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Isolation and Characterization of Antibiotics-resistant Enteric Bacteria from Borehole Waters in PRESCO Campus, Ebonyi State University, Abakaliki, Nigeria

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Abstract

Water is essential for life. An adequate, safe, and accessible water supply must be available to all. Hence, this research aimed to isolate and characterize bacteria from borehole water samples located at the Presco campus, Ebonyi State University, Abakaliki, and test the antibiotic susceptibility patterns of the bacteria isolated. Twelve (12) water samples were collected from six (6) locations in duplicates and analyzed using standard microbiological methods. Serial dilutions were performed on the samples and dilutions of 103 were plated using the pour plate method. After the incubation periods, colonies were counted and expressed in CFU/mL, biochemical tests were carried out and the antibiotics susceptibility profiles of the bacteria isolated were evaluated. From the results, the total microbial counts ranged from 1.0 x 104 to 5.9 x 104 CFU/mL, indicating high contamination of the water samples. The morphology and biochemical tests revealed the presence of Shigella species 5 (45.4 %) and Salmonella species 6 (54.6 %). Shigella species were highly resistant to both Trimethoprim-Sulphamethoxazole and Amoxicillin (80 %) and to Tetracycline (100 %) but were susceptible to Ceftriaxone (100 %) and Ciprofloxacin (80 %). On the other hand, Salmonella species showed a resistant pattern of 83.3 % to Tetracycline, Ciprofloxacin, and Ceftriaxone but were susceptible to Levofloxacin (100 %) and Cefepime (83.3 %). This result is of public health importance as these organisms can threaten individuals utilizing these boreholes as the source of drinking water.

Keywords: Antibiotics, Enteric bacteria, PRESCO, Public health, Water quality.

INTRODUCTION

Water is a valuable commodity which is essential for human existence. The usefulness of water to human life can never be over-emphasized, as it is a natural substance linked to the survival of man and other living things since it is involved in the various functions of the human body (Abaasa *et al.*, 2024). One of the complex challenges facing African rural communities is inadequate supply and lack of safe drinking water. This problem has resulted in several deaths of both children and adults (WHO, 2008). Consequently, various countries have adopted programs to improve water quality, and strong emphasis has been placed on the need for reliance on other water sources, such as groundwater and rainwater, apart from surface water (Okoye *et al.*, 2022a). Over one billion people in the world lack access to safe drinking water, and about 2.5 billion people do not have adequate sanitation services (WHO and UNICEF, 2014). In developing countries such as Nigeria, clean pipe-borne water is unavailable in almost all the states. Due to the inability of the Government to meet the ever-increasing water demand, people resort to groundwater sources such as shallow wells and boreholes as alternative water sources. Natural groundwater is usually of good quality but can deteriorate due to inadequate protection and poor resource management (Sadiya *et al.*, 2018).

In Nigeria, boreholes are dug by individuals public and private entities to overcome the water shortage problem. This has resulted in dependence on boreholes as a readily available water source for drinking and domestic purposes. In most rural communities, boreholes are close to public toilets in market squares, schools, and various compounds. This can contaminate underground water bodies with enteric microorganisms (Ewelike *et al.*, 2022). Faecal bacteria such as *Shigella* sp., *Salmonella* sp., *Escherichia coli*, *Klebsiella* sp., and other pathogenic bacteria such as *Pseudomonas* sp., *Proteus* sp., and *Staphylococcus* sp. have been isolated from groundwater sources (Okoye *et al.*, 2024), and these organisms amongst other factors have led to several water-borne diseases. The World Health Organization (WHO) Guidelines for Drinking Water Quality (GDWQ) identify faecal contamination as the greatest risk to human health associated with drinking water quality (WHO, 2017).

The rapid increase and spread of microorganisms and antibiotic-resistant genes in the environment and the unappealing manifestation of this situation, which is the increasing persistence of bacterial infections among members of a population, is a public health concern (Berendonk *et al.*, 2015; Frieri *et al.*, 2016). This development requires urgent attention from health policymakers and authorities. Earlier studies on antimicrobial resistance focused more on environments and samples considered to be antibiotic resistance hotspots, which include sewage, dairy effluent, municipal wastewater, medical environments, and effluents (Harwood *et al.*, 2001; Li *et al.*, 2001; Brown *et al.*, 2006), but only a few extensive studies exist on the prevalence of antibiotic resistance in borehole water samples from solely residential areas (Dwyer *et al.*, 2017).

Historically, concerns about drinking water's microbial quality have focused on pathogens' occurrence in drinking water distribution systems. Antibiotic-resistant bacteria are emerging biological contaminants of the environment, which originate mainly from hospital, industrial, and community wastewater, animal farms, agricultural lands, and wastewater treatment plants. Hence, this research aimed to isolate and characterize antibiotic-resistant enteric bacteria from boreholes located at the PRESCO campus of Ebonyi State University, Abakaliki, Nigeria.

