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Microbiological And Phenotypic Virulence Assessment Of Coliform Bacteria From Water Sources In Benin City Metropolis

Joy Zitgwai Saidu[1] and Afamefuna Dunkwu-Okafor[1*]

[1]Department of Microbiology, Faculty of Life Sciences,
University of Benin, PMB 1154 Benin City, Edo State, Nigeria.

***Author for Correspondence:** afamefuna.dunkwu-okafor@uniben.edu,

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 °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. 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. (Raimi et al.2019).

Water is typically sourced from either the surface or underground sources. Generally, these sources 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. This crisis is exacerbated in developing nations where public water supply is scarce, posing significant environmental and health risks (Turkarthet 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

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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. (Abiodun et al. 2016).

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.

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.

Physico-chemical tests (Water Quality Test)

Different physicochemical parameters amenable to water quality assessment was done (NSDWQ, 2007; WHO/UNICEF, 2021).

Culture, isolation and identification of *coliforms* 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). The filters were placed on nutrient agar and eosin methylene blue agar plates and were incubated aerobically. Colonies were isolated using different specific media The isolates were subjected to standard identification routine (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)

RESULTS

Table: Physico chemical characteristics of Water Samples

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±(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.

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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

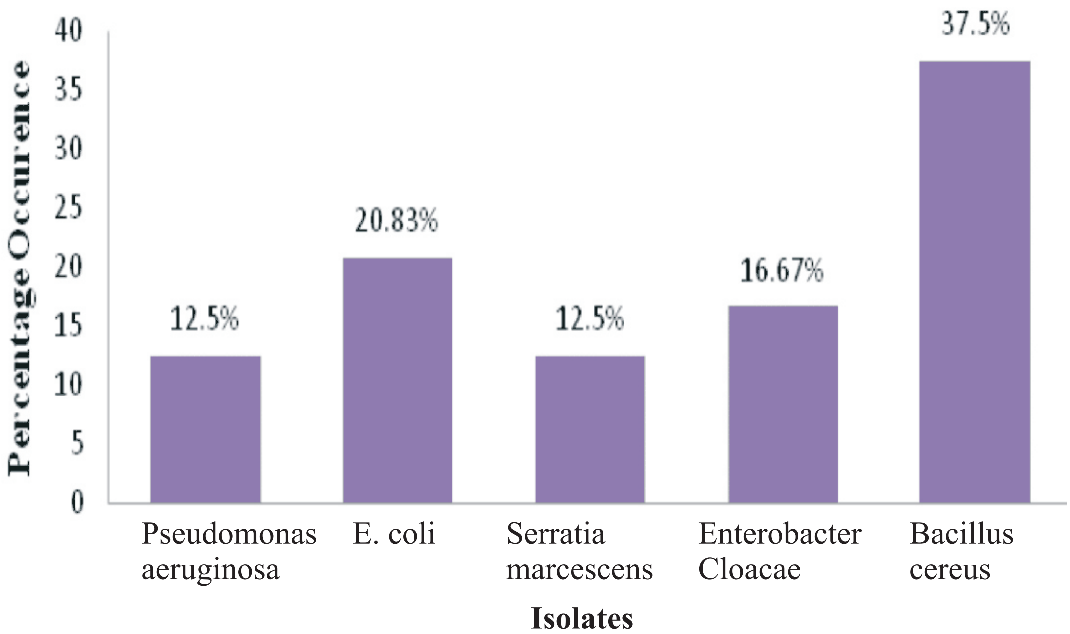

Figure 1: Frequency of occurrence of bacteria isolated from the water samples

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

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

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

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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

Access to adequate safe drinking water is of prime importance to many governmental and international organizations as it is the core component of primary health care a (SOPAC/WHO, 2005). The physico chemical parameters of the different water samples 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). 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 (Trivedeet 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 (Kadiri, 2006).

The results of bacteriological analysis of water samples showed the heterotrophic count obtained from different drinking sources were higher than the stipulated values for bacterial load using the World Health Organization guidelines or standard. The results obtained in the study is similar to the finding of Govindarajan and Senthilnathan (2014) in Ogbomosho, South-western, Nigeria and also Ikeme *et al.* (2014) in study conducted in Owerri metropolis. The presence of these pathogens in such water could account for the incidence of diarrhoea, food poisoning and gastroenteritis especially, among the consumers. Also, presence of these pathogens raises 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.

CONCLUSION

This study 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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