



Assessment of Antibiotic Resistance and Toxigenic Genes of Enteric Bacteria Population from Borehole Water Sources in Diobu, Port Harcourt, Nigeria

Berembo, B. T*, Amadi, L. O, Sampson, T. and Ugboma, C. J.

Department of Microbiology, Rivers State University,
Port Harcourt, Nigeria.

*Corresponding Author: berembo.beremboba@ust.edu.ng

ABSTRACT

This study investigated the enteric bacteria, antibiotic resistance and toxigenic genes profiles of isolates from the borehole water sources in Diobu, Port Harcourt, Nigeria. Water samples were collected at monthly intervals for a period of 12 calendar months (June 2023 to May, 2024), covering both the dry and wet seasons. Water samples were analyzed using standard microbiological methods, leading to the identification of 14 bacteria genera which included pathogenic and opportunistic strains such as *Klebsiella pneumoniae*, *Vibrio cholerae*, *Salmonella typhi*, and *Escherichia coli*. Antibiotic susceptibility testing revealed widespread resistance, with several isolates exhibiting multidrug resistance (MDR) to Fluoroquinolones, β -lactams, and other antibiotic classes. Notably, *K. pneumoniae*, *V. cholerae*, *Yersinia enterocolitica*, *Salmonella typhi*, and *Escherichia coli* showed resistance to multiple agents, while some species, such as *Kluyvera ascorbata* and *Enterobacter aerogenes*, retained susceptibility to aminoglycosides. These findings highlight borehole water as a potential reservoir for MDR bacteria, with significant public health implications due to possible transmission via contaminated water, food, and direct human contact. This highlights the need for continuous environmental surveillance, stricter waste management, and antibiotic stewardship to mitigate the spread of antimicrobial resistance through water.

Keywords: Borehole Water, Enteric Bacteria, Antibiotic Resistance, Water Quality, Public Health.

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 septic tanks, 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* spp., *Salmonella* spp., *Escherichia coli*, *Klebsiella* spp., and other pathogenic bacteria such as *Pseudomonas* spp., *Proteus* spp., and *Kluyvera* spp. 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 presence of such resistant strains in natural water bodies poses a serious public health risk, particularly in communities that rely on untreated surface water for domestic use or recreational activities. Notably, the World Health Organization (WHO) has identified antimicrobial resistance as one of the top ten global public health threats. Compounding these challenges is the role of climate change, which is introducing new stressors into aquatic systems. Changes in temperature, precipitation, and hydrology affect microbial metabolism, community composition, and the mobility and toxicity of pollutants (Doney *et al.*, 2012). Warming waters can enhance microbial growth rates but may also disrupt the balance between microbial groups, enabling pathogenic or resistant strains to dominate. As such, climate variability may exacerbate the ecological impacts of pollution and accelerate the spread of AMR in aquatic habitats. Considering these growing concerns, it is crucial to monitor and assess the microbial diversity, abundance, and antibiotic susceptibility profiles of bacteria in aquatic environments, particularly those experiencing intensive anthropogenic activities. 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.

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, agricultural lands, and wastewater treatment plants.

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).

However, only a few extensive studies exist on the prevalence of antibiotic resistance in borehole water samples from solely residential areas (Dwyer *et al.*, 2017). Hence, this present study aimed to isolate and assess antibiotic resistance, and toxigenic genes of enteric bacterial isolated from borehole water sources in Diobu, Port Harcourt as to ascertain the water quality.

Materials and Methods

Study area

The study area is Diobu community with coordinates 4°47'24"N, 6°59'36"E (Latitude: 4.772152; Longitude: 6.994514) located in Port Harcourt, Rivers State, Nigeria. The bacteria used for this study were the bacteria that were previously isolated from the borehole water sources and identified in the Rivers State University Microbiology Laboratory by the authors. The bacteria were; *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Vibrio cholerae*, *Kluyvera ascorbata*, *Salmonella typhi*, *Escherichia coli*, *Shigella dysenteriae*, *Klebsiella aerogenes*, *Shewanella putrefaciens*, *Citrobacter koseri*, and *Yersinia enterocolitica*.

Collection of water samples

Water samples were collected from fifteen (15) different boreholes in Diobu area of Rivers State, Nigeria in duplicates using sterile containers and labeled 1-30. The sampling protocols previously described by CLSI (2018) were strictly adopted. 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. Samples were collected from each borehole in duplicates into the sterilized container and transported immediately to the Microbiology Laboratory of Rivers State University, Port Harcourt, for bacteriological analysis.

Determination of Enteric bacterial counts

The total enteric count was carried out using the spread 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 10^3 . This was used to complete the ten-fold serial dilution. Exactly 0.1 mL of each appropriate diluent was introduced into different sterile Petri dishes containing the various Media (MacConkey agar, Eosine Methylene Blue, Thiosulphate citrate bile salt and Salmonella-Shigella agar. The plates were incubated at 37°C for 24 hours.

