHO for drinking water. The results of bacteriological analysis (specific to anaerobes) of water samples showed that the anaerobic bacterial count was found to range from 4.50 to 81.00 CFU/100ml, with BHS7 having the highest bacterial count (Figure 1). The percentage of the three different anaerobic bacteria found in the water samples were *Clostridium butyricum* (50%), *Clostridium septicum* (27.8%) and *Clostridium perfringens* (16.7%). *Clostridium butyricum* has the highest percentage of occurrence while the least occurrence was *Clostridium perfringes* as shown on Figure 2. The bacteria possessed hemolysin proteins as well as DNAse enzyme. The antibacterial sensitivity testing revealed that all isolates were susceptible to gentamicin, metronidazole, erythromycin and clindamycin while resistance was widely observed for cefuroxime and colistin as seen on Table 2. It was also evident that they were found to have an MAR index greater than 0.2 which means that the isolates were all pathogens of public health importance. The phenotypic virulence properties of the anaerobic bacterial isolates showed that they had all virulence determinants as evaluated in this study (Table 3).

Table 1: The physiochemical analysis of water samples from different sampling points

Parameter	BHS1	BHS2	BHS3	BHS4	BHS5	BHS6	BHS7	WWS1	WWS2	WHO
pH	7.59±0.15	6.71±0.55	6.66±0.25	6.33±0.25	6.72±0.12	6.18±0.35	6.69±0.55	6.06±0.23	6.63±0.35	6.5-8.5
Tempt. (°C)	25.3±1.50	28.30±1.50	25.3±1.50	26.30±2.15	29.40±1.90	28.3±2.15	26.30±2.50	29.30±2.00	25.30±1.85	< 35
EC (µS/cm)	46.00±3.50	37.00±3.90	48.00±3.00	33.00±3.11	159.00±8.11	26.00±1.25	13.00±2.00	110.00±10.00	16.00±1.55	1000
Turb (NTU)	0.63±0.15	0.27±0.04	1.83±0.25	1.11±0.19	0.90±0.04	0.74±0.04	0.21±0.12	0.84±0.06	1.45±0.7	5
TSS	0.78±0.25	0.98±0.25	0.90±0.02	0.59±0.14	0.65±0.02	0.72±0.03	0.69±0.24	0.87±0.04	0.23±0.02	< 10
Alkalinity	0.21±0.01	0.41±0.11	0.43±0.03	0.40±0.05	0.50±0.05	0.24±0.01	0.12±0.00	0.20±0.00	0.12±0.01	<50
Hardness	1.99±0.22	2.15±0.15	2.67±0.95	2.95±0.23	1.05±0.00	2.50±0.05	2.57±0.09	1.66±0.09	2.54±0.32	100-500
Phosphate	0.12±0.01	0.56±0.04	1.84±0.85	1.99±0.35	0.09±0.00	0.11±0.01	0.10±0.00	0.14±0.00	1.53±0.07	5
Nitrate	0.67±0.01	0.95±0.05	1.05±0.15	1.50±0.05	0.57±0.25	0.66±0.23	0.54±0.03	0.50±0.01	1.12±0.09	40-50
Sulphate	0.75±0.05	0.82±0.03	0.91±0.09	0.79±0.00	1.00±0.00	0.12±0.05	0.13±0.02	0.15±0.05	0.53±0.10	60
BOD	0.02±0.01	0.01±0.00	0.02±0.00	0.03±0.50	0.07±0.00	0.02±0.04	0.03±0.00	0.03±0.00	0.02±0.00	10
COD	0.41±0.05	0.56±0.05	0.50±0.01	0.26±0.05	0.31±0.00	0.36±0.01	0.34±0.04	0.48±0.06	0.02±0.00	10
Copper	0.02±0.00	0.02±0.01	0.02±0.00	0.01±00	0.01±0.00	0.01±0.00	0.01±0.00	0.02±0.00	0.02±0.00	0.02
Lead	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01
Zinc	0.03±0.01	0.03±0.02	0.02±0.01	0.06±0.00	0.28±0.00	0.02±0.00	0.02±0.00	0.01±0.00	0.15±0.00	0.2

