MICROBIOLOGICAL AND PHENOTYPIC VIRULENCE ASSESSMENT OF COLIFORM BACTERIA FROM WATER SOURCES IN BENIN CITY METROPOLIS

ABSTRACT

Access to clean water and adequate sanitation is crucial for maintaining good health; however, numerous individuals face a lack access to clean water. The study was to investigation the physicochemical, assessment of coliform bacteria, phenotypic virulence characteristics and antibiotic susceptibility pattern of the identified organisms from borehole and well water samples. The result obtained showed that pH ranged from 6.06 - 7.59, while temperature (25.3 - 29.4 ^{o}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 World Health Organization for drinking water. The heterotrophic bacterial count range from 138.00±2.83CFU/100ml to 267.50±17.68CFU/100ml, coliform count ranged from 11.00±1.41 CFU/100ml to 147.50±7.78 and the counts were also found to be higher than the values stipulated by World Health Organization guidelines. The bacteria identified were *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Enterobacter cloacae. Bacillus* (37.50%) and *Escherichia* (20.83%) were the most frequently occurring bacterial isolates from water samples in the study. The phenotypic virulence properties of the bacterial isolates showed that they had at least one virulence determinants. The antibacterial sensitivity testing revealed that all isolates were susceptible to Ciprofloxacin (5mcg), with a MAR index greater than 0.2 indicating that the isolates were all pathogens of public health importance. This study therefore highlights the need for continuous monitoring and quality assessment of drinking water sources.

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

Introduction

Water plays a vital role in supporting the cycle of life and must be safeguarded against any form of pollution. Both the human body and other organisms depend on it in its pure, uncontaminated state for sustenance. Access to clean water and adequate sanitation is crucial for maintaining good health; however, numerous individuals face a lack of these fundamental requirements (Parthak, 2013). In communities with limited access to municipal water systems, alternative sources like wells and boreholes are frequently relied upon for domestic use. Water, being the most vital natural resource for sustaining life, poses a challenge as it covers over 75% of the earth's surface but is seldom available in a drinkable state due to various biological, physical, and chemical contaminants (Raimi *et al*., 2019).

Water is typically sourced from either the surface or underground. Surface water, such as rivers, lakes, and reservoirs, contrasts with groundwater, which is extracted via wells or boreholes drilled into aquifers. Generally, these groundwater reservoirs are critical finite natural reservoirs of fresh drinking water on Earth, presumed to be uncontaminated (Goswami *et al*., 2020)

However, groundwater sources often face bacterial contamination due to various factors like watershed erosion, sewage drainage, improper sewage disposal into water bodies, run-offs, and lax enforcement of groundwater investigation or well building standards. Wastewater typically contains high levels of organic material, numerous pathogenic microorganisms, and toxic compounds. This crisis is exacerbated in developing nations where public water supply is scarce, posing significant environmental and health risks (Turkarth *et al*., 2011)

In Nigeria, access to safe drinking water is limited, with only around 48% of urban dwellers and 39% of rural inhabitants having access. Bacterial contamination can lead to various diseases such as gastroenteritis, typhoid fever, cholera, bacillary dysentery, and hepatitis. Waterborne diseases contribute to 80% of illnesses in developing countries. There's a documented high rate of microbe exchange between wells and toilets/septic pits (Galadima *et al*., 2011)

The dependence on groundwater (boreholes and wells) is on the increase as a result of increased surface water contamination. Under proper design and construction practices, boreholes can ensure a proper water supply with minimal risk of local pollution. Currently, the reverse is the case as borehole and well water facilities are poorly managed leading to contamination through the accumulation of physical, chemical, and biological agents in the pipelines and storage tanks of the distribution system (Abiodun *et al*., 2016).

Portability of water cannot be determined solely by appearance; hence a bacteriological examination of the water is required. It is highly cumbersome to analyse for presence of all pathogens within a water sample. Instead, the presence of indicator organisms such as coliform bacsteria is an indication of water contamination. Coliform bacteria are usually derived from the same sources as pathogenic species and exist under the same conditions and are much easy to identify.

This study was to investigation the physicochemical, assessment of coliform bacteria, phenotypic virulence characteristics and antibiotic susceptibility pattern of the identified organisms from borehole and well water samples.

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 clayey 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 borehole 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 qulity involves the aassessment of various physic-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 analysed immediately after collection, for the presence of heterotrophic and total coliforms using membrane filtration method (USEPA, 2009).Aliquots of100ml from each samples 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 MacConkey agar, Salmonella Shigella Agar, Pseudomonas Centrimide Agar, Violet Red Bile Glucose Agar and Bacillus cereus Selective Agar Base 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 (Stager *et al*., 1983; 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, 2015) 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 - Amoxycillin (20+10mcg), 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.