Isolation of Enteric Bacteria

Discrete colonies obtained from the different agar were counted using the colony counter and recorded appropriately; the colonies were sub-cultured onto nutrient agar. The petri dishes were placed in an inverted position in the incubator for 24 hours at 37 °C 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 standards. 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 60° 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, intermediate or resistant using the performance standards of antimicrobial susceptibility testing (CLSI, 2018). The following standard antibiotic discs were used against the isolates: Ceporex (10µg), Ceftriaxone (30µg), Streptomycin (30ug), Cefuroxime (30µg), Ofloxacin (10µg) Amoxicillin Clavulanic acid (Augmentin) (30µg), Reflacine (10µg) Ceftazidime (30µg), gentamycin (30µg) and Ciprofloxacin (30µg).

Results

The morphological and biochemical tests revealed the presence of different rod-shaped bacteria in the borehole water samples (Table 1).

The results revealed there were eight (8) isolates of *Salmonella* spp. (6.7%), eight (8) isolates of *Shigella* spp. (6.7 %), fourteen (14) *Vibrio* spp. (11.7%), fourteen (14) *Klebsiella* spp. (11.7%) ten (10) *Escherichia coli*. (8.3%), seven (7) *Proteus* spp. (5.8%), nine (9) *Citrobacter* spp. (7.5%) and eighteen (18) *Enterobacter* spp (15%) were isolated from the borehole water sampled.

The antibiotics susceptibility testing of enteric bacteria obtained from borehole water samples in the Diobu Community is presented on Table 2. It revealed that some strains were resistant to certain antibiotics while others were sensitive to other antibiotics.

Table 1: Morphological, microscopic, and biochemical characteristics of bacteria isolated from the Boreholes located within Diobu

Cell Morphology	Color	Gram Rxn	MOT	CAT	OX	MR	VP	CIT	Suspected Organism
Rod	Opaque	-	+	+	-	+	-	-	<i>Shigella</i> spp
Rod	Colorless	-	-	+	-	+	+	+	<i>Salmonella</i> spp
Rod	Yellow	-	+	+	-	+	+	+	<i>Vibrio</i> spp
Rod	Red	-	+	+	-	+	-	+	<i>Serratia</i> spp
Rod	Greenish metallic sheen	-	+	+	-	+	-	+	<i>E. coli</i>
Rod	Colorless	-	+	+	-	-	+	+	<i>Citrobacter</i> spp
Rod	Blue	-	+	+	-	+	-	+	<i>Klebsiella</i> spp
Rod	Colorless	-	+	+	-	+	-	+	<i>Proteus</i> spp
Rod	Pink	-	+	+	-	+	+	+	<i>Enterobacter</i> 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 2: Susceptibility Pattern of Enteric Bacterial Isolated from Borehole Water in Diobu

Antibiotics	Concentration (µg)	Resistant n (%)	Susceptible n (%)	Intermediate n (%)
Ceporex (CEP)	30	81 (67.5)	31(25.8)	8(6.7)
Ceftriaxone (TRX)	10	72(60)	35(29.2)	13(10.8)
Streptomycin (S)	30	69(57.5)	45(37.5)	6(5.7)
Cefuroxime(CEF)	30	67(55.8)	43(35.9)	10(8.3)
Ofloxacin (OFX)	10	52(43.3)	51(42.5)	17(14.2)
Augmentin (AUG)	10	73(60.8)	30(25)	7(14.2)
Reflacine (PEF)	10	54 (45)	55 (45.8)	11(9.2)
Ceftazidime (CTZ)	30	62 (51.7)	53 (44.2)	5 (4.1)
Gentamycin (CN)	30	65(54.2)	48 (40)	7 (5.8)
Ciprofloxacin(CPX)	30	75(62.5)	29(24.2)	16(13.3)