Key: BHS1: Borehole Water Site 1, BHS2: Borehole Water Site 2, BHS3: Borehole Water Site 3, BHS4: Borehole Water Site 4, BHS5: Borehole Water Site 5, BHS6: Borehole Water Site 6, BHS7: Borehole Water Site 7, WWS1: Well Site 1, WWS2: Well Water Site 2, WHO: World Health Organization

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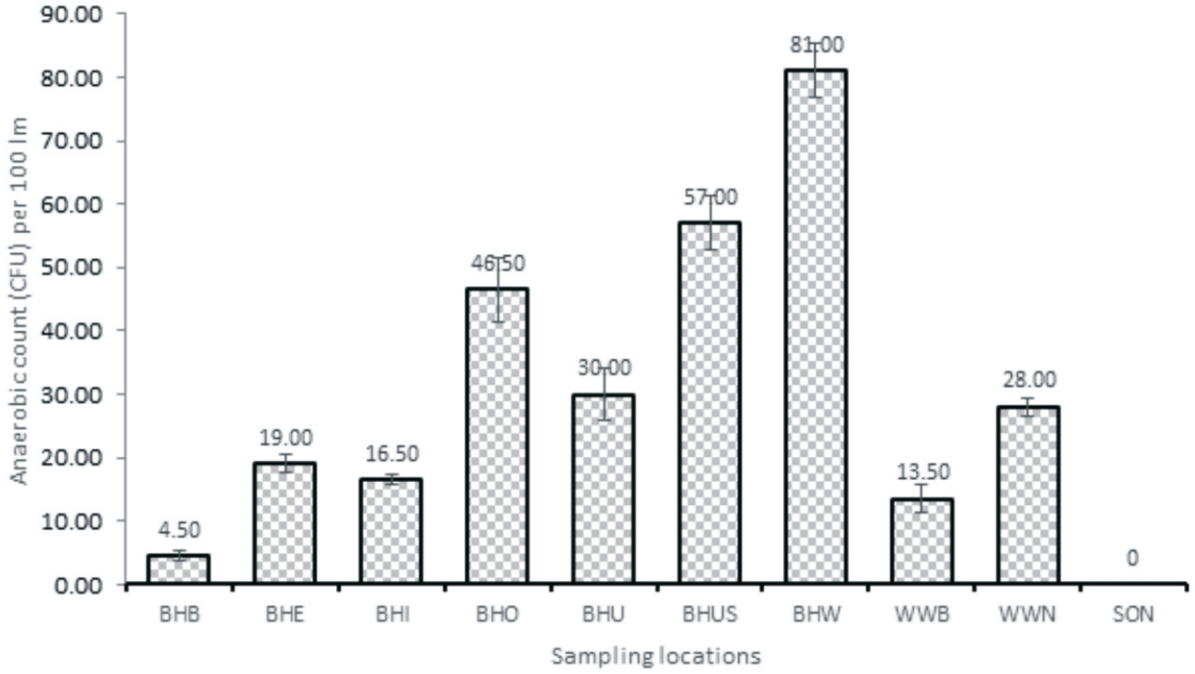

Figure 1: Total anaerobic bacterial count of water samples from different points
Key: BHS1: Borehole Water Site 1, BHS2: Borehole Water Site 2, BHS3: Borehole Water Site 3, BHS4: Borehole Water Site 4, BHS5: Borehole Water Site 5, BHS6: Borehole Water Site 6, BHS7: Borehole Water Site 7, WWS1: Well Site 1, WWS2: Well Water Site 2, WHO: World Health Organization