RESULTS

The results of physiochemical properties of water samples from different sampling points in Benin City metropolis was evaluated as presented on Table 1. The pH ranged from 6.06 - 7.59, while temperature (25.3 - 29.4 ^{o}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 World Health Organization for drinking water.

Table 2, shows the Mean Heterotrophic bacteria and Coliform Counts of water samples from different points. The results showed that the heterotrophic bacterial count range from 138.00±2.83CFU/100ml to 267.50±17.68CFU/100ml. The heterotrophic count obtained from different drinking sources showed that the water samples were found to have higher than the stipulated values for bacterial load using the World Health Organization guidelines or standard. The coliform count ranged from 11.00±1.41 CFU/100ml to 147.50±7.78 and the counts were also found to be higher than the values stipulated for potable water by WHO. The identified and frequency of occurrence of bacterial isolates is presented on Figure 1. The bacteria identified were *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Enterobacter cloacae. Bacillus* (37.50%) and *Escherichia* (20.83%) were the most frequently occurring bacterial isolates from water samples in the study. The phenotypic virulence properties of the bacterial isolates (Table 3) showed that they had at least one virulence determinants. The antibacterial sensitivity testing revealed that all isolates were susceptible to Ciprofloxacin (5mcg), while it was also evident that they were found to have an MAR index greater than 0.2 (Table 4) which means that the isolates were all pathogens of public health importance.

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. (^oC)	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 Water Site 1, WWS2: Well Water Site 2, WHO: World Health Organisation.

Table 2: Mean Heterotrophic bacteria and Coliform Counts of water samples from different points (Log_{10} CFU/100ml)

Sites	Heterotrophic Bacteria counts	Coliform Counts
BHS1	267.5±17.68	147.5±8.36
BHS2	149.0±14.14	11.0±0.33
BHS3	146.0±5.66	37.0±1.23
BHS3	149.5±2.12	147.0±5.65
BHS4	147.5±2.12	131.0±2.67
BHS5	138.0±2.83	52.5±1.05
BHS6	152.5±4.95	169.5±5.24
BHS7	132.0±61	44.0±2.31
WWS1	147.0±1.41	51.0±1.38
WWS2	160.5±2.12	45.0±2.11
WHO	10±0.00	10.0±0.00

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 Water Site 1, WWS2: Well Water Site 2, WHO: World Health Organisation.

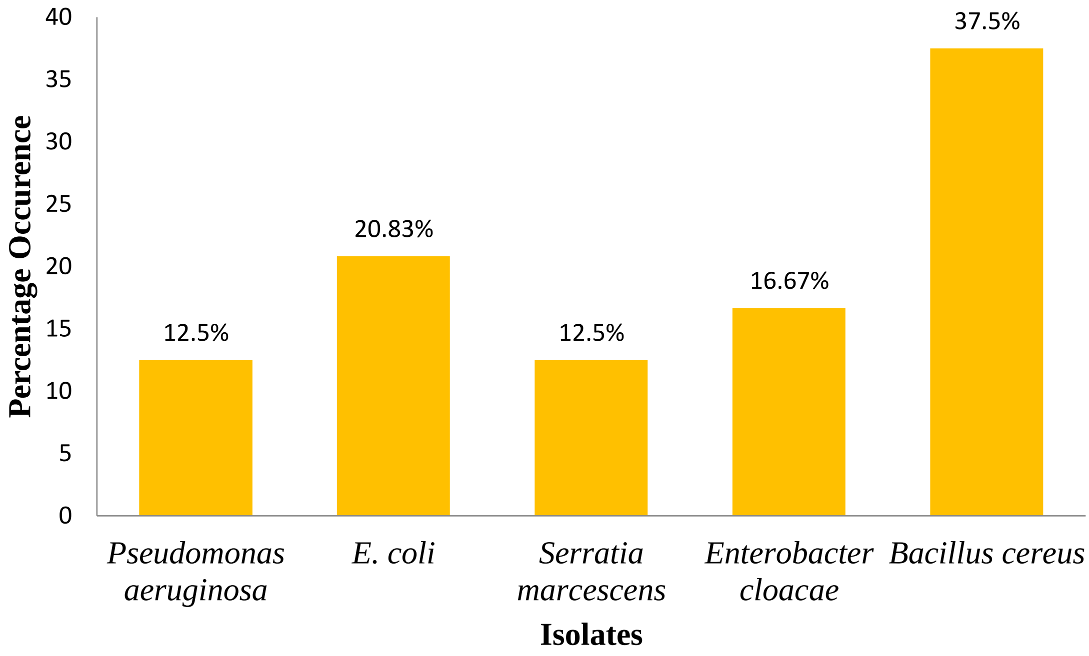

Figure 1: Frequency of occurrence of bacteria isolated from the water samples

Table 3: Phenotypic virulence determinants of bacterial isolates from water sources