Key: n= Number of Species

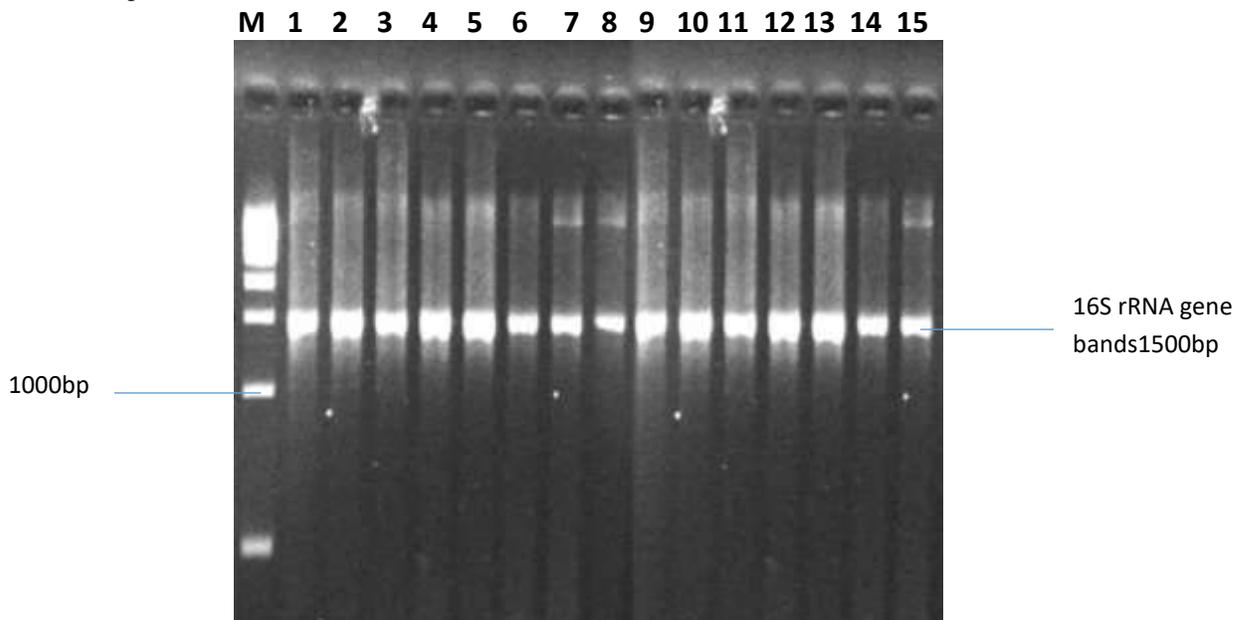


Plate 1: Agarose gel electrophoresis showing the 16SrRNA bands. Lane 1-15 showing the 16SrRNA bands at 1500bp while lane M represents the 1000bp molecular ladder

The 16S rRNA of all the bacteria isolated from Diobu showed percentage similarities to their genBank relatives and to other species at 100% similarity. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the 16S rRNA of the isolate OG to *Vibrio alginolyticus*, TM was closely related to *Proteus mirabilis*, GAM was closely related to *Proteus vulgaris*, AB was closely related to *Salmonella enterica*, NSK, EME, URU was closely related to *Escherichia coli*, AB₂ and TM₂ were closely related to *Vibrio parahaemolyticus* and *Vibrio cholerae*, OG₂, LUM, GAM₂ EME₂ TM₃ were closely related to *Salmonella typhimurium*, *Shigella flexneri*. *Shigella boydii*, *Proteus mirabilis*, and *Shigella sonnei* respectively as shown in the phylogenetic tree presented in Figure 1.

Table 4 presents the molecular identification of some antibiotic-resistant bacteria isolates, their percentage relatedness to GeneBank reference strains, and their accession numbers from the Diobu community. Isolates identified were *Klebsiella* sp (100% related to *Proteus mirabilis* strain WQZ10) with ascension number FN650811; *Vibrio* sp (100% related to *Vibrio alginolyticus* strain ATCC3378) with ascension number CPO13485; *Escherichia* sp.(100% related to *Escherichia coli* strain KSA-15) with ascension number AYO36017; *Salmonella* sp. (100% related to *Salmonella typhimurium* strain 109/9) with ascension number KP899805 and *Shigella* sp. (100% related to *Shigella boydii* strain P288) with ascension number OQ915463.