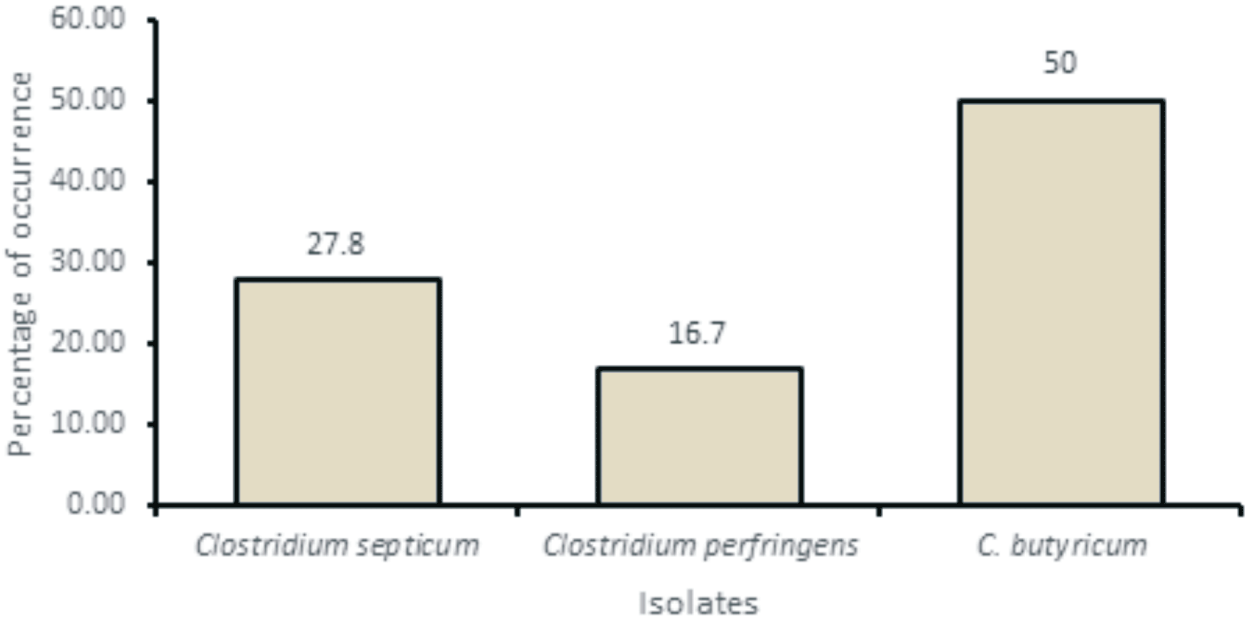

Figure 2 Frequency of occurrence of bacterial isolates from water samples

Table 2 Antibacterial sensitivity of bacterial isolates obtained from water samples

Isolates	GEN	CS	CB	M	AG	E	CIP	TE	CD	MAR index
C. septicum	S	R	R	S	R	S	S	S	S	0.3
C. perfringens	S	R	R	S	S	S	S	S	S	0.2
C. butyricum	S	R	R	S	S	S	S	S	S	0.2

KEY: S - Susceptible, **R –** Resistant, GEN - Gentamycin (10mcg), CS - Colistin (10mcg), CB - Cefuroxime (30mcg), M - Metronidazole (5mcg), AG - Amoxicillin (30mcg), E - Erythromycin (15mcg), CIP - Ciprofloxacin (5mcg), TE - Tetracycline (30mcg), CD - Clindamycin (2mcg),

Table 3 Phenotypic virulence determinants of bacterial isolates from water sources

Isolates	Hemolysin	DNAse	Gelatinase	Lipase
Clostridium septicum	β	+	+	-
Clostridium perfringens	β	+	+	-
Clostridium butyricum	β	+	+	-