MATERIALS AND METHODS

Collection of water samples

Water samples were collected from six (6) different boreholes around the PRESCO campus of Ebonyi State University, Abakaliki, in duplicates using sterile containers and labeled 1-12. The sampling protocols previously described by the Clinical and Laboratory Standards Institute (CLSI, 2018) were strictly adhered to during sample collection. Each sample was collected aseptically in screw cap containers after the water was allowed to waste for 3 to 5 minutes. Care was taken not to allow air bubbles into the bottles during collection. The samples were collected aseptically from each sampling point in duplicates into the sterilized container and transported immediately to the Microbiology Laboratory of Ebonyi State University, Abakaliki, for bacteriological analysis.

Determination of total bacterial counts

The total bacterial count was carried out using the pour plate technique. A ten-fold serial dilution was carried out by measuring 1 mL of each water sample into nine (9) mL of distilled water in a test tube covered with cotton wool and foil and mixed properly to make a dilution of 10^{-1}. This was used to complete the ten-fold serial dilution. Exactly 0.5 mL of each appropriate diluent was introduced into different sterile Petri dishes containing plate count agar (PCA) and Salmonella-Shigella agar. The plates were incubated at 37 ºC for 24 hours.

Isolation of enteric bacteria

Discrete colonies obtained from the plate count agar were counted using the colony counter and recorded appropriately, while the colonies on the Salmonella-Shigella agar were sub-cultured onto nutrient agar. The petri dishes were placed in an inverted position in the incubator for 24 hours at 37 ºC to obtain pure cultures. Presumptive morphological identification of the colonies was done by observing their appearance on the media. The colonies were stored in Peptone water test tubes for cultural/bacteriological identification and biochemical characterization.

Antibiotics susceptibility testing

The antibiotics susceptibility pattern of the identified isolates was performed against a wide range of antibiotics. This was done using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Nassar *et al.*, 2019). A suspension was made from a 24-hour-old culture in sterile water to match the 0.5 McFarland turbidity standard. This was swabbed on the entire surface of solidified Mueller-Hinton agar plates using sterilized swab sticks. The surface of the medium was streaked in four directions while the plates were rotated at approximately 60º to ensure even distribution. The inoculated Mueller-Hinton agar plates were allowed to dry for a few minutes. Sterilized forceps were used to place the antibiotic discs evenly on the inoculated Mueller-Hinton agar plates so that the disc should be about 15mm from the edge of the plate and not closer than 25mm from disc to disc. After 30 minutes, the plates were inverted and incubated at 37 °C for 24 hours. After the incubation period, the diameter of the growth inhibition zones around the discs was measured to the nearest millimeter using a meter rule. The results were interpreted as sensitive or resistant using the performance standards of antimicrobial susceptibility testing (CLSI, 2018). The following standard antibiotic discs were used against the isolates: Vancomycin, Ciprofloxacin, Ceftriaxone, Levofloxacin, Tetracycline, Amoxicillin, Amikacin Cefepime, Doxycycline and Trimethoprim-Sulfamethoxazole.

RESULTS

Total bacterial counts

Total bacterial counts obtained from this study revealed the values ranged from 1.0×10^4 to 5.9×10^4 CFU/mL (Table 1). These values do not agree with the guidelines set by the World Health Organization (WHO, 2017), which recommends that total heterotrophic bacterial counts in 100 mL of drinking water should be < 100 CFU/mL, as counts > 100 CFU/mL indicate a general decrease in water quality. Hence, the borehole water samples used by students residing at the PRESCO campus of Ebonyi State University, Abakaliki, are highly contaminated, which can threaten the students drinking such water.

Table 1: Total bacterial counts of isolates obtained in borehole water samples

S/No	Number of colonies	CFU/mL
1	52	1.0×10^5
2	256	5.1×10^5
3	187	3.7×10^5
4	47	9.4×10^4
5	83	1.7×10^5
6	291	5.8×10^5

Morphological and biochemical characteristics of bacterial isolates

Morphological and biochemical tests revealed the presence of different rod-shaped bacteria in the borehole water samples (Table 2). Morphological characteristics and biochemical test results revealed there were six (6) isolates of *Salmonella* sp. (54. 6%) and five (5) isolates of *Shigella* sp. (45.4 %) isolated from the borehole water sampled.

Antibiotic susceptibility testing

The antibiotics susceptibility testing of *Salmonella* sp. and *Shigella* sp. obtained from borehole water samples in the PRESCO campus are presented in Tables 3 and 4, respectively. It revealed that some strains were resistant to certain antibiotics while others were sensitive to other antibiotics.