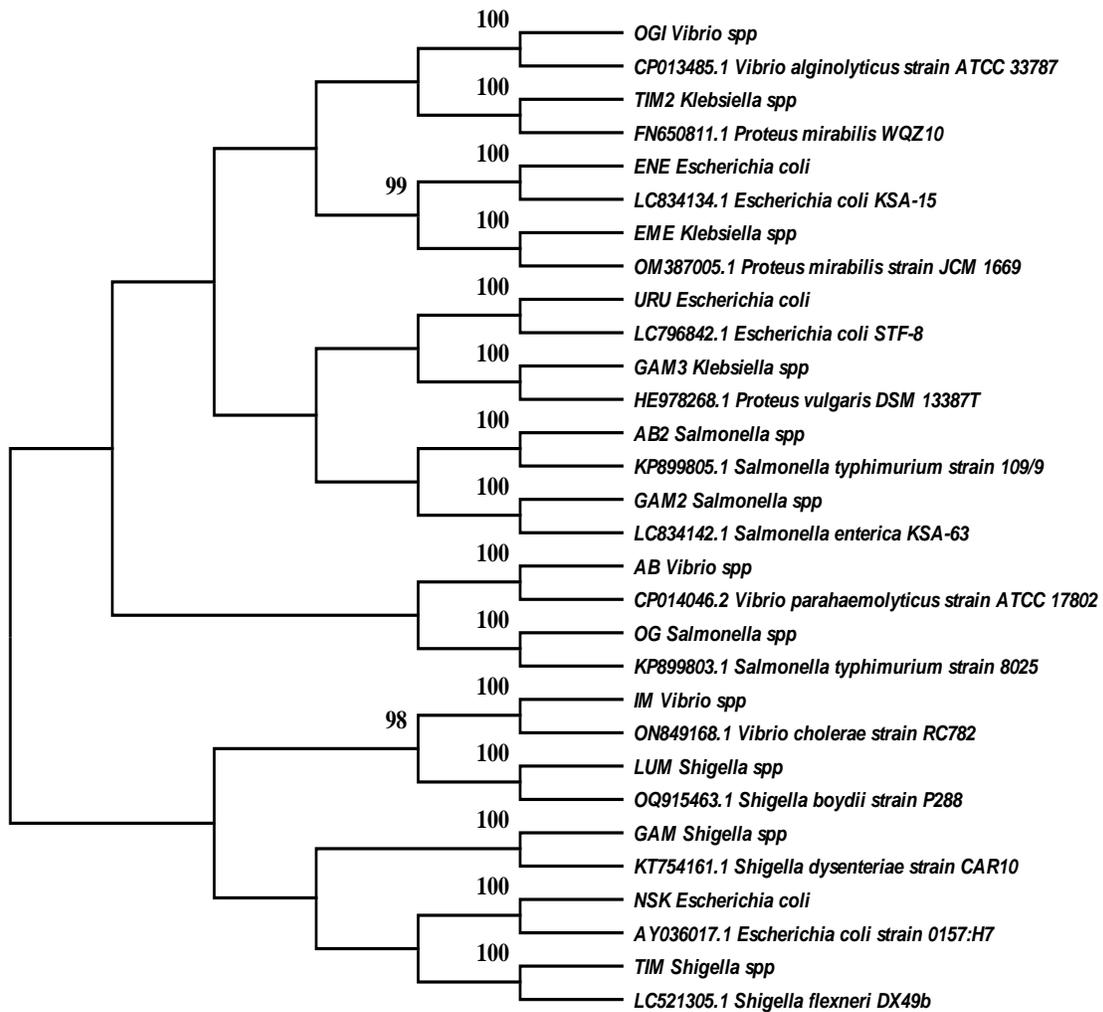


Figure 1: Phylogenetic Tree showing evolutionary distance between bacterial Isolates

Table 3: Molecular Identification of Antibiotic-Resistant Bacteria from Ox-Bow Lake and Percentage Relatedness with Gene Bank Relatives and their Ascension Numbers

Isolate code	Source	Physiological Identification	Molecular Identification /Gene Bank Relative	Percentage (%) Relatedness	Ascension Number
OG	Mile 1	<i>Vibrio</i> sp	<i>Vibrio alginolyticus</i> strain2024 ATCC3378	100	CPO13485
URU	Mile 1	<i>Escherichia</i> sp	<i>Escherichia coli</i> strain KSA-15	100	AYO36017
AB	Mile 1	<i>Salmonella</i> sp	<i>Salmonella tyhimurium</i> 109/9	100	KP899805
OG 2	Mile 1	<i>Salmonella</i> sp	<i>Salmonella typhimurium</i> strain D8025	100	KP899803
LUM	Mile 1	<i>Shigella</i> sp	<i>Shigella boydii</i> P288	100	OQ915463
TIM	Mile 2	<i>Klebsiella</i> sp	<i>Proteus mirabilis</i> strain WQZ10	100	FN650811
EME 2	Mile 2	<i>Klebsiella</i> sp	<i>Proteus mirabilis</i> strain JCM1669	100	OM387005
TM 3	Mile 2	<i>Shigella</i> sp	<i>Shigella sonnei</i> DX49b	100	LC521305
GAM	Mile 2	<i>Klebsiella</i> sp	<i>Proteus vulgaris</i> strain DSM13387T	100	HE978268
GAM 2	Mile 2	<i>Salmonella</i> sp	<i>Salmonella enterica</i> strain KSA -63	100	LC834142

The gel electrophoresis results of the molecular profiling of antibiotic resistance and toxigenic genes in Plate 2 to 6. Plate 2 shows the amplification of the blaCTX-M gene, which is associated with extended-spectrum beta-lactamase (ESBL) production and resistance to third-generation cephalosporins. Lanes 1-15 contains DNA from different bacterial isolates (*Vibrio alginolyticus*, *Proteus mirabilis*, *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhimurium*, *Salmonella enterica*, *Vibrio parahaemolyticus*, *Salmonella enterica*, *Vibrio cholerae*, *Shigella boydii*, *Shigella flexneri*, *Proteus mirabilis* and *Shigella sonnei*). The presence of distinct bands at 500 bp in each lane confirms the successful amplification of the blaCTX-M gene in these bacterial species. The 100 bp molecular ladder serves as a size reference. The comparison confirms that the observed bands align with the expected 500 bp fragment size of the blaCTX-M gene.

The agarose gel electrophoresis results in Plate 3 indicate the presence of the toxR gene in *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, and *Vibrio cholerae* at approximately 1000 bp. Lane 1, 2 and Lane 3 contain DNA samples from *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, and *Vibrio cholerae* respectively.

The presence of distinct bands at 1000 bp suggests successful amplification of the toxR gene, which is associated with quinolone resistance. The 100 bp molecular ladder serves as a reference to estimate the sizes of the amplified DNA fragments. The banding pattern confirms that the observed toxR gene bands correspond to the expected 1000 bp size. The presence of the STX1 gene, a known toxigenic gene, was detected in *Escherichia coli*, *Shigella boydii*, *Shigella flexneri* and *Shigella sonnei* through agarose gel electrophoresis, as shown in Plate 4. The results confirmed distinct bands at 180bp, indicating the successful amplification of the STX1 gene in these bacterial isolates.

The agarose gel electrophoresis results in Plate 5 show the amplification of the represents the artA gene bands at 1200bp which encodes type 1 fimbrial adhesin, a key virulence factor involved in bacterial adhesion and biofilm formation. Lanes 1-3 contain DNA samples from different bacterial isolates (*Salmonella typhimurium*, *Salmonella enterica* and *Salmonella typhimurium*). The presence of distinct bands at 1200bp in all lanes indicates the successful amplification of the artA gene in these bacterial species. The 100-2000 bp molecular ladder serves as a reference for estimating the DNA fragment sizes.