Key: β; Beta, +; Positive, -; Negative

DISCUSSION

Water is very essential to the survival of all organisms and the human body is composed of approximately 70% water by mass (Enger and Smith, 2004). An adequate supply of safe and portable water assist in preventing the spread of gastrointestinal diseases, supports domestic and personal hygiene and improves the standard of living (Kangpe *et al*., 2014). However, much of the world's population does not have access to safe drinking water. This is because dead vegetation, metal leachates from solid waste dumps; leaching of rocks, sewage, industrial wastes and agricultural chemicals return eventually to the river by run offs (Ademola, 2008). As population increases, the need of water for domestic, transport, power, agricultural and industrial purposes also increase (Kangpe *et al*., 2014). Apart from expansion as a result of increase in population, other reasons such as crises has led to the development of settlement towns in Nigeria. Private water sources are not often subjected to any quality standard before usage, thereby resulting in the consumption of contaminated water (Kangpe *et al*., 2014). According to WHO (2012), about 2 million people die annually due to diarrhea diseases, most of them are children less than 5 years of age.

The physicochemical properties of the water samples evaluated in the study revealed that the pH, total suspended solid, turbidity, nitrate, phosphate, sulphate, dissolved oxygen, biological oxygen demand and chemical oxygen demand varied across the different sampling points but were within the stipulated range by WHO. The results obtained in this study agrees with the report of Rajini *et al*. (2010) who opined that pH and other physicochemical parameters of drinking water sources were within the stipulated guidelines of regulatory bodies like the WHO. Seth *et al*. (2014) also reported that mean pH of different water sources had a range of 7.41-7.46 indicating that the water samples was highly buffered. The slight acidic nature of the borehole water sample could be attributed to the buffering properties of some inorganic substances (Trivede *et al*., 2014). Hardness and turbidity of the borehole water samples is an important consideration in determining the suitability of water for domestic and industrial uses. Hardness is caused by multivalent metallic cations and with certain anions present in the water to form scale or undissolved substances. The principal hardness causing cations are the divalent calcium, magnesium, ferrous iron and manganese ions (Sengupta, 2013).

The results of bacteriological analysis (specific to anaerobes) of water samples showed that the anaerobic

bacterial count was found to range from 4.50 to 81.00 CFU/100ml. There are no specific guidelines for the presence of anaerobes in water samples using the world health organization guidelines or standard. The identified anaerobic bacterial isolates were *Clostridium butyricum*, *Clostridium septicum*, and *Clostridium perfringens*. The highest occurring bacterial isolates from the anaerobes obtained from water samples was *Clostridium butryricum* (50.00%) followed by *C. septicum* (27.78%) in the study. The results obtained in this study was at variance with the guidelines stipulated by regulatory bodies. The World Health Organization/Food and Agricultural Organization recommended a maximum permissible limit of 1.0 x 102 CFU/ml for potable water. Standard Organisation of Nigeria (SON) set the maximum permissible level of total coliform as 10 CFU/ml and the values for thermotolerant coliform or *E. coli*, Faecal *Streptococcus,* while *Clostridium perfringens* spore as 0 CFU/100ml (Oludare and Sikiru, 2012; Sunday *et al*., 2014). The findings in this study were consistent with the report of Agbabiaka and Oyeyiola (2012) who isolated some uncommon bacteria including *Clostridium* from water samples in Ilorin Nigeria. The results obtained in this study is also similar to the report obtained about *Clostridium* species, which have also been found to be widely isolated from different environment including water sources (Hengens *et al*., 2012).

The antibacterial susceptibility testing in the present study showed that all isolates were susceptible to gentamicin but were also resistant to erythromycin and tetracycline. It was also evident that all isolates were found to have an MAR index of $\geq$ 0.2 which means that the isolates were all pathogens of public health importance. This was also in line with the study of Oshoma *et al*. (2009) who stated that antibiotic sensitivity test of their study revealed that all isolates (*Clostridium perfringens*, *Clostridium butyricum* and *Clostridium septicum*) found were all susceptible to Gentamicin (10 mcg), metronidazole (5 mcg), Erythromycin (15 mcg), Ciprofloxacin (5 mcg), Tetracycline (30 mcg) and Clindamycin (2 mcg) but on the other hand were resistant to Colistin (10 mcg) and Cefuroxime (30 mcg).