Table 2: Morphological, microscopic, and biochemical characteristics of bacterial isolates from Boreholes located within PRESCO Campus

Cell Morphology	Color	Gram Rxn	MOT	CAT	OX	IND	MR	VP	CIT	Suspected Organism
Rod	opaque	-	-	+	-	-	+	-	-	*Shigella* spp.
Rod	Colorless	-	+	+	-	-	+	+	+	*Salmonella* spp.

KEY: Gram Rxn = Gram reaction, MOT = Motility test, CAT = Catalase test, OX = Oxidase test, IND = Indole test, MR = Methyl red test, VP = Voges-Proskauer test, CIT = Citrate test.

Table 3: Antibiotics susceptibility profile of *Salmonella* sp. isolated from boreholes located within the PRESCO Campus

Antibiotics	Disc potency (µg)	No of isolates	S (%)	R (%)
SXT	25	6	4 (66.7)	2 (33.3)
TE	30	6	1 (16.7)	5 (83.3)
VA	30	6	3 (50)	3 (50)
AM	30	6	0 (00)	6 (100)
DXT	30	6	5 (83.3)	1 (16.7)
AK	30	6	6 (100)	0 (00)
FEP	30	6	5 (83.3)	1 (16.7)
CIP	5	6	1 (16.7)	5 (83.3)
CRO	30	6	1(16.7)	5 (83.3)
LEV	5	6	6 (100)	0 (00)

KEY: SXT= Trimethoprim-Sulfamethoxazole, TE = Tetracycline, VA = Vancomycin, AM = Amoxicillin, DXT= Doxycycline, TOB = Tobramycin, AK = Amikacin, FEP = Cefepime, CIP = Ciprofloxacin, CRO = Ceftriaxone, LEV = Levofloxacin, S = number of susceptible isolates %, R = number of resistant isolates %.

Table 4: Antibiotics susceptibility profile of *Shigella* sp. obtained from boreholes located within the PRESCO Campus

Antibiotics	Disc potency (µg)	No of isolates	S (%)	R (%)
SXT	25	5	1 (20)	4 (80)
TE	30	5	0 (00)	5 (100)
VA	30	5	2 (40)	3 (60)
AML	30	5	1 (20)	4 (80)
DXT	30	5	4 (80)	1 (20)
AK	30	5	2 (40)	3 (60)
FEP	30	5	2 (40)	3 (60)
CIP	5	5	4 (80)	1 (20)
CRO	30	5	5 (100)	0 (00)
LEV	5	5	3 (60)	2 (40)

KEY: SXT= Trimethoprim-Sulfamethoxazole, TE = Tetracycline, VA = Vancomycin, AM = Amoxicillin, DXT= Doxycycline, TOB = Tobramycin, AK = Amikacin, FEP = Cefepime, CIP = Ciprofloxacin, CRO = Ceftriaxone, LEV = Levofloxacin, S = number of susceptible isolates %, R = number of resistant isolates %.

DISCUSSION

This study has shown that borehole water samples contain faecal bacteria. The presence of these bacteria in the sampled water sources is of public health concern because it does not comply with the World Health Organization (WHO) guidelines for coliform bacteria of zero total coliforms per 100 mL of water. The World Health Organization (WHO) sets stringent standards for drinking water quality to ensure public health and safety (WHO, 2017). These standards encompass various parameters, including microbiological, chemical, and physical aspects, to mitigate the risks associated with waterborne diseases.

Total bacterial counts obtained from this study revealed the values ranged from 1.0×10^4 to 5.9×10^4 CFU/mL. These values do not agree with the guidelines set by the World Health Organization (WHO, 2017), which recommends that total heterotrophic bacterial counts in 100 mL of drinking water should be < 100 CFU/mL, as counts > 100 CFU/mL indicate a general decrease in water quality. Hence, the borehole water samples used by students residing at the PRESCO campus of Ebonyi State University, Abakaliki, are highly contaminated, which can threaten the students drinking such water.

Morphological characteristics and biochemical test results revealed there were six (6) isolates of *Salmonella* sp. (54. 6%) and five (5) isolates of *Shigella* sp. (45.4 %) isolated from the borehole water sampled. The presence of *Salmonella* sp. in the present study was also reported by Ekelozie *et al.* (2018), as they recorded a prevalence of *Salmonella* species in borehole water samples in their research, while the isolation of *Shigella* sp. is in line with the study of Olalemi *et al.* (2021), who obtained *Shigella* and other enteric bacteria from two borehole water samples. Similarly, Okoye *et al.* (2022b) reported the presence of enteric bacteria isolated from bottled and sachet water, which come from borehole water. The presence of enteric bacteria in borehole water could be attributed to various causes such as inadequate sanitation, poor hygiene of water handlers, faecal contamination, etc. The presence of enteric bacteria in drinking water has been linked to cholera, typhoid fever, diarrhea, dysentery, gastrointestinal infections, and, in severe cases, death (Abaasa *et al*., 2024; Okoye *et al*., 2024). This is of public health importance, as it mitigates the efforts of the United Nations towards achieving SDG 6 - to ensure access to safe water and sanitation for all.