The alignment of the bands confirms that the observed artA gene bands match the expected 1200bp fragment size. The presence of the hlyA gene bands at 1170bp of, a known virulence factor was confirmed *Proteus mirabilis*, *Proteus vulgaris* and *Proteus mirabilis* through agarose gel electrophoresis. The results, as illustrated in Plate 6, showed distinct bands at 1170bp, indicating the successful amplification of the hlyA gene in these bacterial isolates.

Table 4 presents the resistance and toxigenic gene profiling of the bacterial isolates, highlighting their potential pathogenicity and antibiotic resistance mechanisms. Several isolates, including *Vibrio alginolyticus* strain2024 ATCC3378, *Proteus mirabilis*

strain WQZ10 harbored the BlaCTX-M resistance gene, indicating their resistance to extended-spectrum beta-lactam antibiotics. Similarly, *Salmonella typhimurium* 109/9 carried the BlaCTX-M gene, which is associated with resistance to fluoroquinolones.

The toxigenic genes detected among the isolates included toxR (cholera toxin-producing gene) in *Vibrio alginolyticus*, and *Vibrio cholerae*, Stx1 (shiga-toxin-producing gene) in *Escherichia coli* and *Shigella boydii* suggesting their potential to produce harmful toxins. Additionally, the artA gene, linked to enterotoxin production, was found in *Salmonella typhimurium* and *Salmonella enterica* and hlyA gene in *Proteus mirabilis* and *Proteus vulgaris*.

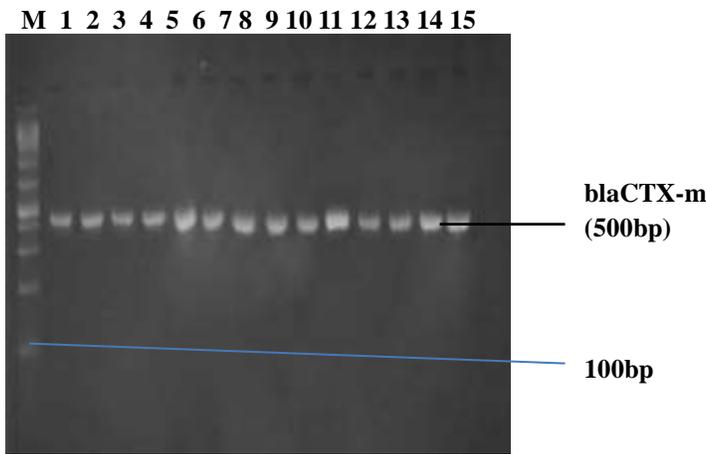


Plate 2: Lane 1-15 represents the blaCTX-m gene bands at 500bp of enteric bacterial while M represents the 100bp molecular ladder.

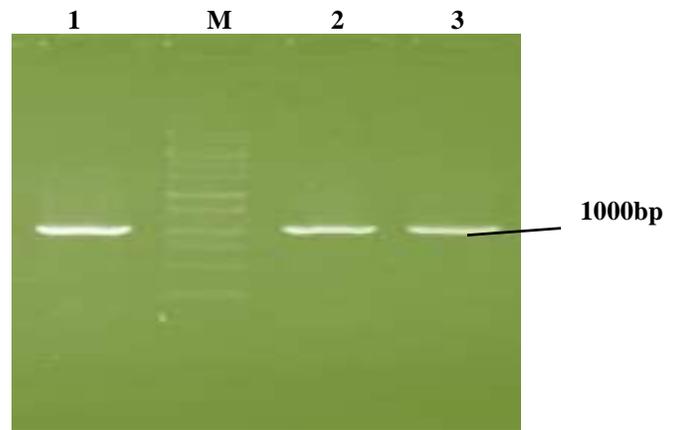


Plate3: Agarose gel electrophoresis of the toxR gene bands. Lane 1-3 represent the toxR gene bands at 1000bp *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, and *Vibrio cholerae*

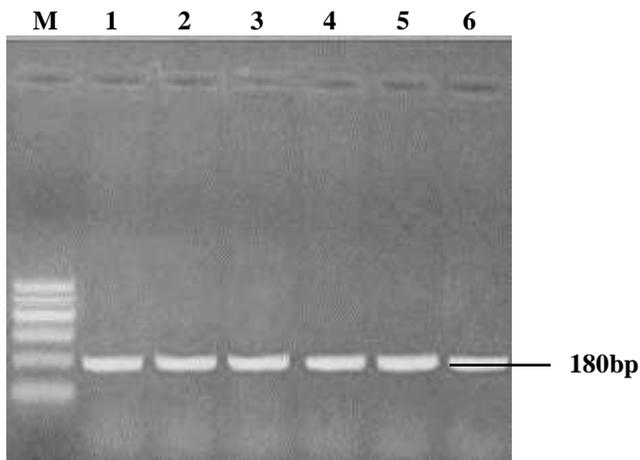


Plate 4: The spvC gene bands at 180bp of *Escherichia coli*, and *Shigella boydii*, *Shigella flexneri*, *Shigella sonnei*