Isolates	Hemolysin	DNAse	Gelatinase	Lipase
Bacillus cereus	β	+	+	+
Enterobacter cloacae	Α	-	-	+
Escherichia coli	Γ	-	-	+
Serratia marcescens	β	+	+	-
Pseudomonas aeruginosa	Γ	+	+	-

Key: β ; Beta, Α; Alpha, Γ; Gamma, +; Positive, -; Negative

Table 4: Antibiogram of bacteria isolated from the water samples

Isolates	GEN	CS	CB	M	AG	E	CIP	TE	CD	MAR index
Bacillus cereus	S	S	R	S	S	R	S	R	S	0.33
Enterobacter cloacae	S	R	R	R	R	S	S	R	S	0.55
Escherichia coli	S	S	S	R	S	R	S	S	R	0.33
Serratia marcescens	R	S	R	R	R	R	S	R	S	0.66
Pseudomonas aeruginosa	S	S	S	R	R	R	S	S	S	0.33

Key; **S -** Susceptible, **R –** Resistant, GEN - Gentamycin (10mcg), CS - Colistin (10mcg), CB - Cefuroxime (30mcg), M - Metronidazole (5mcg), AG - Amoxycillin (20+10mcg), E - Erythromycin (15mcg), CIP - Ciprofloxacin (5mcg), TE - Tetracycline (30mcg), CD - Clindamycin (2mcg),

DISCUSSION

Water is a fundamental resource integral to all environmental and social processes. Access to adequate safe drinking water is of prime importance to many governmental and international organizations since it is undoubtedly the core component of primary health care and a basic component of human development as well as a precondition for man's success in dealing with hunger, poverty and death (SOPAC/WHO, 2005). The pH, total suspended solid, turbidity, nitrate, phosphate, sulphate, dissolved oxygen, biological oxygen demand and chemical oxygen demand varied across the different sampling points showed that they 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, strontium, ferrous iron and manganous ions (Kadiri, 2006).

The results of bacteriological analysis of water samples showed that the bacterial count was observed to range from 138.00±2.83 CFU/100ml to 267.50±17.68 CFU/100ml. The heterotrophic count obtained from different drinking sources showed that the water samples were found to have higher than the stipulated values for bacterial load using the World Health Organization guidelines or standard. The coliform count ranged from 11.00±1.41 CFU/100ml to 147.50±7.78 and the counts were also found to be higher than the values stipulated for potable water by the world health organization. The identified bacterial isolates were *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Enterobacter cloacae.* The results obtained in the study is similar to the finding of Govindarajan and Senthilnathan (2014) in Ogbomosho, South-western, Nigeria, who reported that borehole water was contaminated with *Escherichia coli*. Also similar to the findings in this study was the report of Ikeme *et al.* (2014) in Owerri metropolis who showed that borehole water contained *Escherichia*, *Staphylococcus aureus* and *Pseudomonas* species. The finding of this study was also similar to the work of Thivya *et al.* (2014) in Kebbi state, Nigeria, who showed that borehole water contained *Staphylococcus aureus* and *E. coli*. In a similar study of borehole water samples tested fielded bacteria growth with organisms such as *Klebsiella* sp., *Streptococcus* sp. and *Pseudomonas* sp. (Singh *et al.*, 2012). The presence of these pathogens in such water could account for the incidence of diarrhea, food poisoning and gastroenteritis especially, among the consumers. Also, presence of these pathogens raise public health concerns that need to be addressed and the need for microbial assessment of water for production of drinks should also be emphasized to reduce possible contamination. The antibacterial susceptibility testing in the present study showed that all isolates were susceptible to Ciprofloxacin but were also resistant to erythromycin and tetracyclines. It was also evident that all isolates were found to have an MAR index greater than 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 (*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Enterobacter cloacae*) found were all susceptible to Ciprofloxacin but on the other hand were resistant to Metronidazole and Colistin.

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 WHO standard. However, the microbiological quality did not meet the requirement of WHO standard for potable water quality. The majority of the water samples were contaminated with coliforms and potential bacterial pathogens, amongst which were resistant strains. This study therefore highlights the need for continuous monitoring and quality assessment of drinking water sources for purification processes to enhance the elimination of pathogenic bacteria. Hence environmental agencies should ensure compliance with relevant standards to avoid risks to human health.

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