Antibiotic susceptibility test results of 5 isolates of *Shigella* sp. obtained from borehole water samples revealed that the isolates were susceptible to Ciprofloxacin (80 %) and Ceftriaxone (100 %). This result is consistent with the WHO (2005) recommendation that cases of *Shigella dysenteriae* should be treated with Ciprofloxacin as first-line treatment and Pivmecillinam, Ceftriaxone and Azithromycin as second-line treatment. The isolates were resistant to Trimethoprim-Sulfamethoxazole (80 %), the result of which disagrees with the findings of Nelson *et al*. (1976), who stated that Trimethoprim-Sulfamethoxazole is the best currently available drug for the treatment of shigellosis in areas where multiple antibiotic resistance of *Shigella* sp. is common. This result also contradicts the findings of Lolekha *et al*. (1991), who opined that the antimicrobial agent of choice has been changed from Ampicillin to Trimethoprim-sulfamethoxazole and recently to Fluoroquinolones. Despite the above submissions, research carried out in 2013 revealed that *Shigella* infections have become increasingly resistant, and this was observed in the present study as the isolates showed resistance to several antibiotics, which agrees with the present study. The *Shigella* species in this study were resistant to Tetracycline. This result agrees with the study of Abaasa *et al*. (2024). Tetracycline resistance now occurs in increasing numbers in pathogenic, opportunistic, and commensal bacteria. *Shigella dysenteriae* is the first tetracycline-resistant bacterium (Ali *et al*., 2011). The presence of tetracycline-resistant pathogens limits the use of these agents in treating diseases. Tetracycline resistance is often due to the acquisition of new genes, which code for energy-dependent efflux of tetracyclines or for a protein that protects bacterial ribosomes from the action of tetracycline (Chopra and Roberts, 2001). Resistance to Amoxicillin (80 %) was also found with the *Shigella* species isolated in the present study, and this agreed with the research of Siraj *et al*. (2019), who obtained a resistance rate of 95 %.

Antibiotic susceptibility test of *Salmonella* revealed that the isolates were highly resistant to Tetracycline (83.3 %). Sabrina *et al*. (2021) reported the resistance of *Salmonella* to tetracycline and sulphonamides. According to the PCR studies on *Salmonella*, tetracycline resistance genes most frequently detected from the organism were *tetA* and *tetB* genes, which are associated with plasmids, transposons, or both, and are often conjugative, highlighting the transference potential of these genes to other bacteria, environment, animals, and humans (Sabrina *et al*., 2021). The present study also recorded a resistance rate of 83.3 % each for Ciprofloxacin and Ceftriaxone. This result is comparable with the study of Saeed *et al*. (2019), who reported extended drug-resistant *Salmonella* in Asia and other areas of Africa. The result also corroborates the study of Okoye *et al*. (2024), who obtained *multidrug-resistant Salmonella species as they showed resistance to Amoxicillin-Clavulanic acid and other fourth-*generation antibiotics. This result is of public health concern as the resistant strains transfer resistance to other organisms. In the present study, *Salmonella* species showed a high susceptibility to Cefepime (83.3 %) and Levofloxacin (100 %). This result is similar to the result of Okoye *et al*. (2024), with a susceptibility of 98 % to cefepime, and also mirrors the work of Sjolund-Karlsson *et al*. (2014) who obtained *Salmonella* species that were all susceptible to Levofloxacin. Hence, Cefepime and Levofloxacin can be used as a drug of choice in treating *Salmonella* infections.

CONCLUSION

The presence of *Salmonella* and *Shigella* in borehole water sources located at the students' hostel is of public health concern. This is due to the presence of these pathogenic bacteria, indicating faecal contamination, and most students source their drinking water from the boreholes. Indication of fecal contamination reveals that sewage disposal systems are located at close distances to the location of the boreholes. Some antibiotics tested against the organisms revealed the presence of both sensitive and resistant strains. Many bacteria have emerged with unprecedented resistance to antibiotics, including those isolated from the present study. Hence, successful treatment with these antibiotics depends on the infecting strains.

RECOMMENDATION

It is therefore recommended that:

- ✓ Students and other populace in and around the PRESCO campus should avoid drinking borehole water without proper treatment by boiling it, as enteric organisms heavily contaminate it.
- ✓ The appropriate authorities should carry out enlightenment campaigns on the proper use of antibiotics.
- ✓ Drilling of boreholes should be far away from wastewater/sewage/suck-away disposal channels.

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