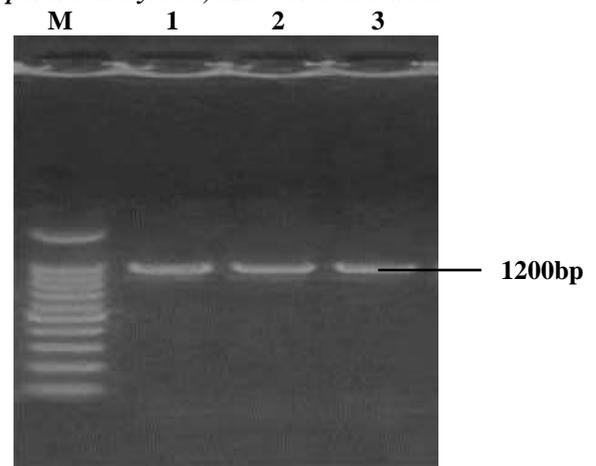


Plate 5: represents the artA gene bands at 1200bp bands of *Salmonella typhimurium*, *Salmonella enterica* and *Salmonella typhimurium*

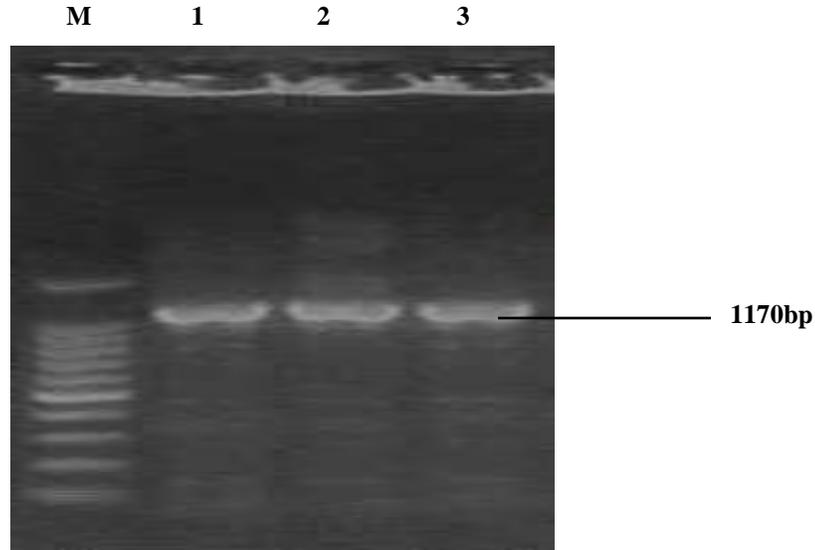


Plate 6: represent the hlyA gene bands at 1170bp *Proteus mirabilis*, and *Proteus vulgaris* while M represents the M represents the 1000bp molecular ladder.

Table 4: Antibiotic Resistance and Toxigenic gene profile of bacteria isolated from Diobu

Isolate code	Molecular Identification /Gene Bank Relative	Number of Base pairs	Resistance Gene(s)	Toxigenic Gene(s)
OG	<i>Vibrio alginolyticus</i> strain2024 ATCC3378	500/1000	BlaCTX-M Positive	toxR positive
TM	<i>Proteus mirabilis</i> strain WQZ10	500/1170	BlaCTX-M Positive	HlyA positive
NSK	<i>Escherichia coli</i> strain STF-8	500/180	BlaCTX-M Positive	stx 1 positive
AB	<i>Salmonella tyhimurium</i> 109/9	500/1200	BlaCTX-M Positive	ArtA positive
TM 2	<i>Vibrio cholerae</i> strain RC728	500/1000	BlaCTX-M Positive	toxR positive
LUM	<i>Shigella boydii</i> P288	500/180	BlaCTX-M Positive	stx 1 positive

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 cells/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.

Morphological characteristics and biochemical test results revealed there were eight (8) isolates of *Salmonella* spp. (6.7%), eight (8) isolates of *Shigella* spp. (6.7 %) fourteen (14) *Vibrio* spp. (11.7%) fourteen (14) *Klebsiella* spp. (11.7%) ten (10) *Escherichia coli* (8.3%), seven (7) *Proteus* spp. (5.8%), nine (9) *Citrobacter* spp. (7.5%) and eighteen (18) *Enterobacter* spp (15%) isolated from the borehole water sampled. The presence of *Salmonella* spp. 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* spp. 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.

The detection of *Vibrio cholerae* signals potential public health risks, as it is the etiological agent of cholera, a severe diarrheal disease linked to water contamination. Its occurrence aligns with reports from Okoh *et al.* (2010) in South Africa, where *Vibrio cholerae* was linked to poor sanitation and runoff from surrounding communities. Likewise, the presence of *Salmonella typhi* the causative agent of typhoid fever indicates fecal contamination and mirrors observations by Odonkor and Ampofo (2013) in Ghana. Antibiotic susceptibility test results of enteric bacterial isolates obtained from borehole water samples revealed that the isolates were susceptible to Reflacine (45.8 %) and Ceftazidime (44.2%). This result is consistent with the WHO (2005) recommendation that cases of *Shigella dysenteriae* should be treated with Ciprofloxacin as first-line treatment, Ceftriaxone and Azithromycin as second-line treatment. The isolates were resistant to Ceporex (67.5 %), the result of which disagrees with the findings of Nelson *et al.* (1976), who stated that Ceporex 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 Ceporex and recently to Fluoroquinolones.