Among the various determinants of virulence in invasive strains, α-hemolysin (HlyA), which hemolyzes red blood cells by forming pores in the erythrocyte membrane assumes significance. The frequency by which hemolytic bacteria strains can be isolated from patient samples increases with the severity of disease (Johnson *et al.,* 1988). In the present study hemolytic activity was seen in the three *Clostridium* spp, which is also in correlation with the work of Suzaki *et al*. (2021).

Gelatinase, an important virulence factor which is capable of hydrolyzing gelatin, collagen, and other bioactive peptides is associated with inflammation (Orskov and Orskov, 1985). This study detected gelatinase producing ability in the three *Clostridium* spp. DNases are enzymes that break down DNA. Many pathogenic bacteria produce DNases that help them to evade the host immune system and to spread through host tissues (Sharma *et al*., 2019). It is used to detect the presence of DNase activity in a bacterial isolate. It was observed that the *Clostridium* spp showed DNAse activity, which is in line with the work of Swlatek *et al*., (1987) who reported that all the *Clostridium* spp isolated showed DNAse activity.

CONCLUSION

The study of the physiochemical and bacteriological analysis of water samples from different sampling points in Benin City indicated no elevated levels of all the physicochemical parameters when compared with SON standard. However, the microbiological quality did not meet the requirement of SON standard for potable water quality. The majority of the water samples were contaminated with anaerobes (*Clostridium* species) amongst which were resistant strains. This study therefore highlights the need for continuous monitoring and quality assessment of drinking water sources for pathogenic bacteria. Hence environmental agencies should ensure compliance with relevant standards to avoid risks to human health.

REFERENCES

Ademola FA. (2008). Baseline heavy metals concentration in river sediments with Okikipopo South East belt of the Nigerian bituminous sand field. *Journal of Chemical Society of Nigeria* **33**:29-38.

Agbabiaka TO. and Oyeyiola GP. (2012). Microbial and physicochemical assessment of Foma River, Itanmo, Ilorin, Nigeria: an important source of domestic water in Ilorin metropolis. *International Journal of Plant, Animal and Environmental Sciences* **21**(1):209–216.

Bergey DH, William B, Whitman Vos P, De George M, Garrity, and D Jones. Bergey's Manual of Systematic Bacteriology: Vol. 3. New York: Springer, 2009. Pp1450.

Enger ED. and Smith, B. F. (2004). *Environmental Chemistry*. Return Press Ltd.

Ezeanah U. (2020). Basic infrastructure provision and its impact on people's experiences of housing quality in Benin City, Nigeria. *Journal of Environmental Science*. ***20*(1):** 77-93.

Graham K, Stack H. and Rea R. (2020). Safety, beneficial and technological properties of enterococci for use in functional food applications–a review. *Critical Reviews in Food Science and Nutrition*. ***60*(22):** 3836-3861.

Johnson JR, Moseley SL, Roberts PL, Stamm WE. (1988). Aerobactin and other virulence factor genes among strains of Escherichia coli causing urosepsis: association with patient characteristics. *Infect Immun*. **56:** 405-412.

Kangpe NS, Egga ES. and Mafuyai GM. (2014). Physico-chemical and microbial assessment of *s*ome well water from Mista-Ali Town, Bassa LGA, Plateau State Nigeria. *Asian Review of Environmental and Earth Sciences*. *1(2): 39-42.*

Kumpel E, Peletz R, Bonham M. and Khush R. (2016). Assessing drinking water quality and water safety management in Sub-Saharan Africa using regulated monitoring data. *Environmental Science & Technology*. ***50*(20):** 10869-10876.