Despite the above submissions, research carried out in 2013 revealed that enteric bacterial 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 enteric bacterial isolates in this study were resistant to Augmentin. This result agrees with the study of Abaasa *et al.* (2024). Augmentin resistance now occurs in increasing numbers in pathogenic, opportunistic, and commensal bacteria. *Shigella dysenteriae* is the first Augmentin-resistant bacterium (Ali *et al.*, 2011).

The presence of Augmentin resistant pathogens limits the use of these agents in treating diseases. The resistance is often due to the acquisition of new genes, which code for energy dependent efflux of antibiotics or for a protein that protects bacterial ribosomes from the action of the drug (Chopra and Roberts, 2001). Resistance to Ceporex (67.5 %) 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 %. The resistance profiles of *K. pneumoniae* and *Salmonella typhi* align with Chanda *et al.* (2019), who documented widespread β -lactam and fluoroquinolone resistance in aquatic bacterial isolates. In contrast, *K. ascorbata*, *Y. enterocolitica*, and *E. aerogenes* exhibited higher susceptibility to aminoglycosides (Gentamycin) and older agents such as Streptomycin and Chloramphenicol. Adelowo *et al.* (2014) suggested that the continued susceptibility of some environmental bacteria to aminoglycosides may be due to their limited use in aquatic contexts.

The detection of MDR pathogens in the borehole water that may be used for drinking or domestic purposes has direct implications for human health. Such bacteria can enter human populations via contaminated food, direct water contact, or through horizontal gene transfer to other pathogens (Baquero *et al.*, 2008). Mobile genetic elements such as plasmids, Integron, and transposons facilitate the spread of resistance genes from environmental bacteria to clinically significant strains, creating a potential One Health challenge.

Communities relying on untreated or inadequately treated water sources are particularly vulnerable to waterborne disease outbreaks, which may be difficult to control when caused by MDR organisms. Additionally, the contamination of food sources prepared with contaminated water serves as another pathway for resistance transmission to humans.

Conclusion

The borehole water in Diobu serve as drinking water sources for the inhabitants of the Diobu area of Port Harcourt, in Rivers State. The presence of *Escherichia coli*, an indicator of faecal contamination reveals that sewage disposal systems located at close distances to the location of the boreholes. The content of septic tanks must have leached into the subsoil. The presence of pathogenic bacteria such as *Escherichia coli*, *Salmonella*, *Klebsiella*, *Enterobacter*, *Vibrio* and *Shigella* species is of major public health concern. 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 using these antibiotics would depend on the infecting strains.

References

- Abaasa, C.N., Ayesiga, S., Lejju, J.B., Andama, M., Tamwesigire, I.K., Bazira, J. & Byarugaba, F. (2024). Assessing the quality of drinking water from selected water sources in Mbarara City, Southwestern, Uganda. *PLoS One*, 19(3), 134–148.
- Adelowo, O. O., Fagade, O. E. & Agersø, Y. (2014). Antibiotic resistance and resistance genes in *Escherichia coli* from poultry farms, southwest Nigeria. *Journal of Infection in Developing Countries*, 8(9), 1103–1112.
- Ali, H., Bacha, K. & Katema, T. (2011). Bacteriological quality and antibiotic susceptibility of some isolates of well waters used for drinking in Jimma Town, Southwest Ethiopia. *Journal of Health Sciences*, 6, 95–108.
- Baquero, F., Martínez, J. L. & Cantón, R. (2008). Antibiotic and antibiotic resistance in water environments. *Current Opinion in Biotechnology*, 19(3), 260–265.
- Berendonk, T.U., Manaia, C.M., Merlin, C., Fatta-Kassino, D., Ctryn, E., Walsh, F., Burgmann, H., Sorum, H., Nostrom, M., Pons, M., Kreuzinger, N., Huovinen, P., Stefani, S., Schwartz, T., Kisano, V., Baquero, F. & Martinez, J. L. (2015). Tackling antibiotic resistance: the environmental framework. *Nature Review in Microbiology*, 13,310–317.
- Brown, K.D., Kulis, J., Thomson, B. & Mawhinney, D. B. (2006). Occurrence of antibiotics in hospital, residential and dairy effluent, Municipal Wastewater and Rio Grande in New Mexico. *Science of the Total Environment*, 366, 772–783.
- Chanda, W., Manyepa, M., Chikwanda, E., Muma, J. B. & Mwansa, J. C. (2019). Antibiotic resistance of *Salmonella enterica* serovar Typhi and other enteric bacteria: A review. *Journal of Infectious Diseases and Therapy*, 7(2), 1000386.
- Chopra, I. & Roberts, M. (2001). Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews*, 65(2), 232–260.
- Clinical and Laboratory Standards Institute (CLSI). (2018). Performance standards for Antimicrobial Susceptibility Testing, 28th Ed., Supplement M100; Clinical and Laboratory Standards Institute: Wayne, PA (USA).
- Doney, S. C., Ruckelshaus, M., Emmett Duffy, J., Barry, J. P., Chan, F., English, C. A. & Talley, L. D. (2012). Climate change impacts on marine ecosystems. *Annual Review of Marine Science*, 4, 11–37.
- Dwyer, J.O., Hynds, P.D., Adley, C. & Ryan, M.P. (2017). Evaluation of levels of antibiotic resistance in groundwater derived *Escherichia coli* isolates in the Mid-West of Ireland and elucidation of potential predictors of resistance. *Journal of Hydrogeology*, 4,939–951.
- Ekelozie, I.S., Ekejindu, I M., Ochiabuto, O.M.T.B., Obi, M.C, Onwuasonya, U.F. & Obeagu, E.I. (2018). Evaluation of Salmonella species in water sources in two Local Government Areas of Anambra State. *Cohesive Journal of Microbiology and Infectious Diseases*, 1(1), 1–9.
- Ewelike, N., Okoli, C.A., Echendu, M.N. & Enekwa, J.U. (2022). Bacteriological assessments of borehole water in some communities in Owerri West,

- Southeastern Nigeria. *GSC Biological and Pharmaceutical Science*, 18(3), 177–181.
- Frieri, M., Kumar, K. & Bouhn, A. (2017). Antibiotic Resistance. *Journal of Infection and Public Health*, 10, 369– 378.
- Harwood, V.J., Brownell, M., Perusek, W. & Whitlock, J. E. (2001). Vancomycin resistant *Enterococcus* spp. isolated from wastewater and chicken feces in the United States. *Journal of Applied Environmental Microbiology*, 10, 4930– 4933.
- Li, Cao, Y., Zhao, Z.X., Lin, S. & Meng. A.M. (2001). Characterization and expression pattern of poull1, a novel class II POU gene in Zebra fish. *Chinese Science Bulletin Kexue Tongbao*. 46(18), 1523– 1527.
- Lolekha, S., Santisuk, V. & Pavit, P. (1991). Response to antimicrobial therapy for shigellosis in Thailand. *Reviews of Infectious Disease*, 13: S342–S346.
- Nassar, M.S.M., Hazzah, W.A. & Bakr, W.M.K. (2019). Evaluation of antibiotic susceptibility test results: How guilty a laboratory could. *Journal of Egyptian Public Health Association*, 94, 4–9.
- Odonkor, S, T. & Ampofo, J. K. (2013). *Escherichia coli* as an indicator of bacteriological quality of water; An overview. *Microbiology Research*, 4(1), e2.
- Okoh, A. I., Sibanda, T. & Gusha, S. S. (2010). Inadequately treated wastewater as a source of human enteric viruses in the environment. *International Journal of Environmental Research and Public Health*, 7(6), 2620–2637.
- Okoye, E.C.S., Onuorah, S.C., Okoye, L.C. & Nwadiogbu, J.O. (2022a). Effect of seasonal variations on the physicochemical characteristics of spring water in Oji River LGA, Enugu State, Nigeria. *Archives of Agriculture and Environmental Sciences*, 7(1), 86–92.
- Okoye, E.C.S., Ezeokoli, M.C. & Okoye, L.C. (2022b). Microbiological examination of bottled and sachet water sold and consumed in Nnewi metropolis, Anambra State, Nigeria. *International Journal of Multidisciplinary Research and Growth Evaluation*, 3(2), 584–588.
- Okoye, E.C.S., Egudu, N.A., Dibua, N.A. & Okoye, L.C. (2024). Seasonal Variation and antibiotics susceptibility patterns of bacteriological parameters in groundwater sources in Oyi LGA, Anambra State, Nigeria. *Journal of Advances in Microbiology*, 24(5), 40–55.
- Olalemi, A.O., Ige, O.M., James, G.A., Obasoro, F.I., Okoko. F.O. & Ogunleye, C.O. (2021). Detection of enteric bacteria in two groundwater sources associated microbial health risks. *Journal of Water Health*, 19(2), 322–335.
- Sadiya, A., Chukwuma, C.O., Olatunbosun, O.A. & Onyinye, F.N. (2018). Comparative study of the physicochemical and bacteriological qualities of some drinking water sources in Abuja, Nigeria. *Global Journal of Pure and Applied Sciences*, 24, 91–98.
- Siraj, H., Getamesay, M. & Zemenu, Y.K. (2019). Prevalence of Shigella species and its drug resistance pattern a systematic review and meta-analysis. *Annals of Clinical Microbiology and Antimicrobials*, 18(22), 12-22.
- WHO (2008). Guidelines for Drinking-water Quality: Third Edition, Incorporating First and Second Addenda. Geneva: World Health Organization.
- WHO (2017). Guidelines for drinking-water quality: fourth edition incorporating the first addendum. Geneva: Licence: CC BY-NC-SA 3.0 IGO.
- WHO & UNICEF (2014). Progress on sanitation and drinking water. WHO Press, Geneva, Switzerland.