Nigerian Standard for Drinking Water Quality (NSDWQ) (2007). Nigerian Standard for Drinking Water Quality. Nigerian Industrial Standard. Retrieved on 1st May, 2023 from chromeextension://efaidnbmnnnibpcajpcglclefindmkaj/https://africacheck.org/sites/default/files/Nigerian-Standard-for-Drinking-Water-Quality-NIS-554-2015.pdf

Oludare A. and Sikiru S. (2012). Microbiological, physicochemical and mineral quality of borehole water in Ijebu land, Ogun state, Nigeria. *International Journal of Science and Advanced Technology*. **2(1):** 23 – 30.

Omorogieva OM, Imasuen OI, Isikhuemen MI, Ehinlaye OA, Anegbe B. and Ikponmwen MO. (2016). Hydrogeology and water quality assessment of Ikhueniro and Okhuahe using water quality index. *Journal of Geography, Environment and Earth Science International*. **6(3):** 1-10.

Orskov I. and Orskov F (1985). *Escherichia coli* in extraintestinal infections. *J Hyg Camb*. **95:** 551-575.

Oshoma CE, Aghimen MO. and Bello ZO. (2009). Growth and survival of *Esherichia coli* in kunu zaki during storage. *World Journal of Agricultural Science. **5*(4):** 447-497.

Prescott LM. (2001). *Introduction to Microbiology*, 5th ed.; McGraw-Hill companies: New York, NY, USA, Volume 20, pp. 919–920.

Rajini K, Roland P, John C. and Vincent R. (2010). Microbiological and physiochemical analysis of drinking water in George. *Journal of Nature and Science*. **8(8):** 261-265.

Sengupta P. (2013). Potential Health Impacts of Hard Water. *International Journal of Preventive Medicine.* **4(8):** 866–875.

Seth ON, Tagbor TA. and Bernard O. (2014). Assessment of chemical quality of groundwater over some rock types in Ashanti region, Ghana. *American Journal of Science Research.* **5:** 1–6.

Sharma S, Bhat GK, Shenoy S. (2007). Virulence factors and drug resistance in Escherichia coli isolated from extraintestinal infections. *Indian J Med Microbiol*. **25:** 369-373.

Soto RP, Deen TA, Nagabhatla N. and Ayala G. (2018). Explaining water pricing through a water security lens. *Water*. ***10*(9):** 1173.

Sunday J.J, Spencer NCO, Kingsley O, Edet AO. and Amaka DD. (2014). Physico-chemical and microbiological properties of water samples used for domestic purposes in Okada town, Edo State, Nigeria. *International Journal of Current Microbiology and Applied Science*. **3(6):** 886-894.

Tille PM. and Forbes BA. (2014). Bailey and Scott's diagnostic microbiology (Thirteenth edition.). St. Louis, Missouri: Elsevier pp1038.

Trivede P, Bajpai A. and Thareja S. (2010). Comparative study of seasonal variations in Trivede-chemical characteristics in drinking water quality of Kanpur, India with reference to 200 MLD filteration plant and groundwater. *National Science.* **8:** 11–17.

United States Environmental Protection Agency (USEPA). (2009). *Method 1603: Escherichia coli (E. coli) in Water by Membrane Filtration using Modified Membrane—Thermotolerant Escherichia coli Agar (Modified mTEC)*; US Environmental Protection Agency office of water (4303 T): Washington, DC, USA, p. 42. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.epa.gov/sites/default/files/2015-08/documents/method_1603_2009.pdf

Uzal FA, Navarro MA, Li J, Freedman, J.C., Shrestha, A. and McClane, B.A., (2018). Comparative pathogenesis of enteric *Clostridial* infections in humans and animals. *Anaerobe*. ***53*:**11-20.

WHO/UNICEF (2021). Joint Monitoring Program for Water Supply, Sanitation and Hygiene (JMP) - Progress on household drinking water, sanitation and hygiene 2000 – 2020. (2021). https://www.unwater.org/publications/who/unicef-joint-monitoring-program-water-supply-sanitation-and-hygiene-jmp-progress-0. [accessed 30 November 2023].